ADVANCED AND CONTEMPORARY STUDIES IN HEALTH SCIENCES

EDITOR
Assoc. Prof. Sadettin DEMİREL

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Chapter 1

USE OF TEA (*Camelia sinensis*) FACTORY WASTE IN LABORATORY ANİMAL BREEDİNG.

Buğra Genç [1]
Cemile Aslı Bilgici[2]

Abstract

A significant amount of factory waste is generated during the production of tea, which is among the beverages and the most consumed beverage in Turkey. There are problems in managing these wastes for different reasons. Storage of these wastes brings extra costs. Methods such as burning, burying in the ground, and throwing into rivers and seas cause various biological and environmentally threatening problems with their chemical structure and phenolic compounds. Therefore, different methods are needed in waste management. To date, some attempts have been made to use these wastes in an area where they can be useful by protecting them from their harmful effects (manure, chipboard, caffeine, pellet fuel, ruminant litter and fish feed production). One of these initiatives is its inclusion in animal diets. However, studies on their inclusion in animal diets have shown that they may have very limited use due to the high amounts of tannins and catechins they contain. According to these results, a method that is sufficiently effective to bring tea factory waste into the economy as a commercial product has not been clearly found in these areas. In this study, the potential of plant wastes produced in tea factories to be included in the diet rations of different animal species by reducing tannin levels without significantly affecting the nutrient profile with a simple and economical method was investigated and the results of a preliminary study were presented. In the light of the data obtained, it was concluded that it is possible to use tea factory wastes in laboratory mortar diets and cages as bedding material.

Keywords: Bedding, diet, laboratory animals, tannin, tea factory waste.

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Introduction

The countries with the highest production and consumption of tea (Camelia sinensis) in the world are China, India, Kenya and Turkey, respectively. It is known that, according to 2021 data, China reached a production level of 13,757 thousand tons in tea production, India reached 5,482 thousand tons and Kenya reached 2,338 thousand tons. In recent years, approximately 50% of the world reserves have been made up of Chinese production. According to the latest tea production data obtained from the Rize Commodity Exchange, 442,490,421 kg of fresh tea was processed in 2022, and 230,044,773 kg of the fresh tea produced was processed by the private sector and 212,445,648 kg by ÇAYKUR. (Çaykur, 2023; Anonym, 2023).

Tea plant, which produces food quality products in Turkey, is a plant that is produced in higher tonnage than many other plant foods due to cultural characteristics. In tea production, physical processes are applied to the plant in factories. During the processes, content-rich waste parts in the form of fiber and dust are released from leaf clippings and woody plant stems. It is known that these wastes contain high amounts of cellulose, acid detergent fiber (ADF) and neutral detergent fiber (NDF). Woody wastes and leaf clippings, which are completely natural and vegetal, can dissolve in nature and turn into soil over time as organic matter. These wastes have a water retention capacity of 2.6 times their own weight and have low salinity and acid character (pH 5.35). (Kütük et al., 1996). Çayın işlenmesi sırasında %3,5 oranında yukarıda tanımlanan şekliyle çay atığı ortaya çıkmaktadır (Yalınkılıç et al., 1996).

According to the latest published data (Çaykur, 2023) for 2022, considering that 3.5% of the 442,490,421 kg of fresh tea is produced as waste during processing, it is seen that serious amounts of waste are generated in the Black Sea Region. Disposal of these wastes with various alternative methods has been evaluated within the scope of previous studies and projects (e.g. Rize Tea Research and Application Center Technical Support Project, BROP CCI No.2007 TR 16 I PO 003).

However, it has been concluded that it is not efficient to use it as fertilizer, furniture raw material, fuel, cattle and sheep feed.

Although it is considered as "harmless" waste as included in the Waste Management Legislation, measures such as storing tea waste, ensuring storage conditions, and ensuring odor management become a burden for small producers and businesses in terms of both financial, environmental and human resources. Some of the waste is converted into low-calorie energy by burning it in a small number of businesses with suitable incinerators. For a material that is harvested, processed and turned into a product in high tonnage on an annual

basis, this waste rate should not be ignored when there are alternative areas of use. These wastes, which cannot be effectively recycled by factories and used as alternatives, also have the potential to cause environmental pollution.

In countries on different continents where annual tea cultivation and production is carried out at high intensity, the wastes produced are buried in the ground, burned or converted into alternative products with different methods in order to dispose of them. These products are used worldwide in the caffeine industry (Konwar, 1988), the pig and poultry feed industry (Chutia et al., 1983; Uuganbayar et al., 2006; Ko and Yang, 2008), the fish feed industry, the organic fertilizer industry (Aşık and Kütük, 2012) products or by-products. However, a very small portion of the 3.5% of the waste generated during tea production in our country is used as pellet fuel, and the rest is disposed of by pouring into water resources, seas and rivers and burning, which has the potential to be harmful to the environment (Yalınkılıç et al., 1996).

Mixing tea waste into the soil and dumping it into rivers and the sea is a practice that harms the environment because the caffeine and phenolic compounds it contains increase soil acidity (Chowdhury et al., 2016) and change water quality. When this practice is carried out in agricultural regions, it may also cause problems in growing other agricultural products as it will increase the amount of tannin in the soil.

Storage of waste generated in tea production poses financial and physical difficulties.

Due to the resulting product being a food processing waste and its environmental impacts, it is very important to manage the waste appropriately to avoid non-compliance with legislation on waste storage and management (Chowdury et al., 2016). In this regard, producers must have a license in accordance with the waste regulation and there must be scientific and environmentally friendly guidelines for the disposal of tea waste. Large and suitable areas for storing the waste under suitable conditions and equipment and labor costs for its transfer are also among the issues that need to be taken into consideration. Although experiments are being carried out on its use as raw materials for alternative products in different sectors (animal feed, chipboard, mushroom cultivation, compost, pellet fuel, etc.), it does not seem possible to talk about a successful and sustainable transformation, especially in our country.

When the research on the use of various varieties of the tea plant in animal nutrition is examined, it is noted that the pulp remaining after consumption of the plant in its fresh or processed form as a beverage is used (Nasehi et al., 2017). There are more than 4000 chemicals in the structure of this plant, which

does not shed its leaves all year round (Kaçar, 1997). Of these substances, flavonoids, flavones, flavanones, isoflavones, flavonols, flavanols and anthocyanins are the most important in terms of nutritional physiology. It is seen that the antioxidant, anticarcinogenic and antiatherosclerotic properties of these substances are cited in many studies (Balentine et al., 1997). In the reports of Konwar et al., (1985), it is seen that the tannic acid level in tea waste is 6.3%. Kunjikutty et al., (1977) found this value to be 1.8%, and Imik et al., (1999) found it to be 7.89%. It is thought that these changes are caused by regional and soil characteristics and plant diversity (İmik et al., 2002). Imik et al., (1999) reported that 7.89% tannin content had a toxic effect on ruminant animals. However, in another study by the same researcher (İmik et al., 2002), it was also reported that 10% tea waste in the diet did not negatively affect animal nutrition parameters and animal health in ruminants. It is noteworthy that the results of these studies examined gave contradictory results. However, these studies reflect the results of studies conducted on ruminants. The fact that the digestive system of ruminants has a microbial population activity that is significantly affected by polyphenolic compounds and especially the tannin level seems to have made the waste product not preferred in this area. For the reasons mentioned above, tea waste has not been brought to a level that can be used economically in the field of animal nutrition. In vivo and in vitro studies using factory wastes of conventionally produced teas (Özyılmaz and Genç, 2019) yield different results. In a study investigating the production of pellets from factory tea waste (Bilgin et al., 2016), it was reported that the pellet form could be achieved with moisture, heat and pressure applications. According to these data, it can be thought that tea factory wastes may be included in the pellet structure, which is the general form of diet for laboratory animals. This will contribute to meeting the local raw material needs in the production of laboratory animal diets. In research using live animals, creating a healthy and accurate animal model is a condition of primary importance in order to achieve results as close to reality as possible. Choosing the most appropriate diet is important for the nutrition of animals, which is vital for them to remain suitable for the conditions of use in research throughout their lives. In the field of animal nutrition, the physical structure of the diet, its nutritional content, easy availability, easy and economical supply of the required raw materials, compliance with transfer and storage conditions, and presentation methods to animals are factors that have the potential to directly affect animal health, research results, economy and workforce. As a matter of fact, for the repeatability feature, which is one of the reliability criteria of research, it is

necessary to provide the nutrient requirements of the models at the most appropriate level and condition (Barnard et al., 2009).

Research in terms of Nutrient Content

Tea factory wastes have been evaluated in some studies in terms of their nutritional composition. In some of these studies, it is seen that trials have been carried out on their use in diet rations used in ruminant (Konwar et al., 1985), poultry (Angga et al., 2018) and fish (Zhou et al., 2016) nutrition. However, there is no research on its use in laboratory animal diets. İmik et al., (2002), in a study in which they determined the use of tea factory wastes in ruminant nutrition and their nutritional values, determined the dry matter (DM), crude protein (CP), ether extract (EE) and crude ash (CA) values (%) as it to be 93.02, 14.38, 1.06 and 4.38 respectively. According to the results, it was emphasized that adding 10% of tea factory waste to ruminant rations could be tolerated in terms of health and productivity performance and that animals could get used to this material rich in tannins. Angga et al., (2018) reported that DM, organic matter (OM), and CP values were 93.59%, 88.08%, and 19.63%, respectively, and that it could be used safely in broiler rations by reducing the amount of tannin without negatively affecting the CP value. Nasehi et al., (2017), in their study, found that DM, CP, CA, OM, EE, neutral detergent fiber (NDF), acid detergent fiber (ADF) and metabolic energy (ME) values were 92.72%, 15.66%, 5.75, 94.24%, 1.16%, 38.47%, 25.87% and 7.7 Mj/kg respectively.

In another study investigating similar parameters, Kunjikutty et al., (1977) reported nutrient values as CP 29%, EE 7.4%, Crude cellulose (CC) 14.1% and CA 3.8%. Zahedifar et al., (2019) found that the DM, CP, NDF, EE, CA and ME values of the products in the waste phase were 94%, 19.3%, 47.6%, 8.4%, 6.18% and 19.6 MJ/kg, respectively. In an in vivo study conducted by Ahmed et al. (2015) with green tea factory wastes, they reported that the DM, CP, EE, CC and CA values in the wastes used were 80.88%, 20.1%, 2.1%, 18.2% and 4.88%, respectively.

Konwar et al., (1985), in a study in which they used tea factory wastes, which they determined as CP 19.8%, EE 1.37%, and CA 7.58%, in goats fed with rations containing tea factory waste at different levels (0%, 0.5%, 1% and 2%). They reported that live weight gain and feed consumption showed a linear increase in proportion to the amount of waste ($p<0.05$). In the same study, it was determined that blood serum glucose and total cholesterol levels decreased significantly ($p<0.05$) with increasing waste rate. This positive effect on live weight and feed consumption was reported as by Tan et al. (2011) in their research, it is believed that it is due to the effectiveness of tea catechins, which

support the development of intestinal tract and rumen microorganisms. Ahmed et al., (2015) supports this finding that tea factory wastes have a positive effect on feed consumption and live weight gain in goats, with assessable CP levels varying between 22-35%.

In a study conducted by Özyılmaz and Genç, (2019) with in vitro gas production technique, the nutritional values of tea factory wastes were determined as DM 95%, OM 90%, CA 3.5%, NDF 47%, ADF 47%, CP 14%, EE%. 1 and ME was determined as 8 MJ/kg DM.

In this form, it has been determined that it can be more valuable than many feed raw materials used in animal nutrition. According to the research results, the nutritional values of tea factory wastes vary. As reported by Fazaeli et al., (2000), geographical differences, differences in cultivation methods, processing techniques and differences depending on the tea variety stand out as the reason for this. It is known that the nutrient profile of the wastes produced may vary due to the fact that tea processing techniques [(Çaykur, Rotervan, Orthodox and CTC (crushing-tearing-curling)] involve heat and pressure applications at different degrees and durations (Nas and Gökalp, 1991). According to the research results referenced above, it can be said that tea factory wastes have a remarkable feature in terms of their nutritional content in animal diets. Especially protein sources, which have the most important share in diet production, cause high costs. Companies producing quality laboratory animal diets resort to using imported raw materials in order not to compromise on quality, which further increases the cost. Tea factory waste, whose crude protein value can be between 14% and 35%, is seen as having the potential to provide an advantage in this regard by being included in the ration at a certain rate.

In addition to ruminant feeding, it is possible to include plant sources in the nutrition of rabbits, which are also called pre-ruminants due to their digestive system physiology characteristics, to meet the need for cecum microbial activity and to ensure crude cellulose (CC) balance in the rations. This situation brings about the necessity of using extra roughage resources for diet production. According to the research results previously referenced, it can be seen that tea factory wastes have an average CC value of 18% and can provide an advantage in meeting this requirement. In this way, the need for extra roughage use can be reduced with balanced rations.

A Preliminary Study on the Reduction of Tannin and Phenolic Compounds

In a study carried out by The Scientific and Technological Research Council of Turkey (TÜBİTAK) with the support of code 2209 (Buğra Genç, Cemile

Aslı Bilgici), it was aimed to reduce the tannin levels of tea factory wastes with a simple and economical method without significantly affecting the nutrient profile. After reducing tannin levels to desired levels, its potential to be included in animal diets was investigated. Tea factory wastes as research material were provided from 5 different factories with high tea processing intensity in Rize Province. Factory wastes were selected from different forms of waste, including thin fibrous wastes and thick fibrous wastes, depending on the plant having different parts containing woody structure and the processing method. Sampling was done using the bulk batch sampling method. The first number of samples to be taken from the bulk lot was calculated as the square root of 20 times the amount of the lot in tons, with the upper limit being 7 samples for lots up to 2.5 tons and 100 samples for those weighing more than 2.5 tons, as reported in British Columbia Ministry of Forests, (1996). The tea factory wastes enhanced were transferred to the laboratories of Ondokuz Mayıs University and after pre-drying, they were ground in the forage grinding machine and made ready for laboratory tests. Aggregate samples obtained from different factories were combined and used.

Research groups were formed as follows:

In thin form and not treated with water: (ThD)

In thick form and not treated with water: (TcD)

In thin form and treated with water: (ThW)

In thick form and treated with water: (TcW)

In order to reduce the polyphenol and catechin levels in the trial group wastes, the wastes were treated with 1/50 non-chlorinated water for 48 hours according to the method reported by Angga et al., (2018). After the treated samples were filtered using gauze, they were kept in the drying cabinet at 65 °C for 48 hours. This procedure was not applied to the control group waste. In the control and research groups, Weende analysis, Soxhelet extraction and metabolic energy calculations (AOAC, 2019) were performed and dry matter (%), crude protein (%), crude fat (%), crude cellulose (%), crude ash (%) ratios were determined. Nutrient profile was revealed. For these analyses, 5 (n) samples from each group were analyzed (Table I). In order to determine to what extent the procedure for reducing the Polyphenol and Catechin levels is effective in the control and research groups, total polyphenol (%), catechin (ECG) (%), caffeine (%) and gallic acid (%) rates were determined through service procurement. Total polyphenol analysis was determined by ISO 14502-1/2005 UV spectrophotometric method, total catechin amount was determined by ISO 14502-2/2005 HPLC method, caffeine and gallic acid content was determined by TS ISO 107207 UV spectrophotometric method, and the amount

of condensed tannin was determined by UV spectrophotometric method. Chemical analyzes were carried out in the Western Mediterranean Agricultural Research Directorate Medicinal and Aromatic Plants Center Laboratory and Rize Tea Research and Application Center (ÇAYMER) Laboratories (Table II).

The following formulas were used to determine the energy amounts of tea factory waste according to animal species:

Metabolic energy formula for rat and mouse: ME,MJ KG/DM = (0,01465xCP) + (0,03558xEE) + (0,01465 x NFE).

NFE (Nitrogen free extract) = DM – (CP+EE+CS+CA).

Digestible energy formula for rabbit: DE = (-1801+7,1xCP+12,01xEE+5,59XNFE) kcal/kgDM.

NFE (Nitrogen free extract) = DM – (CP+EE+CS+CA).

Metabolic energy formula for guinea pig: ME = 15,9-0,219xCP

Table I. Dry matter (DM) (%), crude ash (CA) (%), crude protein (CP) (%), ether extract (EE) (%), crude fiber (CC) (%) NFC (g/kg) and metabolic energy (kcal/kg ME) values of tea factory wastes ,

Parameters	ThD	ThW	TcD	TcW
DM (%)	92.45	92.00	91.00	92.00
CP (%)	18.17	15.50	13.00	10.80
EE (%)	1.16	1.40	0.90	1.00
CC (%)	18.20	17.40	21.00	20.00
CA (%)	3.80	3.80	4.20	4.00
NFC (g/kg)	510.00	540.00	527.30	564.00
ME (Rat-Mouse)	2522	2545	2372	2440
DE rabbit	2482	2470	2165	2244
ME Guinea pig	2844	3000	3102	3228

Table II. Total polyphenol (g/g%), catechin (ECG) (g/g%), caffeine (g/g%) and gallic acid (g/g%) and condensed tannin (mg catechin/mg tea waste%) values of tea factory wastes.

Parameters	ThD	ThW	TcD	TcW
Total polyphenol	9.1100	5.7900	6.9900	3.3800
Catechin (ECG)	0.1135	-	0.0410	-
Caffeine	1.1180	0.3750	1.0125	0.1730
Gallic acid	0.1445	0.0380	0.0560	0.0230
Condensed tannin	23.8300	15.8000	20.4800	10.9000

According to the results obtained, it was concluded that while the soaking process caused a slight decrease in nutrients, it could have a significant reducing effect on tannin and phenolic compounds. It seems that there is a need for further research in which the statistical significance will be examined with a larger number of samples, and it is thought that their use in this field may be useful if similar results are obtained.

In the light of the first results obtained from this preliminary research, basic data has been obtained for the economic recovery of a waste material that is harmful to the environment and costly to store in the livestock industry. These data are also valuable as preliminary findings of a more detailed research at the next stage.

Tea Factory Waste as Animal Bedding

In addition to being included in diets, tea factory waste can also be considered as an alternative, healthy and economical litter for laboratory animals. It is a known fact that laboratory animals do not eat tea factory waste without any processing. Therefore, it can be considered as a bedding material. It is seen that the material preferences to be used as bedding in livestock farming vary from region to region in terms of cost and availability (Gençoğlan and Gençoğlan, 2017). These materials are examined under four general categories: wood, plants, soil and recycled products (Malone, 1992). These products can be listed as chip sawdust, sawdust, gypsum chips, bark, straw/leaf, factory paper waste, grain and other plant wastes and residues. In laboratory animal breeding, wood shavings, paper scraps and corn cob scraps are the most commonly used products as bedding. While these products can be obtained with economical methods for use in conventional cultivation, there are also more costly products produced with more advanced techniques in line with the requirements of the cultivation method (Gençoğlan and Gençoğlan, 2017).

Laboratory animal bedding is basically; It serves to absorb moisture from feces and urine and to provide nesting material. These materials are of importance not only regarding the microenvironment of the animals but also the characteristics of the cage and room in which they live (Hirsjiirvi and Viiliaho 1985, Raynor et al. 1983, Sakaguchi et al., 1989). With these features, bedding for laboratory animals has a very important place in terms of animal welfare.

Since substrate materials have the potential to interact with experimental interventions, they can affect the outcome of experiments on enzyme induction, cytotoxicity, carcinogenic compounds and anesthetics (Törrönen et al., 1989, Potgieter and Wilke 1992). For this reason, in the selection and application methods of the substrates, care should be taken to ensure that they comply with

the standards to ensure consistency and comparability of the test results. It is known that a suitable litter to be used in laboratory animals should have high moisture absorbing properties, be non-traumatic and non-toxic, easily available, economical, not harmful to cage disinfection systems, and free of dust and splinters (Kraft, 1980). The bedding material can be consumed by animals due to behavioral disorders, stress and/or feeding procedure faults. For this reason, if the materials to be used as litter originate from agricultural products, they should not contain harmful chemicals that can cause accumulation in living tissues, such as pesticides used in cultivation (Malone et al., 1983). In the region where tea production takes place in Turkey, there is no obligation to use pesticides in cultivation due to snowfall and fluctuating annual temperature between the Georgian border and Ordu Province (Seyis et al., 2018). This shows that tea factory waste does not pose a potential danger in this respect since there are no pesticide residues. In particular, bedding materials such as wood sawdust, which are prone to disintegration and pollination, can cause respiratory or eye problems with the dust and particles they create. In addition, wood and splinter pieces trapped in sawdust may also have traumatic effects on animals (Smith et al., 2004). Not all trees from which sawdust originates may be suitable for laboratory animal research. It is known that especially cedar and white pine sawdust are not suitable for use in pharmacological studies. There are also studies reporting that clover, which is considered to be used as an alternative bedding source, dyes the fur of animals and is therefore not suitable for use (Smith et al., 2004). Since tea factory wastes have a fibrous structure, they do not cause disintegration and eventual pollination as much as wood sawdust. With these features, it can be thought that tea factory wastes may be useful in preventing respiratory and eye diseases and mechanical traumatic injuries of animals caused by pollination.

To date, there have been very few comparative studies on the animal health of bedding used in mice and rodents (Smith et al., 2004). There are studies reporting that the reproductive efficiency of mice using corn cob bedding was reduced compared to the groups using sawdust (Port and Kaltenbach, 1969), and that conjunctivitis was observed in athymic mice using cotton bedding (Bazille et al., 2001). In a study investigating the effects of bedding on mucosal immunity (Sanford et al., 2002), it was reported that there was an increase in the number of Peyer's patches in the groups where sawdust bedding was used, and the virus-specific IgA level was higher in Peyer's patches and mesenteric lymph nodes. It was reported that liver microsomal enzyme activity was significantly ($P<0.05$) different in inbred and outbred mice tested on different wood sawdust (Vessel, 1967). It has been observed that in animals using bedding prepared

with sawdust obtained from red cedar, white pine and pondoresa pine, sleeping time decreased and liver microsomal enzyme activity, which metabolizes hexobarbital, decreased compared to animals using mixed beech, birch and maple sawdust. It has been reported that liver microsomal enzyme activity, caused by chlorinated hydrocarbon-containing insecticides and eucalyptol-containing aerosol sprays, increased in animals using cedar sawdust bedding containing aromatic hydrocarbons, even at low levels (Vessel et al., 1976). It was also observed that in mice in which red cedar sawdust was used, the duration of anesthesia decreased (Beamer et al., 1981), liver microsomal enzyme activity increased, and the incidence of breast and liver tumors increased (Sabine, 1975). Corning and Lipman (1991) showed that the ammonia level in cages with micro barriers and filter covers, where wood sawdust was used as litter, was significantly higher than in open conventional cages and could reach dangerous levels (>200 ppm) before the weekly litter change period expired. Depending on the quality of the litter, the effects of ammonia accumulation on mice and rats can be demonstrated through research. Increased mortality in rats (Appelman et al., 1982), negative effects on the immune response in guinea pigs (Targowski et al., 1984), lack of concentration in Long-Evans rats and Swiss albino mice in the running ring, and rats being affected more than mice (Tepper et al. ., 1985) results can be given as examples of these studies. According to the National Institute for Occupational Safety & Health (NIOSH, 2023) statement, aerosol ammonia level has the potential to affect human health when exposed to 25 ppm/8 hours or 35 ppm/15 min. These disorders may occur in the form of irritation to the respiratory system, burning in the eyes and difficulty breathing, as well as exposure to unpleasant odors. It is possible for free ammonia gas to reach a level that will disturb humans in laboratory animal units as a result of the water holding capacity and desiccant properties of the bedding being inadequate, cage filters and covers malfunctioning or being opened by animal movements.

Conclusion

It is seen that the use of tea factory wastes in the diets of laboratory animals that require advanced studies in the field of animal nutrition and as bedding material, which is of great importance in terms of animal welfare, will contribute to the economy of a material that does not have a low potential for waste and harm to the environment. In addition, it can be thought that by evaluating this material, a positive step can be taken towards sustainable environmental health.

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Chapter 2

Tritrichomonas foetus cases reported in Turkey between 1951-2022

Burak ŞAHİN[1]

INTRODUCTION

Although protozoans in the Trichomonadidae family are generally found in the digestive system, they can also be seen in the reproductive system and other systems. There are Trichomonas, Tritrichomonas, Tetratrichomonas, Pentatrichomonas and Trichomitus genus. (Vuruşaner and Gülanber 2015).

Tritrichomonas foetus is seen in cattle, cats, zebu, horses, pigs and deer. It settles in the reproductive systems of cattle and causes abortions. It is not zoonotic. It is widely seen in the world. (Vuruşaner and Gülanber 2015, Kanca et al. 2019).

The agent has a direct life cycle. Their final hosts are cattle and they have no intermediate hosts. Since there are no intermediate hosts, the disease is transmitted through the mating of a bull susceptible to the disease with an infected female or by the mating of an infected bull with a female susceptible to the disease. The agent colonizes the male and female reproductive tract and does not spread to the epithelial tissue. When bulls are infected, the agent is localized in the secretions at the distal epithelial border of the prepuce, penis and urethra. Rarely, the disease is asymptomatic with a mild, small amount of purulent discharge in the first 14 days of infection. Apart from natural mating with asymptomatic bulls, the spread of infection can also be seen through vaginal examinations via speculum and not changing surgical or artificial insemination gloves. (Ondrak 2016).

As bulls get older (>3-4 years), the epithelial crypts in the penis and prepuce deepen, creating microaerophilic environments suitable for chronic infections, which causes chronic carriage. For this reason, young bulls are infected for a short time, and by rejuvenating the bulls in the herd, the spread of infection decreases and the success of treatment in young bulls increases. It is important to increase the young bull population in the fight against the disease. (Michi et al. 2016, Ondrak 2016, Kanca et al. 2019).

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Tritrichomonas foetus is closely related to herd size and herd management. Increasing herd sizes cause an increase in the prevalence of *Tritrichomonas foetus* in bull populations. In a large-scale study, the disease prevalence in bulls in extensive beef cattle herds with ≥ 500 females per herd was determined to be 53.9%; In herds with 100-500 female cattle, the prevalence is calculated as 10%. It has been determined that the prevalence of the disease increases significantly as the bull/cow ratio increases in the herd. (Rae 1989).

When female cattle are affected by the disease, a mild vaginitis occurs. During the estrus phase of the sexual cycle, the agent settles in the uterine lumen and settles in the uterus, oviduct, cervix, vagina and vulva within an average of 7-14 days (Kanca et al. 2019). The presence of the agent does not affect the ciliary activity of the oviduct. In general terms, although the infection does not prevent fertilization, it is characterized by catarrhal endometritis, vaginitis and edema in the organs of the reproductive system. It has been stated that removal of the agent from female genital organs may take between 95 days and 22 months. Immunity against the disease is short-lived but progresses slowly. It has been reported that the immunity period does not exceed 450 days. Animals that have recovered from the disease may become susceptible again and infections may reoccur. (Yoo 2010, Corbeil et al. 2005). Fighting the disease in herds is difficult and long-lasting due to the occurrence of reinfections (BonDurant 2005).

Prolongation of the calving interval is the most important reproductive effect of the disease. It has been determined that, on average, the calving interval is extended by 96 to 98 days. A simulation model showed that when the prevalence of bulls in herds with the disease increased from 20% to 40%, the annual number of calves decreased by 14-50% (Villarroel et al. 2004). The first clinical findings that usually attract attention in flocks affected by the disease are abortions and pyometra. However, the incidence of abortions and pyometra is low (≤ 5%). Early abortions and embryonic deaths are common due to the inflammatory response that occurs in the uterus approximately 45-60 days after infection. Embryonic deaths are generally 50-70. It is formed between days and therefore interestrus intervals are lengthened. Following embryonic deaths, the gestational corpus luteum becomes permanent, and pyometra and anestrus occur as a result of intense inflammation in the uterus. Abortions usually occur in the 2nd-4th week of pregnancy. It takes place during the months. (Kanca et al. 2019).

Table 1. Studies on the spread of *Tritrichomonas foetus* in Turkey

Researchers	Country (City)	Animal	Material	Method	n	Prevalence (%)
Mimioğlu (1951)	Turkey	Cow	Uterus Foetus	Direct microscopy	554 25	0
Bıyıklıoğlu and Pişkin (1999)	Turkey (Ankara)	Bull	Preputial fluid	Culture	50	0
Serin et al. (2010)	Turkey (Aydın)	Cow	Vaginal smear	Direct microscopy	164	8,5
Guven et al. (2013)	Turkey (Erzurum)	Cow	Foetus	PCR	246	5,7
Pekmezci and Pekmezci (2017)	Turkey (Samsun)	Cat	Feces	PCR	100	0
Katanalp and Koçhan (2020)	Turkey (Diyarbakır)	Cat	Feces	Inpouch TF Feline Culture – PCR	30	0
Irehan et al. (2022)	Turkey (Elazığ)	Cow	Uterus-Foetus	PCR	55	3,63

Control of trichomoniasis can be achieved by testing bulls before the breeding season (Givens 2006).

The test is performed by culturing the aspirated preputial discharge in microbial culture. Negative results become certain after planting two or three cultures (Peter 1997). Cows become susceptible to the disease again 1 year after recovery. A vaccine is available for *Tritrichomonas feetus*. Vaccines called Trichguard or Trichguard Plus (Fort Dodge Animal Health, Madison, NJ, USA) are administered to cows. There is no vaccine for bulls (Anderson et al. 1996).

It has been reported that *Tritrichomonas foetus* is commonly seen in cats in environments with high environmental stress, such as shelters and cat breeding enterprises, and in young cats that have not reached immunological maturity, in many European countries such as America, Germany, Italy, Greece, Switzerland, Korea, Iceland, and in America (Katanalp and Koçhan 2020). Although the infection does not cause a serious clinical picture in animals, when it settles in the intestine, ileum, cecum and colon, it can cause plasmacytic, lymphocytic inflammations and recurrent diarrhea with gaseous, mucusy, foul-smelling and sometimes bloody stools, thus weight loss, loss of appetite and vomiting may occur. Cats with serious clinical findings may develop fecal lumps and anal inflammation (Tolbert and Gookin 2009, Balkaya and Dumanlı 2013, Tınar and Umur 2015). In young cats, the anus may become erythematous, edematous and painful due to severe diarrhea. As diarrhea becomes more severe, the likelihood of rectal prolapse increases (Katanalp and Koçhan 2020).

Definitive diagnosis of *Tritrichomonas foetus*, which can be confused with giardiosis on microscopic examination, is made by PCR and culture. It is reported that ranidazole, furazolidine and metronidazole are used in its treatment (Pekmezci and Pekmezci 2017, Arranz Solis et al. 2016).

CONCLUSION

As a result, although the number of studies in Turkey is limited in the literature review, it has been seen that protection and control methods should be increased and animal owners should be made aware of the disease, as *Tritrichomonas foetus* causes serious clinical findings, including abortion and pyometra in cows, and fecal clumping and anus inflammation in cats.

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Chapter 3

Online Learning and Teaching Approach Programs Used in Education

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GİRİŞ

To comply with social distancing regulations during COVID-19, many universities had to quickly adjust the way they manage teaching to accommodate online learners. Blended learning (BL) is the combination of traditional face-to-face teaching with technologies that enable learning and teaching (TELT) and is an approach used by many higher education institutions worldwide. Blended learning enables diverse and innovative teaching and learning approaches that can support the delivery of midwifery education online, providing students with high quality education as well as safety. Currently most midwifery education is delivered through. Traditional face-to-face teaching approach through online, digital, electronic learning (eLearning), distance learning as mobile learning (mLearning), electronic portfolios (ePortfolios) or a blended approach. Distance learning is not a new concept, before the development of the internet, distance learning resources were sent through the mail. Currently, distance education has been transformed into digital education; it is delivered directly to students via eLearning or mLearning platforms. Recently, the term mLearning has become popular, but is used interchangeably with eLearning; mLearning refers to learning material that can be viewed on mobile devices (Moore, 1990).

1. Systems Used in Distance Education

1. Electronic Learning (E-Learning)

Electronic learning applications and processes, web-based trainings, computer-based trainings, virtual classrooms and digital collaborations.

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Training content Internet, intranet, extranet, audiovisual or audiotape, mobile devices, satellite television and CDROMs through a mediator. It has a wide range of tools and equipment for its implementation and dissemination. has. In some applications, all of these tools are utilized, while in other applications or a combination of several of them (Coşgun, 2007).

2. Online Learning

The connection of computers to other computers through a network, online is called. For teaching data, computers and communication lines (internet, intranet, interactive electronic learning systems that distribute them to users via telephone) online learning. In an online learning activity, learners content is presented by the computer according to their needs. This learning The basic tool in the model is the computer and computer networks. Access to the system online learners are in effective communication with instructors or other students. Education is usually delivered synchronously (Coşgun, 2007).

3. Web Based Education

Web-based education, as the name suggests, is delivered over the web. This is The Internet is actively used in the system. Today, the Internet comes to mind when it comes to the web. However, web-based education can also be done on local networks. The most important thing here is web surfers. In web-based education, a system that will affect the users and can be accessed quickly is preferred. This is directly related to the Internet or intranet used by the user and where the system is installed. Generally, training sheets are created using HTML, the standardized language of the web. Apart from HTML Using flash, animated or more visual documents can be prepared. This system also facilitates the use of videos and audio files previously uploaded to the system. Web-based education, which is realized without space and time limitations, is generally preferred (Alkan 2003).

