Academic Studies in Health Sciences

EDITORS

Prof. Dr. Engin ŞAHNA
Prof. Dr. Hasan AKGÜL
Prof. Dr. Zeliha SELAMOĞLU

gece kitaplığı

İmtiyaz Sahibi / Publisher • Yaşar Hız
Genel Yayın Yönetmeni / Editor in Chief • Eda Altunel
Kapak & İç Tasarım / Cover & Interior Design • Gece Kitaplığı
Editörler / Edıtors • Prof. Dr. Engin ŞAHNA
Prof. Dr. Hasan AKGÜL
Prof. Dr. Zeliha SELAMOĞLU
Birinci Basım / First Edition • © Haziran 2023
ISBN • 978-625-430-863-5

Gece Kitaplığı / Gece Publishing
Türkiye Adres / Turkey Address: Kızılay Mah. Fevzi Çakmak 1. Sokak Ümit Apt. No: 22/A Çankaya / Ankara / TR
Telefon / Phone: +90 312 384 80 40
web: www.gecekitapligi.com
e-mail: gecekitapligi@gmail.com

Baskı & Cilt / Printing & Volume
Sertifika / Certificate No: 47083

Academic Studies in Health Sciences

June 2023

Editörler

Prof. Dr. Engin ŞAHNA
Prof. Dr. Hasan AKGÜL
Prof. Dr. Zeliha SELAMOĞLU

İÇİNDEKİLER

CHAPTER 1

BIOMATERIALS USED IN THE FIELD OF HEALTH

Nurhan GÜMRÜKÇÜOĞLU[1]

1 Prof. Dr. , ORCID ID https:// orcid.org/0000-0002-9669-6318 Karadeniz Technical University, Vocational School of Health Sciences, Department of Medical Services and Techniques, Trabzon, TURKEY ngumrukcuoglu@ktu.edu.tr

By nature, human is a living organism that struggles with health problems. Although there are systems of protection, prevention, healing properties, there are times when the human body is vulnerable to certain disease-causing organisms. That's why, human beings have always sought ways to overcome their weaknesses, and wanted to replace a lost tooth, leg, arm. While doing this, it aimed to use synthetic material closest to the biological structure.

Synthetic or natural materials that take on the task of living tissue, support it or serve as a part of the system are called biomaterials. Briefly; Structures consisting of a mixture of natural or artificial substances other than drugs used to fulfill a function of the organ or body are defined as biomaterials. Biomaterials are in contact with body fluids continuously or for a certain period of time. Dialysis machines, dental implants, artificial joints, surgical threads, plastic surgery, contact lenses are actually the areas of use of biomaterials that we frequently encounter in our daily lives. It is also used in orthopedic applications as joint prosthesis and bone replacement material, artificial heart parts, heart valve, catheter, fixator material, spinal instrumentation, metal parts, screws, screw washers, eye screws, nails, fixator wires, anatomical plates, hip plates. , angled plates and implantable devices, etc. [1]. One of the most important issues in this area is the selection of suitable biomaterials that will meet the expected requirements. For this purpose, table 1 is considered [2].

Today, intensive researches are carried out in order to develop these artificial and natural materials. Thanks to these R&D studies in the field of biomaterials, materials with superior properties are obtained. Therefore, the types of materials used in different fields are increasing day by day. Thanks to the developments in information technologies, nanotechnology and manufacturing sector, biomaterials with different properties are obtained [3]. The use of biomimetic nanotechnology, which makes use of naturally occurring processes, draws attention in the identification of cells. Thanks to biomimetic approaches, the development of microfluidic devices that enable the identification and separation of cells is provided. These devices achieve results by using the natural process of cell cycles.

Table 1. Use of natural/synthetic biomedical materials [2]

Application Areas Of Materials	Material Types
Sense Organs	
Intraocular Lens	PMMA, Silicon rubber
The inner ear canal	Platinum
Contact Lens	Silicon-acrylate hydrogens
Corneal Bandage	Collagen hydrogels
Skeletal Systems	
Dental Implants	Titanium Titanium Alumina Calcium Phosphate
Joints	Titanium Titanium Alumina Vanadium
Bone filler	Poly (PMMA)
In the treatment of bone deformities	Hydroxyapatite
Thin metal sheets used to fix broken bone ends	Stainless steel, cobalt-chrome alloy
Artificial tendons and ligaments	Teflon, poly
Hearth/Vascular Systems	
Blood vessel prostheses	Poly (ethylene terephthalate), teflon, Polyurethane
Hearth valves	Stainless steel, carbon
Catheters	Silicone rubber, teflon, Polyurethane
Organs	
Artificial heart	Polyurethane

Metals are preferred as biomaterials because they are durable, easily shaped and resistant to abrasion. The disadvantages of metals are low biocompatibility, corrosion in body fluids, being very hard compared to tissues and causing allergic tissue reactions. In the early 1900s, the use of pure metal as implant material was common. The success of these materials increased thanks to the surgical techniques developed in the 1930s, and the use of alloys such as vitalium emerged. The scientists were working on a variety of glass and ceramic materials that could attach to bone tissue due to the corrosion problems of metals. Working in this field, Larry Hench developed bioactive glass or bioglass materials in 1969. Although these materials have high biocompatibility and corrosion resistance, they are hard, brittle, difficult to process, low mechanical properties and high densities. For this reason, orthopedic and dental implants are made of metallic biomaterials and bioceramics, while cardiovascular system and general plastic surgery materials are made of polymers. Non-magnetic polymer composites work in harmony with modern systems such as magnetic resonance and tomography. Since ceramics and metal alloys are radio-opaque, they pose a problem in X-ray radiography. However, radio-transparency

can be adjusted in composite materials. Composites, which are lightweight and exhibit superior mechanical properties, are highly suitable as structural components of imaging devices [4].

Polymer technology has been widely used in fields such as dentistry and cardiovascular surgery thanks to the developments in the 21st century. For example, the use of polymer materials such as PMMA in the treatment of cataracts is still available. Polymers may swell as a result of contact with body fluids or their properties may change during processing. The release of the active substances in the system occurs by the disintegration of the polymer or chemical separation of the drug from the polymer as a result of the osmotic effect or swelling that develops due to the penetration of the solvent into the system. Metals are preferred because of their strength and resistance in areas where the durability of polymer material is weak. However, the disadvantages of metals are their low biocompatibility, corrosion or being much harder than natural tissue. In addition to metals such as titanium with high biocompatibility, polymers with toxic effects are also available. Since materials that come into contact with tissue or blood cause some reactions that pose a potential risk to health, the advantages and disadvantages of the systems should be compared very well before implementation (table 2).

Table 2. Application, advantages and disadvantages of synthetic materials [5]

Materials	Advantage	Disadvantage	Application Area
POLYMERS -Silastic -Teflon -Dacron -Nylon	It is flexible and low density. There is no fabrication difficulty in material production	Since their mechanical strength is low, they disintegrate over time	Surgical threads arterial veins tendons nose ear zygomatic bone
Metals -Titanium Alloy -Vitalyum 316, 316L, S, S	They are durable materials due to their high tensile strength	They have low biocompatibility and high density. They corrode in the body	Orthopedic connectors
Ceramics -Aluminum Oxides -Calsium Aluminates -Titanium Oxides -Carbons	They have good biocompatibility and are inert. Resistant to corrosion and over compression	Due to its low mechanical reliability , it has no stretching feature. They are fabrication difficulties due to the high density	His prosthesis teeth and subcutaneous systems
COMPOSITES Ceramic and carbon coated metals	They have good biocompatibility and are inert. Corrosion resistant and high tensile strength	Material production has fabrication difficulties	Hearth valves, kneecaps and implants

Thanks to the technologies developed in parallel with each other in order to obtain ideal biomaterials, metal, ceramic, polymer and composite materials have been used and continue to be used to undertake many tasks both inside and outside the body. Thanks to the developments in tissue engineering, it is aimed to heal the damaged area by integrating with the patient's own tissue after incorporating living cells into them and placing them in the body, unlike conventional biomaterials. Biomaterials used in the field of biotechnology have various purposes such as replacing damaged tissue, treating, supporting healing or diagnosing a problem. Biomaterials used in biotechnology, waste treatment, industrial biological production and inevitably in the pharmaceutical industry still have insurmountable shortcomings. In this sense, biomaterial science emerges as a versatile and promising field that progresses in cooperation with fields such as medicine, tissue engineering, biochemistry, and physics.

Figure 1. The relationship of biomaterial science with other disciplines

Biomaterials are effective in regenerating materials such as fillers, dental implants and dental tissue. Various filling materials are used to repair dental tissues damaged by dental caries or traumatic reasons. Amalgam alloy is mostly preferred as filling material during restoration of posterior teeth. Amalgam has been used in dentistry as a filling material for over a hundred years. When amalgam powder consisting of copper, silver, tin and zinc is mixed with mercury, a hard and durable amalgam alloy is obtained. The lack of aesthetics and the mercury content are the main negative aspects of this alloy. In this area, studies have been carried out in recent years to develop aesthetic filling materials suitable for the natural color of the tooth.

The first material developed of this type is a composite resin prepared by adding glass-based filler particles into an organic matrix based on Bis-GMA (Bis phenol A-glycidyl dimethacrylate). The most important future goal in composite materials is the development of non-shrinking, even expanding monomers. Organic ceramics called ormoser are used as filling materials with less polymerization shrinkage. Ormoser composite contains inorganic-organic copolymers and inorganic armed filler particles. Aesthetic composite resins are now widely used in restoring anterior and posterior teeth. The bond that composite materials make to tooth enamel is highly resistant. Despite its success in bonding to enamel, it is not a very

suitable texture for bonding filling materials due to its high organic content and water content.

The latest development in this field is the application of acidic adhesives directly to the dentin and enamel tissue without acid pretreatment. This practice is becoming more and more common as it reduces the processing steps. Because composite materials have low modulus of elasticity and high strength, they are widely used in orthopedic applications. In addition, when the composition of the composite material is changed, it becomes easier for the implant to adapt to the mechanical and physiological conditions in the body. For this reason, composite materials are more advantageous in terms of providing structural compatibility compared to homogeneous materials [4].

Biocompatibility

Biocompatibility is the physical, chemical and biological compatibility of a biomaterial to body tissues and the optimum adaptation it provides to the mechanical behavior of the body. A biocompatible material causes inflammation, clot formation, etc., on the surrounding tissues. it is a material that does not have a negative effect [6]. Ceramics, metals, polymers and composite biomaterials can be used as biomaterials and adapt to human health [7]. The reason why ceramics are biocompatible is that they contain ions in the body (calcium, potassium, magnesium, sodium, etc.) and ions that are very few toxins (zirconium, titanium) for the body [8, 9]. Considering the technological developments, although bioinert ceramics cause almost no reaction when they come into contact with the tissue, degradation (chemical dissolution) and mechanical wear are observed in them over time. Alumina, which has high density and purity, is widely used in the construction of hip prostheses and dental implants due to its corrosion resistance, high strength and good biocompatibility [10].

Metallic Biomaterials

Metal and metal alloys, which exhibit superior mechanical properties due to their crystal structures and strong metallic relationships, have a great use in the field of biomaterials. In orthopedic applications, there are areas of use as joint prosthesis and bone replacement material, artificial heart parts, catheters, valves, heart valves in facial and maxillofacial surgery or cardiovascular surgery. The metallic parts of the devices produced for diagnostic and therapeutic purposes in biomedical device technology are made of biomaterial materials. Biomaterials are produced by combining metals such as iron, copper, chromium, cobalt, nickel, titanium, tantalum, molybdenum and vanadium in appropriate amounts. Vanadium Steel is the first metal that has the longest history in the biomedical field and was developed for use in the human body. It has been used as a plate and screw in bone fractures. These

prostheses, which were used until the 1960s, were abandoned after they were corroded in the body, causing serious dangers [11].

The biomaterials produced must be compatible with the body. The suitability of metals to the biological environment is related to their corrosion in the body. Corrosion occurs as a result of metals losing their properties by forming compounds such as oxygen and hydroxide as a result of unwanted chemical reaction with their environment. Fluid in the human body, water, dissolved oxygen, ions such as chloride and hydroxide is a highly corrosive environment for biomaterials. The materials in these environments weaken as a result of corrosion, or the corrosion products that occur enter the cells in the tissue and damage them. In addition, when different metals come into contact with each other in orthopedic applications, a galvanic battery is formed in the body fluid. If the surgical stainless steel wire, cobalt or titanium alloy femoral part in this environment comes into contact with the femur, galvanic battery is formed and galvanic corrosion occurs [12].

The places where orthopedic materials will be used are selected considering the weight, daily activity, mechanical properties and mechanical loads of the person. During their daily activities, living things show a stretching effect of 4 MPa on bones and 40-80 MPa on tendons. The average load on the hip joint is up to 3 times the body weight. During the jumping process, these values can increase up to 10 times the body weight. During daily activity (standing, running, sitting) the stresses in the body are repeated throughout the day. These repetitive movements can cause fatigue, cracking or plastic deformation of biomaterials [1].

Bioceramics

Ceramics designed and produced for use in damaged, diseased or corroded parts of the body in human life are called bioceramics [8, 13]. Bioceramic materials are classified according to their structural functions as bioinert, bioactive and absorbable [14]. Bioinert; They are the ones whose physical and mechanical properties are preserved and resistant to corrosion and wear where they are applied. The most commonly used bioinert materials are alumina, zirconia and some porous ceramics used as supporting implants in bone structure [15]. In particular, zirconia is one of the highest strength ceramic materials suitable for medical use [16]. Bioactive; is the establishment of a direct and strong chemical bond with the tissue. resorbable materials; As it dissolves, it is replaced by host tissue. These materials are used as temporary filling material to bind the tissues together, to temporarily strengthen the weakened tissues and to support the tissues in this way [17, 18]. The most commonly used ceramic materials in this group are; bioactive glasses (BGs), glass-ceramics and calcium phospha-

tase (CaPs), ceramics and cements. The application of these materials to bone replacement began around the 1970s [14]. They are mainly used as filling material in bone injuries because of their ability to connect to the bone without a fibrous connective tissue interface [19, 20].

Ceramics, which form an important group of inorganic materials, are used in a wide variety of applications in the field of health. Tissue culture containers, eyeglasses, diagnostic devices, thermometers, fiber optics used in endoscopy can be counted among these applications. In addition, insoluble porous glasses are used in the field of health as a carrier of enzymes, antibodies and antigens. The resistance of ceramics to microorganisms, solvents, temperature, pH change and high pressure provides a great advantage during application.

Polymeric Biomaterials

One of the most important materials developed for use in the medical field is polymeric biomaterials. Many interactions occur when these biomaterials come into contact with living tissue or physiological fluids. These interactions that occur in biomaterials are frequently encountered in applications in the field of health, as they have bioactivity and antimicrobial properties. In nanotechnological applications made during preclinical and clinical studies, polymers are preferred. When drug-loaded polymeric nanoparticles are examined, features such as prolonged circulation, increased permeability and retention effect, reduced drug side effects, improved drug tolerance and/or better drug bioavailability are encountered. For example, drug-loaded polymeric nanoparticle applications provide increased amounts of drug accumulation in tumor regions [21].

Polymers; It can be prepared from components such as fibers, films, gels, beads, nanoparticles. These components have wide use as biomaterials in applications. For example, polyethylene (PE), polyurethane (PU), polytetrafluoroethylene (PTFE), polyacetal (PA), polymethylmethacrylate (PMMA), polyethylene terephthalate (PET), silicone rubber (SR), polysulfone (PS), polylactic acid (PLA) and polyglycolic acid Many polymer applications such as (PGA) are used in the field of health. There is also the use of polymeric biomaterials for tissue regeneration. It also has various applications such as repair of cartilage, bone, periodontal tissue and nerves. However, their mechanical strength in the field of orthopedics is weak. Mass production is difficult as they are weak against mechanical force. They may swell by absorbing the liquids in their environment or they may secrete toxic products such as unwanted monomers and antioxidants. Also, polymer properties may be affected during sterilization processes (autoclaving, ethylene oxide). When hydrolysis occurs in polymer materials, hydrophilic surfaces are formed. These surfaces cause further

penetration of corrosion products into the polymer. Biocompatibility is very important for biomaterials, it should not cause any reaction, allergy, clot or inflammation in the human body.

Polymeric biomaterials are produced using a wide variety of chemical processes. They can be obtained using a wide variety of sources such as living tissue origin, microbiological origin and petrochemical product. Polymers are grouped according to the properties of their constituent materials. Polysaccharides such as cellulose, polypeptides and nucleic acids such as DNA are natural polymers. Those in the nylon and polystyrene group are synthetic polymers [22].

Natural Polymers

Natural polymers are defined as polymers that are obtained from biological structures found in nature and have unique functional properties. In the ecological environment, proteins such as collagen, gelatin, elastin, actin, polysaccharides such as cellulose, starch, dextran, chitin, and polynucleotides such as DNA and RNA exist as natural polymers. For example, chitin and chitosan are in the polysaccharide group and are found in the exoskeleton of shellfish. Chitin is called the N-acetyl-D-glucosamine polymer chain, while chitosan is a copolymer of D-glucosamine and N-acetyl-D-glucosamine [23].

Since living organisms have complex structures, production costs are quite high. Therefore, large enough quantities are not produced. However, they have different usage areas thanks to their functional features. It is used as a thickener, gelling agent, binder, dispersing agent, lubricant, adhesive and biomaterial. The usage patterns and usage areas of some polymers found in nature are given in table 3.

Polymer and Application Areas

In tissue engineering, research is carried out to create, repair or replace cells, tissues and organs based on the combination of cells and/or cells with biomaterials. Thanks to this approach, it is tried to produce materials that resemble the natural tissue or tissues of the body. Tissue engineering, materials science, medicine and biology are related disciplines [25]. Thanks to these branches of science, the production of synthetic biocompatible materials takes place. Polymers have an important place among these produced materials. Most of the new techniques used in the production phase are obtained by taking advantage of the unique properties of nanoparticles due to large surface area, mass ratio, small size and composition, thanks to these properties, it allows the use of surface ligands to increase the level of detection or to provide faster detection.

Table 3. Usage areas of natural polymers [24]

How polymers are used	Uses of polymers
Solution/gel	Coating of bioprostheses drug delivery systems cosmetic injectable in cosmetic skin defects three-dimensional cell culture
Thin hollow tube	Cell culture matrix nerve cell regeneration tubular tissue material
Sphere/Microsphere	Carrier drug delivery system for cell culture
Membrane	Spinal surgery dialysis membrane tissue guided regenartion cornea protective wound dressing material patches
Sponge	Hemostatic agent Drug deliverysystem Three-dimensional cell culture Wound and skin dressing material
Powder rigid form	Drug delivery system bone filling and repair bone repair

The targeting of nanoparticles is easily functionalized with the help of ligands, and the enhancement of this ability enables the binding and signaling of analytes to provide effective detection. Biosensors are designed by improving these properties of nanoparticles. These studies primarily focused on the use of inorganic nanoparticles, especially metallic or magnetic nanoparticles. Today, gold nanoparticles are used in sensor systems due to their surface structure, especially for signal transduction amplification, these nanoparticles are stated to have unique surface chemistry [26].

Monomers are formed when carbon and hydrogen atoms are properly combined. The simplest of such formations is called ethylene ($H_2C=CH_2$) and many ethylene molecules are linked by covalent bonds to form a polymer chain called polyethylene. Polymers with this hydrocarbon chain are defined as organic polymers. However, there are polymers that consist of atoms other than hydrogen and carbon atoms. For example, polymer chains composed of silicon, nitrogen or phosphorus atoms are called inorganic polymers. Polymer chains can be linear or branched. These structures are formed by the attachment of other chains as side branches to the polymer main chain. When these side branches are linked with another main chain, cross-linked polymers are formed.

High molecular weight compounds in long chain or branched structure are obtained when many same or different atomic groups are held together by chemical bonds with little or no cross-linking, Van der Waals bonds, hydrogen bonds or primary covalent bond strengths. It is the result of this branching that the solubility of these polymers obtained in this way becomes difficult in suitable solvents, they do not dissolve in cross-linked structures, but only absorb the solvent into their structures [27]. It is expected that the polymers in the body are not affected by factors such as temperature and pH, so some chemicals are used in the construction phase. However, the chemicals used have the potential to leak into the human body over time. This creates a disadvantage for polymers. Thanks to its cross-linking properties, there are improvements in its mechanical properties. Thanks to this bonding, materials increase their density, improve their strength and hardness, which causes cross-linked materials to lose their flexibility [28].

The behavior of biomaterials in the biological environment plays an important role in the selection of polymer types to be used. This issue should be taken into account in the process of forming cells, tissues and organs. Otherwise, the expected result from the treatment process cannot be obtained. For example, high-density polyethylene material should be used for polymeric biomaterials to be used in hip prostheses. Screws used in fracture treatments should have a self-degradable polymer structure without showing toxic properties after fulfilling their function [29].

The properties of polymers differ greatly from their constituent monomers. Thanks to the advanced production techniques used today, complex design applications can be easily realized. Thanks to this approach, an advantage is provided in the artificial tissue, organ or device construction phase. For example, materials to be used in orthopedic implant applications are required to exhibit high strength and low stretching properties. This feature is seen in the application forms of polyethylene (PE) with high molecular weight. Polyethylene material can be elastic, flexible or rigid depending on its production. The soft surface and high molecular weight of polyethylene give it low friction properties. It is long-lasting when used in artificial joints. Polytetrafluoroethylene (PTFE), known as Teflon, has low density, elastic modulus, surface tension, friction coefficient and high crystallinity. Thanks to this feature, it is preferred in the production of artificial veins. The hydrophobic form of Teflon known as GoreTex is used in vascular prostheses. Although PTFE has a very stable structure both thermally and chemically, it is a very difficult polymer to process.

This very hydrophobic structure has excellent lubricity. Polyethyleneterephthalate (PET) is preferred in the suturing of heart valves. Polyamides, on the other hand, are used as surgical thread or wound dressing material due to their workability. PMMA (polymethyl methacrylate) is

a hydrophobic, linear chain polymer that is glassy at room temperature. It is commercially known as Lucite and Plexiglas. They are widely used in intraocular lenses, prosthetic eyes and hard contact lenses due to their light transmittance, hardness and stability. In addition, PMMA is used as a filling material in the form of a collagen mixture, lip thickening, hip and breast augmentation in aesthetic operations. Uniform hydrophilic monomer groups, poly(2-hydroxy ethyl methacrylate) (PHEMA), poly(glycerylmethacrylate), poly(3-hydroxy propylmethacrylate) and poly(hydroxy alkyl methacrylate) are examples of such hydrogels [30]. Three-dimensional structures consisting of cross-linked polymeric structures, high molecular weight, swellable in water at physiological temperature and pH, but insoluble are called hydrogels. There are gels that can react in ambient conditions such as ion strength, pH, temperature and electric current. These gels are called smart gels or stimulus-responsive gels. Three-dimensional hydrophilic network gels contain high amounts of water in their structure. They also preserve their structural integrity and flexibility to a certain extent. They consist of hydrophilic functional groups such as OH, COOH, $CONH_2$ and SO_3H. They swell as a result of absorbing the water in the body, but they do not dissolve. They are obtained by utilizing natural or synthetic polymers. Hydrogels obtained from natural polymers show partial mechanical strength. May contain microorganisms that can cause disease. It can trigger factors such as inflammation/immunity in the body. Bioactive properties are not seen in synthetic hydrogels [31]. Hydrogels are inert during the biological reaction. They are highly resistant to degradation in the body, they are not absorbed by the body. They can be sterilized by heat and prepared in different forms and shapes. It also has properties such as high oxygen permeability, good mechanical stability and favorable refractive index. Thanks to its water content, it shows great resemblance to natural textures. We see the first application of these properties in hydrogels in contact lenses. It is also used as artificial kidney membrane, artificial skin, bioadhesive material in wound healing, and biomaterial in aesthetic surgery. Today, one of the most important application areas is controlled drug release systems in pharmacy. For example, the control of insulin secretion is achieved with the help of smart hydrogels that can release more insulin when the glucose level rises.

Synthetic biocompatible polymers have different applications in areas such as dental, eye, orthopedics, cardiovascular, drug release and suture. In these applications, different design requirements are needed as well as different features. Because it is necessary to produce materials with the desired properties in the construction of artificial tissues, organs or devices. Otherwise, unsuitable artificial tissue, organ or device will cause undesirable toxic effects in living organisms. Implant materials used in the body

must be in accordance with the characteristics of the structure to be replaced. For this reason, synthetic biocompatible materials used in the field of health should be carefully selected. Some of the synthetic biocompatible polymers used in the field of health today are given in table 4.

Table 4. Some usage areas of synthetic polymers

Synthetic Biocompatible Polymers		
Applications	Features And Design Requirements	Production Materials
Dental	-Stability and corrison resistance, plasticity -Durable and can be coated -High adhesion/ texture compatibility -Low allergenicity	-Dentures/Filling for PMMA -Polyamides -Poly (Zynakrilatlar)
Eye	-Gel/Film forming ability -Oxygen Permeability	-Polyacrylamide gels -PHEMA and Copolymers
Orthopedic	Ability to resist mechanical restraint and deformation -Biocompatible with bones and muscles	-PE, PL, PG -PMMA -PLG
Heart/Vein	-Deformation resistance -Lubricity -Sterilization	-Silicon -Teflon -Polyurethane -PEO
Drug release	-Favorable drug release profile -Drug biocompatibility -Biodegradability	-Silicon -HEMA -PLG, EVA -PCPP-SA
Seams	-Good tensile strength, holding strength -Flexibility, knot retention, low texture drag	-Nylon -PLG, PTMC-G -PP, PB-TE

CONCLUSION

Let's take a look at the past and the present before giving the targets for the future in biomaterials. In the past, when a tissue was damaged or lost its function, the solution was to remove that tissue. However, later on, different areas of application developed depending on the discovery of new antiseptics, penicillin and other antibiotics, hygiene and vaccinations. In this case, replacing the damaged tissue with a healthy tissue gained importance. In transplantation (transplant), the patient's own tissue and tissues taken from another human or animal are used. Implants (placement) have a limited lifespan. In case of bioactive fixation to tissues, the life of orthopedic prostheses is extended. Reconstruction of tissue structure includes regaining tissue function, metabolic and biochemical behaviors, and biomechanical performance.

In recent years, new biomaterials have been developed and used in

the biomedical field thanks to the researches carried out in line with the needs. Thanks to the combination of different branches of science and the use of advanced technologies, more biocompatible biomaterials are produced. Multidisciplinary studies carried out by branches of science such as material science, tissue engineering, physics, chemistry, biology and medicine will shed light on the future of biomaterials and will positively affect modern medicine. Thanks to different perspectives in the field of health, there will be changes in treatment methods. For example, it will allow the determination of new design criteria in many areas such as early detection, biomaterials and release systems.

This will pave the way for increased production of many biomaterials, from artificial hearts to hip replacements. Many materials, especially restorative materials, should be evaluated for their effects on tissues before using biomaterials such as dentin, pulp, and periodontal. In addition to the physical and mechanical properties of the materials, biocompatibility aspects should also be investigated. Because the success rate in applications increases thanks to the selection of reliable materials. For this reason, the polymeric biomaterials to be used must have superior properties. Studies on this subject; replacing the damaged tissue with a healthy tissue is done for the restructuring of natural tissues by using the body's capacity to renew itself. For this, it is necessary to develop polymeric biomaterials that will enable the restructuring of natural tissues.

Tissue regeneration can be achieved by preparing tissue scaffolds from porous, organic and inorganic porous polymeric materials that can dissolve in the body at controllable rates and have controllable surface properties. This will be carried to a higher level thanks to the development of smart materials. Shape memory materials; shape memory alloys, shape memory ceramics, shape memory polymers, shape memory gels and shape memory hybrid materials are smart materials developed for this purpose. As the number of these materials increases, the treatment stages will rise to much higher levels.

As a result, we can say that biomaterial science is an effort to make our living conditions and the world we live in more livable by producing materials that are compatible with biological structures.

REFERENCES

1. Gümüşderelioğlu, M. (2002). Tıbbın geleceği biyomalzemeler. *Bilim ve Teknik Dergisi*

2.Cömert, Z.Y., Cömert, I. Bakkaloğlu, A. (2004). Toz metalurjisinde kullanılan biyomalzemeler, *11th International Materials Symposium*, 161-165.

3. Myung, J.H., Gajjar, K.A., Saric, J., Eddington, D.T., Hong, S. (2011). Dendrimer-mediated multivalent binding for the enhanced capture of tumor cells. *Angew. Chem. Int. Ed*., *50*, 11769- 11772. DOI: 10.1002/anie.201105508.

4. Bilim Teknik Temmuz 2002

5. documents.tips/documents/biyomateryaller-ve-kullanim-alanlari-bitirme-tezi.html (28.04.2017).

6. Güven, Ş.Y. (2010). Ortopedik malzemelerin biyouyumlulukları ve mekanik özelliklerine göre seçimi. *Ulusal Tasarım İmalat ve Analiz Kongresi*, 472-484.

7. Gür, A.K., Taşkın, M. (2004). Metalik biyomalzemeler ve biyouyum. *Doğu Anadolu Bölgesi Araştırmaları.*

8. Tüylek, Z. (2017). Biomaterials and use their in health. *Bozok Med. J. 7*, 4, 80-89.

9. Cheng, L., Ye, F., Yang, R., Lu, X., Shi, Y. et al. (2010). Osteoinduction of hydroxyapatite/βtricalcium phosphate bioceramics in mice with a fractured fibula. *Acta Biomater.*, *6*, 1569-1574. DOI: 10.1016/j.actbio.2009.10.050

10. Güven, Ş.Y., Çetin, H. (2007). Metalik biyomalzemeler ve implantlar. *S.D.Ü. 15. Yıl Mühendislik Mimarlık Sempozyumu*, 175-181.

11. Park, J.B. Kim, Y.K. (2000). Metallic biomaterials. *The Biomedical Engineering Handbook, Second Edition*, CRC Press LLC.

12. Corces, A. (2004). Metallic alloys. *Medicine Instant Access to the Minds of Medicine*, Section 1 of 11.

13. Fillingham, Y. A., Cvetanovich, G.L., Haughom, B.D., Erickson, B.J., Gitelis, S. (2016). Bioceramic bone graft substitute for treatment of unicameral bone cysts. *J Orthop Surg*., *24,* 222-227. DOI: 10.1177/1602400220

14. El-Ghannam, A. (2005). Bone reconstruction: from bioceramics to tissue engineering. *Expert Rev. Med. Dev*., 2, 87-101. DOI: 10.1586/17434440.2.1.87

15. Williams, D.F (1999). The Williams dictionary of biomaterials. *Liverpool University Press*, Liverpool, p. 368.

16. Villermaux, F. (2000). Zirconia-alumina as the new generation of ceramic-ceramic THP: wear performance evaluation including extreme life conditions. *In Transactions of the Sixth World Biomaterials Congress*, *Minneapolis: Society for Biomaterials*, p. 878.

17. Tamimi, F., Le Nihouannen, D., Eimar, H., Sheikh, Z., Komarova, S., et al.

(2012). The effect of autoclaving on the physical and biological properties of dicalcium phosphate dihydrate bioceramics: brushite vs. monetite. *Acta Biomater*., 8, 3161-3169. DOI:10.1016/j.actbio.2012.04.025

18. Kümbüloğlu, Ö., Oral, O. (2013). Biyomateryaller. *EÜ Dişhek Fak Derg*., 34, 1, 27-33. DOI: 10.5505/eudfd.2013.15238

19. Temenoff, J.S., Mikos, A.G. (2000). Tissue engineering for regeneration of articular cartilage. *Biomaterials*, 21, 431-440. DOI: 10.1016/s0142-9612(99)00213-6

20. Agrawal, C.M., Ray, R.B. (2001). Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. *J. Biomed. Mater. Res*., 55, 141-150. DOI: 10.1002/1097-4636(200105)55:2<141::aid-jbm1000>3.0.co;2-j

21. Deng, C., Jiang, Y.J., Cheng, R., Meng, F.H., Zhong, Z.Y. (2012). Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: promises, progress and prospects. *NanoToday*, 467-480. DOI:10.1016/J.NANTOD.2012.08.005

22. Gibas, I., Janik, H. (2010). Synthetic polymer hydrogels for biomedical applications. *Chem. Chem. Technol*., 4, 4, 298-304. DOI:10.23939/CHCHT04.04.297

23. Jiang, T., James, R., Kumbar, S.G., Laurencin, C.T. (2014). Chitosan as a biomaterial: structure, properties, and applications in tissue engineering and drug delivery, natural and synthetic biomedical polymers. In: Sangamesh Kumbar, CatoLaurencin, MengDeng (ed.), Chapter 5, ElsevierInc. ISBN: 978- 0-12-396983-5, 91-107.

24. Rivron, N.C., Liu, J., Rouwkema, J., De Boer, J., Van Blitterswijk, C.A. (2008). Engineering vascularised tissues in vitro. *Eur Cell Mater*., 27-40. DOI: 10.22203/ecm.v015a03

25. Saha, K., Agasti, S.S., Kim, C., Li, X., Rotello, V.M. (2012). Gold nanoparticles in chemical and biological sensing. *Chem Rev*., 112, 2739-2779. DOI: 10.1021/cr2001178.

26. kimyaca.com/biyouyumlu-polimerik-malzemeler (28.04.2017).

27. Lee, B., Khang, H., Lee, G.L.J., Ed Bronzino, J.D. (2000). Polymeric biomaterials. *Biomed. eng. handb*., Second Edition.

28. Bonfield, W., Taner, E. (1997). Hydroxyapatite composite biomaterials–Evolution and applications. *Materials World*, 18- 20.

29. Keskin, A.O. (2000). Hidroksiapatit seramiklerin mekanik özelliklerinin zirkonya ilavesi ile geliştirilmesi. *Yüksek Lisans Tezi*, İTÜ, İstanbul.

30. NPCS board of consultants & Engineers. Disposable Products Manufacturing Handbook, 2014, ISBN: 9789381039328.

31. Bajpai, A.K., Shukla, S.K., Bhanu, S., Kankane, S. (2008). Responsive polymers in controlled drug delivery. *Prog. Polym. Sci*., 1088-1118. DOI:10.1016/j.progpolymsci.2008.07.005

CHAPTER 2

BODY MASS INDEX, FOOT POSTURE, AND KNEE PATHOLOGIES

Ebrar ATAK[1]

1 PhD, Assistant Professor, Istanbul Gedik University, Faculty of Health Sciences, Department of Physiotherapy and Rehabilitation. Orcid ID: 0000-0001-9426-7010

INTRODUCTION

Body Mass Index (BMI) is a simple, useful, and valid method that is scientifically accepted and used to calculate whether people's body weights are within normal limits. Studies have shown that being overweight is a negative factor in terms of knee pathologies in individuals with high BMI (Burton et al., 1985). When the relationship between BMI increase and knee pathologies is considered, it is suggested that there is an increase in mechanical strain due to excess load on the knee joint, which leads to joint degeneration and pathologies. Additionally, situations where the anatomical alignment in the knee joint is disrupted, such as varus or valgus, can cause some knee pathologies, and conversely, varus or valgus deformities can develop after some knee pathologies (Must et al., 1999). It is believed that these pathologies develop secondary to degenerative changes in the knee. However, it is also known that changes in the foot arch affect knee biomechanics. In deformities such as pes planus or pes cavus that occur in the foot, it can be said that these pathologies easily disrupt the biomechanical alignment in higher-level joints and cause various problems, considering that the body is a closed kinetic chain. When looked at from the perspective of human creation and existence, the foot is one of the most basic indicators of human characteristics. Humans, who still have the quality of possessing the most advanced structure among living forms, stand on two feet and differentiate themselves from other living beings by turning all these advanced functions into action on two feet.

In this context, human foot structure has very distinct features compared to all other living beings since humans spend most of their functional life exerting great effort against gravity on two legs. Additionally, considering static and dynamic loads, the foot needs to withstand all processes and loads while maintaining a balanced, ambulatory, mobile, yet stable structure. Even under normal physiological and anatomical conditions, being able to withstand all of these loads is a magnificent design and existence wonder. However, all of these anatomical and physiological processes may not always be balanced for everyone.

The muscles that affect the foot, the dynamic structures that make up the foot and its arch, the skin that makes up the sole of the foot, congenital or acquired problems, systemic diseases, incorrect shoe choices, and most importantly, the direct effects of the loads on the foot can all cause the foot to be mechanically and dynamically stressed while transferring the vertical loads on the body to the ground, leading to pathological processes related to the foot. Foot arch pathologies have been addressed by many researchers, and detailed classifications have been made regarding their diagnosis, classification, etiology, and pathophysiology. However, there are not many studies in the literature that examine how the knee is affected by foot arch

problems, the role of body weight in this, how much the foot is affected by body weight, and the consequences of this in a specific age group of healthy sedentary individuals. Although there are studies examining foot arch pathologies in children, geriatric individuals, and certain diseases, and it is known that excess weight is a significant factor in knee pathologies, there are no studies on how excess weight affects the foot arch and what kind of results it may lead to in the knee, if any (Barry, R. J., & Scranton, P. E., Jr. 1983). The purpose of this study is to investigate whether the pathology and biomechanical changes that occur in the knee due to BMI are directly related to mechanical stress caused by weight or secondary to changes caused by excess weight in the foot arch (Hintermann, B., & Ruiz, R. 2021).

Body Mass Index

Body Mass Index (BMI) is an indirect method used to measure the relationship between weight and height, and to determine whether body weight is within normal limits. BMI is consistent with methods that directly measure body fat, such as submersion in water or radiological fat measurement, and is calculated by dividing body weight in kilograms by the square of height in meters. Due to its simplicity, affordability, and non-invasiveness, BMI is used more frequently in clinical settings compared to other methods. Studies have also shown that BMI levels are consistent with body fat and potential health problems. Widespread and long-term applications of BMI measurements have contributed to community-based research and provided health professionals with information on the weight of the population and potential risks associated with it, as well as temporal, regional, and statistical subgroups. The clinical limits of BMI should be well-defined. BMI is an indirect method that relates body weight to body fat. Age, gender, ethnic origin, and muscle mass are factors that affect body weight and fat. BMI does not distinguish between excess fat, muscle, or bone mass, nor does it determine individual differences in fat distribution. Nevertheless, studies have shown strong correlation between BMI and radiographic or other laboratory methods that measure body fat. (Lean, M.E., Han, T.S., & Seidell, J.C. 1999).

The BMI scale for adults aged 20 and over is shown in the following table 1.

Table 1. Body Mass Index Scale

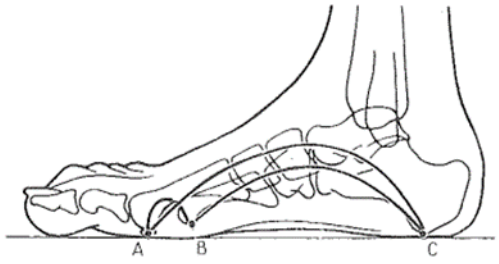
BMI Range	Classification
Below 18.5	Underweight
18.5 - 24.9	Normal weight
25.0 - 29.9	Overweight
30.0 - 34.9	Class I obesity
35.0 - 39.9	Class II obesity
40.0 and above	Class III obesity (severe obesity)

Pes Planus

The foot is a complex structure that needs to be rigid to support body weight, dynamic to facilitate walking, and flexible to adapt to the external environment. The development of body segments, including the foot, is influenced by genetics, nutrition, hormones, and internal/external forces both during intrauterine and postnatal life. The musculoskeletal system, being the most susceptible to mechanical forces, can be affected by abnormal internal and external forces, resulting in various deformities (Bernhardt D. B. 1988).

The foot, which carries body weight and has shock-absorbing properties, resembles a tripod connected by arches and carrying weight at three points during normal development (Figure 1) (Salathé, E. P., Jr, Arangio, G. A., & Salathé, E. P. 1990).

Figure 1. Foot bearing weight from three points.



The foot is a complex structure that has to carry the body weight, provide walking function, and adapt to external conditions. The development of body segments in proportion and synchronized during intrauterine and postnatal life is influenced by factors such as genetics, nutrition, hormones, and internal and external forces. Mechanical forces are most effective on the musculoskeletal system, and it is clear that various deformities can

develop when internal and external forces are less or more than normal.

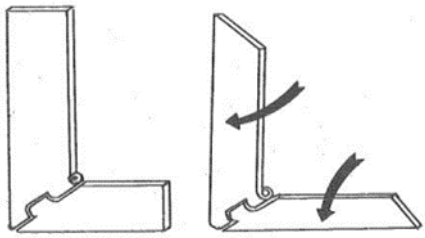
In the normal developmental process, the foot, which carries body weight and has shock-absorbing properties, resembles a tripod connected by arches at three points (Figure 1). The medial longitudinal arch is formed by the calcaneus, talus, cuneiform bones, and the first three metatarsal bones. The lateral longitudinal arch is formed by the calcaneus, talus, cuboid, and the fourth and fifth metatarsal bones. The transverse arch is formed by the heads of the metatarsals. The first and fifth metatarsal heads settle lower than the others, becoming load-bearing (Rodgers M.M. 1988).

When weight is placed on the arch, its shape can be passively maintained by the plantar fascia, ligaments, and muscles. Under static and dynamic conditions, the shape of the medial longitudinal arch is maintained by the plantar fascia, plantar calcaneonavicular ligament, talocalcaneal ligament, peroneus longus, tibialis posterior, flexor digitorum longus, flexor hallucis longus, and abductor hallucis longus muscles. Electromyographic studies have shown that ankle and foot muscles do not have a protective effect on the arch under static conditions, but tibialis posterior and flexor digitorum longus muscles especially assist in maintaining the arch under dynamic conditions (Hintermann, B., & Ruiz, R. 2021).

Dynamic Conditions of the Foot

When weight is loaded onto the foot, the position of the foot changes along with the pelvis through the ankle joint, subtalar joint, and transverse tarsal joint. The oblique axis of motion of the subtalar joint is important for transferring rotations from the tibia and fibula to the foot (Figure 2) (Morris J. M. 1977).

Figure 2. Axis of motion of the subtalar joint.



During walking, there is a 5-degree internal rotation in the tibia with heel strike. This rotation increases just before foot contact and is transferred to the foot through the tibiotalar and subtalar joints. During heel strike, the

part of the heel that contacts the ground remains lateral to the center of the ankle. Therefore, the body weight transferred to the talus creates a pronation moment in the subtalar joint, loading the medial longitudinal arch. The lateral rotation that occurs in the tibia at foot contact provides supination in the foot. This situation increases the necessary stability for bearing body weight in the medial arch and transverse tarsal joint. The greatest effect of the weight borne on the foot appears during the push-off phase, which also requires maximum stability. During the push-off phase, the plantar fascia arch is elevated due to hyperextension of the metatarsophalangeal joints, and the foot becomes supinated, providing maximum stability.

Etiology and Pathomechanics

The loss of the normal height of the medial longitudinal arch, causing it to collapse, is referred to as pes planus or flat foot, due to various reasons (Turner et al., 2020).

Types:

1. Flexible Pes Planus: When weight is placed on the medial longitudinal arch, the arch collapses, and when the weight is lifted, the curve of the arch reappears.

2. Rigid Pes Planus: The shape of the arch remains the same both when weight-bearing and when the weight is removed, and the height of the arch is below normal.

A) Types

Rigid Pes Planus:

a) Congenital Convex Pes Valgus

b) Tarsal Coalition

Flexible Pes Planus:

a) Talipes Calcaneovalgus

b) Talipes Valgus due to Gastro-Soleus muscle contracture

c) Sustentaculum Tali Hypoplasia

B) Acquired Causes

Ligamentous hyperlaxity and genetic causes:

a) Ehlers-Danlos syndrome

b) Down syndrome

c) Marfan syndrome

d) Osteogenesis Imperfecta

Muscle weakness and imbalance:

a) Tibialis Posterior muscle weakness

b) Muscular Dystrophies

c) Peripheral nerve injuries

d) Involvement of the Spinal Cord

e) Cerebral Palsy

C) Arthritic causes:

a) Inflammatory conditions of the subtalar and transverse tarsal joints

b) Traumatic arthritis

D) Causes related to contracture:

a) Myostatic contracture of the Peroneal muscles

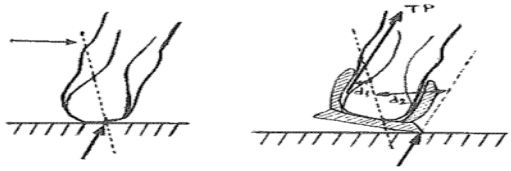
b) Acquired contracture of the Gastro-Soleus muscle.

In pes planus analysis performed using various imaging techniques, the most important finding is the plantar deviation of one or all of the talo-calcaneal, talonavicular, and naviculocuneiform joints that make up the arch. Due to the looseness of ligaments, the weight of the body on the hypermobile foot pushes the calcaneus under the talus into pronation. The anterior part of the calcaneus shifts laterally and dorsally while the talus rotates medially and downwards. As a result of the elongation of the plantar calcaneonavicular ligament over time, the ability to support the head of the talus is lost. Abduction occurs in the navicular with the horizontal movement of the talonavicular joint, and the forefoot follows this movement. Normally, the weight line passes between the second and third toes, but valgus of the foot causes the weight line to pass over the first metatarsal or even more medially. In children, the foot is actively adducted to move the weight line laterally. Some researchers believe that the adduction and supination deformities of the foot are not a primary problem, but rather a secondary development to compensate for pes planus. When viewed from the lateral side, the long axes of the talus, navicular, and first metatarsal align with each other. This axis also makes a perpendicular angle with the line drawn through the middle of the navicular in parallel with the proximal articular surface of the navicular. An increase in the downward angulation between the long axis of the talus and the long axis of the navicular or first metatarsal is one of the findings of pes planus. Normally, the average angle between the long axis of the talus and the horizontal plane is 26.5 degrees, which increases with pes planus.

In a posteroanterior view, medial deviation occurs with plantar flexion

of the talus, while the anterior portion of the calcaneus slides laterally and the talocalcaneal angle rises above normal (figure 3). If the foot were a rigid structure, it would lose function after any deformity. However, due to its flexibility, the foot compensates for many structural abnormalities through the subtalar and midtarsal joints. If the deformity is corrected and these joint compensations persist for a long time, secondary or compensatory problems may occur. Due to varus in the front of the foot, first the 5th metatarsal head and then the 1st metatarsal head make contact with the ground under the effect of body weight. This results in excessive pronation at the subtalar joint, rather than the normal position of the forefoot, and compensates for the varus. Overpronation at the subtalar joint leads to ligamentous damage over time, the talus is not adequately supported, and eventually the talus shifts medially and plantarly (Tiberio D. 1988). In dynamic conditions, the tibialis posterior muscle is the most important supporter of the medial arch. Studies have shown that this muscle produces up to 9 kg of force. Especially in athletes who participate in running sports, the supporting ability of the medial longitudinal arch (MLA) of the tibialis posterior muscle decreases due to fatigue caused by excessive use and injuries over time, leading to collapse of the arch (figure 3).

Figure 3. Vector loads causing arch collapse.



Due to arthritis, the limited movement of the ankle joint is compensated through the subtalar joint, and the medial displacement of the talus head in the plantar direction initiates flat feet. Degenerative changes occurring in the talonavicular and calcaneocuboid joints cause the medial displacement of the talus head, leading to abduction of the front of the foot. This abduction of the forefoot causes the body weight to shift more medially, which accelerates the collapse of the medial longitudinal arch (MLA). Flat feet caused by spastic peroneal muscles are rare. Generally, pain in the subtalar joint occurs due to the hypertonus of the peroneus brevis. Pronation increases in the subtalar joint due to increased pressure in the interosseous and talocalcaneal ligaments. Spasticity causes contractures in the soft tissues, eventually leading to foot pronation and the formation of rigid flat feet over time. Natural flat feet are present in children up to the age of 3-4 years due to the fat tissue under the foot, and this appearance should

disappear by the age of 7. If the MLA does not start to form at these ages, it is indicated that there is a tendency for flat feet. In conclusion, flat feet disturb the mechanical balance of the foot, cause mechanical problems in these regions, and also lead to some compensatory postural misalignments. Due to its various negative effects, flat feet should be evaluated well, foot mechanics and pathomechanics should be well known, and the causes should be well analyzed.

Knee Pathologies

Knee Joint Anatomy

The knee joint is a hinge type of joint that primarily allows flexion and extension movements. Joint stability is provided by static and dynamic structures. Static structures consist of the capsule and ligaments, while dynamic structures consist of muscles and tendons (Landsmeer et al., 2019).

Bones

The knee joint is formed between the femur, tibia, and patella. The fibula is not included in this joint.

Femur

The distal end of the femur that forms the joint surface consists of two condyles that join at the intercondylar notch. The condyles show an asymmetric structure in terms of size and shape. The medial condyle is larger and has a more symmetrical curvature. The curvature of the lateral condyle increases more sharply at the back.

Tibia

The tibial joint surface is composed of the medial and lateral tibial condyles, and the intercondylar prominence that separates them. The medial condyle is concave, while the lateral condyle is slightly convex. The tibial condyles are inclined backwards by about 80-100 degrees. The medial prominence is the starting point of the anterior cruciate ligament (ACL), and the lateral prominence is the starting point of the posterior cruciate ligament (PCL).

Patella

The patella is a sesamoid bone located within the extensor mechanism of the knee, between the quadriceps and patellar tendons. Three-quarters of the back surface articulates with the femur's trochlea, while the remaining quarter does not participate in this joint. The joint surface is divided into medial and lateral facets by a central ridge. The medial facet is small, oblique, and convex, while the lateral facet is larger, wider, and concave. There is a 130-degree angle between the facets.

Menisci

The small contact surface created by the mismatch between the condyles of the femur and tibia in the knee joint is eliminated through the fibrocartilaginous structures called menisci located between the bones. These structures, which are C-shaped and have a triangular cross-section, sit on the tibial condyle and are tightly attached to the surrounding capsule and intercondylar distance with ligaments. Although both menisci show similar shape and structure to each other, they exhibit differences that reflect their functions. The triangular cross-section of the menisci has three surfaces. The upper surface is concave and in contact with the femoral condyles. The lower surface is flat and in contact with the tibial condyle. Menisci are extra-synovial structures and exhibit characteristic nutrition. They are nourished by the superior and inferior branches of the medial and lateral genicular arteries. The vessels that enter the meniscosynovial compound form a "perimeniscal capillary plexus." This plexus nourishes the 25-33% circumferential part of the meniscus. Studies investigating the innervation properties of the menisci show the presence of proprioceptive receptors. Therefore, menisci also function as a proprioceptive sensory organ that protects the joint from excessive stress.

Medial Meniscus

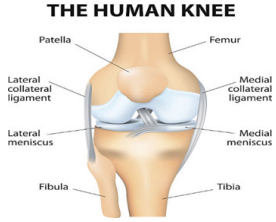
It is approximately 3.5 cm in size and semi-circular in shape. Its posterior horn is tightly attached to the posterior intercondylar area. It also has a strong fibrous connection with the posterior oblique ligament and the semimembranosus tendon. The middle third, about 1/3, is attached to the joint capsule, femur, and tibia on the periphery. The capsular ligaments on the tibial side are also called the coronal ligament. The anterior horn is attached to the anterior intercondylar area. The medial meniscus shows a very tight connection with the tibia and joint capsule. Due to the tight attachment, the medial meniscus is less mobile and more prone to injury.

Lateral Meniscus

The structure of the lateral meniscus is more circular and covers a significant portion of the joint surface. Its attachment points are located at the lateral and posterior intercondylar areas just next to the anterior cruciate ligament for the anterior horn and at the posterior intercondylar area just in front of the attachment area of the posterior horn of the medial meniscus for the posterior horn. Two ligamentous structures extend from the posterior horn of the lateral meniscus to the medial femoral condyle and intercondylar fossa. They are named based on their relationships with the anterior cruciate ligament, with the anterior one being called the anterior meniscofemoral ligament (also known as Humphry's ligament) and the posterior one being called the posterior meniscofemoral ligament (also known as

Wrisberg's ligament). The connection between the lateral meniscus and the joint capsule is disrupted at the posterior horn due to the presence of the popliteus tendon within the joint, which does not have any attachment to the lateral collateral ligament. This makes the lateral meniscus more mobile and less susceptible to injuries.

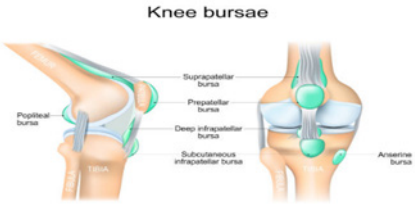
Figure 4: Structure of the bones and ligaments of the knee joint. (www.istockphoto.com/tr)



Synovial Membranes and Bursae

The knee joint is the largest synovial cavity in the body, and its synovial membrane attaches to the front of the patella edge and extends distally from the medial and lateral retinacula. It covers the infrapatellar fat pad, which extends downward and backward from the lower pole of the patella. A synovial fold, also known as the ligamentum mucosum or ligamentum infrapatellare, extends into the intercondylar notch in the middle and front of the joint. Additionally, the synovial membrane lines the joint capsule from the inside of the femoral condyles on both sides. To ensure smooth functioning of the joint capsule and tendons around the knee, several bursae are present. These include the prepatellar bursa, the bursa located under the iliotibial band, the infrapatellar bursa, the bursa located under the lateral collateral ligament and capsule, the bursae under the medial and lateral heads of the gastrocnemius muscle, the biceps and semimembranosus bursae, the bursa located between the superficial and deep layers of the medial collateral ligament, and the pes anserinus bursa.

Figure 5: Appearance of the bursae of the knee joint. (www.istockphoto.com/tr)



Capsules and Ligaments

The fibrous capsule of the knee joint also functions as a ligament in different areas where it thickens. Therefore, the ligaments, which are the most important static stabilizers of the knee joint, are examined together with the joint capsule.

Anterior Complex

1. Quadriceps femoris muscle 6. Vastus medialis obliqus (VMO)
2. Vastus medialis muscle (VM) 7. Patellar ligament
3. Vastus intermedius muscle (VIM) 8. Infrapatellar fat pad
4. Rectus femoris muscle 9. Medial retinaculum
5. Vastus lateralis muscle (VL) 10. Lateral retinaculum

Quadriceps Tendon: It is the tendon formed by the fusion of the four components of the quadriceps muscle. It originates a few centimeters above the patella and extends down to its lower part.

Patellar Tendon: It attaches proximally to the lower edge of the patella and distally to the tibial tuberosity. The superficial fibers of the approximately 6 cm tendon merge with the quadriceps tendon proximally.

Medial and Lateral Retinaculum: The medial and lateral longitudinal retinaculums are fibrous tracts that originate from the VM and VL. They run parallel to the patellar tendon and attach to the tibia. They serve as a reserve extensor mechanism.

Infrapatellar Fat Pad: It is located in the anterior part of the knee joint between the patellar tendon and the synovial membrane. It has a shock absorption function during the most powerful contraction of the quadriceps muscle. The fat pad supports the vascularization of the anterior cruciate ligament and plays a role in its revascularization after repair (Sukerkar, P. A., & Doyle, Z. 2022).

Medial Complex

The structures that provide coverage and support to the medial side of the knee joint can be analyzed in three layers. The first layer is situated in the front and comprises the sartorial fascia. The second layer is composed of the superficial medial collateral ligament, which is at its longest when the knee joint is flexed at a 45-degree angle. The different orientations of the anterior and posterior fibers lead to functional variations. The posterior fibers are taut during extension, while the anterior fibers are taut during flexion. The third layer is formed by the joint capsule, which thickens beneath the superficial medial collateral ligament and gives rise to the deep

medial collateral ligament that has vertically oriented fibers. The deep medial collateral ligament is securely attached to the medial meniscus's central region.

Lateral Complex

Similarly, the capsuloligamentous structures that envelop the lateral side of the knee joint can be studied in three layers. The topmost layer is the lateral retinaculum, while the middle layer includes the lateral collateral ligament, arcuate ligament, and fabellofibular ligament. The deep layer is formed by the joint capsule.

The lateral collateral ligament originates from the lateral femoral epicondyle and runs downward as a genuine ligamentous structure beneath the lateral retinaculum, where it attaches to the fibular head. It also has a connection with the biceps femoris tendon.

Posterior Complex

1. Posterior Capsule
2. Oblique Popliteal Ligament
3. Arcuate Popliteal Ligament
4. Semimembranosus
5. Popliteus
6. Gastrocnemius
7. Biceps Femoris

Posterior Capsule: The posterior capsule of the knee joint is divided into three parts - medial, middle, and lateral. It is taut in extension and lax in flexion.

Gastrocnemius Muscle: The medial and lateral heads of this muscle originate from the postero-superior parts of the femoral condyles. A bursa is often found beneath the medial gastrocnemius tendon, and the fabella is present within the lateral head of gastrocnemius in approximately 30% of the population.

Central Complex: The central complex of the knee joint includes the anterior cruciate ligament, posterior cruciate ligament, Humphry's ligament (anterior meniscotibial ligament), Wrisberg's ligament (posterior meniscotibial ligament), medial meniscus, and lateral meniscus.

Knee Biomechanics

The knee joint's bone and soft tissue structures make flexion-extension and internal-external rotation the most important movements, while axial compression-distract ion and medial-lateral translation have the least amount of movement. Anteroposterior displacement and adduction-abduction movements depend on the cruciate and collateral ligaments' integrity and tension. The lateral femoral condyle's radius is larger than the medial condyle, leading to tibial internal rotation during flexion and external rotation during extension, known as the "screw home" mechanism. As knee

flexion increases, the femur shifts backward, causing femoral roll-back. The instantaneous center of rotation at the intersection of the cruciate ligaments rotates posteriorly during knee flexion, facilitating femoral roll-back. Normal walking puts a load of 2 to 5 times body weight on the knee joint, which can increase up to 24 times during running. For an adult male, the knee bears a load between 1400 and 3500 Newtons when walking. The knee joint has an active range of motion of 0 to 120 degrees in hip extension, increasing to 140 degrees when the hip flexes due to increased hamstring activity. Normally, the knee joint achieves passive flexion of up to 160 degrees (MacDessi et al., 2021).

Patellofemoral Joint

The patella, also known as the kneecap, increases the leverage arm of the quadriceps muscle, which enhances its effectiveness. Additionally, it acts as a functional stabilizer by providing a surface for contact against the trochlea and also serves to shield the femoral condyles during knee flexion. The load on the patellofemoral joint varies depending on the activity, with climbing stairs putting a greater load on the joint compared to sitting. The force on the joint surface is minimal during knee extension but increases with flexion, peaking between 60 and 90 degrees. The stability of the patellofemoral joint is ensured by the muscles, medial and lateral retinacular structures, ligaments, and bone structure. The Vastus Medialis Obliqus (VMO) muscle provides dynamic stability up to 30 degrees of flexion, while the medial patellofemoral ligament provides more than half of the forces to the lateral side, giving static stability during this period. As the patella enters the trochlear groove during more advanced flexion, the bone structure provides stability.

Biomechanical Properties of Ligaments

The medial collateral ligament is made up of two parts - a superficial and a deep segment. The superficial segment is crucial for maintaining medial stability, with its anterior vertical fibers becoming taut during flexion, while the posterior oblique fibers become taut during extension.

The lateral collateral ligament has a different function compared to the medial collateral ligament. It is tight in extension and permits slight rotation during flexion. It is the primary structure that offers stability against varus stresses in all degrees of flexion. (MacDessi et al., 2021)

Posterior Cruciate Ligament is the most important structure that prevents posterior translation of the tibia. PCL provides 90% of the stability in posterior direction.

The anterior cruciate ligament is a crucial structure that primarily prevents the forward movement of the tibia under the femur. Although the

ACL provides some degree of anterior stability in conjunction with the posterior horn of the meniscus and capsule, it may not withstand physiological loads. Additionally, the ACL plays a role in restricting tibial internal rotation, particularly noticeable in the first 30 degrees of flexion (Luvsannyam et al., 2022).

Meniscus Biomechanical Properties

Even though the meniscus lacks blood vessels, it is still an active tissue. Fibrochondrocytes react to changes in pressure by modifying the production of proteoglycans. When the knee joint is subjected to pressure, the triangular shape of the menisci causes them to move outward, resulting in circumferential fibers being subjected to tensile forces. Proteoglycans possess the ability to withstand compressive forces due to their biochemical characteristics. They are hydrophilic and can hold up to 50 times their own weight in water, releasing 20% of it into the surrounding environment when under load. The meniscus adapts to loading in two stages: an elastic deformation caused by sliding between proteoglycans and collagen chains, followed by the release of synovial fluid into the joint. This permits the meniscus to bend slightly under pressure and distribute the force applied to it, then return to its initial form and reabsorb the fluid when the load is removed. This flow of fluid helps to feed the fibrochondrocytes and promote joint lubrication. The meniscus is half as rigid as articular cartilage, making it more pliable. Therefore, it functions as a shock absorber, safeguarding the articular cartilage. Walking transmits 1.3 times body weight to the knees, whereas running transmits 2 times body weight. In loads up to 150 kg, the lateral compartment bears almost all the load, while in the medial compartment, the load is distributed evenly between the meniscus and articular cartilage. In total, both menisci bear 35-50% of the load on the knee. The anterior horns of both menisci are more mobile than the posterior horns.

Meniscal Tears

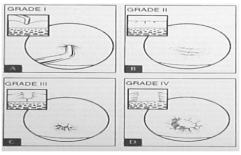
Classically, isolated tears with findings are more common on the medial side, which is three times more frequent than on the lateral side. However, in patients with an acute anterior cruciate ligament tear, longitudinal or oblique tears are usually detected in the posterior horn of the lateral meniscus. Meniscal tears frequently involve the posterior horns on both sides, and tears in the anterior horns are often an extension of tears in the posterior horns. In young patients, tears usually occur after a significant rotational trauma and are vertical, longitudinal, or oblique. In elderly patients, tears occur in previously degenerated menisci and are part of the general wear and tear of the knee joint.