4. Mobile Learning

Education, which is called e-Learning, can be accessed via mobile devices. is called m-Learning (mobile learning). Recently The increasing use of laptops in Turkey makes the concept of mobile learning more popular. to bring it to mind. Mobile devices include laptops and tablets computers, pocket PCs, portable media players, smartphones, etc. is received. Mobile devices can use wireless connectivity, GPRS connection, Bluetooth or can be connected to the educational environment using infrared (Barutay 2009).

5. Internet Supported Education

Internet-supported education, synchronously or asynchronously via the internet is a form of education. The Internet is the largest computer network. Communication It happens over TCP/IP. We can also see it as an extended form of web-based education. Web surfers take an active role in web-based education. Only web surfers play a role on the Internet. the real estate can also be any application that connects over the TCP/IP protocol (Çardak 2006).

6. Virtual Universities

Apart from traditional universities, there are other universities that provide education and training only in the virtual environment. universities are called virtual universities. Virtual universities can be established in Turkey. There is a need for regulation changes to be made by the Council of Higher Education (YÖK). Some segments even distance education is difficult to be accepted by universities, while virtual universities It seems difficult to establish in our country at the moment. Making something virtual means to discover its importance and then to combine it with a new content without losing its old power. is to recreate it. So creating an efficient virtual university means creating a real to explore the power resources of a university and then to make it available in an electronic environment. it means to recreate.

Training is the first part and constitutes the infrastructure of management. brings.Currently, "student unity" is achieved through various means (chat rooms, visiting areas, libraries, game rooms where you can get anything you want) virtual. It is possible to encounter universities. Only a few of them are online, virtualcounselors. These virtual universities also provide a place where students can monitor community activities on campus or on bulletin boards, as well as other important information. they make publications that can be received. and at this rate, in the future, art galleries and other cultural activities can also still be brought online. By carrying out all these components together A powerful learning experience can be created in a corporate environment.A virtual university can thus not only gives us information, but also information about corporate culture and social culture. to the virtual universities.In fact, when the functioning of existing virtual universities is examined one can see what a virtual university looks like. The emerging communication technologies The fastest adoption in education systems is taking place in the USA. Distance America, which has more than

a century of history in education, is now trying to countries' attention (Gökçe, 2008; Kaya, 2002).

2. Platforms Used in Distance Education

Synchronous (Simultaneous) Distance Learning

The learning-teaching activity is a two-way communication between the student and the teacher. is an application. In this regard, the two-way tele-conferencing model, the two-way There are three models: one-way TV and two-way internet-based conferencing. In the system, students and teachers tele-conference with each other over the internet. (Olcay, 2011).

Asynchronous (Asynchronous) Distance Learning

Teacher-student and teacher-student interaction in the space where learning-teaching activities are carried out is an educational model in which communication between student-student groups is one-way. This within the model; students and teachers cannot communicate among themselves and they do not get immediate answers to their questions. Teaching by letter, radio, television and one-way. Internet can be given as an example of this model. In this model, teachers can use They present the information to their students on web-based pages. Students can access this information they can take their time and work. In this model, it is sufficient for users to have internet access (Olcay, 2011).

3.Software Used in Distance Education

Moodle

Moodle is an easy-to-use online course management system. Moodle is free and open source software. Moodle is a "Modular Object Oriented Dynamic Learning Environment" Flexible (Modular) Object-Oriented Dynamic Learning. It stands for Environment. It can be used very easily by anyone (teacher, student). is its biggest feature. Moodle is used in more than a hundred countries. language support is available. Moodle has a wide range of developers and is fast to translate. New versions are developed and used in a very short time. System Windows and Linux systems under which it can operate (Hunte2010).

Adobe Connect

Adobe Connect is a platform that brings teacher and student together is a software that helps make lessons more understandable. Specialized software

through which simultaneous and video chatting is also possible. With this software features of traditional education with file sharing, video and voice chat and web based activities can be realized. It can be a way of engaging and stimulating students' interest. effective content can be designed quickly with adobe connect. Adobe Connect It is used in the infrastructure of many universities providing distance education (Işık, 2009).

Dimdim

Dimdim Moodle is a web-based learning content management system (LCMS- based Learning Content Management System). Moodle is an open source system and can be used for free. It can even be modified and distributed (Ajlan et al, 2008). Dimdim is web-based. Without the need to install any program documents, web tools such as quizzes, lessons, assignments, calendar, gradebook provides a variety of tools. Also open source, moodle is a real tool called Dimdim. It also provides support for a timely web conferencing application (Terry et al., 2009).

Enocta Education Platform

Enocta Education Platform is a platform that enables organizations to realize their training and development projects achieve the desired results of knowledge sharing and behavioral development Enocta is an education management system that helps to monitor and measure whether it is achieving its goals. Enocta Education Platform is today Turkey's most preferred education management systems. Enocta Education Platform comprehensive functions and features offers. With Enocta Education Platform, companies can manage all their training and development activities from a single point. Educational institutions with Enocta Education Platform, collecting requests for training events, planning events, matching training needs create training programs, and quickly and quickly thanks to the online assessment and evaluation system. measurement, tracking and budgeting of training resources from a single center (Aldım, 2013).

Distance Education Formal Education Comparison

A serious questioning in the understanding of education with distance education started. While formal education dominated the education system, its former supporter, distance education education, and nowadays as an alternative to formal education, these two It has caused controversy on all sides of the education system. The discussions range from reaction, Today, it has passed into the dimension of scientific research. Rapidly developing

internet technologies although the majority of these studies are on web-based distance education has been the cause. The results of some studies have shown that distance education is similar to formal education in all aspects. while others believe that both systems have equal pros and cons. that there are pros and cons. These scientific studies also provide a clear conclusion to the problem failed to bring about a clear understanding of which aspects of the two education systems should be looked at. It is important in terms of identifying the headings (Larry 1996).

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Chapter 4

Hypermobility

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Abstract

Hypermobility is the hyperextensibility of the synovial joints. Diagnosing hypermobility can be made through clinical assessment and specific joint mobility tests, such as the Beighton score, which evaluates the flexibility of specific joints. Medical imaging or genetic testing sometimes be necessary to rule out underlying connective tissue disorders. In hypermobile individuals, complaints of joint instability and musculoskeletal pain occur, and an increase in psychological symptoms such as anxiety reduces the quality of life of individuals. Treatment for hypermobility-related issues often involves physical therapy to improve joint stability, strengthen muscles, increase proprioception and balance, and reduce the risk of injuries. Pain management techniques and lifestyle modifications also recommended. In cases where hypermobility is associated with a connective tissue disorder, specialized medical care and ongoing management is necessary. In this review, we tried to compile and present the current literature on hypermobility, from evaluation to treatment.

INTRODUCTION

1- Hypermobility

Hypermobility, also known as joint hypermobility, is a condition in which a person's joints have a greater range of motion than what is considered typical for the general population (Hakim & Grahame, 2003). This means the joints can move beyond the normal range without causing pain or discomfort. Hypermobility is commonly seen in the body's connective tissues, including ligaments, tendons, and the joint capsule. These tissues are responsible for holding the bones together and providing stability to the joints. In individuals with hypermobility, these connective tissues are more elastic and flexible, allowing for increased joint mobility.

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While hypermobility is often a benign and naturally occurring trait, it can be associated with specific health issues:

a. Joint Pain: Some hypermobile individuals may experience joint pain, especially after repetitive or excessive movements (Grahame, 2009).

b. Joint Instability: The increased range of motion in hypermobile joints can lead to joint instability, making them more prone to dislocation or subluxation (partial dislocation) (Grahame, 2009).

c. Soft Tissue Injuries: Hypermobile individuals may be more susceptible to ligament sprains, muscle strains, and other soft tissue injuries (Hakim & Grahame, 2003).

d. Developmental Issues: In children, hypermobility leads to delays in motor skill development, such as walking and coordination (Romeo et al., 2022).

e. Connective Tissue Disorders: In some cases, hypermobility is associated with connective tissue disorders, such as Ehlers-Danlos syndrome (EDS) (Forghani, 2019).

2- The Prevalence of Hypermobility

The prevalence of hypermobility varies depending on the population being studied and the criteria used to define hypermobility. Benign joint hypermobility, not associated with any underlying medical condition, is relatively common in the general population. Studies have shown that hypermobility is more prevalent in certain groups, such as:

a. Children and Adolescents: Hypermobility is more common in children and tends to decrease with age as the connective tissues mature and become less elastic. It is estimated that around 10% to 30% of children and adolescents may exhibit hypermobility (Sobhani-Eraghi, Motalebi, Sarreshtehdari, Molazem-Sanandaji, & Hasanlu, 2020).

b. Females: Hypermobility is more frequently observed in females compared to males.

c. Athletes and Dancers: Certain sports or activities that require increased joint flexibility and range of motion, such as gymnastics, ballet, and contortion, may be associated with a higher prevalence of hypermobility (Skwiot, Śliwiński, Milanese, & Śliwiński, 2019).

d. Certain Ethnic Groups: Hypermobility is more prevalent in certain ethnic groups, predominantly African and Asian populations, but the data is inconsistent across all populations (Lawrence, 2014).

It is crucial to note that while hypermobility is common, hypermobility associated with connective tissue disorders, such as Ehlers-Danlos syndrome (EDS), is much less prevalent. Ehlers-Danlos syndrome is a group of rare genetic

disorders that affect the connective tissues, leading to joint hypermobility, skin abnormalities, and other systemic manifestations. The prevalence of Ehlers-Danlos syndrome varies based on the specific type of EDS. The exact prevalence of hypermobility and hypermobility-related disorders varies depending on the diagnostic criteria, geographical location, and population studied.

3- The Types of Hypermobility

Hypermobility can manifest in different forms, and it is essential to distinguish between benign hypermobility and hypermobility associated with underlying connective tissue disorders. There are several types of hypermobility:

1. Benign Joint Hypermobility: This type of hypermobility is considered a natural variation and is often not associated with any underlying medical condition. Many people have a few hypermobile joints without experiencing any significant issues or symptoms (Lawrence, 2014).
2. Generalized Joint Hypermobility: In this type, multiple joints throughout the body are hypermobile. Generalized joint hypermobility may be seen in conditions like Ehlers-Danlos syndrome (EDS) and Marfan syndrome.
3. Localized Joint Hypermobility: This form is characterized by hypermobility in specific joints or joint groups. For example, someone may have hypermobility only in their fingers, thumbs, or knees.
4. Syndromic Hypermobility: Some connective tissue disorders or genetic syndromes are associated with hypermobility. Conditions like Ehlers-Danlos syndrome, Marfan syndrome, and Loeys-Dietz syndrome fall into this category (Tofts, Elliott, Munns, Pacey, & Sillence, 2009).
5. Acquired Joint Hypermobility: In some cases, hypermobility may develop due to injuries, repetitive strain, or certain medical conditions affecting the joints and connective tissues.

4- Assessment of Hypermobility

Hypermobility can be measured using clinical assessments and specific joint mobility tests. One commonly used tool for evaluating hypermobility is the Beighton score. The Beighton score is a simple and quick assessment that evaluates the flexibility of certain joints in the body. It consists of five tests, each scored from 0 to 1 point, with a maximum score of 9 (Beighton et al., 2012).

The five tests are:

a. Passive dorsiflexion of the fifth finger (pinkie) beyond 90 degrees: 1 point if the finger can bend backward beyond 90 degrees, 0 points if it cannot.

b. Passive apposition of the thumb to the flexor aspect of the forearm: 1 point if the thumb can touch the forearm, 0 points if it cannot.

c. Hyperextension of the elbow beyond 10 degrees: 1 point if the elbow can bend backward beyond 10 degrees, 0 points if it cannot.

d. Hyperextension of the knee beyond 10 degrees: 1 point if the knee can bend backward beyond 10 degrees, 0 points if it cannot.

(The ones above are scored by evaluating right and left separately.)

e. Forward bending with knees straight and palms on the floor: 1 point if the individual can place their palms flat while keeping their knees fully extended, 0 points if they cannot.

If an individual scores 4 or more out of 9 points on the Beighton score, they are considered to have joint hypermobility.

Other Clinical Tests:

1. Carter-Wilkinson Test: This test assesses Hyperextension of the knees. The individual sits on a table with their knees extended over the edge, and the amount of Hyperextension is measured (Juul-Kristensen, Schmedling, Rombaut, Lund, & Engelbert, 2017).
2. Walker-Moder-Score: This test evaluates the range of motion in the thumb, wrist, elbow, and knee joints to assess hypermobility.
3. Rizzoli Foot Index: This test measures hypermobility in the foot and evaluates the range of motion in specific foot joints.
4. Nine-Point Hypermobility Test: This variation of the Beighton score includes additional joints, such as the hips and ankles.
5. Brighton Criteria: The Brighton criteria are clinical criteria used to assess joint hypermobility and distinguish it from hypermobility-related disorders. It considers a joint range of motion, systemic symptoms, and family history.
6. Bulbena Beighton Score: This scoring system evaluates hypermobility in various joints, including the neck, shoulders, hips, and other areas not included in the traditional Beighton score.
7. Hospital del Mar Hypermobility Scale: This scale assesses joint hypermobility and musculoskeletal pain (Schlager et al., 2018).
8. Upper Limb Tension Test (ULTT-1): This test evaluates the flexibility of the nerves and soft tissues in the upper limbs.

5- The Pathogenesis of Hypermobility

The pathogenesis (underlying mechanisms) of hypermobility is not fully understood. However, it is believed to involve a combination of genetic and environmental factors that affect the structure and function of the connective tissues in the body. Connective tissues, such as ligaments, tendons, and the joint capsule, are responsible for providing stability and support to the joints.

a) Genetic Factors: Hypermobility often runs in families, suggesting a genetic component to the condition. Specific genes involved in the formation and maintenance of connective tissues may play a role in determining an individual's joint flexibility and susceptibility to hypermobility. Some connective tissue disorders, such as Ehlers-Danlos syndrome (EDS), are known to be caused by genetic mutations affecting collagen and other structural proteins (Malfait, Hakim, De Paepe, & Grahame, 2006).

b) Collagen Abnormalities: Collagen is the primary structural protein in connective tissues and is crucial in maintaining the integrity and strength of ligaments and tendons. In some cases of hypermobility, there may be alterations in the composition or structure of collagen, leading to increased joint laxity.

c) Elastin Abnormalities: Elastin is another essential protein in connective tissues that provides elasticity and flexibility. Abnormalities in elastin can contribute to hypermobility by allowing joints to move beyond the normal range of motion.

d) Joint Capsule and Ligament Changes: The joint capsule and ligaments are essential components of joint stability. Changes in the structure or laxity of these tissues can contribute to joint hypermobility (Beighton, Grahame, & Bird, 2011).

e) Hormonal Influences: Hormones, particularly female sex hormones like estrogen, have been implicated in the pathogenesis of hypermobility. For example, some women experience increased joint flexibility during pregnancy due to hormonal changes, which usually resolves after childbirth (Hakim & Grahame, 2003).

f) Developmental Factors: Hypermobility is more common in children and tends to decrease with age as the connective tissues mature and become less elastic. The development of hypermobility may be influenced by factors related to growth and tissue development during childhood (Akkaya, Burak, Yildiz, Yildiz, & Elbasan, 2023; Van Meulenbroek et al., 2021).

6- Diagnosis of Hypermobility

Diagnosing hypermobility involves a combination of clinical assessment, physical examination, and, in some cases, additional tests to rule out underlying medical conditions. Here is an outline of the diagnostic process:

a) Medical History: The healthcare professional will start by taking a detailed medical history, which includes asking about any joint-related symptoms (such as pain, instability, or recurrent dislocations), family history of hypermobility or connective tissue disorders, and any other relevant medical conditions.

b) Physical Examination: A thorough physical examination is essential to assess joint flexibility and mobility. The healthcare professional will perform the Beighton score and may use other clinical tests to evaluate specific joints for hypermobility (Simpson, 2006).

c) Joint Assessment: The range of motion of various joints will be assessed to determine if they are hypermobile or have a greater-than-normal range of motion.

d) Additional Tests: In some cases, further tests may be necessary to assess joint structures, rule out other conditions, or evaluate overall joint health. These tests may include:

- Imaging: X-rays, MRI, or ultrasound can be used to examine joint structures, identify any structural abnormalities, and rule out injuries or other joint-related issues.
- Genetic Testing: Genetic testing may be recommended to confirm the diagnosis if there is a suspicion of an underlying connective tissue disorder (such as Ehlers-Danlos syndrome or Marfan syndrome).
- Blood Tests: In certain situations, blood tests may be conducted to rule out inflammatory joint conditions or other systemic disorders that could be causing joint symptoms.

e) Functional Assessment: The healthcare professional may assess how hypermobility affects the individual's daily activities and quality of life, mainly if joint instability or pain is present.

f) Differential Diagnosis: It is important to differentiate benign hypermobility from hypermobility related to an underlying connective tissue disorder. The diagnostic process involves ruling out other potential causes of joint symptoms and considering family history and other clinical findings (Tofts et al., 2009).

g) Fatigue: Poor sleep, muscle weakness, and dysautonomia have all been shown to be associated with worse fatigue in hypermobility(Pacey, Nicholson, Adams, Munn, & Munns, 2010).

7- Genetic Syndromes and Hypermobility

There are several genetic syndromes or connective tissue disorders associated with joint hypermobility. These syndromes are characterized by abnormalities in the structure and function of connective tissues, particularly collagen, which can lead to increased joint flexibility and other systemic manifestations. Here are some of the primary genetic syndromes with joint hypermobility:

a) Ehlers-Danlos Syndrome (EDS): EDS is a group of connective tissue disorders caused by gene mutations responsible for collagen production or

processing. There are several types of EDS, with the hypermobility type (formerly known as EDS Type III) being the one primarily associated with joint hypermobility. In addition to hypermobility, individuals with EDS may have soft, velvety skin, easy bruising, and a tendency to develop joint dislocations and chronic joint pain (Castori & Hakim, 2017).

b) Marfan Syndrome: Marfan syndrome is caused by mutations in the fibrillin-1 gene, leading to abnormal connective tissue and affecting multiple body systems, including the cardiovascular system, eyes, and skeleton. Joint hypermobility is a common feature in Marfan syndrome, and individuals may also have tall stature, long limbs, aortic root dilation, and lens dislocation in the eyes.

c) Loeys-Dietz Syndrome (LDS): LDS is a connective tissue disorder caused by gene mutations in the TGF-beta signaling pathway. Joint hypermobility and arterial tortuosity, aneurysms, and other vascular abnormalities are characteristic features of LDS.

d) Osteogenesis Imperfecta (OI): OI, also known as "brittle bone disease," is caused by mutations in genes that affect collagen production. While joint hypermobility is not a defining feature of OI, it can be present in some individuals with milder forms of the condition.

e) Stickler Syndrome: Stickler syndrome is caused by mutations in genes responsible for collagen synthesis and processing. It is characterized by joint hypermobility, hearing loss, vision problems (such as myopia and retinal detachment), and characteristic facial features.

f) Hypermobile EDS (hEDS): In addition to the classic EDS hypermobility type, a newer classification has been proposed in some medical guidelines. The exact genetic basis for hEDS is not well-defined, and it is primarily diagnosed based on clinical criteria, including joint hypermobility and other related symptoms (Tinkle et al., 2017).

8- Injuries and Hypermobility

The relationship between joint hypermobility and injuries is complex and multifaceted. While joint hypermobility itself is not considered a pathological condition, it can be associated with an increased risk of specific injuries and musculoskeletal issues. The main reasons for this association include:

a) Joint Instability: Hypermobility sometimes lead to joint instability, which means the joints are more susceptible to moving beyond their normal range of motion. This increased laxity in the joint structures may make them more prone to dislocation, subluxation (partial dislocation), and sprains (Nathan, Davies, & Swaine, 2018).

b) Ligament and Soft Tissue Vulnerability: The ligaments and soft tissues surrounding hypermobile joints may be weaker or more prone to stretching and tearing. This can result in ligament sprains, muscle strains, and other soft tissue injuries, especially during physical activities or sports (Pacey et al., 2010).

c) Overuse Injuries: Hypermobile individuals may unknowingly place additional stress on their joints due to an increased range of motion. Repetitive movements or overuse of hypermobile joints lead to wear and tear injuries, such as tendonitis or bursitis (Baeza-Velasco, Gély-Nargeot, Pailhez, & Vilarrasa, 2013).

d) Joint Pain and Compensation: Some hypermobile individuals may experience joint pain or discomfort, which can lead to compensatory movement patterns. Over time, these compensatory movements may strain other body parts, potentially causing secondary injuries.

e) Increased Risk of Falls: In some cases, joint hypermobility can affect balance and coordination, increasing the risk of falls and injuries.

f) Impact on Performance: Joint hypermobility may affect an athlete's performance and skill execution, especially in sports that require precise control of joint movements. Maintaining stability and control over hypermobile joints can be challenging and affect an athlete's overall performance (Russek & Errico, 2016).

g) Proprioception and Balance: Joint hypermobility can influence proprioception (the sense of joint position) and balance, potentially increasing the risk of falls and injuries during sports and physical activities (Palmer, Bailey, Barker, Barney, & Elliott, 2014; Sahin et al., 2008).

h) Dislocation-Prone Joints: Joint hypermobility can predispose individuals to joint dislocations, particularly in weight-bearing joints like the shoulders, knees, and fingers. Some joints, such as the shoulders and fingers, are more commonly affected by joint dislocations in hypermobile individuals. Sports that involve repetitive or forceful movements of these joints, such as gymnastics or martial arts, may carry a higher risk of dislocations (Nathan et al., 2018).

9- Joint Dislocation and Hypermobility

Hypermobility is associated with an increased risk of joint dislocation and subluxation (partial dislocation) due to the excessive range of motion in hypermobile joints. A joint dislocation occurs when the bones that form a joint are forced out of their normal position, resulting in a loss of joint alignment. Below hypermobility is linked to joint dislocation:

a) Joint Instability: Hypermobility is characterized by increased joint laxity, meaning the ligaments and other supporting structures around the joint are looser

and more flexible. As a result, hypermobile joints have less inherent stability, making them more susceptible to displacement or dislocation, especially during sudden or forceful movements (Kirk, Ansell, & Bywaters, 1967).

b) Greater Range of Motion: Hypermobile joints can move beyond the normal range of motion, allowing for a greater degree of movement. While this flexibility can be advantageous in certain activities, it also means that the joint is more likely to move excessively and potentially lead to dislocation.

c) Repetitive Stress: Activities or sports that involve repetitive motions, such as gymnastics or dance, can place repetitive stress on hypermobile joints. Over time, this repetitive stress can increase the likelihood of joint dislocation.

d) Weight-Bearing Joints: Certain joints, such as the shoulders, knees, and fingers, are more prone to dislocation in hypermobile individuals. These joints often bear the brunt of physical activity, and their inherent instability can lead to dislocations.

e) Joint Dislocation Symptoms: Joint dislocation in hypermobile individuals may present with sudden, severe pain, swelling, limited range of motion, and a visible deformity in the joint. In some cases, the dislocated joint may spontaneously return to its normal position, leading to a subluxation.

While joint dislocation is more common in individuals with joint hypermobility, not all hypermobile individuals will experience dislocations. The severity of hypermobility, the specific joints affected, and an individual's lifestyle and physical activities can influence the risk of joint dislocation.

10- Management and Physiotherapy of Hypermobility

The management of hypermobility aims to address any symptoms, prevent injuries, and improve joint stability and function. The specific approach to management will depend on the individual's symptoms, the severity of hypermobility, and whether there is an underlying connective tissue disorder. Here are some general management strategies for hypermobility:

a) Physiotherapy: Physical therapy is a cornerstone of hypermobility management. A physical therapist design a customized exercise program to strengthen the muscles around hypermobile joints, improve joint stability, and enhance overall flexibility and proprioception. This can help reduce the risk of injuries and alleviate joint pain (Keer & Simmonds, 2011).

b) Joint Protection: Individuals with hypermobility should be taught to protect their joints during daily activities to avoid overstretching or causing injury. Learning proper body mechanics and using joint-friendly techniques can be beneficial (Keer & Simmonds, 2011).

c) Pre-participation Screening: Athletes, especially those with known joint hypermobility, should undergo pre-participation screenings to identify specific areas of concern or tailor training programs accordingly.

d) Bracing and Support: In some cases, using orthotic devices or braces can provide additional support to hypermobile joints, particularly during physical activities or if specific joints are prone to dislocation (Keer & Simmonds, 2011).

e) Pain Management: If joint pain is present, pain management techniques such as over-the-counter pain relievers, heat or cold therapy, and rest can be helpful. In severe cases, a healthcare provider may prescribe medications to manage pain.

f) Activity Modification: Some high-impact or high-risk activities may need to be modified or avoided to prevent joint injuries. Low-impact exercises such as swimming and biking are often recommended for individuals with hypermobility (Palmer et al., 2016).

g) Education and Self-Management: Understanding the condition and learning self-management techniques can empower individuals to manage their hypermobility actively. Learning to recognize and respond to joint symptoms can help prevent the worsening of the condition (Boudreau, Farina, & Falla, 2010).

h) Splints and Taping: In certain situations, using splints or taping techniques can provide external support to hypermobile joints and aid in joint stabilization (Tudini, Levine, Healy, Jordon, & Chui, 2023).

i) Genetic Counseling: For individuals with an underlying connective tissue disorder, genetic counseling can help provide information about the condition, its inheritance pattern, and family planning options.

j) Technique and Skill Development: Athletes should focus on proper technique and skill development to minimize excessive joint movement and maintain joint stability.

k) Sport-Specific Training: Sport-specific training can be tailored to accommodate the individual's joint hypermobility and reduce the risk of injury during specific activities.

l) Psychological Support: Some individuals with hypermobility may experience anxiety or depression related to chronic pain or activity limitations. Seeking psychological support can help address these emotional aspects of living with hypermobility.

m) Regular Follow-up: Regular check-ups with a healthcare professional can monitor the condition, track progress, and adjust the management plan as needed (Sundemo, Senorski, & Samuelsson, 2021).

Hypermobile individuals; should avoid activities involving running, jumping, and jumping movements and sports involving close contact, such as basketball

and football. Traction should not be applied to the joints; body stretching exercises should be done for a short time without too much difficulty. Forceful weights should not be lifted, and movements that strain the joints, such as pushing or pulling heavy objects, should be avoided.

CONCLUSION

Once hypermobility is noticed, it should first be evaluated by a specialist team. The Beighton score is the gold standard for evaluation. Short- and long-term goals for treatment should then be determined. Posture correction and body awareness should be increased in a short time. Pain in painful areas (usually low back, hip, and knee pain) should be reduced. Upper and lower extremity muscle strength, endurance, and functional stability should be increased. Our long-term goals are to increase sport-specific functional capacity and cardiovascular fitness by promoting an active lifestyle and protecting against future injuries and pain. Participation in recreational activities should be encouraged. Considering the laxity in the joints, resistance exercises should be performed without damaging the joint space. In addition, individuals with hypermobility need to work closely with a healthcare team, which may include rheumatologists, physical therapists, orthopedic specialists, and other specialists, as necessary, to develop an individualized management plan tailored to their specific needs and challenges.

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Chapter 5

Brain Energy

Gonul GUROL CIFTCI[1]

BRAIN ENERGY

Since the human brain is the most active organ, it consumes 20% of the energy in the body (Balasubramanian, V., 2021). Most of the management of metabolic processes in the brain depends on astrocytes, cells where glycogen is stored (Falkowska et al., 2015, Duran et al., 2019). The primary source is, of course, glucose. Data in the literature shows that the presence and absence of glycogen pave the way for the developing of some diseases (Duran et al., 2019, Hirase et al., 2019). In situations like sleep and hypoglycemia, glycogen decreases, and even excessive decreases negatively affect neuronal survival (Swanson and Choi, 1993). In addition, there is knowledge that astrocytic energy is affected by stress. This is also due to the presence of glucose-sensitive neurons in the brain (Harris and Attwell, 2012). In this case, it reveals to us that astrocytes and neurons in the brain interact with each other and provide vital support to each other. Glycogen stores in the brain are spread in the cortex, striatum and, hippocampus, pons (Brown and Ransom, 2007, Oe et al., 2016, Zhao et al., 2017, Markussen et al., 2023). Glycogen has multiple roles in the brain, such as neurotransmitter release and learning.

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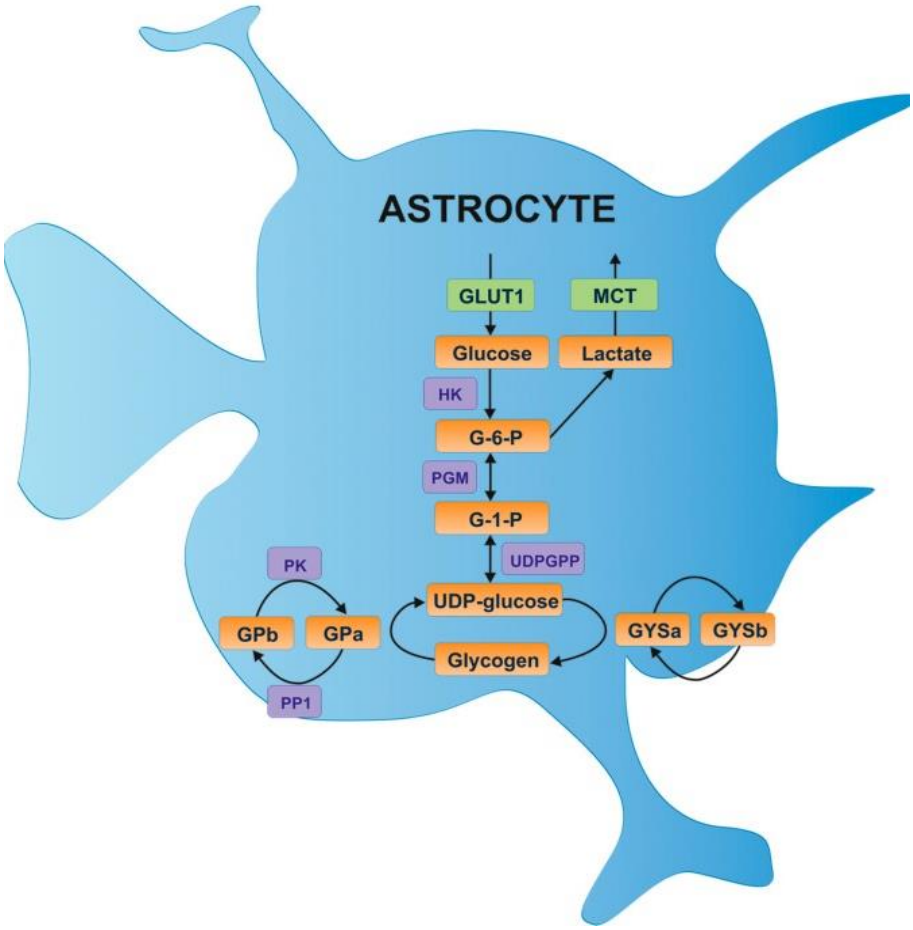

Figure 1. Glycogen synthesis in astrocytes (Falkowska et al., 2015).

In astrocytes, glucose is transported via two transporters (Figure 1): the insulin-sensitive glucose transporter GLUT4 and theglucose transporter GLUT1. Afterward, glucose is converted into glucose-6-phosphate bythe hexokinase enzyme, broken intoglucose-1-phosphate byphosphoglucomutase, and converted into UDP glucose. UDP glucose is then converted into glycogen. Astrocytes have very high and rapid glycolytic activity. This glycogen is presented to neurons via monocarboxylate transporters (MCT) and is the source of lactate. Thus, glucose is metabolized into lactate. It is then oxidized to pyruvate by lactate dehydrogenase and the tricarboxylic acid cycle. As aresult, adenosine triphosphate (ATP) is produced by oxidative phosphorylation (Falkowska et al., 2015, Matsui et al., 2017, Jha and Morrison, 2020).

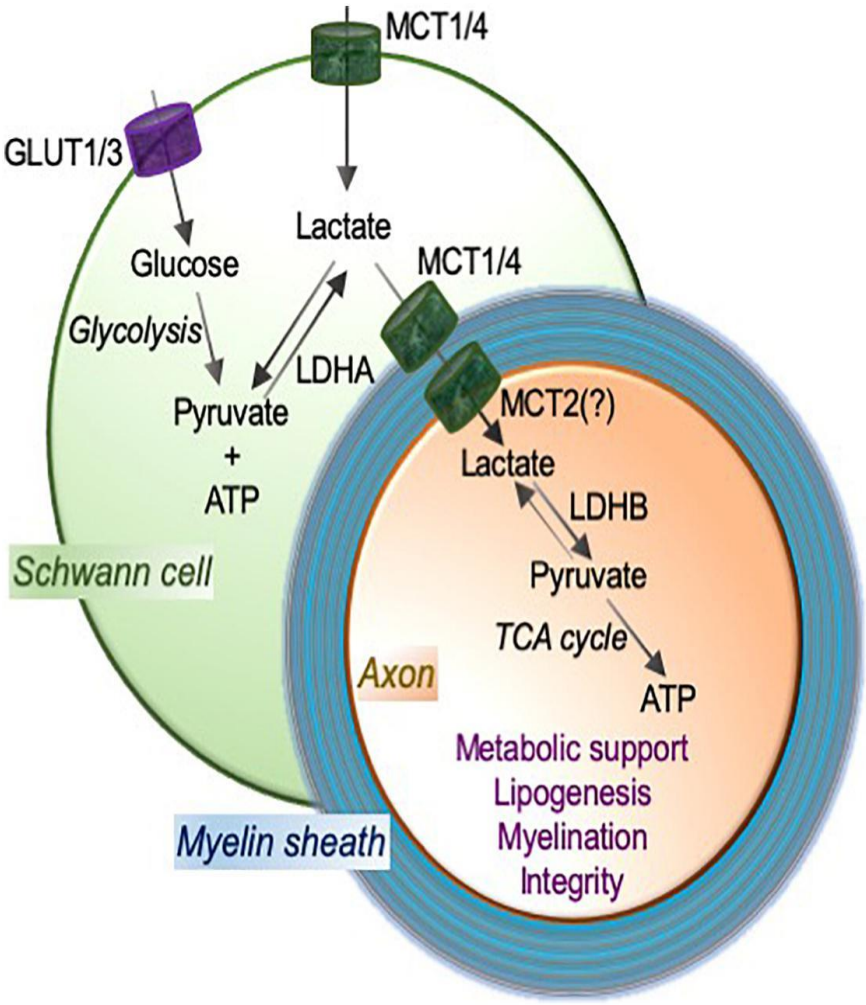

Figure 2. Effects of monocarboxylate transporters (MCT) on lactate transport from Schwann cell to neuron (Jha and Morrison, 2020).