Cartilage Lesions

Chondral lesions can occur after an acute trauma or as a result of repeated mechanical trauma caused by existing instability or meniscal lesions. On examination, there may be localized tenderness and/or effusion in the knee. In particular, Rosenberg et al. proposed that cartilage defects on the weight-bearing surface of the knee joint can be identified by taking posterior-anterior radiographs of the knee in 45° of flexion while standing. Arthroscopy is the most valuable method for diagnosing cartilage lesions. Cartilage lesions can be divided into two types: partial-thickness lesions that do not penetrate the subchondral bone surface and full-thickness lesions that penetrate the subchondral bone and reach the bone marrow, where macrophages, lymphocytes, and pluripotent stem cells are present. The Outerbridge classification (Figure 8), which was initially described to evaluate patellar chondromalacia, has been used to classify other cartilage lesions in the knee joint over time (Slattery et al., 2018).

According to the Outerbridge classification, Stage 1 is characterized by swelling and softening, Stage 2 by fibrillation and fragmentation. Stage 3 is characterized by fragmentation with a fissure of less than 1/2 inch, and Stage 4 is a full-thickness lesion that penetrates the bone.

Figure 6. Outerbridge Classification of Cartilage Lesions.



Osteoarthritis (OA)

Osteoarthritis (OA) is a joint disorder characterized by damage to the joint cartilage, as well as the formation of new bone on the joint surface and edges. While there is a theory that the occurrence of cartilage fibrillation initiates the degeneration that progresses to secondary bone formation, an alternative hypothesis suggests that OA begins with cartilage degeneration due to increased stiffness of the subchondral bone. There is a definite correlation between age and osteoarthritis, with it being very rare in those under the age of 40, but very common in those over the age of 60. A study conducted in America showed that radiological evidence of OA was present in 90% of individuals over the age of 65. While the male-to-female ratio is equal in those under the age of 55, it is more commonly found in females over the age of 55. Age, gender, heredity, acute trauma, chronic

loading, previous joint diseases, and obesity are all factors that play a role in the etiology of OA.

Pathogenesis

The articular cartilage is responsible for the movement of the joint surfaces on each other. Its task is to carry the load and provide a contact surface. It is avascular and alymphatic. Histologically, it consists of extracellular matrix and chondrocytes in different states within the matrix. Chondrocytes synthesize both matrix elements and enzymes that break down the matrix. The matrix consists mainly of collagen fibers and proteoglycans synthesized by chondrocytes. In addition to these, non-collagenous acidic glycoproteins, lipids, and calcium salts are found in the matrix. More than half of the matrix is composed of collagen fibers, primarily type II. In addition to type II, small amounts of type V, IX, and XI are also found in cartilage.

Proteoglycans fill the space between collagen fibers. Proteoglycans are formed by the attachment of a core protein to glycosaminoglycans. There are three types of glycosaminoglycans that vary depending on age and location: chondroitin sulfate-B, chondroitin-4 sulfate, and keratan sulfate. Proteoglycans have a hydrophilic property that provides cartilage elasticity. Changes in cartilage at the onset of osteoarthritis are due to the breakdown of proteoglycans and type II collagen. In cartilage degeneration, cytokines such as interleukin-1 and metalloproteases, and in the development of osteophytes, local growth factors have been shown to be important. Due to the loss of proteoglycans, cartilage softens and its resistance decreases. Since collagen tissue is broken down, it becomes irregular and tears vertically (fibrillation). When cartilage thins, the underlying bone is exposed and osteophyte development is seen peripherally. The subchondral bone thickens and takes on an ivory appearance under pressure. Pressure necroses and microfractures can occur. Non-specific mild synovitis can develop due to the release of breakdown products. As a result of all this, joint pain, stiffness, restricted range of motion, and deformity can occur (Abramoff, B., & Caldera, F. E. 2020).

Classification

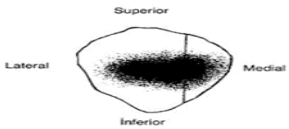
1. Primary Osteoarthritis: Often hereditary. Primary generalized osteoarthritis, which is accompanied by Heberden's nodules, is carried by an autosomal gene that is dominant in females and recessive in males. Generalized osteoarthritis without these nodules shows a polygenic inheritance. The increased frequency of HLA A1 and HLA B8 in generalized osteoarthritis suggests a role for genetic predisposition.

2. Secondary Osteoarthritis: Occurs as a result of trauma or pre-existing joint disease.

Chondromalacia Patella

Chondromalacia patella is a term used to describe degeneration in the patella articular cartilage, first described by Aleman in 1928. It is often used interchangeably with patellofemoral pain. The nerve endings are absent in articular cartilage, so it cannot be a direct source of pain. The synovium and subchondral bone are two areas that can cause pain in chondral defects. Articular cartilage debris that enters the joint can cause chemical irritation of the synovium, leading to pain and swelling. Pathological lesions in chondral defects in the knee joint are not the same as those in traumatic arthritis. In chondromalacia patella, the initial lesion is a change in collagen fibers and the basic substance in the deep levels of the cartilage. Goodfellow et al. used the term basal degeneration to describe this lesion. Chondromalacia patella is a disease of the deep layers of cartilage that only affects the superficial layers in the late stages. In contrast, the initial changes in osteoarthritis occur on the surface of the cartilage. There is a loss of continuity and fibrillation in the transverse fibers. Chondromalacia is associated with a decrease in sulfated mucopolysaccharides in the basic substance. These changes are most commonly seen in two areas of the deep layer of the cartilage: one is a approximately 1 cm area on either side of the prominence that separates the lateral and medial surfaces, and the other is located on either side of the inferior of the central prominence that separates the lateral and medial surfaces. These two areas are very close to each other and sometimes merge. These non-contact surfaces are not subjected to mechanical stresses of the joint, so chondromalacia in these areas is not very important. However, some of these areas can participate in joint motion, especially in advanced ranges of motion. Softened cartilage is insufficient to support the collagen network, and the complex structure begins to break down, leading to the next stage of degeneration, which is fibrillation. These changes can also affect the subchondral bone, as shown in the study by Goodfellow et al.

Figure 7. The most common areas of patellar chondromalacia.



Outerbridge observed a protrusion in varying heights passing through the medial femoral condyle of the osteochondral complex in most human knees. He suggested that friction on the medial surface cartilage during normal knee movements caused by the patella passing over this protru-

sion leads to chondromalacia. Goodfellow made a differential diagnosis between age-related superficial degeneration and basal degeneration. The age-related degeneration develops on the medial facet, does not progress, and includes surface changes. Basal degeneration, on the other hand, develops on the ridge separating the medial and other facets.

1. Degree changes include minimal joint cartilage changes. There may be localized softening without deterioration or minimal irregularities on the cartilage surface.

 (Figure 8. 1st degree cartilage degeneration)

2. Degree changes include irregular surface fibrillation or fissure areas.

 (Figure 9. 2nd degree cartilage degeneration)

3. Degree changes have significant fibrillation with fissures extending to the subchondral bone.

 (Figure 10. 3rd degree cartilage degeneration)

4. Degree changes do not show joint cartilage and lead to erosion of the subchondral bone.

 (Figure 11. 4th degree cartilage degeneration)

Etiology

Etiological factors are grouped into two main categories: biomechanical and biochemical. 1. Biomechanical Causes A. Acute A1. Chondral or osteochondral fracture with patella dislocation A2. Direct trauma A3. Patella fractures causing irregular joint surface B. Chronic B1. Recurrent subluxation or dislocation of the patella B2. Increased Q angle B3. Quadriceps imbalance B4. Malalignment due to femoral shaft fracture B5. Excessive lateral pressure syndrome B6. Meniscus injury associated with patellar movement and instability B7. Reflex sympathetic dystrophy 2. Biochemical Causes A. Diseases 1. Rheumatoid arthritis 2. Recurrent hemarthrosis 3. Alkaptonuria 4. Peripheral synovitis. 5. Sepsis and adhesions B. Iatrogenic 1. Repeated intra-articular steroid injections 2. Prolonged immobilization C. Degenerative 1. Primary osteoarthritis (Dekker et al., 2021).

Clinical Evaluation

The main complaint in patellofemoral joint diseases is pain behind the

patella, medial to the joint line, and sometimes in the popliteal fossa. Pain increases with activities such as climbing stairs or prolonged sitting with the knee flexed (Davenport, M., & Oczypok, M. P. (2020)). Pain may be bilateral and not associated with any trauma. The localization of the pain may lead to incorrect diagnoses. Confusion between meniscal pathologies that cause pain in the anteromedial joint line and patellofemoral pain complaints may result in incorrect treatment methods being applied. The second complaint of a patient with patellar dysfunction includes complaints such as sounds coming from the patellofemoral joint, feeling of emptiness, and locking, which are used to describe interruptions or irregularities in the soft rhythm of normal patellofemoral movement (Gaitonde, D. Y., Ericksen, A., & Robbins, R. C., 2019). The feeling of friction is felt or rarely heard by the patient, especially when the load on the patellofemoral joint increases (such as climbing up and down stairs). Joint effusion is seen in most patients with patellofemoral joint disease. In chronic cases, quadriceps atrophy is also detected.

Classification of Chondromalacia

Closed Chondromalacia: A simple softening of the patellar cartilage in the form of blister formation. The definite diagnosis is made by arthroscopic examination because macroscopic surface continuity is observed. Microscopically, fibrous metaplasia and flattening of cells, and edema in the deep layer of the cartilage are seen.

Open Chondromalacia: There are single or multiple fissures on the joint cartilage surface. Softening in adjacent areas accompanies this. If this softening and fissures are outside the patellofemoral contact areas, they do not cause patellofemoral pain. Fibrillation, fissure, and ulceration continue in the joint cartilage. Subchondral bone has emerged. The subchondral bone, which appears polished, is called eburnation. This is the final stage of cartilage degeneration.

Chondrosclerosis: Unlike softening in the cartilage, hardening is observed. The surface appears yellow. There is a complete loss of cartilage quality. The lesion is in collagen and proteoglycan structures rather than fibrocartilage.

Pseudopatella Formation: Generally localized on the medial facet. The lack of contact, which is essential for cartilage nutrition, forms the basis of the pathology. Superficial changes occur as a result of elevation and tearing of the cartilage lamina layer (Habusta et al., 2022).

Global Chondromalacia: Whereas in previous definitions, the lesion was localized in a single facet, chondromalacia is observed in the entire patellar cartilage due to causes such as patellar fracture and arthrofibrosis.

Assessment and Classification of Cartilage Defects in Knee Joint

Cartilage lesions are the most common lesions, especially in the knee joint. It is known to cause permanent irreversible damage and pave the way for osteoarthritis. Despite all technological and scientific developments, there is still no definitive treatment. Although arthroscopic evaluation is the gold standard for diagnosis in cartilage lesions, it is of great importance to know the presence of cartilage problem before surgical intervention. Advantages such as informing the patient about the possible physiotherapy and rehabilitation process after surgery, and ensuring a healthy balance between patient expectation and treatment yield can be obtained. Cartilage injuries of all degrees, including chondral and osteochondral lesions, have been classified in different ways in the past. At this point, it is necessary to distinguish between cartilage lesions and osteoarthritis. This distinction can be made by looking at the number of lesions and the condition of the opposing surface. It seems appropriate to define cases where the number of lesions is less than three and the opposite surface is normal as cartilage lesions, and the opposite as osteoarthritis (Abramoff, B., & Caldera, F. E. 2020). Cartilage lesions occur as a result of major, minor, or repeated microtraumas, not as a degenerative process as in osteoarthritis.

Patellofemoral Pain Syndrome

Patellofemoral Pain Syndrome (PFPS) is a set of symptoms that adversely affect the daily life activities of individuals, especially young adults, causing functional limitations. In the absence of other pathological conditions, PFPS is defined as anterior knee (retropatellar-peripatellar) pain that increases with activities such as prolonged sitting, going up and down stairs, and squatting. For many years, all patients with anterior knee pain were referred to as patellar chondromalacia. However, chondromalacia requires the softening and fissuring of the patellar cartilage tissue to be demonstrated. Currently, the term chondromalacia patella is used as a clinical diagnosis for a limited group of patients without alignment disorders and often with a history of trauma. Other pathological conditions causing anterior knee pain should be distinguished from PFPS in clinical diagnosis. Differential diagnosis should include pain radiating from the hip and back, inflammatory-degenerative arthritides, neuromas, meniscus tears, patellofemoral joint tumors, osteochondritis dissecans, medial synovial plica syndrome, Osgood-Schlatter syndrome, patellar tendinitis, prepatellar bursitis, and Sinding-Larsen-Johansson syndrome. Although the initial cause and pathogenesis of PFPS are not fully understood, many factors are blamed, such as acute trauma, knee ligament injury, instability, overuse, immobilization, excessive weight, genetic predisposition, congenital anomalies of the patella, and alignment disorders in the extensor mechanism of the knee. Most authors focus on the theory of patellar align-

ment disorder. Abnormal muscle and biomechanical factors are thought to increase patellofemoral contact pressure by changing the relationship between the patella and the femoral trochlear notch, causing pain and dysfunction. It is commonly believed that conservative rehabilitation practices alleviate the symptoms of patients with PFPS (Gaitonde et al., 2019).

Disorders of Alignment

1- Situations where Q angle increases: Q angle is defined as the angle between the patella-tibial tubercle axis and the quadriceps force vector. It increases with femoral anteversion, external tibial torsion, and displacement of the tibial tubercle.

2- Excessive Pronation at the Subtalar Joint: It increases the lateral pull of the quadriceps on the patella.

3- Muscle Tightness: Tight hamstrings increase the reaction force of the patellofemoral joint during the stance phase of gait and also cause compensatory pronation at the subtalar joint by increasing ankle dorsiflexion. Tightness in the gastrocnemius muscle limits dorsiflexion at the ankle joint, resulting in compensatory pronation at the subtalar joint. Tightness in the iliotibial band pulls the patella laterally during knee flexion.

4- Patella Alta: It predisposes to patellar subluxation.

5- Vastus Medialis Oblique Insufficiency: Vastus medialis is divided into two parts by a septum, Vastus Medialis Oblique (VMO) and Vastus Lateralis. While the function of Vastus Lateralis is knee extension, VMO is only a dynamic medial stabilizer of the patella, and its insufficiency causes lateral displacement of the patella (Fulkerson J. P. 2002). As a result, the knee joint mainly consists of the patellofemoral joint. There are many structures in this region, including cartilage, subchondral bone, synovial plica, Hoffa's fat pad (infrapatellar fat pad), retinaculum, capsule, and tendons. Each of these structures alone or together can cause pain in the knee joint. It is important to differentiate them from PFAS (patellofemoral pain syndrome).

CONCLUSION

Considering all the information, it can be assumed that increased BMI primarily and frequently affects the talus via the tibia and then the navicular, leading to the dropping of the navicular bone and the formation of a pes planus deformity in the foot. In addition, the caudal direction in the talus and navicular results in internal rotation, which, through the talocalcaneal ligaments, can also cause an increase in the ankle valgus angle. All these changes in the foot force the distal tibia to translate medially and internally rotate. When this strain exceeds the level that soft tissue and

joint can tolerate, compensatory varus angulation in the knee may occur to compensate for the valgus change in the foot. It is highly likely that the increased varus angle in the knee will affect muscle contracture angle and the patellofemoral joint. Over time, the decreased tissue resistance and weakened compensatory mechanisms due to aging can pose a significant risk for developing knee OA and other pathological processes in the lower extremities. Although excessive weight or obesity, which is the primary factor in the formation of this closed kinetic chain, can be an important factor in indicating the speed and severity of developing pathological processes, reducing weight may not be able to reverse this process. However, weight loss will be beneficial for the treatment of knee pathologies. In addition, in any knee problem, foot posture must be evaluated, and if there is any deformity, measures and therapies to correct it must be applied. Arch-supporting insoles applied to the foot, exercises that correct the concavity of the arch and ankle valgus angle can be used as treatment. In addition, treatments and devices that correct the increased varus angle and reduce the adduction moment can also be used. When faced with any complaints related to the knee, lower extremity alignment, especially foot posture, should not be ignored, and the mechanisms that cause the problem should not be overlooked by focusing only on the problem. A holistic approach should be taken when evaluating the situation. In conclusion, it can be said that excessive weight or obesity negatively affects the lower extremities and increases the speed and severity of pathological processes, but these processes may not be caused directly by the excessive load on the knee joint from excess weight, but rather indirectly due to the impairment of biomechanical alignment as a result of the deformation of the foot arch. Another important point to be remembered in the evaluation is that degeneration of the tissues may not only be caused by mechanical loading, but also by some hormones or enzymes produced in adipose tissue, which can have adverse effects on the tissues and play a role in traumatic processes.

REFERENCES

Burton, B. T., Foster, W. R., Hirsch, J., & Van Itallie, T. B. (1985). Health implications of obesity: an NIH Consensus Development Conference. International journal of obesity, 9(3), 155–170.

Hubert, H. B., Feinleib, M., McNamara, P. M., & Castelli, W. P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation, 67(5), 968–977. https://doi.org/10.1161/01.cir.67.5.968

Haffner, S. M., Mitchell, B. D., Hazuda, H. P., & Stern, M. P. (1991). Greater influence of central distribution of adipose tissue on incidence of non-insulin-dependent diabetes in women than men. The American journal of clinical nutrition, 53(5), 1312–1317. https://doi.org/10.1093/ajcn/53.5.1312

Colditz, G. A., Willett, W. C., Rotnitzky, A., & Manson, J. E. (1995). Weight gain as a risk factor for clinical diabetes mellitus in women. Annals of internal medicine, 122(7), 481–486. https://doi.org/10.7326/0003-4819-122-7-199504010-00001

Han, T. S., van Leer, E. M., Seidell, J. C., & Lean, M. E. (1995). Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. BMJ (Clinical research ed.), 311(7017), 1401–1405. https://doi.org/10.1136/bmj.311.7017.1401

Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G., & Dietz, W. H. (1999). The disease burden associated with overweight and obesity. JAMA, 282(16), 1523–1529. https://doi.org/10.1001/jama.282.16.1523

Lean, M. E., Han, T. S., & Seidell, J. C. (1999). Impairment of health and quality of life using new US federal guidelines for the identification of obesity. Archives of internal medicine, 159(8), 837–843. https://doi.org/10.1001/archinte.159.8.837

Barry, R. J., & Scranton, P. E., Jr (1983). Flat feet in children. Clinical orthopaedics and related research, (181), 68–75.

Rodgers M. M. (1988). Dynamic biomechanics of the normal foot and ankle during walking and running. Physical therapy, 68(12), 1822–1830. https://doi.org/10.1093/ptj/68.12.1822

Morris J. M. (1977). Biomechanics of the foot and ankle. Clinical orthopaedics and related research, (122), 10–17.

Hintermann, B., & Ruiz, R. (2021). Biomechanics of Medial Ankle and Peritalar Instability. Foot and ankle clinics, 26(2), 249–267. https://doi.org/10.1016/j.fcl.2021.03.002

Bernhardt D. B. (1988). Prenatal and postnatal growth and development of the foot and ankle. Physical therapy, 68(12), 1831–1839. https://doi.org/10.1093/ptj/68.12.1831

Salathé, E. P., Jr, Arangio, G. A., & Salathé, E. P. (1990). The foot as a shock absorb-

er. Journal of biomechanics, 23(7), 655–659. https://doi.org/10.1016/0021-9290(90)90165-y

Turner, C., Gardiner, M. D., Midgley, A., & Stefanis, A. (2020). A guide to the management of paediatric pes planus. Australian journal of general practice, 49(5), 245–249. https://doi.org/10.31128/AJGP-09-19-5089

Gould N. (1983). Evaluation of hyperpronation and pes planus in adults. Clinical orthopaedics and related research, (181), 37–45.

Tiberio D. (1988). Pathomechanics of structural foot deformities. Physical therapy, 68(12), 1840–1849. https://doi.org/10.1093/ptj/68.12.1840

Landsmeer, M. L. A., Runhaar, J., van Middelkoop, M., Oei, E. H. G., Schiphof, D., Bindels, P. J. E., & Bierma-Zeinstra, S. M. A. (2019). Predicting Knee Pain and Knee Osteoarthritis Among Overweight Women. Journal of the American Board of Family Medicine : JABFM, 32(4), 575–584. https://doi.org/10.3122/jabfm.2019.04.180302

Sukerkar, P. A., & Doyle, Z. (2022). Imaging of Osteoarthritis of the Knee. Radiologic clinics of North America, 60(4), 605–616. https://doi.org/10.1016/j.rcl.2022.03.004

MacDessi, S. J., Griffiths-Jones, W., Harris, I. A., Bellemans, J., & Chen, D. B. (2021). Coronal Plane Alignment of the Knee (CPAK) classification. The bone & joint journal, 103-B(2), 329–337. https://doi.org/10.1302/0301-620X.103B2.BJJ-2020-1050.R1

Luvsannyam, E., Jain, M. S., Leitao, A. R., Maikawa, N., & Leitao, A. E. (2022). Meniscus Tear: Pathology, Incidence, and Management. Cureus, 14(5), e25121. https://doi.org/10.7759/cureus.25121

Fithian, D. C., Kelly, M. A., & Mow, V. C. (1990). Material properties and structure-function relationships in the menisci. Clinical orthopaedics and related research, (252), 19–31.

Hu, J., Xin, H., Chen, Z., Zhang, Q., Peng, Y., & Jin, Z. (2019). The role of menisci in knee contact mechanics and secondary kinematics during human walking. Clinical biomechanics (Bristol, Avon), 61, 58–63. https://doi.org/10.1016/j.clinbiomech.2018.11.009

Hassebrock, J. D., Gulbrandsen, M. T., Asprey, W. L., Makovicka, J. L., & Chhabra, A. (2020). Knee Ligament Anatomy and Biomechanics. Sports medicine and arthroscopy review, 28(3), 80–86. https://doi.org/10.1097/JSA.0000000000000279

Slattery, C., & Kweon, C. Y. (2018). Classifications in Brief: Outerbridge Classification of Chondral Lesions. Clinical orthopaedics and related research, 476(10), 2101–2104. https://doi.org/10.1007/s11999.0000000000000255

Katz, J. N., Arant, K. R., & Loeser, R. F. (2021). Diagnosis and Treatment of Hip and Knee Osteoarthritis: A Review. JAMA, 325(6), 568–578. https://doi.org/10.1001/jama.2020.22171

Abramoff, B., & Caldera, F. E. (2020). Osteoarthritis: Pathology, Diagnosis, and Treatment Options. The Medical clinics of North America, 104(2), 293–311. https://doi.org/10.1016/j.mcna.2019.10.007

Belyaeva, E. A., & Avdeeva, O. S. (2019). Terapevticheskii arkhiv, 91(5), 96–102. https://doi.org/10.26442/00403660.2019.05.000213

Davenport, M., & Oczypok, M. P. (2020). Knee and Leg Injuries. Emergency medicine clinics of North America, 38(1), 143–165. https://doi.org/10.1016/j.emc.2019.09.012

Habusta, S. F., Coffey, R., Ponnarasu, S., Mabrouk, A., & Griffin, E. E. (2022). Chondromalacia Patella. In StatPearls. StatPearls Publishing.

Dekker, T. J., Aman, Z. S., DePhillipo, N. N., Dickens, J. F., Anz, A. W., & LaPrade, R. F. (2021). Chondral Lesions of the Knee: An Evidence-Based Approach. The Journal of bone and joint surgery. American volume, 103(7), 629–645. https://doi.org/10.2106/JBJS.20.01161

Gaitonde, D. Y., Ericksen, A., & Robbins, R. C. (2019). Patellofemoral Pain Syndrome. American family physician, 99(2), 88–94.

Fulkerson J. P. (2002). Diagnosis and treatment of patients with patellofemoral pain. The American journal of sports medicine, 30(3), 447–456. https://doi.org/10.1177/03635465020300032501

CHAPTER 3

DETRUSOR MUSCLE PHYSIOLOGY AND EMERGENCY MEDICINE

Leyla ÖZTÜRK SÖNMEZ[1]

1 M.D., Department of Emergency Medicine, Beyhekim Training and Research Hospital, Konya, Turkey.

Ph.D. Physiology

Introduction

Smooth muscle cell layers or plates are present in different parts of the body including blood vessels, stomach, intestines, bladder, air tracts, uterus, and sinuses. Smooth muscle is different from skeletal muscle and cardiac muscle in histological terms as it lacks lines (1).

The contraction activity in smooth muscles mainly takes place through the phosphorylation condition of the myosin light chain. The myosin light chain phosphorylation in some smooth muscle cells is kept at a low level unless an external stimulator (receptor or mechanical activation) is present. This activity ends up with smooth muscle tonus and its intensity may change (1). Intracellular Ca^{++} concentration increase is quite important to start smooth muscle contraction (2). Smooth muscle relaxation takes place through the disappearance of a contractile stimulator or the effect of a material preventing contraction mechanism. Regarding relaxation; a decrease in intracellular Ca^{++} concentration and myosin light chain (MLC) phosphatase activity should happen(3,4).

The bladder muscle wall forms the detrusor muscle. Detrusor muscle presents smooth muscle characteristics.

The bladder has constructed of two parts: The body part which lies above the ureteral orifices and the base part including the bladder neck and the trigone. Since the two areas are different but in harmony with themselves regarding morphological, neurological, and pharmacological constructure. The body part's histological examination consists of randomly settled myofibrils. This formation is different from the longitudinal and circular smooth muscle configuration of the ureter and gastrointestinal tract.

The bladder outlet is composed of the external urethral sphincter, urethra, and the base of the bladder. A laminar configuration exists in the bladder base. A longitudinal superficial layer exists beneath the trigone. There exists a muscle layer under the superficial layer which continues with detrusor. The deep muscle layer of the bladder base consists of smaller muscle fibers which have a circular configuration(5).

This review will cover detrusor muscle physiology.

Materials and methods

Search strategy

PubMed, Medline, Excerpta Medica Database (Embase), and Cumulative Index to Nursing and Allied Health Literature (CINAHL) databases had been searched from 1980 to 2022 and "detrusor", "bladder', "muscle", "physiology» words were searched. Also, a search was done amongst the references of the identified articles. The abstract and the full paper were

both assessed. Along with Medical Subject Headings (MeSH) terms and the keywords, we used the Cochrane Highly Sensitive Search Strategy to identify articles in PubMed. Articles published in English focused on the words "detrusor", "bladder', "muscle", and "physiology' were assessed. Nonaccessible full text studies and articles published before 1980 were excluded. Seventy-five original articles, systematic reviews, and meta-analyses were included in total in this review.

Data extraction and management

Based on the selection criteria, one author (LOS) selected all studies accessed from the databases and bibliographies. All studies relevant to "detrusor", "bladder', "muscle", "physiology' and their outcome measures were reviewed. We retrieved full-text copies of the articles identified as potentially relevant by the author.

Bladder

The bladder is a hollow organ constructed of smooth muscles. Bladder muscle wall forms the detrusor muscle. The detrusor is different from trigonal and urethral smooth muscles in terms of structure and function (6). The function of the bladder is the storage and complete discharge of urine (urination, miction). The approximate bladder volume is 400-500 mL (7).

The bladder includes two main parts; its body and base. The body of bladder is located over ureteral holes. The base is a ureterovesical junction including the deep detrusor and anterior wall of bladder. The bladder is surrounded by a mucosal sheath and the external part is partially covered by the serosa and fascia of the peritoneum. Muscle wall forms the detrusor muscle (6). Storage is related to a slight increase in internal pressure with the decrease of bladder wall tension. Discharge happens with the contraction of the detrusor smooth muscle with a force higher than the pressure preventing the outflow (7). Detrusor smooth muscle produces the force required for outflow in the urination stage of urination cycle (sometimes through the increasing of intraabdominal pressure). Bladder neck helps to prevent the involuntary urinary leakage. It also plays a role in resistance formation in the filling phase of urination cycle. Hence bladder neck and urethra have a closer functional relationship compared to detrusor. Bladder neck also has a more intense sympathetic innervation compared to the detrusor (8). In some conditions, this opening-closing cycle can be degraded such as in the overactive bladder, through uncontrolled activity. This activity might be strong enough to cause unintentional urinary loss or cause urinary urgency, pain, and discomfort. Thus, good understanding of how the bladder storage and urination methods are controlled is important to provide treatments minimizing these pathologies (7).

There are two types of contraction in the bladder; phasic contractions and spontaneous contractions. Phasic contractions cause bigger contractions and are triggered by transmitters released from parasympathetic fibers are phasic contractions. Spontaneous contractions have not started primarily by motor nerves. The formation of spontaneous contractions are still unclear. The spontaneous contractions distinguished from the neuro-mediated phasic contractions through their contraction mechanism. The spontaneous contractions have both physiological and pathological effects.

The Mechanism of Spontaneous Contraction Includes:

They are not affected by neurotoxins but are sensitive to Ca^{++},

They can enlarge enormously with the detrusor mucosa,

Micromotions of muscles can reflect as small intravesical pressure fluctuations and can become prominent as small movements,

They can advance and cause over-active bladders (9, 10).

Sympathetic nerves of the bladder are derived from the spinal cond' s thoracolumbar region (Th10-L2). Parasympathetic nerves derived from the sacral 2-4 spinal parasympathetic nucleus. Acetylcholine (ACh) release and muscarinic receptor activation stimulates detrusor contractions which induces the start of emptying the bladder(11).

Basic Characteristics of Detrusor Smooth Muscle Cell

The smooth muscle cells of the bladder wall are shaped as spindles. In relaxed form, they are at several hundred μm- long and 5 to 6 μm diameter. On the other hand, the skeletal muscle fibers are thousands of times longer and 20 times wider. Detrusor smooth muscle is formed of muscle sheets and they are in connection in certain junctions which brings a unique design to its physiologic structure and each cell owns one nucleus(5).

Detrusor muscle presents both single-unit smooth muscle characteristics and multiple unit smooth muscle characteristics. Nerve coordination is functionally needed to provide urination characteristic. Beams of muscle cells with different sizes are surrounded by collagen-rich bond tissue in human detrusor (6). The detrusor layer forms the bladder wall body. It is formed by soft muscle beams aparted by interstitial cells and connective tissue. Excitator entrance is provided by parasympathetic postganglionic nerves (7).

The detrusor surface is formed by urothelium(a tight transitional epithelium), basal membrane, and lower urothelium which is present on vesical face and protected by serosa and mucosa layer. The urothelium is coated by the mucopolysaccharide glycocalyx layer which acts against the destructive effect of the urine. Urothelium consists of three layers; basal

cells, intermediate, and umbrella cells layer. The umbrella cells layer is a superficial layer which formed by large hexagonal umbrella-shaped cells (12). The urothelium's main mission, by consisting a barrier through tight junctions between umbrella cells, is to provide a barrier between urine and urine-contacting tissues (12, 13). Urothelium consists of an extracellular matrix with a lower urothelium separating it from the detrusor, fibroblasts, adipocytes, interstitial cells, blood vessels, a muscle layer called muscularis mucosa and, afferent and efferent nerve endings(7).

Detrusor Smooth Muscle Contraction

Like all smooth muscles, bladder detrusor contraction takes place when the myosin light chain kinase phosphorylates the myosin light chains, as well as the contraction interaction of actin and myosin proteins (14). On the other hand, the phosphorylation of myosin filaments is related to the phasic increase in calcium-free intracellular concentration both with calcium changing place in extracellular space and without calcium through intracellular calcium release (15, 16). The size and rate of bladder pressure zone is related to the degradation of cytosolic ATP through actine and myosin and the active interaction of calcium carried by myosin light chain kinase. In physiological terms, bladder contraction and discharge take place as the result of muscarinic receptor stimulation of acetylcholine released from parasympathetic nerves. The rate and amount of urine flow is related to bladder drainage degree, momentary bladder contraction force, size and rate of pressure zone and the bladder performing the mechanical function in drainage (17). For urination in the presence of intravesical pressure, a gradually increasing force is required for the active drainage of the bladder (18).

Smooth muscle contraction is generally regulated by Ca^{++}. Ca^{++} cytoplasmic concentration generally increases at the beginning of force tension but decreases to basal levels in the continuation of contraction (19). But a Ca^{++} -independent mechanism also exists in detrusor muscle (20).

Muscarinic receptors provide both the extracellular influx of calcium and its discharge from intracellular storages (21, 22). Pressure and tension rate at the bladder are directly related to the rate at the concentration of intracellular free calcium increases. However, the increases in the rate are dependent on the combination of extracellular calcium influx through L-type calcium channels and the discharge of intracellular calcium from the sarcoplasmic reticulum (SR). When the calcium released from the sarcoplasmic reticulum is compared to extracellular calcium translocation, it is closer to smooth muscle filaments. Thus, a higher amount of calcium is released from SR and the pressure zone and tension rate are faster. Stimulation of muscarinic receptors in the bladders of humans and other mammals constitutes a basic mechanism for the contraction of detrusor smooth

muscle (23). This contractile activity can be started through the parasympathetic nerve stimulation and the intrinsic excitatory nerve's electrical field stimulation (EFS) (24). In human detrusor strips, the response to EFS is completely provided through the release of acetylcholine (25) and has an effect on muscarinic M3 receptors of the cell membrane. There is not much depolarization in the cell membrane in response to muscarinic receptor activation (26, 27), however, the action potential frequency has a significant increase. Besides, it increases inositol triphosphate (IP3) concentrations (28). This defines the IP3-mediated release of Ca^{++} from intracellular depots. The muscarinic stimulation and acetylcholine starts Ca^{++} release from SR (29). In the human bladder, the release of Ca^{++} from SR by EFS has not shown a systematic change in the membrane potential (30). To inhibit the contractions, muscarinic receptors, and voltage-dependent L type Ca^{++} channels are both necessary. It is understood that Ca^{++} flow manages these contractions through voltage-dependent Ca^{++} channels (31).

Detrusor Smooth Muscle Relaxation

β- adrenoceptors are considered to constitute most of the receptors meditating human detrusor relaxation (32). β3-adrenoceptor agonists cause the direct dose-related detrusor relaxation in the storage stage of miction (urination) cycle and inhibit detrusor over-activity. This causes an increase in bladder capacity with no change in urination pressure or residue volume (33). The pathway of cyclic adenosine monophosphate (cAMP) activated through the binding of noradrenaline to β1, β2, β3 adrenoceptors primarily mediates the detrusor smooth muscle relaxation(33). β3-adrenoceptors constitute over 95% of all β3-adrenoceptor mRNA in the bladder.

Hypoxia also lowers the tension of smooth muscles as like as skeletal and cardiac muscles. Mitochondrial respiratory inhibitor sodium cyanide (NaCN) significantly inhibits the contraction induced through high K^+ in the bladder (34).

Bladder Receptors

a-Cholinergic Receptors

Acetylcholine is the most important contractile transmitter activating muscarinic receptors in detrusor myocytes. Muscarinic receptors have five sub-types encoded by five different genes and are called M1, M2, M3, M4, and M5, presented in the detrusor layers, urothelium, nerve fibers, and interstitial cells (35). In smooth muscle of detrusor, M2 and M3 sub-types are principally present (36). These receptors also combine functionally. Both sub-types bind to G proteins although they have different signal transduction ways (37). M1, M3, and M5 receptors activate phosphoinositide hydrolysis by combining with Gq / 11. This connection provides the mo-

bilization of intracellular calcium. Muscarinic receptor activation triggers the non-selective cation channels and Rho kinase (38). Through hydrolysis of phosphoinositide, the muscarinic receptors of the bladder cause the contraction of the smooth muscle(39). While muscarinic receptors stimulating IP3 production can operate in muscarinic agonists, M2 muscarinic receptors not stimulating IP3 formation can be activated in concentrations with lower agonist concentrations (29). M3 receptors are regarded as substantial for the contraction detrusor. M3 receptor stimulation is considered to cause contraction generally via phosphoinositide hydrolysis (39).

The M2 receptor's role of function has not been clearly defined yet, but the stimulation of this receptor is considered to oppose symptomatic (β- adrenoceptor) mediated smooth muscle relaxation through adenylate cyclase inhibition (40). Also, M2 receptor stimulation activates non-specific cation channels via the activation of protein kinase C (41) or inhibits K-ATP channels (42). In a healthy bladder, M2 receptors have less contribution to detrusor contraction compared to M3 receptors. The part of M2 receptors in the contraction of detrusor increases in case of some diseases. In damaged rat bladder, M2 receptors only or M2 and M3 receptor combination mediates the contraction (43). In obstructed hypertrophic rat bladders, the total muscarinic receptor and M2 receptor intensity increases and M3 receptor intensity decreases (44). In a healthy detrusor, contractions are mediated by M3 receptors. In neurogenic bladder, M2 receptors contribute partially in bladder contractions (45).

b- Adrenergic Receptors (AR)

Noradrenalin is released from the adrenergic nerves by electrical stimulation in detrusor muscle (46). β-adrenoceptors are dominant on α-adrenoceptors (α-AR). The response of a healthy detrusor to noradrenalin is relaxation. To increase cAMP, adenylyl cyclase is stimulated by β-AR(47).

Alpha (α) Adrenergic Receptors

α1-Adrenoceptors

α1-adrenoceptor mRNA is found in rat, monkey, and human bladders. Using competitive RT-PCR, it is observed that total α-1-adrenoceptor mRNAs are constituted from α-1A at 95%, α-1B at 1%, and α-1D at 4% (48). Using real-time PCR, it was observed that α-1A adrenoceptors constitute 33%, α-1B constitutes 53% and α-1D constitutes 14% of total α1-adrenoceptor mRNAs (49).

α2-Adrenoceptors

Alpha-2 adrenergic receptors spread all through the central and peripheric nervous system (50). The presence of α2-adrenoceptor sub-type

mRNA has not been discovered in the bladder yet. On the other hand, the presence of α2-adrenoceptors at protein level in the detrusor and bladder base/neck of humans detected by radioligand-binding studies (51). Pre-inhibition of neurotransmitter release originating both from postganglionic sympathetic and parasympathetic nerve terminal constitutes the most well-known function of α2-adrenoceptors in most tissues (52). Also, α2-adrenoceptor stimulation inhibits parasympathetic nerve activity in the human (53) bladder through an effect on the vesicular parasympathetic ganglion. Central and peripheric α2-adrenoceptor stimulation may differ.

Beta (β) Adrenergic Receptors

The human bladder has mRNA for these three β-adrenoceptor subtypes (54). β3-adrenoreceptor stimulation decreases basal bladder tension and releases KCI-induced bladder strips (55). In the human bladder, β3-adrenoceptor activation inhibits neurogenic contractions(56), and lowers neuronal stimulated acetylcholine (ACh) release (57). Presence of β3 adrenoceptor in human detrusor was proved in 1998 and its expressions at mRNA level were later confirmed through RT-PCR (58). More than 95% of all adrenoceptor mRNAs in human bladder are constituted by β3-adrenoceptor, and α-adrenoreceptor mRNA constitutes only 3%. The smooth muscle fibers, urothelium and interstitial cells of the human bladder contain β3-adrenoceptors(59, 60). The mechanism through which β3 adrenoceptors conduct the inhibition of stimulated ACh release from the cholinergic nerve terminals in the bladder is still not well identified. The main function of β-adrenoceptors in the bladder is to provide an increase in the bladder in accordance with smooth muscle relaxation and urination cycle (61).

c- Dopamine Receptors (D)

Dopamine Receptors have five sub-types called as D1, D2, D3, D4 and D5. These receptors are coded with DRD1, DRD2, DRD3, DRD4 and DRD5 genes present in humans. The G protein-dependent dopamine receptors D1, D2, D3, D4 and D5, mediate the diversity of physiological functions controlled by dopamine in brain and its periphery (62). They provide all physiological functions of the catecholaminergic neurotransmitter dopamine such as optional movement, hormonal regulation, and hypertension. Dopamine receptors have canonical effects on cAMP-mediated signalization. They can also regulate an infinite number of cellular tasks to manage dopamine-related functions and behaviors(62).

d- Histamine Receptors (H)

Histamine is excreted by mast cell degranulation and effects through its receptors known as H1, H2, H3, and H4 which are founded all over the

body. H4 receptors are distributed less compared to the remainings. H1 receptors are located in the stomach, ileal and colonic lymphoid tissue, smooth muscle, and ganglions. H2 receptors are mainly settled in the stomach mucosa; but are also present in all parts of the gastrointestinal tract. H3 receptors are present in the stomach, colon, ganglions, and ileal and colonic lymphoid tissue. H4 receptors are present in the stomach, colonic mucosal and ileal lymphoid tissue, ganglions, and gastrointestinal tract smooth muscles (63).

Histamine stimulates calcium release effectively in smooth muscle cells (64). Histamine causes the contraction of the bladder. H1 receptors are predominantly present in the bladder and they are located both on muscle and non-cholinergic neural terminals (65).

e-Serotonin Receptors

Serotonin (5-hydroxytryptamine or 5-HT) is a bio amine having multi-functions with significant signal roles in several physiological tracts. Nearly all of the 5-HT in the human body is synthesized by enterochromaffin cells. 5-HT is a biogenic monoamine resembling adrenalin, noradrenalin, dopamine, and histamine. 5-HT is produced by the hydroxylation of tryptophan, an essential amino acid, to 5-hydroxy-tryptophan (5-HTP) by tryptophan hydroxylase.

All monoamine transporters, as well as serotonin transporters (SERT), span the membrane 12 times, and are membrane-embedded sodium-dependent transporters. Transportation of intracellular substrate and Na^+ and Cl^- in the K^+ interchange constitutes their basic mechanism (66). SERTs are present in CNS, gastrointestinal system, pulmonary and peripheric veins, and thrombocytes.

The strongest and most specific anti-depressant drug class, is named as selective serotonin reuptake inhibitors (SSRI). SSRIs specifically bind to SERT and the serotonin accumulates in synaptic junction. SSRIs also inhibit presynaptic autoreceptors to increase serotonin amount in the synaptic cleft (67).

There are some factors determining the signal strength and duration on the postsynaptic serotonin receptor. In the synaptic cleft, the main determiner of the serotonin effect is the amount. Two mechanisms playing a direct role in controlling serotonin presence in synaptic cleft; first the binding of serotonin to its own autoreceptor, and second, the activity of SERTs located on the presynaptic membrane. While the negative reuptake forming through the stimulation of the 5-HT autoreceptor decreases serotonin release, it detracts SERT and serotonin from the synaptic cleft (66, 68).

With the stimulation of 5-HT1 receptor, the smooth muscle relaxation

occurs through the release of nitric oxide from the endothelium (69).

f-Proteinkinase-C

It causes stenosis in arterial smooth muscle cells through[Ca^{++}] increases or voltage-gated Ca^{++} channels or through release from intracellular storage. As a result, blood pressure increases (70). It is related to arterial smooth muscle membrane potential and calcium depolarization. This causes an increase in the activity voltage-gated Ca^{++}channels leading to an increase in the Ca^{++} and vascular tonus. The activity of calcium-active potassium (BK) channels, voltage-gated potassium (KV) channels, ATP-sensitive potassium (K-ATP) channels, and different K^{+} channels containing internal rectifier constitutes the membrane potential of Potassium (KIR) channels (71).

Its activators are phosphatidylserine, calcium ion, and diacylglycerol (72).

Protein kinase C's(PKC) physiological function is controlled through maturation, catalytic activation, and targeting. An unmatured PKC cannot be catalytically activated. It is phosphorylated in three different serine / threonine-phosphorylated areas and thus transforms into a form sensitive to physiological stimulation. Thus, the mature PKC can be catalytically activated through the stimulation of different receptors and a few activators, and may be ready to target to certain cellular divisions such as the plasma membrane, Golgi complex, nuclear membrane, and nucleus. Phosphatidylserine (PS) is substantial for the catalytical activity of PKCs. Although the specific connection location has not been defined yet, they are considered to connect to C1 and C2 areas. Ca^{++} mediates the activity of cPKCs at C2 area. Diacylglycerol (DAG) and phorbol ester activate cPKC and nPKCs through connecting to C1 area (73, 74). The enzymatic activity of PKC is inhibited through tyrosine phosphorylation (75).

The Importance of Detrusor Muscle Physiology in Emergency Medicine:

Understanding the detrusor muscle physiology well will help in the diagnosis of bladder-related symptoms that are frequently referred to the emergency department, such as urinary retention, urinary incontinence, lower urinary tract symptoms, macroscopic hematuria, facilitate the detection of underlying pathologies, and accelerate the treatment process because of knowing the pathophysiology.

Conclusion: Detrusor muscle physiology has complex components. The physiological understanding of detrusor cell activities, mediators, and receptors will provide a better understanding of detrusor muscle-related pathophysiological processes.

References

1- Webb RC. Smooth Muscle Contraction And Relaxation. Adv Physiol Educ. 2003;27(1-4):201-6.

2- Brading AF, Teramoto N, Dass N, and McCoy R. Morphological and physiological characteristics of urethral circular and longitudinal smooth muscle. Scand J Urol Nephrol. 2001;35 Suppl 207: 12–18

3- Morgan K. The role of calcium in the control of vascular tone as assessed by the Ca indicator Aequorin. Cardiovasc Drugs Ther. 1990;4: 1355–1362

4- Somlyo AP, Wu X, Lalker LA, and Somlyo AV. Pharmacomechanical coupling: the role of calcium, G-proteins, kinases and phosphatases. Rev Physiol Biochem Pharmacol. 1999;134: 201– 234.

5- Yoshimura N, Chancellor MB. Physiology and Pharmacology of the Bladder and Urethra Campbell-Walsh Urology, Pages 1786-1833.

6- Andersson KE, Arner A. Urinary bladder contraction and relaxation:physiology and pathophysiology. Physiological Reviews 2004;84:935-86.

7- Fry CH, Vahabi B. The Role of the Mucosa in Normal and Abnormal Bladder Function. Basic Clin Pharmacol Toxicol. 2016 Oct;119 Suppl 3(Suppl 3):57-62.

8- Mitchell BM, Chitaley KC, Webb RC. Vascular smooth muscle contraction and relaxation. In: Hypertension Primer: The Essentials of High Blood Pressure, edited by Izzo JL and Black HR. Dallas, TX: Am. Heart Assoc., 2003, p. 97–99.

9- Moro C, Chess-Williams R. Non-adrenergic, non-cholinergic, nonpurinergic contractions of the urothelium/ lamina propria of the pig bladder. Auto Autocoid Pharmacol. 2012; 32:53–9.

10- Moro C, Leeds C, Chess-Williams R. Contractile activity of the bladder urothelium/ lamina propria and its regulation by nitric oxide. Eur J Pharmacol. 2012; 674:445–9.

11- Lukasz Dobreka–d, Beata Skowronb, Agnieszka Baranowskab, Piotr J. Thore. Spectral Heart Rate Variability Analysis in Experimental Obstructive and Chemical Overactive Bladder Models. f. Adv Clin Exp Med. 2013;22, 3, 337–346.

12- Acharya P, Beckel J, Ruiz WG, Wang E, Rojas R, Birder L, Apodaca G. Distribution of the tight junction proteins ZO-1, occludin, and claudin- 4, -8, and -12 in bladder epithelium. Am J Physiol Renal Physiol. 2004; 287: F305–18.

13- Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol Renal Physiol. 2000;278: F867–74.

14- Ebashi S, Mikawa T, Kuwayama H, Suzuki M, Ikemoto H, Ishizaki Y, Koga R.Calcium regulation in smooth muscle.in:M.J.Seigman, A.P. Somlyo, N.L. Stephens (Eds.)Regulation and Contraction of Smooth Muscle.A.R. Liss,New York. 1987;109–118.

15- Stull JT, Kamm KE, Taylor DA.Calcium control of smooth muscle contractility. Am. J. Med. Sci. 1988; 296:241.

16- Adelstein RS, Sellers JR. Effects of calcium on vascular smooth muscle contraction. *Am J Cardiol*. 1987;59(3):4B-10B. doi:10.1016/0002-9149(87)90076-2

17- Margot ve ark. 1997 Margot S. Damaser, Kap-Byung Kim, Penelope A. Longhurst, Alan J. Wein And Robert M. Levin. Calcium Regulation Of Urinary Bladder Function. The Journal Of Urology Vol. 1997;157, 732-738.

18- Levin ve ark. 1989. Levin R.M, Hypolite J, Ruggieri M.R, Longhurst P.A, Wein A.J.Effects of muscarinic stimulation on intracellular calcium in the rabbit bladder: comparison with metabolic response. Pharmacology. 1989; 39:69.

19- Morgan KG, Suematsu E. Calcium and vascular smooth muscle tone. Am J Hypertens. 1990;3: 2915- 2985.

20- Yoshimura Y, Yamaguchi O. Calcium Independent Contraction of Bladder Smooth Muscle J Urol. 1997; 4:62-67

21- Mostwin JL. Receptor operated intracellular calcium stores in the smooth muscle of the guinea pig bladder. J Urol. 1985; 133: 900–5

22- Kishii KI, Hisayama T, Takayanagi I. Comparison of contractile mechanisms by carbachol and ATP in detrusor strips of rabbit urinary bladder.Jap. J. Pharmacol. 1992; 58:219.

23- Andersson KE. Detrusor contraction – Focus on muscarinic receptors. Scand J Urol Nephrol Suppl. 2004; 215:54–7.

24- Brading AF, Williams JH. Contractile responses of smooth muscle strips from rat and guinea-pig urinary bladder to transmural stimulation: effects of atropine and α, β-methylene ATP. Br J Pharmacol. 1990; 99: 493–8

25- Tagliani M, Candura SM, Di Nucci A, Franceschetti GP, D'Agostino G, Ricotti P, Fiori E, Tonini M. A re-appraisal of the nature of the atropine-resistant contraction to electrical field stimulation in the human isolated detrusor muscle. Naunyn Schmiedebergs Arch Pharmacol. 1997; 356(6): 750–5.

26- Fujii K. Evidence for adenosine triphosphate as an excitatory transmitter in guinea- pig, rabbit and pig urinary bladder. J Physiol. 1988; 404: 39–52

27-) Brading AF, Mostwin JL. Electrical and mechanical responses of guinea-pig bladder muscle to nerve stimulation. Br J Pharmacol. 1989; 98:1083–90.

28- Iacovou JW, Hill SJ, Birmingham AT. Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: evidence that muscarinic and purinergic receptors raise intracellular calcium by different

mechanisms. J Urol. 1990; 144: 775–9.

29- Hashitani H, Bramich NJ, and Hirst GD. Mechanisms of excitatory neuromuscular transmission in the guinea-pig urinary bladder. J Physiol. 2000;524: 565–579

30- Wu C, Bayliss M, Newgreen D, Mundy AR, Fry CH. A comparison of the mode of action of ATP and carbachol on isolated human detrusor smooth muscle. J Urol. 1999; 162:1840–7.

31- Fovaeus ve ark. 1987 Fovaeus M, Andersson KE, Hedlund H. Effects of some calcium channel blockers on isolated human penile erectile tissues. J Urol. 1987; 138: 1267–72.

32- Takeda M, Obara K, Mizusawa T, Tomita Y, Arai K, Tsutsui T, Hatano A, Takahashi K, Nomura S. Evidence for β3-adrenoceptor subtypes in relaxation of the human urinary bladder detrusor: analysis by molecular biological and pharmacological methods. J Pharmacol Exp Ther. 1999; 288: 1367–1373.

33- Sacco E, Bientinesi R. Mirabegron: a review of recent data and its prospects in the management of overactive bladder. Ther Adv Urol. 2012; 4:315–24

34- Kaneda T, Takeuchi M, Shimizu K, Urakawa N, Nakajyo S, Mochizuki-Kobayashi M, Ueda F, Hondo R. Inhibitory mechanism of monensin on high K+ induced contraction in guniea-pig urinary bladder. J. Pharmacol. 2006;100: 133–141.

35- Mukerji G, Yiangou Y, Grogono J, Underwood J, Agarwal SK, Khullar V, Anand P. Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical correlations. Journal of Urology. 2006; 176:367–373.

36- Eglen RM. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. Auton Autacoid Pharmacol. 2006; 26: 219–33

37- Chess-Williams R. Muscarinic receptors of the urinary bladder: detrusor, urothelial and prejunctional. Auton Autacoid Pharmacol. 2002;22: 133–145.

38- Jezior JR, Brady JD, Rosenstein DI, McCammon KA, Miner AS, Ratz PH. Dependency of detrusor contractions on calcium sensitization and calcium entry through LOE-908-sensitive channels. Br J Pharmacol. 2001;134: 78–

39- Harriss DR, Marsh KA, Birmingham AT, Hill SJ. Expression of muscarinic M3- receptors coupled to inositol phospholipid hydrolysis in human detrusor cultured smooth muscle cells. J Urol 1995; 154: 1241–1245

40- Hegde SS, Choppin A, Bonhaus D, Briaud S, Loeb M, Moy TM, Loury D, Eglen RM. Functional role of M2 and M3 muscarinic receptors in the urinary bladder of rats in vitro and in vivo. Br J Pharmacol. 1997; 120: 1409–18.

41- Kotlikoff MI, Dhulipala P, Wang YX. M2 signaling in smooth muscle cells. Life Sci. 1999; 64: 437–42.

42- Nakamura T, Kimura J, Yamaguchi O. Muscarinic M2 receptors inhibit Ca2+

activated K+ channels in rat bladder smooth muscle. Int J Urol. 2002; 9: 689– 96

43- Kumar V, Cross RL, Chess-Williams R, Chapple CR. Recent advances in basic science for overactive bladder. Current Opinion in Urology. 2005; 15:222–226

44- Braverman AS, Ruggieri MR. Hypertrophy changes the muscarinic receptor subtype mediating bladder contraction from M3 toward M2. Am J Physiol Regul Integr Comp Physiol. 2003; 285: R701–8.

45- Pontari MA, Braverman AS, Ruggieri MR Sr. The M2 muscarinic receptor mediates in vitro bladder contractions from patients with neurogenic bladder dysfunction. Am J Physiol Regul Integr Comp Physiol. 2004; 286: R874–80

46- Mattiasson A, Andersson KE, Elbadawi A, Morgan E, Sjogren C. Interaction between adrenergic and cholinergic nerve terminals in the urinary bladder of rabbit, cat and man. J Urol. 1987;137: 1017–1019.

47- Perlberg S and Caine M. Adrenergic response of bladder muscle in prostatic obstruction. Its relation to detrusor instability. Urology. 1982; 20: 524–527.

48- Scofield ve ark. 1995 Scofield M.A, Liu F, Abel PW, Jeffries WB. Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. J. Pha rmacol. Exp. Ther. 1995;275, 1035–1042

49- Nomiya M. & Yamaguchi O. A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. J.U rol. 2003;170, 649–653.

50 - Nguyen V,Tiemann D,Park E,Salehi A. Alpha-2 Agonists. Anesthesiol Clin. 2017;35(2):233-245

51- Levin R.M., Ruggieri M.R. & Wein A.J. Identification of receptor subtypes in the rabbit and human urinary bladder by selective radio-ligand binding. J.U rol. 1988;139, 844–848.

52- Maggi C.A, Santicioli P, Furio M, Meli A. Dual effects of clonidine on micturition reflex in urethane anesthetized rats. J. Pharmacol.E. 1985; 235, 528–536.

53- Kunisawa Y, Kawabe K, Nıujıma T, Honda K, Takenaka T. A pharmacological study of alpha adrenergic receptor subtypes in smooth muscle of human urinary bladder base and prostatic urethra. J.U rol. 1985;134, 396–398.

54- Li G, Zheng XH, Li K, Li ZH, Luo XD, Liu YL, Wang P. Age-dependent changes in β-adrenoceptor function in human detrusor and possible mechanisms. Zhonghua Wai Ke Za Zhi. 2003;41(7):526-9.

55- Wuest M, Eichhorn B, Grimm MO, Wirth MP, Ravens U, Kaumann AJ. Catecholamines relax detrusor through beta 2-adrenoceptors in mouse and beta 3- adrenoceptors in man. J Pharmacol Exp Ther. 2009;328: 213–222.

56- Rouget C, Rekik M, Camparo P, Botto H, Rischmann P, Lluel P, Palea S, Westfall TD. Modulation of nerve-evoked contractions by β3-adrenoceptor agonism in human and rat isolated urinary bladder. Pharmacol Res. 2014; 80:14–20

57-D' Agostino G, Maria Condino A, Calvi P. Involvement of β3-adrenoceptors in the inhibitory control of cholinergic activity in human bladder: direct evidence by [(3)H]-acetylcholine release experiments in the isolated detrusor. Eur J Pharmacol. 2015;758: 115–122.

58 - Igawa Y, Yamazaki Y, Takeda H, Hayakawa K, Akahane M, Ajisawa Y, Yoneyama T, Nishizawa O, Andersson KE. Functional and molecular biological evidence for a possible beta3-adrenoceptor in the human detrusor muscle. Br J Pharmacol. 1999;126: 819–825.

59- Otsuka A, Kawasaki H, Matsumoto R, Shinbo H, Kurita Y, Iwashita T, Ozono S. Expression of β-adrenoceptor subtypes in urothelium, interstitial cells and detrusor of the human urinary bladder. Low Urin Tract Symptoms. 2013;5: 173–180.

60- Limberg ve ark. 2010 Limberg BJ, Andersson KE, Aura Kullmann F, Burmer G, de Groat WC, Rosenbaum JS. β-Adrenergic receptor subtype expression in myocyte and non-myocyte cells in human female bladder. Cell Tissue Res. 2010; 342:295–30

61- Silva I, Costa AF, Moreira S, Ferreirinha F, Magalhães-Cardoso MT, Calejo I, Silva- Ramos M , Correia-de-Sá P. Inhibition of cholinergic neurotransmission by β3- adrenoceptors depends on adenosine release and A1-receptor activation in human and rat urinary bladders. Am J Physiol Renal Physiol. 2017;313(2): F388-F403.

62- Beaulieu JM,Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev. 2011;63(1):182-217.

63- Sullivant A, Mackin A, Pharr T, Cooley J, Wills R, Archer T. Identification ofhistamine receptorsin the canine gastrointestinal tract. Vet Immunol Immunopathol. 2016;182:29-36.

64- Neuhaus J, Oberbach A, Schwalenberg T, Stolzenburg JU. Cultured smooth muscle cells of the human vesical sphincter are more sensitive to histamine than are detrusor smooth muscle cells. Urology. 2006;67(5):1086-92.

65- Poli E, Coruzzi G, Bertaccini G. Pre- and postjunctional effects of histamine on the guinea pig urinary bladder: evidence for heterogeneity in the H1-receptor population. Agents Actions. 1988;23(3-4):241-3.

66- Torres G, Gainetdinov R, Caron, M. Plasma membrane monoamine transporters: structure, regulation and function. Neuroscience. 2003;4, 13–26.

67- Owens M.J. Molecular and cellular mechanisms of antidepressant drugs. Depression and Anxiety. 1996;4, 153–159.

68- Ni W, Watts S. 5-Hydroxytryptamine in the cardiovascular system: focus on

the serotonin transporter (SERT).Clinical and Experimental Pharmacology and Physiology. 2006;33, 575–583.

69- Mohammad- Zadeh ve ark. 2008). Mohammad - Zadeh LF, Moses L, Gwaltney-Brant SM. Serotonin: a review. J Vet Pharmacol Ther. 2008;31(3):187-99.

70- McCarron JG, Chalmers S, Bradley KN, MacMillan D, Muir TC. Ca2+ microdomains in smooth muscle. Cell Calcium. 2006;40: 461–493.

71- Standen NB, Quayle JM. K+ channel modulation in arterial smooth muscle. Acta Physiol Scand. 1998;164: 549–557

72- Kishimoto A, Takai Y, Mori T, Kikkawa U, Nishizuka Y. Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phos phatidylinositol turnover. J Biol. Chem. 1980;255, 2273-2276.

73- Nishizuka Y. The molecular heterogeneity of protein kinase C and implications for cellular regulation. Nature. 1998;334, 661-665

74- Ono Y, Fujii T, Igarashi K, Kuno T, Tanaka C, Kikkawa U, Nishizuka Y. Phorbol ester binding to protein kinase C requires a cysteine-rich zinc-finger-like sequence. Proc. Natl. Acad. Sci. 1989;4868-4871.

75- Denning M.F, Dlugosz A.A, Howett M.K, Yuspa S.H. Expression of an oncogenic rasHa gene in murine kerat inocytes induces tyrosine phosphorylation and reduced activity of protein kinase C delta. J. Biol. Chem. 1993;268,26079-26081

CHAPTER 4

THE FACT THAT SHOULD BE DETECTED EARLY: AMBLYOPIA (LAZY EYE)

Levent DOĞAN[1], İbrahim Edhem YILMAZ[2]

1 Dr., Göz Hastalıkları Uzmanı, Kilis Devlet Hastanesi, ORCID: 0000-0002-4849-3698
2 Dr., Göz Hastalıkları Uzmanı, Kilis Devlet Hastanesi, ORCID: 0000-0003-1154-425X

Amblyopia, popularly known as lazy eye, is a neurodevelopmental disease that can be seen in both eyes, often in one eye, resulting in low vision (Martin, Lee, White, Solomon, & Rüttiger, 2001). In patients with amblyopia, there is no pathology in the eye or the visual pathways that continue behind the eye(McKee, Levi, & Movshon, 2003). In addition, another accepted definition of amblyopia is the presence of at least two lines of visual acuity difference between both eyes according to the Snellen or logMAR charts(Holmes et al., 2001). In patients who develop amblyopia, not only the visual level, but also the factors affecting the visual quality such as three-dimensional visual quality, color vision quality, and position acuity are affected(Holmes et al., 2001). The main risk factors for amblyopia include premature birth, low birth weight, growth retardation, and presence of amblyopia in first-degree relatives(Herbison et al., 2013; Li et al., 2013).

Amblyopia usually occurs because of one of the pathologies of strabismus, anisometropia and deprivation. As a result of strabismus, the image of an object does not fall on the same area of the retina in both eyes, the brain generally suppresses the blurrier image and images from the dominant eye are used. Anisometropia, on the other hand, is the refractive error between the two eyes of different sizes, and as a result, a more blurred retinal image occurs in one of the eyes. Physical barriers such as cataracts, droopy eyelids (ptosis) that prevent the formation of a high-quality retinal image can cause deprivation amblyopia. In addition, symmetrical, high refractive errors in both eyes can cause bilateral deprivation amblyopia.

a) Epidemiology

Amblyopia is the most prevalent cause of monocular vision loss in children, with an estimated population frequency of 2% to 5% (Li et al., 2013). Delays in diagnosing and treating amblyopia can result in a considerable visual impairment in adults, and amblyopia is one of the primary causes of monocular vision loss in adults.

Deprivation ambliopia is less common than other types of amblyopia. A number of clinical studies have shown that one-third of ambliopia is caused by anisometropia, a third by strabismus, and the majority of the remaining group occurs as a result of conditions where strabismus and anisometropia are seen together (Holmes, Leske, Burke, & Hodge, 2003). But strabismus-related ambliopia can be detected by families earlier than other causes of amblyopia, and treatment can be started earlier. Therefore, these rates can vary in different age groups.

b) Diagnosis of Amblyopia

The main element in the diagnosis of amblyopia is visual acuity meas-

urements. The diagnosis of amblyopia can be made when there are pathologies that may cause amblyopia in addition to the decrease in visual acuity in one or both eyes, the visual acuity does not increase even though the refractive error is corrected at the most appropriate level, and this condition cannot be explained by another eye pathology. Amblyopia is the cause of the residual visual impairment that remains as a result of correcting the refractive error with appropriate glasses or surgically correcting conditions such as cataract and ptosis.

For children under the age of two and a half, amblyopia, which is especially present in one eye, can be understood by the fact that the same eye always prefers it when fixing light or small toys. Children with strabismus are easier to detect because they always prefer the same eye for fixation or because their eyes with less vision are outside the fixation angle. "Teller cards", "Kay pictures" and "Cardiff cards" can be used to determine the low vision level in children more concretely (Rydberg, Ericson, Lennerstrand, Jacobson, & Lindstedt, 1999; Wallace, 2005).

For children older than two and a half years, Snellen or logMAR scales with symbols and letters can be used to quantify visual acuity more accurately. However, in scales such as Snellen, which is outside the logMAR test, the letters or symbols do not change in size equally when moving to the lower or upper rows, which may cause us to obtain inconclusive visual acuity results. In children aged five and over, the more scientific "Early Treatment Diabetic Retinopathy Study (ETDRS)" charts can be used in addition to the Snellen chart (Beck et al., 2003).

In many clinical visual acuity tests, it is presented to the patient with an isolated letter and 4 or 5 letters in a row around this letter. Presenting letters that are not in the crowd alone to patients is not correct for the diagnosis and follow-up of amblyopia. The "crowding phenomenon" means a lower visual acuity result when presented with optotypes around letters or symbols, and this is a finding seen both in children with amblyopia and in patients with impaired vision due to the central nervous system.

The expected neural development degrees of children in each age group are different and this condition affects the level of visual acuity. In one study, the mean visual acuity in 4-year-old children was found to be 0.1 logMAR (decimal equivalent of 8/10) with age-adjusted logMAR tests. For example, in these patients, doctors do not need to apply additional amblyopia treatments to bring the vision level to the 10/10 target.

c) Amblyopia Screening

We have said that the most important factor in the diagnosis of ambliopia is the measurement of visual acuity, so it is wise to use the acuity of

vision in the follow-up and scan of amblyopia. Most scanning programs only use visual acuity, while some also use the presence and amount of strabismus by doing autorefraction and automatic photo scans. Some programs also include vision functions such as depth perception.

According to the "Vision in Preschoolers Study" data, in a group of children aged 3-5 years old, the autorefractometer method used for scanning ambliopia was found to be more functional than the visual acuity method obtained using HOTV letters, Lea symbols, the photo scanner method and the depth perception tests. In the United Kingdom, Williams and his colleagues also found that the results obtained by combining the autorefraction method with cycloplegia and the cover test for strabismus detection in children aged 37 months on average were found to be statistically more significant in the detection of amblyopia compared to other methods (Williams, Harrad, Harvey, Sparrow, & Team, 2001).

Another conclusion had shown us by the "Vision in Preschoolers" study is the fact that if autorefraction is preferred as a scanning tool to visual acuity tests, the scan can also be done by nurses and people trained from the public instead of by an ophthalmologist or optometrist (V. i. P. S. Group, 2004). Because the results in visual acuity tests can be affected by the person who applies it, the scale and shape chosen, even the distances at which letters or symbols are shown.

Amblyopia caused by strabismus and anisometropia should be detected and treated before the age of 7. In this context, screening tests should also be done in the first years of life, where visual and neural development is very important. In many states in the United States, screening tests are recommended at the earliest age (average 3 years of age) when a visual acuity test can be performed. To reduce the burden of ambliopia on society, many studies suggest that their tests must be done as early as possible and that their treatments must begin without delay.

d) Amblyopia Treatment

Some public health authorities questioned the necessity of treatment for amblyopia, especially in one eye, and argued that this would create a small functional deficit and even create more psychological burden in children during the treatment period. While there are many studies showing that the quality of life of people with amblyopia in both eyes is seriously affected, there are a few studies showing that the quality of life decreases in people with amblyopia in one eye. Although low visual acuity in one eye does not affect daily activities much, it is known that these people are restricted in their choice of occupation (Membreno, Brown, Brown, Sharma, & Beauchamp, 2002). In a study, it was shown that the university success

rate of people with amblyopia in one eye is lower than healthy individuals.

In individuals with amblyopia in one eye, the slightest decrease in vision in the healthy eye will directly affect the quality of life. Thirty-five of the patients with amblyopia in one eye, whom Tommila and Tarkkanen followed for 20 years, lost their sight in their healthy eyes. More than 50% of these individuals lost their vision in their healthy eyes due to trauma (Tommila & Tarkkanen, 1981). In a study, it was shown that the incidence of vision loss in amblyopic individuals was 1.75/1000 in the healthy eye. In the same period, while the incidence of blindness in children was 0.11/1000, this rate was 0.66/1000 in adults. Based on these data, the possibility of developing blindness in amblyopic individuals is too high to be underestimated. Especially considering that these patients are still children, visual acuity should be increased as much as possible with their expected life expectancy. It should not be forgotten that after a trauma they may experience, their amblyopic eyes will switch to a better seeing eye state.

Prevention

Eye scans are very important for identifying the factors that cause ambliopia. The sooner anisometropia and strabismus are diagnosed and treated, the sooner and greater the increase in visual acuity. Sometimes better treatment results can also be obtained in older children and adolescents.

According to a study by the Pediatric Eye Disease Investigator Group (PEDIG), two-thirds of children with moderate strabismus or anisometropia under the age of 7 had a vision level higher than 20/30 with treatment in a short period of six months (M. X. Repka et al., 2008).

The treatment choice

The chance of success in the treatment of ambliopia decreases with the age of the child. However, at least one treatment method should be recommended to every patient diagnosed regardless of the patient's age. There are many factors that affect the patient's outcome of treatment, such as the age of initiation, the cause, severity, the duration of ambliopia, the results obtained from previous therapies, and the patient's compliance to therapy. Correction of the pathology causing amblyopia, correction of the refractive error, preferring the amblyopic eye to the normal eye in daily life with eye patch are the main treatment methods. The primary goal of treatment is to equalize the acuity of both eyes as much as possible. The treatment protocol is determined according to the child's age, visual acuity, adjustment to previous treatments and the child's physical, social and psychological status.

Main treatments for amblyopia in children;

- Optical correction of refractory defects
- Treatment of eye patch
- Pharmacological treatment
- Refractive Surgery
- Alternative treatments

Optical correction

Even correction of low-grade refractive defects, especially in myopic patients, can significantly increase the patient's visual acuity. With the correction of the refractive defect and ensuring the continuity of this condition, clearer images fall into the fovea of the ambliopian eyes. Especially in patients undergoing optic correction for the first time, only optical correction can improve ambliopia. According to the results of the Pediatric Eye Disease Investigator Group study, children with ambliopia were treated only with glasses until the increases in visual acuity stopped, and in one-quarter of these children, the eyes with poor vision were able to reach the level of vision acuity of well-seeing eyes (Beck, 1998). Correction of refractive defect is the primary treatment method for patients between 0-17 years of age (Group, 2008; Scheiman et al., 2005). Correcting the refractive defect with glasses and enabling patients to adapt to this situation is a very difficult process, especially in patients who see well with one eye. Since it is very difficult to persuade patients to wear glasses continuously in such cases, resolving the refractive defect with surgical methods can yield successful results.

Occlusion therapy

Eye patch treatment is a treatment method that has been used for more than 100 years (Loudon & Simonsz, 2005). Eye patch treatment should be started in children whose vision level cannot be increased by only using glasses (M. Repka et al., 2005; Michael X Repka et al., 2003). By closing the eyes of the children with better vision, it is ensured that they continue their daily activities with their relatively less-sighted eyes. According to the data of the "Amblyopia treatment study", in children under 7 years of age with severe amblyopia (visual acuity between 20/100 and 20/400), 6-hour patching per day provided the same amount of visual improvement as all-day eye-patch treatment (P. E. D. I. Group, 2004). In children with moderate amblyopia (visual acuity between 20/40 and 20/80), eye patching for 2 hours per day provided a similar degree of visual improvement with 6 hours per day (Michael X Repka et al., 2003). In patients up to the age of 15 years, it has been shown that visual acuity can be improved with occlusion therapy. For this reason, this treatment method should also be

applied in older children and adolescents, who have not tried occlusion treatment before (Scheiman et al., 2005).

Before starting the treatment, it is necessary to convince the parents of the necessity and functionality of the treatment. This will reduce the pressure on the patient and family (David Newsham, 2000; D Newsham, 2002). In occlusion therapy, it is necessary to close the eye directly, not to tape the glasses. Because children will continue to see from the periphery of the glasses and they can abuse this situation.

Pharmacological treatment

Cycloplegia

Pharmacological treatment can be used in patients with cycloplegia in the sound eye, especially in patients whose visual acuity cannot be increased with eyeglasses alone, who have low compliance with occlusion therapy, or who have latent nystagmus (Mohan, Saroha, & Sharma, 2004; Michael X Repka et al., 2003). Cycloplegia treatment impairs the accommodation function of the better seeing eye, making image focusing difficult. This treatment is particularly beneficial in patients with hyperopia refractive errors in their sound eyes. It has been shown that pharmacological treatment of cycloplegia can be effective up to the age of 15 (Michael X Repka et al., 2014).

Treatment of amblyopia with daily cycloplegic drops has yielded as successful results as occlusion treatment as an initial treatment (Force, 2011). In children with moderate amblyopia, no significant difference was found between instilling 1% atropine once every other day for 4 months and instilling it every other day in terms of increasing visual acuity (Michael X Repka et al., 2003). It has been shown that atropine, twice a week, has a partial positive effect on visual acuity in children aged 3-12 years with severe amblyopia (Gopal, Kelkar, Kelkar, & Pandit, 2019).

Levodopa- Carbidopa

Iuvone et al. proposed that increasing dopamine levels may improve visual acuity in amblyopia patients. Some investigators have shown that retinal dopamine levels are reduced in patients who develop deprivation amblyopia (Iuvone, Tigges, Fernandes, & Tigges, 1989). In a randomized trial, PEDIG investigated the effects of levodopa and carbidopa in addition to daily eye patching for 2 hours in amblyopic patients. As a result, they noticed that there was no clinically significant increase in visual acuity of the patients. Sofia et al. studied the effect of levodopa and placebo in addition to all-day eye-closure in children who had not previously received any amblyopia treatment other than eyeglasses treatment, in a randomized

period for 1 year. They discovered a statistically significant improvement in vision in patients who received levodopa after a year. However, the dose of levodopa used in this study was three times that of the PEDIG study (Sofi, Gupta, Bharti, & Tantry, 2016).

Citicoline

Citicoline has cholinergic as well as neuroprotective properties. Early studies of citicoline found that using it in conjunction with occlusion therapy resulted in significant increases in vision in adult patients; however, this positive effect did not last after the drug was stopped. Citicoline alone or in combination with occlusion therapy produced promising results in studies with amblyopic children. After occlusion therapy, untreated amblyopic patients were randomized to receive citicoline or a placebo. The citicoline group had a significant increase in visual acuity at the end of 90 days; however, the fact that it did not improve vision in the placebo group despite eye closure treatment has raised concerns about the validity of this study. Furthermore, the duration of studies investigating the effect of citicoline on amblyopic patients did not exceed 3-6 months (Campos, Schiavi, Benedetti, Bolzani, & Porciatti, 1995; Fresina, Dickmann, Salerni, De Gregorio, & Campos, 2008).

Refractive surgery

Refractive surgeries can be used in children who are unable to adapt to glasses and in patients who do not respond to other standard treatment methods. Correction of refractive error with surgery improved not only visual function but also general functions in ametropic children with neurobehavioral disorders who could not wear glasses. Although clear lens extraction has shown some benefits in the treatment of amblyopia, it does not appear to be a powerful or logical alternative to PRK. There aren't a lot of randomized controlled trials that look into and support the effect of refractive surgery methods on amblyopia (Kraus & Culican, 2018).

Alternative treatments

Orthoptic treatments are non-invasive visual physical activities prescribed by a doctor that can improve visual acuity and binocular vision. Computer programs, prisms, filters, metronomes, vergence and accommodation activities, and hand-eye coordination movements are examples of these activities. These treatments can be administered in the office, with the assistance of therapists, or by patients at home. Although there is little scientific evidence to support these treatment techniques, they can be used in conjunction with other amblyopia treatments.

Binocular treatment can be used on patients who do not have strabis-

mus or only have a minor angle shift. A high-contrast image is shown to the amblyopic eye, while a low-contrast image is shown to the sound eye in this treatment method. The binocular treatment method was used in the "Falling blocks" game on iPad® (Apple, Inc., Cupertino, CA) devices, and children wearing red-green anagliptic glasses played the game. Initial research on this treatment method appears promising (Holmes et al., 2016; Kraus & Culican, 2018).

Liquid crystal glasses were developed as an alternative to occlusion treatment in amblyopia, and patients are more likely to comply with liquid crystal glasses than with occlusion treatment. At regular intervals, the glass in front of the sound eye becomes opaque. Spierer et al. demonstrated that this treatment method is equally as effective as occlusion therapy (Spierer et al., 2010).

References

Beck, R. W. (1998). The pediatric eye disease investigator group. In (Vol. 2, pp. 255-256).

Beck, R. W., Moke, P. S., Turpin, A. H., Ferris III, F. L., SanGiovanni, J. P., Johnson, C. A., . . . Blair, R. C. (2003). A computerized method of visual acuity testing: adaptation of the early treatment of diabetic retinopathy study testing protocol. *American journal of ophthalmology, 135*(2), 194-205.

Campos, E., Schiavi, C., Benedetti, P., Bolzani, R., & Porciatti, V. (1995). Effect of citicoline on visual acuity in amblyopia: preliminary results. *Graefe's archive for clinical and experimental ophthalmology, 233*, 307-312.

Force, U. P. S. T. (2011). Vision screening for children 1 to 5 years of age: US Preventive Services Task Force Recommendation statement. *Pediatrics, 127*(2), 340-346.

Fresina, M., Dickmann, A., Salerni, A., De Gregorio, F., & Campos, E. C. (2008). Effect of oral CDP-choline on visual function in young amblyopic patients. *Graefe's Archive for Clinical and Experimental Ophthalmology, 246*, 143-150.

Gopal, S. K., Kelkar, J., Kelkar, A., & Pandit, A. (2019). Simplified updates on the pathophysiology and recent developments in the treatment of amblyopia: A review. *Indian journal of ophthalmology, 67*(9), 1392.

Group, P. E. D. I. (2004). A randomized trial of atropine regimens for treatment of moderate amblyopia in children. *Ophthalmology, 111*(11), 2076-2085. e2074.

Group, P. E. D. I. (2008). Patching vs atropine to treat amblyopia in children aged 7 to 12 years: a randomized trial. *Archives of ophthalmology, 126*(12), 1634-1642.

Group, V. i. P. S. (2004). Comparison of preschool vision screening tests as administered by licensed eye care professionals in the Vision in Preschoolers Study. *Ophthalmology, 111*(4), 637-650.

Herbison, N., Cobb, S., Gregson, R., Ash, I., Eastgate, R., Purdy, J., . . . Foss, A. (2013). Interactive binocular treatment (I-BiT) for amblyopia: results of a pilot study of 3D shutter glasses system. *Eye, 27*(9), 1077-1083.

Holmes, J. M., Beck, R. W., Repka, M. X., Leske, D. A., Kraker, R. T., Blair, R. C., . . . Hertle, R. W. (2001). The amblyopia treatment study visual acuity testing protocol. *Archives of ophthalmology, 119*(9), 1345-1353.

Holmes, J. M., Leske, D. A., Burke, J. P., & Hodge, D. O. (2003). Birth prevalence of visually significant infantile cataract in a defined US population. *Ophthalmic epidemiology, 10*(2), 67-74.

Holmes, J. M., Manh, V. M., Lazar, E. L., Beck, R. W., Birch, E. E., Kraker, R. T., . . . Summers, A. I. (2016). Effect of a binocular iPad game vs part-time

patching in children aged 5 to 12 years with amblyopia: a randomized clinical trial. *JAMA ophthalmology, 134*(12), 1391-1400.

Iuvone, P. M., Tigges, M., Fernandes, A., & Tigges, J. (1989). Dopamine synthesis and metabolism in rhesus monkey retina: development, aging, and the effects of monocular visual deprivation. *Visual neuroscience, 2*(5), 465-471.

Kraus, C. L., & Culican, S. M. (2018). New advances in amblyopia therapy II: refractive therapies. *British Journal of Ophthalmology, 102*(12), 1611-1614.

Li, J., Thompson, B., Deng, D., Chan, L. Y., Yu, M., & Hess, R. F. (2013). Dichoptic training enables the adult amblyopic brain to learn. *Current Biology, 23*(8), R308-R309.

Loudon, S., & Simonsz, H. (2005). The history of the treatment of amblyopia. *Strabismus, 13*(2), 93-106.

Martin, P. R., Lee, B. B., White, A. J., Solomon, S. G., & Rüttiger, L. (2001). Chromatic sensitivity of ganglion cells in the peripheral primate retina. *Nature, 410*(6831), 933-936.

McKee, S. P., Levi, D. M., & Movshon, J. A. (2003). The pattern of visual deficits in amblyopia. *Journal of vision, 3*(5), 5-5.

Membreno, J. H., Brown, M. M., Brown, G. C., Sharma, S., & Beauchamp, G. R. (2002). A cost-utility analysis of therapy for amblyopia. *Ophthalmology, 109*(12), 2265-2271.

Mohan, K., Saroha, V., & Sharma, A. (2004). Successful occlusion therapy for amblyopia in 11-to 15-year-old children. In (Vol. 41, pp. 89-95): Slack Incorporated Thorofare, NJ.

Newsham, D. (2000). Parental non-concordance with occlusion therapy. *British Journal of Ophthalmology, 84*(9), 957-962.

Newsham, D. (2002). A randomised controlled trial of written information: the effect on parental non-concordance with occlusion therapy. *British Journal of Ophthalmology, 86*(7), 787-791.

Repka, M., Wallace, D., Beck, R., Kraker, R., Birch, E., Cotter, S., & Holmes, J. (2005). Pediatric eye disease investigator group two-year follow-up of a 6-month randomized trial of atropine vs patching for treatment of moderate amblyopia in children. *Arch Ophthalmol, 123*(2), 149-157.

Repka, M. X., Beck, R. W., Holmes, J. M., Birch, E. E., Chandler, D. L., Cotter, S. A., . . . Quinn, G. E. (2003). A randomized trial of patching regimens for treatment of moderate amblyopia in children. *Archives of ophthalmology (Chicago, Ill.: 1960), 121*(5), 603-611.

Repka, M. X., Kraker, R. T., Beck, R. W., Holmes, J. M., Cotter, S. A., Birch, E. E., . . . Glaser, S. R. (2008). A randomized trial of atropine vs patching for treatment of moderate amblyopia: follow-up at age 10 years. *Arch Ophthalmol, 126*(8), 1039-1044. doi:10.1001/archopht.126.8.1039

Repka, M. X., Kraker, R. T., Holmes, J. M., Summers, A. I., Barnhardt, C. N.,

Tien, D. R., & Glaser, S. R. (2014). A randomized trial of atropine versus patching for treatment of moderate amblyopia: follow-up at 15 years of age. *Journal of American Association for Pediatric Ophthalmology and Strabismus {JAAPOS}, 18*(4), e9.

Rydberg, A., Ericson, B., Lennerstrand, G., Jacobson, L., & Lindstedt, E. (1999). Assessment of visual acuity in children aged 1½-6 years, with normal and subnormal vision. *Strabismus, 7*(1), 1-24.

Scheiman, M. M., Hertle, R. W., Beck, R. W., Edwards, A. R., Birch, E., Cotter, S. A., . . . Donahue, S. (2005). Randomized trial of treatment of amblyopia in children aged 7 to 17 years. *Archives of ophthalmology (Chicago, Ill.: 1960), 123*(4), 437-447.

Sofi, I. A., Gupta, S. K., Bharti, A., & Tantry, T. G. (2016). Efficiency of the occlusion therapy with and without levodopa-carbidopa in amblyopic children-A tertiary care centre experience. *International Journal of Health Sciences, 10*(2), 249.

Spierer, A., Raz, J., BenEzra, O., Herzog, R., Cohen, E., Karshai, I., & BenEzra, D. (2010). Treating amblyopia with liquid crystal glasses: a pilot study. *Investigative ophthalmology & visual science, 51*(7), 3395-3398.

Tommila, V., & Tarkkanen, A. (1981). Incidence of loss of vision in the healthy eye in amblyopia. *British Journal of Ophthalmology, 65*(8), 575-577.

Wallace, D. K. (2005). Tests of fixation preference for amblyopia. *American Orthoptic Journal, 55*(1), 76-81.

Williams, C., Harrad, R., Harvey, I., Sparrow, J., & Team, A. S. (2001). Screening for amblyopia in preschool children: results of a population-based, randomised controlled trial. *Ophthalmic epidemiology, 8*(5), 279-295.

CHAPTER 5

MAMMARY GLAND IMMUNOLOGY AND PRESENCE OF CYTOKINES IN COW MASTITIS

Tarık ŞAFAK[1], Öznur YILMAZ[2], Ali RİŞVANLI[3]

1 Assistant Professor, Kastamonu University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Kastamonu, Türkiye. https://orcid.org/0000-0002-6178-4641

2 Assistant Professor, Siirt University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Siirt, Türkiye. https://orcid.org/0000-0003-0424-9471

3 Prof. Dr., Fırat University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Elazığ, Türkiye. https://orcid.org/0000-0001-5653-0025

Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Bishkek, Kyrgyzstan. https://orcid.org/0000-0001-5653-0025

Mastitis is one of the most common and costly diseases worldwide. Mastitis is an infection that affects individual or herd health, udder health and milk yield. Mastitis is defined as inflammation of the udder tissue (Halasa et al., 2007; Darbaz et al., 2023). Innate and adaptive immunity is formed in the organism against mastitis. It has been known that cytokines, which are one of the parts of both immune systems, have had an effect on the pathophysiology of mastitis for decades (Sordillo et al., 2018). Immune cells have trouble to eradicate invading pathogens during mastitis. Cytokines are crucial for establishing an immune response against an intramammary infection. Cytokines are a general term describing molecules in the structure of the polypeptide, protein, or mostly glycoprotein, formed by the combination of the words cyto (cell) and kinos (action). So far, scientists have discovered a plethora of cytokines (Dembic, 2015). Cytokines are mostly produced by T and B lymphocytes, macrophages, and dendritic and granulocyte cells (Abbas et al., 2015). Cytokines play important roles in numerous physiological and pathological processes within the organism. These responsibilities include immune system cell differentiation, activation (immunostimulation), and deactivation (immunosuppression). On the other hand, immune system cells play crucial roles in tissue and organ migration (chemotaxis). Cytokines exert their effects by binding to receptors on the target cell's membrane. These receptors are specific for a single cytokine as well as multiple cytokine groups (Dembic, 2015). Cytokines have autocrine (the cytokine acts on the cell where it is secreted), paracrine (the secreted cytokine affects the cell next to it), and endocrine effects (the cytokine is carried through the blood and affects the cell in another part of the body). Cytokines have effects both on their own and in combination with one another. Cytokines have properties such as pleiotropy (one cytokine has different effects in different cells), redundant (various cytokines have the same impact on the same cell), synergistic (cytokines increase the development of each other), antagonistic (one cytokine inhibits the effect of another cytokine) and cascade (cytokines stimulate the production of another cytokine in the form of a chain) (Owen et al., 2013). Even though cytokines are very important in infections, they can also harm the organism. Therefore, there is a delicate balance between the amount and duration of cytokine expressions for cytokines' positive and negative effects on the organism. Nevertheless, significant immunomodulatory abilities on udder functions have been demonstrated according to the roles of cytokines in the organism, the cells they originate from, and the cells they affect. Significant groups of cytokines studied to date include interferons (IFN), Colony Stimulating Factor (CSF), interleukins (IL), and Tumor Necrosis Factor (TNF) (Sordillo and Streicher, 2002; Rişvanlı et al., 2019).

Interferons

It was one of the first cytokine families to be discovered. It has been discovered to prevent pathogen spread by having an antiviral effect in viral infections (Isaacs and Lindenmann, 1957). Interferons are classified into three Types: Type I, II, and III.

Type I IFN includes IFN-alpha (α), IFN-omega (ω), IFN-beta (β), IFN-kappa (κ), and IFN-epsilon (ε). Although they are produced from almost all cells, the primary cell group they are produced from is dendritic cells (de Weerd and Nguyen, 2012; Ivashkiv and Donlin, 2014). Interferon-α is generated by macrophages, dendritic cells, and virus-infected cells in particular. It is essential for preventing the spread of infectious viruses. They are produced well in advance of illness and delay the spread of the virus until immunity is acquired (Male et al., 2012). Interferon-β increases the production of anti-inflammatory cytokines such as IL-4, -10 resulting in the Th2 response. It also leads to the reduction of TNF-α and IFN-γ (Kozovska et al., 1999). Interferon-ω shows the typical features of type I IFNs. In addition, it shows antiviral and antiproliferative effects against the bovine enteric virus (BEV), infectious bovine rhinotracheitis virus (IBRV), and bovine viral diarrhea virus (BVDV), as well as vesicular stomatitis virus (VSV) infection in the mammary cell (An et al., 2017). Interferon-ε and IFN-κ also show antiviral effects like other Type I IFNs (de Weerd and Nguyen, 2012).

Interferon-γ is the only known member of Type II IFNs. The main secreted cell groups are CD8+ T cells, Th1, and Natural Killer (NK) cells. Although IFN-γ does not have a direct antiviral effect, it has essential functions during the cellular immune response (Diker, 2005; de Weerd and Nguyen, 2012). Although it promotes the development of Th1 and B lymphocytes, it inhibits the development of Th9, Th2, and Th17 lymphocytes (Dembic, 2015). Interferon-γ emerges as an indicator of innate and adaptive immunity. On the other hand, excessive secretion of interferon-γ is associated with the pathogenesis of chronic inflammation. Also, IFN-γ has been reported to have the potential to direct inflammatory responses by inhibiting the production of IL-1 and 8, which are proinflammatory cytokines (Mühl and Pfeilschifter, 2003). Increased production of IFN-γ was detected in milk from udders infected with *Staphylococcus aureus (S. aureus*) and *Escherichia coli (E. coli)* (Riollet et al., 2001; Lee et al., 2006). It has been determined that the concentration of IFN-γ in milk is increased in naturally developed coliform mastitis (Hisaeda et al., 2001) and experimental mastitis caused by *Streptococcus uberis* (*S. uberis*)*, E. coli, Klebsiella pneumoniae, S. aureus*, and *Serratia marcescens* (Bannerman et al., 2004a; 2004b; 2004c). Type III IFN, like other types of IFNs, has immunomodulatory and antiviral functions (Dembic, 2015).

Interleukins

Interleukins are a large family of cytokines that are primarily produced by T cells. While their names are IL-1, IL-2, IL-4… etc., many ILs show similarities and differences in source and function (Male et al., 2012).

Interleukin-1

Interleukin-1 is one of the very important proinflammatory cytokines. It plays a very important role in both the systemic and local immune responses. It contributes to the expression of genes in the immune response with other cytokines and the regulation of cell apoptosis and proliferation. As protein structures, there are IL-1α and IL-1β subgroups (Schukken et al., 2011). Interleukin-1 is mainly produced by macrophages that come into contact with bacterial products such as lipopolisakkaritler (LPS) and CD4+ T cells stimulated by TNF and IL-1. Interleukin-1 can affect almost all immune cells. However, Th2 cells are the most important cells that it involves in terms of immune response development. It regulates local inflammatory reactions at low concentrations. When it reaches a high concentration, it enters the circulation and has an endocrine effect. Its systemic effects are characterized by increased body temperature and the release of acute-phase proteins in the liver (Diker, 2005).

It has been reported in different studies that IL-1 is increased in mastitis. In addition, it has been reported that the concentration in milk is increased in intramammary infection caused by *E. coli*. Moreover, mRNA expression of IL-1β and IL-1α has also been isolated in mammary cells derived from mastitis mammary glands (Riollet et al., 2000; Riollet et al., 2001). Although IL-1β concentration reaches its maximum only 4 hours after intramammary infection with *E. coli*, more time is needed to produce IL-1β concentration in mastitis from Gram-positive bacteria (Waller et al., 2003; Rambeaud et al., 2003). A rapid increase in leukocytes in the udder was detected in cows treated with recombinant bovine IL-1β (rboIL-1β) in the dry period to prevent mastitis. In addition, it has been reported that the involution rate of the udder increases, and the lactoferrin concentration increases at the beginning of the dry period in these animals (Wedlock et al., 2004).

Interleukin-2

Although IL-2 was identified as a growth factor for T cells, it was later found to help the growth of many hematopoietic cells, including regulatory T cells (Tregs) (Dembic, 2015). This cytokine's crucial function is to exchange and develop helper and cytotoxic T lymphocytes, B, and NK cells. In addition, Th1 stimulation causes macrophage activation, and Th2 cell stimulation causes antibody synthesis (Diker, 2005). Interleukin-2 is one of the essential cytokines secreted by Th1 helper cells and is responsible

for stimulating cellular immunity against intracellular pathogens (Alnakip et al., 2014).

An increase in IL-2 transcriptional activity has been detected in the bovine udder during the late lactation period (Alluwaimi and Cullor, 2002). After a single dose of IL-2 was administered to the subramamar lymph node following delivery, an increase in leukocyte and epithelial cell activity was observed in the milk (Zecconi et al., 2009). Neutrophils isolated from milk from mammary lobes treated with IL-2 were found to have high phagocytic properties against *S. aureus* in vitro study (Wedlock et al., 2000).

Interleukin-4

Interleukin-4 is mainly secreted by T cells. However, it can also be secreted by B cells, mast cells, dendritic cells, and basophils (Dembic, 2015). It has an impact on B and T cells, macrophages, and mast cells. Interleukin-4, in particular, promotes B cell differentiation and development. As a result, it boosts IgE production (Tizard, 2004). The level of IL-4 was lower in the healthy group compared to the *E. coli* and *S. aureus* mastitis-affected milk (Safak et al., 2022). Fonseca et al. (2009) discovered no difference in the concentration of IL-4 in mastitis and healthy milk. In contrast, IL-4 concentrations were found to be lower in milk with Coagulase-Negative Staphylococci (CNS) mastitis compared to healthy milk (Bochniarz et al., 2017). Based on these findings, it is possible to conclude that the bacterial species had varying effects on the concentration of secreted IL-4. It has been reported that the level of IL-4 in milk from a mastitis udder is lower than that of milk from a healthy udder. Because of this property, IL-4 is classified as an anti-inflammatory cytokine (Bochniarz et al., 2017).

Interleukin-5

Interleukin-5 is generally produced in Th2 cells. Interleukin-5 is the most potent cytokine controlling eosinophils. It plays an essential role in eosinophils' development, growth, maturation, and activation. In addition, it contributes to the production of IgA from B lymphocytes. Therefore, it is known to be involved in the humoral response (Abbas et al., 2015). It is stated that there is an increase in IL-5 level in mastitis caused by *E. coli, S. aureus,* and *Streptococcus agalactiae* (*S. agalactiae*) (Safak et al., 2022). It has been reported that there is an increase in the level of IL-5, and this increase is dependent on the increase in milk somatic cell count (SCC) (Safak and Risvanli, 2022).

Interleukin-6

Interleukin-6 is a cytokine that has pleiotropic effects. It has anti-inflammatory properties as well as pro-inflammatory properties (Scheller

et al., 2014). Although macrophages are the primary producers, T and B lymphocytes also produce it. Interleukin-6 affects hepatocytes, causes the release of acute-phase proteins, and promotes the development of B and T cells. IL-6 stimulates IgM and IgA synthesis with IL-1 and IL-5, respectively (Diker, 2005). Interleukin-6 mRNA transcription is higher in cells isolated from naturally occurring or experimentally induced mastitis mammary cells than in cells isolated from the uninfected mammary gland (Riollet et al., 2001; Lee et al., 2006). It has been reported that blood and milk level of IL-6 in naturally occurring and experimentally induced mastitis range from 10 to 50 ng/ml and 30 to 90 ng/ml, respectively (Dernfalk et al., 2007; Hagiwara et al., 2001). Interleukin-6 concentration was 20 times higher in milk and 2.5 times higher in serum from cows with CNS mastitis compared to healthy cows (Bochniarz et al., 2017). In both natural and experimentally induced mastitis, the blood and serum concentrations of IL-6 are reported to be high only during the infection (Hagiwara et al., 2001; Ma et al., 2011). Interleukin-6 concentration was determined as 5.2 ng/ml, 30.8 ng/ml, and 18.0 ng/ml in healthy milk and milk with CNS-derived subclinical and clinical mastitis, respectively (Osman et al., 2010). In another study, blood IL-6 levels were found to be similar in healthy, subclinical and clinical mastitis groups, while clinical and subclinical mastitis groups in milk were found to be higher than in the healthy group (Kurt et al., 2021). According to reports, the increase in IL-6 concentration in milk occurs before the increase in SCC. As a result, determining milk IL-6 concentration is regarded as a diagnostic parameter that can be used to make an early diagnosis of subclinical mastitis (Sakemi et al., 2010).

Interleukin-10

It is an anti-inflammatory cytokine that regulates inflammation as well as the functions of T cells, NK cells, and macrophages. Its primary source is Th2 cells, which inhibit Th1 cell functions. Macrophages and B cells can also produce it. Interleukin-10 inhibits the production of IL-6, IL-8, TNF- α, IL-1, and IL-12. As a result, it prevents macrophages and T cells from activating their inflammatory functions. It is also known as a cytokine synthesis inhibitor because of this property (Tizard, 2004; Diker, 2005). Interleukin-10 is a well-studied anti-inflammatory interleukin in the immune response in mammary gland. Interleukin-10 has two main functions: it inhibits cytokine synthesis and it decreases the major histocompatibility complex (MHC)-II. Interleukin-10, on the other hand, is involved in antigen uptake, differentiation, and function of B and T lymphocytes. While *E. coli* infection results in a significant increase in the level of IL-10 in milk, *S. uberis* infection results in a delayed immune response. However, it was also stated that no significant increase in IL-10 production was detected in *S. aureus* intramammary infections (Bannerman, 2009). Another study

found lower levels of IL-10 in the serum and milk of cows with CNS-derived subclinical mastitis when compared to healthy cows (Bochniarz et al., 2017).

Tumor Necrosis Factor

Tumor necrosis factor has two subtypes, TNF-β and TNF-α, and the effects of these cytokines are almost similar. Tumor necrosis factor-α, which is stated to be primarily secreted from macrophages, is one of the acute phase cytokines produced in the early stages of infection. Tumor necrosis factor-α has been reported to cause endotoxic shock during peracute coliform mastitis infection. Moreover, high serum and milk concentrations were found in cows that died from acute *E. coli* mastitis in the postpartum period (Sordillo and Streicher, 2002). Tumor necrosis factor-β is mainly secreted from T cells and, like TNF-α, has a systemic pyrogen effect. Moreover, overproduction of TNF-β and TNF-α causes severe tissue destruction; if it continues to increase in severity, it can cause shock and death (Diker, 2005).

As in other infectious diseases, TNF-α is produced in cases of bovine mastitis. High concentrations of cytokinins are detected in both blood and milk after mastitis. While TNF-α was not detected in milk obtained from healthy mammary lobes, it was shown that TNF-α concentration in milk increased in experimentally induced *E. coli* infection (Bannerman, 2009). It has been shown that TNF-α concentrations in milk obtained from cows with naturally developing mastitis due to *E. coli* are significantly increased. Moreover, it was stated that with the increase in the severity of the symptoms of mastitis, the level of TNF-α also increased (Ohtsuka et al., 2001). The concentration of TNF-α was found higher in milk samples with bacterial growth compared to healthy milk samples (Safak and Risvanli, 2021). There is a positive relationship between the increase in clinical findings and TNF- α level in *E. coli*-induced mastitis (Bannerman et al., 2004c).

Colony Stimulating Factor

Colony stimulating factor is a cytokine required for the differentiation and proliferation of various hematopoietic stem cells. This cytokine is produced by various cells, including endothelial cells, fibroblasts, macrophages, and T cells, that bind to cells by a common receptor. In addition, CSF is involved in the development and regulation of the function of granulocytes in cow's milk (Kaçar and Kırşan, 2016; Sordillo and Streicher, 2002). Administration of recombinant bovine granulocyte-macrophage (rbGM)-CSF in cows significantly increases the number of macrophages and neutrophils in the mammary gland. In addition, it both increases the bactericidal capacity of neutrophils in peripheral blood and causes a signif-

icant increase in their chemotactic activity. Because of these effects, applying rbGM-CSF at the initial stage of mastitis caused by *S. aureus* can be used as a potential treatment tool (Wedlock et al., 2004; Takahashi et al., 2004). Pegbovigrastim (bovine granulocyte-CSF), a commercial preparation, increases the production and activity of neutrophils. As a result, Pegbovigrastim provides a 25-35% reduction in clinical mastitis in the first months of lactation. It also reduces new mastitis cases by 35% (Canning et al., 2017; Ruiz et al, 2017).

Transforming Growth Factor

Transforming growth factor (TGF) has two subtypes, TGF-β and TGF-α. Transforming growth factor-β different from the epidermal growth factor family member TGF-α, is a cytokine with well-defined effects on cell growth and differentiation. In addition, TGF-β, which is involved in different stages of mammary gland development, plays a role in developing ductal growth and alveolar functions (Daniel, 2001; Bannerman, 2009). Transforming growth factor-β has three known subtypes in mammals; TGF-β1, β2, and β3. According to reports, only TGF-1 and TGF-2 were found in bovine milk (Bannerman, 2009; Kajdaniuk et al., 2013). The immunomodulatory effects of TGF-β include limiting IFN-γ production, inhibiting nitric oxide production, increasing IL-1 receptor antagonist expression, as well as increasing phagocytosis of damaged parenchymal cells, inflammatory cells, and bacterial debris by macrophages (Bannerman, 2009). Transforming growth factor -α plays a role in tissue repair, epithelial cell proliferation, and improvement of mammary gland morphology. Contrary to other cytokines, it was stated that they were also detected in milk samples obtained from healthy udders. On the other hand, it is stated that there is an increase in TGF-α level in milk with mastitis caused by *E. coli, Mycoplasma bovis, S. agalactiae*, *S. aureus,* and *Pseudomonas aeruginosa* pathogens (Bannerman, 2009). It is also stated that there is no difference in TGF-β level between healthy mammary gland and mastitis cases caused by *E. coli* and *S. aureus* (Lahouassa et al., 2007). In contrast, it is stated that there is a significant increase in TGF-β1 level on in cases of mastitis caused by mixed infection (Safak and Risvanli, 2021). Transforming growth factor-α concentrations typically persist longer after infection. Even in mammary gland infection of *S. aureus* where IL-8 and TNF-α production is not induced, TGF-α production increases. It is possible that TGF- α will play a more significant part in the host's defense against mastitis if there are no other proinflammatory mediators present (Bannerman, 2009).

References

Abbas, A., Lichtman, A., & Pillai, S. (2015). *Basic immunology: functions and disorders of the immune system*. Elsevier.

Alluwaimi, A. M., & Cullor, J. S. (2002). Cytokines gene expression patterns of bovine milk during middle and late stages of lactation. *Journal of veterinary medicine, series B, 49*(2), 105-110.

Alnakip, M., Quintela-Baluja, M., Böhme, K., Fernández-No, I., Caamaño-Antelo, S., Calo-Mata, P., & Barros-Velázquez, J. (2014). The immunology of mammary gland of dairy ruminants between healthy and inflammatory conditions.*Journal of veterinary medicine*, *2014*, 1–31.

An, D., Guo, Y., Bao, J., Luo, X., Liu, Y., Ma, B., ... & Wang, J. (2017). Molecular characterization and biological activity of bovine interferon-omega3. *Research in veterinary science*, *115*, 125-131.

Bannerman, D. D. (2009). Pathogen-dependent induction of cytokines and other soluble inflammatory mediators during intramammary infection of dairy cows. *Journal of animal science*, *87*, 10–25.

Bannerman, D. D., Paape, M. J., Hare, W. R., & Hope, J. C. (2004b). Characterization of the bovine innate immune response to intramammary infection with *Klebsiella pneumoniae*. *Journal of dairy science*, *87*(8), 2420-2432.

Bannerman, D. D., Paape, M. J., Lee, J. W., Zhao, X., Hope, J. C., & Rainard, P. (2004c). *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. *Clinical and vaccine ımmunology*, *11*(3), 463-472.

Bannerman, D., Paape, M., Goff, J., Kimura, K., Lippolis, J., & Hope, J. (2004a). Innate immune response to intramammary infection with *Serratia marcescens* and *Streptococcus uberis*. *Veterinary research*, *35*(6), 681-700.

Bochniarz, M., Zdzisińska, B., Wawron, W., Szczubiał, M., & Dąbrowski, R. (2017). Milk and serum IL-4, IL-6, IL-10 and amyloid A (MAA, SAA) concentrations in cows with subclinical mastitis caused by coagulase-negative staphylococci (CNS). *Journal of dairy science 100* (12), 9674-9680.

Canning, P., Hassfurther, R., TerHune, T., Rogers, K., Abbott, S., & Kolb, D. (2017). Efficacy and clinical safety of pegbovigrastim for preventing naturally occurring clinical mastitis in periparturient primiparous and multiparous cows on US commercial dairies. *Journal of dairy science*, *100*(8), 6504-6515.

Daniel, C. W., Robinson, S., & Silberstein, G. B. (2001). The transforming growth factors beta in development and functional differentiation of the mouse mammary gland. *Bioactive components of human milk*, *501*, 61-70.

Darbaz, I., Ulusoy, B. H., Darbaz, T., Hecer, C., & Aslan, S. (2023). The importance of somatic cell count in dairy technology. *Mljekarstvo*, *73*(2), 75-84.

De Weerd, N. A., & Nguyen, T. (2012). The interferons and their receptors-distri-

bution and regulation. *Immunology and cell biology*, *90*(5), 483-491.

Dembic, Z. (2015). *The cytokines of the immune system the role of cytokines in disease related to immune response*. Mica Haley.

Dernfalk, J., Waller, K. P., & Johannisson, A. (2007). The xMAP™ technique can be used for detection of the inflammatory cytokines IL-1β, IL-6, and TNF-α in bovine samples. *Veterinary immunology and immunopathology*, *118*(1-2), 40-49.

Diker, K. (2005). *İmmünoloji*. Medisan.

Fonseca, I., Silva, P.V., Lange, C.C., Guimarães, M.F.M., Del Cambre Amaral Weller, M.M., Sousa, K.R.S., Lopes, P.S., Guimarães, J.D., & Guimarães, S.E.F. (2009). Expression profile of genes associated with mastitis in dairy cattle. *Genetics and molecular biology, 32* (4), 776-781.

Hagiwara, K., Yamanaka, H., Hisaeda, K., Taharaguchi, S., Kirisawa, R., & Iwai, H. (2001). Concentrations of IL-6 in serum and whey from healthy and mastitic cows. *Veterinary research communications*, *25*, 99-108.

Halasa, T., Huijps, K., Østerås, O., & Hogeveen, H. (2007). Economic effects of bovine mastitis and mastitis management: A review. *Veterinary quarterly*, *29*(1), 18-31.

Hisaeda, K., Hagiwara, K., Eguchi, J., Yamanaka, H., Kırısawa, R., & Iwaı, H. (2001). Interferon-γ and tumor necrosis factor-α levels in sera and whey of cattle with naturally occurring coliform mastitis. *Journal of veterinary medical science*, *63*(9), 1009-1011.

Isaacs, A., & Lindenmann, J. (1957). Virus interference. I. The interferon. *Proceedings of the royal society of london. series b-biological sciences*, *147* (927), 258-267.

Ivashkiv, L. B., & Donlin, L. T. (2014). Regulation of type I interferon responses. *Nature reviews ımmunology*, *14*(1), 36-49.

Kaçar, C., & Kırşan, İ. (2016). Memenin savunma mekanizması. In: Kaymaz, M., Fındık, M., Rişvanlı, A., Köker. A. (Eds). *Evcil hayvanlarda meme hastalıkları*. (1st ed., pp.41-58). Malatya Medipress.

Kajdaniuk, D., Marek, B., Borgiel-Marek, H., & Kos-Kudła, B. (2013). Transforming growth factor beta1 (TGFbeta1) in physiology and pathology. *Endokrynologia polska*, *64*(5), 384-396.

Kozovska, M. E., Hong, J., Zang, Y. C. Q., Li, S., Rivera, V. M., Killian, J. M., & Zhang, J. Z. (1999). Interferon beta induces T-helper 2 immune deviation in MS. *Neurology*, *53*(8), 1692-1692.

Lahouassa, H., Moussay, E., Rainard, P., & Riollet, C. (2007). Differential cytokine and chemokine responses of bovine mammary epithelial cells to *Staphylococcus aureus* and *Escherichia coli*. *Cytokine*, *38*(1), 12-21.

Lee, J. W., Bannerman, D., Paape, M., Huang, M. K., & Zhao, X. (2006). Characterization of cytokine expression in milk somatic cells during intramamma-

ry infections with *Escherichia coli* or *Staphylococcus aureus* by real-time PCR. *Veterinary research*, *37*(2), 219-229.

Ma, J. L., Zhu, Y. H., Zhang, L., Zhuge, Z. Y., Liu, P. Q., Yan, X. D., ... & Wang, J. F. (2011). Serum concentration and mRNA expression in milk somatic cells of toll-like receptor 2, toll-like receptor 4, and cytokines in dairy cows following intramammary inoculation with *Escherichia coli*. *Journal of dairy science*, *94*(12), 5903-5912.

Male, D., Brostoff, J., Roth, D. B., & Roitt, I. (2012). *Immunology*. China.

Mühl, H., & Pfeilschifter, J. (2003). Anti-inflammatory properties of pro-inflammatory interferon-γ. *International immunopharmacology*, *3*(9), 1247-1255.

Ohtsuka, H., Kudo, K., Mori, K., Nagaı, F., Hatsugaya, A., Tajıma, M., ... & Kawamura, S. I. (2001). Acute phase response in naturally occurring coliform mastitis. *Journal of veterinary medical science*, *63*(6), 675-678.

Osman, K. M., Hassan, H. M., Ibrahim, I. M., & Mikhail, M. M. (2010). The impact of staphylococcal mastitis on the level of milk IL-6, lysozyme and nitric oxide. *Comparative immunology, microbiology and infectious diseases*, *33*(1), 85-93.

Owen, J., Punt, J., Stranford, S. A., & Jones, P. (2013). Cytokines and chemokines. In: Owen, J., Punt, J., Stranford, S. A. (Eds). *Kuby immunology*. (7th ed., pp. 105-140), H. Freeman and Company.

Rambeaud, M., Almeida, R. A., Pighetti, G. M., & Oliver, S. P. (2003). Dynamics of leukocytes and cytokines during experimentally induced *Streptococcus uberis* mastitis. *Veterinary immunology and immunopathology*, *96*(3-4), 193-205.

Riollet, C., Rainard, P., & Poutrel, B. (2000). Differential induction of complement fragment C5a and inflammatory cytokines during intramammary infections with *Escherichia coli* and *Staphylococcus aureus*. *Clinical diagnostic laboratory ımmunology*, *7*(2), 161-167.

Riollet, C., Rainard, P., & Poutrel, B. (2001). Cell subpopulations and cytokine expression in cow milk in response to chronic Staphylococcus aureus infection. *Journal of dairy science*, *84*(5), 1077-1084.

Rişvanlı, A., Doğan, H., Şafak, T., & Öcal, H. (2019). Memenin savunma sistemi: Meme savunmasında meme başı ve meme başı kanalının rolü. In: Öcal, H. (Ed.). *İneklerde mastitis dışındaki meme, meme başı ve meme derisinin hastalıkları*. (1st ed., pp.1-10). Ankara, Türkiye Klinikleri.

Ruiz, R., Tedeschi, L. O., & Sepúlveda, A. (2017). Investigation of the effect of pegbovigrastim on some periparturient immune disorders and performance in Mexican dairy herds. *Journal of dairy science*, *100*(4), 3305-3317.

Safak, T., & Risvanli, A (2021). Changes in somatic cell count, composition and cytokine levels in milk from cows with mastitis due to mixed infections. *Acta scientiae veterinariae, 49*, 1830.

Safak, T., & Risvanli, A. (2022). Udder defense system: Effect of milk SCC level on Th1/Th2 cytokine balance. *Journal of the hellenic veterinary medical society, 73*(2), 4135–4140.

Safak, T., Risvanli, A., & Asci-Toraman, Z. (2022). Th1/Th2 cytokine polarization in milk according to different pathogens causing subclinical mastitis in cows. *Mljekarstvo*, *72*(2), 105-113.

Sakemi, Y., Tamura, Y., & Hagiwara, K. (2011). Interleukin-6 in quarter milk as a further prediction marker for bovine subclinical mastitis. *Journal of dairy research*, *78*(1), 118-121.

Scheller, J., Garbers, C., & Rose-John, S. (2014). Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. *Seminars in immunology, 26*, 1-11.

Schukken, Y. H., Günther, J., Fitzpatrick, J., Fontaine, M. C., Goetze, L., Holst, O., ... & Seyfert H. M. (2011). Host-response patterns of intramammary infections in dairy cows. *Veterinary immunology and immunopathology*, *144*(3-4), 270-289.

Kurt, S., T., Eski, F., Mis, L., & Demir, P. A. (2021). Evaluation of oxidative stress, immune system and mineral concentrations in milk and serum of cows with clinical and subclinical mastitis naturally infected by Staphylococcus aureus. *Kafkas üniversitesi veteriner fakültesi dergisi*, *27*(6), 755-762.

Sordillo, L. M. (2018). Mammary gland immunobiology and resistance to mastitis. *Veterinary clinics: food animal practice*, *34*(3), 507-523.

Sordillo, L. M., & Streicher, K. L. (2002). Mammary gland immunity and mastitis susceptibility. *Journal of mammary gland biology and neoplasia*, *7*, 135-146.

Takahashi, H., Odai, M., Mitani, K., Inumaru, S., Arai, S., Horino, R., & Yokomizo, Y. (2004). Effect of intramammary injection of rboGM-CSF on milk levels of chemiluminescence activity, somatic cell count, and *Staphylococcus aureus* count in Holstein cows with *S. aureus* subclinical mastitis. *Canadian journal of veterinary research*, *68*(3), 182-187.

Tizard, I. R. (2004). *Veterinary immunology*. Saunders Company. Philadelphia.

Waller, K. P., Colditz, I. G., Lun, S., & Östensson, K. (2003). Cytokines in mammary lymph and milk during endotoxin-induced bovine mastitis. *Research in veterinary science*, *74*(1), 31-36.

Wedlock, D. N., Doolin, E. E., Parlane, N. A., Lacy-Hulbert, S. J., Woolford, M. W., & Buddle, B. M. (2000). Effects of yeast expressed recombinant interleukin-2 and interferon-γ on physiological changes in bovine mammary glands and on bactericidal activity of neutrophils. *Journal of dairy research*, *67*(2), 189-197.

Wedlock, D. N., McCarthy, A. R., Doolin, E. E., Lacy-Hulbert, S. J., Woolford, M. W., & Buddle, B. M. (2004). Effect of recombinant cytokines on leucocytes

and physiological changes in bovine mammary glands during early involution. *Journal of dairy research*, *71*(2), 154-161.

Zecconi, A., Piccinini, R., Fiorina, S., Cabrini, L., Daprà, V., & Amadori, M. (2009). Evaluation of interleukin-2 treatment for prevention of intramammary infections in cows after calving. *Comparative immunology, microbiology and infectious diseases*, *32*(5), 439-451.

CHAPTER 6

OMENTIN: A NOVEL UNDERRECOGNIZED ADIPOKINE

Serpil AYGÖRMEZ[1], Şaban MARAŞLI[2], Emine ATAKİŞİ[3]

1 Research Assistant PhD, Kafkas University Faculty of Veterinary Medicine Department of Biochemistry Kars/TURKEY, ORCID: 0000-0002-5675-5096, e-mail: serpilaygormez@hotmail.com
2 Professor Doctor, Kafkas University Faculty of Veterinary Medicine Department of Biochemistry Kars/TURKEY, ORCID: 0000-0003-0182-6712, e-mail: sabanmarasli@gmail.com
3 Professor Doctor, Kafkas University Faculty of Veterinary Medicine Department of Biochemistry Kars/TURKEY, ORCID: 0000-0002-5685-1870, e-mail: et_tasci@hotmail.com

1. Adipokines

Adipose tissue insulates the body thermally and stores energy in the form of triacylglycerol (Ntaios et al. 2013). Cell signaling mediators secreted by this tissue are called adipokines. Adipose tissue has been identified as a multifaceted tissue that releases numerous adipokines, which can serve as humoral or immunomodulatory agents (Alsaif et al. 2015). Approximately one third of adipose tissue are comprised of adipocytes, with fibroblasts, macrophages, stromal cells, and monocytes comprising the remaining portion (Leal and Mafra. 2013). Among the molecules considered adipokines, leptin, resistin, omentin-1, vaspin, chemerin, ghrelin, visfatin, apelin, retinol binding protein-4, plasminogen activator inhibitor-1, serum amyloid A, and adiponectin are the most abundant ones and constitute 0.05% of plasma proteins. Molecules such as proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), and interleukin-6 produced by macrophages in adipose tissue are also considered adipokines (Leal and Mafra 2013, Ntaios et al. 2013, Alsaif et al. 2015, Azas et al 2017). Elevated body fat levels impact the adipokine production, leading to metabolic modifications that seem to influence labor through their effect on the uterus (Ntaios et al. 2013, Alsaif et al. 2015, Azas et al 2017, Shibata et al. 2017). Adipokines or adipose tissue products play an important role in endocrine and metabolic disorders (Alipoor et al 2018). Adipokines indirectly influence arteriosclerosis by acting as hormones or growth factors that modulate insulin resistance and fat and glucose metabolism. Additionally, adipokines directly affect endothelial function, vascular homeostasis, and atherogenesis (Ntaios et al. 2013). Dysfunctional adipose tissue in obesity can affect glucose and lipid metabolism. Therefore, adipokines may have a critical role in the development of obesity-related complications and inflammatory conditions (Alsaif et al. 2015). Research on the role of adipokines in the development of obesity and arteriosclerosis has been extensive, with a focus on their potential as therapeutic targets to decrease cardiovascular morbidity and mortality (Ntaios et al. 2013). Adipocyte-derived hormones have structural homology with cytokines, which are actively involved in the regulation of numerous biological processes. Adipose tissues release several adipokines such as adiponectin, visfatin, and omentin. These adipokines play a vital role in insulin sensitivity, adipocyte differentiation, proliferation, and neuropeptide Y regulation (Nway et al. 2016).

2. Omentin Hormone

Omentin was first identified as intelectin-1 in 2001 and was named "omentin" in 2004 because it was highly expressed in the omentum tissue (Yang et al. 2006, Sittichoroon ve ark. 2014). Omentin is a novel fat depot adipocytokine isolated from omental visceral adipose tissue in 2003 and

identified in cdeoxyribo nucleic acid (cDNA) (Yang et al. 2003, Pan et al. 2010, Briana et al. 2011, Aktas et al. 2014, Elsaid et al. 2018). In 2005, Omentin was discovered to act as a receptor for Intelectin-1 and intestinal lactoferrin in the Paneth cells of the intestine. Furthermore, omentin was detected in endothelial cells and reported as endothelial lectin (Tan et al. 2015). Initially, its expression was detected in the heart, lung, ovary, small intestine, and placenta, and to a lesser degree in muscle and kidney, apart from adipose tissue. Omentin was subsequently expressed in adipose tissue (Yang et al. 2006, Ohashi et al. 2014, Boron et al. 2015, Elsaid et al. 2018). Additionally, the expression of omentin-1 was detected in both endothelial cells and syncytiotrophoblasts within the human placenta, i < 1ndicating a potential maternal origin for fetal serum levels (Garces et al. 2015).

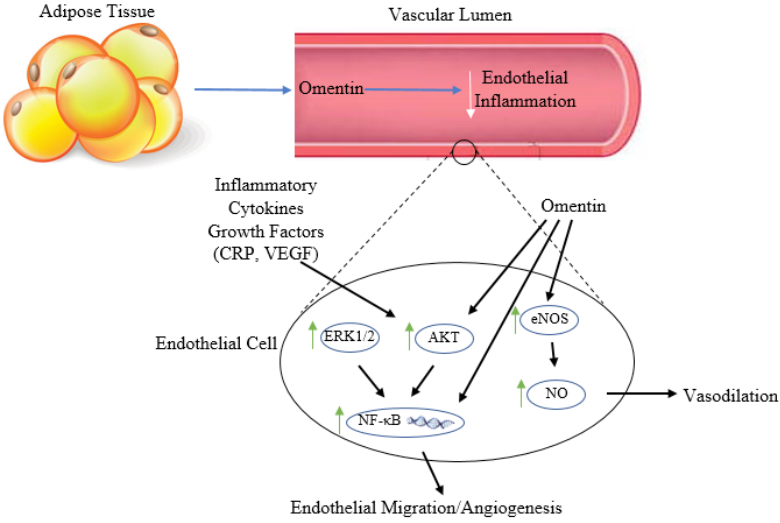


Figure 1: *Physiological effects of omentin molecule (Tan et al. 2010).*

2.1. Structure

Omentin participates in defense mechanisms by binding to galactofuranoses on bacteria as intelectin-1 (Kafalidis et al. 2013, Ohaski et al. 2014). Protein sequence analysis shows that the omentin cDNA encodes a 313-amino-acid protein. The part of the protein located at the amino-terminal end is predominantly hydrophobic. Studies have indicated that it is typically cleaved around amino acids 17 and 18 from this particular end (Yang et al. 2006, Boron et al. 2015, Tan et al. 2015, Elsaid et al. 2018).

Omentin has two homologous isoforms, omentin-1 and omentin-2. Omentin-1 is the most abundant circulating isoform (Pan et al. 2010, Kafalidis et al. 2013, Luis et al. 2017, Lin et al. 2021). Omentin-1 and omentin-2 are proteins that are secreted specifically by adipose tissue and synthesized by visceral stromal vascular cells. However, these proteins are not categorized as those belonging to the adipocyte class (Brunetti et al. 2011). In humans, omentin-1 and omentin-2 genes are localized adjacent to each other (Brunetti et al. 2013). Omentin-1 and omentin-2 share 83% similarity in their amino acid sequences (Tan et al. 2015). The omentin-1 and omentin-2 genes are adjacent to each other at the 1q22–q23 chromosome region, which is linked to type II diabetes mellitus (T2DM) in several populations (Pan et al. 2010, Brunetti et al. 2011, Tan et al. 2015, Elsaid et al. 2018). Omentin-1 is a 33-40 kDa adipokine. It consists of 8 exons and 7 intron regions (Auguet 2011, Tohidi et al. 2012, Boron et al. 2015, Tan et al. 2015, Karabulut et al. 2016, Shen et al. 2016, Li et al. 2017, Luis et al. 2017). Omentin-1, with uniprot code Q8WWAQ and gene bank expression number AY549722, is more intensively studied than omentin-2 (Tan et al. 2015).

2.2. Metabolic Effects

Adipokines are involved in the pathogenesis of insulin resistance, hypertension, dyslipidemia, and cardiovascular disease (Luis et al. 2017). Omentin-1 is an adipokine that regulates insulin activity (Yang et al. 2006). *In vitro* studies show that omentin-1 increases insulin-mediated glucose uptake by stimulating subcutaneous and omental adipocytes (Krysiak et al. 2012, Kafalidis et al. 2013). Other studies supporting the view that omentin-1 increases the efficiency of insulin in glucose metabolism have reported that circulating omentin-1 concentration increased after weight loss (Merono-Navarrate et al. 2010, Brunetti et al. 2011, Brunetti et al. 2013). Omentin-1 level is thought to be inversely correlated with obesity and insulin resistance (Briana et al. 2011).