The roles of monocarboxylate transporters are still not fully understood. As a result of animal studies, the locations of MCT1, MCT2, and MCT4 in brain tissue (Figure 2) have been demonstrated by molecular techniques. While MCT4 and MCT1 are present in Schwann cells, MCT2 is more abundant in the peripheral nervous system (Jha and Morrison, 2020). While the GLUT1 transporter is expressed in astrocytes and endothelial cells, theexpression of GLUT3 is in neurons (Wu et al., 2023). There is increasing evidence that this traffic established between neurons and glial cells through MCTs may constitute the key to the energy cycle processes in the brain. The

number of promising studies in which MCTs are designed as target molecules for developing new diagnoses and treatments in neurodegenerative diseases, where their effectiveness in synaptic activity may vary, is increasing daily.

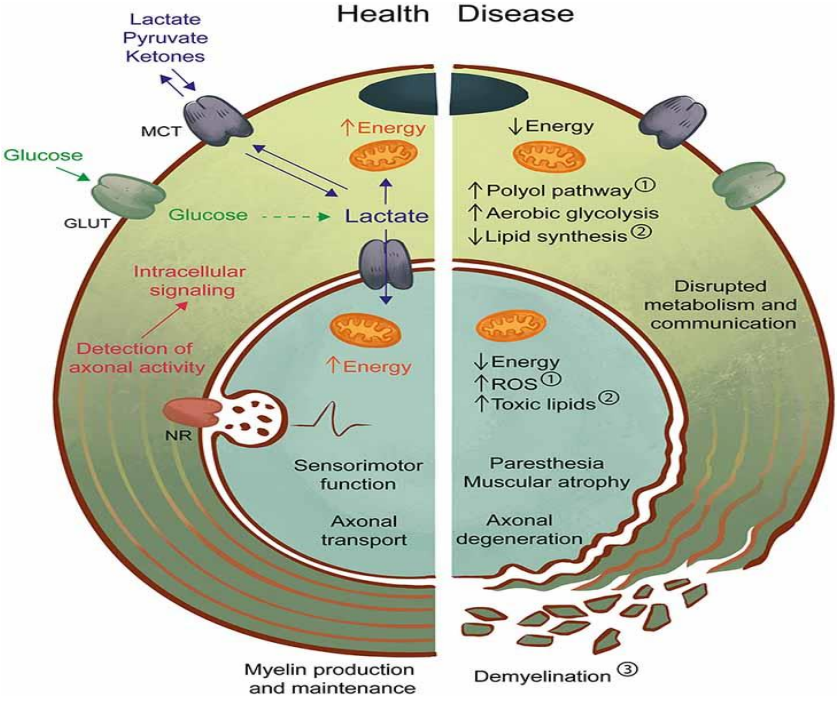

Figure 3. Glucose metabolism in healthy and diseased Schwann cells (Bouchanova and Chrast, 2020).

In addition to aging, evidence is emerging that metabolic interactions between Schwann cells and neurons are impaired in various neurodegenerative diseases such as Alzheimer's, Parkinson's, dementia, and amyotrophic lateral sclerosis (ALS). Mitochondrial functions are impaired in hyperglycemic conditions, and oxidative stress is induced (Figure 3). Over time, these result in neuropathic symptoms and axon degeneration (Bouchanova and Chrast, 2020). As a result of advanced molecular studies on lactate as well as MCTs in maintaining neuronal balance, it is also stated that genes related to synaptic plasticity and cell death are regulated by lactate (Margineanu et al., 2018). While the genes regulated by lactate and related to cell death were Txnip, Apaf, Bcl2111, and Hrk, the genes active in synaptic plasticity were found to be Yay Bdnf c-Fos, Zif268, Atf4, Nr4a1, Gadd45b, Gadd45g, Harika3k11, Dusp4, Dusp6, Dusp10. Bdnf, Grfa2, Nr4a2, and

Vegfa have been identified in neuronal protection. Additionally, the hydroxycarboxylic acid receptor HCAR1, defined as the lactate receptor, is expressed in pial fibroblast-like cells lining vessels, pericyte-like cells along intracerebral microvessels, and neurons (Margineanu et al., 2018, Briquet et al., 2022, Wu et al., 2023). In experimental investigations, this information was determined visually (Figure 4) on the monomeric red fluorescent protein (mRFP) expression of the HCARR1 promoter using different techniques such as using a 2-photon microscope, anti-mRFP immunohistochemistry, in situ hybridization (RNAscope™) and from the data presented in the literature (Briquet et al., 2022).

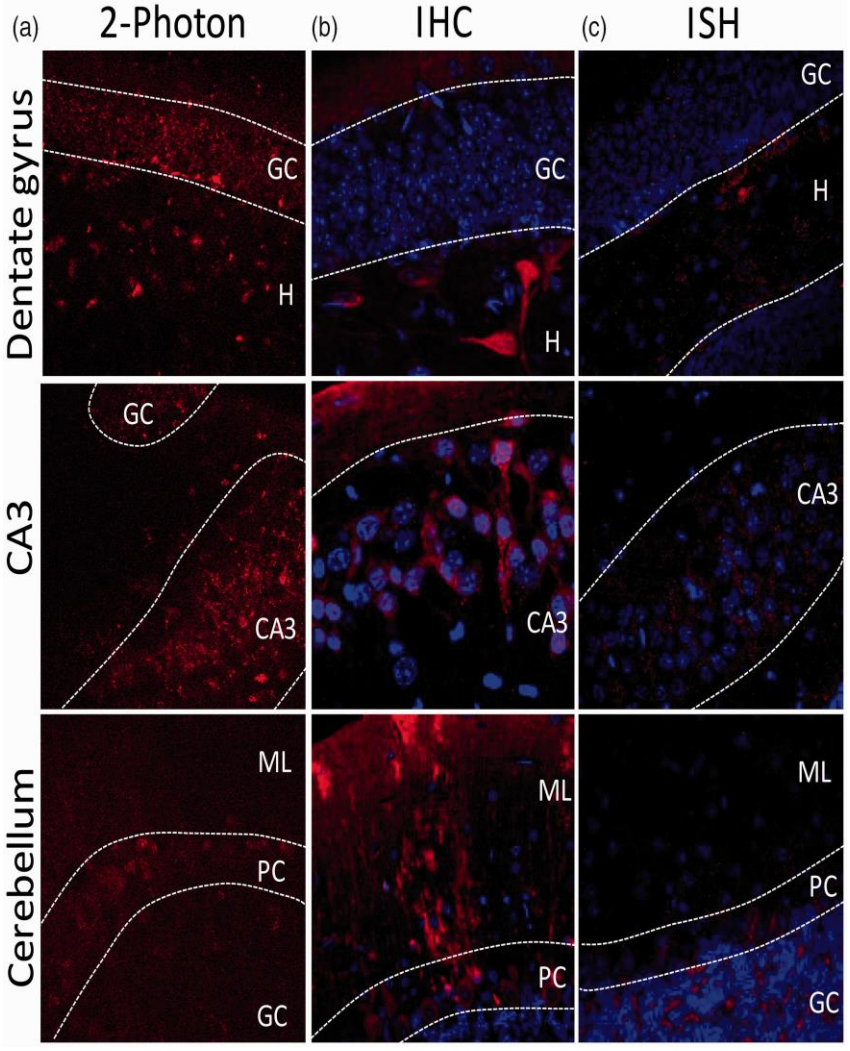

Figure 4. Distributions of mRFP-positively stained HCAR1 in various mouse brain regions (Briquet et al., 2022)

HCAR1 transcript is shown in red and nuclei (DAPI) in blue. GC = granule cell layer, H = hilus, ML = molecular layer, PC = Purkinje cell, ISH= in situ hybridization assay, IHC= immunohistochemistry

Today, it is tried to be demonstrated that keeping the glycogen content of the brain at an adequate level is very important for hemostatic balance. The effects of hypoglycemia, as well as hyperglycemia, are significant for neuronal survival, cognitive functions, and stress-related emotional reactions. In this context, there is a need for the use of new molecular and immunohistochemical data and techniques that include further detailed analyses in which specific antibodies and MCT isoforms are discovered to investigate the effectiveness of astrocytic metabolites and energetics in various pathophysiological conditions of the brain

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Chapter 6

The Importance Of The Warburg Effect in Homeostasis

Gonul GUROL CIFTCI[1]

Examining many studies on the Warburg effect in oncogenetic approaches brings to mind the path this effect follows in diseases involving a multicellular structure, which can be summarized as metabolic regeneration or programming. We can think of this effect, which can be considered fuel for growing cells, as a metabolic change. The real question here is what cells need and under what conditions change how they produce energy, or more precisely, how this occurs in the disease formation process (Vander et al., 2009).

It paves the way for oxidative stress, which is triggered in situations such as stress, depression, sadness, low quality of life, genetic factors, autoimmune diseases, and neurodegenerative diseases. As aresult, reactive oxygen species(ROS) are produced, and the uncontrolled proliferation of cancer cells is triggered. In light of the information obtained from pharmacological and molecular studies, we can say that the course of cancer cells may vary depending on the location and type of cancer and even the person's psychosocial state and environmental conditions, which explains the differences in response to treatment between individuals. The presence of cells under hypoxic conditions can trigger several homeostatic mechanisms, depending on the needs and for how long they remain (Figure 1). Sometimes, hypoxia may occur as aresult of pathophysiological immune activity. In addition, it is stated that physiologically occurring moderate and constant hypoxia mediates many processes, such as regulating the activity of immune cells, supporting anatomical structuring and development, antibody quality, immune tolerance, and B cell development (Taylor et al., 2016, Chen and Gaber, 2021).

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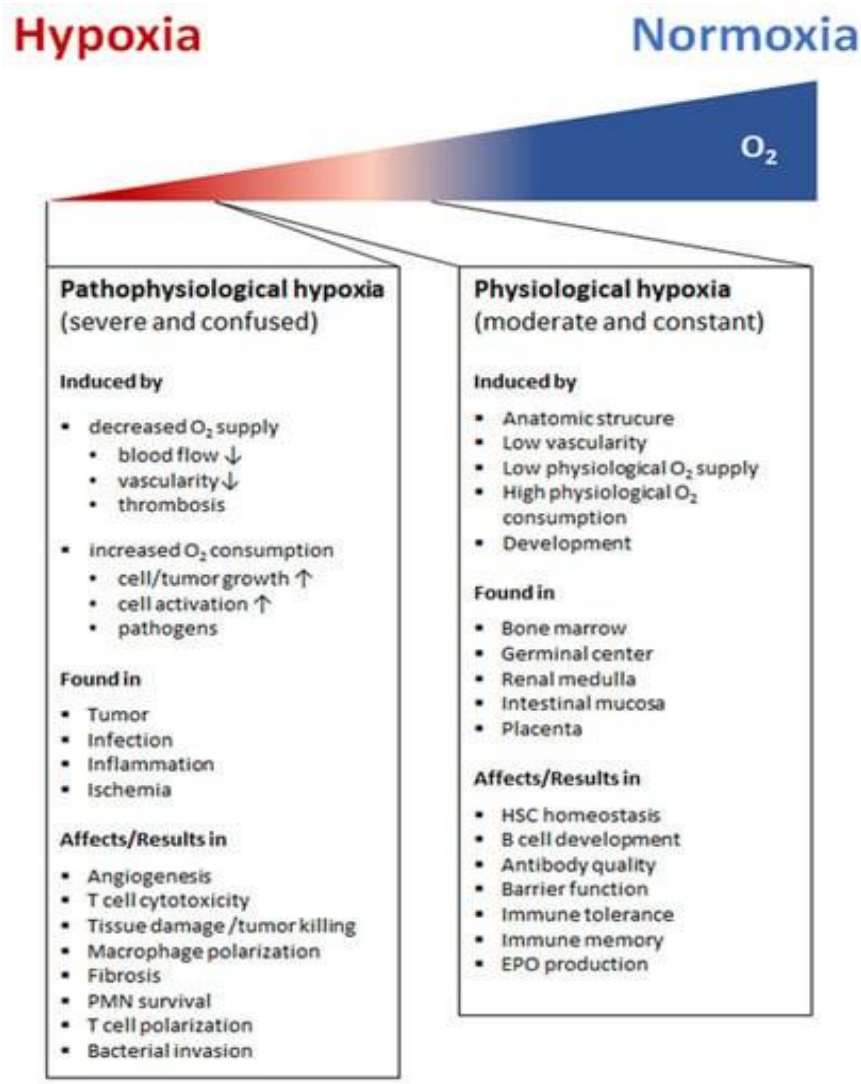

Figure 1. Physiological and pathophysiological effects of hypoxia (Chen and Gaber, 2021).

Among the mechanisms triggered by several factors is the maintenance of Adenosine triphosphate (ATP) production in mitochondria. Suppose people with this condition already suffer from diseases that are rapidly affected by hypoxic conditions, such as kidney disease, lung disease, heart disease, ischemic disease, liver disease, and obstructive sleep apnea syndrome. This seriously complicates homeostatic processes (Georgakilas, A. G., 2012, Parabhakar et 2020, Taylor and Scholz, 2022, Drochioiu, G., 2023). Studies show that Hypoxia-inducible factor-1 alpha(HIF-1α) is a vital regulator of the

Warburg effect and an activator of aerobic glycolysis (Figure 2). It also acts as a transcription agent (Reiter et al., 2021). HIF-1α is stabilized by ROS production, which increases in hypoxic conditions, and HIF-1α triggers the expression of glucose transporters and glycolytic enzymes. Subsequently, the conversion of pyruvate to acetyl coenzyme A in themitochondria decreases while promoting the pentose phosphate pathway and increasing lactate flow (Yu et al. , 2017, Reiter et al., 2021).

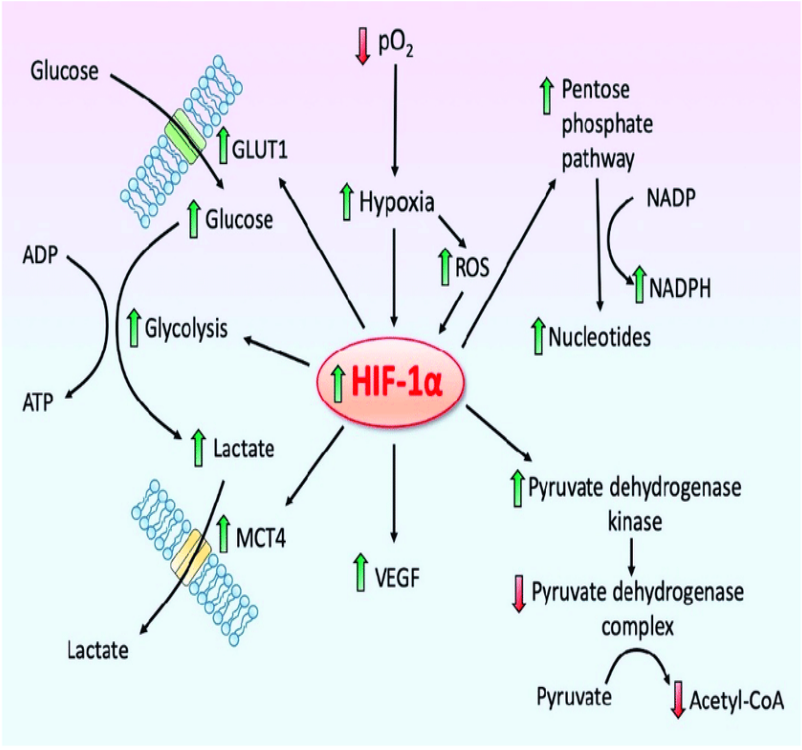

Figure 2. The effect of hypoxia-inducible factor-1 alpha (HIF-1α) on Warburg-type metabolism (Reiter et al., 2021).

Activated inflammatory immune cells, like tumor cells, meet the need for ATP through aerobic glycolysis under the Warburg effect (Palsson-McDermott and O'neill, 2013). While healthy cells obtain ATP following a process that combines the tricarboxylic acid (TCA) cycle with glycolysis and mitochondrial respiration, cells such as tumor cells and activated macrophages receive energy through a pathway called the "Warburg effect" in which metabolism switches from oxidative phosphorylation to aerobic glycolysis (Figure 3). (Viola et al., 2019, Soeters et al., 2021). In the initial discovery of this effect, it was

hypothesized that aerobic respiration was impaired due to a defect in themitochondria of cancer cells. Then glycolytic dependence emerged, but today, studies have come to light that this is not the case, that cancer cells also have an alternative pathway for aerobic glycolysis. Such a metabolic regulation appears due to a homeostatic response (Vander et al., 2009).

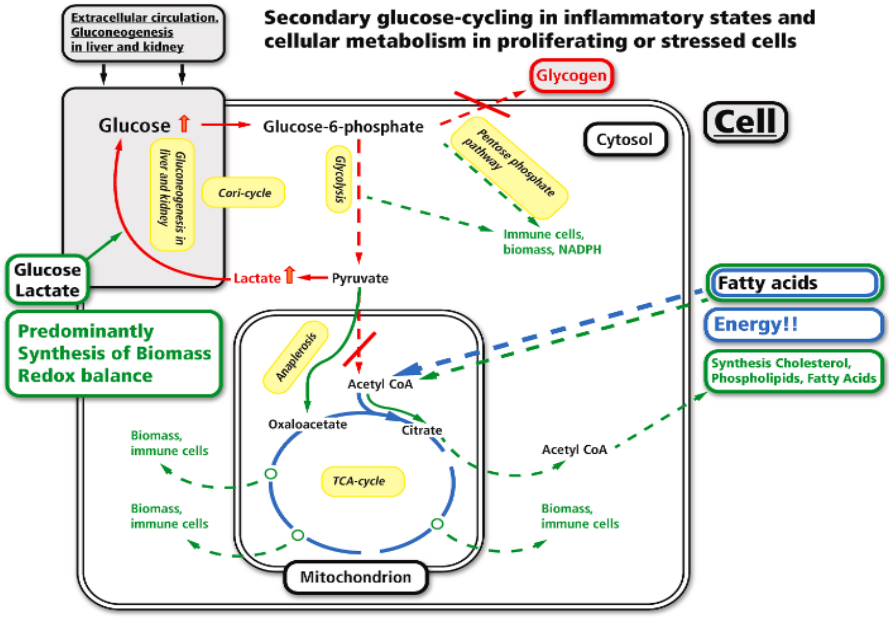

Figure 3. Significant and cataplerotic effects of the glycolytic pathway, TCA cycle, and Pentose-phosphate cycle (Soeters et al., 2021).

Red lines indicate the Cori cycle that preserves glucose for cells and inhibits glucose oxidation and glycogen synthesis, while green lines represent anapleros

The increase in the activity of HIF-1α, which is affected by hypoxic conditions, in a sense, directs the metabolism of immune cells. Due to inflammation's challenging and rapidly triggered nature, the inflammation zone generally remains hypoxic (Campbell et al., 2014). In order not to be affected by this hypoxic condition and to ensure the continuity of cell production, immune cells turn to the ATP production pathway under oxygen-independent conditions (Gaber et al., 2019). There is evidence that HIF-1α affects the function of innate and adaptive immune cells (Figure 4), including neutrophils, macrophages, dendritic cells, T and B lymphocytes, natural killer(NK) cells, and innate lymphoid cells (Taylor and Colgan, 2017, Taylor and Scholz, 2022). While HIF-1α has been shown to mediate survival, trained immunity, and bacterial killing effects on granulocytes, knowledge that it serves T cell

stimulation, M1/M2 differentiation, and cytokine and chemokine production in macrophages is obtained from both experimental and cell culture studies (Colgan et al. al., 2020, Kolliniati et al., 2022, Marrocco and Ortiz, 2022, He et al., 2023, Zhang et al., 2023). Therefore, understanding that HIF-1α contributes to regulating immunometabolism has paved the way for many pharmacological studies on controlling inflammation through HIF-1α (Taylor and Colgan, 2017, Taylor and Scholz, 2022).

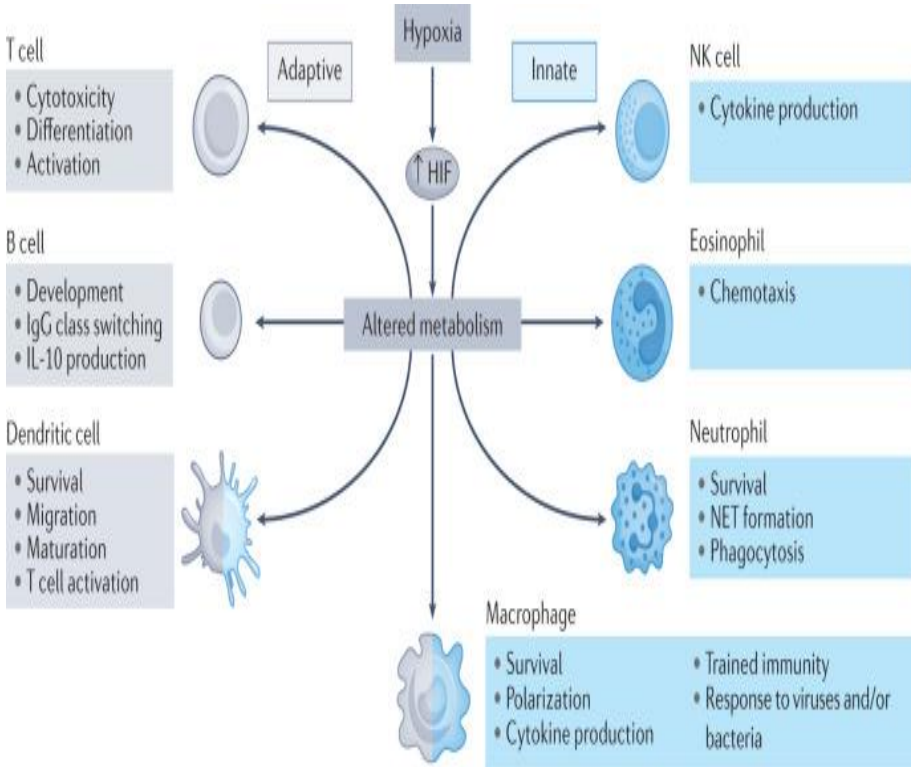

Figure 4. Effects of HIF-1α on immunity (Taylor and Scholz, 2022).

The question mark that remains in mind about the Warburg effect is whether it was a choice, a chain cycle, or whether it emerged by chance (Figure 5). Namely, although there is a predominant view that cells can choose normal metabolic pathways if there are healthy environmental conditions, alternatively, there is an opinion that the effect of some mutagens can trigger these chain reactions, and as a result, this metabolic tendency may occur. It is also suggested that some conditions lead to the deterioration of mitochondrial functions, and aging may be a factor of chance. In other words, while environmental conditions can activate metabolic selection, it is thought that

chain reactions triggered by some mutations or metabolic processes disrupted by aging may play an active role in this selection (Burns and Manda, 2017).

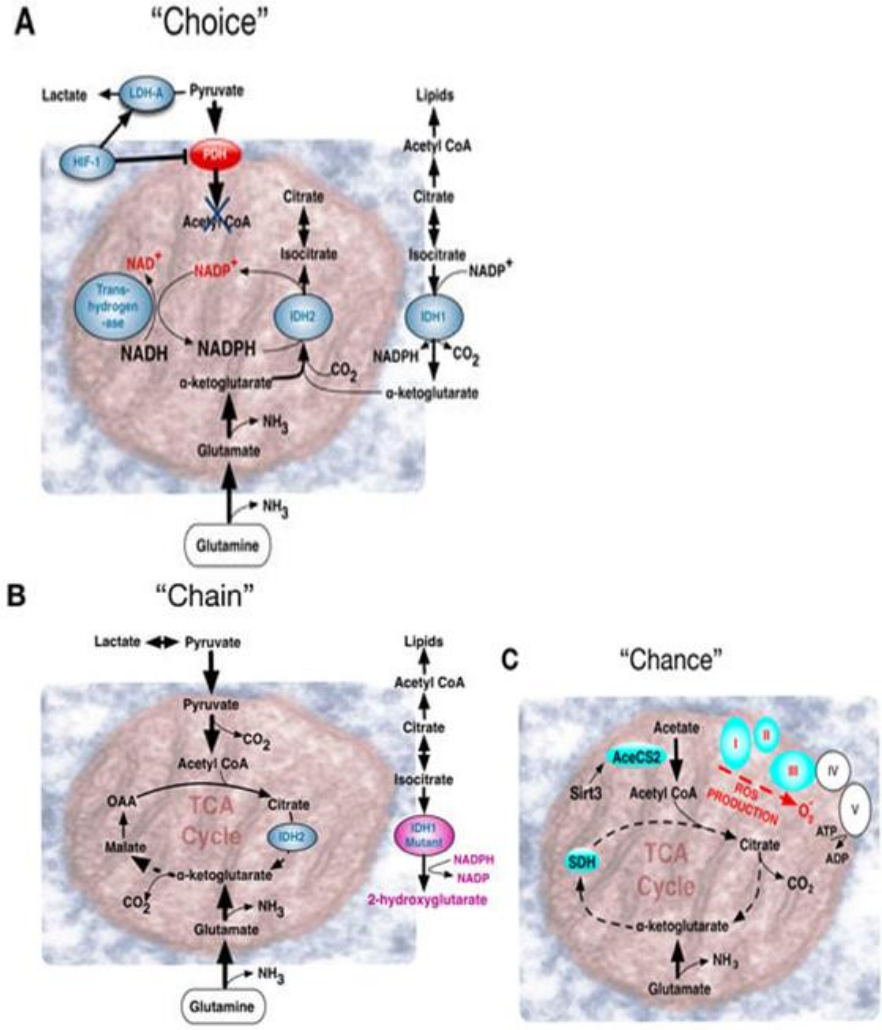

Figure 5. Mechanisms influencing the mitochondrial metabolism and the Warburg effect (Chose, chain or chance?). (Burns and Manda, 2017).

The complex and multifaceted interactions of the Warburg effect and its interactions in both the cascade of inflammatory processes and metabolic events force the discovery of common or intersecting molecules. However, despite the limited data, it can be hoped that critical pathways can be analyzed by

identifying joint metabolic promoters. Considering that immunometabolism has an underlying causality in various diseases, elaborating the reasons for the changing choices of homeostatic mechanisms under hypoxic conditions seems to shed light on new therapeutic approaches.

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Chapter 7

Embryotoxicity and Teratogenicity

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ABSTRACT

Human beings have been continuing their generation by sexual reproduction since the day they existed. The newborn, which is formed by the mating of male and female individuals, is born by normal birth or cesarean delivery. But especially in old times, births either do not result in success or the newborn individual is born with defects. With the development of technology and science, it has been realized that there are factors affecting this situation. As a result of the researches, it has been shown that the use of some drugs and non-medicinal substances during pregnancy causes babies to be born with anomalies. This situation necessitated the application of embryotoxicity and teratogenicity tests to medicinal products that are likely to be used in pregnancy. For this purpose, teratogenicity tests are applied to all kinds of substances that are likely to be used by pregnant women during pregnancy, and the system in which drugs and other substances are classified according to their toxic effect risks during pregnancy is constantly updated according to the results of the tests. This text provides information on the embryotoxic and teratogenic effects of various drugs and products on animals.

Keywords: Chicken Embryotoxicity Screening Test, Embryotoxicity, Hen's Eggs Test, Teratogenicity, Toxicity Test

INTRODUCTION

Humans have been reproducing through sexual reproduction since its existence. In this process, the sperm of male individuals fertilize the eggs of female individuals, and after the zygote is formed because of successful fertilization and completes its development in the female uterus, birth occurs, and new individuals are born. However, sometimes, even if

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fertilization is successful, the resulting individual dies before completing its development or after birth. Cases of abortion or abnormal birth have occurred since the beginning of recorded history. The current state of technological development and facilities reduces the number of these cases.

Nowadays, with the development of technology and science, a cause-effect relationship has begun to be sought in cases of abortion and birth anomalies. With the development of technology and science, a cause-and-effect relationship has begun to be sought in cases of miscarriage and birth anomalies. For example, it has been determined that anomalies such as Down syndrome, hemophilia, and color blindness develop as a result of genetic disorders. On the other hand, the food and drinks consumed, the medications taken, and even the nutritional supplements during pregnancy affected the fetus in the womb, causing miscarriages or abnormal births. This situation brings up the debate about how beneficial or harmful technological developments are. As technology develops, the number of useful or harmful products in the market increase, and people are negatively affected by these products as a result of unconscious consumption. In parallel with the development of technology, the number of drugs on the market is increasing day by day, and pregnant women consume these drugs without consulting a doctor or pharmacist and harm themselves and their unborn babies. Undoubtedly, most of the negative effects are due to negligence.

Women are exposed to certain drugs and chemicals, willingly or unintentionally, during pregnancy, and both women and babies are affected differently by this situation. The reasons why women use medication during pregnancy are as follows (Aydoğdu, 2019):

• The woman uses medication because she is not aware that she is pregnant.

• Due to the decrease in various blood parameters during pregnancy, women use nutritional supplements.

• The pregnant woman has a chronic disease and therefore uses medication specific to her disease.

• The woman got sick during pregnancy and uses medication because she needs it.

In these cases, the baby in the womb is negatively affected by the medicines and foods used. Negative effects on the embryo or fetus in the womb are explained by the concepts of embryotoxicity and teratogenicity (Kayaalp and Akıcı, 2013).

What are embryotoxicity and teratogenicity?

These two concepts are used to explain the negative effects on the embryo or fetus during pregnancy and are terms related to the science of toxicology, also known as toxicology.

Embryotoxicity is a term whose dictionary meaning is “pre-uterine abrasion.” In medical terms, it refers to the poisoning of the baby in the womb, called the embryo.

Teratogenic: disrupts the morphological development of the embryo or fetus when exposed to it at the embryological or fetal stage. It is the name given to bacteria, viruses, drugs, etc. that cause various diseases and anomalies.

Teratogenicity; It is the fact that various teratogenic substances encountered during pregnancy in women pass through the placenta into the circulation of the fetus, causing developmental or deformity disorders and death in the fetus.

The science of "teratology" examines births with anomalies after exposure to teratogens (Kayaalp and Akıcı, 2013).

Teratogenicity Epidemiology

It has been reported that 3% of all births in the United States (USA) are abnormal, that is, 120,000 babies with anomalies are born annually (Ornoy and Arnon, 1993). Although medication use is rarely the cause of birth defects, some medications can increase the rate of birth defects (Brent, 2004). The findings obtained as a result of the research show that the cause of the fetal anomalies cannot be fully known, but it has been reported that 25% of the anomalies are due to genetic factors, 10% to environmental factors, 3% to chromosomal anomalies and 3% to factors such as drugs (Brent, 2004). The incidence of drug-related anomalies in the first year after birth has been reported to be 1% (Kadıoğlu and Kalyoncu, 2006). The reasons for the anomalies that develop in the first year after birth are given in the table below (Table 1).

Table 1: Possible causes of anomalies seen in the first year after birth (Brent, 2004; Demir, 2008)

Possible causes	**Incidence (%)**
Unknown Causes	65-75
Genetic factors	15-25
Environmental factors	10

The effects of teratogens to which women are exposed during pregnancy on the embryo or fetus depend on the stage of pregnancy when exposure occurs. Since the gestation period in humans is 280 days, the zygote formed as a result of fertilization develops continuously until birth, and its sensitivity to teratogens varies at different stages. In order to detect the teratogenic effects of teratogenic substances, it is necessary to know the developmental stages of the embryo (Demir, 2008; Queenan et al., 2005).

Changes in Susceptibility to Teratogens during the Developmental Periods of Embryos

The average first 60 days of the gestation period, which is 280 days in humans, is the embryonic period and is the period in which organ development, called organogenesis, is most observed (Kayaalp and Akıcı, 2013).

The embryo stages seen in the embryonal period are:

1. Blastocyst Formation: It is the first 5-8day period following fertilization (Demir, 2008)

2. Implantation: 8-13 days after fertilization (Queenan et al., 2005)

3. Early Post-implantation: It is the period covering 14-17 days of pregnancy and continues until the formation of the neural plate. Since it is the period when cell division and differentiation are most active and abundant, teratogenic effects are most visible in this phase (Demir, 2008)

4. Organogenesis Phase: 3rd-8th months after fertilization. It is the period covering the period between weeks. Since differentiation in many organ systems occurs in this period, it is the most sensitive period to teratogens (Kayaalp and Akıcı, 2013). 17-30. Inability to close the teratogen neural tube taken between days; 6.5-8. Teratogen taken between weeks causes heart anomalies (Aydoğdu, 2019).

5. Fetal Period: It is the period from the eighth week of pregnancy to the end. In case of exposure to teratogens, different deformations are observed depending on the time of exposure (Freyer, 2008). Pregnancy periods and teratogenic effects are shown in the figures below (Figure 1). (In figure 1, the risk of anomaly development is high in the red stripes and low in the yellow stripes).

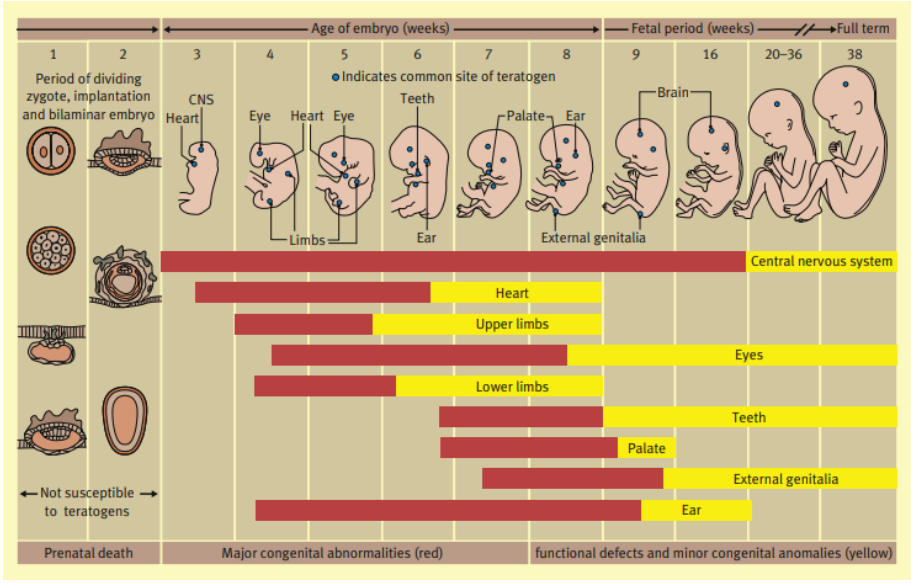

Figure 1: Gestation periods and teratogenic effects (8)

Mechanisms of Teratogenic Effects

Possible teratogenous effects that may occur after exposure to teratogens during pregnancy vary depending on the stage of gestation and the duration of exposure. In addition, the exposed teratogen must pass through the placenta and reach the embryo or fetus for a teratogenic effect. As with most material passages, teratogenic substances pass through the placenta by passive diffusion and reach the embryo or fetus. This is because large, non-ionized compounds with a lipid/water partition ratio pass easily through the placenta. (Kayaalp and Akıcı, 2013; Demir, 2008).

The six generally accepted rules of teratology in this context were published by Wilson, J. (1977) in the Manual of Teratology. The six teratology principles identified (Friedman, 2010) are: 1. Teratogenesis sensitivity depends on the genotype of the embryo and how it interacts with environmental factors.

2. Sensitivity to teratogenic agents varies depending on the stage of development during exposure.

3. Teratogenic agents act in specific ways on cell and tissue development to initiate abnormal embryogenesis.

4. The final signs of abnormal development are functional impairment, growth retardation malformation, and death.

5. Exposure to adverse environmental effects on developing tissues depends on the structure of the agent.

6. Symptoms of abnormal development increase as the dose increases.

Pregnancy

Pregnancy is a process that has a complex physiology inherent in female individuals and must be experienced for human reproduction. In this process, in order to know the possible teratological effects of drugs or other substances, the physiology of pregnancy must first be known. In this context, information will be given about the physiological changes seen during pregnancy, drug use during pregnancy, and pregnancy categories of drugs.

Physiological Changes in Pregnant Women

The gestation period lasts 280 days in humans and many physiological adaptations occur in this process. These are mainly cardiovascular, pulmonary, renal and gastrointestinal changes. Although these changes do not develop randomly, they occur to meet the increased needs of the fetus. Physiological changes seen during pregnancy are briefly as follows (Atasü et al., 1984; Doğan et al. 2008):

1.Gastrointestinal motility decreases and bowel movements decrease, increasing the transit time from the intestine. Since the time of passage from the intestine will be prolonged, it will change the absorption and blood concentration of the drug used.

2.The concentration of serum albumin decreases by 1/3, and therefore there are differences in blood concentrations of compounds carried with albumin.

3.Increased plasma and extracellular fluid volume affect the transport of compounds by disrupting their concentration.

4.While there is some increase in kidney size, approximately 30% increase in bilateral kidneys occurs. Glomerular filtration rate increases by 50.

5.Liver blood flow and size remain constant during pregnancy.

6.Cardiac blood flow and speed begin to vary in the level of sensation at the 8th week of pregnancy, but left ventricular function does not change.

7.Changes in pulmonary function and capacity also occur. Oxygen consumption increases tidal volume and inspiratory capacity, while total lung capacity, residual volume and functional residual capacity decrease.

Drug Use in Pregnancy

Pregnancy is an important physiological condition in which drug use requires special attention. Drugs taken during pregnancy may pose a risk and

often the level of risk and possible consequences cannot be determined due to the complexity of pregnancy physiology.

Pregnancy is an important physiological condition where drug use requires special attention. Because medications taken during pregnancy can pose risks, and often due to the complexity of gestational physiology, the level of risk and possible consequences cannot be determined.

In this context, research on drug use during pregnancy is now available in the literature, and according to this research, a very large percentage of pregnant women today, 80-90%, use at least one drug during pregnant. (Mitchell et al., 2008; Kaplan et al., 2014; Oray, 2014). Another study found that the most commonly used drugs in pregnancy are antibiotics and painkillers. The percentage of women who encountered a single drug during pregnancy was to be 10% in all pregnancies, 83.6% during the first trimester, and almost all pregnant women in this segment were unaware that they were pregnant (Olukman et al., 2006).

While there is not enough clinical study on drug use during pregnancy, studies in the literature have documented that the abnormalities caused by drug use account for less than 1% of the total anomalous births. (Brent, 2004). However, considering that the abnormalities associated with drug use can be prevented, it seems that the rate is not as small as it appears.

Table 2 shows the rates of anomalies caused by drugs determined to be teratogenic in pregnant women (Aydoğdu, 2019).

Table 2: Teratogens that cause anomalies in pregnant women and the rates of births with anomalies they cause.

DRUG USED	ANOMALY BIRTH RATE (%)
Androgens and androgenic progestatives	0.3-18
Antiepileptics	
Phenytoin	10
Valproate	1
Trimethadione 60	60
Antineoplastics	
Folate antagonists	30
Others	17
Synthetic Retinoids	20
Thalidomide	20
Warfarin and other anticoagulants	7

In Table 3, drugs with toxic effects on the fetus are given together with their effects (Nahum, 2006).

Table 3: Toxic drugs and their toxic effects on the fetus.

MEDICINE	EFFECT
Trimethorphine	Neural tube defect (NTD)
Thalidomide	The most common heart abnormality is ventricular septal defect (VSD). It also causes deafness, phocomelia and gastrointestinal developmental defects.
Propylthiouracil	Goiter in newborn
Progestin therapy	Masculinizing effects at high doses
Penicillamine	Collagen disorder, cutis lasa, hyperflexibility in joints
Misoprostol	Atherosclerosis, limb development disorders, Mobius syndrome
Methimazole	Aplasia cutis
Minoxidil	Hirsutism in newborns
Lithium	Ebstein Anomaly and other anomalies in chronic use
Ionizing radiation	Microcephaly, IUGR, mental retardation at high levels
Ethanol	Fetal alcohol syndrome, microcephaly, mental retardation, IUGR, typical facial dysmorphogenesis, ear anomalies
Diethylstilbestrol (DES)	Genital anomalies, adenosis, clear cell carcinoma of the vagina in adolescence
Coumarin derivatives	Nasal hypoplasia, IUGR
Cocaine	Pregnancy losses
Caffeine	It has not been proven to be associated with teratogenicity in normal use. However, it is stated that the risk of abortion increases with high exposure.
Isoniazid (INH) and Para-aminosalicylic acid (PAS)	Increase in some central nervous system (CNS) anomalies.
ACE inhibitor	Fetal hypotension syndrome, which ends with decreased fetal renal blood flow in the 2nd and 3rd trimester periods, anuria, oligohydramnios, pulmonary developmental disorder, and underdevelopment of cranial bones.
Androgens	In high doses, deepening of the voice, hair growth, muscular body structure in the female fetus and hypospadias in the male fetus may occur.
Aminopterin, Methotrexate	Growth failure, microcephaly, meningomyelocele, mental retardation, hydrocephalus, cleft palate

Pregnancy Categories of Drugs

Drugs used during pregnancy have different effects on the embryo or fetus depending on their structure. In 1962, 10,000 children were exposed to thalidomide and were born with anomalies, and this necessitated the creation of a classification system to be used as a reference in drug use during pregnancy. After this case, studies on the classification of drugs began and a

classification system consisting of five letters was created by the FDA in 1979. In this system consisting of categories A, B,

C, D and X, drugs are classified from the least risky (A) to the riskiest (X). Table 4 contains information about pregnancy risk categories determined by the FDA (Kaplan et al., 2014; Zorlu and Arı, 2006).

Table 4: FDA pregnancy risk categories and definitions

CATEGORY	DEFINITION
A	In controlled studies conducted in pregnant women, it was determined that the drug has no risk of teratogenic effects on the fetus in the first trimester and there is no evidence of risk in the following trimesters.
B	Although studies in animals have shown that the drug has no risk of teratogenic effects on the fetus, there are no controlled studies in humans or no risk of teratogenic effects on the fetus has been found in controlled studies in animals.
C	Although the risk of teratogenic effects of the drug has been detected in studies conducted on animals, there are no controlled studies conducted in pregnant women or studies on this subject in animals or pregnant women.
D	Drugs that can be used in pregnant women when safer drugs cannot be used or are not effective in life-threatening diseases, although there is clear evidence of the risk of teratogenic effects on the fetus.
X	These are drugs whose teratogenic effect risk has been definitively proven in studies conducted in animals and humans, and whose teratogenicity risk outweighs their benefits.

Risk-benefit decisions regarding the use of drugs during pregnancy were phased out in 2015 due to insufficient category definitions (Pernia and DeMaagd, 2016), but this classification continues to be used in our country. With the improvement of technological possibilities, ADEC (Australian Drug Evaluation Committee) and FASS (Pharmaceutical Specialties in Sweden) classification systems were developed as a contribution to the classification system created by the FDA. In a study, 236 drugs were examined and 61 of these drugs were determined to be in the same risk category in all classification systems (Çelik et al., 2000). Table 5 shows the risk categories and definitions in the classification made by ADEC (Yiğiter, 2012).

Table 5: A, D, E, C pregnancy risk categories and definitions

GROUPS	DEFINITION
Group 1	Extensive human testing and animal studies do not indicate that the drug is embryotoxic/teratogenic.
Group 2	Extensive human testing does not indicate that the drug is embryotoxic/teratogenic
Group 3	Extensive human testing does not indicate that the drug is embryotoxic/teratogenic, but the drug appears to be embryotoxic/teratogenic in animals.
Group 4	There are no adequate and well-controlled studies on the effects of the drug on humans. Animal studies show that it has no embryotoxic/teratogenic effects.
Group 5	There are no adequate and well-controlled studies on the effects of the drug on humans.
Group 6	There are no adequate and well-controlled studies on the effects of the drug on pregnant women, but animal studies show that it has an embryotoxic/teratogenic effect.
Group 7	The drug has a risk of embryotoxic/teratogenic effects on the human fetus, at least in the first trimester.
Group 8	The drug has a risk of embryotoxic/teratogenic effects on the human fetus during the second and third trimesters.
Group 9	There is a risk of the drug causing prenatal complications and abnormalities.
Group 10	There is a risk that the drug may cause specific hormonal activities in the human fetus.
Group 11	The mutagenic/carcinogenic risk of the drug is known.

Embryotoxic Agents

As a result of controlled studies, the existence of drugs that are definitely embryotoxic has been determined. In Table 6, drugs that have been confirmed to be embryotoxic by causing developmental toxicity in humans and their effects in humans are presented (Mattison, 1992; Jelovsek, 1989).

TOXICITY TESTS

The purpose of toxicity tests is not only to identify the toxic effects of chemical substances on living organisms, but also to determine the doses at which the toxic effects of chemical substances on living organisms will not be seen. If the effects of a substance that is exposed for a long time are to be examined, it is necessary to apply substances and conditions with equivalent properties within the period in which the study is carried out. In tests to see the expected toxic effect, a substance known to produce the expected effect should be applied to the positive control group, and thus it should be tested to see if the experiment is working properly (Saygı, 1991; Committee for

Proprietary Medicinal Products, 2000). All substances that come into contact with the human body through internal consumption or external use are tested for toxicity before being put into use (Loomis, 1978). Toxicity tests can be classified as follows according to the length of the test period (Saygı, 2003):

Table 9: Lethal dose (LD_{50}) values of some chemicals in humans and rodents

Substance	**Human LD_{50}**	**Rat LD_{50}**	**Mouse LD_{50}**	**Rabbit LD_{50}**
Lindane	840mg/kg	125mg/kg	-	130mg/kg
Caffeine	192mg/kg	192mg/kg	620mg/kg	-
Boric acid	640mg/kg	2660mg/kg	3450mg/kg	-
Amytal	43mg/kg	560mg/kg	-	575mg/kg

In Table 10, the lethal dose values of some chemicals in humans and rodents are evaluated according to the Hodge and Sterner Scale (Saygı, 2003).

Table 10: Grading of toxicity according to the Hodge and Sterner Scale

Toxicity degree	**Oral LD_{50} (mg/kg; rat, single dose)**	**Inhalation LC_{50} (ppm; rat, 4 h exposure**	**Dermal LD_{50} (mg/kg; rabbit, single application to skin**	**Possible LD_{50} for humans**
Extremely toxic	<1	<10	<5	1 drop or less
Severely toxic	1-50	10-100	5-43	4ml
Moderately toxic	50-500	100-1000	44-340	30ml
Less toxic	500-5000	1000-10,000	350-2810	600ml
Practically non-toxic	5000-15,000	10,000-100,000	2820-22.590	1 liter
Relatively harmless	>15,000	>100,000	>22,600	1 liter

Subacute Toxicity Tests

In these tests, the xenobiotic agent is applied to experimental animals one or more times every day. Rodent or non-rodent animals can be used as test subjects. The test lasts one or three weeks, and during the experimental period, 3 different dose levels are tested on two different animal species. In determining the doses, the predetermined LD50 value is used to determine the safe values for the 3 doses to be selected.

Subchronic Toxicity Tests

In subchronic toxicity tests, the study period is 3 months and rats and dogs are preferred as experimental animals. With toxicity tests, information

is obtained about the effects of xenobiotics on main organs such as liver, kidney, brain, heart and bladder. In addition, thanks to these tests, the toxicity risks of substances we encounter in daily life can be determined. There are four steps in determining toxicity risk: identification of the chemical substance, dose-response relationship, exposure and risk characterization. Tests can be used to determine the lowest observed effect level (LOEL), the lowest level of adverse effects (LOAEL), no observed effect levels (NOEL), total daily intake (TDI), Benchmark Concentration (BMC) value (value between NOEL and LOAEL) without any health problem.

Chronic Toxicity Tests

These are toxicity tests designed to detect the long-term toxic effects of exposure to xenobiotics. If the substance is expected to be used in humans for a lifetime, this substance must be tested throughout the life of the animal used in the study. However, in terms of both cost problems and animal ethics, it is accepted that these tests be performed for a maximum of 6 months in rodents and a maximum of 9 months in non-rodent animals. In these tests, the dose administered to the subjects is expressed as the maximum tolerated dose (MTD). Animals surviving at the end of the test are subjected to complete histopathological investigations, and the results are evaluated.

Special Toxicity Tests

Tests in this group are classified according to their purposes as "interaction with other chemicals, effects on reproduction, teratogenicity tests, carcinogenicity tests, mutagenicity tests, acute dermal and eye irritation tests, skin sensitivity tests and effects on behavior tests" (Saygı, 2003).

The Importance of Experimental Animals in Drug Development

Experimental animals are used to determine the effectiveness of a newly developed drug against a specific disease and to determine the degree of safe use in patients (Saygı et al., 1997). First of all, the disease to be studied against the drug is created in experimental animals, and then the substance whose effect will be examined is applied and the results are evaluated. In addition, newly developed drugs are subjected to short- and long-term toxicity tests using experimental animals before they are put into clinical use. In order to investigate the therapeutic effect of the substance, the disease

that is expected to be treated must first be established in experimental animals. It is very difficult to put newly developed drugs into clinical use without studying on experimental animals, therefore experimental animals are important in drug development (Stoewsand, 1996).

Despite all the preclinical and clinical tests applied, the safety of the drugs for use in humans cannot be fully determined. It has been reported in various examples that some serious side effects and even cases resulting in death may occur as a result of the widespread use of the drug, and the reason for this has been determined to be that living species respond to a xenobiotic with various mechanisms (Saygı, 2003).

ALTERNATIVE TOXICITY TESTS

Alternative tests are being developed to investigate the toxic effects of xenobiotics on humans. For this purpose, various cell cultures are used in studies instead of experimental animals. For example, the American National Cancer Institute abandoned the use of mice in cancer research and started using 60 types of human cell cultures instead of mice, and it was reported that the results they obtained from cell cultures were more realistic. Diseases in which human cell cultures are used in research include AIDS, hepatitis and epilepsy (Saygı, 2003).

Uses of Cell Cultures

The areas of use of cell cultures are as follows (Saygı, 2003):

Identifying enzyme systems that metabolize drugs

Determination of factors that may affect the activity of enzymes responsible for the metabolization of drugs

Determining the metabolism products of drugs and the toxicity risk of these products

Determining the metabolism time of drugs

Determining the degree of interaction of drugs with each other

Investigation of the effects of various factors such as genetics, age, environment and disease on the metabolization process of drugs

Predicting whether people have the potential for drug allergy

Human liver cell cultures are most commonly used to investigate the conditions mentioned above (Guillouzo, 1993). The advantages and disadvantages of human cell cultures in toxicity studies are given in Table 11 (Guanaratna, 2000).

Table 11: Advantages and disadvantages of human cell cultures in toxicity

ADVANTAGES	DISADVANTAGES
Species differences disappear	Since it is a single system, it is not suitable for multifactorial drug metabolism.
It enables research on specific tissue cells such as skin and liver, where xenobiotics are thought to have a possible toxic effect.	Technological infrastructure requires cost and experience
Allows the elucidation of toxicity mechanisms on cells	The life cycle of cells in the in vitro environment is short
Animals do not suffer or die	Enzymes have limited stability
	The penetration ability of the culture medium is limited
	Needs cofactors

Stem Cells in Embryotoxicity Studies

Due to the complex structure of embryo or fetus development and the complex relationships between mother and fetus during pregnancy, some in-vitro methods have been developed in order to obtain rapid results, reduce costs and reduce animal consumption in experiments when determining the teratogenicity potential of substances. Since the experimental use of human stem cells (hESC) is unethical, the mouse embryonic stem cell test (EST) is one of the in-vitro tests that can be used appropriately. The most important factor in embryotoxicity tests is that ESCs mimic in-vivo early stage embryonic development and have tissue-specific expression profiles (Wobus and Löser, 2011). EST, whose reliability has been officially accepted by the European Center for the Evaluation of Alternative Methods (ECVAM), was first developed on mouse embryo cells by Horst Spielman and his colleagues in 1997 (Spielmann et al., 1997). The principle of the mouse EST technique is based on the evaluation of 3 toxicological endpoints. These:

1. Evaluation of growth inhibition of 3T3 fibroblasts representing differentiated cells

2. The 3-(4,5-dimethylthiazol-2-yl)-diphenyl tetrazolium bromide (MTT) method is used to evaluate the cytotoxicity of undifferentiated embryonic cells 10 days after exposure. This substance is transported to living cells by active transport and is reduced to blue-violet colored, water-insoluble formazone by the mitochondrial succinate dehydrogenase enzyme. The resulting color is measured spectrophotometrically and the values found are related to the number of living cells.

3. It is the evaluation of the inhibition of differentiation of embryonic cells into myoblasts, the precursors of cardiac muscle, 10 days after exposure (ECVAM, 2001).

Nowadays, EST is used to examine the embryotoxic effects of many types of substances. Zhou et al. evaluated the developmental toxicity risk of perfluorooctane sulfonate, perfluorooctanoic acid and bisphenol A, which are endocrine disrupting chemicals, using the EST technique (Zhou et al., 2017). Dimopoulou et al. investigated toxicokinetic and toxicodynamic studies on embryotoxicity tests of some azole derivatives based on their rate of passage through the placenta. They reported that all of the chemicals had a good correlation between embryoculture testing and the potentially toxic effects obtained by the EST technique (Dimopoulou et al., 2018). In a study conducted by Imai in 2016, embryonic stem cell culture and hepatocyte culture were combined and a hybrid system was created (Imai, 2016). The sex-specific effects of chemicals can be categorized as sensitive to males, sensitive to females and sensitive to both sexes. Embryotoxic and especially developmental effects related to male and female sex can be examined by chromosome karyotyping of embryonic stem cells (Cheng et al., 2016). In addition, the investigation of signaling pathways has become a new perspective in the mechanistic investigation of embryotoxicity (Yang, 2016).

Use of Chicken Embryos in Embryotoxicity and Teratogenicity Tests

Humans are exposed to increasing amounts of xenobiotics of unknown embryotoxic and teratogenic potential. The embryotoxic and teratogenic effects of this type of xenobiotics need to be tested in detail on rodents such as rats or mice or rabbits. However, it is not possible to test every new chemical compound produced. Therefore, it would be beneficial to develop inexpensive and rapid alternative screening techniques that can accurately predict the effects of a xenobiotic on the mammalian embryo. Various in vivo and in vitro test systems using mammalian and non-mammalian animal species are used for this purpose. There are many studies in the literature in which the embryotoxic and teratogenic effects of different chemical compounds were determined using poultry embryos and especially chicken embryos (Özparlak, 2015).

Embryotoxicity and Teratogenicity Tests Using Chicken Embryos

Jelinek (1977) developed the Chicken Embryotoxicity Screening Test (CHEST) using fertilized chicken eggs. In CHEST-I; The test substances, prepared in various concentrations in a geometric series of ten in the ratio of 1/102 - 1/106 in the appropriate solvent, are injected under the caudal region of healthy embryos at stages 10-11 according to the H&H scale, with a special microinjector under a microscope. After another 24 hours of

incubation, the eggs are opened and the distance between the yolk arteries and the end of the tail is measured on a dissecting microscope with an ocular micrometer and abnormal embryos are detected. The area between the last ineffective dose and the first effective dose is determined as the embryotoxic area. In CHEST-II, the doses in the embryotoxic area reached in CHEST-I are injected subgerminally into embryos at stages 11-14., and intraamniotically into embryos at stages 17-20. and 21-24. according to the H&H scale. Incubation continues until the 8th day and teratogenic evaluations are made. Although CHEST-I is quite sensitive, it has difficulties such as difficulty in manipulation and inability to achieve perfect sterilization. In addition, since only one part of the embryo is evaluated, there is a high probability that effects on other body parts will be missed. Jelinek and her colleagues evaluated the toxic effects of 130 substances with CHEST-I and CHEST-II methods and reported the test results of these compounds (Jelinek et al., 1985).

It is also possible that the results obtained from CHEST-I can be adapted to mammals. The value obtained by multiplying the dilution concentrations in the determined embryotoxic range by 10-2 is accepted as the toxic limits per kg of live weight of the pregnant mammalian mother. Although the problem of species differences arises in the application of CHEST results to mammals and especially humans, morphogenetic events and their course are similar in all species (Jelinek, 1977).

In addition to CHEST, Kemper and Luepke (Kemper and Leupke, 1986) used the Hen's Eggs Test (HET) and Nishigori et al. (Nishigori et al. 1992) used the Hen's Fertile Egg Screening Test (HEST) with various modifications using fertilized chicken eggs have developed. All these tests are easy, cheap, provide reproducible results and can be performed in a short time. Knowing the developmental stages of the chicken embryo very well is another important advantage. The ability to use a large number of chicken embryos provides an advantage over studies with mammalian species in the statistical evaluation of toxicity. At the same time, it reduces the number of subjects and trials to be used later in toxicological studies on mammals, minimizes the pain and suffering that can be inflicted on a living organism, and does not violate ethical rules, legal restrictions, and animal rights. It has also been reported that the results obtained from CHEST and HET are largely compatible with the results obtained from mammals (Jelinek, 1977). However, in these models using chicken embryos, the lack of placenta and mother-fetus relationship in mammals, and the fact that some compounds may show non-specific sensitivity and give false positive results are

considered disadvantages (Bozkurt et al., 2021). Kemper and Luepke (1986) stated that mortality rate, growth retardation, teratogenicity, systemic effects and immunopathological effects can be evaluated with the HET method.

In addition to these mentioned tests, Luepke (1985) developed the Hen's Egg Test-Chorioallantoic Membrane, HET-CAM. This test aims to determine the eye-related irritation potential by evaluating the hemorrhage, lysis and coagulation effects of chemical substances such as cosmetic products on the chorioallantoic membrane. Rosenbruch and Holst (1990) developed a similar model with the yolk sac blood vessels system as an alternative to HET-CAM. Similarly, Neumann et al. (1997) developed the Photo Hen's Egg Test (PHET) as an alternative to the rabbit eye irritation test.

Jelinek and Marhan (1994) subjected 50 xenobiotics with different pharmacological properties to routine rat and rabbit tests to demonstrate the validity of CHEST. They stated that the results were approximately 80% consistent with CHEST, however, alternative testing methods cannot replace routine testing methods but can be helpful. Rosenbruch (1997) drew attention to the use of the chicken egg model, especially in eye and mucosa-related toxicity studies, tumor biology, and studies on the effects of heavy metals and the effects of drugs on the cardiovascular system. He also drew attention to the use of chicken eggs, a model in experimental biology and medicine, in the early stages of the incubation period, when the embryo's sensitivity to pain has not developed, and thus reported that this test could be a real alternative to animal experiments. Hashizume et al. (1992) conducted a study highlighting the importance of the development stage of chicken embryos at the time of injection, the injection site, and the type of solvent in teratogenicity studies.

In *in ovo* embryotoxicity tests, the injection site and time, the volume and pH of the test solution used, the doses applied and the number of eggs used for each dose group, and the type and concentration of the solvent used are important factors to consider. In embryotoxicity studies conducted on chicken embryos, injection methods were used to administer the substance to be tested into the air chamber, caudal region of the embryo, albumin and egg yolk. The air chamber is considered the ideal injection site because of its ease of application, the lowest risk of infection of the eggs, the homogeneous and rapid diffusion of the given solution, and the elimination of mechanical damage that may occur to the embryo due to the increase in intra-egg pressure, which is the case in other methods (Figure 8). In studies conducted with various pesticides, the air chamber injection method was preferred on

different days of incubation. Despite all these advantages, the inability to determine whether the entire substance reaches the embryo during the injection into the air chamber is considered a significant disadvantage of this method (Özparlak, 2015).

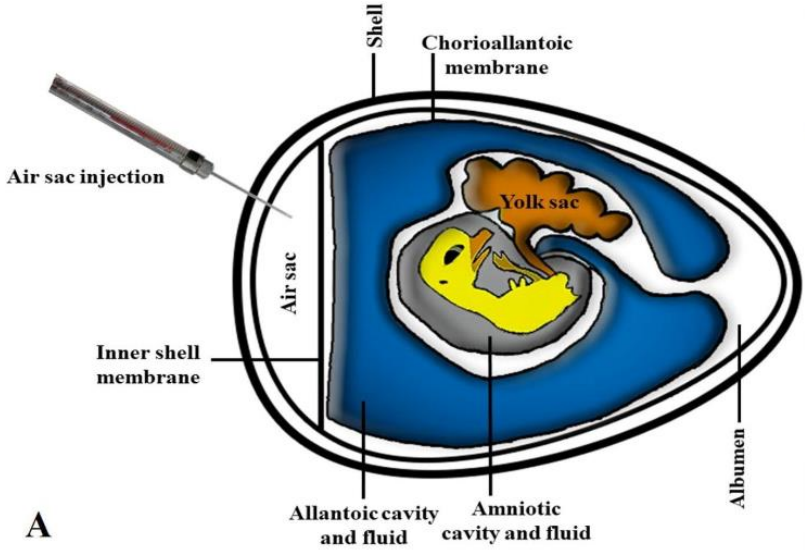

Figure 8: Injection technique into the chicken egg air space (Bozkurt, 2021)

For the injection time, it has been reported that if it is aimed to determine the embryotoxic effects of the natural form of the substance to be tested, the very early embryonic period should be preferred, and if it is aimed to determine the effects of the metabolites that will be formed as a result of the metabolism of the tested substance in the liver, the injection to be made in the later period should be preferred (Jelinek, 1985).

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Chapter 8

Peptide *N*-glycosidase F Production for *N*-glycan Release

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INTRODUCTION

Glycans, intricate molecules composed of sugars, represent an indispensable fraction among the fundamental constituents of cellular architecture and stand as one of nature's most abundant and diverse biopolymers (Lowe & Marth, 2003). These molecular entities exhibit versatility in their existence, manifesting either as independent oligosaccharides or integrated forms within glycoconjugates. The intricate process of glycosylation, wherein oligosaccharides intricately bind to proteins to create glycoproteins, emerges as a prevalent post-translational modification witnessed within cellular mechanisms. This glycosylation phenomenon profoundly influences the development and functionality of specific brain regions, crucial segments of the gastrointestinal system, and pivotal operations within the hepatic, visual, and immune systems, thereby underlining its paramount importance in biological processes.

Moreover, the landscape of eukaryotic proteins is significantly shaped by the prevalence of glycoproteins, constituting more than half of these vital cellular components (Freeze et al., 2015). The formation of glycoproteins, functioning as glycoconjugates, ensues through the attachment of one or multiple glycans to proteins, typically occurring through *N*- or *O*-linked manners, conjoined with a polypeptide structure (Varki & Lowe, 2009). Particularly within eukaryotic secretory pathways, the regulation of crucial proteins predominantly hinges on

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the orchestration conducted by *N*-linked glycans (*N*-glycans). Furthermore, the intricate ballet of glycoprotein folding is modulated by the delicate equilibrium orchestrated by *N*-glycans, as elucidated by studies delving into glycoprotein balance (Helenius & Aebi, 2001).

The exploration of glycoprotein balance not only enriches our understanding of cellular dynamics but also holds substantial promise in steering the development of novel pharmaceutical interventions, envisaged to potentially combat and mitigate diverse diseases on the horizon (Hebert et al., 2014).

Current Challenges in Deglycosylation

In the pursuit of characterizing glycans, their separation from the proteins they are intricately linked to becomes imperative. Diverse deglycosylation methodologies are employed to accomplish this intricate separation process. These methodologies traverse the realms of chemical techniques as well as enzymatic applications. However, chemical approaches, notably β-elimination and hydrazinolysis, carry the burden of recognized carcinogenic potential. Additionally, these methods often leave residual salts post-reaction, rendering glycan analysis via mass spectrometry cumbersome and altering the inherent structure of glycans and the residual polypeptide, thereby constricting their biological functionality pertaining to glycans (Patel et al., 1993). The glycans produced via chemical means often face commercial constraints due to an amalgam of technical intricacies and environmental concerns. The consequential generation of substantial chemical waste during these processes further accentuates their environmental unfriendliness. Contrastingly, enzymatic processes present a more environmentally conscious avenue for glycan production, as highlighted in studies (Chen et al., 2010). Consequently, the utilization of glycans generated via chemical methods finds limitations within sectors like food and healthcare, necessitating high-efficiency processes and stringent purity criteria for their viable application. A promising solution to mitigate these limitations lies in the employment of deglycosylating enzymes. Among the repertoire of enzyme-based techniques, Peptide-*N*-Glycosidases (PNGases) emerge as the prominent choice (Altmann et al., 1995). PNGases facilitate the detachment of glycans from proteins by catalyzing the hydrolysis of the bonds linking them to asparagine residues (Nuck et al., 1990; Takahashi, 1977). Specifically, the PNGase F enzyme enjoys significant popularity due to its efficacy in liberating *N*-glycan structures from glycoproteins. Despite successful purification of PNGase F through a multifaceted and prolonged process derived from *Flavobacterium Meningosepticum* bacterial culture, obtaining sizable quantities of pure protein remains a persistent challenge

within this methodological approach (Loo et al., 2002). This ongoing challenge necessitates further refinement and optimization for enhanced efficiency in the production of pure proteins.

Glycans and Their Roles

Glycans, ubiquitous carbohydrate-based polymers synthesized across the spectrum of living organisms, including plants, animals, and various microbial sources, manifest a remarkable diversity and indispensability in biological systems. These intricate macromolecules primarily exist as covalent linkages between saccharides and proteins or lipids, wielding substantial influence over the structural and mass variations within living systems. The interdisciplinary domain of Glycobiology, rooted in understanding the intricacies of glycans and their derivatives, delves into exploring their structural nuances, chemical properties, biosynthetic pathways, and biological functionalities (Landsteiner & Van Der Scheer, 1931).