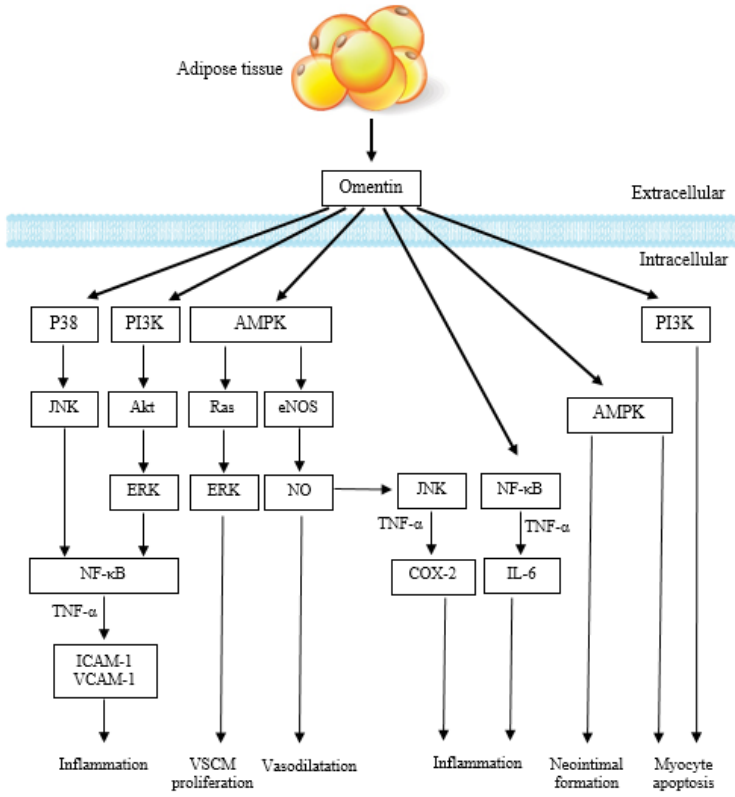


Figure 2: *Schematic presentation of the roles of omentin in intravascular and intracellular signal transduction pathways (Tan et al. 2015).*

In addition to its other effects, omentin-1 displays anti-inflammatory properties as an adipokine. Owing to its ability to enhance insulin signaling, it elevates insulin sensitivity and promotes glucose metabolism within the surrounding omental adipose tissue. It modulates body fat distribution between visceral and subcutaneous adipose tissue. Omentin-1 accelerates insulin-mediated glucose transport. It enhances insulin sensitivity and glucose metabolism in distant regions of the body, such as muscle, liver, and subcutaneous fat. In human visceral omental tissue, omentin-1 levels are decreased in individuals with obesity. Omentin-1 can be a biomarker of several metabolic diseases (Boronet al 2015, Nway et al. 2016, Elsaid et al. 2018, Asal et al. 2022). Obesity causes chronic low-grade inflammation and serum omentin-1 concentration may be associated with inflammatory conditions. However, the relationship between omentin-1 and inflammatory cytokines has not been fully elucidated (Pan et al. 2010). Omentin-1 is negatively correlated with metabolic risk factors. Serum omentin-1 levels are significantly reduced in individuals with obesity and other related met-

abolic disorders such as insulin resistance, glucose intolerance, and T2DM (Souza et al. 2007, Pan et al. 2010, Saremi et al. 2010, Kafalidis et al. 2013, Panagiotou et al. 2014, Garces et al. 2015). Omentin-1 plasma levels and gene expression correlate negatively with obesity and insulin resistance, systolic blood pressure, hemoglobin A1C, body mass index, waist circumference, leptin, triglyceride, and total cholesterol levels and positively with adiponectin and high density lipoprotein levels. (Panagiotou et al. 2014, Pan et al. 2010, Boron et al. 2015, Luis et al. 2017). A negative relationship between omentin-1 and oxidative stress has been reported (Asal et al. 2022). The phase known as the "transition period" marks the most crucial point in the lactation cycle of dairy cows, encompassing the shift from pregnancy to lactation. This period covers the last 3 weeks before calving and the first 3 weeks after calving (Abuelo et al. 2015, Kabir et al. 2022). The transition period is characterized by several metabolic and endocrine changes regulated as a result of increased nutrient demands aimed at supporting milk production. (Abuelo et al. 2015). It is reported to be a sensitive period for dairy cows (Drackley 1999, Abeyta et al. 2023). A study on periparturient cows reported that the elevated levels of omentin-1, which were detected for the first time at time of birth, may indicate its potential as a biomarker for metabolic diseases during this period (Aygormez & Atakisi, 2021).

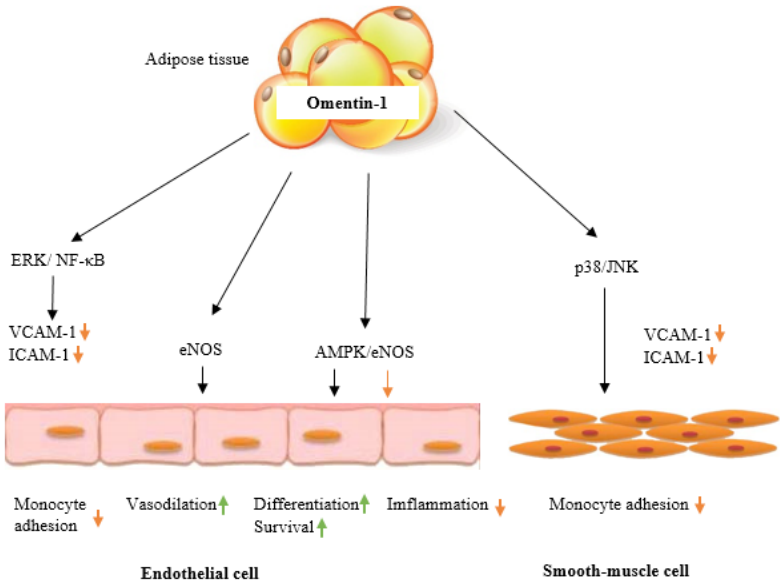


Figure 3: *Protective function of omentin-1 in vessels (Ohashi et al. 2014).*

2.3. Pathogenesis

A negative correlation has been observed between circulating omentin-1 and anthropometric parameters. Therefore, various strategies are being explored to achieve negative energy balance by decreasing secondary body weight, including reducing caloric intake, utilizing medications, and undergoing surgical interventions. This weight loss strategy can be used to reduce proinflammatory adipokine levels and increase circulating anti-inflammatory adipokines. Some studies have evaluated the effect of bariatric surgery and dietary interventions on omentin-1 serum concentrations (Luis et al. 2017). Omentin-1, which has the highest expression in visceral adipose tissue, is an independent marker of fatty liver disease and its level is increased in non-alcoholic fatty liver disease (Ebrahimi et al. 2018). Omentin-1 plays an anti-inflammatory role by preventing TNF-α-induced expression of VCAM-1 through inactivation of p38 and JNK and inhibition of superoxide production in vascular smooth muscle cells. Omentin-1 inhibits TNF-α-mediated induction of proinflammatory molecules such as cyclooxygenase and endothelial nitric oxide synthase in vascular endothelial cells. Therefore, it regulates the immune reaction by exerting an anti-inflammatory effect. The fact that omentin-1 was significantly lower in the synovial fluid of patients with knee osteoarthritis explains its effect on the immune system (Li et al. 2017). The role of omentin-1 in various types of cancer is reportedly controversial. Serum omentin levels increase in patients with colorectal or prostate cancer, but decrease those with breast, kidney, and endometrial cancers (Asal et al. 2022). In addition to these, Shen et al. (2016) reported the absence of any association between metabolic risk factors and circulating serum omentin levels in renal cell carcinoma patients. Local regulation within adipose tissue can cause it to exhibit endocrine effects in addition to its autocrine and paracrine effects. The paracrine effect is to increase insulin sensitivity and thereby stimulate glucose metabolism, which affects the distribution of adipose tissue. Their effects in muscle, liver or subcutaneous adipose tissue cells are similar (Boron et al 2015). Omentin mRNA is mostly expressed predominantly in the stromal vascular fraction of visceral adipose tissue. It can hardly be detected in subcutaneous adipose tissues and mature adipocytes (Pan et al. 2010). Moreover, omentin-1 is a novel type of Ca^{+2}-dependent lectin with affinity for galactofuranosyl residues. Therefore, it plays a role in identifying particular pathogens and components of bacteria (Tan et al. 2015).

3. Conclusion

Adipokines are secreted throughout the organism by various tissues. They show cytoprotective effects in numerous pathological conditions such as cardiovascular disease, osteoporosis, cancer, and diabetes. Ac-

cordingly, it appears highly likely that omentin-1 could be developed into agents that have the potential to modify diseases. Furthermore, the specific receptor for omentin-1 has not been elucidated thus far. We conclude that comprehensive studies on omentin-1 and its receptor may lead to promising advances in the diagnosis and treatment of diseases.

References

Abeyta, M. A., Horst, E. A., Goetz, B. M., Rodriguez-Jimenez, S., Mayorga, E. J., Al-Qaisi, M., & Baumgard, L. H. (2023). Effects of hindgut acidosis on inflammation, metabolism, and productivity in lactating dairy cows fed a high-fiber diet. *Journal of Dairy Science,* 21(23),73-75.

Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2015). The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. *Journal of Animal Physiology and Animal Nutrition,* 99(6),1003-1016.

Aktas, G., Alcelik, A., Ozlu, T., Tosun, M., Tekce, B. K., Savli, H., Tekce, H., & Dikbas, O. (2014). Association between omentin levels and insulin resistance in pregnancy. *Experimental and Clinical Endocrinology & Diabetes,* 122,163-166.

Alipoor, E., Mohammad, H. F., & Hosseinzadeh, A. M. J. (2018). Adipokines in critical illness: A review of the evidence and knowledge gaps. *Biomedicine & Pharmacotherapy,* 108,1739-1750.

Alsaif, S., Mumtaz, S., & Wray, S. (2015). A short review of adipokines, smooth muscle and uterine contractility. *Life Sciences,* 125,2-8.

Asal, D. M., Mesbah, N. M., Abo-Elmatty, D. M., Fathy, H., & Abdel-Hamed, A. R. (2022). Association of vaspin rs2236242 and Val109Asp omentin genes polymorphism and their serum levels with increased risk of breast cancer. *Meta Gene,* 31,101016.

Auguet, T., Quintero, Y., Riesco, D., Morancho, B., Terra, X., Crescenti, A., Broch, M., Aguilar, C., Olona, M., Porras, J. A., Hernandez, M., Sabench, F., Castillo, D., & Richart, C. (2011). New adipokines vaspin and omentin. circulating levels and gene expression in adipose tissue from morbidly obese women. *BMC Medical Genetics,* 12,60.

Aygormez, S. & Atakisi, E. (2021). Investigation of omentin-1 and metabolic parameters in periparturient cows. *Journal of the Hellenic Veterinary Medical Society,* 72,2733-2740.

Azas, H., Leroy, A., Ghesquiere, L., Deruelle, P., & Hanssens, S. (2017). Effects of adipokines and obesity on uterine contractility. *Cytokine & Growth Factor Reviews,* 34,59-66.

Boron, D., Czerny, B., Bartkowiak-Wieczorek, J., Sieron, D., & Wolski, H. (2015). Omentin polymorphism and its relations to bone mineral density in women. *Archives of Current Medical Research,* 46,173-180.

Briana, D. D., Boutsikou, M., Baka, S,. Gourgiotis, D., Marmarinos, A., Liosi, S., Hassiakos, D., & Malamitsi-Puchner, A. (2011). Omentin-1 and vaspin are present in the fetus and neonate, and perinatal concentrations are similar in normal and growth-restricted pregnancies. *Metabolism-Clinical and Experimental,* 60,486-490.

Brunetti, L., Di Nisio, C., Recinella, L., Chiavaroli, A., Leone, S., Ferrante, C., Orlando, G., & Vacca, M. (2011). Effects of vaspin, chemerin and omentin-1 on feeding behavior and hypothalamic peptide gene expression in the rat. *Peptides,* 32,1866-1871.

Brunetti, L., Orlando, G., Ferrante, C., Recinella, L., Leone, S., Chiavaroli, A., Di Nisio, C., Shohreh, R., Manippa, F., Ricciuti, A., & Vacca, M. (2013). Orexigenic effects of omentin-1 related to decreased CART and CRH gene expression and increased norepinephrine synthesis and release in the hypothalamus. *Peptides,* 44,66-74.

Drackley, J. K. (1999). Biology of dairy cows during the transition period: the final frontier?. *Journal of Dairy Science,* 2,2259-2273.

Ebrahimi, S., Gargari, B. P., Izadi, A., Imani, B., & Asjodi, F. (2018). The effects of Ramadan fasting on serum concentrations of vaspin and omentin-1 in patients with nonalcoholic fatty liver disease. *European Jouenal of Integrative Medicine,* 19,110-114.

Elsaid, N. H., Sadik, N. A., Ahmed, N. R., Fayez, S. E., & El-Ghaffar Mohammed, N. A. (2018). Serum omentin-1 levels in type 2 diabetic obese women in relation to glycemic control, insulin resistance and metabolic parameters. *Journal of Clinical & Translational Endocrinology,* 13,14-19.

Garces, M. F., Ruíz-Linares, C. E., Vallejo, S. A., Peralta, J. J., Sanchez, E., Ortiz-Rovira, A., Curtidor, Y., Parra, M. O., Leal, L. G., Alzate, J. P., Acosta, B. J., Dieguez, C., Nogueiras, R., & Caminos, J. E. (2015). Maternal serum omentin-1 profile is similar in humans and in the rat animal model. *Cytokine,* 75,136-141.

Kabir, M., Hasan, M. M., Tanni, N. S., Parvin, M. S., Asaduzzaman, M., Ehsan, M. A., & Islam, M. T. (2022). Metabolic profiling in periparturient dairy cows and its relation with metabolic diseases. *BMC Research Notes,* 5,231.

Kafalidis, G., Boutsikou, T., Briana, D. D., Boutsikou, M., Marmarinos, A., Baka, S., Hassiakos, D., Gourgiotis, D., & Malamitsi-Puchner, A. (2013). Adipokines vaspin and omentin-1 are up-regulated in large for gestational age infants at term. *Cytokine,* 62,70-74.

Karabulut, S., Afsar, C. U., Karabulut, M., Alis, H., Bozkurt, M. A., Aydoğan, F., Serilmez, M., & Tas, F. (2016). Clinical significance of serum omentin-1 levels in patients with pancreatic adenocarcinoma. *BBA Clinical,* 6,138-142.

Krysiak, R., Handzlik-Orlik, G., & Okopien, B. (2012). The role of adipokines in connective tissue diseases. *European Journal of Nutrition,* 51,513-528.

Leal, VdeO, & Mafra, D. (2013). Adipokines in obesity. *Clinica Chimica Acta,* 419,87-94.

Li, Z., Liu, B., Zhao, D., Wang, B., Liu, Y., Zhang, Y., Li, B., & Tian, F. (2017).

Omentin-1 prevents cartilage matrix destruction by regulating matrix metalloproteinases. *Biomedicine & Pharmacotherapy,* 92,265-269.

Lin, S., Li, X., Zhang, J., & Zhang, Y. (2021). Omentin-1: Protective impact on ischemic stroke via ameliorating atherosclerosis. *Clinica Chimica Acta,* 517,31-40.

Luis, D. A., Izaola, O., Primo, D., & Aller, R. (2017). Modifications of serum levels of omentin-1 and other cardiovascular risk factors following weight loss secondary to a mediterranean hypocaloric diet. *Clinical Nutrition,* 5614,31407-31403.

Moreno-Navarrete, J. M., Catalán, V., Ortega, F., Gómez-Ambrosi, J., Ricart, W., Frühbeck, G., & Fernández-Real, J. M. (2010). Circulating omentin concentration increases after weight loss. *Nutrition & Metabolism,* 9,7-27.

Ntaios, G., Gatselis, N. K., Makaritsis, K., & Dalekos, G. N. (2013). Adipokines as mediators of endothelial function and atherosclerosis. *Atherosclerosis,* 227,216-221.

Nway, N. C., Sitticharoon, C., Chatree, S., & Maikaew, P. (2016). Correlations between the expression of the insulin sensitizing hormones, adiponectin, visfatin, and omentin, and the appetite regulatory hormone, neuropeptide Y and its receptors in subcutaneous and visceral adipose tissues. *Obesity Research & Clinical Practice,* 10,256-263.

Ohashi, K., Shibata, R., Murohara, T., & Ouchi, N. (2014). Role of anti-inflammatory adipokines in obesity-related diseases. *Trends in Endocrinology & Metabolism,* 25,348-355.

Opatrilova, R., Caprnda, M., Kubatka, P., Valentova, V., Uramova, S., Nosal, V., Gaspar, L., Zachar, L., Mozos, I., Petrovic, D., Dragasek, J., Filipova, S., Büsselberg, D., Zulli, A., Rodrigo, L., Kruzliak, P., & Krasnik, V. (2018). Adipokines in neurovascular diseases. *Biomedicine & Pharmacotherapy,* 98,424-432.

Pan, H. Y., Guo, L., & Li, Q. (2010). Changes of serum omentin-1 levels in normal subjects and in patients with impaired glucose regulation and with newly diagnosed and untreated type 2 diabetes. *Diabetes Research and Clinical Practice,* 88,29-33.

Panagiotou, G., Mu, L., Na, B., Mukamal, K. J., & Mantzoros, C. S. (2014). Circulating irisin, omentin-1, and lipoprotein subparticles in adults at higher cardiovascular risk. *Metabolism,* 63,1265-1271.

Saremi, A., Asghari, M., & Ghorbani, A. (2010). Effects of aerobic training on serum omentin–1 and cardiometabolic risk factors in overweight and obese men. *Turkish Journal of Sports Science,* 28,993-998.

Shen, X. D., Zhang, L., Che, H., Zhang, Y. Y., Yang, C., Zhou, J., & Liang, C. Z. (2016). Circulating levels of adipocytokine omentin-1 in patients with renal cell cancer. *Cytokine,* 77,50-55.

Shibata, R., Ouchi, N., Ohashi, K., & Murohara, T. (2017). The role of adipokines in cardiovascular disease. *The Anatolian Journal of Cardiology,* 70,329-334.

Sitticharoon, C., Nway, N. C., Chatree, S., Churintaraphan, M., Boonpuan, P., & Maikaew, P. (2014). Interactions between adiponectin, visfatin, and omentin in subcutaneous and visceral adipose tissues and serum, and correlations with clinical and peripheral metabolic factors. *Peptides,* 62,164-175.

Souza, B. C. M., Yang, R. Z., Lee, M. J., Glynn, N. M., Yu, D. Z., Pray, J., Ndubuizu, K., Patil, S., Schwartz, A., Kligman, M., Fried, S. K., Gong, D. W., Shuldiner, A. R., Pollin, T. I., & McLenithan, J. C. (2007). Omentin plasma levels and gene expression are decreased in obesity. *Diabetes,* 56,1655-1661.

Tan, B. K., Adya, R., & Randeva, H. S. (2010). Omentin: A novel link between inflammation, diabesity, and cardiovascular disease. *Trends in Cardiovascular Medicine,* 20,143-148.

Tan, Y. L., Zheng, X. L., & Tang, C. K. (2015). The protective functions of omentin in cardiovascular diseases. *Clinica Chimica Acta,* 448,98-106.

Tohidi, M., Akbarzadeh, S., Larijani, B., Kalantarhormozi, M., Ostovar, A., Assadi, M., Vahdat, K., Farrokhnia, M., Sanjdideh, Z., Amirinejad, R., & Nabipour, I. (2012). Omentin-1, visfatin and adiponectin levels in relation to bone mineral density in Iranian postmenopausal women. *Bone,* 51,876-881.

Ucgun, T., Başar, C., Memişoğulları, R., Demirin, H., Turker, Y., & Aslantaş, Y. (2014). Serum visfatin and omentin levels in slow coronary flow. *Revista Portuguesa de Cardiologia,* 33,789-794.

Yang, R., Xu, A., Pray, J., Hu, H., Jadhao, S., Hansen, B., Shuldiner, A., Mc-Lenithan, J., & Gong, D. (2003). Cloning of omentin, a new adipocytokine from omental fat tissue in humans. *Diabetes,* 1,13-17.

Yang, R. Z., Lee, M. J., Hu, H., Pray, J., Wu, H. B., Hansen, B. C., Shuldiner, A. R., Fried, S. K., McLenithan, J. C., & Gong, D. W. (2006). Identification of omentin as a novel depot-specific adipokine in human adipose tissue: possible role in modulating insulin action. *American Journal of Physiology-Endocrinology and Metabolism,* 290,1253-1261.

CHAPTER 7

GHRELIN HORMONE; ITS PHYSIOLOGICAL SIGNIFICANCE AND EFFECT PATHWAYS

Hamit USLU[1], Gözde ATİLA USLU[2]

1 Assoc. Prof. Dr. Department of Physiology, Erzincan Binali Yıldırım University, Faculty of Medicine, Erzincan – Türkiye. hamit_uslu@hotmail.com Orcid Id: 0000-0002-3974-5814
2 Assoc. Prof. Dr. Department of Physiology, Erzincan Binali Yıldırım University, Faculty of Medicine, Erzincan – Türkiye. gzd.gozde@hotmail.com Orcid Id: 0000-0002-2328-9164

Introduction

The name of the hormone Ghrelin a 28-amino acid (aa) hunger hormone, is a combination of the Proto-Indo-European words "ghre" meaning "grow" and "relin" meaning to secrete, referring to its ability to stimulate the release of Growth hormone (GH) (Kojima and Kangawa, 2007). Ghrelin, which is produced in humans from the precursor preproghrelin, which has 117 amino acids, is found at locus 3p25–26 of chromosome 3. It has five exons and four introns. The 1st exon of 23 aa is separated as a residual portion and proghrelin of 94 aa is formed. With the separation of the C-terminus of 66 aa from proghrelin, ghrelin of 28 aa is formed (İlhan and Erdost, 2009; Date et al., 2000). The majority of circulating ghrelin is produced by X/A-like cells in the oxyntic mucosa of the stomach (the highest content is in the gastric fundus), it is mostly synthesized in the X/A-like cells of the small intestine after the stomach, but in small amounts, pancreas, kidney, placenta, lymphatic tissue, gonads, thyroid. Ghrelin is thought to be produced in a different organs, including the pituitary, heart, lungs, and hypothalamus (Date et al., 2000; Gnanapavan et al., 2002). When its receptors were investigated, it was first identified as the GH secreting receptor (GHS-R) as a specific G-protein coupled receptor, but it was soon discovered that its endogenous ligand was ghrelin. When ghrelin receptors are examined, two types of receptors, GHS-R1a and GHS-R1b, draw attention. GHS-R1a is reported to have 366 aa and seven transmembrane domains that allow it to bind ghrelin, whereas GHS-R1b has 289 aa and five transmembrane domains plus a carboxyl-terminal region, and ghrelin does not bind GHSs (Guan et al., 1997; Camina et al., 2006). According to research, GHS-R1a is strongly expressed in the pituitary, the hypothalamus (particularly in the arcuate nucleus), and the hippocampus. Additionally, it is present in the substantia nigra pars compacta, dorsal-medial raphe, and the ventral tegmental area. According to reports, GHS-R1a is released in a lot of organs and tissues in the periphery, such the thyroid, adrenal glands, pancreas, spleen, heart muscle, testicles, ovaries, stomach, and bowels (Gnanapavan et al., 2002; Guan et al., 1997). GHSR1b's functional role is not yet completely understood. However, in a study, it was suggested that GHSR1b caused a weakening in the signaling capacity of GHRS-la in human embryonic kidney 293 cells expressing GRLN-R, and it did not produce a detectable response both intracellularly and extracellularly in Ca^{2+} mobilization experiments (Chan and Cheng, 2004). Additionally stimulating the secretion of GH-releasing hormone (GHRH), ghrelin has an orexigenic effect, stimulates intestinal motility and gastric acid secretion, regulates sleep, sense of taste and reward-seeking behavior, energy and glucose homeostasis, modulation of stress and anxiety, cell It has been found to be effective in proliferation, bone physiology and improvement of

cardiovascular functions (Müller et al., 2015).

Ghrelin - Growth Hormone (GH) Release

Ghrelin was first identified in the stomach of rats in 1999 as a particular GH secretagogue (GHS) (Kojima et al., 1999). The GH inhibiting hormone (GHIH-somatostatin), GHRH, and ghrelin work together to control GH secretion. After connecting to its receptors in the somatotroph cells located in the anterior part of the pituitary gland, GHRH increases GH secretion via raising cAMP levels. It is stated that ghrelin affects both GHRH-secreting neurons and GHIH-secreting neurons, which act on the somatotrophs of the anterior pituitary gland. After ghrelin binds with GH-SR-1a, phospholipase C is activated, catalyzing the generation of secondary messengers IP3 and DAG. After binding to the IP3 receptor, it triggers the release of Ca^{2+} into the cytoplasm. It causes the release of Ca^{2+} into the cytoplasm after connecting to the IP3 receptor. Ca^{2+} interact with the vesicular membrane, causing fusion of GH-secreting vesicles with the cell membrane, which leads to exocytosis and increases transcription of genes involved in GH synthesis. Exogenously administered GHS predominantly antagonizes somatostatin and stimulates GHRH release (Kojima and Kangawa, 2007; Khatib et al., 2014). In a study on fish, it was reported that central and peripheral injections of ghrelin stimulate Luteinizing Hormone (LH), GH release and mRNA expression, therefore it is a significant marker in the regulation of growth and reproductive physiology (Unniappan and Peter, 2004). In another study, it was stated that ghrelin caused weaker GH secretion than GHRH, and did not have an additive or synergistic effect on GH secretion when applied together with GHRH, therefore ghrelin indirectly affected GH release from the pituitary gland (Yamazaki et al., 2002).

Ghrelin – Food Intake and Orexigenic Effect

Appetite; It can be defined as the desire to eat as a result of the feeling of hunger. Both central and peripheral mechanisms control it. Central neural connections link the hypothalamus's nuclei to the brainstem and higher areas. These areas regulate calorie intake, stomach motility, gastric emptying, and reward-based and sensory-pleasant food ingestion. The hypothalamus's paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventromedial hypothalamic nucleus (VMC), dorsomedial hypothalamic nucleus (DMC), and arcuate nucleus (ARC) all contribute significantly to the centrical adjustment of appetite and satiety. The orexigenic neuropeptides NPY and AGRP are co-localized with one group of neurons, whereas the anorexigenic neuropeptides proopiomelanocortin (POMC), leptin receptors, cocaine and amphetamine-regulated transcript (CART), and -melanin stimulating hormone (-MSH) are co-localized with

another group of neurons (Elias et al., 1998; Harrold et al., 2012). According to research, ghrelin applications increase food intake and body weight gain. However, there is a decrease in secretion after eating in conditions like hyperglycemia and obesity, as well as increases after fasting, hypoglycemia, cachexia, and anorexia (Toshinai et al., 2001; Shiiya et al., 2002). Shiiya et al. (Shiiya et al., 2002) showed that ghrelin secretion increased under negative energy balance conditions and decreased in positive energy balance conditions. They also stress the significance of ghrelin as a marker for the control of eating habits and energy homeostasis. When the orexigenic effect pathway of ghrelin was investigated, the most striking one was the study conducted by Shintani et al. (18) in 2001. In this study, it was revealed that after central application of ghrelin, the expression of hypothalamic AGRP mRNA increased significantly, this increase contributed to the inhibition of the hypothalamic melanocortin system, and it antagonizes the effect of leptin through the activation of the hypothalamic NPY/Y1 receptor pathway, and as a result of these results, it achieves its orexigenic effect. In a study conducted in 2020, it was determined that as a result of ghrelin receptor activation in the lateral parabrachial nucleus, an increase in food consumption and a change in consumption behaviors such as food choice occurred, but it did not cause any changes in appetitive behaviors related to food reward and motivation (Bake et al., 2020). In another study, inhibition of nicotinamide phosphoribosyltransferase (NAMPT), which is effective in the adjustment of energy balanced, significantly reduced fasting and ghrelin-induced food consumption and NAD^+ levels, increased ROS levels, and influenced in the expression of genes related to AGRP, POMC and mitochondrial function. As a result of these results, it was proposed that this enzyme could be a useful marker for controlling fasting and ghrelin-induced food consumption (De Guia et al., 2020). Ghrelin is also effective in mechanisms such as gastric motility and acid secretion, which are effective in the peripheral regulation of appetite, and as mentioned before, it is synthesized in the fundus, body and antrum of the stomach, where ghrelin-generating cells are encountered in all sections of the digestive tract, as well as in the duodenum, ileum, cecum and colon. However, it was reported that the stomach is where ghrelin is most intensely produced, and that as one moves toward the lower gastrointestinal tract, fewer cells are secreted ghrelin (Sakata and Sakai, 2010). It has been determined that ghrelin applications cause an increase in gastric acid secretion and motility (Masuda et al., 2000). Yang et al. (23) suggested that ghrelin receptor deficiency attenuates gastric motility in mice, and although the mechanism is still not completely appreciated, this deficiency might affect the development and cell number of nerve cells in the gastric plexus, and decrease gastric motility with the loss of nerve cells in the gastric plexus. In another study, it was found that ghrelin accelerates gastric myoelectric stimulation

and gastric emptying, and since this effect cannot be completely blocked by atropine, it may exert its effect not only through the vagal pathway but also through local pathways by increasing the activity of non-cholinergic excitatory neurotransmitters in the gastrointestinal tract (Tümer et al., 2008).

Ghrelin – Energy Homeostasis

Ghrelin plays a significant part in maintaining energy homeostasis by regulating body weight and glucose metabolism, additionally its orexigenic effect during negative energy balance. In a study where ghrelin was chronically administered by the central route, it was found that the sympathetic nervous system's central control of the adipocyte metabolism, independent of its hyperphagic effect, and lipoprotein lipase, acetyl CoA carboxylase α, which promotes fat storage in the WAT, increased the glucose usage rate of white (WAT) and brown (BAT) adipose tissue without affecting the skeletal muscle. It has been determined that various enzymes such as acid synthase and stearoyl-CoA desaturase-1 increase mRNA expression and cause a decrease in the expression of mitochondrial UCP 1 and 3 related to thermogenesis in BAT (Theander-Carrillo et al., 2006). According to investigation on the effects of ghrelin on energy metabolism in the hypothalamus, this hormone increased food consumption by way of NPY/AgRP neurons in the ARC and increased body weight and adiposity by way of GHSR-containing neurons in the PVN; it has been proposed that these two mechanisms are important for the regulation of hypothalamic energy (Briggs and Andrews, 2011). Ghrelin causes the switch from lipid to carbohydrate for energy needs, enhances carbohydrate utilization, and promotes fat accumulation, according to research on its effects on glucose metabolism. Human studies have shown that acylated ghrelin elevates glucose and insulin levels while unacylated ghrelin has the opposite effect (Van der Lely, 2009). A separate investigation evaluated the outcomes of acute delivery of acylated, unacylated, and a mixture of both ghrelins. The results indicated that acylated ghrelin caused an immediate elevated in glucose and insulin levels, which was stopped by when the two are applied in combination. It has been shown to reduce insulin sensitivity for up to 6 hours (Gauna et al., 2004). According to Tong et al. (29) ghrelin antagonists might be a new pharmacological target for the therapy of T2D because they can increase cell activity and reduce the first-phase insulin and C-peptide responses to glucose after intravenous delivery in healthful persons. In addition, in a study on mice, it was determined that ghrelin inhibited glucose-stimulated insulin secretion with a direct effect on the islets of Langerhans (Reimer et al., 2003). According to a study from 2020, pancreatic ghrelin expression and circulating ghrelin concentrations are lower in people having type two diabetes (T2D), and ghrelin has direct, glucose-dependent effects on

suppressing insulin in the islets of Langerhans (Lindqvist et al., 2020). It is underlined that pharmacologically inhibiting ghrelin signaling offers therapeutic potential for treating insulin resistance and T2D, but further experimental and clinical investigation is need to completely understand the mechanism of action and route.

Ghrelin – Inflammation and the Immune System

Although it was thought that the production and secretion of ghrelin was mainly due to the stomach, this idea changed after it was determined that ghrelin mRNA and protein expression were also carried out in the brain, adipose tissue, placenta, jejunum, duodenum, colon, lungs, liver, and lymphoid organs (Taub et al., 2010; Hattori, 2009; Smith et al., 2005; Van der Lely et al., 2004; Baatar et al., 2011). Immune system cells are extremely sensible to modifications in energy status and metabolism. These cells are highly correlated with hormones and nutrients in the blood and are impacted by their levels even though they are able to sense changes in their surroundings (Baatar et al., 2011). Different immune cells, particularly B and T lymphocytes, neutrophils, and monocytes, express both GH and ghrelin, an orexigenic hormone. Additionally, these immune cells have hormone receptors. Both the GH releasing receptor (GHS-R) for ghrelin and the GH receptor (GHR) for GH are present in these cells. Just like in the anterior pituitary gland, ghrelin stimulates GH secretion in these cells (Hattori, 2009). GHS-R and ghrelin were discovered to be expressed in human T lymphocytes and monocytes by Dixit et al. (37). Additionally, they stated that ghrelin particularly suppresses the production of cytokines that cause inflammation like IL-1, IL-6, and TNF-α by acting through GHS-R. According to Baatar et al. (36) ghrelin decreases pro-inflammatory cytokines as IL-1, IL-6, IL-8, TNF-α, MCP1, HMGB1, Th1, Th17, and VCAM-1 and adhesion molecules while also increasing the level of IL-10, a cytokine that fights inflammation. They reported that it has positive effects by stimulating cell migration and that it can be a promising resource in the prevention of various inflammations, cachexia and some autoimmune diseases and tissue damage. Intratracheal administration of lipopolysaccharide (LPS) by instillation shows that intravenously administered ghrelin in acute lung injury induced by lipopolysaccharide (LPS) alleviates acute lung inflammation and suppresses the formation of proinflammatory cytokines caused by LPS, which is mediated partially by increased nitric oxide production in lung macrophages (Chen et al., 2008). In mice with acute kidney injury brought on by intraperitoneal injection of LPS, subcutaneous (SC) ghrelin administration has been shown to provide renal protection throughout endotoxemia-induced acute kidney damage by importently lowering elevated serum TNF-α, IL-1β and IL-6 levels as well as endothelin-1 levels (Wang et al., 2009). Also, it has been noted that

ghrelin, when given sc for four weeks following a myocardial infarction at a dose of 100 g/kg, dramatically lowers consantration of TNF-α and IL-1β and prevents neural remodeling (Yuan et al., 2009). Ghrelin may have vasodilatory effects in people, enhance heart health, and lower peripheral vascular resistance in those with chronic heart failure (Gruzdeva et al., 2019).

Human ghrelin is thought to have a substantial role in restoring CD4 cell proliferation, making it a potential therapy option for sepsis (Zhou et al., 2018). Ghrelin prevents age-related thymic involution by promoting lymphocyte growth in the bone marrow and thymus. Additionally, ghrelin prevents apoptosis by altering the stress-induced apoptotic signaling pathway in the thymic apoptosis brought on by restriction. It has been reported that it can stimulate thymic function again in immunocompromised individuals by promoting thymopoiesis, which decreases with aging (Baatar et al., 2011; Lee et al., 2016; Himmerich and Sheldrick, 2010).

Ghrelin – Sleep and Memory

It has been suggested that ghrelin has a positive effect on sleep, making sleep more effective and quality, may have an effect on synaptic plasticity in memory-related regions, may improve memory capacity and may produce anti-depressive effects. It was even reported that ghrelin levels decreased after anti-depressant intake (Morin et al., 2018). Prolonged ghrelin therapy, according to Eslami et al. (Eslami et al., 2018), not only enhanced memory processing and recall in healthy rats while passive avoidance learning, but it also improved faulty synaptic plasticity and reduced memory loss in Alzheimer's patients. It is additionally claimed that postsynaptic processes may have an impact in longtime ghrelin-strengthened memory without significantly influencing presynaptic transmitter release. In a study of patients with obstructive sleep apnea, however, there was no important distinction in ghrelin concentrations while comparing to the healthy control (Zhang et al., 2018). In a meta-analysis study that supported this research, it was discovered that there was no difference in plasma/serum ghrelin levels between those suffering from obstructive sleep apnea-hypopnea syndrome and controls, nor was there any change after continuous positive airway pressure treatment (Sun et al., 2021). According to a different study, ghrelin lacks a circadian rhythm and sleep deprivation has no impact on the hormones cortisol and ghrelin's 24-hour release patterns (Zareian et al., 2018). In their 2020 study, Hornsby et al. (50) investigated circulating concentrations of unacylated-ghrelin (UAG) and acyl-grelin (AG) in Parkinson's patients with dementia, cognitively intact Parkinson's patients and controls. According to the results obtained in this study, hippocampal ghrelin-receptor expression stayed constant while the plasma AG:UAG proportion and the amount of GOAT+ cells only decreased in

Parkinson's patients who were also diagnosed with dementia. The regulation of hippocampal-dependent flexibility and spatial memory may use the AG:UAG ratio as a diagnostic biomarker, according to studies. However, they emphasised that whether the decrease in AG:UAG ratio is specific to PDD should be tested in different dementia phenotypes. Li et al. (51) found that activation of GHS-R1a and subsequent activation of PI3K/Akt/GSK3β signalling cascades and administration of ghrelin suppressed the intrinsic excitability of dCA1 pyramidal neurons by activating GHS-R1a, and administration of the PI3K inhibitor LY294002 blocked the effect of ghrelin. In a study where rats were used as test subjects for passive avoidance response acquisition, it was proposed that SC application of morphine impaired memory and caused amnesia, while pre-injection of intra-CA1 ghrelin prevented the amnesic effect of morphine and advanced memory, and that ghrelin performs the avoidance task through CA1 nicotinic receptors (Nazari-Serenjeh et al., 2019). According to Steriger et al. (53), the effect of ghrelin involves much more than increasing nutrition and appetite, and it could have a role in memory and central nervous system disorders. In addition, it was stated that ghrelin is effective in the regulation of sleep and wakefulness and nonREM sleep was promoted in men and mice after systemic ghrelin treatment; it was revealed that this effect was also affected by conditions such as gender, time of administration and depression. It is interesting that the effect pathway has not yet been fully clarified, despite the fact that the literature contains contradictory results on the effectiveness of ghrelin on sleep and memory.

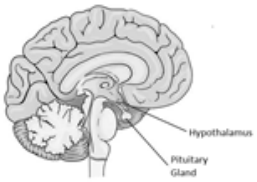
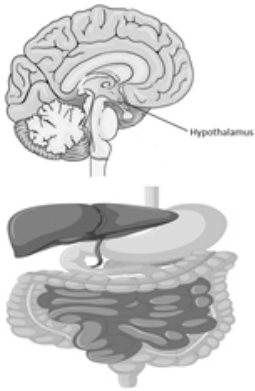
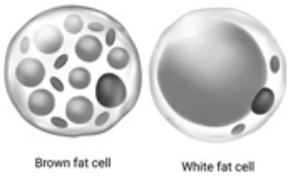
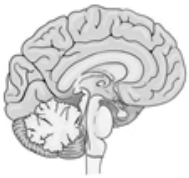
Ghrelin - Effects on Reproduction and Stress Hormones

Ghrelin may also be useful in the physiological regulation of the female reproductive system by influencing the synthesis and secretion of reproductive hormones from the hypothalamus and pituitary. Increased levels of active ghrelin and GH were observed during pregnancy, suggesting that this may be important for both maternal metabolism and fetus. (Szczepankiewicz et al., 2010). In a study of cyclic and pregnant rats, Caminos et al. (55) found that while the ghrelin gene is expressed in the rat ovaries during the period of estrous, the relative levels of mRNA differ depending on the phase of the cycle. (proestrus; lowest, diestrous d 1, i.e. luteal phase; highest). In pregnant rats, ghrelin mRNA expression was statement to be higher in the early period and lower expression in the later period, but was detected in the rat ovary throughout pregnancy. They also showed that the corpus luteum is the main site for ghrelin expression in ovarian tissue. Elevated circulating ghrelin levels may be partly responsible for changes in the reproductive axis during negative energy balance, and this impact may be mediated by inhibition of GnRH release via the hypothalamic-pituitary pathway and suppression of gonadal LH (Fernández-Fernández et

al., 2004; Fernández-Fernández et al., 2007). Moreover, ghrelin was found to have inhibitory activity on luteinising hormone (LH) secretion in prepubertal males and gonadectomised male and female rats, whereas it didn't affect follicle stimulating hormone (FSH) secretion. Later research, however, showed that ghrelin also had an impact on FSH secretion; this effect, however, depended on the cycle phase and was only inhibited in oestrus (Fernández-Fernández et al., 2004; Fernández-Fernández et al., 2005). In another study, it was determined that LH response increased in response to naloxone under saline infusion and inhibitory and/or retarding effects emerged after acylated ghrelin infusion (Lanfranco et al., 2008). It was also discovered that LH frequency decreased after short-term peripheral ghrelin injection, which increased plasma ghrelin levels 2.9-fold compared to the beginning in the ovariectomized Rhesus monkeys (Vulliémoz et al., 2004). Since they are both potent stimulators of food intake and gonadotrophin release, ghrelin and GnIH are acknowledged as two very significant agonistic peptides. GnIH suppresses either GnRH neurons or gonadotropes by binding to the GnIH-R, GPR147, which would be found on both human gonadotropes and GnRH neurons. However, it has been proposed that ghrelin may inhibit gonadotropin synthesis and secretion by acting on the hypothalamus and pituitary via the GnIH-GPR147 system (Celik et al., 2016). Together with there are conflicting findings on this issue, another study claimed that acute injection of ghrelin into healthy women did not affect basal and GnRH-induced LH and FSH secretion, suggesting that ghrelin may not have a significant physiological effect on gonadotropin release in women (Messini et al., 2009). Some of the other hormones directly or, more likely, indirectly affected by ghrelin are adrenocorticotropic hormone (ACTH) and corticosterone. The repetitive central application of ghrelin to normal rats elevated circulating ACTH and corticosterone levels, as well as the volume and density of ACTH cells. Even though the precise mechanism of action is unknown, it was put forward to a spike in ghrelin levels during fasting may contribute to neuroendocrine and behavioral alterations during fasting-induced stress, leading to a rise in ACTH and corticosterone concentrations (Stevanović et al., 2007). The functional integrity of the hypothalamus-pituitary-adrenal axis is stated that responsible for the ACTH/cortisol reaction to ghrelin. Stress-induced plasma levels of ACTH and ghrelin were evaluated in an investigation on SPD rats (low anxiety type) and WKY rats (high anxiety type), and it was defined that ACTH and ghrelin concentrations elevated in both rat species. In response to acute stress, they hypothesized that psychological stress enhanced ghrelin secretion in the occintic mucosa as well as circulating ghrelin levels in both rat strains (Kristenssson et al., 2006). In another study, it was discovered that the stress group had significantly higher levels of active ghrelin. The sympathetic pathway mediated delayed gastric emptying during the acute

phase of continuous stress, but increased active ghrelin release caused accelerated gastric emptying during the chronic phase (Ochi et al., 2008).

Table 1. *Physiological Effects of Ghrelin Hormone*

Target Tissue / Organ	Physiological Effects	Physiological Pathway
	Stimulates GH secretion	✓ After binding to GHSR-1a, phospholipase C is activated, which catalyses the formation of inositol triphosphate (IP3) and diacylglycerol (DAG). After binding to the IP3 receptor, it triggers Ca^{2+} release into the cytoplasm. Ca^{2+} causes fusion of GH-secreting vesicles with the cell membrane, followed by exocytosis.
	It plays a role in both the central and perpherall regulation of food consumption.	✓ Increases AGRP mRNA expression. This increase inhibits the hypothalamic melanocortin system. ✓ Antagonises the effect of leptin through activation of the NPY/Y1 receptor pathway. ✓ In the lateral parabrachial nucleus, it results in the activation of ghrelin receptors. ✓ Increases stomach and intestinal motility. ✓ Increases gastric acid secretion. ✓ Stimulates gastric emptying.
	Regulates energy homeostasis	✓ Lipoprotein lipase stimulates lipogenesis through the enzymes acetyl CoA carboxylase α, fatty acid synthase and stearoyl-CoA desaturase-1. ✓ Inhibits lipolysis. ✓ Without affecting skeletal muscle, it increases the rate at which WAT and WAT uses glucose.
	Improving the quality of sleep and memory capacity.	✓ The mechanism of action and pathway are not fully known.

Conclusion

The underlying physiopathological mechanisms must first be clarified in order to combat and/or control metabolic and neurodegenerative disorders, which are now a major global public health issue. In this review, we emphasize the several physiological roles played by ghrelin and briefly discuss its mechanisms of action in order to enrich our knowledge and experiences. As a result of the literature review, it has been determined that the pathways of action of ghrelin hormone in some physiological functions have not been fully determined. To ascertain whether it has a beneficial effect on sleep and memory and its effect pathway, it is seen that more research in both experimental and clinical areas is necessary.

References

Baatar, D., Patel, K., Taub, D.D. (2011). The effects of ghrelin on inflammation and the immune system. *Molecular and cellular endocrinology, 340*(1), 44–58.

Bake, T., Le May, M.V., Edvardsson, C.E., Vogel, H., Bergström, U., Albers, M.N., Skibicka, K.P., Farkas, I., Liposits, Z., Dickson, S.L. (2020). Ghrelin receptor stimulation of the lateral parabrachial nucleus in rats increases food intake but not food motivation. *Obesity, 28*(8), 1503–1511.

Briggs, D.I., Andrews, Z.B. (2011). Metabolic status regulates ghrelin function on energy homeostasis. *Neuroendocrinology*, *93*(1), 48–57.

Camina, J.P. (2006). Cell biology of the ghrelin receptor. *J Neuroendocrinol, 18*(1), 65–76.

Caminos, J.E., Tena-Sempere, M., Gaytan, F., Sanchez-Criado, J.E., Barreiro, M.L., Nogueiras, R., Casanueva, F.F., Aguilar, E., Dieguez, C. (2003). Expression of ghrelin in the cyclic and pregnant rat ovary. *Endocrinology, 144*(4), 1594–1602.

Celik, O., Celik, N., Aydin, S., Aygun, B.K., Haberal, E.T., Kuloglu, T., Ulaş, M., Aktun, L.H., Acet, M., Celik, S. (2016). Ghrelin action on GnRH neurons and pituitary gonadotropes might be mediated by GnIH-GPR147 system. *Hormone Molecular Biology and Clinical Investigation, 25*(2), 121–128.

Chan, C.B., Cheng, C.H. (2004). Identification and functional characterization of two alternatively spliced growth hormone secretagogue receptor transcripts from the pituitary of black seabream Acanthopagrus schlegeli. *Mol Cell Endocrinol, 214*(1-2), 81–95.

Chen, J., Liu, X., Shu, Q., Li, S., Luo, F. (2008). Ghrelin attenuates lipopolysaccharide-induced acute lung injury through NO pathway. *Medical Science Monitor, 14*(7), BR141–BR146.

Date, Y., Kojima, M., Hosoda, H, Sawaguchi, A., Mondal, M.S, Suganuma, T., Matsukura, S., Kangawa, K., Nakazato, M. (2000). Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology, 141*(11):4255–4261.

De Guia, R.M., Hassing, A.S., Skov, L.J., Ratner, C., Plucińska, K., Madsen, S., Diep, T.A., Cruz, G.V.D., Trammell, S.A.J., Sustarsic, E.G., Emanuelli, B., Gillum, M.P., Gerhart-Hines, Z., Holst, B., Jonas, T. (2020). Treebak Fasting-and ghrelin-induced food intake is regulated by NAMPT in the hypothalamus. *Acta Physiol, 228*(4), e13437.

Dixit, V.D., Schaffer, E.M., Pyle, R.S., Collins, G.D., Sakthivel, S.K., Palaniappan, R., Lillard, J.W., Taub, D.D. (2004). Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest, 114*(1), 57–66.

Elias, C.F., Saper, C.B., Maratos-Flier, E., Tritos, N.A., Lee, C., Kelly, J., Tatro, J.B., Hoffman, G.E., Ollmann, M.M., Barsh, G.S., Sakurai, T., Yanagisawa, M., Elmquist, J.K. (1998). Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol, 402*(4), 442–459.

Eslami, M., Sadeghi, B., Goshadrou, F. (2018). Chronic ghrelin administration restores hippocampal long-term potentiation and ameliorates memory impairment in rat model of Alzheimer's disease. *Hippocampus, 28*(10), 724–734.

Fernández-Fernández, R., Tena-Sempere, M., Aguilar, E., Pinilla, L. (2004). Ghrelin effects on gonadotropin secretion in male and female rats. *Neuroscience letters, 362*(2), 103–107.

Fernández-Fernández, R., Tena-Sempere, M., Navarro, V.M., Barreiro, M.L., Castellano, J.M., Aguilar, E., Pinilla, L. (2005). Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: in vivo and in vitro studies. *Neuroendocrinology, 82*(5-6), 245–255.

Fernández-Fernández, R., Tena-Sempere, M., Roa, J., Castellano, J.M., Navarro, V.M., Aguilar, E., Pinilla, L. (2007). Direct stimulatory effect of ghrelin on pituitary release of LH through a nitric oxide-dependent mechanism that is modulated by estrogen. *Reproduction, 133*(6), 1223–1232.

Gauna, C., Meyler, F.M., Janssen, J.A.M.J.L, Delhanty, P.J.D., Abribat, T., Van Koetsveld, P., Hofland, L.J., Broglio, F., Ghigo, E., Van Der Lely, A.J. (2004). Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. *J Clin Endocrinol, 89*(10), 5035–5042.

Gnanapavan, S., Kola, B., Bustin, S.A., Morris, D.G., McGee, P., Fairclough, P., Bhattacharya, S., Carpenter, R., Grossman, A.B., Korbonits, M., (2002). The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. *J Clin Endocrinol Metab, 87*(6), 2988–2991.

Gruzdeva, O.V., Borodkina, D.A., Belik, E.V., Akbasheva, O.E., Palicheva, E.I., Barbarash, O.L. (2019). Ghrelin physiology and pathophysiology: focus on the cardiovascular system. *Kardiologiia, 59*(3), 60–67.

Guan, X.M., Yu, H., Palyha, C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J.S., Smith, R.G., Van der Ploeg, L.H.T., Howard A.D. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res, 48*, 23–29.

Harrold, J.A., Dovey, T.M., Blundell, J.E., Halford, J.C. (2012). CNS regulation of appetite. *Neuropharmacology, 63*(1), 3–17.

Hattori, N. (2009). Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. *Growth Horm. IGF Res, 19*, 187–197.

Himmerich, H., Sheldrick, A. (2010). TNF-α and ghrelin: opposite effects on immune system, metabolism and mental health. *Protein & Peptide Letters, 17*(2), 186–196.

Hornsby, A.K., Bundwal, L., Carisi, M.L., Santos, V.V., Jhonson, F., Roberts, L.D., Sassi, M., Mequinon, M., Stark, R., Reichenbach, A., Lockie, S.H., Siervo, M., Howel, O., Morgan, A.H., Wells, T., Andrews, Z.B., Burn, D.J., Davies, J.S. (2020). Unacylated-ghrelin impairs hippocampal neurogenesis and memory in mice and is altered in Parkinson's dementia in humans. *Cell Rep, 1*(7), 100120.

İlhan, T., Erdost, H. (2009). Ghrelin. *Uludag Univ J Fac Vet Med, 28*(1), 67–74.

Khatib, N., Gaidhane, S., Gaidhane, A.M., Khatib, M., Simkhada, P., Gode, D., Zahiruddin, Q.S. (2014). Ghrelin: ghrelin as a regulatory Peptide in growth hormone secretion. *J Clin Diagn Res, 8*(8), MC13–MC17.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature, 402*(6762), 656–660.

Kojima, M., Kangawa, K. (2005). Ghrelin: structure and function. *Physiol Rev, 85*, 495–522.

Kristenssson, E., Sundqvist, M., Astin, M., Kjerling, M., Mattsson, H., De La Cour, C.D., Håkanson, H., Lindström, E. (2006). Acute psychological stress raises plasma ghrelin in the rat. *Regulatory peptides, 134*(2-3), 114–117.

Lanfranco, F., Bonelli, L., Baldi, M., Me, E., Broglio, F., Ghigo, E. (2008). Acylated ghrelin inhibits spontaneous LH pulsatility and responsiveness to naloxone, but not that to GnRH in young men: evidence for a central inhibitory action of ghrelin on the gonadal axis. *J Clin Endocrinol Metab, 93*, 3633–3639.

Lee, J.H., Kim, T.J., Kim, J.W., Yoon, J.S., Kim, H.S., Lee, K.M. (2016). The anti-apoptotic effect of ghrelin on restraint stress-induced thymus atrophy in mice. *Immune network, 16*(4), 242–248.

Leite-Moreira, A.F., Soares, J.B. (2007). Physiological, pathological and potential therapeutic roles of ghrelin. *Drug Discov Today, 12*(7-8), 276–288.

Li, N., Xiao, K., Mi, X., Li, N., Guo, L., Wang, X., Sun, Y., Li, G., Zhou, Y. (2021). Ghrelin signaling in dCA1 suppresses neuronal excitability and impairs memory acquisition via PI3K/Akt/GSK-3β cascades. *Neuropharmacology, 203*, 108871.

Lindqvist, A., Shcherbina, L., Prasad, R.B., Miskelly, M.G., Abels, M., Martínez-Lopéz, J.A., Fred, R.G., Nergård, B.J., Hedenbro, J., Groop, L., Hjerling-Leffler, J., Wierup, N. (2020). Ghrelin suppresses insulin secretion in human islets and type 2 diabetes patients have diminished islet ghrelin cell number and lower plasma ghrelin levels. *Mol Cell Endocrinol*, *511*, 110835.

Masuda, Y., Tanaka, T., Inomata, N., Ohnuma, N., Tanaka, S., Itoh, Z., Hosoda, H., Kojima, M., Kangawa, K. (2000). Ghrelin stimulates gastric acid secretion and motility in rats. *Biochem Biophys Res, 276*(3), 905–908.

Messini, C.I., Dafopoulos, K., Chalvatzas, N., Georgoulias, P., Messinis, I.E. (2009). Effect of ghrelin on gonadotrophin secretion in women during the menstrual cycle. *Human Reproduction, 24*(4), 976–981.

Morin, V., Hozer, F., Costemale-Lacoste, J.F. (2018). The effects of ghrelin on sleep, appetite, and memory, and its possible role in depression: a review of the literature. *L'encephale, 44*(3), 256–263.

Müller, T.D., Nogueiras, R., Andermann, M.L. (2015). Ghrelin. *Mol metab, 4*(6):437–460.

Nazari-Serenjeh, F., Darbandi, N., Majidpour, S., Moradi, P. (2019). Ghrelin modulates morphine-nicotine interaction in avoidance memory: Involvement of CA1 nicotinic receptors. *Brain Res, 1720*, 146315.

Ochi, M., Tominaga, K., Tanaka, F., Tanigawa, T., Shiba, M., Watanabe, T., Fujiwara, Y., Oshitani, N., Higuchi, K., Arakawa, T. (2008). Effect of chronic stress on gastric emptying and plasma ghrelin levels in rats. *Life sciences, 82*(15-16), 862–868.

Reimer, M.K., Pacini, G., Ahrén, B.O. (2003). Dose-dependent inhibition by ghrelin of insulin secretion in the mouse. *Endocrinology, 144*(3), 916–921.

Sakata, I., Sakai, T. (2010). Ghrelin cells in the gastrointestinal tract. *Int J Pept*, 2010, 945056.

Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S., Hosoda, H., Kangawa, K., Matsukura, S. (2002). Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab, 87*(1), 240–244.

Shintani, M., Ogawa, Y., Ebihara, K., Aizawa-Abe, M., Miyanaga, F., Takaya, K., Hayashi, T., Inoue, G., Hosoda, G., Kojima, M., Kangawa, K., Nakao, K. (2001). Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes*, *50*(2), 227–232.

Smith, R.G., Jiang, H., Sun, Y. (2005). Developments in ghrelin biology and potentialclinical relevance. *Trends Endocrinol Metab, 16*, 436–442.

Steiger, A., Dresler, M., Schüssler, P., Kluge, M. (2011). Ghrelin in mental health, sleep, memory. *Molecular and Cellular Endocrinology, 340*(1), 88–96.

Stevanović, D., Milošević, V., Starčević, V.P., Severs, W.B. (2007). The effect of centrally administered ghrelin on pituitary ACTH cells and circulating ACTH and corticosterone in rats. *Life sciences, 80*(9), 867–872.

Sun, M.L., Niu, X., Xiao, X.Y., Chen, X. (2021). The differences in plasma/serum ghrelin levels between obstructive sleep apnea-hypopnea patients and

controls: A protocol for systematic review and meta-analysis. *Medicine, 100*(8), e24368.

Szczepankiewicz, D., Skrzypski, M., Pruszynska-Oszmalek, E., Zimmermann, D., Andralojc, K., Kaczmarek, P., Wojciechowicz, T., Sassek, M., Nowak, K.W. (2010). Importance of ghrelin in hypothalamus–Pituitary axis on growth hormone release during normal pregnancy in the rat. *Journal of Physiology and Pharmacology, 61*(4), 443.

Taub, D.D., Murphy, W.J., Longo, D.L. (2010). Rejuvenation of the aging thymus: growth hormone-mediated and ghrelin-mediated signaling pathways. *Curr. Opin. Pharmacol. 10,* 408–424.

Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueras, R., Perez-Tilve, D., Pfluger, P., Casteneda, T.R., Muzzin, P., Schürmann, A., Szanto, I., Tschöp, M.H., Rohner-Jeanrenaud, F. (2006). Ghrelin action in the brain controls adipocyte metabolism. *J Clin Investig*, *116*(7), 1983–1993.

Tong, J., Prigeon, R.L., Davis, H.W., Bidlingmaier, M., Kahn, S.E., Cummings, D.E., Tschöp, M.H., D'Alessio, D. (2010). Ghrelin suppresses glucose-stimulated insulin secretion and deteriorates glucose tolerance in healthy humans. *Diabetes*, *59*(9), 2145–2151.

Toshinai, K., Mondal, M.S., Nakazato, M., Date, Y., Murakami, N., Kojima, M., Kangawa, K., Matsukura, S. (2001). Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem Biophys Res, 281*(5), 1220–1225.

Tümer, C., Oflazoğlu, H.D., Obay, B.D., Kelle, M., Taşdemir, E. (2008). Effect of ghrelin on gastric myoelectric activity and gastric emptying in rats. *Regul Pept*, *146*(1-3), 26–32.

Unniappan, S., Peter, R.E. (2004). In vitro and in vivo effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. *Am J Physiol Regul, 286*(6), R1093–R1101.

Van der Lely, A.J. (2009). Ghrelin and new metabolic frontiers. *Horm Res in Paediatr*, 71(1), 129–133.

Van der Lely, A.J., Tschop, M., Heiman, M.L., Ghigo, E. (2004). Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endoc. Rev, 25*, 426–457.

Vulliémoz, N.R., Xiao, E., Xia-Zhang, L., Germond, M., Rivier, J., Ferin, M. (2004). Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the ovariectomized rhesus monkey. *The Journal of Clinical Endocrinology & Metabolism, 89*(11), 5718–5723.

Wang, W., Bansal, S., Falk, S., Ljubanovic, D., Schrier, R. (2009). Ghrelin protects mice against endotoxemia-induced acute kidney injury. *American Journal of Physiology-Renal Physiology, 297*(4), F1032–F1037.

Yamazaki, M., Nakamura, K., Kobayashi, H., Matsubara, M., Hayashi, Y., Kanga-

wa, K., Sakai, T. (2002). Regulational effect of ghrelin on growth hormone secretion from perifused rat anterior pituitary cells. *J Neuroendocrinol, 14*(2), 156–162.

Yang, C.G., Wang, W.G., Yan, J., Fei, J., Wang, Z.G., Zheng, Q. (2013). Gastric motility in ghrelin receptor knockout mice. *Mol Med Rep, 7*(1), 83–88.

Yuan, M.J., Huang, C.X., Tang, Y.H., Wang, X., Huang, H., Chen, Y.J., Wang, T. (2009). A novel peptide ghrelin inhibits neural remodeling after myocardial infarction in rats. *European journal of pharmacology, 618*(1-3), 52–57.

Zareian, P., Abtahi, S.R., Serenjeh M.N. (2018). Influence of Sleep Deprivation on the 24-Hour Ghrelin Secretion Pattern. *Shiraz E Medical J,19*(10), e66327.

Zhang, D.M., Pang, X.L., Huang, R., Gong, F.Y., Zhong, X., Xiao, Y. (2018). Adiponectin, omentin, ghrelin, and visfatin levels in obese patients with severe obstructive sleep apnea. *BioMed research international, 2018*, 3410135.

Zhou, M., Aziz, M., Ochani, M., Yang, W.L., Sharma, A., Wang, P. (2018). The protective role of human ghrelin in sepsis: restoration of CD4 T cell proliferation. *PLoS One, 13*(7), e0201139.

CHAPTER 8

LENTINAN: NEW MEDICAL TREATMENT AS IMMUNOMODULATORY AND VACCINE ADJUVANT

Irmak DiK[1], Burak DiK[2]

1 Ass.Prof.Dr.; Department of Virology, Veterinary Faculty, University of Selcuk, Konya, Turkey. irmakdik@selcuk.edu.tr, ORCID: 0000-0003-2516-9489

2 Asc.Prof. Dr.; Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey. burakdik@selcuk.edu.tr, ORCID: 0000-0003-2738-6911

1. Introduction

For many years, people have utilized mushrooms as food and for therapeutic purposes. Polysaccharides, which have been proven to have antiviral, anticancer, and immune-system boosting properties, are the primary active components in mushrooms. One of the most popular mushrooms, Lentinus edodes (L. edodes), is widely grown in China, Japan, and other Asian nations, partly because of its flavor and nutritional benefits. Lentinus edodes is a kind of fungus that is commonly cultivated and consumed in Asian nations. (Zhang et al., 2011). Lentinula edodes (shiitake) mushrooms have been used medicinally for many years in China (Zhang et al., 2019). Lentinula edodes is a medicinal mushroom species used as a food, and lentinan (LNT) extracted from L. edodes has various therapeutic effects (Kumar et al., 2023).