Exploration into the roles of glycans within cellular mechanisms, particularly their involvement in cell adhesion and receptor activation, bolsters the concept of glycoproteins functioning as crucial sentinels, fortifying the host against microbial and viral incursions (Barboza et al., 2012). Protein glycosylation, a fundamental process, encompasses three main categories: *N*-glycans, *O*-glycans, and glycosaminoglycans (proteoglycans) (Schachter, 2000).

O-glycans predominantly form bonds with hydroxyl groups of serine or threonine residues within polypeptide chains, establishing *N*-acetylgalactosamine linkages, while *N*-glycans attach to specific asparagine residues within the Asn-X-Ser/Thr motif via *N*-acetylglucosamine bonds (HexNAc) (Varki & Lowe, 2009). The core structure of *N*-glycans comprises two HexNAc and three mannose residues, occasionally accommodating fucose within the HexNAc residue, thereby influencing the core structure's functional diversity and complexity (Varki & Lowe, 2009; Yanagidani et al., 1997). This core *N*-glycan synthesis initiates in the endoplasmic reticulum, extended by the sequential addition of other monosaccharides modulated by glycosyltransferases and glycosidases, crucially determining the branching patterns and linkage configurations (Ohtsubo & Marth, 2006). The extension process, enriched with fucose and sialic acid, intricately diversifies and complicates the landscape of *N*-glycan structures.

Based on sialylation and fucosylation patterns, *N*-glycans stratify into three primary classes: high mannose, complex, or hybrid, exemplified in Figure 1, delineating their structural diversity and functionality (Varki & Lowe, 2009). Moreover, *N*-glycans actively engage in recognition and binding events with

cell membrane lectins from microorganisms (Nwosu et al., 2012). Beyond their structural roles, glycans wield substantial influence over protein behavior, impacting their structural properties, conformational dynamics, solubility, immunogenicity, antigenicity, and resistance to proteolytic degradation (Spik et al., 1994).

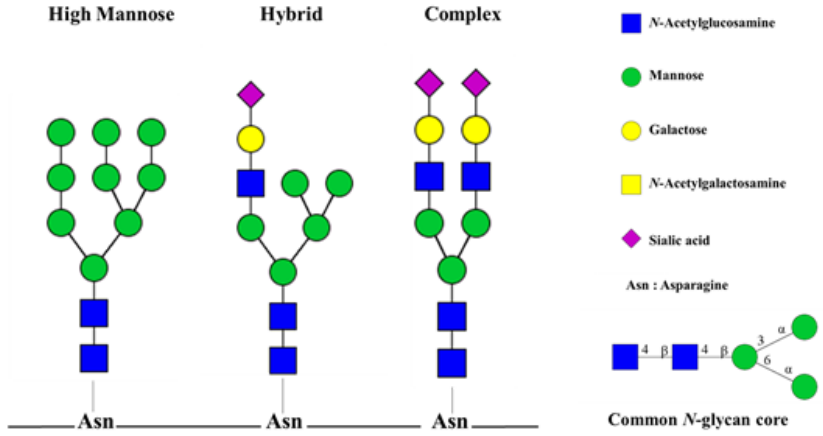

Figure 1: Basic *N*-glycan structures (Varki & Lowe, 2009).

Figure 1: Basic *N*-glycan structures (Varki & Lowe, 2009).

Recent investigations unveiled the role of *N*-glycans derived from lactoferrin, contributing to inhibiting *Pseudomonas aeruginosa* bacteria responsible for bacterial keratitis and the invasion of corneal epithelial cells, underscoring the pivotal roles played by glycans in host-pathogen interactions (Kautto et al., 2016). The multifaceted impacts of glycans extend to shaping protein folding pathways, dictating their conformational preferences, and ultimately influencing their functional roles within biological systems (Wormald et al., 2002).

Peptide *N*-glycosidase F

Amidases (EC 3.5.1.X) represent a versatile class of biocatalysts renowned for their multifaceted role in enzymatic activities, specifically characterized by their involvement in hydrolytic and acyl transfer processes targeting carbon-nitrogen (C-N) bonds, thus rendering them pivotal in the intricate synthesis pathways of chiral carboxylic acids (Sharma et al., 2009). This diverse enzymatic cohort finds its ubiquitous presence across various biological domains, thriving within the cellular milieu of bacteria, yeast, fungi, plants, and diverse animal tissues, underscoring their fundamental role in a plethora of

biological systems (Kang et al., 2019). Among these, the N-glycosidase F (PNGase F, EC 3.5.1.52), belonging to the asparagine amidase category, has emerged prominently, exhibiting a broad spectrum of enzymatic specificity and demonstrating remarkable efficacy in the selective liberation of diverse *N*-glycan structures from glycoproteins, thereby establishing itself as a cornerstone in the realm of glycoanalytical methodologies (Vilaj et al., 2021). This specific enzyme, PNGase F, selectively targets the hydrolysis of bonds tethering *N*-acetylglucosamines on an array of high-mannose, hybrid, and complex oligosaccharides anchored to *N*-linked glycoproteins and glycopeptides' asparagine residues, showcasing its precision in glycan cleavage processes (Tarentino et al., 1985). Initial endeavors in isolating PNGase F showcased its robust enzymatic activity, subsequently leading to successful purification from diverse species, each demonstrating promising levels of efficacy (Barsomian et al., 1990). The naturally secreted PNGase F by *Flavobacterium meningosepticum*, a gram-negative bacterium, was meticulously isolated employing laborious, multi-step methodologies from bacterial cultures, ultimately yielding minute quantities (ranging from 0.1 to 0.5 mg/L culture) of pure protein, underscoring the intricate challenges in its purification process (Mussar et al., 1989). Characterized by a molecular weight approximately around 35 kDa, this isolated PNGase F comprises a polypeptide chain spanning 314 amino acids (Loo et al., 2002). Moreover, its enzymatic activity peaks at a pH of 8.5, further underlining its optimal biochemical conditions for functionality (Tarentino et al., 1985). Advanced efforts in efficient cloning methodologies have significantly enhanced its production and purification within the confines of Escherichia coli (Barsomian et al., 1990). Furthermore, studies conducted by Loo et al. have elucidated enhanced efficiency following the removal of the enzyme's signal sequence, showcasing continuous advancements in enhancing its enzymatic efficacy (Loo et al., 2002).

Utilization of PNGase F remains unparalleled as the preeminent method for the comprehensive removal of nearly all *N*-glycans from glycoproteins, barring instances where the glycan lacks a core α(1 → 3)-fucose, thereby allowing PNGase F to efficaciously liberate an extensive repertoire of asparagine-linked complex, hybrid, or high-mannose oligosaccharides (Trimble & Tarentino, 1991). Remarkably, a tripeptide containing asparagine linked to an oligosaccharide serves as the minimal substrate for PNGase F, wherein the cleavage event leads to the deamination of the attached asparagine residue, facilitating the retrieval of the intact oligosaccharide structure. However, endoglycosidases such as F1, F2, and F3 exhibit subdued activities upon natural glycoproteins, their release of glycan structures remains limited, warranting the

sustained relevance and efficiency of PNGase F in glycan cleavage for diverse analytical and biotechnological applications (Altmann et al., 1995).

Production of PNGase F

One model molecular cloning and production method of PNGase F is The Expresso Rhamnose Cloning and Expression System. This aims to clone this enzyme with an N-His-SUMO tag for glycan separation. It provides a faster and more reliable application compared to other conventional cloning systems. In this system, also known as an *in-vivo* cloning system, the PCR product can be cloned by mixing it with the vector and competent cells provided by the kit without the need for additional enzymatic applications such as restriction enzymes. The system utilizes SUMO (small ubiquitin-related modifier) protein as a fusion partner. This protein can covalently bind and detach from specific protein substrates in eukaryotic cells. Additionally, even when expressed at high levels in prokaryotic cell systems, it exhibits rapid folding and relatively swift characteristics. The presence of the SUMO protein, a tag that is 100 amino acids in length, enhances the purification, stability, solubility, and expression of recombinant enzymes (Sucu et al., 2021).

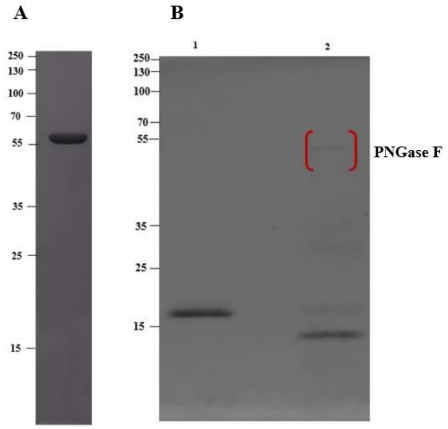

Figure 2: (A) SDS-PAGE (4-12%) gel of Recombinant PNGase F. (B) Enzymatic deglycosylation of denatured RNase B by PNGase F Lane 1: Glycosylated RNase B (17kDa). Lane 2: Denatured RNase B deglycosylated by PNGase F.

Conclusion

Characterization of glycans, which are important participants in biological systems, necessitates effective separation from proteins, which is accomplished by a variety of deglycosylation methods. Chemical procedures, while powerful,

have environmental and safety concerns, making enzymatic methods, particularly those utilizing PNGases, an appealing option. PNGase F is notable for its accuracy in releasing *N*-glycan structures from glycoproteins. However, difficulties in the purifying process persist, preventing large-scale production. While purifying issues exist, advancements in cloning methods and signal sequence removal have increased manufacturing efficiency. Its unparalleled ability to extract nearly all *N*-glycans makes it an essential tool for glycoprotein analysis.

To enable PNGase F to be produced on a wide scale, future research should concentrate on improving the purifying procedure. Enhancing enzymatic procedures and investigating the possibilities of other deglycosylating enzymes may also offer substitute alternatives. A promising direction for future research is suggested by the ongoing investigation of glycan functions in many biological processes and medicinal applications.

To summarize, the path to efficient glycan characterization entails overcoming hurdles in enzymatic techniques, particularly in the context of PNGase F purification, while also examining the larger landscape of glycan functions and uses. This multidisciplinary approach holds the key to realizing glycans' full potential in a variety of domains ranging from healthcare to biotechnology.

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Chapter 9

Telemedicine and Current Developments

İlknur PALAZ[1]

INTRODUCTION

Telehealth involves the use of information and communication technologies to protect and improve the health of individuals and communities in critical situations where access to healthcare services is crucial. Its objectives include providing disease diagnoses and treatments, as well as addressing healthcare research or educational needs. Telehealth is recognized as a tool that enables healthcare professionals to deliver high-quality healthcare services in their respective fields rapidly and effectively (Otero et al., 2014). Currently, telehealth applications include one of the oldest practices known as teleradiology. Teleradiology involves the use of technologies established for the evaluation of radiology images by expert radiologists located in a specific center. Teledermatology utilizes information and communication technologies for the examination of images related to skin health. Telepathology involves the use of technology support for the remote diagnosis of pathological findings. Teleintensive care focuses on the remote monitoring and tracking of intensive care patients using technology. Tele-nursing is the use of communication technology to provide nursing services. Similar technologies continue to find their place in healthcare (saglik teknoloji.com).

The concept of telemedicine has its roots in the invention of communication tools. As early as 1879, a Lancet article suggested that the use of telephones could reduce unnecessary visits to medical offices. Almost a century ago, in 1922, the American inventor Hugo Gernsback described the idea of a device he named "teledactyl." This device was envisioned to allow doctors to see distant patients on a screen and treat them using robot arms. The first application that could be termed telemedicine emerged in the 1950s when some hospitals in the United States began sharing information and images over telephone lines. A notable early success in this field occurred when two hospitals in Pennsylvania managed to share X-ray images over telephone lines. Initially used for doctors to consult with specialists in other locations, this method proved particularly

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beneficial for patients in rural areas to access expert doctors. The early success paved the way for the development and expansion of telehealth. Telehealth applications have continued to advance and innovate in the field of healthcare. They have become mainstream and are accepted as a preferred method of providing care in various healthcare domains. A search using the keywords "telemedicine" and "telehealth" in the PubMed database from 2010 to 2021 revealed over 27,000 published articles, indicating the widespread adoption and ongoing development of telemedicine applications. This example illustrates that telemedicine applications are becoming more prevalent and are continuously being enhanced. The full potential of this growing healthcare application area is still being explored (Ucael et al., 2021).

Mobile applications have become an indispensable technological development and are present in every aspect of life. Mobile apps provide services in various areas such as shopping, health, travel, communication, entertainment, and education (Çelik and Taş, 2021a). Organizations that understand the importance of reaching everyone at all times effectively utilize mobile applications and social media tools to connect with individuals directly (Çelik and Taş, 2021b). With new mobile health technologies, it is possible to remotely monitor and store health data such as blood pressure and heart rate for future use. Individuals with chronic illnesses can use their smartphones for medical records, storing data about themselves and providing it to healthcare providers when needed. This can particularly contribute to improving healthcare services in low- and middle-income countries where the healthcare system may not be well-developed, and the number of healthcare providers may be limited (Sweileh et al., 2017). Advancements in wireless communication and mobile network technologies have enabled the remote monitoring of medical data, such as heart rate and blood oxygen levels, for individuals working in distant locations like astronauts (Işık and Güler, 2010). Especially during the COVID-19 pandemic, when there is a possibility of contact with the patient (Demirağ and Hintistan, 2020), mobile applications in the healthcare sector have become even more crucial.

Telemedicine applications are supported by some segments of the population for various reasons, while others may not be in favor. According to those who support these applications, telemedicine facilitates easier and faster access to healthcare, promoting high medical care standards at lower costs. As an alternative method to reduce healthcare costs, telemedicine can decrease the likelihood of unnecessary diagnostic or treatment procedures and enhance communication between healthcare units. Additionally, it can bring significant improvements in the quality of medical care by directing patients from their

location to specialist physicians. Through online shared resources, it ensures continuity in health education, enhancing information exchange and communication between healthcare professionals and institutions. Moreover, by expanding the scope of healthcare beyond national borders, it enables patients to receive treatment from globally renowned healthcare professionals online (Toader et al., 2011). However, the transfer of health information to electronic platforms is thought to raise ethical concerns and potentially weaken the doctor-patient relationship. Despite the emergence of new technologies and care models, the fundamental ethical responsibilities of healthcare professionals, especially doctors, remain unchanged. Medical practice is a moral activity based on trust between the patient and the doctor. Regardless of the healthcare model, physicians must prioritize patient welfare above all other interests, respect patient privacy, and ensure the continuity of care (Chaet et al., 2017).

Telemedicine has been shown to enhance the continuity and quality of healthcare services (Darkins et al., 1996). It holds significant potential for globally delivering healthcare services by reducing diagnostic variability and improving access, efficiency, and cost-effectiveness (Craig, 2005).

In addition to saving time and workforce, the quality of healthcare services is enhanced through telemedicine applications. Communication, consultation, and continuous training between major urban health centers and rural healthcare facilities significantly reduce medical errors. Telemedicine encompasses meeting the need for specialists in interpreting patient examinations, ensuring accurate and prompt diagnosis in complex cases, providing easy access to patient data, remote patient monitoring, and offering remote education and research opportunities to healthcare personnel. The result of these actions is cost-effectiveness within the scope of telemedicine (Paksoy, 2017).

Advantages and Disadvantages of Telemedicine

Telemedicine applications are stated to reduce unnecessary hospital admissions. They also decrease the costs associated with hospital transfers and medical care, facilitate access to care, provide continuous support to patients and their family members, enhance the chances of living in a home environment, improve access to various healthcare professionals for patients in rural or remote areas, reduce waiting times for appointments, and prevent infection-related issues encountered in hospital environments and waiting areas (Botsis and Hartvigsen, 2008).

Despite the many defined benefits, there are some barriers to using telehealth from the perspective of the elderly. Older individuals tend to adopt this new form of healthcare relatively slowly, and they have limited confidence

in successfully using new technologies. Elderly individuals surrounded by friends and family members who use telemedicine are more likely to adopt this application. Additionally, trying out these applications with the recommendation of their physicians seems more feasible. An important point to consider is that the elderly want to perceive telehealth as safe and reliable; they need to believe that their personal health information will be kept private and secure.

1. Applications Within the Scope of Telemedicine

In today's rapidly advancing technological landscape, we observe the widespread use of artificial intelligence applications in various fields. In the upcoming period, an increase in the use of artificial intelligence in the healthcare sector is expected (Taş, 2022). Telemedicine applications have become increasingly prevalent, especially in areas such as neurology, cardiology, psychiatry, pediatrics, where the number of specialists is insufficient, or specialists are unevenly distributed geographically. Various studies are being conducted to enhance the usability of artificial intelligence in telemedicine applications with the growing prevalence of artificial intelligence in various fields. Trends in telemedicine usage focus on areas such as patient monitoring, health information technology, smart assistant diagnostic aid, and information analysis. Artificial intelligence encompasses various areas such as automatic diagnosis and treatment recommendations, image recognition, and interpretation. Artificial intelligence and deep learning algorithms provide rapid analysis, guiding healthcare professionals and making significant contributions to the diagnosis of diseases (Mansur and Aydın, 2021).

1.1. Telepsychiatry

Telepsychiatry applications provide a suitable alternative for mental health services that are currently available and highly inadequate. The primary factor driving the advancement of telepsychiatry is the inability of the number of expert physicians working in the field of psychiatry, especially child and adolescent psychiatrists, to meet the demand (Bal et al., 2015). Telepsychiatry is used for direct clinical case management, education, consultation, and supervision. In the 1950s, doctors from the University of Nebraska Psychiatric Institute used video conferencing for the first time to provide consultation, group therapy, long-term therapy, and medical student education between the psychiatric institute and the state hospital (Chen et al., 2020). Some patients report feeling more comfortable, open, and honest, especially when discussing difficult topics through telepsychiatry, as the virtual space of the session creates

a sense of protection. Another advantage of telepsychiatry is the opportunity for special patients such as immigrants, refugees, and asylum seekers to receive psychiatric help in their native languages without the need for an interpreter (Di Carlo et al., 2021). Concerns exist about the suitability of telepsychiatry for some patients with psychotic symptoms or a risk of self-harm. Additionally, patients with auditory, visual, or cognitive impairments may not be suitable for telepsychiatry applications (Cowan et al., 2019).

1.2. Teledermatology

Teleradiology emerged as one of the pioneering branches of telemedicine in its early years. Generally accepted, teleradiology involves the electronic transmission of medical images and related information from one geographical location to another. The increasing demand for radiology services and the growing workload posed a significant challenge. The most significant advantage of teleradiology has been its ability to facilitate workflow, offering the crucial benefit of working independently of location. Other advantages include the ability to provide faster reports and obtaining consultations in emergency services or cases requiring subspecialty expertise (Şenol, 2021).

1.3. Teledermatoloji

The use of telemedicine in dermatology is referred to as "teledermatology." Teledermatology is an alternative examination method that allows the diagnosis or evaluation for a second opinion, with the aid of clinical information, through the digital or video camera images of patients at a certain distance without traditional face-to-face patient examination. This can be done using either the "live video-conferencing method" or the "store and forward method" in a computerized environment (Warshaw et al., 2011).

1.4. Telepathology

Telepathology is a remote pathology application that transmits macroscopic and/or microscopic images using telecommunication connections. This communication tool can be used to establish remote diagnoses, consult with experts in the field, share knowledge and experience, or facilitate collaborative work among pathologists working in geographically distant locations (Weinberg, 1996).

1.5. Remote patient monitoring

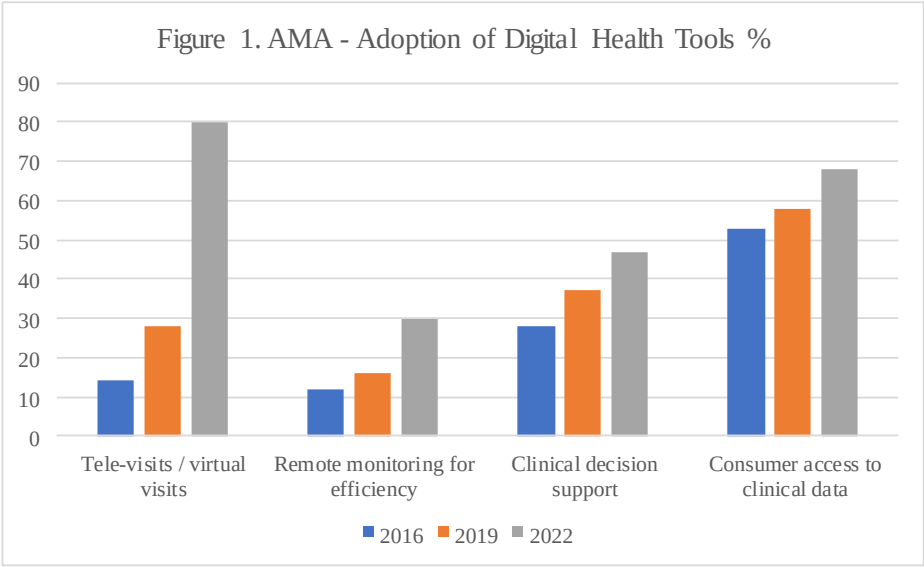
Thanks to the monitoring systems developed in the field of healthcare, the dependence of patients on hospitals has significantly decreased, allowing them to continue their normal life processes at home. This leads to a reduction in the

overcrowding experienced in hospitals, and problems such as insufficient personnel, limited bed numbers, and hospital-acquired infections can be prevented. Additionally, hospital expenses are eliminated. For these reasons, providing healthcare services outside of healthcare institutions has become a necessity (Küçüköner and Yavuz, 2016).

2. American Medical Association (AMA) 2022 Telehealth Data

Between 2016 and 2022, the AMA has released the report results of three regular intervals of telehealth studies. Here are some of the data:

- The percentage of physicians who believe that digital health tools are an advantage for patients increased from 85% in 2016 to 93% in 2022, regardless of age or specialization.
- The average number of digital health tools used by a physician increased from 2.2 in 2016 to 3.8 in 2022.
- While the percentage of physicians using teleconsultation was 14% in 2016, it rose to 80% in 2022. The percentage of physicians using remote monitoring devices increased from 12% in 2016 to 30% in 2022.
- More than 80% of participants (with a 12% increase from 2016) indicate better access to care since patients started using telehealth.
- 62% of participants believe that patients have experienced higher satisfaction since the introduction of telehealth.
- 44% of participants stated that telehealth has reduced healthcare costs.
- Pathology, Radiology, and Psychiatry have been the top three specialties in overall telemedicine usage.
- Since 2019, there has been a general increase in the adoption of all digital health tools, with the most significant increase occurring in teleconsultations.
- In teleconsultations, 80% of doctors prefer the clinic, while 95% of patients prefer home environments.
- The areas of use for telehealth services are 77% for treatment and care, 72% for diagnosis, screening, and assessment, 53% for continuous monitoring, and 41% for triage.
- The most commonly used telehealth platforms are reported as voice and video phone calls and the Zoom application.



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CONCLUSIONS

The rapid development of communication technology in today's world enables people to communicate with each other more quickly and effectively. In this context, telemedicine applications enrich the communication experience by offering users various communication options such as instant messaging, voice, and video calls. One of the most significant advantages of telemedicine applications is that messaging and calls take place over the internet, allowing users to communicate with each other from any point worldwide. Additionally, telemedicine applications are often free or low-cost, helping users to maintain their budgets. These applications also play a crucial role in the business world. Employees can easily communicate, collaborate, and effectively manage project management through instant messaging and meeting tools, thereby accelerating business processes and increasing efficiency. However, with the increasing use of telemedicine applications, some security concerns arise. Particularly, careful attention should be paid to the privacy of personal data and the security measures of the applications. In conclusion, telemedicine applications have gained a significant place in the modern communication world and come with many advantages. Nevertheless, using these applications correctly and securely will enable users to make the most of these benefits.

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Chapter 10

Epigenetic Alterations in Food Allergy

Meliha Merve HIZ ÇİÇEKLİYURT[1]

INTRODUCTION

Food allergy is an adverse immunological reaction to a specific food protein. It is crucial to differentiate food allergy from other non-immune-mediated adverse reactions to foods. More than 20% of both adults and children change their diet due to perceived food allergies (Waserman & Watson, 2011).

Food allergy is defined as an unanticipated reaction of the body to a foodstuff or foodadditive following oral ingestion which can result unexpected syptoms. Allergic reactions can originate from a variety of immunological mechanisms, either immunoglobulin E (IgE)-mediated, non-IgE mediated, or a combination of both.

Food allergies mediated by IgE typically occur shortly after ingestion and may cause symptoms such as hives, skin redness, vomiting, and in severe cases, anaphylaxis. These symptoms are caused by the release of mediators resulting from cross-linking of antigens to mast cell-bound IgE antibodies, leading to mast cell degranulation (Calvani et al., 2021; Mastrorilli et al., 2023).

In contrast, non-IgE-mediated allergies have a delayed onset and typically cause reactions in the gastrointestinal tract (Anvari et al., 2019). The mechanism for non-IgE allergic reactions primarily involve T lymphocytes and show symptoms after consumption of the allergenic food within a time frame ranging from one hour to seven days[3]. Mixed-type allergic reactions combine symptoms from both IgE and non-IgE-mediated reactions.

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Figure 1 Symptom of food allergies

Could food trigger our allergies epigenetically?

Certain food substances can cause epigenetic modifications that influence immunomodulation and, in turn, affect mast cell function, potentially triggering allergic reactions (Cañas et al., 2021; Wu et al., 2023).

Basicly, epigenetic modulations comprise DNA methylation, histone modifications, and non-coding RNA modifications. Among MicroRNAs (miRNAs) are small non-coding RNAs that play a significant regulatory role in the post-transcriptional control of gene expression.

DNA methylation and Allergy

DNA methylation is a crucial epigenetic mechanism that plays a vital role in various cellular processes, including, chromosome stability regulation of transcription and chromatin structure, X chromosome inactivation together with embryonic development, and genomic imprinting. Due to its involvement in such critical processes, meticulous control of methylation is necessary. Improper functioning of the epigenetic control system is often at the root of many diseases. Improper establishment or maintenance of epigenetic modifications may lead to pathological consequences associated with these processes (Robertson, 2005; Rodenhiser & Mann, 2006)

DNA methylation is a widely studied epigenetic modification tightly regulated during haematopoiesis (Martino et al., 2014; Trowbridge et al., 2009). DNA methylation occurs in regions of the genome where CpG sequences are concentrated. Only about 10% of these sequences are found in regions called

CpG islands, which are larger than 500 base pairs and have a GC content of more than 55%. Conserved CpG islands are typically located in gene promoter regions, particularly in housekeeping and regulatory genes that require continuous expression. These regions are resistant to DNA methylation. In contrast, CpG sequences in heterochromatin regions, such as repeated sequences and transposons, have a high rate of DNA methylation. Methylation of these regions represses transcription and maintains chromosome stability by preventing the movement of these elements within the genome (Smith & Meissner, 2013; Takai & Jones, 2002).

DNA methylation determines which genes will be expressed in specific cells. It involves the addition of a methyl group of cytosine to DNA, which usually results in the silencing of the gene of interest. Methylation in promoter regions, particularly CpG islands, is the most studied aspect of DNA methylation.

The relationship between promoter region methylation and gene silencing was first described in the 1970s. Methylation of CpG islands in promoter regions was found to have a repressive effect on gene expression, shaping the general perception of the function of DNA methylation. However, the relationship between DNA methylation and gene silencing remains unclear. The developing genome-wide methylation mapping has enabled the analysis of DNA methylation in transcription initiation sites, exon and intron regions, regulatory regions and repeated sequences (Jones, 2012).

Martino (2014) examined DNA methylation profiles on CD4+T cells of 12 allergic and 12 non-allergic children with IgE-mediated food allergy diagnosed in the twelfth month. In the DNA methylation process, they stated that differential methylation was associated with gene expression profiles and allergy. As a result, early CD4+T cell development was found to be dysregulated in DNA methylation by MAPK signalling and reported to be effective in the development of food allergy (Martino et al., 2014). Later in 2017, Weronica et al. conducted a study at the epigenome level to clearify the relationship between DNA methylation and IgE levels, in 728 allergic individuals; and identified 15 CpG regions associated with IgE, which are linked to important genes such as ACOT7, ILR5A, KCNH2, PRG2, and EPX(Ek et al., 2017). In addition, they found 331 loci associated with allergen-specific IgE. However, none of these CpG regions could be linked to self-reported allergies or immune diseases.

Histon Modifications and Allergy

Histone modifications are chemical alterations, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation, that occur in the structure of histone proteins. These modifications are responsible for packaging DNA and are among the epigenetic mechanisms that regulate gene expression by changing the chromatin structure. For instance, chromatin has an open structure that is favourable for transcription due to the reduced affinity of acetylated histones for DNA. Histone methylation more complex process that involves the methylation of specific amino acid residues and varying degrees of methylation. While lysine 9 methylation at the N-terminal end of histone H3 is a silent DNA indicator, lysine 4 methylation is found in the promoter region of active genes.

Histone modification has been shown to play important roles in immune cells which cause allergical reactions among airway smooth muscle cells, lung epithelial cells, and fibroblasts (Alaskhar Alhamwe et al., 2018).

MicroRNA's and Allergy

MicroRNAs (miRNAs) are small RNA molecules, approximately 18-25 nucleotides in size, that regulate gene expression post-transcriptionally. miRNAs regulate gene expression by recognising sequences of the same origin and interfering with transcriptional, translational or epigenetic processes (Chen et al., 2019). The study of miRNAs in allergy is accelerating because miRNAs are critical modulators of gene expression and potential biomarker candidates (Ogulur et al., 2021; Pattarayan et al., 2018; Sastre et al., 2017; Specjalski & Jassem, 2019). Due to their potential to regulate the immune system and cause tissue inflammation in allergic diseases, MiR-21, miR-146a and miR-155 are the most studied miRNAs. (Hervé et al., 2023; Rebane, 2015). By acting through target genes to regulate gene expression networks, miRNAs affect various aspects of immune cell function that are critical for type 2 immune responses(Weidner et al., 2021) . These facets include cell survival, proliferation, differentiation, and effector functions (Pua & Ansel, 2015).

RESULTS

The current evidence and previous research has led us to focus on DNA methylation status in allergy and asthma, particularly in relation to T cell immune responses. This may help us understand the role of methylation levels of specific genes in the occurrence of allergic reactions and the development of diseases such as asthma (Alaskhar Alhamwe et al., 2018)]. This may lead to a better understanding of the impact of epigenetic regulations on disease pathophysiology and the development of future treatment or diagnostic methods.

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Chapter 11

Evaluation of Cancer Microarray Data by Artificial Intelligence

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Introduction

Cancer is a malignant cell formation caused by genetic changes, which is the result of cells destroying the main cellular control mechanisms, multiplying independently and living and spreading according to the needs of a single type of cell in central. Cancer's seriousness is emphasised by its rank as the second most common cause of death globally, after cardiovascular diseases(Miller et al. 2021).

Updated statistics on cancer incidence and outcomes are prompting new studies, focusing mainly on the cause and cure of cancer. The comparability of incidence rates enables us to identify the risk factors, to plan and prioritise cancer control resources, and to monitor and evaluate the impact of specific primary prevention.

The massive amounts of data from health authorities, hospitals and laboratories with clinical data need to be evaluated more comparatively to find a cure for cancer. In contrast, none of the clinal or epidemiological data were helpful to identify the neoplasms mechanism. Yet, gene expression analysis enables comprehension and intervention in cell activity at a genetic level, particularly in tackling challenges posed by cancer diagnosis, treatment, and drug discovery(Munkácsy, Santarpia, and Gy\Horffy 2022) , (Brewczyński et al. 2021). Furthermore, this analytical approach can enhance the creation of more efficient therapies by aiding comprehension of the role of various genes in cancer initiation and progression. Accordingly, changes in gene expression can function as critical indicators for early cancer detection and identification of tailored treatment methods. Therefore, gene expression analysis holds the potential to offer more personalized, preventative, and predictive healthcare services in the battle against cancer.

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Misregulation of gene expression is significant for comprehending biological variations between disease and healthy states. In this context, transcriptome analyses of the illness play a pivotal role in identifying pathogenesis and contributing to the identification of a disease-specific target pathway or drug. Gene expression analysis is utilized to conduct a methodical evaluation of genetic alterations that arise within a cell or tissue under specific circumstances. This analysis supplies data concerning which genes are expressed and to what extent, such as through quantifying the quantity of DNA transcripts within a tissue or cell.

The employment of microarray analysis is achieving momentum in understanding and countering this intricate condition. At least on a genetic or epigenetic basis, artificial inteligence enables comprehension of high-throughput DNA sequencing data(Shendure and Ji 2008). RNA sequencing (RNA-seq) has also adopted these sequencing technologies, allowing for the identification and quantification of various RNA populations, such as mRNA and total RNA, related to gene expression. RNA-seq has revolutionised biomedical research by enhancing researchers' potential to analyse a diverse array of biological data (Kukurba and Montgomery 2015).

RNA-seq analysis enables the probing of genes and their corresponding transcripts with the aim of detecting new exons or entire transcripts, evaluating gene expression and alternative transcripts, and examining alternative splicing structures. This method allows for identifying new candidate drugs or unresolved points in the pathogenesis of diseases. RNA-Seq is an NGS method characterized by its ability to determine the nucleotide sequences of RNA molecules and offers greater specificity, resolution and sensitivity compared to DNA microarrays. Furthermore, RNA-Seq is a versatile tool that can be used to determine the amount of RNA at a given time and to study the transcriptome.