Lentinan (LNT) is a (1,3)-D-glucan that was isolated from this mushroom. Initial research indicated that LNT was thought to have anti-cancer properties when studied in a sarcoma-bearing mice model in the 1960s. Later research revealed it to be a physiologically active macromolecule having immunomodulatory, antiviral, antitumor, anticancer, and anticoagulant activities (Liu et al., 1999; Zhang et al., 2011; Zi et al., 2020). Additionally, it has been claimed that LNT aids with the body's immune system, including T-cells, natural killer cells, and macrophages. LNT has also been used in the therapy of COVID-19 (Kumar et al., 2023). Lentinan has recently gained popularity as a nutritional supplement and complementary medicine across the globe. Despite the powerful anti-cancer and anti-viral activity of LNT has been known for nearly 40 years, problems in its extraction and purification, chain structure, and structure-effect relationship are an obstacle to the development of LNT as a therapeutic agent (Zhang et al., 2011).

In recent years, naturally sourced immunomodulatory supplements have stand out in the food and drug industries (Liu et al., 1999; Zhang et al., 2011). Also, adjuvants can be obtained from simple natural extracts and polysaccharide-based adjuvants have emerged safer and more biocompatibility (He et al., 2020; Talarico et al., 2005).

2. Structural and Pharmacological Features

Structural Features

LNT has isolated from the body and mycelium of Lentinula edodes mushroom in the 1970s. The aqueous solution of LNT shows a triple helical structure, while its in dimethyl sulfoxide (DMSO) shows a random helical structure. The addition of water to dissolved LNT in DMSO, the helix structure of LNT and the aggregation process can be disrupted. The link

between physico-chemical features and biological effects of LNT is very important. For example, if the triple helix structure of LNT is disrupted, the anticarcinogenic effect is reduced (Vetter, 2023).

LNT should be extracted from the fungal cell wall by an appropriate method to be used actively in practice. LNT is extracted in systems such as hot water, polyethylene glycol, alkaline solution, ultrasonic, and ultra-high pressure. The use of high-energy processes such as ultrasonic and ultra-high pressure reveals that the activity of LNT is not impaired (Liu & Huang, 2019; Zhang et al., 2011). In recent years, it has been reported that LNT extraction using high-energy processes has more active components than alkali and temperature processing (Kumar et al., 2023). The polysaccharide fraction of LNT consists of both the β-(1–3)-D glucan and the side chain of β-(1–6)-linked glucopiranoside (Zhang et al., 2008).

LNT has used as a drug carrier nanomaterial (biomedical) with biological/immunological activities and in the treatment of various diseases. However, its immunological and pharmacological beneficial properties should be extensively investigated. (Kumar et al., 2023).

Pharmacological Features

LNT has approved as an adjuvant in cancer chemotherapy in Japan in 1985 and in China in 1995 (Barton et al., 2016). LNT has been shown to have positive effects in the treatment of many cancers such as colon, lung and breast cancer in different studies (Kumar et al., 2023). It is also approved for the treatment of many cancer types and some diseases such as hepatitis. LNT can be produced as capsules, tablets and injections (Zhang et al., 2019).

LNT is pharmacologically biological response modifier. In addition to the immune response of LNT against the tumor, it provides a serious immunomodulator contribution to chemotherapy because of its low-toxic effects (Yiran Zhang et al., 2018).

LNT restrains cell proliferation by stopping the G2/M phase in the division of cancerous cells in breast cancer (Xu et al., 2017). It also suppresses the antiapoptotic genes of cancer cells (Gu et al., 2022). LNT reduces colon cancer by inhibiting TLR-4-mediated NF-kB signaling pathways and activates intrinsic and extrinsic pathways (J. Wang et al., 2017; Zhang et al., 2021). Moreover, β-glucan structure in the LNT has anti-tumor and immunomodulatory activities by inducing cytokines (Meng et al., 2016; Zhang et al., 2019). LNT has found to act synergistically with paclitaxel through the ASK1/p38 MAPK signaling pathway in the lung cancer treatment (Liu et al., 2015). LNT induces apoptosis on tumor cells via intracellular reactive oxygen species (ROS) *in vitro* (Bao et al., 2015). It also

induces cytotoxicity on S180 cells by upregulating Bax and downregulating Bcl-2 (Zhang et al., 2015). In an *in vivo* study, LNT has significantly suppressed breast tumor growth by showing a synergistic effect with Herceptin (Cheung et al., 2002).

The triple helix structure and high molecular weight of LNT determine its biological effectiveness. In addition, it has been reported that it shows dose-dependent activity in different cancer types (Liu et al., 2015; Y. Wang et al., 2017). The dose-related anticancer activity of LNT has been reported as 25–800 μg/ml in vitro, while it has been reported as 25–200 mg/kg in vivo (Zhang et al., 2015). However, some authors explained that its anticancer effect is not dose dependent, its high molecular weight has immunomodulatory effect and strengthens anti-tumor activity (Zhang et al., 2005).

In addition to anticancer effects of LNT, it has anticoagulant, anti-viral, anti-tumor, anti-cancer, antidiabetics and immunomodulators effects for the treatment of various diseases (Liu et al., 1999; Vannucci et al., 2017; Zhang et al., 2011). Sulfated modification of LNT inhibited replication of infectious bronchitis virus in vitro (Wang et al., 2010).LNT has shown a potential to treat sepsis and lung injury in *Klebsiella pneumoniae* infection as it effectively alleviates bacterial load and inflammation in the blood and bronchoalveolar lavage fluid, increases oxygen saturation, and improves lung physiological parameters (Masterson et al., 2019). LNT has significantly prevented cisplatin-induced kidney injury by activating the NRF2-ARE signaling pathway in vivo. (Chen et al., 2016).

LNT has been notified that is absorbed by approximately 46.59% after oral administration and reaches its maximum concentration within 1 hour and detected in blood during8 hours. It has not reach very high concentrations in other organs outside the gastrointestinal organs and is excreted in large amounts in feces and in small amounts in urine (Zheng, Pan, et al., 2021). Intravenous use of LNT is recommended instead of oral administration due to poor intestinal absorption. It was investigated that LNT was more distributed in the liver (50.60%) and spleen (20.72%) in 5 minutes after the injection. Its level was 31.66% and 12.68% in the liver and spleen, respectively, 24 hours after the injection (Yu Zhang et al., 2018). LNT is metabolized by CYP2D6 and CYP2C9 enzymes within the CYP450 enzyme family (Zheng, Zhang, et al., 2021).

The side effects of LNT are rare, however the side effects such as low blood pressure, allergies, acute asthma, dizziness, and shock have been reported. The symptoms occur in the first 60 minutes following the use of LNT. It should not be mixed with other drugs and should only be diluted with glucose solution or 0.9% sodium chloride (Zhang et al., 2019). It has

been reported that LNT causes vasodilation and bleeding by affecting the vascular system (Suzuki et al., 1994).

3. Immunoregulatory Effects of Lentinan

The fungal polysaccharides containing β-glucan and their derivatives have stronger immunomodulatory activity than plant polysaccharides (Jiang et al., 2010). β-glucans are resistant to gastric acid. They bind to macrophage receptors in the intestinal wall and then spread to the spleen, lymph nodes, and bone marrow (Vetter, 2023).

The relationship between β-glucans and immune cells is not fully known. However, β-glucan is the main component of LNT, and it is important for the immune regulation. LNT downstream certain toll like receptors, Dectin-1 receptor, complement receptor, and pathways such as MAPK-NFκB and Syk-PKC. It activates immunocytes (NK, macrophage, T cells) and stimulates the various cytokines by regulating the signal pathway and receptors (Vetter, 2023; Zhang et al., 2019). In addition, the glucans increase cellular and humoral response through different receptors (Vetter, 2023).

LNT inhibits cancer cells in humans by increasing the activities of some immune cells and cytokines that act as signal messengers between cells. It has also been shown that LNT treatment contributes nitric oxide and stimulates the immune system (Vetter, 2023).

LNT enhances the response of antigen-specific cytotoxic T-lymphocytes and IL-2 level. IL-2 induction by LNT causes NK and lymphokine-activated killer cell activation. LNT increases macrophage and macrophage-activating factor reactivity. Moreover, it also induces the macrophages to produce IL-1 and IL-6 (Suzuki et al., 1994).

LNT stimulates NF-κB activation in A549(human alveolar epithelial) cells and decreased proinflammatory cytokine production (IL-2, IL-6, IL-8, TNF-a, IL-22), TGF-β and IL-10 levels. However, it suppresses apoptosis by reducing oxidative stress. Its immunomodulatory and pulmonary cytoprotective effects may have been important for the treatment of COVID-19 as in vitro (Murphy et al., 2020).

In addition, LNT has upregulated the expression of TNF-α, TLR4, and TLR9 via T-lymphocytes in mice. It has promoted IgG secretion by B-lymphocytes and increased the phagocytic activity of macrophages in mice. LNT has been reported to activate natural killer cells (NK) and enhanced in TNF-α, IL-12 and IFN-γ concentrations. It has also provided the regulation of Th1 and Th2 cells (Zhang et al., 2019). In addition, molecular docking and western blotting data have revealed that LNT provides immunomodulation by activating the JAK2/STAT3 signaling pathway (Guan et

al., 2023).

Another important effect of LNT in regulating immunity is "trained immunity". β-glucan plays an immunoregulatory role by creating a non-specific immune response (Yiran Zhang et al., 2018).

4. Vaccine Adjuvant of Lentinan

The development of vaccines is undoubtedly one of the greatest contributions to human health. The total eradication of smallpox, the global elimination of polio, and the vaccines have reduced the death and morbidity rates of numerous infectious diseases in many different countries. In world, the vaccinations are a cornerstone of public health, and much care is taken to ensure that the populace has access to safe and efficient vaccines (Facciolà et al., 2022; Vanderslott & Marks, 2021). They are one of the most crucial preventative measures against infectious diseases. Numerous vaccination types have been created over time for many different antigen components A strong immune response and long-term protection are the desired outcomes of immunizations (Pulendran & Ahmed, 2011). Since live vaccines have antigenic proliferation, the vaccine usually has the capacity to create immunity in a single dose and there is no need for immune system-activating auxiliary substances. However, new vaccines like inactivated and recombinant vaccines are preferred because of the risks of causing disease and the many difficulties encountered in the creation of live vaccines. These vaccinations, which stand out in this context and are regarded as safer, have modest immune system stimulant qualities and require strong adjuvants for this (Clem, 2011). Adjuvants have developed in the early 20th century in response to the failure of conventional vaccines to provide adequate protection. They are used in combination with the antigen to elicit stronger immune responses compared to the antigen alone (Pérez et al., 2013). Adjuvants can be obtained from simple natural extracts as well as from artificial synthetic compounds (Aguilar & Rodriguez, 2007). Adjuvants are the primary components that boost the efficacy of vaccination applications with a variety of outcomes, particularly by serving as carriers, depots, and immune response stimulators. Few adjuvants were used in vaccinations for a very long time, and aluminum salts were the most often used adjuvant. However, the current text draws attention to new substances with beneficial adjuvant properties and increased safety. The manufacture of both antigen and adjuvant components has been heavily influenced by contemporary technologies like nanotechnology and molecular biology, boosting the effectiveness of vaccines. The development of vaccines using microparticles, emulsions, and immunostimulants is currently a top priority for effectively vaccination. Although some vaccination adjuvant side effects, such as the recently recognized ASIA syndrome, have been reported in studies, there is no disputing the immunizations' immense use-

fulness. The recent COVID-19 pandemic has brought the value of vaccines back into focus, particularly for the control of any future pandemics. This area of adjuvant research has the potential to significantly contribute to the development of vaccinations that are more effective (Facciolà et al., 2022).

In order to prevent and eradicate viral infections, vaccination is crucial (Sen et al., 2010). Many methods and medication trials are conducted to increase the immunological potential of the vaccination and to extend the period of action. (Mohamed et al., 2013; O'Hagan, 2007). Unlike traditional adjuvants, polysaccharide-based adjuvants have emerged as new and safer candidates due to their high biocompatibility and low toxicity (He et al., 2020; Talarico et al., 2005).

Lentinus edodes is a traditional Chinese herbal medicine. It is widely used to increase resistance against bacterial, fungal, viral or parasitic infections and tumors (He et al., 2020; Zi et al., 2020). LNT is a β-(1,3)-D-glucan isolated from Lentinus edodes, a mushroom species. It is defined as a biologically active macromolecule that activates the immune system and immunomodulatory properties (Liu et al., 1999; Zhang et al., 2011; Zi et al., 2020). Studies have shown that LNT can be used as an immunomodulator by stimulating various immune cells such as macrophages, dendritic cells, NK cells and lymphocytes (Chen et al., 2012; Wang et al., 2005). LNT can to function as a vaccine adjuvant and enhance the immune response (McCormack et al., 2010). Intranasal administration of LNT in combination with a vaccine against tuberculosis called Bacillus Calmette-Guerin (BCG) has been reported to induce the activation of immune cells at high levels in lung tissue. It has also been reported that the combination of BCG with LNT increases the local immune response to BCG in the lung, while reducing the vaccine-related side effects (Zhang et al., 2011). Co-administration of LNT with Newcastle virus vaccine increased the antibody titer and LNT is a new T-cell-directed adjuvant candidate compared with other well-known immunostimulants such as Corynebacterium parvum and lipopolysaccharide (LPS) (Chihara et al., 1987; Guo et al., 2009).

The high prevalence of viral infections, especially COVID-19, which has been the pandemic in recent years, and the H5N1 subtype, which is important for poultry and humans, has become an important public health problem. (He et al., 2020; Kumar et al., 2023; Murphy et al., 2020). In viral infections, ideal protection rather than treatment is required. Therefore, very effective vaccinations are required to prevent the spread of viral infections. The purpose of vaccination is to gain active immunity against infectious diseases. Adjuvants increase the potency of the vaccine mainly by improving immunogenicity and maintaining long-term immune responses (Kumar et al., 2023).

Liu et al. (2020), showed experimentally that LNT-CaCO3 significantly increased lymphocyte proliferation and increased the frequency of CD69+ B cells in spleen lymphocytes and the ratio of CD4+ to CD8+ T cells in their study. In addition, LNT-CaCO3 has been reported to induce the secretion of IgG and Th-related cytokines (IL-2, IL-4, IFN-γ and TNF-α) in immunized mice. Therefore, LNT-CaCO3 microspheres have been shown to induce potent cellular and humoral immune responses and have potential use as vaccine delivery systems (Liu et al., 2020).

In recent years, the high prevalence of avian influenza viruses isolated from poultry and humans, particularly the H5N1 subtype, has become a major public health concern He et al. (2020) is reported that LNT-modified calcium carbonate (CaCO3) microparticles as an adjuvant for the H5N1 vaccine can induce a stronger cellular and humoral immune response and may be a potential adjuvant for H5N1 vaccine.

LNT has shown encouraging results as a vaccine adjuvant, as it has been observed that it has a strengthening effect on Th-1 type and Th-2 type immune responses. However, further studies are needed to explore the clinical applicability of LNT as a vaccine adjuvant in the prevention of infectious diseases.

5.Conclusion

Traditional herbal drog treatments have been used for thousands of years and β-glucans have been contributed to strengthening the immunity. The Th1/Th2 regulatory effect of LNT is important for its immunological effect. The low toxic effect, the potential to strengthen the effects of drugs and vaccines, and the low cost of LNT are advantages.

In this text, the general properties of LNT, its immunomodulatory effects, and its adjuvant properties have been elaborated. LNT has been revealed to raising the quality of life and cancer treatment in pharmacological research, in recent years. The anticancer phase studies of LNT and its immunomodulatory and vaccine adjuvant ability have led to a new glimmer of hope from a pharmacological point of view.

The research on the immunomodulatory effects of LNT against infective diseases and on vaccine adjuvants are limited, despite the anticancer effects of LNT are frequently studied. In the future, these research topics will reveal new indication areas for phase studies of LNT.

References

Aguilar, J. C., & Rodriguez, E. (2007). Vaccine adjuvants revisited. *Vaccine*, *25*(19), 3752-3762.

Bao, L., Wang, Y., Ma, R., Ren, X., Cheng, R., & Agula, B. (2015). Apoptosis-inducing effects of lentinan on the proliferation of human bladder cancer T24 cells. *Pakistan journal of pharmaceutical sciences*, *28*(5).

Barton, C., Vigor, K., Scott, R., Jones, P., Lentfer, H., Bax, H. J., Josephs, D. H., Karagiannis, S. N., & Spicer, J. F. (2016). Beta-glucan contamination of pharmaceutical products: How much should we accept? *Cancer immunology, immunotherapy*, *65*, 1289-1301.

Chen, G., LianShun, C., Lei, L., Sheng, B., Ju-Xiang, S., Yue, D., & Shiwei, C. (2012). Lentinan has a Regulatory Effect Th Immune Response in BALB/c mice Infected with Toxoplasma Gondii. *Chin. J. Microecol*, *12*, 1064-1070.

Chen, Q., Peng, H., Dong, L., Chen, L., Ma, X., Peng, Y., Dai, S., & Liu, Q. (2016). Activation of the NRF2-ARE signalling pathway by the Lentinula edodes polysaccharose LNT alleviates ROS-mediated cisplatin nephrotoxicity. *International Immunopharmacology*, *36*, 1-8.

Cheung, N.-K. V., Modak, S., Vickers, A., & Knuckles, B. (2002). Orally administered β-glucans enhance anti-tumor effects of monoclonal antibodies. *Cancer immunology, immunotherapy*, *51*, 557-564.

Chihara, G., Hamuro, J., Maeda, Y., Shiio, T., Suga, T., Takasuka, N., & Sasaki, T. (1987). Antitumor and metastasis-inhibitory activities of lentinan as an immunomodulator: an overview. *Cancer detection and prevention. Supplement: official publication of the International Society for Preventive Oncology, Inc*, *1*, 423-443.

Clem, A. S. (2011). Fundamentals of vaccine immunology. *Journal of global infectious diseases*, *3*(1), 73.

Facciolà, A., Visalli, G., Laganà, A., & Di Pietro, A. (2022). An overview of vaccine adjuvants: current evidence and future perspectives. *Vaccines*, *10*(5), 819.

Gu, S., Xu, J., Teng, W., Huang, X., Mei, H., Chen, X., Nie, G., Cui, Z., Liu, X., & Zhang, Y. (2022). Local delivery of biocompatible lentinan/chitosan composite for prolonged inhibition of postoperative breast cancer recurrence. *International Journal of Biological Macromolecules*, *194*, 233-245.

Guan, Z., Liu, Y., Liu, P., Yin, L., Chen, L., & Duan, W. (2023). Lentinan regulates the immune efficacy of macrophage for lung metastasis in triple negative breast. *Journal of Functional Foods*, *105*, 105560.

Guo, Z., Hu, Y., Wang, D., Ma, X., Zhao, X., Zhao, B., Wang, J., & Liu, P. (2009). Sulfated modification can enhance the adjuvanticity of lentinan and improve the immune effect of ND vaccine. *Vaccine*, *27*(5), 660-665.

He, J., Liu, Z., Jiang, W., Zhu, T., Wusiman, A., Gu, P., Liu, J., & Wang, D. (2020).

Immune-adjuvant activity of lentinan-modified calcium carbonate microparticles on a H5N1 vaccine. *International Journal of Biological Macromolecules*, *163*, 1384-1392.

Jiang, M.-H., Zhu, L., & Jiang, J.-G. (2010). Immunoregulatory actions of polysaccharides from Chinese herbal medicine. *Expert opinion on therapeutic targets*, *14*(12), 1367-1402.

Kumar, A., Paliwal, R., & Gulbake, A. (2023). Lentinan: An unexplored novel biomaterial in drug and gene delivery applications. *Journal of Controlled Release*, *356*, 316-336.

Liu, F., Ooi, V., & Fung, M. (1999). Analysis of immunomodulating cytokine mRNAs in the mouse induced by mushroom polysaccharides. *Life sciences*, *64*(12), 1005-1011.

Liu, W., Gu, J., Qi, J., Zeng, X. N., Ji, J., Chen, Z. Z., & Sun, X. L. (2015). Lentinan exerts synergistic apoptotic effects with paclitaxel in A549 cells via activating ROS-TXNIP-NLRP 3 inflammasome. *Journal of cellular and molecular medicine*, *19*(8), 1949-1955.

Liu, Y., & Huang, G. (2019). Extraction and derivatisation of active polysaccharides. *Journal of enzyme inhibition and medicinal chemistry*, *34*(1), 1690-1696.

Liu, Z., Yu, L., Gu, P., Bo, R., Wusiman, A., Liu, J., Hu, Y., & Wang, D. (2020). Preparation of lentinan-calcium carbonate microspheres and their application as vaccine adjuvants. *Carbohydrate Polymers*, *245*, 116520.

Masterson, C., Murphy, E., Major, I., Gonzalez, H., O'toole, D., McCarthy, S., Laffey, J., & Rowan, N. (2019). Purified beta-glucan from the Lentinus edodes mushroom attenuates antibiotic resistant Klebsiella pneumoniae-induced pulmonary sepsis. In *Bacterial and Viral Lung Infections and Pathogenesis* (pp. A1222-A1222). American Thoracic Society.

McCormack, E., Skavland, J., Mujić, M., Bruserud, Ø., & Gjertsen, B. T. (2010). Lentinan: hematopoietic, immunological, and efficacy studies in a syngeneic model of acute myeloid leukemia. *Nutrition and cancer*, *62*(5), 574-583.

Meng, X., Liang, H., & Luo, L. (2016). Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities. *Carbohydrate Research*, *424*, 30-41.

Mohamed, E. M., Ibrahim, E. E.-S., & Mohamed, F. S. (2013). Effect of administration of ultra-corn with bivalent Foot and Mouth disease oil vaccine in calves. *Veterinary World*, *6*(8), 486.

Murphy, E. J., Masterson, C., Rezoagli, E., O'Toole, D., Major, I., Stack, G. D., Lynch, M., Laffey, J. G., & Rowan, N. J. (2020). β-Glucan extracts from the same edible shiitake mushroom Lentinus edodes produce differential in-vitro immunomodulatory and pulmonary cytoprotective effects—Implications for coronavirus disease (COVID-19) immunotherapies. *Science of*

the Total Environment, *732*, 139330.

O'Hagan, D. T. (2007). MF59 is a safe and potent vaccine adjuvant that enhances protection against influenza virus infection. *Expert review of vaccines*, *6*(5), 699-710.

Pérez, O., Romeu, B., Cabrera, O., González, E., Batista-Duharte, A., Labrada, A., Pérez, R., Reyes, L. M., Ramírez, W., & Sifontes, S. (2013). Adjuvants are key factors for the development of future vaccines: lessons from the Finlay adjuvant platform. *Frontiers in immunology*, *4*, 407.

Pulendran, B., & Ahmed, R. (2011). Immunological mechanisms of vaccination. *Nature immunology*, *12*(6), 509-517.

Sen, A., Saravanan, P., Balamurugan, V., Rajak, K. K., Sudhakar, S. B., Bhanuprakash, V., Parida, S., & Singh, R. K. (2010). Vaccines against peste des petits ruminants virus. *Expert review of vaccines*, *9*(7), 785-796.

Suzuki, M., Takatsuki, F., Maeda, Y. Y., Hamuro, J., & Chihara, G. (1994). Antitumor and immunological activity of lentinan in comparison with LPS. *International Journal of Immunopharmacology*, *16*(5-6), 463-468.

Talarico, L., Pujol, C., Zibetti, R., Faría, P., Noseda, M., Duarte, M., & Damonte, E. (2005). The antiviral activity of sulfated polysaccharides against dengue virus is dependent on virus serotype and host cell. *Antiviral research*, *66*(2-3), 103-110.

Vanderslott, S., & Marks, T. (2021). Charting mandatory childhood vaccination policies worldwide. *Vaccine*, *39*(30), 4054-4062.

Vannucci, L., Sima, P., Vetvicka, V., & Krizan, J. (2017). Lentinan properties in anticancer therapy: A review on the last 12-year literature. *American Journal of Immunology*, *13*(1), 50-61.

Vetter, J. (2023). The Mushroom Glucans: Molecules of High Biological and Medicinal Importance. *Foods*, *12*(5), 1009.

Wang, D., Guo, Z., Ma, X., Hu, Y., Huang, X., Fan, Y., Yang, S., & Guo, L. (2010). Effects of sulfated lentinan on cellular infectivity of avian infectious bronchitis virus. *Carbohydrate Polymers*, *79*(2), 461-465.

Wang, D., Hu, Y., Sun, J., Kong, X., Zhang, B., & Liu, J. (2005). Comparative study on adjuvanticity of compound Chinese herbal medicinal ingredients. *Vaccine*, *23*(28), 3704-3708.

Wang, J., Li, W., Huang, X., Liu, Y., Li, Q., Zheng, Z., & Wang, K. (2017). A polysaccharide from Lentinus edodes inhibits human colon cancer cell proliferation and suppresses tumor growth in athymic nude mice. *Oncotarget*, *8*(1), 610.

Wang, Y., Han, X., Li, Y. D., Wang, Y., Zhao, S. Y., Zhang, D. J., & Lu, Y. (2017). Lentinan dose dependence between immunoprophylaxis and promotion of the murine liver cancer. *Oncotarget*, *8*(56), 95152.

Xu, H., Zou, S., & Xu, X. (2017). The β-glucan from Lentinus edodes suppress-

es cell proliferation and promotes apoptosis in estrogen receptor positive breast cancers. *Oncotarget*, *8*(49), 86693.

Zhang, L., Li, X., Xu, X., & Zeng, F. (2005). Correlation between antitumor activity, molecular weight, and conformation of lentinan. *Carbohydrate Research*, *340*(8), 1515-1521.

Zhang, M., Zhang, Y., Zhang, L., & Tian, Q. (2019). Mushroom polysaccharide lentinan for treating different types of cancers: A review of 12 years clinical studies in China. *Progress in molecular biology and translational science*, *163*, 297-328.

Zhang, Y., Li, Q., Shu, Y., Wang, H., Zheng, Z., Wang, J., & Wang, K. (2015). Induction of apoptosis in S180 tumour bearing mice by polysaccharide from Lentinus edodes via mitochondria apoptotic pathway. *Journal of Functional Foods*, *15*, 151-159.

Zhang, Y., Li, S., Wang, X., Zhang, L., & Cheung, P. C. (2011). Advances in lentinan: Isolation, structure, chain conformation and bioactivities. *Food hydrocolloids*, *25*(2), 196-206.

Zhang, Y., Liu, Y., Zhou, Y., Zheng, Z., Tang, W., Song, M., Wang, J., & Wang, K. (2021). Lentinan inhibited colon cancer growth by inducing endoplasmic reticulum stress-mediated autophagic cell death and apoptosis. *Carbohydrate Polymers*, *267*, 118154.

Zhang, Y., Xu, X., & Zhang, L. (2008). Gel formation and low-temperature intramolecular conformation transition of a triple-helical polysaccharide lentinan in water. *Biopolymers: Original Research on Biomolecules*, *89*(10), 852-861.

Zhang, Y., Zhang, M., Jiang, Y., Li, X., He, Y., Zeng, P., Guo, Z., Chang, Y., Luo, H., & Liu, Y. (2018). Lentinan as an immunotherapeutic for treating lung cancer: a review of 12 years clinical studies in China. *Journal of cancer research and clinical oncology*, *144*, 2177-2186.

Zhang, Y., Zheng, Z., Yang, X., Pan, X., Yin, L., Huang, X., Li, Q., Shu, Y., Zhang, Q., & Wang, K. (2018). A sensitive and rapid radiolabelling method for the in vivo pharmacokinetic study of lentinan. *Food & function*, *9*(6), 3114-3125.

Zheng, Z., Pan, X., Wang, H., Wu, Z., Sullivan, M. A., Liu, Y., Liu, J., Wang, K., & Zhang, Y. (2021). Mechanism of lentinan intestinal absorption: Clathrin-mediated endocytosis and macropinocytosis. *Journal of Agricultural and Food Chemistry*, *69*(26), 7344-7352.

Zheng, Z., Zhang, Y., Liu, Y., Wang, J., Cui, Z., Pan, X., Liu, Y., Tang, W., & Wang, K. (2021). Metabolic degradation of lentinan in liver mediated by CYP450 enzymes and epoxide hydrolase. *Carbohydrate Polymers*, *253*, 117255.

Zi, Y., Jiang, B., He, C., & Liu, L. (2020). Lentinan inhibits oxidative stress and inflammatory cytokine production induced by benzo (a) pyrene in human keratinocytes. *Journal of Cosmetic Dermatology*, *19*(2), 502-507.

CHAPTER 9

VITAMINS

Gamze GÖK[1]

1 Uz. Dr., T.C. Sağlık Bakanlığı Ankara Bilkent Şehir Hastanesi Tıbbi Biyokimya Laboratuvarı, Orchid No:0000-0002-2804-5548

Vitamins are the essential substances that take place in metabolic reactions in the human body. They have vital functions in human metabolism, and serious diseases occur in cases of missing or excess intake. Vitamins can be classified into two groups. These groups are water-soluble vitamins and fat-soluble vitamins (Yaşar & Melek, 2014).

1. Water Soluble Vitamins

There is not any structural similarity in the organic compounds of water-soluble vitamins.' They play essential roles in normal cellular growth, development, and functions. Water-soluble vitamins exist in the diet in minute quantities. Deficiency or existing sub-optimal levels of water-soluble vitamins affect human health negatively (Said, 2011).

After entering the body, the water-soluble vitamins dissolve in water, and due to the dissolving in water properties, humans are not able to store surplus amounts of water-soluble vitamins. (Lykstad & Sharma, 2019)

Vitamin C and Vitamin B complex vitamins are members of the water-soluble vitamins. Vitamin B1, B2, B3, B5, B6, B7, B9, and B12 are the memebers of Vitamin B complex vitamins. For avoiding the deficiency of water-soluble vitamins, water-soluble vitamins must be taken regularly. Many foods including fruits, vegetables, fortified grains, cereals, meat, peas, and eggs contain water-soluble vitamins (Lykstad & Sharma, 2019).

1.1 Vitamin B1 (Thiamin):

Vitamin B1i s also called Thiamin. Vitamin B1 is in the vitamin B complex group. This vitamin is a substantial vitamin for the human and takes place in various biological processes. In the deficiency of Thiamin, an increased risk of morbidity and mortality occurs. If the patient is untreated or inadequately treated the mortality rates approach 20%. Irreversible neurological situations may occur in nearly 85% of the survivors (Latt & Dore, 2014). For a lot of enzymes, Vitamin B1 acts as a cofactor. Some of these enzymes involve pyruvate dehydrogenase, branched-chain ketoacid dehydrogenase, alpha-ketoglutarate, and transketolase. These enzymes take place in generating energy in the metabolism of glucose (Sica, 2007).

Vitamin B1 deficiency causes an activity decrease of the enzymes and a reduction in pyruvate oxidation may occur. Lactate accumulation in the brain and blood occurs due to Vitamin B1 deficiency. During this process, adenosine triphosphate (ATP) production decreases. Accumulation of lactate causes lactic acidosis. Furthermore, the decrease in the pH level of the brain may lead to neurological symptoms accompanying Vitamin B1 deficiency. Also, the reduction of enzyme activity can cause a decrease in neurotransmitter synthesis (Manzetti, Zhang, & van der Spoel, 2014).

In Vitamin B1 deficiency; ATP depletion effects the aerobic tissues including the heart, nerves, and the brain. If the Vitamin B1 deficiency symptoms involve the heart symptoms it is named Wet Berberi. Wet beriberi can be characterized by edema, dyspnea on exertion and also high output heart failure. If the Vitamin B1 deficiency symptoms involve nervous system symptoms it is named Dry Beriberi. Wernicke encephalopathy occurs when damage happens in mammalian bodies and the thalamus's medial dorsal nucleus in the brain. Wernicke encephalopathy has a triad including confusion, ataxia, and, ophthalmoplegia. Wernicke-Korsakoff syndrome comes out with personality change, confabulation, and memory loss accompanying the symptoms (Lykstad & Sharma, 2019).

1.2 Vitamin B2 (Riboflavin)

Vitamin B2 is in the vitamin B complex group. It is known as Riboflavin. Vitamin B2 has important roles in metabolic pathways. This vitamin is the precursor of the cofactors. These cofactors are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FAD and FMN take place in numerous enzymatic reactions. In oxidation-reduction processes, flavocoenzymes can catalyze one and two-electron transfer reactions.

In various critical metabolic durations involving the citric acid cycle, nitric oxide synthases, the mitochondrial port chain of the electron transport, catabolism of the branched-chain amino acids, and also redox homeostasis flavoenzymes are the key players. They also have important roles in apoptosis, DNA repair, chromatin remodeling, and protein folding (Joosten & van Berkel, 2007).

Vitamin B1 is not synthesized endogenously; it is an essential nutrient that must be provided through dietary intake. Flavocoenzyme metabolism's defects include inherit genetic disorders. These genetic disorders involve disorders of Vitamin B2 or flavocoenzyme transport and also involve the synthetic pathway enzymes of essential cofactors FAD and FMN (Balasubramaniam, Christodoulou, & Rahman, 2019).

Deficiency of Vitamin B2 leads to corneal vascularization and cheilosis (Lykstad & Sharma, 2019). Flavin homeostasis's impairment in humans may cause multisystem dysfunction involving anemia, cardiovascular disease, abnormal fetal development, and neuromuscular disorders. Vitamin B2 therapy may be beneficial in the deficiencies of the flavoenzymes deficiencies so early recognition is crucial (Balasubramaniam et al., 2019).

1.3 Vitamin B3 (Niacin)

Vitamin B3 is in the water-soluble vitamin of the B complex group. It is also known as Niacin. This vitamin is necessary for the cell's metabolism and functions (Hegyi, Schwartz, & Hegyi, 2004).

NAD, nicotinamide adenine dinucleotide phosphate (NADP+), nicotinamide adenine dinucleotide phosphate (NAD(P)H), and nicotinamide adenine dinucleotide phosphate (NADP+) are the cofactors obtained from Vitamin B3(Makarov, Trammell, & Migaud, 2019). NAD and NADP are important coenzymes. They have important roles in amino acid, protein, pyruvate, fatty acid, glycerol metabolism, high energy phosphate bonds synthesis, and glycolysis (Hegyi et al., 2004). The deficiency of Vitamin B3 can occur as Pellagra. Pellagra is known as 3D's: dementia, dermatitis, and diarrhea. Vitamin B3 deficiency is rare but it can be seen in alcoholics. Vitamin B3 also has a medication role. It can be used for dyslipidemia treatment (Banka et al., 2017).

1.4 Vitamin B5 (Pantothenic acid)

Vitamin B5 is in the water-soluble vitamin B complex group. This vitamin is known as Pantothenic Acid. It is found in various animals and plants. Milk, eggs, beef, chicken, vegetables, and whole grains contain Vitamin B5. Since many food sources involve Vitamin B5, its deficiency is not common. It is demanding to identify Vitamin B5 deficiency. A patient with a deficiency of Vitamin B5 usually has deficiencies in other nutrients (Sanvictores & Chauhan, 2020).

Vitamin B5 plays the precursor role in the synthesis of coenzyme A (CoA) so it affects CoA's metabolic pathway. CoA plays roles in numerous human biochemical reactions including intermediary metabolism, cell growth, and neurotransmitter synthesis (Leonardi & Jackowski, 2007).

If the pantothenate kinase 2 gene has a mutation, this may cause Vitamin B5 inadequency by reducing the activity of pantothenate kinase 2. Since adequate pantothenate kinase 2 activity deficiency is potentially able to lessen pantothenic acid conservation to CoA, CoA levels may reduce (Dezfouli et al., 2012).

1.5 Vitamin B6 (Pyridoxine)

Vitamin B6 is in the Vitamin B vitamin complex (Hellmann & Mooney, 2010). It is also known as Pyridoxine. This vitamin cannot be synthesized by humans thus Vitamin B6 must be taken with the diet. Fish, poultry animals, nuts, legumes, and potatoes are rich food sources of Pyridoxine.(Morris, Picciano, Jacques, & Selhub, 2008)

Vitamin B6 is an enzymatic co-factor that takes place in numerous biochemical reactions involving replacement reactions, cleavages of the aldols, transaminations, β-elimination, γ- elimination, and α-decarboxylations. Vitamin B6-related reactions exist in biosynthesis and degradation metabolic reactions of the amino acids, fatty acids, and sugars (Percudani & Peracchi, 2009). Vitamin B6 includes different pyridine derivatives and

these are; pyridoxine, pyridoxal, and pyridoxamine (Hellmann & Mooney, 2010). Vitamin B6 exists in six basic forms (Kall, 2003).

Pyridoxine takes part in; sodium-potassium balance, t red blood cell production, monoamine, transmitters, serotonin, dopamine, epinephrine, and norepinephrine production. It is the aromatic amino acid decarboxylase enzyme's cofactor (Hartvig, Lindner, Bjurling, Långström, & Tedroff, 1995). Pyridoxine deficiency causes nervous system symptoms. In the deficiency of Vitamin B6, hyperacusis, excessive irritability, and also impaired alertness are seen in humans. Vitamin B6' active form deficiency, causes convulsions and epileptic encephalopathy (Ahmad, Mirza, Qadeer, Nazim, & Vaid, 2013).

1.6 Vitamin B7 (Biotin)

Vitamin B7 also called vitamin H or biotin is in the Vitamin B complex vitamin group. One of the Vitamin B7 chains contains a ureido group that binds to avidin, and the other chain contains a valeric acid-added tetrahydrothiophene group. Vitamin B7 has eight stereoisomers, but the only biologically active isomer is the D-biotin form (Said, 2012)

For all living things Vitamin B7 is essential. No synthesization occurs in animal cells, but plant cells, yeast, and bacteria are able to synthesize Vitamin B7. The source of biotin in humans is dietary products, it is abundant in offal, while it is less in lean meat, cereals, and fruits (Leon-Del-Rio, 2019). Another source of biotin in humans is bacteria that live symbiotically in the intestines. The biotin produced by these bacteria is absorbed, but its role in the human body is not clear (Said, 2011). Biotin deficiency due to insufficient intake is very rare. Vitamin B7 deficiency develops in people with biotin metabolism disorders, long-term anticonvulsant use, continuous egg white feeding, and long-term total parenteral nutrition (Said, 1999).

Vitamin B7 is a coenzyme for carboxylases (Kim, 1997). Vitamin B7 also has important duties in chromatin structure, epigenetic regulation of genes, and cell signaling (Zempleni, Wijeratne, & Hassan, 2009).

1.7 Vitamin B9 (Folate)

Folate is the term that indicates water-soluble organic compound groups which are essential players in the synthesis of deoxyribonucleic acid (DNA). This vitamin is also called Vitamin B9. Folate's synthetic form is folic acid. In metabolic reactions, folate transforms into tetrahydrofolic acid. Tetrahydrofolic acid is in charge of various transfer/methylation reactions. These transfer/methylation reactions are needed for red blood cell maturation and are important for synthesizing nitrogenous bases in ribonucleic acid (RNA) and DNA. In the kidney and liver, there are small

reserve pools for folate. Folic acid deficiency can lead to macrocytic megaloblastic anemia. Macrocytic megaloblastic anemia usually arises from hemolytic anemia, chronic alcoholism, increased requirement during pregnancy, or malabsorption disorders. Folate is found naturally in some kind of food sources. Humans are not able to synthesize folate so it must be regularly ingested by consuming green leafy vegetables, lettuce, broccoli, meats, milk, and eggs (Merrell & McMurry, 2023)

In the case of folic acid deficiency, red blood cells are produced and their function is impaired. This leads to anemia. This is called "folic acid deficiency anemia" or megaloblastic-hyperchromic anemia. Tongue-tingling, tongue burning, inflamed red, "slippery" tongue, paleness, difficulty in breathing, inflammation of the mucous membranes (especially the intestinal mucosa), diarrhea, appetite, diseases of the cardiovascular system, increased bleeding tendency, fatigue, depression, weight loss, and irritability are the symptoms of folic acid deficiency (Khan & Jialal, 2018).

Fetuses who take very little folic acid from their mothers during pregnancy are at risk of nervous system development disorders. In the condition called spina bifida, part of the spine remains open and children become severely physically disabled. Folic acid deficiency rarely occurs, and this happens under certain conditions. These conditions are increased need for folic acid during pregnancy and lactation, alcohol, malnutrition, chronic inflammatory bowel diseases, Long-term antibiotic therapy, and treatment with methotrexate (Mahajan & Aundhakar, 2015).

1.8 Vitamin B12 (Cobalamin)

Vitamin B12 consists of a corrin ring, which is produced mainly by microorganisms and takes part in important reactions in the body. Vitamin B12 is also called cobalamin or corrinoid because it contains cobalt atoms in its structure (Aslan, 2005). In 1948, Dr. E. Lester Smith and colleagues isolated cobalamin from liver tissue and named it vitamin B12 (Smith et al., 1952). Cobalamin is named aquacobalamin, deoxyadenosylcobalamin, methylcobalamin, hydroxycobalamin, and cyanocobalamin according to the difference in axial ligands attached to cobalt. Serum contains the methylcobalamin form most while tissue contains the deoxyadenosyl cobalamin form most (Klee, 2000). The most durable form among them is cyanocobalamin (Leal, 2004).

Vitamin B12 is synthesized by molds, archaea, fungi, and bacteria and is found in soil, polluted water, and animal gut. A very small amount of vitamin B12 produced by some digestive system bacteria in the small intestine is absorbed here, but vitamin B12 which is synthesized in the large intestine cannot be used by the human body. Since the absorption in the small intestine is also very low, people should take vitamin B12 with food

(Baysal, 2004). Plants do not contain Vitamin B12. This vitamin is rich in animal livers, and also red meat, kidney, cheese, milk, oil, and seafood contain Vitamin B12 (Adkins & Lönnerdal, 2003).

Although there are sufficient Vitamin B12 levels in animal foods for the need of a person, Vitamin B12 deficiency is pervasive in the world, and its incidence varies between 3% and 40% (Kalem et al., 2016). Generally, people who do not have any gastrointestinal disease and have adequate animal food intake do not have vitamin B12 deficiency (Babior & Bunn, 1996).

In cases where transcobalamin I and transcobalamin II levels are increased, such as liver diseases, lymphoma, myeloproliferative diseases, and autoimmune diseases, deficiency findings may be observed even though the normal or high levels of vitamin B12. Although there is no vitamin B12 deficiency, in cases such as Multiple myeloma, folic acid deficiency, and excess vitamin C taking the serum levels of vitamin B12 can be measured as low (Nasreddine, Hwalla, Sibai, Hamzé, & Parent-Massin, 2006).

Vitamin B12 deficiency's clinical symptoms show the effects on different systems depending on the decrease in the vitamin level. In addition, in terms of disease severity Vitamin B12 deficiency may contain differences. Vitamin B12 deficiency symptoms occur on the degree of Vitamin B12 deficiency and may differ depending on the duration. In the case of mild deficiency of Vitamin B12, fatigue, and anemia occur. In case of moderate deficiency of Vitamin B12; some mild neurological findings such as glossitis, distal sensory dysfunction, and macrocytic anemia may occur.

In a severe deficiency of Vitamin B12, bone marrow suppression, neurological disorders, and cardiomyopathy risk may occur (Hunt, Harrington, & Robinson, 2014).

1.9 Vitamin C (Ascorbic Acid)

Vitamin C, is also known as ascorbic acid or L-ascorbic acid or ascorbic acid. This vitamin is a water-soluble vitamin and it is a monosaccharide derivative. Vitamin C's structure is similar to glucose and other six-carbon monosaccharides (Lykkesfeldt & Tveden-Nyborg, 2019). Humans cannot synthesize vitamin C because they do not have the enzyme 'L-gluconolactone oxidase'. Humans must take Vitamin C in their diet (Nishikimi, Fukuyama, Minoshima, Shimizu, & Yagi, 1994).

Vitamin C is rich in fresh fruits, fresh vegetables, and raw meat. Among the fruits, especially citrus fruits, and vegetables such as rosehip, spinach, pepper, and parsley, vegetables are the richest in terms of ascorbic acid (Rumsey & Levine, 1998). After ascorbic acid is taken into the body through food, it is absorbed into the blood by active transport in the gastro-

intestinal tract, especially by the oral mucosa and duodenum. Meanwhile, its concentration in the blood rises in a short time. It is transported to the tissues by the blood circulation and when the body's pool is saturated, the surplus is excreted unchanged through the kidneys through the urine (Griffiths & Lunec, 2001).

Symptoms of scurvy begin to appear; if the daily intake of 10 mg or less of vitamin C continues for a month (Zemel & Shi, 2000). Early symptoms of scurvy are fatigue, exhaustion, and gingivitis. As scurvy progresses, disruption in collagen synthesis and weakness in connective tissue occurs. In addition, depending on this situation, petechiae, ecchymosis, and purpura may be seen. Delays in wound and fracture healing may occur. If scurvy is not treated and progresses further; iron absorption decreases and iron deficiency anemia may occur (Li & Schellhorn, 2007). Moreover, as a result of consuming Vitamin C at doses of 1 g or higher per day for several weeks; fatigue, metabolic acidosis, kidney stone formation, oxaluria, gastrointestinal system disorders, renal tubular diseases, and infertility may occur (Jacob, 2002).

2. Fat-Soluble Vitamins

Vitamin A, Vitamin D, Vitamin E, and Vitamin K are in the fat-soluble vitamins group. These vitamins have important roles in a lot of physiological duration including vision, coagulation, bone metabolism, and immune system functions (Reddy & Jialal, 2021). These vitamins are laid up in tissue, so they are retained by the body for a longer time. (Traber, 2014).

2.1 Vitamin A

Vitamin A also called retinol is one of the fat-soluble vitamins. It is an alcohol derivative, found mainly in the liver in the form of all-trans or 11-cis isomers (Winterburn, Montgomery, Dryer, Conway, & Spector, 1982). Vitamin A's precursor is Beta-carotene. Beta-carotene is a member of the carotenoid family, a plant pigment. Beta-carotene scavenges superoxide radicals, it can also suppress singlet oxygen and interact directly with peroxy radicals, and exhibits antioxidant properties (Akkuş, 1995).

Vitamin A is responsible for growth. Vitamin A can be in 3 forms: aldehyde, alcohol, and acid. Its aldehyde form is retinal, its alcohol form is retinol and its acid form is retinoic acid. Vitamin A naturally exists in two forms and these structures are; retinol and 3-dehydroretinol. Mammals cannot actively synthesize vitamin A. Vitamin A, taken in the form of a provitamin in mammals, turns into its active form after ingestion (Mert, Bildik, Ertekin, & Dede, 1999) Vitamin A' hydrolyzation happens by intestinal and pancreatic enzymes and Vitamin A's emulsification happens by bile acids and dietary fats. After these processes, Vitamin A is absorbed in

the duodenum (Hodge & Taylor, 2023).

Some species have small amounts of carotenoids in the muscles. Plants with chlorophyll in their structure contain plenty of carotenoids. Carotenoids are provitamins of Vitamin A and are synthesized by plants. Retinol is found in the liver of mammals and living in saltwater fish; while 3-dehydroretinol is found in freshwater fish. Grass, milk, green, clover, colostrum, egg yolk, and butter contain plenty of Vitamin A (Mert et al., 1999).

Inadequate intake causes Vitamin A deficiency. This deficiency is a common health concern and it is associated with both morbidity and mortality. Vitamin A deficiency is most effective in young children and it occurs in impoverished areas of the world (Hombali, Solon, Venkatesh, Nair, & Peña-Rosas, 2019).

This vitamin takes part in immune function, visual pigment generation, and the maintenance of mucosal membranes. Vitamin A deficiency can cause night blindness because of the retinal rods' visual pigments' poor regeneration. If Vitamin A deficiency persists, degeneration in the rods occurs, and also xerophthalmia may develop (Saari, 2016).

2.2 Vitamin D

Vitamin D is also called the "sunshine vitamin" due to its production in the skin when sun exposure happens. Sun exposure is this vitamin's primary source. In the skin, 90% of Vitamin D is synthesized endogenously, the remaining part is taken with foods of animal and plant origin (Jeon & Shin, 2018). Vitamin D functions in many of the systems in the human body.

This vitamin behaves as a hormone and (or) hormone precursors (prohormone). Prohormones are precursors that turn into hormones in the body. Different vitamins are taken with food or supplements, but vitamin D is produced when our skin is exposed to the sun's UV rays (Ellison & Moran, 2021). The structure of vitamin D is composed of three solid steroid rings that are the same as cholesterol and steroid hormones. Vitamin D belongs to a subclass of the steroid family called 'secosteroids' (Subramanian et al., 2022).

Vitamin D has two forms and these are vitamin D3 and D2. Vitamin D3's synthesis is in the skin. This synthesis happens endogenously. High concentrations of Vitamin D3 exist in cod liver oil and fish oil. Vitamin D2 exists in yeast and fungi and this form is synthesized from ergosterol. After Vitamin D3's formation is completed, it leaves the cutaneous tissue and enters the circulation. Then. In the liver; Vitamin D's metabolization to 25-hydroxy vitamin D by 25-hydroxylase occurs. Then, in the kidney; 1,25-dihydroxy vitamin D occurs. Vitamin D and its metabolites are ina-

ctivated by 24-hydroxylase and their excretion occurs via bile (Charoenngam, Shirvani, & Holick, 2019).

Vitamin D is important for bone metabolism and calcium homeostasis. In children, a deficiency of Vitamin D may lead to osteomalacia and rickets. In adults, Vitamin D deficiency may cause osteomalacia. In the 1930s milk was fortified with Vitamin D and due to this situation effectiveness in eradicating rickets was successful. But subclinical Vitamin D deficiency is still an important problem in the world. The subclinic Vitamin D deficiency is related to fragility fractures and osteoporosis. Moreover, this vitamin is also associated with cancer, cardiovascular diseases, depression, and diabetes (Sizar, Khare, Goyal, Bansal, & Givler, 2021).

2.3 Vitamin E

Vitamin E is a fat-soluble vitamin(Kemnic & Coleman, 2022). Alpha-tocopherol form of Vitamin E is the need for humans. In the small intestine; Vitamin E's all forms are absorbed. Only alpha-tocopherol is metabolized in the liver. Then, the remaining vitamin E forms are removed and excreted by the liver (Suzuki, Kume, & Herbas, 2018).

Vitamin E has immunomodulator, antioxidant, and antiplatelet effects. Vitamin E takes part in the decrease of prostaglandin E2 and lipid peroxidase. This vitamin has a role in the LDL's oxidative changes' presentation. It inhibits protein kinase C and platelet adhesion (Kemnic & Coleman, 2022).

In developing countries, Vitamin E deficiency occurs due to insufficient intake but it is unlikely in developed countries. In developed countries, deficiency of Vitamin E occurs through Crohn's disease, abetalipoproteinemia, chronic cholestatic hepatobiliary, short-bowel syndrome, and cystic fibrosis (Kemnic & Coleman, 2022)

2.4 Vitamin K

Vitamin K is an effective organic compound in the synthesis of coagulation proteins' active forms. This vitamin is essential and of vital importance in the normal coagulation process (Ferland, 1998). In 1929, Danish scientists Henrik Dam and Edward Adelbert Doisy, determined the bleeding in the intestines, subcutaneous fat layer, and brain of chicks which were fed by an artificial diet. In 1943; as a result of their work, they were awarded the Nobel Prize for isolating the natural form of vitamin K (Fieser, 1939).

Vitamin K stores in the body are sufficient for one week in healthy individuals in the absence of dietary intake (Costakos, Greer, Love, Dahlen, & Suttie, 2003). The main source of this vitamin is diet. Leafy green vege-

tables, cauliflower, cabbage, green peas, beans, green tea, olives, olive oil, soybean seeds, and dairy products are rich resources for Vitamin K (Sarah L Booth & Suttie, 1998).

Vitamin K and its dependent proteins and may play roles in inflammation, energy metabolism, and regulation of calcification (Sarah L. Booth, 2009).

REFERENCES

Adkins, Y., & Lönnerdal, B. (2003). Potential host-defense role of a human milk vitamin B-12–binding protein, haptocorrin, in the gastrointestinal tract of breastfed infants, as assessed with porcine haptocorrin in vitro. *The American journal of clinical nutrition, 77*(5), 1234-1240.

Ahmad, I., Mirza, T., Qadeer, K., Nazim, U., & Vaid, F. H. (2013). Vitamin [B. sub. 6]: deficiency diseases and methods of analysis. *Pakistan journal of pharmaceutical sciences, 26*(5), 1057-1070.

Akkuş, İ. (1995). Serbest radikaller ve fizyopatolojik etkileri. *Mimoza Yayınları, Konya, 1*, 57-63.

Aslan, D. (2005). Tietz Klinik Kimyada Temel İlkeler. *Beşinci baskıdan çeviri. Ankara: Palme Yayıncılık*.

Babior, B., & Bunn, H. (1996). Megaloblastic Anemias. Kurt JI, Eugene B, Jean DW et al. *Harrison's Principles of Internal Medicine. Thirteenth Edition. New York*, 1726-1732.

Balasubramaniam, S., Christodoulou, J., & Rahman, S. (2019). Disorders of riboflavin metabolism. *Journal of inherited metabolic disease, 42*(4), 608-619.

Banka, S. S., Thachil, R., Levine, A., Lin, H., Kaafarani, H., & Lee, J. (2017). Randomized controlled trial of different aspirin regimens for reduction of niacin-induced flushing. *American Journal of Health-System Pharmacy, 74*

Baysal, A. (2004). *Beslenme*: Hatiboğlu Yayınevi.

Booth, S. L. (2009). Roles for Vitamin K Beyond Coagulation. *Annual review of nutrition, 29*(1), 89-110. doi: 10.1146/annurev-nutr-080508-141217

Booth, S. L., & Suttie, J. (1998). Dietary intake and adequacy of vitamin K. *The Journal of nutrition, 128*(5), 785-788.

Charoenngam, N., Shirvani, A., & Holick, M. F. (2019). Vitamin D for skeletal and non-skeletal health: What we should know. *Journal of clinical orthopaedics and trauma, 10*(6), 1082-1093.

Costakos, D. T., Greer, F. R., Love, L. A., Dahlen, L. R., & Suttie, J. W. (2003). Vitamin K prophylaxis for premature infants: 1 mg versus 0.5 mg. *American journal of perinatology, 20*(08), 485-490.

Dezfouli, M. A., Jaberi, E., Alavi, A., Rezvani, M., Shahidi, G., Elahi, E., & Rohani, M. (2012). Pantothenate kinase 2 mutation with eye-of-the-tiger sign on magnetic resonance imaging in three siblings. *Iranian Journal of Neurology, 11*(4), 155.

Ellison, D. L., & Moran, H. R. (2021). Vitamin D: vitamin or hormone? *Nursing Clinics, 56*(1), 47-57.

Ferland, G. (1998). The vitamin K-dependent proteins: an update. *Nutrition reviews, 56*(8), 223-230.

Fieser, L. F. (1939). Synthesis of vitamin K1. *Journal of the American Chemical Society, 61*(12), 3467-3475.

Griffiths, H., & Lunec, J. (2001). Ascorbic acid in the 21st century–more than a simple antioxidant. *Environmental Toxicology and Pharmacology, 10*(4), 173-182.

Hartvig, P., Lindner, K., Bjurling, P., Långström, B., & Tedroff, J. (1995). Pyridoxine effect on synthesis rate of serotonin in the monkey brain measured with positron emission tomography. *Journal of Neural Transmission/General Section JNT, 102*, 91-97.

Hegyi, J., Schwartz, R. A., & Hegyi, V. (2004). Pellagra: dermatitis, dementia, and diarrhea. *International journal of dermatology, 43*(1), 1-5.

Hellmann, H., & Mooney, S. (2010). Vitamin B6: a molecule for human health? *Molecules, 15*(1), 442-459.

Hodge, C., & Taylor, C. (2023). Vitamin A Deficiency.[Updated 2023 Jan 2]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*.

Hombali, A. S., Solon, J. A., Venkatesh, B. T., Nair, N. S., & Peña-Rosas, J. P. (2019). Fortification of staple foods with vitamin A for vitamin A deficiency. *Cochrane Database of Systematic Reviews*(5).

Hunt, A., Harrington, D., & Robinson, S. (2014). Vitamin B12 deficiency. *Bmj, 349*.

Jacob, R. (2002). i Sotoudeh G., Vitamin C function and status in chronic disease. *Nutrition in Clinical Care, 5*, 66-74.

Jeon, S.-M., & Shin, E.-A. (2018). Exploring vitamin D metabolism and function in cancer. *Experimental & molecular medicine, 50*(4), 1-14.

Joosten, V., & van Berkel, W. J. (2007). Flavoenzymes. *Current opinion in chemical biology, 11*(2), 195-202.

Kalem, P., Benli, A. R., Koroglu, M., Benli, N. C., Koyuncu, M., Cesur, O., & Dane, P. B. K. (2016). The effect of ferritin, vitamin B12 and folic acid on pregnancy outcomes. *Int J Clin Exp Med, 9*(11), 22413-22417.

Kall, M. A. (2003). Determination of total vitamin B6 in foods by isocratic HPLC: a comparison with microbiological analysis. *Food Chemistry, 82*(2), 315-327.

Kemnic, T., & Coleman, M. (2022). Vitamin E Deficiency. 2021 Jul 18. *StatPearls. Treasure Island (FL): StatPearls Publishing*.

Khan, K. M., & Jialal, I. (2018). Folic acid deficiency.

Kim, K.-H. (1997). Regulation of mammalian acetyl-coenzyme A carboxylase. *Annual review of nutrition, 17*(1), 77-99.

Klee, G. G. (2000). Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. *Clinical chemistry, 46*(8), 1277-1283.

Latt, N., & Dore, G. (2014). Thiamine in the treatment of W ernicke encephalopathy in patients with alcohol use disorders. *Internal medicine journal, 44*(9), 911-915.

Leal, N. A. (2004). *B12 metabolism in humans*: University of Florida.

Leon-Del-Rio, A. (2019). Biotin in metabolism, gene expression, and human disease. *Journal of inherited metabolic disease, 42*(4), 647-654.

Leonardi, R., & Jackowski, S. (2007). Biosynthesis of pantothenic acid and coenzyme A. *EcoSal Plus, 2*(2).

Li, Y., & Schellhorn, H. E. (2007). New developments and novel therapeutic perspectives for vitamin C. *The Journal of nutrition, 137*(10), 2171-2184.

Lykkesfeldt, J., & Tveden-Nyborg, P. (2019). The pharmacokinetics of vitamin C. *Nutrients, 11*(10), 2412.

Lykstad, J., & Sharma, S. (2019). Biochemistry, water soluble vitamins.

Mahajan, S. K., & Aundhakar, S. C. (2015). A study of the prevalence of serum vitamin B12 and folic acid deficiency in Western Maharashtra. *Journal of family medicine and primary care, 4*(1), 64.

Makarov, M. V., Trammell, S. A., & Migaud, M. E. (2019). The chemistry of the vitamin B3 metabolome. *Biochemical Society Transactions, 47*(1), 131-147.

Manzetti, S., Zhang, J., & van der Spoel, D. (2014). Thiamin function, metabolism, uptake, and transport. *Biochemistry, 53*(5), 821-835.

Merrell, B., & McMurry, J. (2023). Folic Acid.[Updated 2022 Dec 21]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*.

Mert, N., Bildik, A., Ertekin, A., & Dede, S. (1999). Biyokimya, YY Ü. *Veteriner Fakültesi Yayını, Van*, 162-164.

Morris, M. S., Picciano, M. F., Jacques, P. F., & Selhub, J. (2008). Plasma pyridoxal 5′-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003–2004. *The American journal of clinical nutrition, 87*(5), 1446-1454.

Nasreddine, L., Hwalla, N., Sibai, A., Hamzé, M., & Parent-Massin, D. (2006). Food consumption patterns in an adult urban population in Beirut, Lebanon. *Public health nutrition, 9*(2), 194-203.

Nishikimi, M., Fukuyama, R., Minoshima, S., Shimizu, N., & Yagi, K. (1994). Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *Journal of biological chemistry, 269*(18), 13685-13688.

Percudani, R., & Peracchi, A. (2009). Address: Department of Biochemistry and Molecular Biology, University of Parma, 43100 Parma, Italy. *BMC Bioinformatics, 10*, 273.

Reddy, P., & Jialal, I. (2021). Biochemistry, Fat Soluble Vitamins.[Updated 2020 Sep 21]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www. ncbi. nlm. nih. gov/books/NBK534869*.

Rumsey, S. C., & Levine, M. (1998). Absorption, transport, and disposition of ascorbic acid in humans. *The Journal of Nutritional Biochemistry, 9*(3), 116-130.

Saari, J. C. (2016). Vitamin A and vision. *The Biochemistry of Retinoid Signaling II: The Physiology of Vitamin A-Uptake, Transport, Metabolism and Signaling*, 231-259.

Said, H. M. (1999). Biotin bioavailability and estimated average requirement: why bother? (Vol. 69, pp. 352-353): Oxford University Press.

Said, H. M. (2011). Intestinal absorption of water-soluble vitamins in health and disease. *Biochemical Journal, 437*(3), 357-372.

Said, H. M. (2012). Biotin: biochemical, physiological, and clinical aspects. *Water Soluble Vitamins: Clinical Research and Future Application*, 1-19.

Sanvictores, T., & Chauhan, S. (2020). Vitamin B5 (pantothenic acid).