How to Performed MicroArray?

Microarray data are provided by means of tools containing a two-dimensional array obtained by laboratory technique. These tools are often referred to as chips or slides and each spot is reserved for a single DNA sequence or gene. DNA samples are bound to the microarray slide by hybridisation and the expression of each gene is measured by colour scanning. In microarray data, rows represent gene expression levels and columns represent samples. Microarrays can be used to identify translatable or non-translatable DNA or RNA, thus contributing to the understanding of cellular processes by correlating genome-wide expression profiles with

specific conditions or diseases. It also provides important information for the discovery of new drugs in pharmacogenomics research and therapeutic drug development. While the advantages of microarrays include the ability to measure thousands of genes simultaneously, there are also limitations such as relatively low accuracy, precision and specificity, and sensitivity to changes in hybridisation temperature, purity of genetic material; all these factors can affect gene expression. Figure 1 shows the experimental protocol of the microarray data.

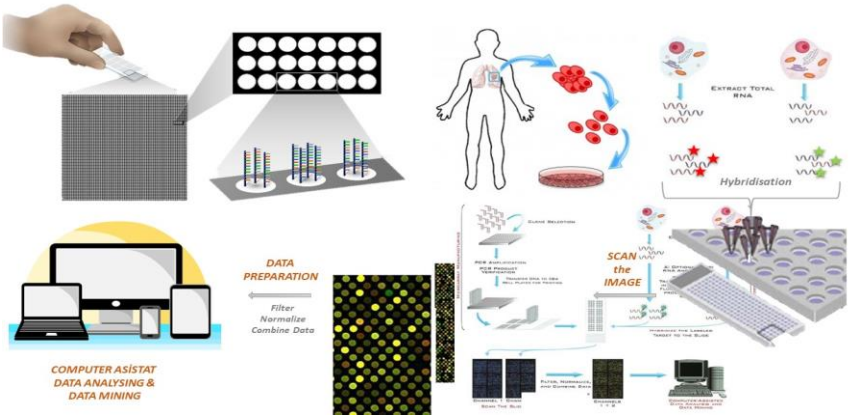

Figure 1. Microarray experiment protocol(Adapted from Macgregor and Squire 2002).

In experimental studies, databases that share microarray data provide an important resource in gene expression analysis and bioinformatics studies, offering researchers a large data pool. These databases contain gene expression profiles from different organisms and various conditions, giving scientists the opportunity to study biodiversity and condition-specific gene expression changes. Gene Expression Omnibus (GEO), a platform managed by the National Centre for Biotechnology Information (NCBI), hosts a large data repository containing gene expression, microarray and genome chip data. ArrayExpress is another important resource operated by EMBL-EBI and hosts microarray data from various biological samples. The Cancer Genome Atlas (TCGA), which focuses on cancer research, is known for containing comprehensive cancer genome data for gene expression analysis. These databases support research and discovery processes in the scientific community by providing a shareable and accessible infrastructure, enabling large-scale gene expression analyses.

Machine Learning In MicroArray Data Evaluation

The use of machine learning on microarray data is very important. Microarray data usually refers to data measured over a large number of small time intervals. This data is usually in the form of time series and can be found in many fields, such as sensor data, financial market data or health monitoring data. Machine learning is a powerful tool for analysing such data and predicting future events. Machine learning techniques such as time series analysis, regression, classification and clustering can be applied on microarray data. For example, in a healthcare application, using a person's heart rate, blood pressure and other biometric data, we can make predictions about that person's health status. In financial markets, predicting future price movements by analysing microarray data can provide strategic advantages to investors. In addition, applications such as detecting equipment failures in advance and optimising maintenance processes by analysing sensor data in production facilities are also possible. Machine learning algorithms can recognise patterns in microarray data and predict future events using these patterns. However, working with this type of data often brings some challenges. Factors such as data size, noise, missing data and time dependency can affect machine learning applications. Therefore, correct model selection, data preprocessing and model training processes are of great importance.

In addition to traditional methods for microarray target prediction, machine learning methods have been developed to analyse data that enable rapid evaluation of biological data and cannot be obtained by classical methods. When computer science is used in the evaluation of biological data, the understanding, evaluation and target prediction of microRNAs becomes more effective. Many tools are available for microRNA target prediction, and most of these tools work using machine learning techniques. Machine learning is an application of artificial intelligence that provides systems with the ability to automatically improve on their own experience. This method extracts and learns information from sample data sets and uses this information to make predictions on unknown data points. The dataset obtained from microarray research is not suitable for direct use and preliminary steps are performed on these data with machine learning technologies. Microarray-based microRNA target prediction is usually performed as shown in Figure 2.

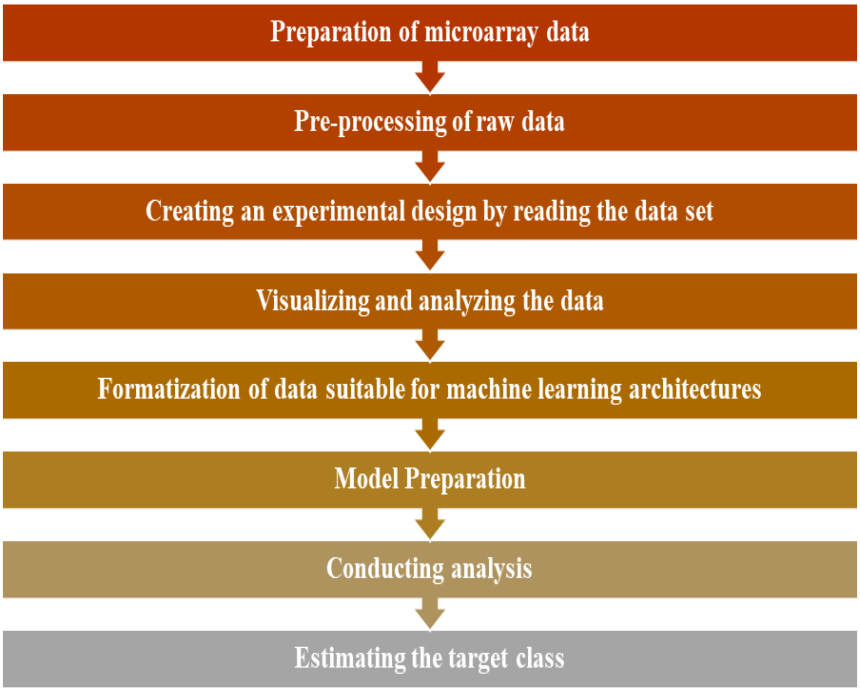

Figure 2. Microarray analysis process

There are many tools and programming languages used for bioinformatics analyses, with a choice of both paid and free ones. The most widely used tools in this field are usually open source and free. Some free tools that are frequently preferred in bioinformatics applications are as follows:

BioPython: It is a library based on the Python language and supports a wide range of functionality for bioinformatics applications. It can perform various analyses on DNA, RNA and protein data.

Bioconductor: An open source project focusing on the R programming language. It includes a number of packages for microarray data, genomic analyses and other bioinformatics applications.

BioJava: Another library based on the Java programming language and used to perform various operations on bioinformatics data.

Python: It was created in 1991 by Dutch software developer Guido van Rossum. Python's simple language structure makes it easy to learn and code. In addition, the indented code writing scheme simplifies the understanding of the language by increasing the readability of the code. Python's interpreted code structure and operating cross-system compatibility makes this language an ideal choice for rapid application development.

R/Studio: The R language is a programme specifically developed for statistical calculations, graphics and data analysis. The language was created by Robert Gentleman and Ross Ihaka in the Department of Statistics at the University of Auckland, New Zealand. The R language allows complex mathematical problems to be easily solved on software. Thanks to its simple structure, the R language is very easy to learn. This feature allows mathematicians and statisticians to quickly create their own algorithms on the R language. R language is not a package programme, it is a software development platform. Those who want to do programming using the R language can access the R language and R Studio tool for free. The R language and R Studio facilitate programming processes by providing users with a unique software and interpretive environment.

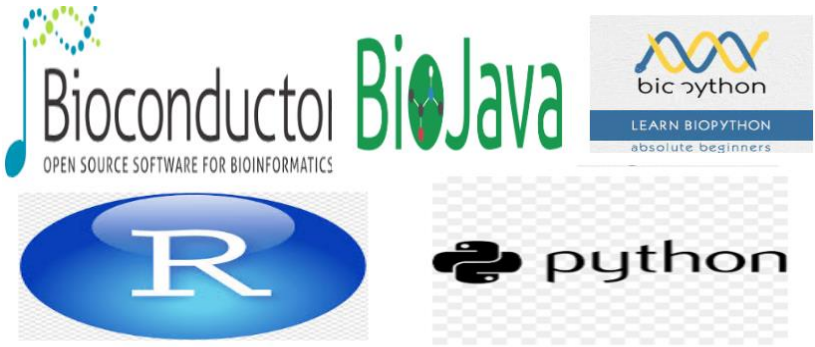

Figure 3. Bioinformatics programming

What we known About Cancer by AI

In this section, studies in the literature on applications on microarray data are presented. When we look at the literature, it is observed that analyses have been made on many diseases using bioinformatics and machine learning. Kılıçarslan and Dönmez (2023) reported a machine learning based analysis for disease detection using eight microarray datasets of liver, lung, kidney, pancreas, prostate, breast, colorectal and brain tumours. In the study, firstly, the most descriptive features in the dataset are selected and the size is reduced. In order to increase the classification success, the new dataset was classified using machine learning methods such as support vector machine (SVM), an artificial neural network (ANN) and the nearest neighbour (k-NN) algorithm(Kiliçarslan and Dönmez 2023).

Akalın and Yumuşak (2023) used a microarray dataset to accurately and efficiently distinguish ALL, AML and MLL leukaemia types. The first step

of the study is to adopt the whale optimisation algorithm to reduce the computational cost on the microarray dataset, which has a multidimensional structure, and to quickly reach the most accurate result. This algorithm was used to identify potential disease-associated genes in the dataset. These selected specific genes were then classified using the LSTM neural network architecture. This approach provides a simple hierarchy and low computational complexity. As a result of the classification, 100% success was achieved compared to the existing approach(AKALIN and Yumuşak 2023).

Lee et al. (2003) reported that they performed a machine learning-based analysis for the detection of the disease using gene expression data from blood cancer microarray dataset. In the study, they performed multiple classification with the help of support vector machines, which is a machine learning method(Lee and Lee 2003).

Haznedar et al. (2017)liver microarray gene expression data related to cancer were classified using an Adaptive Neuro-Fuzzy Inference System (ANFIS) with Backpropagation (BP), Hybrid algorithms, and Genetic Algorithm (GA) optimization techniques. The performance of these algorithms was compared, revealing that the GA-based approach for training ANFIS yielded greater success. (Haznedar, Arslan, and Kalınlı 2017).

Liu et al. (2002) performed a comparative performance analysis of classification methods such as k-nearest neighbour, naive bayes, support vector machines and C4.5 decision tree by taking microarray profiles from Acute Lymphoblastic Leukaemia (ALL) patients and proteomic microarray patterns from ovarian cancer patients (Liu, Li, and Wong 2002).

Kılıçarslan et al. (2019) performed a machine learning-based analysis for disease detection using a prostate cancer microarray dataset. In the study, firstly, genes that will facilitate the detection of the disease were selected from a very high-dimensional microarray dataset, and then classification was performed using machine learning methods, Support Vector Machine and k-Nearest Neighbourhood classifier methods(KILIÇARSLAN, Kemal, and Cömert 2019).

Turgut et al. (2018) applied machine learning methods for disease detection using breast cancer microarray dataset on two different microarray breast cancer datasets and performed data classification. Random logistic regression and iterative feature elimination feature selection methods were used for high accuracy cancer diagnosis. After applying two different feature selection methods, support vector machines performed best on two microarray breast cancer datasets (Turgut, Dağtekin, and Ensari 2018).

Furey et al (2000) used support vector machines to classify cancer tissue samples using microarray expression data of ovarian cancer tissue and ovarian normal tissue. The main challenge in detection is that microarray datasets represent thousands of genes, both related and unrelated to cancer. Therefore, this study demonstrates that accurately detecting cancer on microarray cancer data is a challenging process (Furey et al. 2000).

The above literature review showed that the analysis of microarray data obtained from cancerous tissues utilized data mining techniques and showed the detection of cancer type.The main difficulty in detection is that microarray data sets represent thousands or even millions of genes, both related and unrelated to cancer. Therefore, it has been revealed by examining the above studies that it is a difficult process to accurately detect cancer through microarray cancer data. First of all, it has been observed that meaningless genes are removed from the data set by using feature selection, i.e. gene selection algorithms from cancerous microarray data. Then, studies have been carried out to detect cancer by using machine learning techniques with the obtained meaningful very clusters.

CONCLUSİON

Analysing microarray data with machine learning aims to understand genetic expression profiles and gain important insights in biomedical research. Studies in this field provide valuable information for deeper understanding of various cancer types, gene expression profiles and cellular processes. The use of machine learning algorithms is an important step towards addressing the complexity and high-dimensional nature of microarray data. Deep learning models, especially methods such as multilayer perceptrons, convolutional neural networks and recurrent neural networks, have attracted attention for their ability to extract meaningful patterns from gene expression data and achieve high success in complex tasks such as cancer classification. The results obtained from these studies show that gene expression profiles can play an important role in the diagnosis and prognosis of diseases, the development of therapeutic strategies and the adoption of personalised medical approaches. Among machine learning applications, methods such as k-Nearest Neighbour (kNN), Support Vector Machines (SVM), Naive Bayes, Deep Linear Models (DLM) and Convolutional Neural Networks (CNN) stand out. This diversity offers the flexibility to choose appropriate methods for different data sets and analysis requirements. As a result, the analysis of microarray data with machine learning adds a new dimension to biomedical research and reveals

the potential for significant progress in understanding and managing genetic-based diseases. Limitations such as relatively low accuracy, precision and specificity emphasise the need for improved methodologies in future research. Future directions include the use of larger and more diverse datasets, the integration of different machine learning algorithms, and the development of open-source tools. Furthermore, further integration of research in this area into clinical applications could be an important step towards early diagnosis of genetically based diseases and more effective treatments. The impact of machine learning algorithms on the analysis of microarray data is very important.

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Chapter 12

Health Problem Pre-Application System Recommendation for International Transportation Personnel and Passengers and Evaluation of The Proposed System

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Bengisu OLGUN BEKMAN[2]

1. INTRODUCTION

Workers of the international transportation sector, passengers and other personnel in these vehicles may need to receive health care in their own country or in foreign countries. Many problems were identified (Gonel, 2023) and possible problems were predicted with academic methods related to achievement of proper health care. A system and a program as the core of the system is proposed in order to eliminate these problems and bring the health service received to a better level. Also, specifications of this program have been identified.

Main aim of the system is to create a health problem statement in order to request proper health care. An international and uniform statement that is free from all possible failures will provide better understanding of the health problem of the patient. Better understanding of the problem will lead to directing the patient to the appropriate healthcare service. The system in question was developed for the benefit of personnel working in the international transportation sector, passengers and other people in these vehicles. It is often a problem for patients for to reach healthcare services during the voyages. But, since the system eliminates language problems, it can be used by a much wider audience such as tourists, immigrants and refugees. And due to the additional benefits of the system, it can be used even in cases where patients and healthcare personnel speak the same language.

The experienced and possible problems and the security barriers of the system proposed to be developed were compared with the Swedish Cheese Model (SCM). Results of the study revealed that barriers of the system prevent all failures.

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1.1 Aim of the Study

A passenger, crew member or another person may get into a situation that requires them to receive health care during a voyage in a transportation vehicle. In a situation like this it is necessary to report to the authorities by the responsible personnel or travelers to ensure that health care is received. The thing to be done by the responsible personnel of these vehicles or other people is to report this situation to the necessary authorities and ensure that health care is received. If we consider that this incident took place on a ship,

- A report will be prepared
- The report will be sent to the relevant authorities
- The report will be evaluated
- Efforts will be made to arrange the most appropriate health service according to the conditions.

The most appropriate healthcare service will be arranged based on the assessment and circumstances (refer to Sections 3.1 and 3.2 for details). The aim of this study is to ensure that all patients' complaints are fully understood and that they reach the most appropriate healthcare service as soon as possible.

1.2 Determined Problems

This section includes the problems experienced by patients who may or may not speak the same language as the healthcare personnel or who try to receive healthcare services with an interpreter. The term ‘problem’ refers to ‘failure’ in SCM.

Table 1. Problems Encountered in Medical Translations (Gonel, 2023)

No	Determined Problem
1	Incomplete and/or inadequate translation carried out by interpreter
2	Incorrect translation carried out by interpreter
3	Translation errors occurred when the interpreter is not qualified to act as a professional medical interpreter
4	Translation errors caused by the use of digital translation platforms
5	Difficulty in accessing the internet or online translator (If online medical translator is to be used)
6	The patient being in a state that he cannot express himself (due to pain, suffering and similar reasons)
7	The patient being in a unconsciousness state
8	Inability of the patient and the health personnel to speak the same language

9	The patient's limited ability to speak the same language as the medical staff but inability in speaking about medical issues
10	The patient that can verbally express himself may not able to read the patient forms written in a complicated medical format.
11	Unavailability of failure to access previous health records of the patient
12	Misunderstanding and or inability to understand the patient's previous health records due to language difference

Problems 1, 2 and 3 in Table 1 are the problems experienced during the translation of the patient's health problems to the healthcare personnel by a person who acts as an interpreter. In Articles 1 and 2, these are situations where a person who undertakes the task of interpreting and is not a professional translator (such as the patient's relative, another seafarer) conveys the patient's complaints incompletely, inadequately or incorrectly. Problem 3 is the translation of the patient's health complaints by a professional translator whose expertise is general translation but not medical translation. In this case, the interpreter may not be able to translate medical terms and complex sentences related to medicine. These problems have been observed to occur if a professional medical translator is not used, and it is possible that they may occur again in the future.

Problems Number 4 and 5 are problems that occurred as a result of translation of the patient's complaints over the internet. The difference is that problem 4 is about the use of internet-based general translation programs and problem 5 is about use of online medical translation services accessed over the internet. To access both types of services, it is necessary to have an internet connection, computer, tablet and similar equipment. For problem 5, additional equipment such as microphone, speaker and preferably a camera are is required. Translation services from digital translation platforms can be obtained from any point connected to the internet, but former studies Gonel (2023) showed that, a sentence used to explain a medical complaint was translated on 7 different websites and 5 different translations were encountered. It should be noted that online medical translation service is not always available and may have some additional difficulties.

Problems 6, 7, 8, 9 and 10 are the health problems experienced when the patient communicates one-on-one with the healthcare personnel without using an interpreter. It may be the case that the patient does not speak the same language and cannot communicate with the healthcare personnel (problem 8). Even though the patient and the healthcare personnel can speak the same language, the patient being in an unconscious state (problem 7) or inability to communicate due to extreme pain, suffering, shock and similar situations (problem 6) is also possible.

If the patient and healthcare personnel speak the same language at a limited level, some communication can be established, but problems may arise regarding the details (problems 9 and 10).

There are also problems regarding the submission and translation of records describing the patient's past health status and may have negative effects on the patient's treatment (problems 11 and 12).

1.3 Anticipated Problems

The patient cannot be expected to know the information that healthcare personnel need in order to make an accurate preliminary assessment. The information that the patient provides and thinks is important to explain his or her health problem may cause the healthcare personnel to fail to make the correct preliminary assessment (Table 2).

Table 2. Possible Problems to be Encountered in Medical Translations

No	Anticipated Problem
13	Failure to declare required health information when creating a statement/report regarding a health problem

2. METHOD

In this study, it is aimed to ensure all problems are to be avoided. The method used in this study is based on the SCM however, it differs from standard SCM.

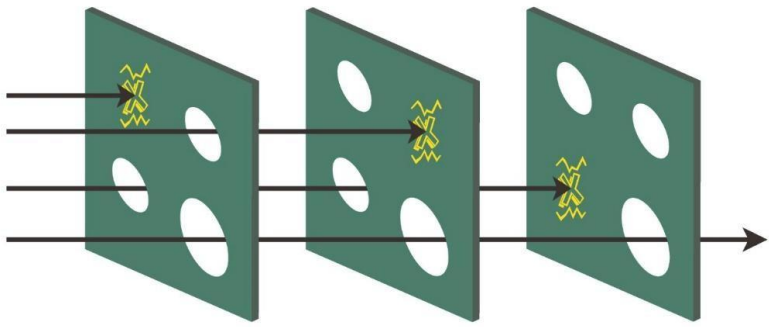

Figure 1. A Basic Illustration of the Swiss Cheese Model

The SCM was first introduced by James Reason in 1990 (Shappell & Wiegmann, 2001). Basically, it is used to reduce or stop all accidents, hazards and any sort of harm. On one side there are hazards/hazard vectors and on one side there are defense layers. Defense layers may not stop all hazards on their

own. But when all defense layers are aligned, hazards can be prevented. The method is generally viewed as being related to accident prevention and modeling. But it is also used to assess risks (Pumar et al., 2023). A basic and standard illustration of the method is shown in Figure 1.

Illustration in Figure 1 is a very generic example of SCM and often used to visually explain SCM. To be more specific and distinguish occurrence and prevention of the failure, Figure 2 and Figure 3 are used. In Figure 2, slices as barriers failed to prevent a failure from happening. However, in Figure 3, although failure managed to overcome several barriers, it was prevented from occurring by another barrier.

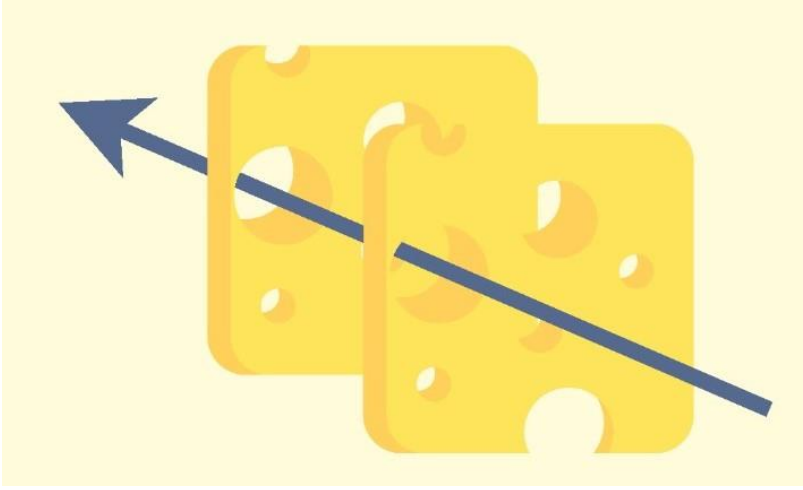

Figure 2. A Failure Occurred

Over time, the method has been developed by researchers and different versions and applications have been created. 'Hot cheese: a processed Swiss cheese model' by Li and Thimbleby (2014) is an example for this version. Model may be both applied as Swiss Cheese (Puthillath, et al., 2021) and Reverse Swiss Cheese (Kirwan, et al., 2022) in different aspects. Since the Swiss Cheese model is explained in detail in many academic studies (Wiegmann et al, 2021; Feinstein et al, 2022) no additional explanation related to origins of the model is included in this study. But key components of the system are explained in the following subsections.

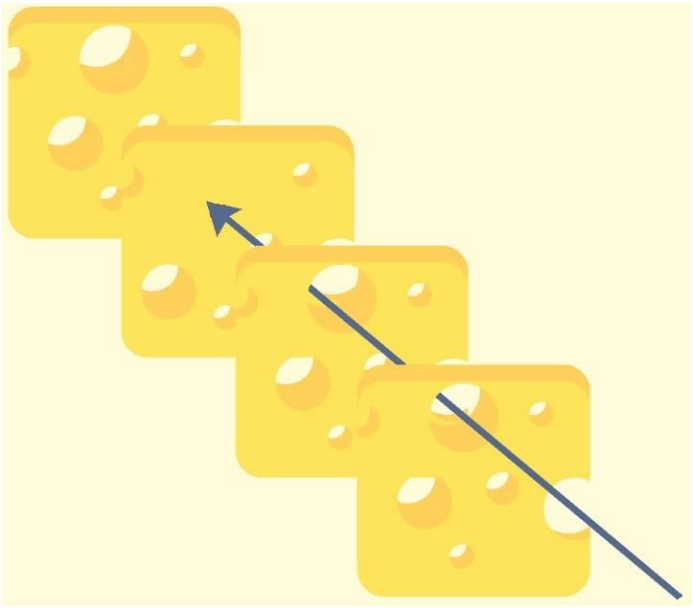

Figure 3. A Failure Prevented

2.1 Eyes and Slices

Any system has defense mechanisms to prevent problems. Generally, the defense mechanism consists of small parts, and these parts also have flaws. The parts of this defense mechanism are identified as ‘slices’ of Swiss cheese, and the defects in the defense mechanism parts are identified as ‘eyes’.

2.2 Active and Latent Failures

In the Swiss cheese method, failures are divided into two as 'active' and 'latent'. Term ‘error’ is used instead of ‘failure’ in some studies. After a certain period of time the model was introduced, the term 'latent conditions' began to be used for a clear definition (Larouzee & Coze, 2020). Active failures are the acts that are leading to accidents in a more direct way. Latent failures on the other hand are more contributory and have less direct effect to accident. An example given by Li and Thimbleby (2014) is, ‘*administering the wrong drug is an active failure, but the latent condition might have been the confusingly similar names of two different drugs*’.

2.3 Some Examples of Swiss Cheese Methods in Academic Studies

Pumar et al. (2023) described slices as homeostatic defenses (age, sex, and hormonal balance and others) and failures as tumor-promoting mechanisms (insults) in their study. Suryoputro et al, (2015) had only one failure in their study

– ‘Train collision’ – and five layers as ‘Fallible decisions’, ‘Line management deficiencies’, ‘Psychological precursors of unsafe acts’, ‘Unsafe acts’ and ‘Inadequate defenses’. As seen in examples, a number of failures and layer may change in different studies and there is no standard in the Swiss Cheese Model.

A very important issue that emerged from the examination of the studies using SCM is that the failures were not divided into 'active' and 'latent' in all studies.

2.3 Method Applied in This Study

In this study, it is aimed to prevent possible failures from occurring rather than analyzing an existing failure or accident. Additionally, barriers specifically created to prevent these failures were used instead of the barriers of an existing system. For these reasons Reverse Swiss Cheese model is applied in this study. Also, failures were not divided into 'active' and 'latent'.

The method was used in Section 4.4 to determine whether the proposed system solves the identified problems.

3. PROPOSED SYSTEM

In this section, the purpose, definition and features of the proposed system are mentioned in order to avoid the problems identified. The current system is also explained here.

3.1 Current System

The system proposed in this study was initially created to find solutions to the problems experienced by seafarers while receiving health care onboard and abroad. It was later modified and developed for use in other international transportation sectors due to the system's advantages. But the existing system is explained and examples are given through seafarers.

If a person needs to receive health care in a normal workplace, the patient reaches the place where he will receive health care either by his own means or by the means of health institutions, depending on the urgency of the health problem. In some cases, health service is brought to the patient. The patient is given the necessary health care or referred to the appropriate place if initial treatment means are not enough. Same system cannot be exercised onboard ships. When a patient requires to receive health care on ships the actions taken are,

- Receiving medical assistance or medical advice services through the Global Maritime Distress Safety System (IMO, 2000),
- For Turkish flagged and other flagged ships with Turkish crew, 'Tele Sağlık' service procurement (T.C. Sağlık Bakanlığı, 2023a),
- Transferring the patient to another sea craft or aircraft (MAIB, 2011),

- Receiving health care when the ship docks at the port (with medical personnel coming on board) (ATSB, 2016),
- Transferring the patient to a shore-based health facility. (MAIB, 2013).

Definitely for the first option and preferably for the second option, communication will be provided by the ship's GMDSS operators. The customary practice for the fifth option is that the patient is accompanied by an officer, preferably with relatively higher foreign language skills (Gonel, 2023). If the ship is under sail, health care is usually requested via a written message, preferably to the ship's agency, port authorities and other relevant parties. In most scenarios for receiving proper healthcare following two are involved;

- Interpreter (or a person transferring health issues),
- A written text.

Current system in a commercial ship is displayed in Figure 4. There are two important problems in the current system. First, when and where the patient will receive health care may not be determined based on sufficient medical data. In this case, it may be delayed for the patient to receive the necessary medical service, and large expenses may be incurred due to misdirection.

The second problem is that the patient may not be properly pre-triaged. Once the patient first reaches medical healthcare, he or she may be referred back to the appropriate healthcare facility. In this case, there will also be a serious loss of time.

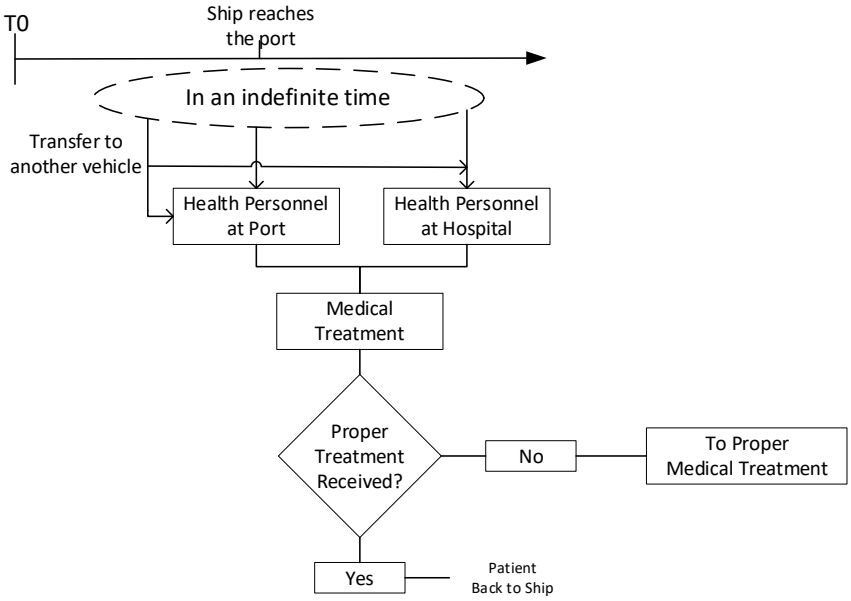

Figure 4. Current System in Commercial Ships

There are online based pre-triage sites already available on the internet. However, the programs used by these sites operate through simple algorithms. Based on the answers given, predetermined results are automatically generated. The patient's health declaration is not determined by medical personnel. Relevant services cannot evaluate a new variant of a known disease, an epidemic disease that has begun to appear in a certain region, or any current situation. In addition, these services work in a single language. A bigger potential problem here is that web browsers translate such pages across languages within their own interfaces. Since such translations are not made by medical translators, it is quite possible for translation errors to occur. 'NeyimVar?' provided by the T.C. Ministry of Health service is one of the best examples of such sites (T.C. Sağlık Bakanlığı, 2023b). The system has detailed visuals and a user-oriented interface. It currently provides services in three languages. It can be thought that there is a similarity between the 'NeyimVar?' and the system proposed in this study. However, 'NeyimVar?' serves for the purpose of guiding the patient, and the system that is the subject of this study is for present the patient's complaints to the healthcare personnel in the most appropriate and accurate way.

There is another version of an online based pre-triage site. These sites provide 'Online Consultant Service' but requires payment. Some of such service providers even provide online consultation by medical personnel. Translation is not included in the service provided in any of the systems examined[3]. A similar service is provided by family medicine physicians in Turkey. Patients can have examinations via video call from their family medicine physicians. In addition, the 'International Patient Support Unit Translation and Call Center' of Health Ministry of Turkey provides health consultancy services in six languages over the phone 24/7 (T.C. Sağlık Bakanlığı, 2019). These services also are not an alternative to the system that is the subject of this study.

3.2 Purpose of the Proposed System

The purpose of the proposed system is to replace the 'written text' described in the previous section (Section 3.1). Proposed system will create a universal health form that will act only as a pre-triage application. Notification will reach the healthcare personnel more quickly and conveniently. In this way, the most appropriate treatment method and place for the patient will be determined and the patient can be directed to the most appropriate health service. The proposed

[3] Some of the services mentioned above are provided by commercial service providers. Publishing permission was not requested from any of such sites that were randomly identified through an internet search. To avoid copyright and similar problems, these sites are not referenced.

system will prevent all errors mentioned in Section 1.2, especially translation errors. It will also ensure that the patient receives health care without an interpreter.

3.3 Area of Use of the Proposed System

The main envisaged areas of use of the proposed system are:

- Personnel working on ships, aircraft, trains and similar transportation sector vehicles
- Passengers and all other persons in these vehicles
- Workers working in foreign countries
- Tourists
- Immigrants, refugees, asylum seekers and those in similar situations
- All patients who need to receive healthcare services - regardless of the language.

Although the system was initially developed for use in places with language differences, it can be used wherever healthcare services will be received. It will direct patients to the right healthcare service and save time and labor. It will eliminate the difficulties experienced when a translator is needed.

3.4 Boundaries of the Proposed System

The proposed system was developed only as a pre-triage application form. This form is intended to be used only for pre-triage and direct the patient to the appropriate healthcare service. This form can neither replace a doctor's examination nor triage service.