Sica, D. A. (2007). Loop diuretic therapy, thiamine balance, and heart failure. *Congestive heart failure, 13*(4), 244-247.

Sizar, O., Khare, S., Goyal, A., Bansal, P., & Givler, A. (2021). Vitamin D Deficiency.[Updated 2021 Jul 21]. *StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*.

Smith, E. L., Fantes, K., Ball, S., Waller, J., Emery, W., Anslow, W., & Walker, A. (1952). B12 vitamins (cobalamins). 1. Vitamins B12c and B12d. *Biochemical Journal, 52*(3), 389.

Subramanian, S., Rhodes, J. M., Taylor, J. M., Milan, A. M., Lane, S., Hewison, M., . . . Nitchingham, D. (2022). Vitamin D, vitamin D—binding protein, free vitamin D and COVID-19 mortality in hospitalized patients. *The American journal of clinical nutrition, 115*(5), 1367-1377.

Suzuki, H., Kume, A., & Herbas, M. S. (2018). The potential of vitamin E deficiency, induced by inhibition of α-tocopherol efflux, in murine malaria infection. *International Journal of Molecular Sciences, 20*(1), 64.

Traber, M. G. (2014). Vitamin E inadequacy in humans: causes and consequences. *Advances in nutrition, 5*(5), 503-514.

Winterburn, P., Montgomery, R., Dryer, R., Conway, T., & Spector, A. (1982). Biochemistry: a Case-Oriented Approach: Wiley Online Library.

Yaşar, H., & Melek, S. (2014). Beslenme ve besinler. *Hatipoğlu Yayınları Ankara, 171*(8), 17-43.

Zemel, M. B., & Shi, H. (2000). Pro-opiomelanocortin (POMC) deficiency and peripheral melanocortins in obesity. *Nutrition reviews, 58*(6), 177-180.

Zempleni, J., Wijeratne, S. S., & Hassan, Y. I. (2009). Biotin. *Biofactors, 35*(1), 36-46.

CHAPTER 10

PROBIOTICS IN THE MAINTENANCE OF ORAL HEALTH

Ezgi Cahide AYDAŞ[1], Nuran BAYRAMOV[2], Şebnem ERÇALIK YALÇINKAYA[3]

1 Res. Assist. Marmara University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology ORCID ID: 0000-0002-9894-8750
2 Asst. Prof., Istanbul Aydın University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology ORCID ID: 0000-0002-8217-8819
3 Prof., Marmara University Faculty of Dentistry, Department of Oral and Maxillofacial Radiology ORCID ID: 0000-0003-2924-1935

History of Probiotics

The history of probiotics parallels the evolution of humanity and may be traced back to ancient times, almost 10,000 years ago, thanks to current sophisticated techniques (Ozen and Dinleyici, 2015). The modern history of probiotics began in the early 1900s, when Dr. Metnikov in Russia found that some people in Bulgaria lived longer, without pain or disease (Gasbarrini *et al.,* 2016). His explanation for their healthy longevity was their diet. Yoghurt, sourdough, bread and buttermilk were part of their diet. Dr. Metnikoff found out that these fermented foods involved beneficial, friendly bacteria capable of digesting decaying food and releasing nutrient-rich by-products that destroyed the oral malodor. These friendly bacteria, which prevented the potential pathogenic agents from causing illness, became known as probiotic bacteria (Bhardwaj and Bhardwaj, 2012). German scientist Werner Kollath introduced the word probiotic (meaning "for life") to describe "substances essential to healthy life development". It has been used by Lilly and Stillwell (1965) as material secreted by one organism and it stimulates the growth of another. In 1992, Fuller described probiotics as "live microbial feed supplements that beneficially affect host animals by improving gut microbial balance" (Gasbarrini *et al., 2015*). The definition of "probiotic" changed numbers of time until 2002, when the WHO and the Food and Agriculture Organization (FAO) developed a new definition that is generally recognized as "a live microbial agent that, when ingested at an adequate dose, may have a beneficial effect on the host" (Zhang *et al.,* 2002). The International Life Science Institute (ILSI) Europe proposes a definition that a probiotic is "a viable microbial food supplement that beneficially affects host health" (Salminen *et al*., 1998). Despite some variations, all definitions require probiotics to be live and to exert health benefits (Vrese and Schrezenmeier, 2008). The first probiotic species found in research was *Lactobacillus acidophilus* by Hull and colleagues in 1984, *Bifidobacterium* was the second probiotic species found by Holcomb and colleagues in 1991 (Bhardwaj and Bhardwaj, 2012). The probiotic strains that belong to the genera Streptococcus, Lactobacillus and Bifidobacterium are the most commonly used types. Species of Lactobacillus include *L. casei, L. acidophilus, L. salivarius, L. lactis, L. helveticus, L. plantarum, L. bulgaricus, L. reuteri, L. rhamnosus, L. fermentum, L. del-brueckii, L. johnsonii.* The strains of Bifidobacterium include *Bifidobacterium longum, Bifidobacterium infantis* and *Bifidobacterium bifidum. Other strains include Enterococcus faecium, Enterococcus faecalis, Saccharomyces boulardii* and *Streptococcus thermophilus* (Mishra *et al.,* 2020).

Oral cavity and microbiome

The oral cavity is one of the most significant entry points to the human body. Food enters the mouth through chewing and saliva as it moves to

the stomach and intestines. Air passes through the nose and mouth before entering the trachea and lungs (Dewhirst *et al,* 2010). There are many different microbial habitats in the oral cavity, including the teeth, gingival sulcus, attached gingiva, hard palate, soft palate, tongue, lip and cheek. Adjacent to the oral cavity are the eustachian tube, middle ear, nasal passages, sinuses, tonsils, pharynx, oesophagus, trachea and lungs. Oral microbiota, oral microflora, and more current oral microbiome are terms used to describe the microorganisms found on or in the human oral cavity and its adjacent extensions, up to the distal oesophagus (Dewhirst *et al.,* 2010). The term "microbiome" is used by Joshua Lederberg "to describe the ecological community of commensals, symbionts, and pathogens that literally share our body space and are largely neglected as factors in health and disease" (Lederberg and McCray, 2001). The oral microbiome is composed of roughly 700 usually found phylotypes, of which about half are likely to be present in any individual at any given time (Palmer, 2014). Microorganisms that colonize one area of the oral cavity have a significant probability of spreading to adjacent sites on adjacent epithelial surfaces. These microorganisms are nourished by gingival crevicular fluid and saliva. This enables the maintenance of the oral ecosystem. The general health and dietary habits of the host have a significant effect on the oral microbiome. The oral microenvironment is influenced by a wide range of pH, saliva and crevicular fluids, nutrient availability, abutment and nonabutment surfaces. (Chugh *et al*, 2020). A number of oral diseases including caries, periodontitis, endodontic infections, alveolar osteitis and tonsillitis have been associated with oral microorganisms. There is increasing evidence linking oral bacteria to many different types of systemic diseases, such as diabetes, cardiovascular disease, stroke, pneumonia and preterm birth (Dewhirst *et al,* 2010).

Mechanisms of probiotic action

The mechanism of probiotics as a therapeutic tool for the control of oral and dental pathologies may perform direct or indirect action in the oral environment according to earlier research in the gastrointestinal tract. There are direct interactions inside dental plaque (colonisation resistance). Mechanism of direct interaction could include prevention of plaque biofilm formation by competing for binding sites on host tissue and other bacteria, and through competition for nutrients. Probiotic species may also produce antimicrobial compounds which are anti-adhesive molecules, hydrogen peroxide, organic acids, peptides and bacteriocins in this process. These compounds inhibit other oral microorganisms. Probiotics have also indirect effects on the oral cavity by affecting the function of both the innate and adaptive immune systems. Lactobacillus can affect immunocompetent cells, including macrophages and T cells and they may cause an alteration

in cytokine production and subsequent effects on overall immunity. Some probiotic species are shown to enhance production of mucin and barrier function, to up-regulate host defence peptides and to promote wound healing and angiogenesis, in addition to the modulation of the immune response. (Meurman, 2005).

Probiotics and oral health

Probiotic bacteria are a growing dental research area (Saiz *et al,* 2021). Oral probiotics are beneficial and safe, favourably affect the oral microbiota, and provide benefits to the oral ecosystem in caries, periodontal disease, endodontics, orthodontics, halitosis, oral cancer, oral candidiasis, recurrent aphthous stomatitis and in managing oral mucositis associated with cancer therapy.

Probiotics and dental caries

Dental caries is considered a multifactorial disease caused by an ecological imbalance between hard dental tissue inorganic components and biofilms (Conrads and About, 2018). Typically, the human microbiota is balanced -symbiotic- with its host, the human body but the use of antibiotics appears to result in serious negative effects such as pathogen resistance, damage to the desired oral microbiome and increased oral cavity susceptibility to caries (Qui *et al.,* 2020). Consequently, probiotic therapy has gained popularity in recent years as a new and preventative technique (Amargianitakis *et al*., 2021). The reason of probiotics use in the treatment of caries is that the potential of probiotic microorganisms to replace cariogenic microorganisms and colonize the oral cavity (Voidarou *et al.,* 2020). However additional studies in vivo and in vitro are required to understand how oral probiotics work and their interaction with host cells and the host microbiome. Probiotics should be used as a preventive reatment rather than as a caries treatment within this period (Amargianitakis *et al.,* 2021).

Probiotics in periodontal health and periodontal diseases

Periodontal diseases are chronic inflammatory diseases that destroy periodontal tissues and the bone that support the teeth. The most common types are gingivitis and periodontitis (Zhang *et al.*, 2022). Periodontitis is caused by periodontopathogenic bacteria (*Aggregatibacter actinomycetancomitans, Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*), which are organised in biofilms at the subgingival and supragingival levels in susceptible hosts (Hajishengallis, 2015). The mechanical removal of the pathogenic biofilm and the use of antiseptics or antibiotics are part of the treatment (Socransky, 2013). The main aim of this treatment is preventing the recolonization of harmful microorganisms

(Saiz *et al.,* 2021). In addition to mechanical therapy, probiotics may alter and occupy the subgingival niche that is susceptible to recolonization by pathogenic bacteria, allowing a new equilibrium with the oral environment (Nadkerny, 2015). Probiotics may also modify the bacterial composition of the biofilm adjacent to implants (Hallström, 2016). They could act as oral immunomodulators, reducing proinflammatory cytokines TNF-α, IL-1β, and matrix metalloproteinases (MMPs) and increasing TGF-β1, IL-10 and tissue inhibitor of metalloproteinases (TIMPs) (Jasberg, 2018).

Probiotics in endodontics

Periradicular pathology is destruction and inflammation of the periradicular tissues, usually as a result of pulpal inflammation or necrosis, with transfer of necrotic by-products and inflammatory mediators through the apical foramen to cause a periapical lesion. Insufficent elimination of the microorganisms and toxins may result in prolonged inflammation and poor healing. More than 90% of the microbiota in root canals with necrotic pulp and diseased periapical areas are obligate anaerobes, usually belonging to the genera *Porphyromonas, Fusobacterium, Peptostreptococcus, Eubacterium* and Prevotella. (Chaurasiya *et al*., 2016).

It is challenging to achieve total sterility inside any root canal system due to complex root canal morphology, such as the existence of multiple accessory and lateral canals. However, studies have shown that at least considerable bioload reduction can be achieved (Bohora and Kokate, 2017). A new concept in endodontics is bacteriotherapy, which replaces pathogenic bacteria with healthy bacteria and normal flora. The function of probiotics in endodontic therapy has not been broadly evaluated. Nevertheless, the potential antimicrobial activity of probiotics against endodontic pathogens has been demonstrated several in vitro studies such as there is the antimicrobial activity against E. faecalis and C. albicans of commercial probiotics composed of different species of Lactobacillus, Bifidobacterium and Streptococci. Studies have also revealed that even the cell-free supernatant containing metabolic by-products of the probiotic samples could potentially inhibit the growth of endodontic pathogens, and thus possibly replace the probiotic microorganisms themselves (Pradeep *et al*., 2017). In addition to eradicating endodontic pathogens, probiotic microflora may also prevent recolonisation of the root canal system, thereby reducing the likelihood of endodontic failure and improving the long-term success of root canal treatment. The literature on probiotics cannot be generalised as it varies according to frequency, dosage, strain, experimental models and methods used. Although probiotic support appears promising, in vitro and in vivo studies are essential to evaluate the efficacy of probiotic use endodontics, maintaining the periapical health and to derive evidence-based treatment outcomes.

Probiotics in orthodontics

Orthodontic tooth movement is provided by applying continued, controlled mechanical forces to the tooth, which casuses remodelling of the alveolar bone by creating compression and tension zones in the periodontal ligament and alveolar bone (Gameiro *et al.,* 2008). The bone remodelling mechanism resulting in tooth movement can be explained by the transient inflammatory process by leukocytes and bone cells (Taddei *et al.,* 2012). Bone resorption and deposition occurs from the designed action of these bone cells, which is caused by stress and mechanical loading (Hadjidakis and Androulakis, 2006). Orthodontic appliances affect the oral microflora. For instance, they increase the number of periodontitis- and caries-related bacteria in plaque and saliva. (Wang *et al.,* 2019 Probiotics may be helpful as supplements to prevent bacterial colonization and make dental biofilm less pathogenic in patients with fixed or removable orthodontic appliances (Saiz et al., 2021). The reduction of osteoclastic activity by probiotics in a murine orthodontic movement model has also been demonstrated to have positive effects on oral health (Pazzini *et al*., 2017).

Probiotics and halitosis

Halitosis is a condition in which an unpleasant odour, caused by oral microorganisms, is emitted from a person's mouth when they breathe out. This can cause discomfort to others. It is a widespread condition, with prevalence rates around the world ranging from around 5% to 65.9% (Lee *et al*, 2021). The aetiology of halitosis is diverse and can result from poor oral hygiene, periodontitis, tobacco use, the consumption of certain foods, espiratory infections, genetic predisposition, dry mouth and oral microbial dysbiosis. Volatile sulphur compounds (VCSs) that cause bad breath include hydrogen sulphide, methyl mercaptan and dimethylsulphide. These are produced by oral bacteria in the mouth cavity during their metabolic activity. Several factors contribute to the production of these compounds, including the predominance of Gram-negative anaerobes, the alkaline pH of saliva, the low redox potential and the presence of sulphur substrates like cysteine and methionine (Saiz *et al.,* 20-21). Findings from previous studies suggest that probiotic consumption of *S. salivarius* (Benic et al., 2019), *L. reuteri, L. salivarius* (Penala *et al.,* 2016) and *W. cibaria* (Lee *et al.,* 2021) may complement mechanical oral care to control halitosis.

Nevertheless, the available evidence is quantitatively and qualitatively inadequate to make further recommendations regarding dosing strategies and pretreatment (Chugh *et al.,* 2020).

Probiotics and oral cancer

One of the most prevalent malignancies in the world is oral cancer.

Each year, there are around 350,000 new cases recorded (Vigneswaran and Williams, 2014). Oral cancer typically occurs on the lips, tongue, buccal mucosa, and mouth floor. Alcohol and cigarette use have been demonstrated to be major etiological risk factors (Santos *et al*., 2016). The mortality rate of oral cancer continues to increase because there is the lack of effective treatment. Secondary oral carcinomas may reappear among treated patients, leading to a poor survival rate of only 50 to 60 % (Zini *et al*., 2010). Health benefits associated with probiotic consumption include producing antimicrobial and antiadhesive substances against pathogens to help modulate the immune system (Collado *et al.,* 2010). Numerous in vivo animal studies have been conducted, although there is no direct evidence for the use of probiotics to prevent or treat malignant cancers in humans (Kumar, 2022). These have shown that probiotics are able to reduce the incidence of tumour formation and aberrant crypt formation. They also suppress bacterial enzyme activities and reduce DNA damage. (Kumar *et al,* 2010). This suggests that they may have potential as chemoprotectants, but further research will be needed before their beneficial effects on human cancer prevention can be quantified (Kamaluddin *et al.,* 2020).

Probiotics in oral wound healing and oral mucositis associated with cancer therapy

Oral mucosal wound healing involves multiple inflammatory mediators/molecules and oral microbiome, age, diet all have an effect on wound healing of oral mucosal (Saiz *et al,* 2021). Cancer therapy interferes with the quality of saliva and decreases salivary glycoproteins which coat and protect the oral mucosa from microbial attachment and irritation. It also induces oral mucositis, which may favour the occurrence of opportunistic infections, severe pain, fever, bleeding, dysphagia, anorexia and dysgeusia (Lalla *et al.,* 2019). Oral mucositis is a common side effect of cancer therapy, such as chemotherapy, radiation therapy, concurrent chemo-radiation therapy and haematopoietic stem cell transplantation (Elad *et al.,* 2020). Probiotics do not appear to have direct effects on oral wound healing, but they may help to lessen oral mucositis and improve quality of life in patients receiving cancer therapy. Some clinical trials have investigated the role of topical probiotics in oral wound healing and in the treatment of oral mucositis in patients receiving chemoradiation therapy (Saiz *et al.,* 2021). The efficacy of probiotics in preventing and alleviating cancer therapy-induced oral mucositis was demonstrated by Liu et al. Future research is needed to investigate the appropriate dosage and possible effects of different species and strains of bacteria in cancer patients (Liu *et al.,* 2022).

Probiotics in oral candidiasis

Oral candidiasis is an opportunistic infection of the oral mucosa, sus-

tained by different yeasts of the genus Candida that overgrow and become virulent under specific conditions of oral dysbiosis, secondary to changes in the diversity and composition of the oral microbiome driven by predisposing systemic and local factors (Contaldo *et al*., 20-22). It is reasonable to approach them by administering beneficial bacteria to re-establish a local flora that can compete with Candida colonisation and infection, since infections sustained by Candida species exploit local dysbiosis, both transient, as after prolonged treatment with broad-spectrum antibiotics, and chronic, as in immunodeficiency (Mundula *et al.,* 2019). The most commonly used probiotics are *Bifidobacterium spp, Lactobacillus spp, Saccharomyces spp* and *Bacillus spp* (Contaldo *et al.,* 2022). Increasingly consistent and clear evidence supports the advantages of probiotics in the treatment of fungal infections. Although there are few reports in the literature on their use in human oral candidiasis, the good effect of this alternative treatment is intuitive (Archambault and Dongari-Bagtzoglou, 2022).

Probiotics in recurrent aphthous stomatitis

Recurrent aphthous stomatitis (RAS) is characterized by round or ellipsoid recurrent ulcers that can appear anywhere in the oral cavity and they are most prevalent in non-keratinized epithelial areas, including buccal, labial mucosae and the lingual margin (Koybasi *et al*, 2006). Although probiotics alone are effective in reducing oral pain, they are ineffective in reducing ulcer severity. Combining probiotics with anaesthetic antiseptic gel or steroids are more effective than anaesthetic antiseptic gel or steroids alone in reducing oral pain and ulcer severity in RAS patients (Cheng *et al*, 2020). Probiotics have shown promise in the treatment of recurrent aphthous stomatitis (RAS). However, more well-designed clinical trials are needed due to the limited amount of data and the quality of the studies (Aggour *et al.,* 2021).

Conclusion

In light of recent in vitro and in vivo researches, probiotics have been shown to have a significant role in the prevention of a wide range of oral health problems, from dental caries to halitosis and periodontal disease. Studies declare a direct connection between the probiotics and the alteration of the oral microenvironment, which is the oral pathogens inhibited by the probiotics. In addition, choosing the most proper probiotic for improving oral health requires more study. Before consalidating probiotics into daily oral health regimes, more data are needed to analyze the mechanisms of probiotic effects on oral health, and continuing clinical trials are required.

REFERENCES

Aggour, R. L., Mahmoud, S. H., & Abdelwhab, A. (2021). Evaluation of the effect of probiotic lozenges in the treatment of recurrent aphthous stomatitis: a randomized, controlled clinical trial. *Clin Oral Investig*. 25(4):2151-2158.

Amargianitakis, M., Antoniadou, M., Rahiotis, C., & Varzakas, T. (2021). Probiotics, Prebiotics, Synbiotics and Dental Caries. New Perspectives, Suggestions, and Patient Coaching Approach for a Cavity-Free Mouth. *Applied Sciences*. 11(12):5472.

Anusha, R. L., Umar, D., Basheer, B., & Baroudi, K. (2015). The magic of magic bugs in oral cavity: Probiotics. *J Adv Pharm Technol Res*. 6(2):43-47.

Archambault, L. S., & Dongari-Bagtzoglou, A. (2022). Probiotics for Oral Candidiasis: Critical Appraisal of the Evidence and a Path Forward. *Front Oral Health*. 3:880746.

Benic, G. Z., Farella, M., Morgan, X. C., Viswam, J., Heng, N. C., Cannon, R. D., & Mei, L. (2019). Oral probiotics reduce halitosis in patients wearing orthodontic braces: a randomized, triple-blind, placebo-controlled trial. *Journal of breath research*, *13*(3), 036010.

Bhardwaj, A. K., & Bhardwaj, S. V. (2012). Role of Probiotics in Dental Caries and Periodontal Disease. *Archives of Clinical and Experimental Surgery, 1*, 45-49.

Bohora, A., & Kokate, S. (2017). Evaluation of the Role of Probiotics in Endodontic Treatment: A Preliminary Study. *Journal of International Society of Preventive & Community Dentistry*, *7*(1), 46–51.

Pradeep, C., Khedkar, S. U., Karde P. A., Patil, A. G., & Dani, N. H. (2017). Potential antibacterial activity of probiotics against endodontic pathogens: An in-vitro pilot study. *International Journal of Scientific Research*. 6(9), 23-25.

Chaurasiya, S., Yadav, G., Tripathi A. M., & Dhinsa, K. (2016). Endodontic Failures and its Management: A Review. *Int J Oral Health Med Res.* 2(5):144-148.

Cheng, B., Zeng, X., Liu, S., Zou, J., & Wang, Y. (2020). The efficacy of probiotics in management of recurrent aphthous stomatitis: a systematic review and meta-analysis. *Scientific reports*, *10*(1), 21181.

Chugh, P., Dutt, R., Sharma, A., Bhagat, N., & Dhar, M.S. (2020). A critical appraisal of the effects of probiotics on oral health. *Journal of Functional Foods, 70*, 103985.

Collado, M. C., Gueimonde, M., & Salminen, S. (2010). Probiotics in adhesion of pathogens: mechanism of action. Bioactive Foods in Promoting Health. 1st ed. Elsevier, 353.

Conrads, G., About, I. (2018). Pathophysiology of Dental Caries. *Monogr Oral Sci*. 27:1-10.

Contaldo, M., Di Stasio, D., Romano, A., Fiori, F., Della Vella, F., Rupe, C., Lajolo, C., Petruzzi, M., Serpico, R., & Lucchese, A. (2023). Oral Candidiasis and Novel Therapeutic Strategies: Antifungals, Phytotherapy, Probiotics, and Photodynamic Therapy. *Current drug delivery*, *20*(5), 441–456.

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010). The human oral microbiome. *Journal of bacteriology*, *192*(19), 5002–5017.

Elad, S., Cheng, K. K. F., Lalla, R. V., Yarom, N., Hong, C., Logan, R. M., Bowen, J., Gibson, R., Saunders, D. P., Zadik, Y., Ariyawardana, A., Correa, M. E., Ranna, V., Bossi, P., & Mucositis Guidelines Leadership Group of the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO) (2020). MASCC/ISOO clinical practice guidelines for the management of mucositis secondary to cancer therapy. *Cancer*, *126*(19), 4423–4431.

Gameiro, G. H., Nouer, D. F., Pereira-Neto, J. S., Urtado, M. B., Novaes, P. D., de Castro, M., & Veiga, M. C. (2008). The effects of systemic stress on orthodontic tooth movement. *Australian orthodontic journal*, *24*(2), 121–128.

Gasbarrini, G., Bonvicini, F., & Gramenzi, A. (2016). Probiotics History. *Journal of clinical gastroenterology*, *50 Suppl 2, Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting held in Rome, Italy on September 13-15, 2015*, S116–S119.

Hajishengallis G. (2015). Periodontitis: from microbial immune subversion to systemic inflammation. *Nature reviews. Immunology*, *15*(1), 30–44.

Hadjidakis, D. J., & Androulakis, I. I. (2006). Bone remodeling. *Annals of the New York Academy of Sciences*, *1092*, 385–396.

Hallström, H., Lindgren, S., Widén, C., Renvert, S., & Twetman, S. (2016). Probiotic supplements and debridement of peri-implant mucositis: a randomized controlled trial. *Acta odontologica Scandinavica*, *74*(1), 60–66.

Jäsberg, H., Tervahartiala, T., Sorsa, T., Söderling, E., & Haukioja, A. (2018). Probiotic intervention influences the salivary levels of Matrix Metalloproteinase (MMP)-9 and Tissue Inhibitor of metalloproteinases (TIMP)-1 in healthy adults. *Archives of oral biology*, *85*, 58–63.

Koybasi, S., Parlak, A. H., Serin, E., Yilmaz, F., & Serin, D. (2006). Recurrent aphthous stomatitis: investigation of possible etiologic factors. *American journal of otolaryngology*, *27*(4), 229–232.

Kumar S. S. (2022). Can probiotics stop oral cancer progression?. *Evidence-based dentistry*, *23*(1), 22–23.

Kumar, M., Kumar, A., Nagpal, R., Mohania, D., Behare, P., Verma, V., Kumar, P., Poddar, D., Aggarwal, P. K., Henry, C. J., Jain, S., & Yadav, H. (2010). Cancer-preventing attributes of probiotics: an update. *International journal of food sciences and nutrition*, *61*(5), 473–496.

Lalla, R. V., Brennan, M. T., Gordon, S. M., Sonis, S. T., Rosenthal, D. I., & Keefe, D. M. (2019). Oral Mucositis Due to High-Dose Chemotherapy and/or Head and Neck Radiation Therapy. *Journal of the National Cancer Institute. Monographs*, *2019*(53), lgz011.

Lederberg, J., & McCray, A., T. (2001). 'Ome Sweet 'Omics- A Genealogical Treasury of Words. *Genealogical Treasury of Words. Scientist.* 15(7):8.

Lee, D. S., Kim, M., Nam, S. H., Kang, M. S., & Lee, S. A. (2021). Effects of Oral Probiotics on Subjective Halitosis, Oral Health, and Psychosocial Health of College Students: A Randomized, Double-Blind, Placebo-Controlled Study. *International journal of environmental research and public health*, *18*(3), 1143.

Liu, Y. C., Wu, C. R., & Huang, T. W. (2022). Preventive Effect of Probiotics on Oral Mucositis Induced by Cancer Treatment: A Systematic Review and Meta-Analysis. *International journal of molecular sciences*, *23*(21), 13268.

Llewellyn, C. D., Johnson, N. W., & Warnakulasuriya, K. A. (2001). Risk factors for squamous cell carcinoma of the oral cavity in young people--a comprehensive literature review. *Oral oncology*, *37*(5), 401–418.

Meurman J. H. (2005). Probiotics: do they have a role in oral medicine and dentistry?. *European journal of oral sciences*, *113*(3), 188–196.

Mishra, S., Rath, S., & Mohanty, N. (2020). Probiotics-A complete oral healthcare package. *Journal of integrative medicine*, *18*(6), 462–469.

Mundula, T., Ricci, F., Barbetta, B., Baccini, M., & Amedei, A. (2019). Effect of Probiotics on Oral Candidiasis: A Systematic Review and Meta-Analysis. *Nutrients*, *11*(10), 2449.

Nadkerny, P. V., Ravishankar, P. L., Pramod, V., Agarwal, L. A., & Bhandari, S. (2015). A comparative evaluation of the efficacy of probiotic and chlorhexidine mouthrinses on clinical inflammatory parameters of gingivitis: A randomized controlled clinical study. *Journal of Indian Society of Periodontology*, *19*(6), 633–639.

Ozen, M., & Dinleyici, E. C. (2015). The history of probiotics: the untold story. *Beneficial microbes*, *6*(2), 159–165.

Palmer R. J., Jr (2014). Composition and development of oral bacterial communities. *Periodontology 2000*, *64*(1), 20–39.

Pradeep, C., Khedkar, S. U., Karde, P. A., Patil, A. G., & Dani, N., H. (2017). Potential antibacterial activity of probiotics against endodontic pathogens: An in-vitro pilot study. *International Journal of Scientific Research*. 6(9).

Pazzini, C. A., Pereira, L. J., da Silva, T. A., Montalvany-Antonucci, C. C., Macari, S., Marques, L. S., & de Paiva, S. M. (2017). Probiotic consumption decreases the number of osteoclasts during orthodontic movement in mice. *Archives of oral biology*, *79*, 30–34.

Penala, S., Kalakonda, B., Pathakota, K. R., Jayakumar, A., Koppolu, P., Lakshmi, B. V., Pandey, R., & Mishra, A. (2016). Efficacy of local use of probiotics as an adjunct to scaling and root planing in chronic periodontitis and halitosis: A randomized controlled trial. *Journal of research in pharmacy practice*, *5*(2), 86–93.

Nair P. N. (2004). Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical reviews in oral biology and medicine: an official publication of the American Association of Oral Biologists*, *15*(6), 348–381.

Qiu, W., Zhou, Y., Li, Z., Huang, T., Xiao, Y., Cheng, L., Peng, X., Zhang, L., & Ren, B. (2020). Application of Antibiotics/Antimicrobial Agents on Dental Caries. *BioMed research international*, *2020*, 5658212.

Saiz, P., Taveira, N., & Alves, R. (2021). Probiotics in Oral Health and Disease: A Systematic Review. *Applied Sciences*. 11(17):8070.

Santos, H. B., dos Santos, T. K., Paz, A. R., Cavalcanti, Y. W., Nonaka, C. F., Godoy, G. P., & Alves, P. M. (2016). Clinical findings and risk factors to oral squamous cell carcinoma in young patients: A 12-year retrospective analysis. *Medicina oral, patologia oral y cirugia bucal*, *21*(2), 151–156.

Salminen, S., Bouley, C., Boutron-Ruault, M. C., Cummings, J. H., Franck, A., Gibson, G. R., Isolauri, E., Moreau, M. C., Roberfroid, M., & Rowland, I. (1998). Functional food science and gastrointestinal physiology and function. *The British journal of nutrition*, *80 Suppl 1*, 147–171.

Socransky, S. S., Haffajee, A. D., Teles, R., Wennstrom, J. L., Lindhe, J., Bogren, A., Hasturk, H., van Dyke, T., Wang, X., & Goodson, J. M. (2013). Effect of periodontal therapy on the subgingival microbiota over a 2-year monitoring period. I. Overall effect and kinetics of change. *Journal of clinical periodontology*, *40*(8), 771–780.

Taddei, S. R., Moura, A. P., Andrade, I., Jr, Garlet, G. P., Garlet, T. P., Teixeira, M. M., & da Silva, T. A. (2012). Experimental model of tooth movement in mice: a standardized protocol for studying bone remodeling under compression and tensile strains. *Journal of biomechanics*, *45*(16), 2729–2735.

Wan Mohd Kamaluddin, W. N. F., Rismayuddin, N. A. R., Ismail, A. F., Mohamad Aidid, E., Othman, N., Mohamad, N. A. H., & Arzmi, M. H. (2020). Probiotic inhibits oral carcinogenesis: A systematic review and meta-analysis. *Archives of oral biology*, *118*, 104855.

Wang, Q., Ma, J. B., Wang, B., Zhang, X., Yin, Y. L., & Bai, H. (2019). Alterations of the oral microbiome in patients treated with the Invisalign system or with fixed appliances. *American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics*, *156*(5), 633–640.

Vigneswaran, N., & Williams, M. D. (2014). Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral and maxillofacial surgery clinics of North America*, *26*(2), 123–141.

Voidarou, C., Antoniadou, M., Rozos, G., Tzora, A., Skoufos, I., Varzakas, T., Lagiou, A., & Bezirtzoglou, E. (2020). Fermentative Foods: Microbiology, Biochemistry, Potential Human Health Benefits and Public Health Issues. *Foods (Basel, Switzerland)*, *10*(1), 69.

de Vrese, M., & Schrezenmeir, J. (2008). Probiotics, prebiotics, and synbiotics. *Advances in biochemical engineering/biotechnology*, *111*, 1–66.

Zhang, Y., Ding, Y., & Guo, Q. (2022). Probiotic Species in the Management of Periodontal Diseases: An Overview. *Frontiers in cellular and infection microbiology*, *12*, 806463.

Zini, A., Czerninski, R., & Sgan-Cohen, H. D. (2010). Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. *Journal of oral pathology & medicine: official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology*, *39*(4), 299–305.

CHAPTER 11

REPRODUCTION AND UROGENITAL SYSTEM IN MALE RATS

Saadet BELHAN[1]

1 Assoc. Prof. Dr. , Van Yuzuncu Yıl University, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Van, Turkey. ORCID ID: http://orcid.org/0000-0002-8115-2051

INTRODUCTION

Experimental animals are preferred in in vivo experimental studies for the development of diagnosis and treatment methods in pathological conditions, as well as investigating the physiological process in humans, due to the lack of an alternative situation. In experimental studies on male reproduction, rats are preferred over other experimental animals. Although many factors (care, feeding, reproduction) have an impact on this preference, their easy availability and easier semen retrieval come first. The larger body size of rats compared to mice may facilitate blood collection and also provide an opportunity for time-course sampling. Considering the situation only in terms of body size, it can be thought that large animal models (dog, pig, sheep, etc.) can also be used in this sense. However, the high maintenance costs, longevity, and small number of offspring of large breeds are not conducive to completion of studies. Rats are popular animals as research models in experimental studies, especially with their metabolic similarities with humans (Johnson and Shayne, 2007).

The rat is a good model for investigating the endocrine system because the endocrine organs are of normal size, neither too small nor too large. This situation facilitates both the removal of organs and the application of chemical agents in experimental studies. In this sense, understanding the reproductive behavior of rats can provide convenience in reproductive studies. In addition, it is necessary to know the reproductive process in order to evaluate the physiological and pathological conditions of reproduction.

1. UROGENITAL SYSTEM IN MALE RATS

1.1. Penis

The anatomy of the ratta penis (length: 20-28 mm, width: 3.6 mm, height: 2.8 mm) is similar to that of a human, but contains a bone and the glans penis is more anatomically complex. There is no clear information about the full function of the os penis. However, it is thought that it may have a role in force transmission during mating. The glans penis is covered with hairless skin with short cornified spines pointing towards the base of the penis. The preputial contains the preputial glands and covers the glans (Hebel and Stromberg, 1976; Kelly, 2000).

1.2. Testicle and Epididymis

Ratta testis (length: 20 mm, diameter: 14 mm) is dense, collagenous, whitish-blue in color and oval in appearance. The visceral layer of the tunica vaginalis (the part of the processus vaginalis that surrounds the testis) is in contact with the tunica albuginea (mean thickness: 20 40 μm), but

does not completely enclose the testis. On the dorsal side is a stalk of connective tissue called the mesorchium, which contains blood vessels and efferent ducts from the seminiferous tubules to the epididymis. Under the tunica albuginea is a layer of vessels called the tunica vascularis. Each testis contains approximately 20-30 tightly packed seminiferous tubules without division by fibrous tissue (Russell, 1992).

Testes begin to descend into the scrotum 15 after birth (Russell, 1992). The descent into the scrotum is completed between the 30th and 40th days of life. However, they can travel between the scrotal sacs and the abdominal cavity throughout life through the open inguinal canal (Picut and Remick, 2016).

The epididymis is a long tubular canal surrounding the dorsal surface of the testis and absorbs approximately 90% of the fluid secreted by the seminiferous tubules. It consists of 3 parts: head, body and tail. The long curved canal of the epididymis, which is 600 cm long in humans, is approximately 400 cm in rats (Hebel and Stromberg, 1976).

1.3. Blood-Testicular Barrier

It has been shown that the Blood-testis barrier formed by Sertoli cells begins to form in postnatal days 15-21, which coincides with the onset of the postnatal cell development period. However, this is not a fully functional barrier, and the functional blood-testis barrier was not established in Sprague-Dawley rats until the 20th postnatal day (Mok et al., 2011).

1.4. Accessory Glands

It has been reported that the accessory glands are developed in male rats. These; ductus deferens gland, seminal vesicles, prostate gland and coagulation gland (part of the prostate), bulbourethral glands (Cowper), urethral glands and preputial glands of the penis (Hebel and Stromberg, 1976). Bulbourethral glands, which are among these glands, are located at the base of the penis and open to the dorsal part of the urethral flexure, while the prostate gland is located in the abdominal part and attracts attention with its lobular structure (Fox, 2015). These glands provide most of the fluid content of the ejaculate. In rats, the fluid coagulates and forms a mating plug that closes the female reproductive tract after mating.

There is still no clarity in the classification of accessory glands in rats. This condition has been reported to be caused by the location of the coagulant gland. Because “coagulant gland; is a lobe of the prostate gland or a separate gland” is still unclear (Greene, 1935).

1.5. Spermatic Cord

The main structures that make up the spermatic cord are; ductus deferens, processus vaginalis, testis and arteries feeding ductus deferens, pampiniform plexus, lymphatic vessels and nerves.

2. SEX DISCRIMINATION IN MALE RATS

Sex discrimination in adult rats is easier than in juvenile rats. In men, the scrotum can be easily seen in the perineal region between the anus and the urethral opening (Fox, 2015; Korenbrot et al., 1997). However, when evaluating the anogenital patency, it should be considered whether the testicles are retracted into the abdomen. Anogenital opening in males is greater than in females (Russell, 1992). Another important issue in sex discrimination is to observe the presence of the penis. The penis is larger than the female urethral papilla (Fox, 2015; Korenbrot et al., 1997). In juvenile rats, sex discrimination can be made by looking at the distance between the anus and the genital tract (Fox, 2015). Evaluation of anogenital patency is more meaningful than scrotal protrusion, especially in rats younger than 2 weeks. Because the descensus testis is completed on the 15th day after birth (Russell, 1992). As in adults, the anogenital distance is longer in juvenile male rats than in females. Finally, the absence of nipples in male rats is also a criterion that can be used for sex discrimination (Cowie, 1984).

3. REPRODUCTIVE FEATURES IN MALE RATS

3.1. Puberty

Age at puberty in rats may vary due to factors such as the number of siblings, hormones and nutrition. It has been reported to age of puberty be between days 65-110 postpartum (Nagatani et al., 2000). Rats with fewer siblings in the same litter and well-fed rats reach puberty earlier. Although rats reaching half their mature weight are considered pubertal, body length measurement (148-150 mm) has been accepted to be more reliable in reaching puberty (Bennett and Vickery, 1970). However, the strongest determinant of puberty is descensus testis and the beginning of spermatogenesis. Sperm production starts from the 45th day (Russell, 1992). Age and experience play a role in the emergence of sexual behaviors. For example, while they do not show interest even to fiery females in the pre-pubertal period, their sexual orientation to females decreases after a period of 150 days. The decrease in sexual orientation in these processes is probably due to the age-related decrease in testosterone hormone (Smith et al., 1992).

Puberty in the male rat occurs with the testes descending into the scrotum and the onset of spermatogenesis. Sperm are first produced in the testicles at about day 45, but optimal production does not occur until about day 75 (Rus-

sell, 1992; Marty et al., 2003). Accelerated testicular growth has been reported in the offspring of pups weaned early (16 days postpartum) compared to pups weaned at an average of 21 and 26 days (Crispel et al., 2013).

3.2. Birth and Adult Body Weight

Rats with an average birth weight of 5-6 grams have an adult body weight of 450-520 grams (Harkness and Wagner, 1989; Fox and Laird, 1970).

3.3. Spermatogenesis

A large number of primary germ cells are present at birth and approximately 75% of them degenerate in the first week of life (Russell, 1992). Spermatogonial cells migrate into the seminiferous epithelium of the testis on the 4th day after birth (Bennett and Vickery, 1970). Existing spermatogenic cycles normally begin earlier. However, these cycles are incomplete and irregular before puberty (Russell, 1992).

Spermatogenesis occurs in the seminiferous tubule (Setchell, 1982). In order to understand the cycle in the seminiferous tubules during the spermatogenesis process, the transverse sections of the seminiferous tubules were examined. In the examination, it was determined that certain combinations of cell types in the basement membrane of the seminiferous tubules occur repeatedly. Particularly in sections taken from rats and guinea pigs, a specific pattern sequence was detected, which seemed to travel along the tubule and created a repetitive wave at intervals. The essence of this model is that at each point along the tubule, cells go through a series of developmental stages and progress from layer to layer (Leblond and Clermont, 1952). In this putative model, it was thought that there could be 4 different stages, each involving a certain combination of cell types. These stages are listed as follows. Stage 1: The stage consisting of spermatogonia; Stage 2: Stage consisting of spermatocytes, Stage 3: Stage consisting of early round spermatids and Stage 4: Stage consisting of mature long spermatids. In order for spermatogenesis to occur in a healthy way, these stages must occur sequentially. In the rat, it was assumed that each stage would last an average of 12.9 days. It was also stated that the number of stages in the rat could be 4.5 (Creasy, 1997).

First sperm production starts on days 45-46. But it is significant in terms of fertility starts on days 62-65 (Russell, 1992). During spermatogenesis in rats, spermatogonia are located outside the blood testicular barrier. At the end of the 8th stage and the beginning of the 9th stage, they cross the blood testicular barrier and migrate to the base of the adjacent compartment of the seminiferous epithelium (Mruk and Cheng, 2004).

At high ambient temperature, the epithelium of the seminiferous tubules in the testicles is damaged. This leads to decreased fertility and infertility. It has been reported that such negative situations are experienced when puppies are exposed to high temperatures (26.6oC), especially during the period until they are 4 days old (Maloy and Hughes, 2013).

3.4. Ejaculation

Ejaculation typically occurs after 3-44 intromissions. Mating lasts up to 3 hours intermittently. During this time, 3-10 ejaculation occurs (Baker, 1979). The amount of semen in rats is 1-2 drops and its density is up to 50-60x10^6 spermazoa per ejaculate (Bennett and Vickery, 1970).

3.5. Semen Collection

In rats, semen can be collected by 2 methods. One of them is the electro-ejaculation method. However, in this method, when ejaculation occurs, the coagulation of the secretions of the accessory glands causes a serious problem in semen retrieval. In addition, since this method requires anesthesia and urine contamination may occur, semen quality may be adversely affected. As a result, fertility may also be adversely affected, as the quality of both fresh and frozen semen may be adversely affected.

Another method is semen retrieval by slicing the cauda epididymis (Fox, 2015). With this method, semen retrieval is easier. However, since this method is done postmortem, it is not possible to collect semen again from the rat whose semen we have taken or to keep the animal alive.

Small rodent sperm are longer than human and elephant sperm. Human sperm: 53 μm and elephant sperm; While 50 μm, rat sperm: approximately 190 μm in length. Rat sperm have a sharply hooked head (Bishop and Walton, 1960).

3.6. Artificial Insemination

Storage and freezing of rat semen at low temperatures results in failure due to the width of the acrosome structure (Bennett and Vickery, 1970). Rats can be inseminated by surgical or non-surgical techniques. The spermatozoa to be used in insemination are left on the upper part of the uterus or inside the bursa of the ovary. It is possible to achieve pregnancy with both techniques (Saito et al., 1996).

3.7. Mating and Breeding

In rat production, usually 1 male rat is placed in the same cage with 4 female rats. When females become pregnant, they are separated individually and each is left in a separate cage. After giving birth, the man must be

separated. Otherwise, not being separated from the mother and offspring causes canibalismus and/or weaning (Bennett and Vickery, 1970; Harris and Kesel, 1990).

It is known that coitus occurs more intensely in the dark environment. After an average of 2-9 intromissions (0.3-0.6 seconds each), the first ejaculation (average 1 second) occurs. It is likely that the pregnancy rate will decrease in single ejaculations with a lower number of intromissions (Fox, 2015). Testosterone and pheromones are effective in mating behavior in male rats (Meisel and Sachs, 1994). Sexual intercourse can be detected by detecting sperm in vaginal smear preparations or by shaping the vaginal plug. This situation can be understood much more easily by observing sexual behavior.

Their average lifespan is 2.5-3.5 years. They are expected to be 65-110 days old to be used in reproduction (Harkness and Wagner, 1989; Fox and Laird, 1970).

3.8. Pheromones

The main pheromone molecules in male rats are squalene, 2 heptanone, and 4-ethylphenol. It has been reported that male rats with excess pheromones are more attractive to females (Zhanget al., 2016).

3.9. The Bruce Effect (Mothers who kill their offspring for alpha males)

It is defined as the termination of pregnancy of a female rodent exposed to a foreign male. It has been reported that this effect occurs under the control of pheromones. The pheromones secreted in men cause a decrease in the production of prolactin, the hormone necessary for the production of progesterone. Since progesterone is an essential hormone for the continuation of pregnancy, a decrease in progesterone will cause a miscarriage during pregnancy. However, ovulation occurs in the female in the next process, and then the embryonal life begins normally.

4. HORMONAL MECHANISM

Neuroendocrine cells in the hypothalamus synthesize hypothalamic hormones in the anterior pituitary gland. These hormones are transported to the anterior pituitary gland via the pituitary portal system, where they regulate the synthesis and secretion of anterior pituitary hormones. GnRH induces the secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH). During puberty, LH causes testosterone levels to increase. LH stimulates Leydig cells to achieve this effect. While LH plays such an important role, the role of FSH in the male hormonal mechanism is not fully known. Although pubertal changes do not occur in the case of

hypophysectomy before puberty, administration of LH, FSH, and testosterone after hypophysectomy can reinitiate spermatogenesis (Setchell, 1982).

4.1. Gonadotropin Releasing Hormone

GnRH releases two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), from the pituitary gland. In all species, GnRH transitions to the pituitary portal veins system pulsatilely (Clarke and Cummins, 1982; Moenter et al., 1992). GnRH is also known as LH releasing hormone (LHRH).

4.2. Anterior Pituitary Hormones

4.2.1. Luteinizing Hormone

LH stimulates the synthesis of androgens by affecting the gonads (ovarium, testis) in both males and females. In all species, including the rat, LH secretion occurs in a pulsatile manner (Gay and Sheth, 1972).

4.2.2. Follicle Stimulating Hormone

FSH, like LH, has significant effects on the gonads. FSH, which is effective in follicular development in females, plays a very important role in stimulating spermatogenesis in males (Sharpe, 1988). In addition to these two important functions, FSH also plays a role in stimulating aromatase activity in both males and females. (Androgens are converted to estrogen by aromatase activity). Inhibin and activin also play a role in regulating the secretion of FSH.

4.3. Gonadal Steroids

Gonadal steroids are effective in stimulating gamete production, the development of reproductive organs and the formation of secondary sex characters and the emergence of sexual behavior. Gonadal steroids are synthesized under the control of LH and FSH secretion in both sexes. It has been reported that steroid hormones are synthesized from cholesterol. The source of cholesterol in rats is different from humans, being high-density lipoproteins (HDL) found in plasma.

Androgens stimulate secondary sexual characteristics in men. It is also necessary for spermatogenesis. It takes part in the development of male auxiliary reproductive organs and helps to fulfill the function of these organs. They are also responsible for male sexual behavior and protein anabolism (Meisel and Sachs, 1994). Androgens have a negative feedback effect on gonadotropin secretion. In rats, the main androgen is testosterone. In the male rat, testosterone is synthesized in Leydig cells via A4 (Hall, 1994). The synthesis and secretion of testosterone occurs by LH (Baird, 1984).

REFERENCES

Baird DT. Reproduction in Mammals. In: Austin CR, Short RV (Eds) Hormonal Control of Reproduction. Cambridge: Cambridge University Press. 1984:91–114.

Baker DEJ. Reproduction and breeding. In: Baker HJ, Lindsey JR, Weisbroth SH (Eds). The Laboratory Rat. Volume 1, New York: Academic Press. 1979;153–168.

Bennett JP, Vickery BH. Rats and mice. In: Hafez ESE (Ed). Reproduction and Breeding Techniques for Laboratory Animals. Lea and Febiger, Philadelphia, PA. 1970:299–315.

Bishop MWH, Walton A. Spermatogenesis and the Structure of Mammalian Spermatozoa. Chapter 7 In: Parkes AS (Ed), Volume 1: Part 2 of Marshall's Physiology of Reproduction. Longmans Green and Co Ltd, London. 1960:1–129.

Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology.* 1982;111(5):1737–9. https://doi.org/10.1210/endo-111-5-1737.

Cowie AT. Lactation. In: Austin CR, Short RV (Eds). Reproduction in Animals. Volume 3, Cambridge University Press, New York, NY. 1984:195–231.

Creasy DM. Evaluation of testicular toxicity in safety evaluation studies: the appropriate use of spermatogenic staging. *Toxicologic Pathology.* 1997;25(2):119–31. https://doi.org/10.1177/019262339702500201.

Crispel Y, Katz O, Ben-Yosef D, Hochberg. Effects of breastfeeding on body composition and maturational tempo in the rat. *BMC Medicine.* 2013;11:114–122. https://doi.org/10.1186/1741-7015-11-114.

Fox JG. Laboratory animal medicine. In: Otto G, Pritchett-Corning KR, Whary MT, Anderson LC (Eds). A Volume in American College of Laboratory Animal Medicine. Elsevier. 2015.

Fox RR, Laird JW. Sexual Cycles. In: Hafez ESE (Ed). Reproduction and Breeding Tecniques for Laboratory Animals. Lea &Febiger, Philadelphia, Chapter 5, 1970.

Gay VL, Sheth NA. Evidence for a periodic release of LH in castrated male and female rats *Endocrinology.* 1972;90(1):158–62.

Greene EC. Anatomy of the Rat. Transactions of the American Philosophical Society. New Series, Hafner Publishing Company. New York and London. 1935.

Hall EF. Regulation of Spermatogenesis. In: Knobil E, Neill JD (Eds). The Physiology of Reproduction. 2nd Edition, Raven Press, New York. 1994;1335–1362.

Harkness JE, Wagner JE. The Biology and Medicine of Rabbits and Rodents. Third ed. Philadelphia, London. Chapter 2–4. 1989.

Harris MA, Kesel M. An improved method for accurately timed mating in rats. Laboratory Animal Science. 1990;40(4):424–425.

Hebel R, Stromberg MW. Anatomy of the Laboratory Rat. The Williams & Wilkins Company, Baltimore. 1976.

Johnson MDG, Shayne C. The rat. In: Gad SC (Ed). Animal Models in Toxicology. Second ed. Chemical Rubber Company Press, Boca Raton FL. 2007;150

Kelly DA. Anatomy of the baculum-corpus cavernosum interface in the Norway rat (Rattus norvegicus), and implications for force transfer during copulation. *Journal of Morphology.* 2000;244:69–77. https://doi.org/10.1002/(SICI)1097-4687(200004)244:1<69:AID-JMOR7>3.0.CO;2-#.

Korenbrot CC, Huhtaniemi IT, Weiner RI. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction*. 1997;17(2):298–303. https://doi.org/10.1095/biolreprod17.2.298.

Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. *Annals of the New York Academy of Sciences*. 1952;55(4):548–573. https://doi.org/10.1111/j.17496632.1952.tb26576.x.

Maloy S, Hughes K. Brenner's Encyclopedia of Genetics. Second ed. Elsevier. 2013.

Marty MS, Chapin RE, Parks LG, Thorsrud BA. Development and maturation of the male reproductive system. *Birth Defect Research. Part B, Developmental and Reproductive Toxicology*. 2003;68(2):125–136. https://doi.org/10.1002/bdrb.10015.

Moenter SM, Brand RM, Midgley AR, Karsch FJ. Dynamics of gonadotropin-releasing hormone release during a pulse. *Endocrinology*. 1992;130(1):503–10. https://doi.org/10.1210/endo.130.1.1727719.

Mok KW, Mruk DD, Lee WM, Cheng CY. A study to assess the assembly of a functional blood-testis barrier in developing rat testes. *Spermatogenesis*. 2011;1(3):270–280. https://doi.org/10.4161/spmg.1.3.17998.

Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-Germ Cell Interactions and Their Significance in Germ Cell Movement in the Seminiferous Epithelium during Spermatogenesis. *Endocrine Reviews*. 2004;25(5):747–806. https://doi.org/10.1210/er.2003-0022.

Nagatani S, Guthikonda P, Foster DL. Appearance of a Nocturnal Peak of Leptin Secretion in the Pubertal Rat. Hormones and Behavior. 2000;37(4):345–352.

Picut CA, Remick AK. Male reproductive system. In: Parker GA, Picut CA (Eds). Atlas of Histology of the Juvenile Rat. Academic Press, London. 2016;227–256.

Russell LD. Normal development of the testis. In: Mohr U, Dungworth DL, Capen CC (Eds). Pathobiology in the Aging Rat. Volume 1, ILSI Press, Washington, DC. 1992:395–405.

Saito TR, Hokao R, Wakafuji Y, Igarashi N, Agematsu Y, Takahashi KW. Parachloroamphetamine (PCA) induced ejaculation in aged rats, semen analysis and artificial insemination. *Laboratory Animals*. 1996;30(4):332–336. https://doi.org/10.1258/002367796780739844.

Setchell BP. Spermatogenesis and spermatozoa. In: Austin CR, Short RV (Eds). Reproduction in Mammals. Volume 1, Cambridge University Press, New York, NY. 1982:63–101.

Sharpe RM. Regulation of Spermatogenesis. In: Knobil E, Neill JD (Eds). The Physiology of Reproduction. Raven Press, New York. 1988;1363-1434.

Smith ER, Stefanick ML, Clark JT, Davidson JM. Hormones and sexual behavior in relationship to aging in male rats. *Hormones and Behavior*. 1992;26(1):110–35. https://doi.org/10.1016/0018-506x(92)90035-t

Zhang YH, Liang HC, Guo HL, Zhang JX. Exaggerated male pheromones in rats may increase predation cost. *Current Zoology*. 2016;62(5):431–437. https://doi.org/10.1093/cz/zow047.

CHAPTER 12

DIAGNOSIS AND MANAGEMENT OF NERVE INJURIES IN MAXILLOFACIAL REGION

Nuri Mert TAYSİ[1], Aysegul Erten TAYSİ[2]

1 Asst. Prof. Dr., Istanbul University- Cerrahpaşa, Dentistry Faculty, Department of Oral and Maxillofacial Surgery. Orcid ID: 0000-0002-5595-9302, nurimert.taysi@iuc.edu.tr, mtaysi23@hotmail.com

2 Asst. Prof. Dr., Altinbas University, Dentistry Faculty, Department of Oral and Maxillofacial Surgery. Orcid ID: 0000-0002-9156-9109, aysegul.erten@altinbas.edu.tr, denterten@gmail.com

Introduction

Nerve injuries related to oral and maxillofacial area could occur as a consequence of several factors including trauma, neoplasms, local infection, overfilling of the root canals in endodontic treatment, local anesthesia administration and surgical procedures such as orthognathic, pre-prosthetic surgery, tooth extraction and dental implant operation (Sivolella et al., 2014; Steinberg et al., 2015). Many nerve injuries related to dentoalveolar surgery are related to inaccurate assessment and can be avoided with proper imaging techniques, and thorough preoperative examination (Steinberg et al., 2015); however, the injuries can be an inevitable due to the surgical proximity to the nerves. Nerve injuries can be catastrophic for patients, resulting in loss or changes of sensation, paralysis, and pain. Moreover, permanent deficits may have serious functional, psychological, and socioeconomic consequences for their victims (Bage and Power, 2021). Therefore, early diagnosis and evaluation of nerve injuries in oral and maxillofacial region is crucial in order to manage the severe consequences. As healthcare providers need to understand the serious consequences of the nerve injuries, and to eliminate the risk of injuries, aims of this chapter are (1) understanding of potential injury mechanisms, specific anatomical sites, (2) risk-reduction strategies to prevent from the injuries, and (3) to review of the treatment methods.

1. Recalling the nerve anatomy in maxillofacial region

To discuss the nerve injuries in dentistry, it is essential to become full knowledge of the nerve anatomy in the cranial region. Major cranial nerves innervating the maxillofacial region are; trigeminal, facial, glossopharyngeal and hypoglossal nerve. The trigeminal and facial nerve, which are close functional and anatomical relationships in both their sensory and motor divisions, are mostly affected ones from nerve afflictions situation that lead to the most problematic consequences in the field of dentistry with serious medical and legal implications (Poorian et al., 2016; Renton, 2018). The trigeminal nerve, also named as cranial nerve-V, innervate the facial sensation and masticatory muscles, while the facial nerve, also named as cranial nerve-VII, primarily controls facial expression muscles as well as the sense of taste. Within the taste sensation, electrophysiological studies exhibited that the trigeminal nerve, which is responsible for somato-sensation on the tongue, regulates the gustatory neurons originating from solitary nucleus of facial nerve located in the medulla and lower pons (Sanders et al., 2010; Park et al., 2022).

There are three divisions of the trigeminal nerve, which separate at the trigeminal ganglion near the cavernous sinus, including ophthalmic nerve as the first division, maxillary nerve as the second division, and mandib-

ular nerve as the third division, which exit the skull through different foramina such as superior orbital fissure, foramen rotundum, and foramen ovale, respectively (Yang and Tuffaro, 2020). There is sparse information available regarding injuries to the first two divisions of the trigeminal nerve compared to the data available on the third division (Kaleem et al., 2020). However, the terminal branch of the second division emerges in the midface through the infraorbital foramen as the infraorbital nerve lying between levator labii superioris and levator anguli oris muscles and it is regularly affected in the case of midface injuries as well as orbital floor fractures (Yang and Tuffaro, 2020). The mandibular nerve, as the third division and the largest branch of the trigeminal nerve, is the only one to convey motor fibres. After exiting the skull from foramen ovale, it extends into the infratemporal region for a short distance as a single trunk prior to dividing into a small anterior and a larger posterior trunk that lie down to the pterygo-mandibular space (Khoury and Townsend, 2011). The anterior trunk gives off a sensory branch, known as the buccal nerve, and motor branches, known as the masseteric, deep temporal nerve, and lateral pterygoid nerve, supplying the muscles of the same name. The posterior trunk, which is principally sensory, divides into three branches known as the lingual, auriculotemporal and inferior alveolar nerve (Yang and Tuffaro, 2020).

Considering the third division injuries, the lingual and inferior alveolar nerve injures are the most common (Kaleem et al., 2020). Renton (2018) stated that the injury incidence of the former has hovered around the same percentage in the United Kingdom over the last thirty years whereas that incidence of the latter has increased, primarily attributed to more dentists performing dental implant operations and endodontic treatments. Given the importance of recalling anatomical knowledge for effectively managing nerve afflictions, we have provided the essential anatomical details concerning these two nerves as follows:

The lingual nerve runs downwards between the lateral pterygoid and tensor veli palatini muscle, after it travels downwards and forwards into the pterygomandibular region between the medial pterygoid muscle and the mandibular ramus positioning itself anterior side of the inferior alveolar nerve. It runs forwards immediately adjacent to the lingual cortex of the inner mandibular angle (temporal crest), the retromolar trigone, and the third molar region. Then it turns medially across the floor of the mouth to innervate lingual mucosa. It is also in intimate association with submandibular duct and it innervates the anterior two-thirds of the tongue with preganglionic parasympathetic fibrils originating from the chordo thypani (Atkinson, 2013).

The inferior alveolar nerve, located posterior to the lingual nerve in the pterygomandibular space passes between the sphenomandibular liga-

ment and the ramus of the mandible en route to mandibular foramen (Bagheri and Meyer, 2012; Lee et al., 2015; Wolf et al. 2016). Once it enters the mandibular foramen, the nerve commences a long course throughout the mandibular canal until it divides into two division, the incisor branch and the mental branch, nearby the mental foramen. During its travel inferiorly in the mandibular canal until the mental foramen, it gives off loads of branches that form an alveolar plexus below the roots of posterior teeth supplying their innervation. The incisor branch runs anteriorly within the body of the mandible and innervated the anterior teeth such as canines and incisors. When it passes through the foramen mentale, it receives the name "mental nerve" and provides sensory innervation to the lower lip and corresponding gingival surface (Waldman, 2014). As the mental foramen is mostly located at the level of the apex of the second premolar or between the premolars, it is crucial to keep in mind that it is not far from the operating area of the oral and maxillofacial surgeon (Bagheri and Meyer, 2012; Lee et al., 2015; Wolf et al., 2016). Moreover, it is clear that the mental nerve course branching out to the mental foramen have many variations. Injury to the mental nerve by any blunt or sharp surgical instruments, dental implant placement, and cyst or tumor enucleation during dental operations may be a consequence of compression, laceration, or even transection of the neurovascular bundle (Hui et al., 2022).

Regarding the facial nerve, it is unique in traversing of parotid gland, a fact of considerable importance to the surgeon (Ellis and Mahadevan, 2013). Marginal mandibular nerve as a division of the facial nerve was the commonly involved nerve in cases of trauma related to the region of oral and maxillofacial surgeon (Poorian et al., 2016). Moreover, this branch passes behind the angulus of the mandible rather less than 1 inch from it before arching upwards mandibular body to innervate the depressor of the lip. Therefore, in order to preserve this nerve, it is of utmost importance that the skin incision must be performed rather more than 2.5 cm below the angulus of the mandible (Ellis and Mahadevan, 2013).

2. Procedures implicated in the nerve injury

Nerve injuries could infrequently, but certainly not exclusively, occur within the broad of maxillofacial surgery. On the whole, the highest incidence of nerve injuries was associated with third molar surgeries, followed by endodontic therapy, other surgical procedures, and lastly dental implant operation (Kaleem et al., 2020). Renton and Yılmaz (2011) emphasized that when inferior alveolar nerve injury is specifically concerned third molar surgery is the most related cause (60%), followed by local anesthetic injection, dental implant operation, and endodontic surgery (19, 18, and 18%, respectively). The same authors also stated that when lingual nerve injury concerned, the third molar was the major cause (73%), followed by

local anesthesia injections (17%).