3.5 Working Principle of the Proposed System

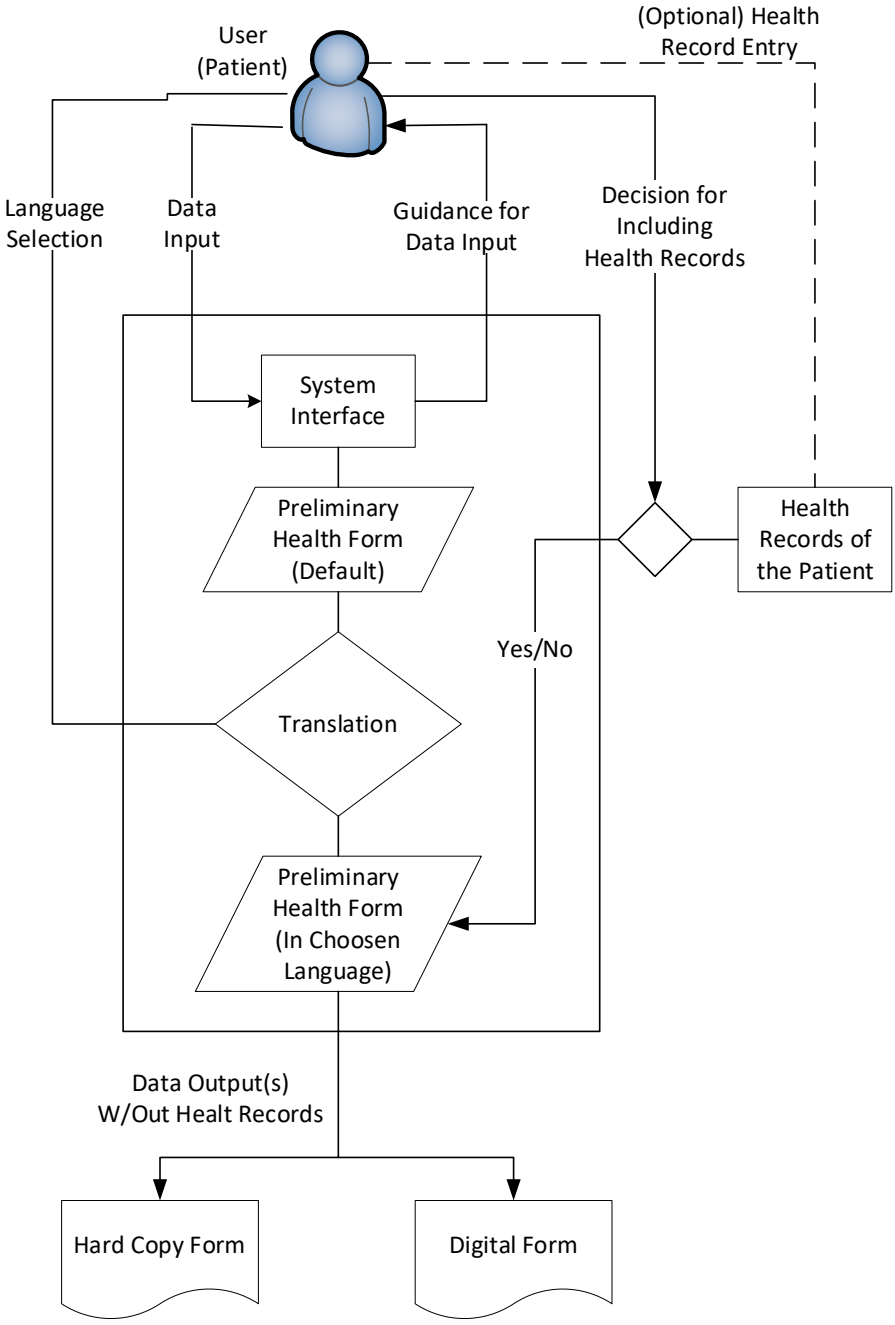

Figure 5. Working Principle of the System

The working principle of the system is explained in detail in Figure 5. The user (patient) logs into the program via a computer, tablet or similar device. A

complaint record begins to be created by following the system's guidance and options. For example, one or more of many options such as 'pain', 'wound' and 'pain' will be selected. The location of the complaint in the body is selected from different human body maps (such as muscle map, bone map). The most important issue at this stage is that the questions that come in order are determined by the physicians and the questions that follow change according to the answers given.

Once all questions are answered, an application draft is created and displayed on the screen for the patient to review. If the patient approves the pre-application registration, the pre-registration will be created and saved on the system. If the patient has entered his past health records into the system at a previous stage or enters them at this stage, he can choose to add his past health records to the health application form at his own discretion.

The patient can translate the record created through the system into the language or languages of his/her choice. He can receive this record digitally in different file types, or he can also print these files if he wishes. All features of the system are explained in detail in Section 4.5.

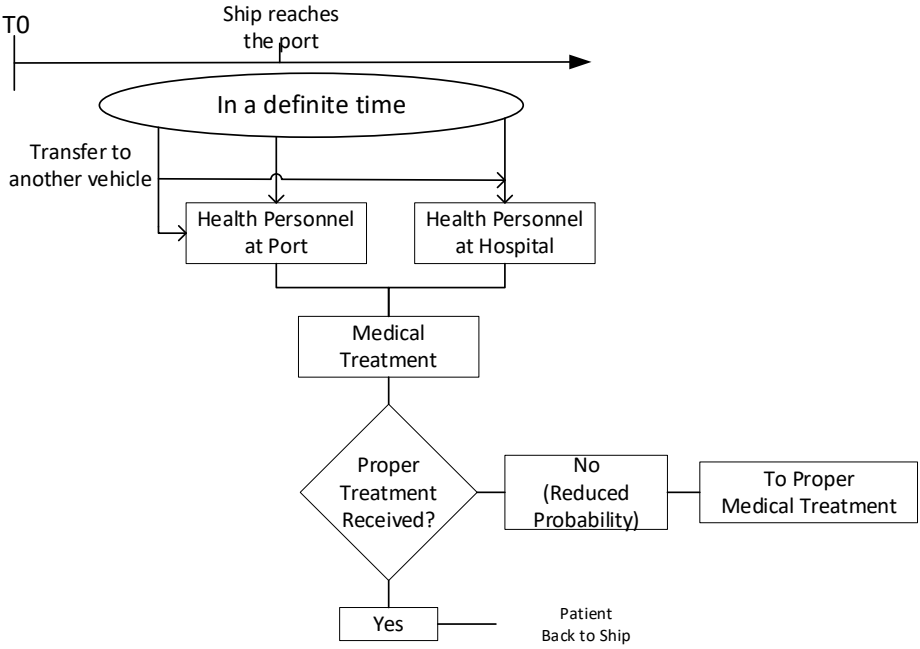

Figure 6. Proposed System in Commercial Ships

Current system and proposed system can be compared by examining Figure 4 and Figure 6.

Proposed system has two major benefits compared to the current system. The first is, the patient is routed to proper medical treatment more properly based on

accurate medical information. This will avoid both unnecessary time loss and expanses. Secondly it will be less likely that the patient will be rerouted for another medical treatment due to pre-triage.

4. EVALUATION OF THE SYSTEM

In this section, SCM was used and it was evaluated whether the proposed system could find solutions to the identified problems.

4.1 Categorization of Identified Problems

In Section 1.1, the problems encountered when applying for health care are mentioned in detail. In this section, these problems are divided into categories. Table 1 shows these identified problems, and Table 3 shows the categories of these problems. These categories correspond to failures in SCM and are not divided into 'active' and 'latent'. Possible problems (Table 2) are not added to Table 3.

Table 3. Categories of the Problems Encountered in Medical Translations

Cat. Code	Categories of the Problems
C1	Communication problems due to language differences
C2	Inability of the patient to express himself due to consciousness and other factors
C3	Translation errors
C4	Internet based errors
C5	Problems related to health records

In both Category C1 and C2, the patient cannot express himself. The difference is that in category C1, the patient is in a state that he can communicate but cannot express himself due to the language difference. In category C2, the patient is unable to express himself due to shock, loss of consciousness and similar reasons. In this case, language is not an issue.

It has been observed that incorrect translations made by non-professional translators can cause many medical issues including death of the patient. And even medical translations made by professional interpreters (interpreters who are not medical translators) cause similar medical issues (Gonel, 2023). Category C3 includes all health problems experienced when the patient's health problems are translated by the translator to the medical staff.

An internet connection and other necessary equipment are needed to use general online translation programs and receive professional medical translation services online. All problems experienced in the provision of relevant connections and services are included in category C4.

Health problems experienced due to failure to submit health records or misunderstanding due to language differences are designated as category E.

4.2 Defense Layers

A multi-layered solution network was modeled. Solution layers Lay A, B1, B2, C1, C2, D and E, identified problem categories were produced as solutions (Table 4). Each of the layers provided solutions to each problem category. The auxiliary layer (Aux Lay A) is an additional layer that was not created as a solution to determined problems (Section 1.2) but for anticipated problems (Section 1.3). Solution layers correspond to slices in SCM.

Table 4. Defense Layers

Lay. Code	Defense Layer
A	Interface where the patient can use any language he wants
B1	Creating health form at the time of complaint occurs or complaint is expected
B2	Creating health for the unconscious or uncapable patients by other people
C1	Translations made only by medical doctors and medical translators.
C2	Creating the patient form without entering text
D	Using an offline interference
E	Keeping past health records ready by entering them into the system in advance

The first layer (Lay A) is use of an interface that allows each patient to log in to the system using their native language or any language of their preference. The system interface has also been prepared in a way that does not require any text writing (Lay C2). Translations between languages will be made by medical doctors and medical translators (Lay C1). Another feature of the interface is working without the need for an internet connection (Lay D).

The system in question was developed for personnel and passengers of ships, planes, trains and similar transportation vehicles. Since there is likely to be a time difference between the occurrence of a health complaint and access to healthcare, pre-registration will be created as soon as a health problem occurs during the voyage. Thus, in cases where the patient loses consciousness or similar, the patient's complaints will be conveyed directly to the healthcare personnel through the patient's statement (Lay B1). In cases where the patient suddenly faints, loses consciousness as a result of an accident, etc., the patient record can be filled in by another person (Lay B2). Performing this process as soon as possible will contribute to the more accurate collection of information about the patient's condition.

If the people who are in question to use the system voluntarily upload their past health records to the system, in case of any emergency, healthcare personnel will be able to access the patient's health records in the language of their choice (Lay E).

4.3 Auxiliary Defense Layers

An additional security layer has been added to the system for the anticipated problem (I13) that is not detected from the actual events but is predicted to occur. The patient will not know the information the medical staff needs for a correct diagnosis. For this reason, each question that the system asks the patient varies according to the previous questions and questions, and all questions and the order of the questions are determined by the physicians (Aux Lay A).

4.4 Evaluation of the System

The identified problems (Section 1.2) were divided into categories (Section 4.1) and the solution layers developed to solve these problems (Section 4.2) were compared with the SCM (Figure 7). As a result, it has been seen that solution layers prevent all problems (detected and potential problems) in theory.

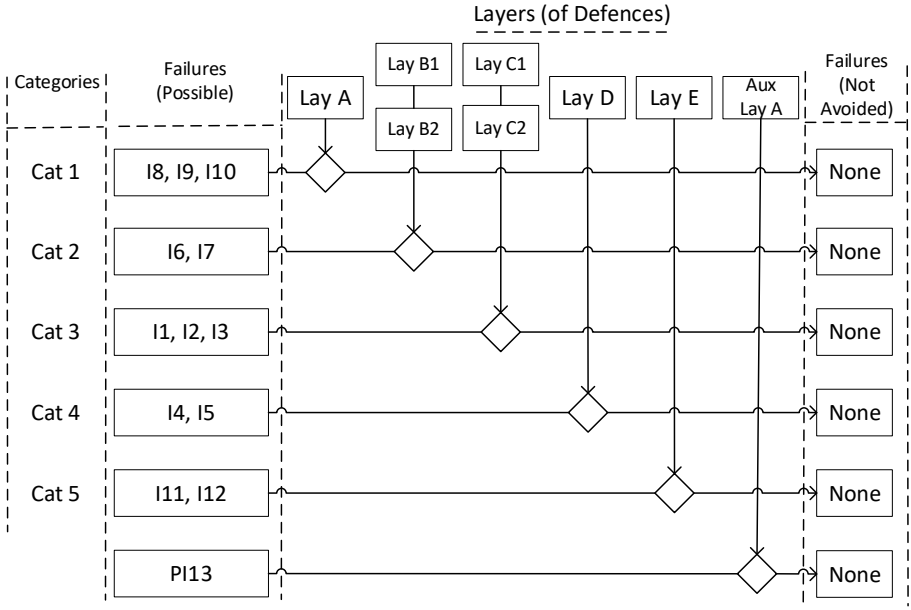

Figure 7. Effects of Defense Layers on Failures

4.5 Benefits of the System

Since the benefits of the system are explained in detail (Gonel, 2023), they are not included in this study to avoid repetition.

5. CONCLUSION

It is quite possible that especially seafarers and also international transport workers and passengers may require healthcare. In these moving vehicles, such as ships, it may not be possible for patients to reach healthcare services in a short time. The patient must be guided in the most appropriate way with the correct preliminary diagnosis. Moreover, correct guidance of the patient is not the only problem. Many problems may occur even when the patient reaches the healthcare personnel and is about to receive healthcare services. Failures may occur due to many different reasons, especially language-related problems. Proposed system will avoid all these problems and thus provide reduced expenses and time loss.

The benefits and additional benefits of the system show that the system can be used not only for transport sector workers and passengers, but also for refugees, immigrants, tourists and many other groups. In addition, it will be appropriate and beneficial to use the system for all patients who will receive healthcare services.

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Chapter 13

Hormonal Regulation of Estrous Cycle in Cats

Özkan ŞİMŞEK[1]

INTRODUCTION

The ovaries are intricate organs that contain a multitude of specialized cell types responsible for regulating various physiological functions associated with reproduction. Within the ovary, each follicle consists of an oocyte, which is a developing egg cell, surrounded by a group of specialized cells. The primary objective of these specialized cells is to support the growth and maturation of the oocyte, ultimately preparing it to potentially become an ovule (Richards and Pangas, 2010).

In cats, the process of follicle ovulation differs from that of other species. In these animals, ovulation is triggered by vaginal stimulation. Cats, for instance, are considered polyestrous animals, meaning they undergo multiple estrous cycles throughout the mating season unless they become pregnant or experience pseudopregnancy (Little, 2012).

The ovary undergoes distinct changes during the estrous cycle, which can be separated into two primary phases; (1) the follicular stage, (2) the luteal stage. The follicular stage is distinguished by the growth and maturation of ovarian follicles, while the luteal phase involves the formation and function of the corpus luteum, a transient endocrine organ that performs a vital role in supporting early pregnancy (Johnson, 2022). This chapter aims to provide concise and informative details regarding the effective hormones in the follicular and luteal stages of the estrous period.

The Role of Cholesterol

Cholesterol has a vital function in the production of steroid hormones (progesterone, estrogen, testosterone) and acts as a precursor. Cells obtain cholesterol from two sources. These are; (1) denove cholesterol synthesis in the cell, (2) high density lipoprotein (HDL) and low density lipoprotein (LDL) (O'Shaughnessy and Wathes 1985). Cholesterol is transported to the ovary by

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the bloodstream as HDL or LDL, depending on the animal species (Grummer and Carrol 1988, Masumura et al., 1992, Clevidence and Bieri 1993).

Cholesterol is transformed to pregnenolone through cytochrome P450, a mitochondrial enzyme, and used in progesterone production (Juengel et al., 1995; Sandhoff et al., 1996a). Studies have shown that the steroidogenic acute regulator (StAR) protein plays a vital function in this stage of stereoidogenesis (Sandhoff et al., 1996b, Townson et al., 1996). The protein has a vital function in facilitating the movement of cholesterol from the outer membrane to the inner membrane of mitochondrial, where it undergoes conversion into pregnenolone (Kiriakidou et al., 1996, Pescador et al., 1996, Stocco 1997). A part of pregnenolone is transformed to progesterone by the enzyme 3β-HSD in the endoplasmic reticulum. The other part is first transformed to 17-hydroxy-pregnenolone through the enzyme 17α-hydroxylase and then to dehydroepiandrosterone by the enzyme desmolase. Dehydroepiandrosterone is then converted to androstenedione by the enzyme 3β-HSD (Luo and Wiltbank. 2006).

Androstenedione is the precursor steroid hormone used in the synthesis of estradiol and is produced by theca cells. Androstenedione not involved in estradiol synthesis binds to androstenedione receptors in granulosa cells (Vallée et al., 2001, Luo and Wiltbank 2006).

The Role of Gonodatropins

The differentiation and growth of follicles on the ovary is a intricate process. This process is largely dependent on the hormones Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH), gonadotropins secreted through the pituitary gland (Richards 1994). However, follicular development can be maintained independently of FSH and LH from the primordial phase to the early pre-antral phase. Follicle stimulating hormone and LH are heterodimeric glycoproteins consisted of two subunits: α and β. The α subunit is the same in both LH and FSH, while the β subunit is unique to each hormone and is responsible for its biological activity (Webb et al., 1999).

During the maturation of the primary follicle, the initiation of granulosa cell differentiation is facilitated by FSH. Concurrently, FSH promotes the development of LH receptors and enhances aromatase activity (Hsueh et al., 1984, Dahl and Hsueh 1988). Pre-antral follicles respond to FSH stimulation by increasing growth in vitro (Webb et al., 1999). Receptors for LH are expressed in both granulosa and theca cells, whereas FSH receptors are only expressed in granulosa cells (Richards et al., 1987, Hillier 1991). Luteinising hormone stimulates the release of androgens from theca cells and progesterone from the

luteal cells. Follicle stimulating hormone induces the production of progesterone and estrogen in granulosa cells (Richards et al., 1987).

Estrogen and FSH work together to induce granulosa cell proliferation and the formation of LH receptors on granulosa cells. The formation of LH receptors on the granulosa cells allows the cells to respond to the synthesis of aromatase stimulated by both FSH and LH, resulting in more estrogen synthesis (Richards 1994). Luteinising hormone has a very important role in the reproductive cycle of all species where ovulation can be stimulated spontaneously or through mating. Ovulation during oestrus is dependent on the release of sufficient levels of LH. The release of sufficient levels of LH is associated with the number of matings and the timing of the oestrous period. Each mating causes another stimulation of LH and an increase in its level. In most cats, four or more matings are sufficient for ovulation to occur (Wildt et al., 1980, Johnson and Gay 1981, Johnson, 2022).

The Role of Estrogen and Progesterone

Estrogen and progesterone are important factors involved in the development of follicles. While estrogen is synthesised by the granulosa cells, progesterone is synthesised by both the granulosa cells and the theca cells. Estrogen production is an marker that shows the cell has reached follicular maturity (Hsueh 1984). Estrogen enhances the action of both FSH and LH by inducing the granulosa cell proliferation within the follicles (Richards 1980). The basic stereoidogenic cells in the antral follicles have histogenetic features essential for estrogen synthesis (Short 1962, Bjersing 1967, Ryan 1979).

In order to explain estrogen synthesis, Amstrong et al. (1979) established the "two cell-two gonodotropin" model. This model includes four main features: 1) Granulosa cells have FSH receptors, 2) Theca cells have LH receptors, 3) FSH stimulates aromatase activity in granulosa cells, 4) LH stimulates androgen synthesis in theca cells.

During the synthesis of steroid hormones in theca cells, cholesterol is converted to pregnenol under the influence of LH and androgens are produced after a series of precursor processes. According to the two-cell-two gonodotropin hormone model, androgens produced by theca cells are carried to the granulosa cells where they are transformed into estrogens (Drummond 2006).

While estradiol level is less than 60-70 pmol/ml during anoestrus and postoestrus periods, it is 150-300 pmol/L during oestrus. If ovulation does not occur, estradiol level decreases to basal level within 5-10 days. However, if ovulation occurs, the amount of estradiol will decrease within 2-3 days. In the

first part of the luteal phase, during pregnancy and false pregnancy, estradiol levels are usually below 70 nmol/L (Verstegen 2004).

In response to FSH, granulosa cells in preantral follicles produce progesterone at low levels. This effect is achieved by the increased release of the enzyme P450, which is stimulated by FSH (Richards et al., 1995, Hillier 2001). The P450 enzyme plays a role in the conversion of cholesterol to pregnenolone, a first step in the mechanism of progesterone production. In addition, FSH also induces the production of 3β-HSD enzyme which enables the conversion of pregnenolone to progesterone (Hsueh 1984, Richards 1988). Progesterone is necessary for ovulation and suppression of FSH and LH hormones released from the pituitary gland by negative feedback mechanism. It has been observed that ovulation does not occur in mice whose progesterone receptor is blocked. Progesterone is also essential for the continued development of the embryo and is the primary hormone produced by the corpus luteum (Lydon et al., 1995).

During the pre-ovulatory period, plasma progesterone levels remain at a basal level. In pregnant and pseudo-pregnant cats, progesterone level starts to increase between 24-50 hours after ovulation. After the first mating, the progesterone level can reach a maximum of 100-200 nmol/L within 20-25 days. In pregnant cats, progesterone level starts to decrease after 25-35 days of pregnancy and remains constant between 15-30 nmol/L. In pseudo-pregnant cats, the progesterone level starts to decrease approximately from the 25th day and reaches the basal value between the 30th and 40th days. This slow decrease in progesterone levels is characteristic for pseudo-pregnant cats (Verstegen 2004).

CONCLUSION

In conclusion, it is known that the stimulation of gonadotropins produced from the anterior pituitary gland under the effect of GnRH results in the synthesis of progesterone and estrogen hormones, which play an vital role in both the follicular and luteal phases. Cats exhibit differences in their reproductive physiology compared to other animals, except for rabbits, as they undergo provoked ovulation. Despite numerous studies attempting to elucidate the physiological changes and mechanisms occurring in the ovaries of cats, the findings have remained limited. Therefore, it is believed that an increase in cell culture studies on feline reproductive cells would enable a more comprehensive understanding of the physiological mechanisms during the follicular development phase.

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Chapter 14

Cardiovascular Diseases and Healthy Eating Recommendations

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INTRODUCTION

Cardiovascular diseases (CVD) encompass a range of pathological conditions affecting the cardiovascular system, which includes disorders affecting the heart and blood vessels, such as hypertension, stroke, atherosclerosis, peripheral artery disease, and venous disorders. The likelihood of developing CVD is linked to unhealthy dietary habits characterized by excessive sodium, processed foods, added sugars, and unhealthy fats intake. This is compounded by insufficient consumption of fruits, vegetables, whole grains, fiber, legumes, fish, and nuts. Contributing factors also include a sedentary lifestyle, overweight and obesity, elevated stress levels, alcohol consumption, and smoking behaviors (see Figure 1). Additionally, CVD often coexists with comorbidities such as obesity, diabetes, hypertension, or dyslipidemia, collectively representing four of the top 10 risk factors for global all-cause mortality (Casas et al., 2018).

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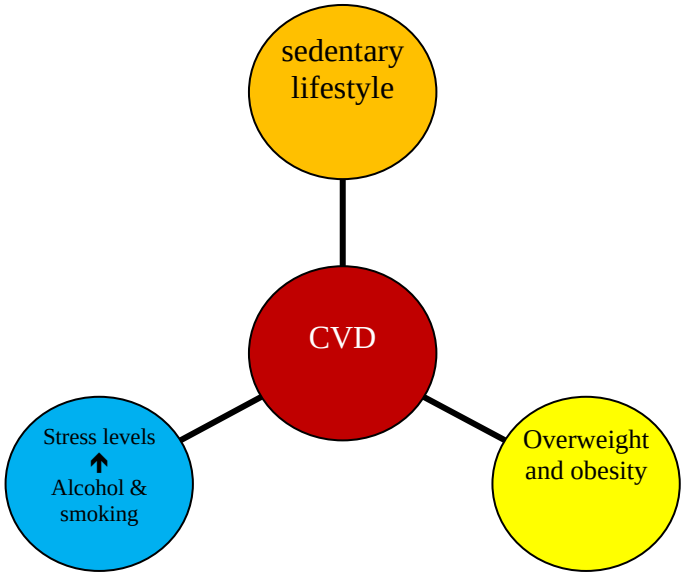

Figure 1. Cardiovascular disease risk factors.

CVD stand as the predominant cause of global mortality, significantly impacting the quality of life and posing substantial challenges to health systems (Giosuè et al., 2022). Epidemiological studies suggest that the incidence of cardiovascular diseases (CVD) could be reduced to a certain extent by addressing or avoiding risk factors such as hyperlipidemia, arterial hypertension, type 2 diabetes, smoking, overweight and obesity, physical inactivity, and inadequate consumption of fruits and vegetables. The analysis utilizes prevalence rate estimates gathered from diverse sources, including data from the Ankara Chronic Diseases and Risk Factors Survey conducted by the Ministry of Health (Balbay et al., 2018). In this context, epidemiological investigations and randomized clinical trials (RCTs) have demonstrated that the promotion of lifestyle modifications, encompassing the adoption of a healthy dietary pattern, is advisable for the prevention of CVD (Salas-Salvadó et al., 2018). Global disparities in dietary patterns are associated with notable variations in the prevalence of chronic diseases (Lordan et al., 2018). The identification and subsequent targeting of dietary factors possessing the highest potential for reducing cardiovascular disease, diabetes, and obesity are of paramount significance from both scientific and public health perspectives (Lordan et al., 2018). While earlier nutritional studies primarily concentrated on

preventing nutrient deficiencies, contemporary research underscores the pivotal role of nutritional strategies in fostering overall health and diminishing the prevalence of non-communicable diseases (Rychter et al., 2020). A primary approach in the management of cardiovascular disease involves the regulation of lifestyle habits, emphasizing the advantageous properties of foods (Rychter et al., 2020).

Cardiovascular diseases (CVD) comprise various conditions impacting the heart and blood vessels, with risk factors encompassing unhealthy dietary habits, a sedentary lifestyle, and comorbidities like obesity and diabetes. Given that CVD stands as the foremost global cause of mortality, it presents substantial challenges to health systems. This underscores the crucial role of preventive measures, including lifestyle modifications and specific dietary interventions, in alleviating the impact of these diseases.

The aim of this review is to identify potential targets for preventing cardiovascular diseases (CVD), whether they take the form of dietary patterns, individual foods, or specific nutrients. The review also seeks to quantify the extent of the observed beneficial effects associated with these targets.

NUTRITION in CARDIOVASCULAR HEALTH

Numerous studies have established an association between healthy dietary patterns and reduced plasmatic concentrations of pro-inflammatory markers, while a Western-type diet, characterized by a preponderance of meat-based dietary patterns, is linked to elevated levels of low-grade inflammation (Casas et al., 2018). The Western diet, characterized by excessive consumption of high-energy-density foods rich in fats, sugars, and animal proteins, coupled with inadequate intake of fruits and vegetables, along with sedentary behavior, induces inflammation and predisposes individuals to conditions such as obesity, heart disease, type 2 diabetes, and metabolic syndrome (Meslier et al., 2020). Recommendations from reputable health organizations emphasize dietary approaches associated with cardiovascular health. The American Heart Association (AHA) advocates for the DASH (Nutritional Approaches to Stop Hypertension) diet, characterized by low sugar and saturated fat content and high levels of vegetables, fruits, and whole grains. Similarly, the European Society of Cardiology (ESC) recommends transitioning from saturated fats to polyunsaturated fats, augmenting fiber, fruit, vegetable, and fish intake, abstaining from alcohol consumption, and adopting a Mediterranean-type diet. Such dietary regimens have demonstrated significant reductions in cardiovascular disease risk (Stewart et al., 2017). Healthy eating patterns, derived from studies on nutrient intake and health outcomes (e.g., DASH and

Mediterranean diets), share common characteristics (Figure 2.) (Cena & Calder, 2020).

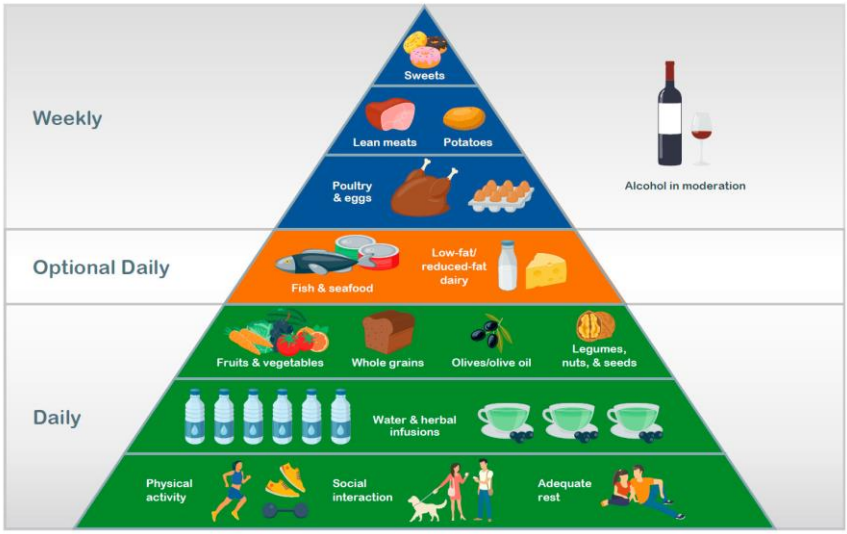

Figure 2. A generalized healthy diet and lifestyle pyramid (Cena & Calder, 2020).

Current evidence highlights that healthy eating patterns share essential characteristics, including increased consumption of fiber, antioxidants, vitamins, minerals, polyphenols, monounsaturated, and polyunsaturated fatty acids (MUFA and PUFA, respectively). Simultaneously, there is a reduced intake of salt, refined sugar, saturated and trans fats. Notably, these patterns involve a high intake and low consumption of specific food groups such as fruits, vegetables, legumes, fish, seafood, nuts, seeds, whole grains, vegetable oils, and dairy products. In contrast, they minimize the intake of pastries, soft drinks, and red and processed meats (Carro & Panisello, 2019; Casas et al., 2018).

Extensive investigations into the cardiovascular outcomes of Mediterranean and DASH dietary interventions have been conducted. These studies reveal the capacity of these interventions to reduce the incidence of cardiovascular diseases by mitigating low-grade inflammation, improving weight management, and ameliorating other risk factors. Consequently, this leads to a diminished occurrence of clinical events (Carro & Panisello, 2019; Casas et al., 2018).

Following healthy dietary patterns is correlated with decreased inflammation, whereas a Western-style diet, characterized by excessive consumption of high-energy-density foods, is associated with heightened levels of low-grade inflammation. This predisposes individuals to conditions like obesity, heart disease, and type 2 diabetes. Recommended dietary approaches, such as the DASH and Mediterranean diets, prove effective in reducing the risk of cardiovascular disease by influencing inflammation, weight management, and other pertinent risk factors.

The DASH Diet

The Dietary Approaches to Stop Hypertension (DASH) diet, originating in the 1990s, has demonstrated efficacy in reducing blood pressure, total cholesterol, and LDL-Cholesterol, thereby significantly lowering cardiometabolic risk (Carro & Panisello). The recommended components of the DASH diet include a rich assortment of fruits, vegetables, legumes, whole grains, and low-fat protein sources, with an emphasis on minimizing or avoiding processed foods, trans fats, and sugary drinks (Diab et al., 2023). The DASH diet, rich in nutrients like potassium, magnesium, calcium, fiber, and antioxidants, promotes the restriction of unfavourable foods. This includes limiting intake of saturated fat, cholesterol, red and processed meats, sweets, sodium, added sugars, and sugar-sweetened beverages (Challa et al.). Saturated fat, cholesterol, red and processed meats, sweets, sodium, added sugars, and sugar-sweetened beverages should be limited, as they belong to the group of unfavorable foods (Rychter et al.). A pivotal aspect of the DASH diet's blood pressure-lowering mechanism is its sodium restriction, with the standard recommendation limiting salt consumption to 2,300 mg per day (Diab et al., 2023). This comprehensive dietary approach contributes synergistically to the underlying pathophysiology of heart failure by reducing pro-inflammatory cytokines and reactive oxygen species, enhancing endothelial function, improving micronutrient status, and mitigating malnutrition (Lim et al.).

Typical serving guide for a patient following the DASH diet: vegetables, about five servings per day; fruit, about five meals a day; carbohydrates, about seven servings a day ; low-fat dairy products, about two servings per day ; Lean meat products are about two servings or less per day (Challa et al., 2022). Further guidelines for adhering to the DASH diet include restricting the intake of added sugars to less than 10% of daily calories, limiting saturated fats to less than 10% of daily calories, keeping sodium consumption below 2300 mg per day, and moderating alcohol intake to one drink per day for women and two drinks per day for men (Cena & Calder, 2020). Despite the well-acknowledged

cardiovascular benefits associated with the DASH dietary pattern, ongoing research is essential to further ascertain and affirm its positive effects in clinical practice (Rychter et al., 2020).

The DASH diet, originating in the 1990s, proves effective in reducing blood pressure and cardiometabolic risk through its emphasis on a nutrient-rich composition, including fruits, vegetables, and limited intake of unfavorable foods. This dietary approach, particularly notable for its sodium restriction, contributes synergistically to heart failure mitigation by addressing pro-inflammatory markers, enhancing endothelial function, and improving overall nutritional status.