2.1. Local Anesthesia Administration

Nerve injuries due to the local anesthesia administration could be caused by a blunt or barbed needle usage and neurotoxic mechanism of local anesthetic agents (Kim et al., 2020; Schiavone et al., 2021). If a blunt or barbed needle were used in multiple injections, there would be quite frankly risk for nerve injury depending on withdrawal from the intraoral soft tissues resulting in intraneural hematoma, and subsequent paresthesia (Schiavone et al., 2021). Multiple administrations of local anesthesia with a blunt or barbed needle for inferior alveolar nerve anesthesia may also cause injury upon withdrawal from intraoral soft tissues, leading to an intraneural hematoma and subsequent paresthesia (Schiavone et al., 2007; Ozen et al., 2006). The estimated incidence of nerve injuries related to local anesthesia administration ranges from 1:26,762 to 1:160,571 (Pogrel et al., 2007; Schiavone et al., 2021). It is stated that most of the cases (85%) undergo spontaneous recovery within 8 to 10 weeks, while some (5%) may require longer recovery time and spontaneous recovery time (Kaleem et al., 2020). However, 10% of these injuries might be sustained as permanent sensory deficits corresponding to an incidence of 1 in 25,000 injection local anesthesia for the inferior alveolar nerve blocks (Kaleem et al., 2020; Pogrel, 2007).

Considering the chemical injury to the nerve due to local anesthetic injection, there is a difficulty in the determining of such neurotoxic effect of local anesthetics directly as the perioperative nerve injury could emerge from various clinical and confounding risk factors (Kim et al., 2020). Relevant to the clinical setting, when the clinicians directly injected local anesthetics into intrafascicular space, there could occur a consequence of axonal damage due to exposure of peripheral nerve (Hogan et al., 2008).

Local anesthetic agents have a complex structure, defining as a lipophilic aromatic or a hydrophilic group, and an amide or an ester linkage chain; so they are categorized into two distinct types such as amino-amide and amino-ester. Considering the dental cartridges containing local anesthetics, the amide group of local anesthetics, which is the predominantly preferred anesthetics in dentistry, includes lidocaine, articaine, mepivacaine, bupivacaine and prilocaine while the ester class includes benzocaine (Kim et al., 2020). When analyzing the scientific literature about the anesthetic type and its concentration, it should be considered that there is a greater risk developing prolonged paresthesia after administration of either articaine or prilocaine (Aquilanti et al., 2022). Hillerup and Jensen (2011) reported that articaine was associated with a higher frequency of paresthesia compared to the administration of 2% lidocaine, 2% or 3%

mepivacaine, and %3 prilocaine, in particular with mandibular blocks. In another study, the prilocaine was related to 34% of cases of nerve injury with a sign of paresthesia, followed by articaine and lidocaine, with 33% and 25%, respectively (Pogrel, 2012). In line with that point, Piccini et al. (2015) also stated a significant overrepresentation of paresthesia and dysesthesias when using articaine and prilocaine.

2.2. Endodontic treatment

Endodontic treatment of posterior mandibular teeth might have the risk to be responsible for inferior alveolar or mental nerve injury via pressure, direct trauma or neurotoxicity (Renton, 2018). Injuries of the inferior alveolar and mental nerves have been reported mostly in second mandibular molars, mandibular premolars and to a lesser occurring in other mandibular teeth as well (Rosen, 2017). Relatively few scientific studies exist about nerve injuries due to endodontic treatment. It should be pointed out that these injuries may not be limited to those teeth close to the IAN canal but may also occur in maxillary teeth as well (Renton, 2018). There are several reports related to maxillary nerve paresthesia through extravasation of either paraformaldehyde-based root canal sealer or sodium hypochlorite-based root canal irrigation solution into the periradicular tissues during the endodontic treatment of maxillary teeth (Pelka and Petschelt, 2008; Alves et al., 2014).

In a comprehensive systematic review of the literature conducted by Rosen et al. (2017) which focuses an altered sensation caused by apically extruded root canal filling materials during endodontic procedure, a total of 84 cases within 28 articles that met the inclusion criteria were assessed. In this systematic review, the authors reported that the majority of patients had recovered fully or partially over time, provided that the root filling material did not consist of paraformaldehyde-based components or that a timely mannered intervention was performed following the occurrence of nerve injuries. As both early diagnosis and immediate intervention are essential for enabling prospective recovery, clinicians should be aware of that symptom of nerve injuries would usually manifest postoperatively, when the anesthesia effect wore out (Pogrel, 2011; Rosen et al., 2014). As highlighted in a case series and literature review, endodontic injuries should be managed within 24- 30 hours to optimize resolution of sensory function. Moreover, when a nerve injury is possible, consuming of anti-inflammatory drugs may be beneficial as a preventive or faster therapeutic method.

There are numerous *in vivo* studies reporting the persistent postendodontic pain without any sign of either unsuccessful endodontic treatment or incomplete radiographic healing (Polycarpou et al., 2005, Renton, 2018).

At this point, the persistent pain might be of a nonodontogenic origin with a neuropathic basis initiated after prolonged nociceptive pain. According to a study, some certain factors were found to be related with persistent postendodontic pain, including prolonged preoperative pain (more than 3 months), female patients, and some chronic pain symptoms prior to dental procedure (Polycarpou et al., 2005, Renton, 2018). In a study consisted of 221 patients with trigeminal nerve injuries related to dentistry, most of them were reported to occur chronic neuropathic pain state due to abnormal and long activation of the nociceptive system previously (Camphell et al., 1990; Renton, 2018). The chronic neuropathic pain state initiates after a lesion of peripheral or central nervous structures that are normally involved in signaling, and requires different therapeutic approaches such as anticonvulsants, which are ineffective for nociceptive pain.

2.3. Local İnfection

Local infection, including osteomyelitis, periapical, and peri-implant infections, may result in sensory discomfort such as paresthesia, anesthesia, hypoesthesia, and hyperesthesia present in the oral cavity (Ozkan et al, 2008). Infection related sensory disturbances is usually arisen from mechanical pressure on the nerve as a result of the accumulation of inflammatory exudate and ischemia due to inflammatory process. In addition, the sensory disturbances, as an alternative explanation, could potentially be attributed to either the toxic metabolites generated by bacteria or the inflammatory byproducts released from tissue damage (Censi et al., 2016). The sensory disturbances caused by local infections typically subside between 4 and 8 weeks after the infection has been eliminated. The sensory disturbances resulting from local infections usually resolves following to the elimination of infection between 4 and 8 weeks (Genc Sen and Kaplan, 2015; Büttner et al., 2018).

2.4. Trauma

Maxillofacial fractures, as a leading cause of maxillofacial trauma, may involve nerves and cause some complications such as sensory disturbance and neurosensory dysfunction (Poorian et al., 2016). Those kinds of complications can also be persistent due to surgical manipulation, surgical dissection, or combination of these factors. In addition, previous studies depicted that those kinds of complications occurred in near to 8-66.7% of patients with mandibular fracture and 15-46% with midface fracture (Poorian et al., 2016; Khan et al., 2022). On the other hand, Chuong et al. (1983) emphasized the extraoral approaches to mandibular fractures were more related to some form of complication, such as prolong recovery time, infection, dehiscence, malocclusion, and nerve damage as well**.** Moreover, an extraoral approach to fractures in proximity to the facial nerve can lead

to motor deficits at a frequency of 3% (de Matos et al., 2010).

In a recent study, the primary causes of nerve injury were observed with firearm injuries, followed by traffic accidents and sports related injuries (Khan et al., 2022). This study reported that while the trigeminal nerve injury was observed at a frequency of 5.5% within the consequences of maxillofacial trauma, it was reported as %1.6 for the facial nerve injury. Considering that the trigeminal nerve injury, the inferior alveolar nerve was identified as the most commonly involved branch at the frequency of 39.1% followed by infraorbital nerve at that 27.2%. As for facial nerve, Poorian et al. (2016) stated that the most effected branch was the marginal branch whereas Khan et al. (2022) reported the involvement of the temporal branch was the most common.

2.5. Orthognathic surgery

The frequency of nerve injuries is reported to be 50% of the complications subsequent to orthognathic surgery, followed by other injuries such as temporomandibular disorder, hemorrhage, hearing problem, infections, and relapse (Jedrzejewski et al., 2015). Neurologic deficits related to orthognathic surgery primarily affect the inferior alveolar nerve, but also may include lingual, mental, infraorbital and the facial nerve (Kim, 2017). Postoperatively, irreversible or reversible changes in sensory mechanism of the peripheral nerve branches are usual as an expected part of the patient's recovery process according to the nerves' intimate proximity to the surgical area (Bagheri et al., 2012). Therefore, the numerous studies examined the correlation between the orthognathic surgery and neurosensory disturbances.

The LeFort I is one of the most frequently utilized and well-known procedures in order to treat midface deformities allowing a correction in three dimensions via advancement, elongation, shortening, and retrusion. This technique depends on two principles, which are pterygomaxillary disfunction and down fracture, which allow maxillary mobility and is associated with vascular and nervous complications in the intraoperative and postoperative period (Dos Santos Alves et al., 2019). The procedure is mostly preferred for the correction of facial asymmetry, obstructive sleep disorder, atrophy of the maxillary bone, and Class II or Class III malocclusion. The most frequent complications related to the nerve disturbance are observed in the maxillary teeth and the surrounding mucosal areas, as well as the facial cutaneous sensation. The most persistent complication due to this operation seems to be sensory deprivation in the upper teeth (Thygesen et al., 2009; Kim, 2017). Nerve injuries are not rare and may also occur in more serious way, as in amaurosis (Dos Santos Alves et al., 2019).

Regarding the management of prognathism, sagittal split ramus osteotomy (SSO) and intraoral vertical ramus osteotomy (IVO) are the two most common and widely preferred surgical techniques. These procedures carry a risk of sensory disturbance in the mental region due to damage of the inferior alveolar nerve during the surgical operation. Whereas IVO offers numerous advantages, one of which is the induction of fewer neurosensory disturbances compared to SSO, IVO requires an extended period of intermaxillary fixation, which makes it not favored in Western developed countries (Wong et al., 2023). It is also stated by previous studies, as neurological problems tend to exhibit a slower recovery in SSO compared to IVO (Yoshioka et al., 2008; Bagheri et al., 2012; Kim, 2017). On the other hand, Wong and Leung(2023) reported that the patients who underwent IVO seemed to have more oral and general health burden in early postoperative period. However, no statistically significant differences was reported between IVO and SSO operations regarding patients' quality of life levels in the late postoperative period. The frequency of inferior alveolar nerve damage during sagittal splint osteotomy ranges from 1.3% to 18%, while sensory disturbances in lower lip and chin region following to the operation have been reported to be 9% to 85% of operated sides (Agbaje et al., 2015). Verweij et al. (2016) indicated that prevalence of altered sensation following SSO increased significantly by age. On the other hand, SSO also carries the risk of facial nerve injury, with a rate ranging from 0.17% to 0.75%. The risk of facial nerve paralysis or hypoesthesia in SSO depends on several factor listed below (Koh et al., 2011; Kim, 2017):

- To inject deeply of local anesthetics with vasoconstrictors
- Mechanical injury by surgical instruments such as chisel or osteotome, particularly during the segmental separation,
- Styloid process fracture during the posterior displacement,
- Damage to soft tissues in the perimandibular region due to slipping of a surgical highspeed instruments either drill or bur during medial osteotomy,
- Facial nerve compression resulting from posterior displacement of segment, and the use of surgical retractors,
- Hematoma.

Medication with steroids during or following to orthognathic surgery is effective on injuries by diminishing pressure due to the postoperative edema. However, if expected recovery does not observed within the first 4-8 months, a second surgical intervention with the aim of grafting the nerve should be considered (Yang and Hwang, 2014; Kim, 2017).

2.6. Tooth extraction

The extraction of the third molars (wisdom teeth) is one of the most frequent procedures performed in the routine clinical practice of oral and maxillofacial surgeon (Roccuzzo et al. 2021; Lee et al., 2022). Regarding its complications, inferior alveolar and lingual nerve damages are the two most serious and notorious complications after impacted third molar extraction as their anatomic position exhibits close proximity to the surgical area (Leung, 2019). The complication incidence of lower third molar operations related to nerve damage was described to be within a wide range, which varies from 0.2% to 8.4% for IAN deficit and 0.1% to 22% for LN deficit (Leung, 2019; Kaleem et al., 2020). In a previous study, examining at 11,599 lower third molar extractions in 6803 patients, Nguyen et al. (2014) reported the incidence of IAN injury as almost 0.7% and that of LN injury as 0.15% in their study. During these kinds of surgeries, both inferior alveolar and lingual nerve can be traumatized by the tooth itself or in an iatrogenic way by the surgical instruments. However, the injury and recovery pathways of inferior alveolar and lingual nerve are quite distinct from one another.

The most likely risk of inferior alveolar nerve injury is from the proximity of the tooth roots, whereas that of lingual nerve injury usually depends on the position of this nerve within the soft tissue medial to the lingual region of alveolar process (Leung, 2019). For the inferior alveolar nerve, when the root of the tooth is directly in contact with the nerve, the force applied by the surgical instruments while elevating the tooth may be transferred to the nerve bundle which in turn may cause compression injury (Ghai et al., 2018). For the lingual nerve, both distally impacted lower third molars and inappropriate flap design, particularly in case of the incision being placed too lingually, greatly affect the injury risk (Tojyo et al., 2019). Considering the cadaver study on determining the anatomical variation of lingual nerve, Behnia et al. (2000) indicated that the lingual nerve was located in its usual position for 85.80% of the samples. Additionally, it was above the lingual crest for 14.05% of samples, and only for one sample it was located in the retromolar pad, particularly just on the surface of the mandible. In their study, the mean horizontal and vertical distances between the nerve from the lingual crest of the alveolar process were reported as just 2.06 ± 1.10 mm and 3.01 ± 0.42 mm, respectively. Additionally, the nerve showed a direct connection with the lingual crest for 26% of the cases.

When compared for their recovery mechanism, lingual nerve injuries appear to pose a greater risk of neurotmesis and mostly repaired with microsurgical techniques (Leung, 2019). Moreover, there is no protective bony canal like the mandibular canal for the lingual nerve, so the regener-

ation time of nerve fiber tends to exhibit random expansion within the soft and cicatrical tissue. Therefore, recovery period of an injured lingual nerve typically needs a significantly much more time than that of the inferior alveolar nerve (Jerjes et al., 2010). Furthermore, since the inferior alveolar nerve runs within the mandibular canal, routine assessment methods such as orthopantomography and computed tomography can be preferred to assess its position. However, magnetic resonance imaging, which is not commonly employed by dental practitioners, is necessary technique to efficiently evaluate the lingual nerve, particularly prior to the third molar surgery (Miloro et al., 1997; Tojyo et al., 2019).

2.7. Dental implant operation

The use of endosseous implants for tooth replacement has gradually gained increased popularity since the 1980s. In the beginning of 1990s, the first preliminary investigations on the incidence rate of nerve injuries due to implantation began to be carried out. It is evident that almost three million people just in the United States have dental implants, and the number of people undergoing that operation is increasing by around 500,000 per year (Renton and Van der Cruyssen, 2023). Depending on the frequency of dental implant placement rising, there was a corresponding increase in clinical trials investigating nerve injuries related to implant fixture day by day. Similar to studies on nerve injuries caused by removal of third molar, investigations of nerve disturbances related to oral implantology revealed a significant variation in reported incidence rates (Steinberg and Kelly, 2015).

Mandibular endosseous implant placement can cause injuries in the peripheral divisions of the trigeminal nerve such as the inferior alveolar and the mental nerve, even with the proper preoperative planning and surgical dexterity during intraoperative management. Listed below are some of the potential reasons of nerve damages related to dental implant operation (Steinberg and Kelly, 2015; Diakonoff and Moreau, 2022).

- The nerve cut or damaged by the drill during the implant bed preparation
- Compression of the neurovascular bundle or the bony canal housing of the nerve as a consequence of improper spacing between the implant fixture apex and superior border of the canal
- Direct pressure on the nerve
- Local anesthesia injection
- Mental nerve damage due to flap incision and/or reflection
- Deposition of bone debris into the mandibular canal, particularly during bone drilling process

- Thermal injury- induced osteitis as a consequence of insufficient irrigation during bone drilling

Unlike nerve injuries caused by most oral surgical procedures, which are typically temporary, implant-related nerve disturbances prone to be more permanent (Kim et al., 2013). Despite the advancements in technology (imaging system used for presurgical planning and computer-guided surgery) and training, the prevention of nerve injuries remains elusive in the oral and maxillofacial surgery field (Renton and Van der Cruyssen, 2023). Libersa et al. (2007) reported on temporary versus permanent nerve injuries sustained during various procedures; where %75 of implant-related nerve injuries were permanent and had poor prognosis for recovery. Several previous studies have reported a prevalence of up to 24% for temporary nerve injuries and 11% for permanent nerve injuries, which result in significant functional changes and disturbances in daily life during speaking, eating, kissing, grooming, applying makeup, or difficulty with cold-induced pain, preventing enjoyment of sports and outdoor activity after dental implant placement (Diakonoff and Moreau, 2022). A recent meta-analysis revealed that most patients' symptom returned to normal as the incidence of nerve damage following to the placement of implant fixture was 13% in the short term (ten days after implant placement) and 3% in the long term (one year after implant placement) (Lin et al., 2016). In another meta-analysis (Padmanabhan et al., 2020), the risk of neurosensory disturbance related to implant placement was defined as 13.50 per 100 person-years. In that study, the authors mentioned that the placement of implant fixture in the anterior site of mandible body had a greater risk depending on the anatomical location of the mental nerve. Numerous *in vivo* studies emphasized the importance of cone-beam computed tomography evaluation in pre-surgical process for determining the length of the anterior and caudal loops of mental nerve as anatomical variants of the existing mandible at the mental foramen region (Mishra et al., 2021; Hui et al., 2022).

It is noteworthy that early assessment of nerve injury symptoms is substantial and critical to improving the quality of dental implant therapy. Importantly, when a patient reports any sign of implant-related nerve injury, the removal or backing out of the implant fixture within the first 24-30 hours is imperative since there is no benefit in removing the dental implant, and additional surgery in the region with neuropathic pain, which can intensify after the relevant time (Renton and der Cruyssen, 2023).

3. Symptoms and assessments of nerve injuries in maxillofacial region

An emphasis should be placed on importance of timely mannered approach in case of iatrogenic trigeminal nerve injuries. Clinicians should

be aware of that sensory nerve injuries associated with dentistry exhibit a recovery rate of 75% exclusively for local anesthetic-related injuries and most of the sensory nerve injuries related to other reasons in dentistry are irreversible. Therefore, it is critical to trace the changes of altered sensation in the postoperative period and it is also imperative for prompt intervention within a 30-hour timeframe as such injuries tend to be permanent beyond that period (Renton, 2018).

From clinical standpoint, inferior alveolar nerve deficit may cause altered sensation in the cutaneous somatic innervation of the lower lip, whereas lingual nerve injury affects the innervation of the anterior two-third of the tongue, and lingual mucoperiosteum of the ipsilateral side. On the other hand, when there is a trauma in the lingual nerve, it may result in a possibly detrimental effect on the taste sensation due to its course running with the chorda tympani as one of the division of facial nerve (Leung et al., 2019). The patient can refer any subjective and verbal descriptions associated with neurosensory disturbances. These descriptive terms and their characteristic features are considered in the list below.

- Allodynia: Painful sensation following to an innocuous stimulus
- Analgesia: Feeling no pain to noxious stimuli
- Anesthetic dolorosa: Painful response in a region that is under anesthesia
- Dysesthesia: Not a normal sensation (either spontaneous or evoked) that is considered unpleasant; there can be an explanation with some definitive words such as tender, prickling, stinging, burning, electric shocks, cold
- Causalgia: Persistent burning pain
- Hyperalgesia: Increased pain to noxious stimuli
- Hyperpathia: Severe and sudden abnormal pain that outlasts noxious stimuli
- Hypoesthesia: Reduced sensation to stimulation; there can be an explanation with some definitive words such as numb, rubbery, swollen, wooden
- Neuralgia: Pain in the distribution of a specific nerve
- Neuropathic pain: Spontaneous pain caused by a lesion or any disease of the somatosensory nervous system. Involves sharp paroxysmal pain, even there is no painful stimuli; there can be an explanation with some definitive words such as throbbing, electric shock, burning, excruciating, wrenching

- Paresthesia: Abnormal sensation occured spontaneously or evocatively that is not unpleasant; there can be an explanation with some definitive words such as tingling, tickling, itching, crawling

Peripheral nerve injuries can be defined using some specific classifications. The first classification system was introduced by Seddon in 1943, which consisted of three types of injury, i.e., neurapraxia, axonotmesis, and neurotmesis, whose definitions varied depending on the extent of damage to axons and coating tissues. In neurapraxia, there is a transient functional loss protecting the nerve continuity. In axonotmesis, the axon is disrupted in surrounding myelin sheats along with preservation of perineurium and epineurium. In neurotmesis defined a situation with complete functional loss because of disconnection or rupture of a nerve and neuroma formation may occur. In 1945, Sunderland classified nerve injuries to five categories according to severity. Sunderland's classification, which is based on the histopathology of peripheral nerve injury and is most useful to anatomists, physiologists, and researchers in neurology field, consisted of adding two more types of axonotmesis to Seddon's classification (Bhandari, 2019; Miloro et al., 2021). A first-degree injury is corresponding to Seddon's neurapraxia, while the second and third-degree involve axon disruption as similar to Seddon's axonotmesis. In fourth-degree injury, disruption of axon, endoneurium, and perineurium is observed. Finally, in fifth degree, there is a complete loss of continuity of nerve corresponding to Seddon's neurotmesis. In 1989, Dellon and Mackinnon introduced a new classification adding a 6^{th} degree of nerve injury to Sunderland's classification and this additional grade combined several of these degrees of injuries per fascicle and is probably more appropriate to the clinical management (MacKinnon and Dellon, 1988; Bhandari, 2019; Evans, 2001).

In 1992, Zuniga and Essick honored a basic testing algorithm, which can be carried out at chairside with minimal equipment, for evaluating the severity of nerve injuries using clinical neurosensory testing methods (Chandan et al., 2021). Zuniga et al. (1995) determined the statistical efficacy of the clinical neurosensory test using surgical findings as the gold standard. On the other hand, a variety of clinical instruments and a scoring system has been developed to measure recovery from trigeminal nerve injury. Clinical measurement instruments include Semmes-Weinstein monofilament (SWMF), static (s) and moving (m) 2-point discrimination, vibratory sensation test, Ten test, shape/texture identification, area localization, and more (Chen et al., 2021). The Medical Research Council (MRC) sensory scale and the Rosén score sensory domain, which were two most comprehensive scoring system related to nerve injuries, can be utilized combining 2 or more of the individual instruments to represent the concept of sensibility as a whole (Chen et al., 2021). The Medical Research

Council sensory scale, originally intended for upper extremities and modified by Mackinnon and Delon for grading trigeminal nerve injuries, allows a global evaluation of neurosensory function, utilizing a combination of aforementioned measurements (Susarla et al., 2007). On this scale, there are four scores ranging from S0 (no recovery) to S4 (complete recovery by objective testing). Moreover, there are also numerous grading scales, particularly for reporting facial nerve function. Those are primarily: The House- Brackmann grading scale (1983), Burres-Fisch system (1986), Nottingham system (1994), and Sunnybrook Scale (1996).

Be that as it may, while it is crucial to track the process of clinical recovery with the methods described above, it is equally important to implement a holistic approach referring the patient for consultation to psychology, social work, neurology, and pain specialists to ensure positive effect on patient's quality of life (Markiewicz et al., 2021).

4. Management of nerve injuries

Despite meticulous preoperative planning and proper surgical technique, nerve injuries may still occur. In this situation, early identification and diagnosis of this complication is utmost importance (Steinberg and Kelly, 2015). Nonetheless, it should be known that all injuries are not managed in exactly the same manner. Currently recommended nerve injuries management strategies can be examined in mentioned below. A strategy for management of nerve injuries in maxillofacial region should be symptom driven. Patient with neurosensory disturbance where either neuropathic pain or complex regional pain syndrome and dysesthesia should be handled differently than those with only neurosensory disturbance (Markiewicz et al., 2021).

As a non-invasive and initial therapy, pharmacologic management of nerve injuries treatment consists of a challenge for suppressing the inflammatory process. Anti-inflammatory drugs should be prescribed for the initial pharmacologic management. If the clinician observed any nerve trauma during surgery, corticosteroids should be considered as they may prevent a neuroma. In this acute phase, topically applied of 1 to 2 ml of dexamethasone in a volume of 4 mg/ml for 1 to 2 minutes may help to reduce neural inflammation and swelling that cause an additional compression on the nerve (Juodzbalys et al., 2011; Markiewicz et al., 2021). During the surgical intervention, if a transection is observed intraoperatively, micro neurosurgical treatment should be immediately considered as an indication as there is no reason to sit and wait for exploration and repair of the nerve (Hillerup, 2008; Renton, 2018). When the clinician is unable to perform micro neurosurgical treatment him/herself and need to refer the patient for specialist care, at least, it would be valuable to tag the nerve ends with a

prolene suture to facilitate its identification at the secondary repair (Yang and Tufaro, 2020).

Considering the prescription of oral corticosteroid after nerve injuries in order to prevent postoperative sequelae, Juodzbalys et al. (2011) stated that two of the 4 mg of dexamethasone tablets, taken for 3 days and one tablet for next 3 days or oral prednisolone 1 mg/kg/day with a maximum dose of 80 mg in total can be prescribed. In their article, it is also mentioned that a high dose of NSAIDs (such as 600 and 800 mg ibuprofen) taken every 8 hours in a day during 3 weeks could be considered as an alternative treatment or be used in addition to corticosteroid treatment. Moreover, Renton and Cruyssen (2023) reported the initial medical management, which includes a short course of high-dose oral NSAIDs, corticosteroids 5-day step-down course, and vitamin B complex for three months. In the literature, it is also mentioned that capsaicin, a substance P depletion medication may be used for treatment of traumatic injury of in trigeminal nerve injury (Markiewicz et al., 2021). As a rule, after 1 week of initial investigation for nerve injury, oral and maxillofacial surgery specialists should continue to trace the neurosensory disturbance on each week during 3 weeks and later on every 2 or 3 weeks during 12 weeks. There are also numerous additional non-invasive therapy methods for nerve injury (Juodzbalys et al., 2011). Those can be listed as; cryotherapy, physiologic therapies including transcutaneous electric nerve stimulation (Bates et al., 1980), acupuncture (Sung et al., 1977), or low-level laser therapy (Poole et al., 1993), and prescription of antidepressants, anticonvulsants. If neurosensory deficit after 12 weeks does not show any resolution, the patient should be given the option for surgical repair. For surgical methods, nerve anastomosis (in terms of a nerve conduit or end-to-end repair) or nerve graft including autologous nerve (e.g., sural nerve or greater auricular nerve) and allogenic nerve (e.g. decellularized human nerve grafts) are the gold standard for the treatment of nerve injury (Yang and Tuffaro, 2020).

Many authors advised referral of injuries before 4 months; however, this may be too late for many peripheral sensory nerve injuries as inferior alveolar nerve injuries related to dentistry require early intervention with permanency preferably after 24–30 hours (Renton, 2018). Risk of nerve injury being permanent greatly increases after this period. Local anesthesia-, endodontic- and implant-related nerve injuries also warrants early intervention. In any event, clinicians who are responsible for the nerve injury must be honest and caring with the patient. Therefore, a home check within 6- 24 h with a phone call after any dental procedure with a high risk, makes the clinicians to ensure that if there is any consequence associated with the nerve injury (Renton, 2013).

Regarding the nerve injuries related to endodontic procedures, neuropathy can be delayed, in so that, the clinicians should inform the patient the importance of reporting any altered sensation up to 3-4 days in postoperative healing process. Once neuropathy is identified, the clinician must reassure the patient prescribe steroids (Prednisolone step down 15 mg 5 days, 10 mg 5 days and 5 mg 5 days, high-dose NSAIDs, 600 mg Ibuprofen 6 hourly, and vitamin B complex). If the reason of changes sensation is an endodontic material into the IAN canal, removal of the apex and or tooth must be performed within 48 h in order to maximize recovery from nerve injury. If the patient prefers to preserve the natural tooth, it may necessary to refer the patient for mandibular decompression and saline irrigation of the inferior alveolar canal. Moreover, if there is no evidence of the endodontic material in proximity of the nerve, the reason may be related to apical inflammation, chemical nerve injury from irrigation solution or filler material and thermal damage (Renton, 2018).

On the other hand, when the nerve injury related to dental implant operation is considered, Steinberg et al. (2015) emphasized that not all implant-related nerve injuries are managed in exactly the same manner. According to the algorithm reported by Steinberg et al. (2015);

- If the implant is not close to vital structures and patient complaints include numbness, a regimen of anti-inflammatory medication (steroids or nonsteroidal medications) continued observation is recommended.

- If it can be shown through radiographic evidence that implant impinges on the canal (but not through the canal), complete removal of the implant or backing out until the impingement is alleviated is recommended. During this process, anti-inflammatory medications may be used while observing for recovery. In case, in which the canal has been transected, removing or backing out the implant will not undo or improve the prognosis. In many cases the IAN is able to be repaired with the implant still left in place. Therefore, early referral for evaluation and possible nerve repair should be considered.

Lastly, considering the management of local anesthesia injury, while it is only known that one fourth of these injuries are permanent, clinicians should not neglect to carry on early medical intervention. Renton et al. (2021) reported the medical management of this injury, prescribing a high-dose oral NSAIDs (400, 600 or 800 mg Ibuprofen) for 2 days. As an alternative treatment, consuming of prednisolone for 5 days with a step down regimen in dosage of 50-40-30-20-10 mg orally can be considered. Additionally, either under NSAIDs or prednisolone treatment, vitamin B complex (Riboflavin 400 mg once daily for maximum of 3 months plus another Vit B complex) should be prescribed.

REFERENCES

Agbaje, J. O., Salem, A. S., Lambrichts, I., Jacobs, R., & Politis, C. (2015). Systematic review of the incidence of inferior alveolar nerve injury in bilateral sagittal split osteotomy and the assessment of neurosensory disturbances. *International journal of oral and maxillofacial surgery*, 44(4), 447-451.

Aquilanti, L., Mascitti, M., Togni, L., Contaldo, M., Rappelli, G., & Santarelli, A. (2022). A Systematic Review on Nerve-Related Adverse Effects following Mandibular Nerve Block Anesthesia. International journal of environmental research and public health, 19(3), 1627. https://doi.org/10.3390/ijerph19031627

Alves, F. R., Coutinho, M. S., & Gonçalves, L. S. (2014). Endodontic-related facial paresthesia: systematic review. *Journal (Canadian Dental Association)*, 80, e13.

Atkinson, M. E. (2013). Anatomy for dental students (4th ed.). Oxford University Press.

Bage, T., & Power, D. M. (2021). Iatrogenic peripheral nerve injury: a guide to management for the orthopaedic limb surgeon. *EFORT open reviews*, *6*(8), 607–617.

Bagheri, S. C., & Meyer, R. A. (2012). Management of trigeminal nerve injuries. *Current therapy in oral and maxillofacial surgery*, 224-237.

Bates JAV, Nathan PW. (1980). Transcuaneous electrical nerve stimulation for chronic pain. *Anaesthesia*, 35:817-824.

Behnia H, Kheradvar A, Shahrokhi M. (2000) An anatomic study of the lingual nerve in the third molar region. *J Oral Maxillofac Surg*, 58:649–653.

Bhandari, P. S. (2019). Management of peripheral nerve injury. *Journal of clinical orthopaedics and trauma*, 10(5), 862-866.

Büttner, R., Schulz, A., Reuter, M., Akula, A. K., Mindos, T., Carlstedt, A., & Morrison, H. (2018). Inflammaging impairs peripheral nerve maintenance and regeneration. *Aging cell*, 17(6), e12833.

Campbell RL, Parks KW, Dodds RN. (1990). Chronic facial pain associated with endodontic therapy. *Oral Surg Oral Med Oral Pathol,* 69: 287–90.

Censi, R., Vavassori, V., Borgonovo, A. E., & Re, D. (2016). Infection Related Inferior Alveolar Nerve Paresthesia in the Lower Premolar Teeth. *Case reports in dentistry*, 2016, 2623507.

Chandan, S. N., Shetty, S. K., Shetty, S. K., & Shah, A. K. (2021). Inferior alveolar nerve dysfunction in mandibular fractures: a prospective cohort study. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, 47(3), 183-189.

Chen, L., Ogalo, E., Haldane, C., Bristol, S. G., & Berger, M. J. (2021). Relationship Between Sensibility Tests and Functional Outcomes in Patients With Traumatic Upper Limb Nerve Injuries: A Systematic Review. *Archives of*

rehabilitation research and clinical translation, 3(4), 100159.

Chuong R, Donoff RB, Guralnick WC. (1983) A retrospective analysis of 327 mandibular fractures. *J Oral Maxillofac Surg*, 41: 305–309.

de Matos, F. P., Arnez, M. F., Sverzut, C. E., & Trivellato, A. E. (2010). A retrospective study of mandibular fracture in a 40-month period. *International journal of oral and maxillofacial surgery*, 39(1), 10–15.

Diakonoff, H., & Moreau, N. (2022). Inferior alveolar nerve injury following dental implant placement: A medicolegal analysis of French liability lawsuits. *Journal of Stomatology, Oral and Maxillofacial Surgery*, 123(2), 158-162.

Dos Santos Alves, J. M., de Freitas Alves, B. W., de Figueiredo Costa, A. C., Carneiro, B. G. D. S., de Sousa, L. M., & Gondim, D. V. (2019). Cranial nerve injuries in Le Fort I osteotomy: a systematic review. *International journal of oral and maxillofacial surgery*, 48(5), 601–611.

Ellis, H., & Mahadevan, V. (2013). Clinical anatomy: applied anatomy for students and junior doctors. John Wiley & Sons.

Evans, G. R. (2001). Peripheral nerve injury: a review and approach to tissue engineered constructs. *The Anatomical Record: An Official Publication of the American Association of Anatomists*, 263(4), 396-404.

Genc Sen, O., & Kaplan, V. (2015). Temporary Mental Nerve Paresthesia Originating from Periapical Infection. *Case reports in dentistry*, 457645.

Ghai S, Choudhury S. (2018). Role of panoramic imaging and cone beam CT for assessment of inferior alveolar nerve exposure and subsequent paresthesia following removal of impacted mandibular third molar. *J Maxillofac Oral Surg*, 17:242-7.

Hillerup S. (2008). Iatrogenic injury to the inferior alveolar nerve: etiology, signs and symptoms, and observations on recovery. *Int J Oral Maxillofac Surg*, 37(8):704-9.

Hillerup, S., Jensen, R. H., & Ersbøll, B. K. (2011). Trigeminal nerve injury associated with injection of local anesthetics: needle lesion or neurotoxicity?. *Journal of the American Dental Association*, 142(5), 531–539.

Hogan, Q. H. (2008). Pathophysiology of peripheral nerve injury during regional anesthesia. *Regional anesthesia and pain medicine*, 33(5), 435-441.

Hui, L., Hung, K.F., Bornstein, M.M. et al. (2022). Assessment of the prevalence and length of the anterior and caudal loops of the mental nerve as anatomical variants of exiting the mandible at the mental foramen using cone-beam computed tomography: a systematic review and meta-analysis. *Clin Oral Invest*, 26, 6423–6441.

Jędrzejewski M, Smektała T, Sporniak-Tutak K, Olszewski R. (2015). Preoperative, intraoperative, and postoperative complications in orthognathic surgery: a systematic review. *Clin Oral Investig*, 19:969–977.

Jerjes W, Upile T, Shah P, Nhembe F, Gudka D, Kafas P et al. (2010). Risk factors

associated with injury to the inferior alveolar and lingual nerves following third molar surgery—revisited. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 109:335–345

Juodzbalys, G., Wang, H. L., & Sabalys, G. (2011). Injury of the inferior alveolar nerve during implant placement: a literature review. *Journal of oral & maxillofacial research*, 2(1), e1.

Kaleem, A., Amailuk, P., Hatoum, H., & Tursun, R. (2020). The Trigeminal Nerve Injury. *Oral and maxillofacial surgery clinics of North America*, 32(4), 675–687.

Khan, T. U., Rahat, S., Khan, Z. A., Shahid, L., Banouri, S. S., & Muhammad, N. (2022). Etiology and pattern of maxillofacial trauma. *PloS one*, 17(9), e0275515.

Khoury, J., & Townsend, G. (2011). Neural blockade anaesthesia of the mandibular nerve and its terminal branches: rationale for different anaesthetic techniques including their advantages and disadvantages. *Anesthesiology research and practice*, 307423.

Kim, Y. T., Pang, K. M., Jung, H. J., Kim, S. M., Kim, M. J., & Lee, J. H. (2013). Clinical outcome of conservative treatment of injured inferior alveolar nerve during dental implant placement. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, 39(3), 127.

Kim, E. J., Kim, H. Y., & Ahn, J. H. (2020). Neurotoxicity of local anesthetics in dentistry. *Journal of dental anesthesia and pain medicine*, 20(2), 55–61.

Kim, Y. K. (2017). Complications associated with orthognathic surgery. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, 43(1), 3-15.

Koh KM, Yang JY, Leem DH, Baek JA, Ko SO, Shin HK. (2011). Facial nerve palsy after sagittal split ramus osteotomy: follow up with electrodiagnostic tests. *J Korean Assoc Maxillofac Plast Reconstr Surg*., 33:190–197.

Kulkarni, V., Sahoo, N. K., Roy, I. D., & Ghosh, S. (2021). Neurosensory evaluation of inferior alveolar nerve following mandibular fracture fixation using modified Zuniga and ESSICK'S protocol. *Advances in Oral and Maxillofacial Surgery*, *4*, 100171.

Lee J, Park J, Moon SY, Lee K. (2022). Automated Prediction of Extraction Difficulty and Inferior Alveolar Nerve Injury for Mandibular Third Molar Using a Deep Neural Network. *Applied Sciences*, 12(1):475.

Lee MH, Kim HJ, Kim DK, Yu SK. (2015). Histologic features and fascicular arrangement of the inferior alveolar nerve. *Arch Oral Biol*, 60(12):1736-41.

Leung, Y. Y. (2019). Management and prevention of third molar surgery-related trigeminal nerve injury: time for a rethink. *Journal of the Korean Association of Oral and Maxillofacial Surgeons*, 45(5), 233-240.

Libersa P, Savignat M, Tonnel A. (2007). Neurosensory disturbances of the inferior alveolar nerve: a retrospective study of complaints in a 10-year period. *J*

Oral Maxillofac Surg, 65(8):1486–9.

Lin, C. S., Wu, S. Y., Huang, H. Y., & Lai, Y. L. (2016). Systematic Review and Meta-Analysis on Incidence of Altered Sensation of Mandibular Implant Surgery. *PloS one*, 11(4), e0154082.

MacKinnon SE, Dellon AL. 1988b. Classification of nerve injuries as the basis for treatment. In: Mackinnon SE, Dellon AL, editors. Surgery of the peripheral nerve. New York: Thieme. p 35–63.

Markiewicz, M. R., Callahan, N., & Miloro, M. (2021). Management of traumatic trigeminal and facial nerve injuries. *Oral and Maxillofacial Surgery Clinics*, 33(3), 381-405.

Miloro M, Halkias LE, Slome HW, Chakeres DW. (1997). Assessment of the lingual nerve in the third molar region using magnetic resonance imaging. *J Oral Maxillofac Surg*, 55:134–137.

Mishra, S.K., Nahar, R., Gaddale, R. et al. (2021). Identification of anterior loop in different populations to avoid nerve injury during surgical procedures—a systematic review and meta-analysis. *Oral Maxillofac Surg,* 25, 159–174.

Nguyen E, Grubor D, Chandu A. (2014). Risk factors for permanent injury of inferior alveolar and lingual nerves during third molar surgery. *J Oral Maxillofac Surg*, 72:2394–401.

Ozen T, Orhan K, Gorur I, et al. (2006). Efficacy of low level laser therapy on neurosensory recovery after injury to the inferior alveolar nerve. *Head Face Med*, 2:3.

Ozkan, B. T., Celik, S., & Durmus, E. (2008). Paresthesia of the mental nerve stem from periapical infection of mandibular canine tooth: a case report. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics*, *105*(5), e28–e31.

Padmanabhan, H., Kumar, A. V., & Shivashankar, K. (2020). Incidence of neurosensory disturbance in mandibular implant surgery - A meta-analysis. *Journal of Indian Prosthodontic Society*, 20(1), 17–26.

Park, S. Y., Jaiswal, M. S., Jang, Y. S., Choi, J. H., Kim, G. C., Hong, J. W., & Hwang, D. S. (2022). Effect of No-ozone Cold Plasma on Regeneration after Crushed Mental Nerve Injury in rats. *International journal of medical sciences*, 19(12), 1732–1742.

Pelka, M., & Petschelt, A. (2008). Permanent mimic musculature and nerve damage caused by sodium hypochlorite: a case report. *Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics*, 106(3), e80–e83.

Piccinni C., Gissi D.B., Gabusi A., Montebugnoli L., Poluzzi E. (2015). Paraesthesia after Local Anaesthetics: An Analysis of Reports to the FDA Adverse Event Reporting System. *Basic Clin. Pharmacol. Toxicol,* 117:52–56.

Pogrel M. A. (2017). Nerve damage in dentistry. *General dentistry*, *65*(2), 34–41.

Pogrel M.A. (2012). Permanent Nerve Damage from Inferior Alveolar Nerve

Blocks: A Current Update. *J. Calif. Dent. Assoc*, 40:795–797

Pogrel MA, Jergensen R, Burgon E, Hulme D. (2011). Long-term outcome of trigeminal nerve injuries related to dental treatment. *J Oral Maxillofac Surg*, 69(9):2284–8.

Pogrel, M. A. (2011). Nerve damage in dentistry. Current Therapy In Oral and Maxillofacial Surgery, 271.

Polycarpou, N., Ng, Y. L., Canavan, D., Moles, D. R., & Gulabivala, K. (2005). Prevalence of persistent pain after endodontic treatment and factors affecting its occurrence in cases with complete radiographic healing. *International endodontic journal*, 38(3), 169–178.

Poole TE, Holland I, Peterson LJ. (1993). Clinical efficacy of low level laser treatment of oro-facial neurosensory deficits. *J Oral Maxillofac Surg*, 51(suppl3): 182-6.

Poorian, B., Bemanali, M., & Chavoshinejad, M. (2016). Evaluation of Sensorimotor Nerve Damage in Patients with Maxillofacial Trauma; a Single Center Experience. *Bulletin of emergency and trauma*, 4(2), 88–92.

Renton, T. (2013). Nonsurgical management of trigeminal nerve injuries. *Trigeminal nerve injuries*, 213-227.

Renton, T. (2018). Trigeminal nerve injuries. *Australian Endodontic Journal*, *44*(2), 159-169.

Renton, T. (2021). Trigeminal Nerve Injuries. In: Bonanthaya, K., Panneerselvam, E., Manuel, S., Kumar, V.V., Rai, A. (eds) Oral and Maxillofacial Surgery for the Clinician. Springer, Singapore.

Renton, T., & Van der Cruyssen, F. (2023). Posttraumatic Trigeminal Neuropathic Pain in Association with Dental Implant Surgery. *Dental Clinics*, 67(1), 85-98.

Renton, T., & Yilmaz, Z. (2011). Profiling of patients presenting with posttraumatic neuropathy of the trigeminal nerve. *Journal of orofacial pain*, 25(4), 333.

Roccuzzo A, Molinero-Mourelle P, Ferrillo M, Cobo-Vázquez C, Sanchez-Labrador L, Ammendolia A, Migliario M, de Sire A. (2021). Type I Collagen-Based Devices to Treat Nerve Injuries after Oral Surgery Procedures. A Systematic Review. *Applied Sciences*, 11(9):3927.

Rosen E. Nerve injury during endodontic surgical procedures. In: Tsesis I, editor. Complications in endodontic surgery. Berlin Heidelberg: Springer-Verlag; 2014. p. 137–51.

Rosen, E. (2017). The diagnosis and management of nerve injury during endodontic treatment. *Evid.-based endod*, 2, 7.

Sanders R. D. (2010). The Trigeminal (V) and Facial (VII) Cranial Nerves: Head and Face Sensation and Movement. Psychiatry (Edgmont (Pa.: Township)), 7(1), 13–16.

Schiavone, M., & Ziccardi, V. B. (2021). Trigeminal nerve injuries in oral and maxillofacial surgery: a literature review. *Frontiers of Oral and Maxillofacial Medicine*, 3, 28.

Sivolella, S., Brunello, G., Ferrarese, N., Della Puppa, A., D'Avella, D., Bressan, E., & Zavan, B. (2014). Nanostructured guidance for peripheral nerve injuries: a review with a perspective in the oral and maxillofacial area. *International journal of molecular sciences*, *15*(2), 3088–3117.

Steinberg, M. J., & Kelly, P. D. (2015). Implant-related nerve injuries. *Dental clinics of North America*, *59*(2), 357–373.

Sung YF, Kutner MH, Cerine FC, Frederickson EL. (1977). Comparison of the effects of acupuncture and codeine on postoperative dental pain. *Anesth Analg*, 56(4):473-8.

Susarla, S. M., Kaban, L. B., Donoff, R. B., & Dodson, T. B. (2007). Functional sensory recovery after trigeminal nerve repair. *Journal of oral and maxillofacial surgery*, 65(1), 60-65.

Thygesen, T. H., Bardow, A., Norholt, S. E., Jensen, J., & Svensson, P. (2009). Surgical risk factors and maxillary nerve function after Le Fort I osteotomy. *Journal of oral and maxillofacial surgery*, 67(3), 528-536.

Tojyo, I., Nakanishi, T., Shintani, Y. et al. (2019). Risk of lingual nerve injuries in removal of mandibular third molars: a retrospective case-control study. *Maxillofac Plast Reconstr Surg*, 41, 40.

Verweij JP, Mensink G, Fiocco M, van Merkesteyn JP. (2016). Incidence and recovery of neurosensory disturbances after bilateral sagittal split osteotomy in different age groups: a retrospective study of 263 patients. *Int J Oral Maxillofac Surg*, 45:898–903.

Waldman, S. D. (2014). Atlas of Interventional Pain Management E-Book. Elsevier Health Sciences.

Wolf KT, Brokaw EJ, Bell A, Joy A. (2016). Variant Inferior Alveolar Nerves and Implications for Local Anesthesia. *Anesth Prog*, 63(2):84-90.

Wong, N. S. M., & Leung, Y. Y. (2023). Comparison of the quality of life changes of patients receiving sagittal split ramus osteotomy or intraoral vertical subsigmoid osteotomy for mandibular prognathism. *Clinical oral investigations*, 27(4), 1435–1448.

Yang HJ, Hwang SJ. (2014). Contributing factors to intraoperative clockwise rotation of the proximal segment as a relapse factor after mandibular setback with sagittal split ramus osteotomy. *J Craniomaxillofac Surg*, 42:e57–e63.

Yang, R., & Tufaro, A. P. (2020). Secondary Nerve Reconstruction. In Facial Trauma Surgery (pp. 514-521). Elsevier.

Yoshioka, I., Khanal, A., Tominaga, K., Horie, A., Furuta, N., & Fukuda, J. (2008). Vertical ramus versus sagittal split osteotomies: comparison of stability after mandibular setback. *Journal of oral and maxillofacial surgery: of-*

ficial journal of the American Association of Oral and Maxillofacial Surgeons, *66*(6), 1138–1144.

Zuniga, J. R., Meyer, R. A., Gregg, J. M., Miloro, M., & Davis, L. F. (1998). The accuracy of clinical neurosensory testing for nerve injury diagnosis. *Journal of oral and maxillofacial surgery*, 56(1), 2-8**.**

CHAPTER 13

THE ELEMENTS IN CLINICAL LABORATORY MEDICINE

Gamze GÖK[1]

1 Uz. Dr., T.C. Sağlık Bakanlığı Ankara Bilkent Şehir Hastanesi Tıbbi Biyokimya Laboratuvarı, Orchid No:0000-0002-2804-5548

According to National Committee for Clinical Laboratory Standards (NCCLS), elements can be divided into four groups. The first group is the essential major elements. This group's members are; Sodium, magnesium, potassium, calcium, phosphorus, sulfur, and chlorine. The second group is the essential trace elements including these members; vanadium, chromium, manganese, molybdenum, cobalt, copper, zinc, selenium, iron, and iodine. The third group is non-essential toxic elements involving these members: aluminum, beryllium, arsenic, cadmium, mercury, thallium, and lead. The fourth group is therapeutic elements including these members: lithium, platinum, and gold (Parsons & Barbosa Jr, 2007).

1. Essential Major Elements

1.1 Sodium

The extracellular fluid's dominant cation in the body is sodium (Barma et al., 2018). Sodium has various roles in the body. It helps regulate the cell membrane's potential and takes place in the maintenance of the extracellular fluid's volume. In the cell membrane during the active transport sodium exchanges through potassium (Ferrannini, 2017). When the level of serum sodium is lower than 135 mmol/L hyponatremia occurs. Some of the hyponatremia symptoms are headache, nausea, and confusion. When the level of serum sodium is higher than 145 mmol/L hypernatremia occurs. Some of the hypernatremia symptoms are restlessness, sleeping difficulties, and tachypnea (Buffington & Abreo, 2016). The kidneys regulate sodium levels. (Palmer & Schnermann, 2015)

1.2 Magnesium

Magnesium is a cation located in the intracellular part of the cell. Magnesium takes place in the metabolism of adenosine triphosphate (ATP), and the functions of muscles and neurons (Jahnen-Dechent & Ketteler, 2012). When the serum magnesium level is lower than 1.46 mg/dL hypomagnesemia arises. Hypomagnesemia may occur due to omeprazole medication, alcohol-consuming disorders, excessive losses through the kidneys, and gastrointestinal system conditions. Hypomagnesemia symptoms involve ventricular arrhythmias (Hansen & Bruserud, 2016).

1.3 Potassium

Potassium is an ion located in the intracellular part of the cell. The Sodium-potassium homeostasis is regulated by the sodium-potassium ATPase pump. Sodium is pumped out of the cell and potassium is entered in the cell by this pump. Potassium filtration takes part in the glomerulus of the kidneys. In the proximal tubule and the Henle loop, potassium reabsorption occurs (Gumz, Rabinowitz, & Wingo, 2015).

Potassium imbalance may cause arrhythmia. When the level of serum potassium is lower than 3.6 mmol/L hypokalemia arises. Hypokalemia symptoms involve fatigue and weakness (Stedwell, Allen, & Binder, 1992) When the level of serum potassium is higher than 5.5 mmol/L hyperkalemia arises. Hyperkalemia may cause arrhythmias. Hypercalemic symptoms include rhabdomyolysis, cramps and weakness in the muscles, and myoglobinuria (Viera & Wouk, 2015).

1.4 Calcium

Calcium is an important element for physiological metabolic reactions. Calcium takes place in the mineralization of the bones, muscle contraction movements, coagulation reactions, and hormone secretion. The major calcium source is the diet. Calcium is a cation located in the extracellular part of the cell. Parathyroid hormone, calcitonin, and vitamin D have roles in calcium regulation (Shrimanker & Bhattarai, 2019).

When the corrected levels of serum calcium are lower than 8.8 mg/dL hypocalcemia arises. In Hypocalcemia it is recommended to check the serum albumin levels for correction of total calcium. In hypoparathyroidism, hypocalcemia occurs and post-thyroidectomy patients' serum calcium levels must be checked. Vitamin D deficiency is also related to hypocalcemia (Cooper & Gittoes, 2008). When the corrected levels of serum calcium are higher than 10.7 mg/dL hypercalcemia arises. Primary hyperparathyroidism occurs with hypercalcemia. Also, hypercalcemia may present in some kinds of malignancies (Turner, 2017).

1.5 Phosphorus

Phosphorus is a cation located in the extracellular fluid of the cell. In the body, 85% of the phosphorus is in the hydroxyapatite form. The teeth and the bones have hydroxyapatite form in their structure. In the body, 15 % of the phosphorus is in the soft tissues. Phosphate is a key player in metabolic reactions. Nucleotide metabolism and ATP metabolism are related to phosphate. Parathyroid hormone, calcitonin, and vitamin D have roles in phosphate regulation. Excretion of phosphorus takes place in the kidneys (Berkelhammer & Bear, 1984). Dysregulation of serum sa, potassium reabsorption occurs.

1.6 Sulfur

Sulfur exists in the environment throughout the growth of the lifeforms. The entrance of sulfur into the body can be by organic and inorganic compounds. sulfur is an essential element for the structure of the body. It has a crucial role in the intermediary metabolism and energy mechanism. Sulfur

is involved in the defense system, It has roles against the reactive species. During a healthy balanced diet usually, there is no additional sulfur intake is recommended. Because humans are taking adequate levels of sulfur in their food, but for those who are preferring restrictive and imbalanced diets supplements including sulfur may be beneficial (Mitchell, 2021).

1.7 Chlorine

Chloride is an anionic element of the extracellular fluid of the cell. Serum chloride levels are regulated by the kidneys. Glomerulus filters the chlorine and its reabsorption occurs in the tubules by the active and passive transports (Walker, Hall, & Hurst, 1990).

Bicarbonate loss through the gastrointestinal system, vomiting, and heart failure may cause hypochloremia. The saline infusion may cause hyperchloremia (Shrimanker & Bhattarai, 2019).

2. Essential Trace Elements

In general, trace elements are found in amounts of micrograms or picograms in biological samples. In the body trace elements' metabolic process from absorption to excretion is under a tight control mechanism (Bethesda, 2012). It is hard to diagnose deficiency of the trace elements. The validated methods for the diagnosis are limited. For trace element measurement serum, urine, hair, lymphocytes, erythrocytes, and leukocytes can be used. Some enzymes involve trace elements in their core. for these kinds of enzymes enzyme activity measurement can be performed. Moreover, some function tests such as electroretinogram, taste, and dark adaptation can be performed. Furthermore, there are balance studies for many elements but this method is not practical (Osamu, 2004).

2.1.Vanadium

Vanadium exists in the human body and some food sources. In the body vanadium regulates some proteins which are linked to phosphate. Some vanadium including formulations, organic or inorganic compounds is converted to vanadate or oxi vanadium. Some studies have shown vanadium compounds' potential effect against type 2 Diabetes Mellitus, cancer, human immunodeficiency viruses, and some tropical and bacterial ailments including amoebiasis, trypanosomiasis, and pneumonia. Moreover, vanadium medications are supposed to be efficient in the protection of cardiovascular and neuronal systems but vanadium preparations have not got any permission for use as drugs (Rehder, 2016).

2.2. Chromium

Chromium oxidation conditions are related to its stability in the existence of water and oxygen. Chromium(6+) involving combined molecu-

les can form mutagenesis and carcinogenesis if these molecules enter the body by inhalation or oral ingestion in abundant quantities. Chromium can enhance the efficacy of insulin hormone and by this property, it has the regulator role for the metabolism of carbohydrates, lipids, and protein. Chromium was known as an essential element but in 2014 it was declared that no persuasive evidence exists. Nearly 1% of dietary chromium intake is absorbed in the body. The chromium binds to transferrin and it is supposed that transferrin has a role in chromium delivery to tissues. Up to now there isn't any animal model including chromium deficiency has been set, and also there is not any chemical compound that has the effect of chromium role in glucose tolerance has not been defined yet either (Vincent & Lukaski, 2018).

2.3. Manganese

Manganese is a coenzyme existing in many metabolic reactions. Manganese is an important element for the metabolism of macronutrients, neurotransmitters, and antioxidant systems. Manganese also takes place in the cleaning of ammonia and the development of the bones. Manganese is located in concentrated values in the tissues that are abundant in mitochondria. The liver and the pancreas are the organs having high levels of manganese during normal status. Manganese may have toxic properties when manganese exposure in over dosages occurs. Manganese toxicity may take place in working in industry or consuming polluted water containing manganese. Manganese may accumulate in the brain and may cause neurological disorders **(**Erikson & Aschner, 2019**).**

Manganese metalloproteins have roles in oxidoreductase, transfer, hydrolysis, ligase, and lyase reactions. Moreover, arginase and manganese superoxide dismutase contain manganese (Aschner & Aschner, 2005; Horning, Caito, Tipps, Bowman, & Aschner, 2015).

2.4. Molybdenum

Molybdenum is a trace element and it is necessary for plants, animals, and some kind of micro-organisms. Karl Scheele discovered molybdenum in 1778. Molybdenum word meaning comes from the lead-like. Molybdenum's role as a part of some enzymes was first defined in the 1950s. Sulfite, aldehyde, and xanthine oxidases require molybdenum. The sulfite oxidase enzyme is located in the mitochondria and takes place in the oxidation reaction of sulfur-containing amino acids. In the conversation reaction of xanthine, xanthine oxidase takes place. Xanthine oxidase can prevent mutations in DNA. Aldehyde oxidase is located in the liver in plenty of amounts and this enzyme is crucial for the metabolism of the drugs (Novotny, 2018).

Molybdenum cofactor deficiency is defined as myoclonic encephalopathy and MRI changes in the cerebrum that are similar to hypoxic and ischemic lesions. Molybdenum cofactor deficiency may occur in the reason of the activity absence of the sulfite oxidase enzyme (Johannes, Fu, & Schwarz, 2022).

2.5.Cobalt

Cobalt is an essential element and it is the coenzyme of Vitamin B12. Cobalt is usually found in +2 and +3 oxidation status forms. Cobalt is found in both inorganic and organic salts (Jomova et al., 2022). Some of the cobalt's features make cobalt derivatives promising agents of medication. Cobalt-containing compounds take place in the activity modification of the therapeutic agents and inhibition of the proteins (Heffern, Yamamoto, Holbrook, Eckermann, & Meade, 2013). Cobalt +3 is a strong and cobalt+2 is a borderline acidic compound (Jomova et al., 2022).

2.6. Copper

Copper is a trace element that takes place in vital organisms. Copper is found in +1 and +2 status. Copper has catalytic properties in protein metabolism and the growth of the body. Adult individuals' copper level intakes change between nearly 0.6 mg/dL to 1.6 mg/dL (Linder, 2013). Copper sources are liver, seeds, beans, and nuts. In normal conditions drinking water has nearly no contribution to copper levels(Halliwell & Gutteridge, 1984).

Copper takes place in respiration of the mitochondrial, absorption of iron. It is a scavenger for free radicals. Moreover copper has roles in elastin formation. But excess free copper ions may be harmful to the cells' components and cellular damage may occur. Copper levels indicate their homeostasis. Some proteins including glutathione, Menkes proteins, metallothionein, copper chaperones, and Wilson proteins take place in the homeostasis of the copper (Tapiero, Townsend, & Tew, 2003).

The kidney, the liver, and the brain contain copper at high levels. Bones and muscles are big in size in the body and due to this situation, they include much of the copper. Copper binding to ceruloplasmin reaction takes place in the liver, by this compound, copper can transport to the tissues of the peripheral side of the body. For many proteins of the body copper serves as a cofactor but surplus copper levels may be harmful for the cell and its components. Copperiedus means copper toxicity. Copperiedeus can occur due to exposure to surplus copper levels by food or water (Royer & Sharman, 2020).

2.7. Zinc

Zinc is one of the essential trace elements and it has an important role

as a micronutrient in nutrition (Akdeniz, KINIK, Yerlikaya, & Ecem, 2016). After zinc is taken with food; Approximately 15-30% of its absorption takes place in the duodenum and is excreted through urine and sweat, mostly with feces. The liver is the main organ involved in zinc metabolism. Zinc deficiency can be seen in rapid growth processes, pregnancy, old age, liver diseases, and prolonged parenteral nutrition. Zinc deficiency may result in some clinical findings such as intrauterine growth retardation, growth retardation, hypogonadism, hepatosplenomegaly, parakeratosis, alopecia, delayed wound healing, congenital anomalies, increased susceptibility to infections, impaired neurophysiological performance, and impaired sense of smell and taste (Saner, Neyzi, & Ertuğrul, 2002). Acrodermatitis enteropathica is a rare inherited disease that occurs as a result of a primary defect in zinc metabolism in humans. There are some studies stating that in case of excess zinc; apoptosis is inhibited and stimulated in case of zinc deficiency. TNF-alpha production is decreased in zinc deficiency. Zinc takes place in DNA synthesis. Zinc is essential for normal fetal growth and development. Zinc, which is in the structure of more than 200 enzymes, proteins, hormones, and neuropeptides, increases transcription in the cell and is also necessary for cell division, growth, and differentiation (Belgemen & Nejat, 2004).

2.8. Selenium

Selenium is an essential element and it has a fundamental importance for human life. Selenium is found in the structure of many enzymes. It serves as a cofactor. It plays a role in thyroid hormone metabolism, antioxidant enzyme defense mechanism, and regulation of the immune system. In addition, some studies; showed that selenium deficiency may be associated with aging, cancer, insulin resistance, diabetes, cardiovascular and neurodegenerative diseases, and even increased risk of mortality. The health effects of dietary selenium intake above the recommended dose are not clear. Selenium plays a role in protecting endothelial cells from peroxynitrite damage. Selenium reduces the effect of reactive oxygen species, and many other compounds such as selenium, hydrogen peroxide, and lipid hydroperoxide. It is involved in the regulation of many antioxidants. Moreover, it protects immune cells from oxidative stress and decreases cytokine release (KANGALGİL & YARDIMCI, 2017).

2.9. Iron

Iron is found in some food sources including; meat products, dried fruits, grain products, green leafy vegetables, and eggs. Iron is involved in the production of ATP and hemoglobin, cell respiration, and the production of new cells. In iron deficiency; anemia, fatigue, decreased learning ability, and delayed wound healing are seen. An increase in the number of red

blood cells, some gastric secretions, a decrease or depletion of iron stores in the body, and ascorbic acid increase the absorption of iron in the body (Ceren & ÇAĞINDI).

2.10 Iodine

İodine is an essential element and it is important for thyroid hormone metabolism. İt is taken to the body by iodized salt. Due to iodine deficiency; hypothyroidism, and cretinism result. Also, disorders in cognitive systems may occur. impaired cognitive development (Niwattisaiwong, Burman, & Li-Ng, 2017).

The severity of iodine deficiency depends on its grade. During pregnancy and the lactation period women's requirements for iodine increase (Rodriguez-Diaz & Pearce, 2020).

Iodine toxicity is not common in humans, this toxicity may occur due to nuclear events that cause the release of radioactive iodine. İodine dependent toxicities may be reversed by bromide therapy (Baker, 2004).

3. Non-Essential Toxic Elements

3.1Aluminum

When the most abundant metals on earth are examined, aluminum appears in the third place. The use of aluminum is preferred in various products and some kinds of technical works (Inan-Eroglu & Ayaz, 2018) The aluminum contained in the diets allows aluminum to enter the body. Also, some deodorants and vaccines contain aluminum. Aluminum concentrations can be measured in urine and blood (Klotz et al., 2017). Aluminum toxicity is encountered in kidney patients and those who are occupationally exposed to aluminum. Aluminum toxicity can affect the lungs, bones, and central nervous system. It is thought that aluminum toxicity may play a role in neurodegenerative diseases in the brain (Sipahi, Palabıyık, Balcı, & Şahin, 2015).

3.2. Beryllium

Beryllium exits in soil and dust due to volcanic activities and mining. It has been defined as a danger to health. Exposure to beryllium may be due to different kinds of industries including electronics, and nuclear. Beryllium toxicity can occur through both acute and chronic poisoning. Acute beryllium toxicity comes with inflammation in the airways. The symptoms include edema (Stearney, Jakubowski, & Regina, 2022). Chronic beryllium toxicity cause berylliosis (Fontenot, Falta, Freed, Newman, & Kotzin, 1999).

3.3 Arsenic

Arsenic, which is a toxic element, exists in different chemical forms. It is seen in nature in trivalent or pentavalent forms. The most easily existing forms in nature are arsenic trioxide, sodium arsenite, and arsenic trichloride (Curtis, 2013).

Arsenic toxicity may occur worldwide. Arsenic toxicity is a problem that can affect millions of humans. Arsenic contamination may exist due to some natural sources. Arsenic involving water and industry may cause arsenic poisoning. In the human body, arsenic is absorbed by the small intestine. In arsenic toxication, nearly two hundred enzymes are inactivated. Some of these enzymes have roles in the repair and synthesis of DNA. Vomiting, pain in the abdominal side of the body, diarrhea, and nausea occur in acute arsenic poisoning. Some multisystem disorders occur in chronic poisoning (Ratnaike, 2003).

3.4. Cadmium

Cadmium is not an essential element it is a toxic element. It is risky for humans. In nature, cadmium is found due to pollutants in the industry. Cadmium including food, cadmium including water, and cigarettes play roles in cadmium exposure. Cadmium accumulation may last nearly thirty years in animals and herbs. Cadmium exposure due to occupational conditions or environmental factors may cause breast cancer, pancreas cancer, prostate cancer, and kidney cancer. Cadmium involves a risk for osteoporosis. Toxic effects of cadmium may be seen in both the liver and the kidney. These organs can produce metallothioneins that can be induced by cadmium. Moreover, cadmium may cause oxidative stress. Cadmium exposure may cause a functional loss in mitochondria and a decrease in energy production. Fermentation has shown as a promising way to cadmium removing (Genchi, Sinicropi, Lauria, Carocci, & Catalano, 2020)

3.5. Mercury

Mercury is liquid at room temperature and has silver-white color. Mercury has been preferred in various professions. Mercury and its derivates have been used as catalysts in the production of synthetic industrial materials such as acetaldehyde and vinichloride, as an electrode in the production of sodium hydroxide and chlorine from sodium chloride, and in the production of thermometers and electrical tools (Atsdr, 1999). In the health industry, mercury including medical preparation was used for antihelmintic therapy. Moreover, there were toothpaste preparations including mercury (Bayrakçı, 2001). 10 mg/m^3 of metallic mercury vapor is life-threatening. Mercury chloride is among the most toxic inorganic mercury compounds. Oral intake can be fatal depending on the dose.10–60 mg/kg

of organic mercury can be lethal to the human body. Serious mercury poisoning can affect the nervous system, affects the kidneys, the respiratory system, and the immune system. Mercury and mercury-containing compounds have toxic properties for the fetus and infant. Mercury exposure during pregnancy is serious and can cause congenital defects and damage to myelin (Akcan & Dursun, 2008).

3.6. Thallium

Thallium is an element that can not be seen in nature by itself. Thallium's color changes with exposure to the air. Thallium entrance to the body may be through the gastrointestinal system, skin absorption, and inhalation. Exposure to thallium is limited. Talium has no taste and no odor either. It can dissolve in water. Because of these properties, its poisonings may accidental and/or criminal. Tallium including preparations used for the treatment of dermatophytosis and thallium's radioactive isotope was used for nuclear scanning tests (Kemnic & Coleman, 2018)**.**

3.7.Lead

Lead is not an essential element for the human body. The body can not degrade lead (Mitra, Sharma, Purohit, & Sharma, 2017). Lead is a commonly used metal in industry and occupational lead exposure may occur (Halmo & Nappe, 2019).

Clinical findings in lead poisoning generally manifest themselves in the nervous system, hematopoietic system, renal system, and cardiovascular system. There is a typical triad in acute lead poisoning, which includes pain in the form of colic, anemia, and central nervous system depression. If the blood lead level rises rapidly, acute encephalopathy occurs. This table is accompanied by cerebral edema, persistent vomiting, fluctuating consciousness, ataxia, and seizures. In chronic lead exposure; weakness, prolonged abdominal pain, nausea, weight loss, fatigue, headache, and loss of cognitive functions may occur (Karcioglu, 2019).

Lead can accumulate in the body's organs after absorption. Lead Depending symptoms may change from one person to another. Lead toxicity is related to lead dosage and exposure time. Due to lead exposure blood pressure may increment. Conduction of the nerves may slow down. Moreover, headaches and concentration disorders may occur. Death may occur in intense conditions (Charkiewicz & Backstrand, 2020).

For lead toxicity; an explanation of blood lead levels and using chelation medication and nutritional supplementation is recommended by the World Health Organization. This organization aims to assist physicians in their diagnosis and treatment decisions (Organization, 2021).

4. Therapeutic Elements

4.1. Lithium

Lithium is one of the first-choice agents in the treatment of acute exacerbations and long-term prophylaxis of bipolar disorder. Its therapeutic range is narrow, therefore, low-dose lithium use is recommended, especially in maintenance therapy (Grandjean & Aubry, 2009).

Lithium is usually prescribed for mania and unbalanced mood situations. But it is also used for major depression, neutropenia, and headaches depending on vascular disorders. But for these uses are not approved as medication therapy. Lithium's action metabolism is not defined exactly. It is thought that lithium may modify catecholamine metabolism (Chokhawala, Lee, & Saadabadi, 2021).

4.2 Platinum

Cisplatin and other platinum-derived drugs show therapeutic efficacy by binding to DNA. It is known that as a result of the binding of cisplatin to DNA, the transcription and replication mechanisms are inhibited and the cancer cell dies (Shen, Pouliot, Hall, & Gottesman, 2012).

Cisplatin can be used alone in the treatment of cancer, as well as in combination with radiotherapy and other chemotherapeutics. Cisplatin, which can provide 90-95% treatment success in the treatment of early-diagnosed testicular cancer, is an anticancer drug used in the treatment of other solid tumors, especially ovarian cancer (Kartalou & Essigmann, 2001).

The first platinum involving medical preparation in clinical use was cisplatin. But, cisplatin has dose-limiting side effects and resistance may occur against cisplatin use. Due to these reasons, some new preparations including cisplatin were made (Khokhar, Al-Baker, Shamsuddin, & Siddik, 1997).

4.3 Gold

Some of the gold nanoparticles in ultrasmall size are herpetic agents. These gold-involving preparations have low systemic toxicity and they can accumulate in tumors. The kidney can clear this preparation fast. They are promising agents for cancer therapy (Fan et al., 2020).

Gold involving nanoparticles affect a different kind of cancers depending on the material used in their synthesis. Gold involving nanoparticles synthesized from Scutellaria barbata have anticancer effects on PANC-1 cancer cells (Wang, Xu, Yan, Liu, Karunakaran, et al., 2019). Gold involving nanoparticles synthesized from Panax notoginseng induces cytotoxicity and apoptosis in PANC-1 cancer cells successfully (Wang, Xu, Yan, Liu, & Li, 2019). Gold involving nanoparticles synthesized from Catha-

ranthus roseus increases apoptosis in HeLa cells (Ke et al., 2019). Gold involving nanoparticles synthesized from Portulaca grandiflora reduces the viability of the C6 glioma cell line by up to 50% by apoptosis or reactive oxygen species production (Ashokkumar, Arockiaraj, & Vijayaraghavan, 2016) Gold involving nanoparticles synthesized from Cystoseira baccata shows strong cytotoxicity against Caco-2 and HT-29 cell lines and also nanoparticles induced apoptosis by extrinsic and mitochondrial pathways (González-Ballesteros, Prado-López, Rodríguez-González, Lastra, & Rodríguez-Argüelles, 2017). Moreover, some of the gold-involving preparations are used for medical therapy in rheumatoid arthritis. (Bethesda, 2012).

REFERENCES

Akcan, A. B., & Dursun, O. (2008). Civa zehirlenmeleri. *Güncel Pediatri, 6*(3), 72-75.

Akdeniz, V., KINIK, Ö., Yerlikaya, O., & Ecem, A. (2016). İnsan sağlığı ve beslenme fizyolojisi açısından çinkonun önemi. *Akademik Gıda, 14*(3), 307-314.

Aschner, J. L., & Aschner, M. (2005). Nutritional aspects of manganese homeostasis. *Molecular aspects of medicine, 26*(4-5), 353-362.

Ashokkumar, T., Arockiaraj, J., & Vijayaraghavan, K. (2016). Biosynthesis of gold nanoparticles using green roof species Portulaca grandiflora and their cytotoxic effects against C6 glioma human cancer cells. *Environmental Progress & Sustainable Energy, 35*(6), 1732-1740.

Atsdr, U. (1999). Toxicological profile for mercury. *Atlanta: Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services*.

Baker, D. H. (2004). Iodine toxicity and its amelioration. *Experimental Biology and Medicine, 229*(6), 473-478.

Barma, M. A., Soiza, R. L., Donnan, P. T., McGilchrist, M. M., Frost, H., & Witham, M. D. (2018). Serum sodium level variability as a prognosticator in older adults. *Scandinavian journal of clinical and laboratory investigation, 78*(7-8), 632-638.

Bayrakçı, B. (2001). Kronik zehirlenmeler. *Katkı Pediatri Dergisi, 22*, 431-449.

Belgemen, T., & Nejat, A. (2004). ÇİNKONUN YAŞAMSAL FONKSİYONLARI VE ÇİNKO METABOLİZMASI İLE İLİŞKİLİ GENLER. *Ankara Üniversitesi Tıp Fakültesi Mecmuası, 57*(3).

Berkelhammer, C., & Bear, R. (1984). A clinical approach to common electrolyte problems: 3. Hypophosphatemia. *Canadian Medical Association Journal, 130*(1), 17.

Bethesda, L. (2012). Clinical and Research Information on Drug-Induced liver injury [Internet]. *National Institute of Diabetes and Digestive and Kidney Diseases*.

Buffington, M. A., & Abreo, K. (2016). Hyponatremia: a review. *Journal of intensive care medicine, 31*(4), 223-236.

Ceren, İ., & ÇAĞINDI, Ö. Demir Minerali: Fonksiyonları, Gıda İşlemenin Biyoyararlılığı Üzerine Etkileri ve Biyoaktif Bileşenler ile İnteraksiyonları. *Çukurova Tarım ve Gıda Bilimleri Dergisi, 35*(2), 151-164.

Charkiewicz, A. E., & Backstrand, J. R. (2020). Lead toxicity and pollution in Poland. *International journal of environmental research and public health, 17*(12), 4385.

Chokhawala, K., Lee, S., & Saadabadi, A. (2021). Lithium. StatPearls: StatPearls

Publishing LLC Treasure Island (FL).

Cooper, M. S., & Gittoes, N. J. (2008). Diagnosis and management of hypocalcaemia. *Bmj, 336*(7656), 1298-1302.

Curtis, D. (2013). Casarett and Doull's TOXICOLOGY The Basic Science of Poisons Eighth Edition.

Erikson, K. M., & Aschner, M. (2019). Manganese: its role in disease and health. *Met Ions Life Sci, 19*(10.1515), 9783110527872-9783110527016.

Fan, M., Han, Y., Gao, S., Yan, H., Cao, L., Li, Z., . . . Zhang, J. (2020). Ultrasmall gold nanoparticles in cancer diagnosis and therapy. *Theranostics, 10*(11), 4944.

Ferrannini, E. (2017). Sodium-glucose co-transporters and their inhibition: clinical physiology. *Cell metabolism, 26*(1), 27-38.

Fontenot, A. P., Falta, M. T., Freed, B. M., Newman, L. S., & Kotzin, B. L. (1999). Identification of pathogenic T cells in patients with beryllium-induced lung disease. *The Journal of Immunology, 163*(2), 1019-1026.

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., & Catalano, A. (2020). The effects of cadmium toxicity. *International journal of environmental research and public health, 17*(11), 3782.

González-Ballesteros, N., Prado-López, S., Rodríguez-González, J., Lastra, M., & Rodríguez-Argüelles, M. (2017). Green synthesis of gold nanoparticles using brown algae Cystoseira baccata: Its activity in colon cancer cells. *Colloids and Surfaces B: Biointerfaces, 153*, 190-198.

Grandjean, E. M., & Aubry, J.-M. (2009). Lithium: updated human knowledge using an evidence-based approach: part III: clinical safety. *CNS drugs, 23*, 397-418.

Gumz, M. L., Rabinowitz, L., & Wingo, C. S. (2015). An integrated view of potassium homeostasis. *New England Journal of Medicine, 373*(1), 60-72.

Halliwell, B., & Gutteridge, J. (1984). Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal, 219*(1), 1.

Halmo, L., & Nappe, T. M. (2019). Lead toxicity.

Hansen, B.-A., & Bruserud, Ø. (2016). Hypomagnesemia as a potentially life-threatening adverse effect of omeprazole. *Oxford medical case reports, 2016*(7), 147-149.

Heffern, M. C., Yamamoto, N., Holbrook, R. J., Eckermann, A. L., & Meade, T. J. (2013). Cobalt derivatives as promising therapeutic agents. *Current opinion in chemical biology, 17*(2), 189-196.

Horning, K. J., Caito, S. W., Tipps, K. G., Bowman, A. B., & Aschner, M. (2015). Manganese is essential for neuronal health. *Annual review of nutrition, 35*, 71-108.

Inan-Eroglu, E., & Ayaz, A. (2018). Is aluminum exposure a risk factor for neu-

rological disorders? *Journal of research in medical sciences: The official journal of Isfahan University of Medical Sciences, 23*.

Jahnen-Dechent, W., & Ketteler, M. (2012). Magnesium basics. Clin Kidney J 5: i3–i14.

Johannes, L., Fu, C.-Y., & Schwarz, G. (2022). Molybdenum Cofactor Deficiency in Humans. *Molecules, 27*(20), 6896.

Jomova, K., Makova, M., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., . . . Valko, M. (2022). Essential metals in health and disease. *Chemico-Biological Interactions*, 110173.

KANGALGİL, M., & YARDIMCI, H. (2017). SELENYUMUN İNSAN SAĞLIĞI ÜZERİNE ETKİLERİ VE DİYABETES MELLİTUSLA İLİŞKİSİ Effects of Selenium on Human Health and its Relationship With Diabetes Mellitus. *Bozok Tıp Dergisi, 7*(4), 66-71.

Karcioglu, O. (2019). *Kurşun zehirlenmesi nedir? Tanı ve tedavisi nasıldır? Lead poisoning*.

Kartalou, M., & Essigmann, J. M. (2001). Recognition of cisplatin adducts by cellular proteins. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 478*(1-2), 1-21.

Ke, Y., Al Aboody, M. S., Alturaiki, W., Alsagaby, S. A., Alfaiz, F. A., Veeraraghavan, V. P., & Mickymaray, S. (2019). Photosynthesized gold nanoparticles from Catharanthus roseus induces caspase-mediated apoptosis in cervical cancer cells (HeLa). *Artificial cells, nanomedicine, and biotechnology, 47*(1), 1938-1946.

Kemnic, T. R., & Coleman, M. (2018). Thallium toxicity.

Khokhar, A. R., Al-Baker, S., Shamsuddin, S., & Siddik, Z. H. (1997). Chemical and biological studies on a series of novel (trans-(1 R, 2 R)-, trans-(1 S, 2 S)-, and cis-1, 2-diaminocyclohexane) platinum (IV) carboxylate complexes. *Journal of medicinal chemistry, 40*(1), 112-116.

Klotz, K., Weistenhöfer, W., Neff, F., Hartwig, A., van Thriel, C., & Drexler, H. (2017). The health effects of aluminum exposure. *Deutsches Ärzteblatt International, 114*(39), 653.

Linder, M. C. (2013). *Biochemistry of copper* (Vol. 10): Springer Science & Business Media.

Mitchell, S. C. (2021). Nutrition and sulfur *Advances in food and nutrition research* (Vol. 96, pp. 123-174): Elsevier.

Mitra, P., Sharma, S., Purohit, P., & Sharma, P. (2017). Clinical and molecular aspects of lead toxicity: An update. *Critical reviews in clinical laboratory sciences, 54*(7-8), 506-528.

Niwattisaiwong, S., Burman, K. D., & Li-Ng, M. (2017). Iodine deficiency: Clinical implications. *Cleve Clin J Med, 84*(3), 236-244.

Novotny, A. (2018). J.; A Peterson, C. *Molybdenum. Adv. Nutr, 9*, 272-273.

Organization, W. H. (2021). WHO guideline for clinical management of exposure to lead.

Osamu, W. (2004). What are Trace Elements? *Trace Elements, 351*.

Palmer, L. G., & Schnermann, J. (2015). Integrated control of Na transport along the nephron. *Clinical Journal of the American Society of Nephrology, 10*(4), 676-687.

Parsons, P. J., & Barbosa Jr, F. (2007). Atomic spectrometry and trends in clinical laboratory medicine. *Spectrochimica Acta Part B: Atomic Spectroscopy, 62*(9), 992-1003.

Ratnaike, R. N. (2003). Acute and chronic arsenic toxicity. *Postgraduate medical journal, 79*(933), 391-396.

Rehder, D. (2016). Perspectives for vanadium in health issues. *Future medicinal chemistry, 8*(3), 325-338.

Rodriguez-Diaz, E., & Pearce, E. N. (2020). Iodine status and supplementation before, during, and after pregnancy. *Best Practice & Research Clinical Endocrinology & Metabolism, 34*(4), 101430.

Royer, A., & Sharman, T. (2020). Copper toxicity.

Saner, G., Neyzi, O., & Ertuğrul, T. (2002). Mikroelementler çinko, Pediatri Cilt 1, 3. *Baskı, Nobel Tıp Kitabevleri, İstanbul*, 174-175.

Shen, D.-W., Pouliot, L. M., Hall, M. D., & Gottesman, M. M. (2012). Cisplatin resistance: a cellular self-defense mechanism resulting from multiple epigenetic and genetic changes. *Pharmacological reviews, 64*(3), 706-721.

Shrimanker, I., & Bhattarai, S. (2019). Electrolytes.

Sipahi, H., Palabıyık, Ş., Balcı, S., & Şahin, G. (2015). Alüminyum toksisitesi ve nörodejeneratif hastalıklardaki rolü. *Türkiye Klinikleri J Neur, 10*(1), 6-14.

Stearney, E. R., Jakubowski, J. A., & Regina, A. C. (2022). Beryllium Toxicity *StatPearls [Internet]*: StatPearls Publishing.

Stedwell, R. E., Allen, K. M., & Binder, L. S. (1992). Hypokalemic paralyses: a review of the etiologies, pathophysiology, presentation, and therapy. *The American journal of emergency medicine, 10*(2), 143-148.

Tapiero, H., Townsend, D. á., & Tew, K. (2003). Trace elements in human physiology and pathology. Copper. *Biomedicine & pharmacotherapy, 57*(9), 386-398.

Turner, J. J. (2017). Hypercalcaemia–presentation and management. *Clinical Medicine, 17*(3), 270.

Viera, A. J., & Wouk, N. (2015). Potassium disorders: hypokalemia and hyperkalemia. *American family physician, 92*(6), 487-495.

Vincent, J., & Lukaski, H. (2018). Chromium. Adv Nutr 9 (4): 505–506.

Walker, H. K., Hall, W. D., & Hurst, J. W. (1990). Clinical methods: the history, physical, and laboratory examinations.

Wang, L., Xu, J., Yan, Y., Liu, H., Karunakaran, T., & Li, F. (2019). Green synthesis of gold nanoparticles from Scutellaria barbata and its anticancer activity in pancreatic cancer cell (PANC-1). *Artificial cells, nanomedicine, and biotechnology, 47*(1), 1617-1627.

Wang, L., Xu, J., Yan, Y., Liu, H., & Li, F. (2019). Synthesis of gold nanoparticles from leaf Panax notoginseng and its anticancer activity in pancreatic cancer PANC-1 cell lines. *Artificial cells, nanomedicine, and biotechnology, 47*(1), 1216-1223.

CHAPTER 14

ARGINASE 2, FASTING INTERMITTENTLY, AND GROWTH HORMONE: INVESTIGATING CROSS-TALK IN THE NEUROENDOCRINE SYSTEM

Ebru BARDAS OZKAN[1]

1 Prof.Dr., Erzincan Binali Yildirim University, Faculty of Medicine, Department of Physiology Erzincan-TÜRKİYE, drebrubardas@gmail.com ORCID: https://orcid.org/0000-0002-7089-8771

INTRODUCTION

The protection of human health is greatly influenced by eating behaviors, sleeping patterns, and eating frequency (Aksungar FB vd., 2005). Since ancient times, individuals all across the world have engaged in the practice of intermittent fasting, which involves voluntary abstention from food and drink. Numerous types and practices of famine are discussed in books on religion and ethnology (Patterson RE vd., 2015). Numerous types of intermittent fasting practices have also been the subject of scientific study (Moro T vd., 2016). Time-restricted feeding, a sort of intermittent fasting, entails following a daily schedule that calls for fasting at specific times of the day and consuming at other times. This makes it more like a regular eating routine than other forms of intermittent fasting. Many people mistakenly miss breakfast and don't eat anything after dinner each day, which brings them fairly close to this eating pattern. The key distinction between time-restricted feeding and other fasting methods, though, is that it never permits you to eat anything you want all day long but instead only for a few hours each day. The maximum number of calories that a person will consume in a short period of time to satisfy hunger will be smaller the shorter the allowable feeding interval is (Zhang Y vd., 201; Rahbar AR vd.,2019). In a typical population, prolonged fasting is challenging to adhere to, but studies have shown that intermittent fasting has a higher rate of compliance (Rahbar AR vd.,2019). Time-restricted feeding is seen as a more stable intermittent fasting approach because the feeding interval is fairly long.

Treatment options for obesity, insulin resistance, and its consequences include calorie restriction and intermittent fasting. The mechanisms behind this response, though, are not yet fully understood. In a recent study, arginase-2 (Arg2) was discovered to be a fasting-induced hepatocyte factor that shields obese mouse models from developing insulin and glucose intolerance, accumulating hepatic and peripheral fat, and responding inflammatory responses in the liver. In diabetic mice models, it was discovered that overexpressing hepatocyte-specific Arg2 increased basal thermogenesis and prevented weight gain, insulin resistance, glucose intolerance, hepatic steatosis, and hepatic inflammation. Studies on how hepatocyte Arg2 is regulated in nutrient-rich and fasting stages, as well as in healthy and diseased states, continue to pique interest (McCue MD, 2010).

In the growth and development of people, insulin-like growth factor-1 (IGF-1) is crucial. In addition to controlling many intracellular signaling pathways, this factor directly stimulates the IGF-1 receptor (IGF-1R). Additionally, it has been suggested that IGF-1R may play a role in a number of life-threatening conditions, including cancer, cardiovascular disease, age-related macular degeneration, and diabetic retinopathy (Chakravarthy MV and Booth FW, 2004; McCue MD, 2012). There are few research ex-

ploring the impact of intermittent fasting on IGF-1 in humans, however one recent study reported that a group of male participants who fasted for 30 days during Ramadan had considerably lower IGF-1 levels (Scheen AJ, 2008). It has been determined, based on the results of past animal and human studies, that adult lifestyles that incorporate intermittent fasting diets have a great deal of potential to foster optimal health and lower the risk of many chronic diseases, particularly for overweight and sedentary people (Anton SD vd., 2017).

Numerous studies (Safdie FM vd., 2009; Varady KA vd., 2016; Panda S, 2016; McAllister MJ vd., 2020; Longo VD and Panda S, 2016) on intermittent fasting have found benefits for human health, but more research is required to understand the processes underlying these benefits. It is envisaged that intermittent fasting will significantly improve public health if it proves to be a complementary strategy for the treatment of some diseases and the prevention of some diseases.

This study sought to determine how intermittent fasting affected arginase-2, which is thought to be linked to insulin resistance, glucose intolerance, obesity, and inflammation, and IGF-1 levels, which are linked to diabetes, cardiovascular disease, and cancer.

HUNGER

'Hunger' is the state in which an organism's energy gains are unable to satisfy its energy requirements. According to its intensity and form, hunger brought on by endogenous and external factors is split into two categories (Sutton EF vd., 2018).

When someone is fasting, they stop eating even when they are physically able to do so. Although food can be provided, the time required to consume food for endogenous reasons (such as exercising willpower...) is better used for other activities (Johnson JB vd., 2017).

Starvation: Although this sort of hunger is present, it is impossible to obtain food due to extrinsic factors (such as food deprivation...) (Klempel MC vd., 2013).

People frequently link hunger to losing weight or having hungry pangs. The involuntary demand for food by a person who is in danger of long-term food deprivation forms the basis of the physiology of hunger. However, the sedentary lifestyle brought on by global change and the need to continually consume food with the emotional hunger that results from the psychology of being without meals for an extended period of time prepare the way for the establishment of many chronic diseases like obesity and diabetes (Parvaresh A. vd., 2019). Full and partial hunger have drawn physiologists' attention for a very long time. Between 1870 and 1890, the

first human scientific research were done, and between 1900 and 1930, the first studies with experimental animals were carried out (Panda S., 2016).

The earliest studies generally looked at variables including body weight loss, urine excretion, and an increase in ketone bodies in the blood, which are produced when liver glycogen stores are depleted. The initial investigations on hunger in humans focused in particular on subjects who had been severely malnourished for an extended period of time (30, 40, 50, and more days). These investigations showed that, during prolonged fasting, the body used carbs before using fat or protein as a source of energy. It has been shown that severe weight loss (up to 60%), complete depletion of fat reserves, and even a 50% reduction in body protein during protracted hunger can result in a mammal's mortality (Longo VD and Panda S, 2016).

The central nervous system is one of the tissues that is shielded against prolonged deprivation. From 1913 to 1922, human patients with diabetes were treated with short-term total starvation, also referred to as therapeutic starvation or long-term low-calorie diet. Up until the time that insulin was used, this persisted. Later, convulsive illnesses like epilepsy were also treated with this method of care. According to a report, it was first employed in 1915 as a weight-loss remedy. In recent years, research have been conducted at the level of biomolecules as well as tissues. Medical and biological sciences are still interested in studying the fundamental processes behind hunger and nutritional metabolism (McCue MD, 2013).

CONTINUOUS FASTING METHODS

In order to lower body weight and visceral fat mass, dietary restrictions are typically advised as the first line of treatment. Calorie restriction is the type of dietary restriction that is most frequently utilized. These diets cut the amount of energy consumed by 15% to 60% of the daily requirement. Limiting calories helps people live longer and lose weight. It has been demonstrated that this practice, even in overweight individuals, greatly improves mitochondrial functioning, eliminates various cadiovascular disease risk factors, and reduces insulin resistance in just six months. Additionally, research has shown that this application can help people who are overweight lose weight and treat a variety of chronic ailments. However, it is a reality that the majority of obese people find it challenging to sustain daily calorie restriction for an extended period of time. Due to the fact that many obese people find it difficult to cut back on their portions while on a diet, excessive food intake persists and weight reduction is inhibited (Fontana L, 2008). The propensity to gain back the weight lost a year following the diet is one drawback of this technique. Therefore, it is essential to create energy restriction measures that are both sustainable and successful. When eating intermittently, no or very few calories are con-

sumed for at least 12 hours. Instead than being a diet, intermittent fasting (IF) modifies everyday eating patterns. The changeover between feeding and fasting intervals is more significant in intermittent fasting than calorie restriction. Contrary to other diets, intermittent fasting is the best plan for weight loss that keeps muscle mass intact and lengthens life. Additionally, intermittent fasting, which entails cycles of eating and fasting, contributes to the control of circadian rhythm. During intermittent fasting, eating and drinking are not restricted, thus insulin levels are high and the body does not use fat stores as a source of energy. When a person fasts for 12 hours or longer, their insulin levels fall and they start to burn fat. By lowering oxidative stress and inflammation and improving energy metabolism, IF aids in cellular defense (Varady KA, 2011).

However, in contrast to calorie restriction (CR), IF focuses more on modifying the frequency of eating. Additionally, IF may have the ability to slow down aging, treat disorders associated with old age, and lessen the negative effects of chemotherapy. Clinical studies evaluating the long-term effects of intermittent fasting on health, disease, and longevity in people are scarce, and it is still unclear what mechanisms underlie the varied intermittent fasting techniques' metabolic impacts. For the treatment of age-related diseases and ailments as well as for maintaining good health, intermittent fasting may offer novel therapeutic approaches (Varady KA, 2011).

All-day fasting, alternate-day fasting, and time-restricted feeding are the three basic types of intermittent fasting. However, there are numerous variations of these approaches that allow for limitations based on personal needs (Fontana L, 2008).

FASTING PRACTICE DAILY

This type of intermittent fasting involves a 24-hour food fast. This type of fasting should only be practiced twice a week and once a month. This method necessitates complete or modified fasting one or two days a week because it entails all-day fasting (Gotthardt JD vd., 2016).

A SECOND DAY OF FASTING

The research on this type of intermittent fasting is the most extensive. It is also the best method for losing weight. It is a type of fasting in which the person eats a meal of his or her choosing at midday or dinner and receives 25% of the energy needed for the whole day. The time spent fasting varies between 30 and 40 hours during alternate-day fasting (ADF) (Jahani M, 2018). The fasting period is 30 hours, for instance, if a person eats their last meal at noon on Sunday (when eating on day 1) and at 6 pm the following day (on day 2). The time would be 40 hours if the last meal on Monday was eaten at 15:00 and the first meal on Wednesday was taken

at 11:00. Alternate-day modified fasting (ADMF) is another type of alternate-day fasting. In order to maintain energy balance, voluntary nutrition is provided five days per week while calorie intake is restricted to one-fourth of the daily need on the other two days (Bhutani S1 vd., 2018).

TIME-LIMITED RESOURCES

The goal of time-restricted feeding (TRF) is to keep the circadian rhythm's regular daily cycle of eating and fasting. It entails a daily fasting window of between 12 and 21 hours. The goal of this diet is to consume fewer calories each day while still eating the same meals. Calorie restriction refers to not eating outside of a predetermined window of time each day (Bhutani S1 vd., 2018). Recent research suggests that by controlling lipid, carbohydrate, and protein metabolism, it may be feasible to synchronize a metabolic clock responsible for these mechanisms when the TRF is produced in accordance with the circadian rhythm. The body's usage of energy to preserve vitality throughout fasting times of the day is balanced by this synchronization. It may be possible to sustain physiological hunger for longer periods of time by reducing daily energy intake (Klempel MC vd., 2013).

In conclusion, it is emphasized that long-term stabilization of hunger physiology will occur under both energy restriction and time restriction paradigms. The circadian rhythm is said to be affected by irregular and prolonged eating patterns throughout the day. Long-term circadian rhythm disruption has been linked to the development of metabolic syndrome symptoms as obesity, hypertension, insulin resistance, and inflammation. It has been demonstrated that irregular eating patterns are positively correlated with the likelihood of developing the metabolic syndrome and other cardiometabolic disorders (Longo VD, Panda S, 2016). In a study examining the connection between the circadian rhythm and biological clock and three types of time-restricted feeding (TRF)—early TRF, in which the last meal of the day is placed between dinner and lunch; mid-day TRF, in which the meal time is placed in the middle of the day; and late TRF, in which food intake is started after 16.00—it was demonstrated that timing breakfast and dinner to the circadian rhythm decreased food intake and hunger, improved glycaemia (Sutton EF vd., 2018).

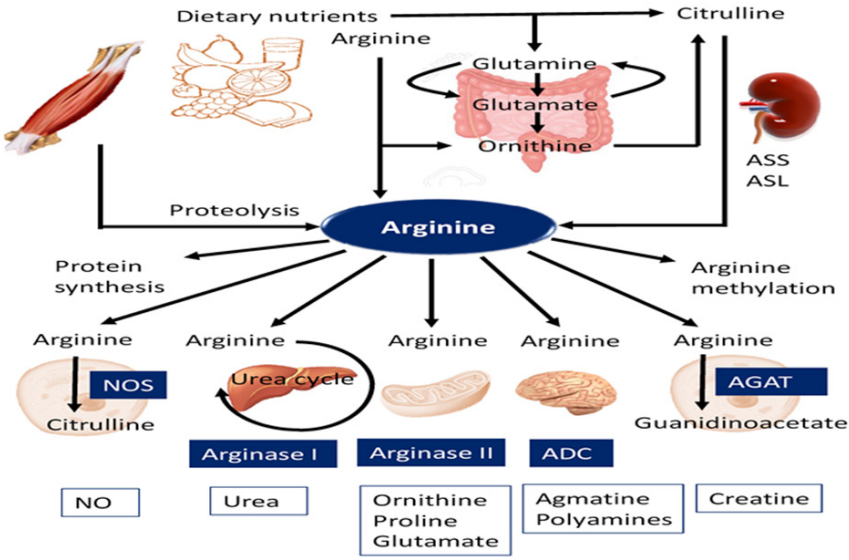
Table 1. Types of time-restricted feeding according to fasting duration
(Panda S, 2016)

16/8 TRF	16 hours fasting, followed by 8 hours of feeding each day characterised
12/12 TRF	12 hours of fasting followed by 12 hours of eating duration.
20/4 TRF	A 20-hour fasting period followed by 4 is characterised by an eating time of one hour. Food consumption is limited to 1-2 meals a day.

ARGININE

Arginine is a "conditionally essential" amino acid because it can be synthesized by the human body naturally. However, endogenous production might not be enough if arginine demand rises. Arginine needs to be given as a supplement from outside since they rise at the time of rapid growth, development, and cellular turnover. In some stressful situations (such injury, inflammation of the small intestine and kidney, burns, etc.), the demand in an adult person increases, necessitating external supplementation. Humans receive their arginine from three main sources: as a component of dietary protein (dietary arginine), as a byproduct of metabolism and the recycling of the endogenous protein cycle (converted arginine), and as arginine de novo (endogenously created spontaneously in the body) (Shatanawi A vd., 2019). The most typical dietary sources of arginine include seeds, nuts, shellfish, and meats. There are two mechanisms to synthesize endogenous arginine. The first is endogenous arginine, which is produced in the kidney-small intestine axis. The second comes from the citrulline-nitric oxide cycle and is called arginine. Epithelial cells in the small intestine create this kind of arginine, which is then delivered to the kidney. This pathway's mechanism is yet not completely known (Hsu CN and Tain LY,2016).

Figure 1. Arginine biosynthesis and metabolism. (*Hsu CN and Tain LY, 2016***)**



Arginosuccinate Synthetase (ASS), Arginosuccinate lyase (ASL), Nitric oxide, Nitric oxide synthase (NOS), Arginine-glycine amidinotransferase is also known as AGAT or arginine decarboxylase.

The liver's arginase enzyme catabolizes the arginine the liver produces. The metabolites of arginine include urea, ornithine, nitric oxide, proline, glutamate, and polyamine, which has a short half-life (1.06) in adults. Arginine has an extremely quick half-life in adults, which is 1.06 hours. Arginase-1, one of the two forms of arginase isozymes known in mammals, is expressed in the liver and can be found in the cytoplasm. It plays a significant part in the urea cycle. Low levels of arginase-2, a mitochondrial enzyme, are expressed in extrahepatic organs like neurons, kidney, muscle, and vascular cells (Li H, 2001). Arginase also controls the amounts of intracellular arginine and citrulline, which are connected to the creation of nitric oxide and polyamines. Arginase activity is linked to polyamine production and cellular proliferation, according to recent studies (Morris SM, 2007).

An essential enzyme called nitric oxide synthase (NOS) has three distinct isoforms and functions in various ways in various tissues. Nitric oxide synthase and arginase both use arginine as their common substrate. By inhibiting inducible nitric oxide synthase, arginine also boosts the efficacy of endogenous nitric oxide inhibitors (Jahani M vd., 2018). Hormones, cytokines, nutrition, and endotoxins all play a significant part in controlling arginine metabolism. The metabolism of arginine is impacted and the need rises as lysine content in the diet rises. However, through raising arginase expression in enterocytes and hepatocytes, glucocorticoids support arginase metabolism (Wu G, Meininger CJ, 2002). Arginase expression can also be greatly boosted by cytokines. Additionally, it is understood that arginine is crucial for spermatogenesis, fetal development, neonatal growth, tissue damage, and long-term metabolic disorders (Hsu CN vd., 2018). Recent research (Wu M, 2020) has demonstrated that arginine, which is known to affect cardiovascular, neurological, and endocrinological functions, also supports intestinal health through intestinal microbiota and cytokines, and participates in the metabolism of immune cells and severe depression.

ARGINASE AND INTERMITTENT FASTING IN RELATION

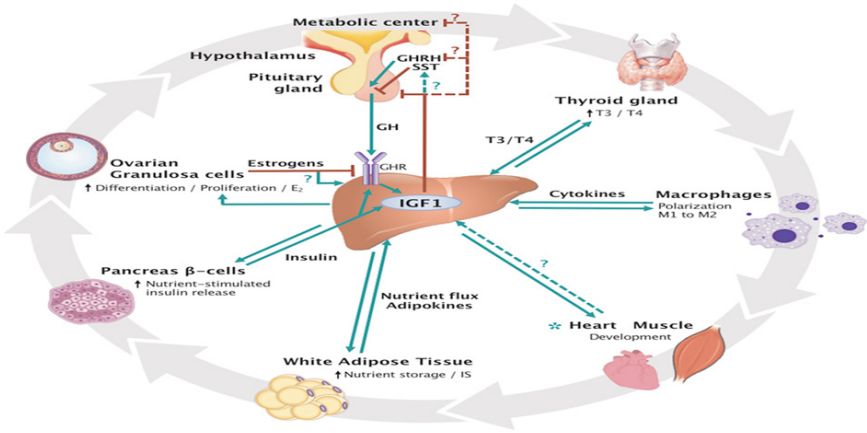
There are not many studies available on this topic. According to a research (), fasting during Ramadan led to a small but non-significant drop in arginase levels. The protection of NOS levels may make use of this application, according to the statement. Another study found that fasting enhanced the production of Arg2 (a protein that metabolizes arginine unique to hepatocytes), which may increase basal thermogenesis. Particularly in diabetic mice, this enzyme has been demonstrated to be protective against weight gain, insulin resistance, glucose intolerance, hepatic steatosis, and hepatic inflammation (Flynn NE vd., 2009; Wu G, 2002). As a result, it is possible to use hepatocyte Arg2 as a therapeutic target to reduce the effects

of obesity and nonalcoholic fatty liver disease. Hepatocyte Arg2 is thought to be a key effector of the hepatic glucose deprivation response (Wu M, 2020).

A GROWTH FACTOR LIKE INSULIN

Insulin-like growth factor-1, a growth hormone factor that mediates somatic growth and shares characteristics with insulin, was initially discovered in the 1970s. IGF-1 is a polypeptide with structurally 70 amino acids and a 40% resemblance to insülin (Giustina A vd., 2018).

Figure 2. IGF-1's metabolic activities (Giustina A vd., 2018)



Growth hormone, growth hormone receptor, growth hormone releasing hormone, and somatostatin are all abbreviations for hormones.

This substance (IGF-1) is an anabolic hormone, with 90% of its synthesis occurring in the liver. Growth hormone regulates the expression of this hormone through a negative feedback loop in peripheral tissues. Additionally, IGF-1 is found in 5% of muscle tissue and 10% of adipose tissue, respectively. Although it contributes very little to the quantity of circulating IGF, bone tissue is one of the richest tissues in IGF-1 (Werner H. vd., 2016). Human fetal connective tissues and cells with a mesenchymal ancestry express the IGF-1 gene. In addition to paracrine and autocrine effects, IGF-1 also has endocrine effects. The expression of the IGF-1 gene in the liver, heart, lungs, and pancreas is primarily regulated by growth hormone. In their respective target tissues, adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) all promote paracrine synthesis of IGF-1 (Pollak MN, 2004).

IGF-1 synthesis at all ages is regulated by nutrition in addition to growth hormone (Beaune B vd., 1997). The blood IGF-1 level cannot be kept at a level greater than 800 calories per day, and this protection necessitates an average daily energy consumption of 1500 calories. This is a crucial result showing that IGF-1 needs sufficient protein and energy intake everyday. IGF-1 acquires high values during childhood and adolescence from low levels before birth (Kineman RD vd., 2018). The IGF-1 receptor (IGF1R) controls the biological activity of IGF-1. IGFBP, a ligand-binding protein found in circulation and extracellular fluids, modulates IGF-1 by binding to it (Pollak MN vd., 2004). IGF-1 controls somatic growth and ensures that growth and development proceed in a way that is consistent with diet. A well-preserved endocrine system that controls the organism's lifespan is made up of growth hormone and IGF-1 (Rahmani J vd., 2019). The relationship between IGF-1 and growth hormone is crucial for maintaining anabolic processes in adults and for growth in children. prevents apoptosis, which stimulates cell proliferation. After puberty, IGF-1 levels start to decline in an age-related manner. This affects anabolic metabolism, or "Somatopause," in adults, which is linked to changes in body composition and metabolism. First off, it has been demonstrated that conditions such as decreased bone and muscle mass and strength, increased fat mass, dyslipidaemia, arterial hypertension, and cardiovascular illnesses lower IGF-1 levels. Therefore, metabolic, biochemical, and functional alterations that promote aging and disease have been observed when the growth hormone/IGF-1 parameter is compromised. Additionally, the central nervous system's release of IGF-1 promotes the growth, viability, and differentiation of glia cells. IGF-1, a powerful neurotrophic factor, promotes the growth and synthesis of myelin while protecting neurons from death. Studies on IGF-1's therapeutic potential are still under progress because it plays a significant part in the pathways that lead to cancer. Understanding the function and control of the IGF-1 system in the body still requires basic and clinical research (Guevara-Aguirre J vd., 2011).

INTERMITTENT FASTING AND IGF-1 ARE ASSOCIATED

Intermittent fasting reduces IGF-1 synthesis by improving muscle and liver cell insulin sensitivity. Age-related declines in IGF-1 levels are a significant component in the acceleration of the rise in insulin and glucose levels (Guevara-Aguirre J vd., 2011). Blood insulin and glucose levels drop by more than 30% and IGF-1 levels drop quickly after a fast lasting longer than three days (Rahmani J vd., 2019). Additionally, it has been noted that in humans, a five-day fast reduces IGF-1 by more than 60% and increases IGFBP1 by at least fivefold. It has been suggested that protein limitation, particularly restriction of essential amino acids, may be the cause of this effect of fasting on IGF-1. Additionally, it is said that fasting is beneficial for

both the prevention and treatment of cancer. In numerous tissues and organs, including the liver and kidney, cell death and atrophy are known effects of substantial reductions in glucose, insulin, and IGF-1 levels brought on by famine. In these tissues, a phase of intense cell multiplication starts with the start of the re-feeding period (Pollak MN vd., 2004). Once more, hunger reduces the ability of cancer cells to adapt to harsh conditions and prevents the development of the tumour by lowering glucose levels, increasing IGF-1 levels, and IGFBP1 levels. IGF-1 has a substantial correlation with numerous disorders, including cancer, aging, and diabetes, hence more thorough research is required (Kineman RD vd., 2018).

DISCUSSION

Humans who fast go without food and liquids for anywhere between four hours and three weeks. Before and after surgery or before various laboratory tests, intermittent fasting is used (Gabel K, vd., 2018). Ramadan fasting is the most well-known example of intermittent fasting, which has its roots in religious and spiritual traditions (Sundaram S, and Yan L., 2016). Numerous studies (Rothschild J, vd., 2014) have shown how intermittent fasting improves human health, including weight loss, body fat reduction, and the prevention and treatment of specific diseases. Excessive energy intake is linked to mortality rates rising, disease occurrence, weight rise, and subsequent fat gain. Randomized controlled trials have demonstrated the positive effects of voluntary weight loss on type 2 diabetes, all-cause mortality, and mental and physical health (Varady KA, vd., 2009). Numerous rat studies have shown the positive effects of CR and weight loss in human clinical trials. However, there are numerous applications relating to the length, nature, and format of IF, and it is still unclear which way of application is the most efficient or how the advantages associated with IF application are realized (Harvie M, and Howell A, 2017).

The metabolic syndrome is a term used to describe a number of irregularities. Insulin resistance is the most fundamental metabolic syndrome indicator. Dyslipidemia, increased visceral fat storage, and hypertension are also seen as indicators of metabolic syndrome. Reduced calorie intake from weight loss contributes to a decrease in these metabolic risk variables (Dulloo AG, and Montani JP, 2012). Although 20% of people do not follow the practice, intermittent fasting has been shown to be an effective way to lower the risk of metabolic illnesses. TRF, an alternative to IF, was created as a result. The benefits of time-restricted feeding (TRF) on weight loss were consistent, according to a recent evaluation of the findings of a meta-analysis of 11 research that have been conducted on the subject. The results of both the observational trial and the randomised study indicated a significant reduction in weight loss (Wei T vd., 2016). There aren’t many

studies looking at how time-restricted feeding affects body fat mass. In a recent animal experiment (Sundaram S, and Yan L., 2016), mice were fed AIN93 and a high-fat diet ad libitum, and the body fat percentage of the mice on the high-fat diet was found to significantly increase. In the same study, mice fed a high-fat diet were subjected to 12/12 and 16/8 hours of TRF. In these mice, a substantial reduction in body fat mass was found. In another study, it was shown that switching high-fat and high-fructose ad libitum fed mice to chronic 9-hour, 12-hour, and workday TRF programmes resulted in a substantial decrease in body fat mass in each group (p0.05, p0.01, and p0.001, respectively). In a 12-week 16/8 TRF application on obese patients, a non-significant time-dependent decrease in body fat mass and visceral fat mass was seen. The same study found that while body weight and energy consumption significantly decreased, there was no significant change in muscle mass. When a group was requested to forego the breakfast meal was compared to the control group in a study looking at the impact of time-restricted feeding on AKSC, it was discovered that there was no change in AKSC levels in the morning hours, but there was a large increase in the evening hours. There was no discernible change in AK levels when the same application was carried out on obese individuals (Betts JA, vd., 2014).

There aren't many research looking into how humans' IGF-1 levels are affected by intermittent fasting (Anderson LJ, vd., 2017). It was found that there was a non-significant increase in IGF-1 levels in the group that was restricted 24 hours a week for 8 weeks compared to the control group in a study looking at the impact of alternate day fasting on plasma IGF-1 levels in humans (Kessler CS, vd., 2018). Alternate day fasting is regarded as an IF application like TRF, however it differs significantly from TRF in terms of how it is applied. Because individuals following TRF diets may have variations in calorie intake. IGF-1 levels may change as a result of variations in protein and calorie consumption, particularly in the absence of restriction. Additionally, skeletal muscle is gradually overloaded during resistance training, which modifies tissue anabolism and protein synthesis in favor of tissue growth. Ramadan fasting considerably reduced caloric and protein consumption in a different trial with 9 male volunteers who underwent submaximal activity, but it had no significant impact on IGF-1 levels (Bouhlel E., vd., 2008). A recent study of fit adult men found that Ramadan fasting dramatically reduced IGF-1 levels, calorie intake, and protein intake. These findings suggest that dietary protein, calories, and exercise all have a big impact on IGF-1 levels (Harder-Lauridsen NM, vd., 2017). There aren't many findings in the literature currently available about how intermittent fasting affects human serum arginase-2 levels. In one study, 44 male university students who fasted throughout Ramadan

had non-significantly lower serum arginase levels. Another study found that in diabetic mice models, hepatic Arginase-2 expression was enhanced by fasting, increasing basal thermogenesis (Oh HS vd., 2017).

CONCLUSION

Given this data, it can be concluded that time-restricted feeding, a form of intermittent fasting, is a successful way to lower body weight and body fat percentage. In this regard, it is believed that time-restricted feeding may have some practical applications, particularly for the treatment of modern-day disorders and their comorbidities like obesity and metabolic syndrome. The effectiveness of calorie-restricted diets or time-restricted nutrition models in preventing or treating certain metabolic illnesses by lowering body weight and body fat percentage, however, is not well established. In this regard, extensive investigations are required. These studies also help to highlight the efficacy of time-restricted nutrition models used in place of the conventional calorie restriction approach.

Time-restricted eating causes a rise in serum IGF-1 levels. This rise in IGF-1 levels is believed to have resulted from the subjects consuming more protein and energy than usual, particularly during the unrestricted time. More sensitive investigations can be carried out with subjects whose food consumption quantities and types are relatively similar or whose food consumption is recorded in order to ascertain the reason for this rise in IGF-1. The effects of the time-restricted feeding model on growth hormone and the GH/IGF-1 system can also be observed through thorough investigations in which GH, IGF1R, and IGFBP can be studied. These investigations may also help to clarify the mechanisms through which the time-restricted feeding paradigm works. Serum arginase-2 levels were marginally elevated by time-restricted eating. To more fully illustrate this effect, larger, more in-depth investigations are required.

REFERENCES

1.Aksungar FB, Eren A, Ure S, Teskin O, Ates G. Effects of intermittent fasting on serum lipid levels, coagulation status and plasma homocysteine levels, Ann. Nutr. Metab., 2005, 49(2):77-82.

2. Patterson RE, Laughlin GA, LaCroix AZ, Hartman SJ, Natarajan L, Senger CM,Martínez ME, Villaseñor A, Sears DD, Marinac CR, Gallo LC. Intermittent Fasting and Human Metanbolic Effects, J. Acad. Nutr. Diet., 2015, 115(8): 1203–1212.

3. Moro T, Tinsley G, Bianco A, Marcolin G, Pacelli QF, Battaglia G, Palma A, Gentil P, Neri M, Paoli A. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males, J. Transl. Med., 2016, 14(1):290.

4. Zhang Y, Higgins CB, Fortune HM, Chen P, Stothard AI, Mayer AL, Swarts BM, De Bosch BJ. Hepatic arginase 2 (Arg2) is sufficient to convey the therapeutic metabolic effects of fasting, Nat. Commun., 2019, 10(1): 1-16.

5. Rahbar AR, Safavi E, Rooholamini M, Jaafari F, Darvishi S, Rahbar A. Effects of Intermittent Fasting during Ramadan on Insulin-like Growth Factor-1, Interleukin 2, and Lipid Profile in Healthy Muslims, Int. J. Prev. Med., 2019, 10(1): 7.

6. McCue MD. Starvation physiology: reviewing the different strategies animals use to survive a common challenge, Comparative Biochemistry and Physiology, Part A, 2010, 156:1-18.

7. Chakravarthy MV, Booth FW. Eating, exercise, and "thrifty" genotypes: connecting the dots toward an evolutionary understanding of modern chronic diseases, J. App. Physiol., 2004, 96:3-10.

8. McCue MD. Comparative Physiology of Fasting, Starvation, and Food Limitation, 1st ed. Berlin, Springer-Verlag Berlin and Heidelberg, 2012:1-25.

9. Scheen AJ. The future of obesity: new drugs versus lifestyle interventions, Expert. Opin. Investig. Drugs., 2008, 17(3):263-367.

10. Anton SD, Moehl K, Donahoo WT, Marosi K, Lee SA, Mainous AG, Leeuwenburgh C, Mattson MP. Flipping the Metabolic Switch: Understanding and Applying the Health Benefits of Fasting, Obesity, 2017, 26(2):254-268.

11. Safdie FM, Dorff T, Quinn D, Fontana L, Wei M, Lee C, Longo VD. Fasting and cancer treatment in humans: a case series report, Aging, 2009, 1(12), 988–1007.

12. Varady KA, Hoddy KK, Kroeger CM, Trepanowski JF, Klempel MC, Barnosky A, Bhutani S. Determinants of weight loss success with alternate day fasting, Obes. Res. Clin. Pract., 2016, 10(4):476-480.

13. Panda S. Circadian physiology of metabolism, Science, 2016, 354(6315):1008-1015.

14. McAllister MJ, Pigg BL, Renteria LI, Waldman HS. Time-restricted feeding improves markers of cardiometabolic health in physically active college-age men: a 4-week randomized pre-post pilot study, Nutr. Res., 2020, 75:32-43.

15. Longo VD, Panda S. Fasting, Circadian rhythms, and time-restricted feeding in healthy lifespan,Cell Metab., 2016, 23(6):1048-1059.

16. Sutton EF, Beyl R, Early KS, Cefalu WT, Ravussin E, Peterson CM. Early time-restricted feeding ımproves ınsulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes, Cell Metab., 2018, 27(6):1212-1221.

17. Johnson JB, Summer W, Cutler RG, Martin B, Hyun DH, Dixit VD, Mattson MP. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma, Free Radical Biology and Medicine, 2007, 42(5):665–674.

18. Klempel MC, Kroeger CM, Varady KA. Alternate day fasting (ADF) with a highfat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet, Metabolism., 2013, 62(1):137-143.

19. Parvaresh A, Razavi R, Yaghooblo K, Hassanzadeh A, Mohammadifard N, Abbasi B, Clark CCT. Modified alternate-day fasting vs. calorie restriction in the treatment of patients with metabolic syndrome: a randomized clinical trial, Complement. Ther. Med., 2019, 47(1):1-19.

20. Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake, Proc. Natl. Acad. Sci. USA., 2003, 13;100(10):6216-6220.

21. Gotthardt JD, Verpeut JL, Yeomans BL, Yang JA, Yasrebi A, Roepke TA, Bello NT. Intermittent fasting promotes fat loss with lean mass retention, ıncreased hypothalamic norepinephrine content, and ıncreased neuropeptide y gene expression in diet-ınduced obese male mice, Endocrinology, 2016, 157(2):679-691.

22. Bhutani S1, Klempel MC, Berger RA, Varady KA. Improvements in coronary heart disease risk indicators by alternate-day fasting involve adipose tissue modulations, Obesity, 2010, 18(11):2152-2159.

23. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Marc Rhoads J, Carey Satterfield M, Smith SB, Spencer TE, Yin Y. Arginine metabolism and nutrition in growth, health and disease, Amino Acids, 2009, 37(1):153-68.

24. Jahani M, Noroznezhad F, Mansouri K. Arginine: Challenges and opportunities of this two-faced molecule in cancer therapy, Biomed. Pharmacother., 2018, 102(1):594-601.

25. Hsu CN, Tain LY. Impact of arginine nutrition and metabolism during pregnancy on offspring outcomes, Nutrients, 2019, 11(7):1452.

26. Morris SM. Arginine metabolism: boundaries of our knowledge, J. Nutr., 2007, 137(6):1602-1609.

27. Li H, Meininger CJ, Hawker JR, Haynes TE, Kepka-Lenhart D, Mistry SK, Morris SM Jr, Wu G. Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells, Am. J. Physiol. Endocrinol. Metab., 2001, 280(1):75-82.

28. Flynn NE, Bird JG, Guthrie AS. Glucocorticoid regulation of amino acid and polyamine metabolism in the small intestine, Amino Acids, 2009, 37(1):123-129.

29. Wu G, Meininger CJ. Regulation of nitric oxide synthesis by dietary factors, Annu. Rev. Nutr., 2002, 22(1):61-86.

30. Wu M, Xiao H, Shao F, Tan B, Hu S. Arginine accelerates intestinal health through cytokines and intestinal microbiota, Int. Immunopharmacol., 2020, 81(1): 1-10.

31. Shatanawi A, Gharaibeh AM, Gharaibeh MN. The Effect of Ramadan Fasting on Arginase Activity in Healthy Volunteers, Fasebj., 2019, 33(1).

32. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton, Endocr. Rev., 2008, 29(5): 535–559.

33. Werner H, Sarfstein R, LeRoith D, Bruchim I. Insulin-like growth factor 1 signaling axis meets p53 genome protection pathways, Front. Oncol., 6(1):1-13.

34. Kineman RD, Rio-Moreno M, Sarmento-Cabral A. Understanding the tissuespecific roles of IGF1/IGF1R in regulating metabolism using the Cre/loxP system, Journal of Molecular Endocrinology, 2018, 61(1): 187-198.

35. Beaune B, Blone S, Fellmann N, Bedu M, Coudert J. Serum insulin like growth factor-I and physical performance in prepubertal Bolivian girls of a high and low socio-economic status, Eur. J. Appl. Physiol., 1997, 76(1):98-102.

36. Pollak MN, Schernhammer ES, Hankinson SE. Insulin-like growth factors and neoplasia, Nat. Rev. Cancer., 2004, 4(7):505-518.

37. Rahmani J, Kord Varkaneh H, Clark C, Zand H, Bawadi H, Ryan PM, Fatahi S, Zhang Y. The influence of fasting and energy restricting diets on IGF-1 levels in humans: a systematic review and meta-analysis, Ageing Res. Rev., 2019, 53:1-8.

38. Guevara-Aguirre J, Balasubramanian P, Guevara-Aguirre M, Wei M, Madia F, Cheng CW, Hwang D, Martin-Montalvo A, Saavedra J, Ingles S, de Cabo R, Cohen P, Longo VD. Growth hormone receptor deficiency is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans, Sci. Transl. Med., 2011, 3(70):70-83.

39. Wei T, Ye P, Peng X, Wu LL, Yu GY. Circulating adiponectin levels in various malignancies: an updated meta-analysis of 107 studies, Oncotarget, 2016, 7(30):48671-48691.

40. Dulloo AG, Montani JP. Body composition, inflammation and thermogenesis in pathways to obesity and the metabolic syndrome: an overview, Obes. Rev., 2012, 13(2):1–5.

41. Harvie M, Howell A. Potential benefits and harms of ıntermittent energy restriction and ıntermittent fasting amongst obese, overweight and normal weight subjects—a narrative review of human and animal evidence, Behav. Sci., 2017, 7(1):4.

42. Varady KA, Bhutani S, Church EC, Klempel M. Short-term modified alternateday fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults, Am. J. Clin. Nutr., 2009, 90:1138–1143.

43. Rothschild J, Hoddy KK, Jambazian P, Varady KA. Time-restricted feeding and risk of metabolic disease: a review of human and animal studies, Nutrition Reviews, 2014, 72(5): 308-318.

44. Sundaram S, Yan L. Time-restricted feeding reduces adiposity in mice fed a highfat diet, Nutrition Research, 2016, 36(6): 603-611.

45. Chaix A, Zarrinpar A, Miu P, Panda S. Time-Restricted Feeding is a preventative and therapeutic intervention against diverse nutritional challenges, Cell Metab., 2014, 20(6): 991-1005.

46. Gabel K, Hoddy KK, Haggerty N, Song J, Kroeger CM, Trepanowski JP, Panda S, Varady KA. Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study, Nutrition and Healthy Aging, 2018, 4(4): 345-353.

47. Betts JA, Richardson JD, Chowdhury EA, Holman GD, Tsintzas K, Thompson D. The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults, Am. J. Clin. Nutr., 2014, 100(2): 539-547.

48. Harder-Lauridsen NM, Rosenberg A, Benatti FB, Damm JA, Thomsen J, Mortensen EL, Pedersen BK, Krogh-Madsen R. Ramadan model of intermittent fasting for 28 d had no major effect on body composition, glucose metabolism, or cognitive functions in healthy lean men, Nutrition, 2017, 37: 92-103.

49. Kessler CS, Stange R, Schlenkermann M, Jeitler M, Michalsen A, Selle A, Raucci F, Steckhan N. A nonrandomized controlled clinical pilot trial on 8 wk of intermittent fasting (24 h/wk), Nutrition, 2018, 46:143-152.

50. Anderson LJ, Tamayose JM, Garcia JM. Use of growth hormone, IGF-I, and insulin for anabolic purpose: pharmacological basis, methods of detection, and adverse effects, Mol. Cell Endocrinol., 2017, 464:65-74.

51. Bouhlel E, Zaouali M, Miled A, Tabka Z, Bigard X, Shephard R. Ramadan fasting and the GH/IGF-1 axis oftrained men during submaximal exercise,

Ann.Nutr. Metab., 2008, 52:261–266.

52. Oh HS, Oh SK, Lee JS, Wu C, Lee SJ. Effects of L-arginine on growth hormone and insulin-like growthfactor 1, Food Sci. Biotechnol., 2017, 26(6):1749–1754