Mediterranean Diet

The term "Mediterranean diet" denotes the dietary pattern prevalent in olive tree-growing regions, particularly Greece/Crete and southern Italy, during the 1960s (Mazzocchi et al., 2019) his plant-centered nutritional approach is characterized by a high consumption of vegetables, fruits, whole grains, and legumes, with distinctive features including the extensive use of olive oil and moderate intake of red wine (Muscogiuri et al., 2022; Rychter et al., 2020). Owing to its abundance of legumes, nuts, vegetables, and fruits, the Mediterranean Diet has the potential to modulate gut microbiota, influencing the production of metabolites with implications for cardiovascular disease risk (Salas-Salvadó et al., 2018). Monounsaturated fats present in olive oil, nuts, and avocados contribute to the prevention of CVD (A. Locke et al., 2018). While all types of olive oil contain oleic acid, unrefined varieties (virgin and extra virgin) additionally feature tocopherols, monounsaturated fatty acids (MUFA), and various bioactive polyphenols (Carro & Panisello, 2019). Omega-3 and monounsaturated fats, found in fish and plants, are recognized as particularly beneficial nutrients (A. Locke et al., 2018). A distinctive aspect of the Mediterranean Diet is its low consumption of red meat and sweets. Studies suggest a negative association between the Mediterranean Diet and metabolic syndrome and diabetes risk factors. Additionally, this dietary pattern has been linked to reductions in low-density lipoprotein and triglyceride levels, body weight, and beneficial effects on blood pressure-(Rychter et al., 2020).

The Mediterranean diet underscores health-promoting dietary patterns, emphasizing the consumption of fruits, vegetables, whole grains, lean protein sources, legumes, dairy, nuts, and healthy fats, while restricting energy-dense sugars and processed foods (Wickman et al., 2021). Essential factors for the positive impact of the Mediterranean Diet on human health include the preference for locally and sustainably grown foods, minimal processing, fresh

preparation at home, mindful consumption, moderate wine intake, post-meal rest, and regular physical activity. Moreover, the geographical and lifestyle characteristics of Mediterranean populations, situated in environments featuring the sea, coasts, mountains, and a longstanding historical tradition of daily exercise, contribute significantly to the favorable effects of this dietary pattern on health (Mazzocchi et al., 2019).

The Mediterranean diet promotes health through an emphasis on diverse, nutrient-rich foods while discouraging energy-dense sugars and processed items. Its positive impact on human health is further attributed to lifestyle factors such as locally sourced and minimally processed foods, home preparation, moderate wine consumption, post-meal rest, and the historical context of daily exercise in Mediterranean populations living in sea, coast, and mountainous environments.

Dietary Fats

Fats, essential for cell membrane structure and energy provision, are categorized into monounsaturated fats, polyunsaturated fats, saturated fats, and trans fats. Unsaturated fats, found in foods such as fish, various vegetable oils, nuts, and seeds, differ from saturated fats, which are more prevalent in animal products and certain plant-derived fats. Polyunsaturated fatty acids, including omega-3 and omega-6, are considered vital for normal growth and reproduction, requiring dietary intake since the body cannot produce them internally (Cena & Calder, 2020).

Rich sources of saturated fatty acids include foods like fatty meats, butter, full-fat dairy products, and tropical oils such as coconut and palm oil, with meat and dairy being prominent contributors to saturated fat intake in Western diets. Trans fatty acids, associated with adverse cardiovascular outcomes, impact serum lipid levels and endothelial function, thereby influencing cardiovascular disease (CVD) risk factors (Jiménez-Cortegana et al., 2021; E. Yu et al., 2018). Increased mono- or polyunsaturated fat intake, in contrast to saturated fats, is linked to reduced CVD risk, and certain plant-derived saturated fats may exhibit more favourable effects on lipid profiles (A. Locke et al., 2018).

The two primary types of polyunsaturated fatty acids (PUFAs), omega-3 (Ω-3 PUFAs) and omega-6 (Ω-6 PUFAs), exhibit distinct effects on cardiovascular health (Jiménez-Cortegana et al., 2021). Very long-chain n-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), derived from marine sources like fish, fish oil, and algae, contribute to cardiovascular health (Trautwein & McKay, 2020). Diets characterized by low saturated fats, high antioxidants, carbohydrates, fiber, monounsaturated fatty acids, and n-3 PUFAs,

particularly from olive oil and certain regions, are associated with cardiovascular benefits (Mazzocchi et al., 2019).

It's crucial to note that individual dietary needs may vary, and consulting with a healthcare professional or a dietitian is recommended for personalized advice based on specific health conditions and goals.

Dietary Cholesterol

Dietary cholesterol, an essential steroid derived from animal tissues (Soliman, 2018), constitutes a significant component of the human body, with approximately 25% of serum cholesterol originating from dietary sources like egg yolks, shrimp, meat, poultry, cheese, and butter. Despite individual variations, the majority of cholesterol is produced endogenously, emphasizing its crucial role in the body (Blesso & Fernandez, 2018). Notably, meat contributes 42%, and eggs contribute 25% to total cholesterol intake, with other food groups comprising the remaining third on average (Carson et al., 2020). Although dietary cholesterol elevates fasting lipids with individual differences, its association with saturated fats underscores the importance of considering their detrimental effects on cholesterol (Spence, 2019). Historically, dietary guidelines recommended limiting cholesterol intake to less than 300 mg/day due to its lipid/lipoprotein-boosting properties. However, the 2015-2020 Dietary Guidelines Advisory Committee found no apparent relationship between diet and serum cholesterol, leading to the removal of the dietary cholesterol intake limit, stating that cholesterol is not a nutrient of concern for overconsumption in the U.S. based on population average intake (Bowen et al., 2018). Cholesterol balance is influenced by the rate of synthesis of cholesterol and bile acids and their excretion from the body, with dietary cholesterol often associated with increased intake of saturated fatty acids, known to increase LDL cholesterol and cardiovascular disease risk (Blesso & Fernandez, 2018). Eggs stand out as a unique dietary source low in saturated fatty acids, nutrient-dense, affordable, and affordable (Soliman, 2018). The impact of dietary cholesterol on fasting lipid levels varies among individuals, and it is crucial to note that its consumption often coincides with saturated fats, which can have adverse effects on cholesterol. On average, meat, including poultry, mixed meals, red meat, processed meat, and seafood, contributes 42% to total cholesterol intake, while eggs contribute 25%, and other food groups collectively contribute the remaining third (Carson et al., 2020). A prospective cohort study that met specific inclusion and exclusion criteria investigated the relationship between dietary cholesterol and cardiovascular disease outcomes, encompassing conditions such as coronary heart disease (CHD) and stroke (Jiménez-

Cortegana et al.). Irrespective of the dietary assessment method used, the study did not identify any significant association between stroke, CHD events, or CHD death and dietary cholesterol. The research displayed notable variability in population, design, sociodemographic characteristics, dietary cholesterol intake ranges, and statistical covariate controls (Carson et al., 2020).

Dietary cholesterol, sourced from animal tissues, holds a substantial role in the human body, with approximately a quarter coming from items such as eggs, meat, and dairy. Despite its influence on fasting lipids and past dietary recommendations, recent research indicates that the connection between dietary cholesterol and cardiovascular outcomes is not substantial. This underscores the intricate interplay of factors influencing cholesterol balance.

Dairy and Dairy Products

Milk and dairy products play a crucial role in providing essential nutrients, including vitamins, minerals, macronutrients, and micronutrients, vital for growth, development, and tissue maintenance (Lordan et al., 2018). Despite their nutritional significance, concerns have arisen regarding their potential impact on cardiovascular disease risk, particularly due to the saturated fat content present in products such as dairy, butter, whole milk, yogurt, and most cheeses (Butler et al., 2020). Rich in amino acids, notably leucine, which stimulates muscle growth, as well as calcium and phosphorus, dairy products have been associated with cardio-metabolic benefits, including positive effects against health issues such as diabetes, obesity, and metabolic syndrome. Nevertheless, the connection between consuming dairy products and cardiovascular health is intricate, and recommendations differ among dietary guidelines. This highlights the importance of exercising caution when interpreting studies involving dairy products, especially when considering factors like full-fat dairy, ice cream, and similar sweet products that have high sugar and fat content (Lordan et al., 2018).

Eggs

Early observational studies indicated a potential association between dietary cholesterol and cardiovascular disease risk(Blesso & Fernandez, 2018). However, the unique cholesterol content of eggs, as highlighted in studies like Ignarro et al. (Ignarro et al., 2007), complicates this relationship. Initial investigations lacked consideration of confounding variables, limiting their findings, while recent epidemiological studies generally demonstrate no significant association between dietary cholesterol or egg consumption and cardiovascular disease risk in the broader population (Blesso & Fernandez,

2018). Despite their cholesterol content, eggs are recognized as an economical and highly nutritious food source, with a medium-sized boiled egg containing various macronutrients, including 186 mg of cholesterol (Kuang et al., 2018; Zhong et al., 2019). Recommendations advise healthy individuals to limit daily egg intake, with up to one egg deemed acceptable. Special caution is advised for specific subgroups, including vegetarians, dyslipidaemia patients (especially those with diabetes or heart failure risk), and older normocholesterolemic individuals, who, under a heart-healthy dietary pattern, may consume up to 2 eggs per day (Carson et al., 2020).

Carbohydrates and Sweeteners

Sugars, refined carbohydrates, high-fructose corn syrup, starches, and trans fats pose a higher risk of dyslipidaemia and coronary heart disease compared to saturated fatty acids. Sweeteners contribute to an increased likelihood of obesity, weight gain, metabolic syndrome, Type 2 Diabetes (T2DM), and coronary heart disease. Their adverse impact encompasses interference with established responses crucial for glucose and energy homeostasis, detrimental effects on the microbiome, modulation of leptin levels, and diminished satiety (Houston, 2018). Leptin, with peripheral effects encompassing the stimulation of inflammatory reactions, oxidative stress, atherogenesis, and thrombosis, contributes to endothelial dysfunction, arterial stiffness, and the development and fragility of atherosclerotic plaques. Additionally, leptin plays a regulatory role in bone homeostasis, reproduction, and angiogenesis, prompting investigations into the impact of rolleptin on the presence, severity, and prognosis of both cardiac and non-cardiac vascular diseases (Katsiki et al., 2018).

MICRONUTRIENTS AND CARDIOVASCULAR HEALTH

Vitamin B6

Vitamin B6 is often explored for its role in cardiovascular health and its potential to lower blood pressure. Inadequate plasma levels of pyridoxal phosphate (PLP) in humans are associated with an elevated risk of atherosclerosis, stroke, and thrombosis. Although severe vitamin B6 deficiency is rare, suboptimal levels or mild deficiency are more common. Research indicates that supplementation with vitamin B6 can reduce IL-6 levels and improve total lymphocytes in individuals with chronic diseases (Stach et al., 2021).

Vitamin D

Vitamin D is vital for the survival of most vertebrates, as it acts as the precursor to the biologically active steroid hormone 1,25-dihydroxy vitamin D (1,25(OH)2D). This hormone undergoes activation through hydroxylation reactions in the liver and kidney, respectively (Driggin et al., 2022). Across evolutionary processes, intricate regulatory systems have evolved to uphold narrow limits of circulating concentrations of the active vitamin D hormone, even amid alterations in the precursor molecule 25(OH)D (Latic & Erben, 2020). Vitamin D deficiency has been linked to several cardiovascular risk factors. While there is limited information on the regulation of local production of 1,25(OH)2D in cardiovascular target cells, it is acknowledged that the local concentration of 1,25(OH)2D within the target cell plays a crucial role in the biological activity of vitamin D signalling. Furthermore, the direct effects of vitamin D on smooth muscle calcification and proliferation may contribute to its influence on cardiovascular health (Kheiri et al., 2018).

Sodium

Major dietary guidelines, including recommendations from Nestel and Mori (Nestel & Mori, 2022), universally advise both the general population and individuals with hypertension to decrease their salt intake. The specified target for salt consumption typically ranges from 4 to 5 grams or 1550-2000 mg of daily sodium concentration. In the context of heart failure, specific guidelines, such as those proposed by Ravera et al. (2016) (Ravera et al., 2016), may suggest even more stringent salt restrictions, limiting intake to 1-2 g per day in cases of advanced symptoms. The existing body of evidence supports a direct and positive correlation between sodium intake and the regulation of blood pressure. The groundbreaking INTERSALT (International Sodium, Potassium, and Blood Pressure Study) study stands as the initial international investigation into this relationship. Beyond epidemiological studies, numerous randomized controlled trials, as evidenced by research conducted by Jaques et al. (Jaques et al., 2021). have consistently affirmed the impact of dietary sodium on blood pressure values and the effective management of hypertension. Ongoing randomized trials seek to further elucidate the comprehensive effects of salt on various cardiovascular disease (Ravera et al., 2016).

Potassium

The INTERSALT study brought to light a negative correlation between urine potassium excretion and blood pressure levels across diverse populations. Increased dietary potassium intake within the range of approximately 60-80

mmol/d has shown a notable and inverse correlation with the incidence of stroke mortality in women. While the established protective effect of dietary potassium on blood pressure and cardiovascular disease is well recognized, there is insufficient evidence to endorse the prolonged use of potassium supplements for cardiovascular protection. The beneficial effects of fruits and vegetables strongly advocate for their regular inclusion in daily diets to ensure an adequate intake of potassium (Ignarro et al., 2007).

Dietary Fiber

Dietary fiber, defined as indigestible polysaccharides primarily composed of complex carbohydrates, encompasses plant-based carbohydrates resistant to digestion by human genome-encoded enzymes like amylase (Cronin et al., 2021; Prasad & Bondy, 2019). Categorized into soluble and insoluble types, each exerts distinct health effects (Amy Locke et al., 2018). Recognized as a crucial element in a healthy diet, dietary fiber positively influences the digestive system by interacting with the gastrointestinal tract, with whole grains, fruits, vegetables, and legumes serving as notable sources (Lin et al., 2021; Amy Locke et al., 2018). Grain-based foods, particularly bread and breakfast cereals, significantly contribute to dietary fiber intake, along with substantial contributions from vegetables, potatoes, and fruits (Trautwein & McKay, 2020). Approximately half of dietary fiber is derived from cereals and a third from fruits and vegetables, with higher intakes inversely linked to cardiovascular disease (CVD) risk, premature death, lower blood pressure, reduced low-density lipoprotein (LDL) cholesterol levels, and decreased breast cancer risk, as well as improved insulin sensitivity (Evans, 2020; Amy Locke et al., 2018; Edward Yu et al., 2018). Recent research indicates an elevated CVD risk associated with the consumption of ultra-processed foods, often characterized by reduced dietary fiber content (Barber et al., 2020). The abundant presence of dietary fiber in whole grains, protein foods, fruits, and vegetables positions them as compelling targets for disease prevention, reducing the risk of atherosclerosis and cardiovascular disease (Soliman, 2019). While fiber intakes are generally high in several countries, they often fall below optimal levels, especially in high-income countries (Evans, 2020).

The USDA dietary guidelines recommend a minimum of 14 g of fiber per 1,000 calories per day, while the Dietary Reference Intake (DRI) sets the daily target at 38 g for men aged 19-50 years, 25 g for women, 31 g for men above 51 years, and 21 g for women aged 51 and above (Amy Locke et al., 2018; Soliman, 2019).

CONCLUSION

CVD present a formidable and intricate challenge to global public health, manifesting as a spectrum of conditions affecting the cardiovascular system. The etiological landscape of CVD is intricately interwoven with factors such as unhealthy dietary practices, sedentary lifestyles, and various comorbidities. As the predominant cause of worldwide mortality, the imperative to address and mitigate the impact of CVD necessitates nuanced strategies grounded in comprehensive lifestyle modifications, with a particular emphasis on dietary patterns. Epidemiological evidence underscores the pivotal role of dietary choices in cardiovascular health, elucidating associations between adverse diets rich in sodium, processed foods, added sugars, and unhealthy fats, and an elevated susceptibility to CVD. Conversely, the adoption of healthful dietary paradigms, exemplified by the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean diets, demonstrates efficacy in ameliorating cardiovascular risk factors. A nuanced understanding of specific nutrients, encompassing beneficial fats, micronutrients, and dietary fiber, further enhances the precision of dietary recommendations for cardiovascular health. Therefore, fostering cardiovascular health mandates a holistic paradigm encompassing lifestyle modifications, comprising the cultivation of healthful dietary patterns replete with nutrient-dense foods, routine physical activity, and the avoidance of deleterious habits. Sustained endeavors in refining dietary guidelines and augmenting public awareness are imperative for alleviating the global burden of cardiovascular diseases.

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Chapter 15

Mediterranean Diet and Cancer

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INTRODUCTION

Cancer, a malignant tumor resulting from the uncontrolled division and multiplication of cells in an organ or tissue, poses a significant challenge to global health, being a leading cause of death worldwide and impeding efforts to increase life expectancy (Yin et al., 2021). The scale of the issue is considerable, with an estimated 14.9 million cases, causing 8.2 million deaths and 196.3 million disabilities (El-Sherif et al., 2021). According to the World Health Organization (WHO) in 2019, cancer ranks as the primary or secondary cause of death before the age of 70 in 112 out of 183 countries and third or fourth in the remaining 23 countries (WHO, 2020).

The worldwide burden of cancer incidence and mortality is on the rise, reflecting population aging, growth, and shifts in the prevalence of key cancer risk factors (Sung et al., 2021). Cancer risk factors primarily stem from individual and environmental characteristics such as genetics, substance use, hormone disorders, or lifestyle choices. Physical activity, sedentary behavior, and dietary habits play pivotal roles in cancer development. Key contributors to cancer include high body mass index, low fruit and vegetable consumption, alcohol and tobacco use, genetic predisposition, and chronic infections like Helicobacter pylori, Human papillomavirus, Hepatitis B, Hepatitis C, and Epstein-Barr virus (Ricceri et al., 2017; Hodge et al., 2016).

Changes in dietary patterns are speculated to contribute to cancer prevention. In Western countries, dietary habits account for 30% of all cancer types (El-Sherif et al., 2021). The World Cancer Research Fund (WCRF) suggests that a diet high in fruits, vegetables, whole grains, legumes, and low in red and processed meat can reduce cancer risk (Maximova et al., 2020). Considering dietary variety, incorporating diverse vegetables and fruits introduces various phytochemicals into the body (Martínez-González et al., 2017).

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A well-established dietary pattern in literature for preventing non-communicable diseases are the Mediterranean diet (Morze et al., 2021). Originating from the work of Angel Keys, the Mediterranean diet's key features include regular consumption of fiber and vitamin-rich fruits and vegetables, limited meat intake, and moderate consumption of dairy products and alcohol. Combining this evidence with emerging medical recommendations for healthier eating, the Mediterranean diet (MD) emerges as an optimal dietary model, embodying characteristics of an ideal healthy diet (Davis et al., 2015).

CANCER EPIDEMIOLOGY AND RISK FACTORS

Genetic and epigenetic factors play a crucial role in the initiation of cancer. The introduction of various environmental elements to these factors contributes to an escalation in the risk rate. Beyond environmental considerations, individual factors also amplify the probability of cancer. According to a study by the World Health Organization, 35% of cancer-related deaths stem from preventable or modifiable risk factors. These factors include the use of alcohol and cigarettes, the economic status of society, infections, parasites, exposure to ultraviolet light, and individuals' dietary habits (Lewandowska et al., 2019).

GASTROINTESTINAL SYSTEM CANCERS

Every cell in the body requires nutrients for its functions. These nutrients encompass proteins, fats, carbohydrates, vitamins, minerals, cellulose fibers, and non-nutritional plant substances. Nutrient intake into the body occurs through the gastrointestinal tract (GIS) (Abramowicz et al., 2015). The gastrointestinal tract comprises the mouth, pharynx, esophagus, stomach, small intestines, large intestines, rectum, and anus, spanning approximately 9 meters. Salivary glands, pancreas, liver, and gallbladder constitute its auxiliary organs and glands (Ogobuiro et al., 2023). The gastrointestinal tract is responsible for breaking down nutrients into their molecular structures, releasing small molecules produced by digestion into the bloodstream, and eliminating undigested nutrients and waste products from the body. Upon food consumption, various secretions and enzymes aid in the movement, digestion, absorption, and excretion of food through the gastrointestinal tract (Abramowicz et al., 2015; Luxner, 2005).

TYPES OF GIS CANCER

Esophageal Cancer

Esophageal cancer, with over 570 thousand cases in 2018, ranks as the seventh most prevalent cancer globally (Yang et al., 2020). Its incidence exhibits significant regional variations, with a high prevalence in East Asia, East and South Africa, and Southern Europe (Huang & Yu, 2018). Gender, obesity, smoking, alcohol use, papillomavirus (HPV), gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), and Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the risk factors for esophageal cancer (Abbas & Krasna, 2017; Corley et al., 2008; Hongo et al., 2009; Kubo & Corley, 2006).

Stomach Cancer

Geographical variations in gastric cancer incidence suggest a significant role of environmental influences, prompting attention to the correlation between diet and gastric cancer risk. The decline in gastric cancer incidence has been theorized to be partly due to widespread food refrigeration, leading to increased consumption of fresh produce and reduced dependence on food preservation. While several studies propose a protective effect of diets rich in fresh fruits and vegetables, the evidence becomes less robust when considering only case-control and prospective data (Forman & Burley, 2006).

Data from a recent large European prospective study failed to establish an overall association between fruit or vegetable consumption and stomach cancer risk (González et al., 2006). Nevertheless, a statistically significant relationship was observed between total dietary vegetable content and gut histological subtype. A non-significant negative association was noted between cardiac cancer risk and citrus consumption. Interestingly, the impact of fruit and vegetable intake on H. Pylori. It was observed to be independent of its condition.

Vitamin C and other antioxidant nutrients have garnered attention as potential mediators of any dietary effect on stomach cancer risk. Vitamin C, with reduced levels in the serum of individuals infected with H. pylori, serves as a promising candidate by reducing the formation of nitroso compounds and acting as a free radical scavenger (Woodward et al., 2001). Case-control studies also provide evidence of a negative association between dietary vitamin C and stomach cancer risk (Mayne et al., 2001). Data from the EPIC cohort indicate a negative correlation between gastric cancer risk and serum vitamin C, while no such association exists with dietary vitamin C intake, suggesting that H. pylori infection does not influence its condition (Jenab et al., 2006). A Cochrane Collaboration review, incorporating high-quality randomized trials, concluded that there is no evidence supporting the reduction of stomach cancer risk through dietary supplementation with antioxidants, including vitamin C

(Bjelakovic et al., 2008). Salt and nitrite are additional dietary components implicated in stomach cancer risk, often used in food preservation. Pickled and smoked foods may contain nitroso compounds and potential carcinogens such as benzopyrene. Notably, the Japanese diet, rich in salted fish and pickled vegetables, underscores the role of dietary nitrate, partly sourced from water, which can be converted to nitrite by bacteria that synthesize nitrate reductase. The presence of H. pylori Infection and hypochloritis facilitates the growth of such bacteria, creating a synergistic effect in gastric carcinogenesis. While case-control studies show a positive association between gastric cancer risk and salt, dietary nitrate, and nitrite intake, prospective study data present conflicting results (Tokui et al., 2005).

Colorectal Cancer

Colorectal cancer constitutes 10% of new cancer cases globally and is the most common cancer type after prostate and lung cancers in men and breast and thyroid cancers in women (Mármol et al., 2019). Numerous nutrition-related risk factors, including diabetes, cholecystectomy, obesity, insulin resistance, and a diet high in fat, as well as excessive consumption of processed and red meat, contribute to the etiology of colorectal cancer (Gao et al., 2017).

Red meat consumption stands out as a prevalent nutrition-related risk factor for colorectal cancer. Studies indicate that a daily intake of 100g of red meat or 50g of processed meat increases the risk of colorectal cancer by 20%, with red meat consumption influencing the formation of colorectal adenomas. Micronutrients such as calcium, folic acid, and vitamin D also affect colorectal cancer risk, where calcium intake below 700-1000 mg/day is associated with an increased risk. Dietary fibers and their primary metabolites, short-chain fatty acids (SCFA), exhibit a protective effect against colorectal cancer (Lucas et al., 2017).

A meta-analysis study revealed that a daily intake of ten grams of supplemental dietary fiber reduces colorectal cancer risk by 10% (Ben et al., 2014). Adequate intake of antioxidants and vitamins diminishes colorectal cancer risk (Grazioso et al., 2019). Consumption of anti-inflammatory foods like turmeric, rosemary, garlic, ginger, onion, thyme, and saffron reduces the risk of colorectal cancer (Thanikachalam & Khan, 2019). The Mediterranean diet, recognized as a healthy dietary pattern, is associated with a decreased risk of colorectal cancer (Isabel & Luis, 2020).

Gallbladder Cancer

Gallbladder carcinoma is classified among biliary tract tumors and stands out as one of the most malignant cancers due to its frequent late diagnosis and low survival rate, often in advanced stages. Determining the incidence proves challenging for these reasons. However, adopting a diet rich in fresh fruits and vegetables while minimizing the consumption of sodium-rich pre-packaged foods can potentially influence the onset of the disease. Improved diet quality has been linked to a reduction in oxidative and inflammatory processes, mitigating carcinogenic effects and impacting the occurrence of gallstones. Both gallstones and reduced obesity are considered initial risk factors for this type of cancer (Larsson et al., 2017).

Pancreatic Cancer

Pancreatic cancer currently ranks as the fourth leading cause of cancer-related deaths in developed countries. Projections indicate that it could become the second leading cause within the next decade unless outcomes improve (Kleeff et al., 2016). The pancreas encompasses two distinct cell groups with endocrine and exocrine functions. Endocrine cells secrete insulin and glycogen hormones, regulating blood sugar levels. Exocrine cells release pancreatic sap to aid digestion, delivering it to the duodenum (Ermaya, 2022).

The pancreas produces enzymes necessary for digesting proteins, carbohydrates, and fats, the three fundamental nutrients. Enzymes in pancreatic fluid include lipase for fat digestion, amylase for carbohydrates, carboxypeptidase for proteins, and nuclease for nucleic acids. Three fundamental substances—acetylcholine, cholecystokinin, and secretin-regulate the secretion of both enzymes and secretions. Primarily, the exocrine function involves producing pancreatic fluid that enters the duodenum along with bile (Chandra & Liddle, 2014).

NUTRITION IN CANCER

Cancer, recognized as one of the leading global causes of death, is a complex disease arising from multiple interactions between genes and the environment. Malnutrition is observed in 40-80% of cancer patients on average (Ravasco, 2019). Consequently, nutrition assumes a vital role in cancer treatment, contributing to infection risk reduction, quicker recovery, enhanced tolerance of treatment-related side effects, sustained energy and strength, maintenance of nutrient stores and weight, and an overall improved sense of well-being (Bazzan et al., 2013).

Ketogenic Diet

The ketogenic diet, characterized by adequate protein, low carbohydrates, and high fat (Minzer, 2021), has emerged as a potential metabolic therapy in cancer. It aims to reduce insulin secretion and convert fatty acids into ketone bodies, promoting a shift to fat oxidation for fuel (Wang et al., 2020). This metabolic therapy is employed as a complementary or alternative approach in various cancer types (Woolf & Scheck, 2015). Aligned with preclinical studies, the ketogenic diet demonstrates the potential to slow down tumor development, halt progression, and prolong patient survival. Positive outcomes also include a reduction in metastatic potential. Additionally, the diet may incorporate fatty acids like MCT and omega 3, showcasing promising results in suppressing tumor growth, as evidenced in neuroblastoma patients (Weber et al., 2020). Long-term application of ketogenic diet therapy has shown improved prognoses, significantly enhancing patient survival rates. Cumulative research suggests its acceptance as a safe treatment method (Hagihara et al., 2020).

Neutropenic Diet

Neutropenia, frequently encountered in hematological malignancies and chemotherapy, occurs in approximately 80% of cancer patients during treatment. Neutropenic diet, also known as a sterile, low microbial, or low bacterial diet, plays a crucial role in non-pharmacological interventions to prevent infection in neutropenic patients. The diet aims to exclude foods abundant in pathogens, including various microorganisms found in fresh vegetables and fruits, uncooked animal secretions, dried fruits, and spices. Proper washing, storage, and cooking of foods become imperative in this context (Sözeri & Kutlutürkan, 2017).

Western Diet

Referred to as the "Western diet" or "supermarket diet," this dietary pattern prevalent in many modern Western countries involves frequent consumption of red and processed meats, sugar-sweetened foods, fried items, and refined grains. Recognized as an obesity-inducing diet, it is characterized by high energy and fat content, with macronutrients exhibiting heterogeneity in quantity and quality. Compared to balanced diet models defined by WHO, the Western diet is believed to be high in saturated fat, low in fiber, rich in simple carbohydrates, and contributes to chronic metabolic inflammation, a precursor to various non-communicable diseases, including cancer, responsible for 80% of deaths in Western societies (Christ et al., 2019).

Mediterranean Diet

The Mediterranean diet stands out for its high consumption of vegetables, fruits, fish, legumes, and unrefined grains, coupled with low amounts of red meat, milk, and dairy products. Olive oil serves as the primary fat source. Rich in low-energy, high-fiber, high-antioxidant and phenolic compounds, and unsaturated fat while low in saturated fat, the Mediterranean diet is employed as a nutritional therapy in preventing, managing, and treating chronic diseases. The diet recommends eight servings of whole grain products, 4-6 servings of fruit, 2-3 servings of vegetables, and 4-5 servings of fish per week. Its high fiber content exerts a cholesterol-lowering effect, regulates glucose levels, and demonstrates a protective effect against cancer (Barbaros & Kabaran, 2014).

The positive association between the Mediterranean diet and cancer stems from its antioxidant and anti-inflammatory properties. Regular consumption of fruits and vegetables rich in antioxidants, such as carotenoids, vitamins C and E, folates, and flavonoids, offers protective effects against cancer by preventing DNA damage. This diet has shown to reduce the risk of breast cancer, colorectal cancer, prostate cancer, and lung cancer by 60-70% (Castelló et al., 2017). Low consumption of red meat in the Mediterranean diet avoids the harmful effects associated with meat cooked at high temperatures, leading to reduced animal fat intake. Consequently, adherence to the Mediterranean diet is often associated with a lower risk of malignancies (Li et al., 2014). The diet's inclusion of omega-3 fatty acids from fish and oilseeds, particularly sardines and mackerel, can slow cancer development by influencing cell proliferation, inflammation, and metastasis (Castelló et al., 2017). Polyphenols found in olive oil, wine, and vegetables further contribute to cancer cell proliferation reduction and protection against metastasis (Praud et al., 2014).

In a meta-analysis conducted by Schwingshackl and fellow researchers, adherence to the Mediterranean diet revealed a substantial inverse correlation between cancer mortality and colorectal cancer risk. This beneficial outcome was predominantly attributed to increased consumption of fruits, vegetables, and whole grains. The meta-analysis, incorporating data from seven different cohort studies, reported a minor reduction (6%) in breast cancer risk for individuals following this dietary pattern (Schwingshackl et al., 2017).

In a study conducted by Erdrich et al., the adherence of 20 men with prostate cancer to the Mediterranean diet exhibited a notable decrease in reactive oxygen species. Compared to the baseline, there was a significant reduction in DNA damage, emphasizing potential protective effects (Erdrich et al., 2015). An epidemiological study in Spain demonstrated that high adherence to the Western diet heightened the risk of breast cancer in both premenopausal and postmenopausal women. Conversely, the Mediterranean diet exhibited a

potential protective effect against breast cancer, particularly in postmenopausal women (Solans et al., 2018).

CONCLUSION

Cancer stands as a prominent global health concern, prompting increased exploration into the impact of lifestyle factors on cancer risk. The Mediterranean diet is gaining considerable attention in the realm of healthy eating and cancer risk reduction. Research suggests that this diet may play a pivotal role in mitigating cancer risk, primarily through the presence of antioxidants, anti-inflammatory components, and high fiber content. Key elements such as olive oil, omega-3 fatty acid-rich fish, antioxidant-packed fruits and vegetables, and whole grains contribute significantly to the effectiveness of the Mediterranean diet in combatting cancer. These nutrients operate through mechanisms like controlling the development of cancer cells, preventing cellular damage, and reducing inflammation.

While the Mediterranean diet, when coupled with healthy eating habits, emerges as a crucial factor in diminishing cancer risk, its full implementation necessitates individuals to evaluate and adjust their lifestyles. Health professionals should underscore the Mediterranean diet's role in cancer prevention, guiding patients toward appropriate and sustainable nutrition plans.

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Chapter 16

Applications of *N*-glycosidases to Produce *N*-glycans from *Spirulina* Proteins

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INTRODUCTION

As the global population continues to expand, the growing concern over the dwindling supply of animal-derived proteins has become more pronounced. This has ignited a surge in discussions emphasizing the importance of plant-based protein sources and the urgent need for discovering novel plant-derived protein alternatives (Aiking, 2014). Recent findings have pointed out that specific algae species boast a protein content of up to 47% of their dry weight (Černá, 2011; Fujiwara-Arasaki et al., 1984), spotlighting them as potential protein sources.

Microalgae, due to their ability to thrive in barren soils with CO_2 and sunlight in saline water, have attracted substantial attention. Their unique growth requirements position microalgae as a promising and sustainable source for various industries, including fuel, food, chemicals, textiles, polymers, and pharmaceuticals (Viegas et al., 2015). Across Eastern nations like China, Korea, and Japan, seafood, notably seaweeds and invertebrates, has long played a significant role in daily diets, continuing to do so today (Cian et al., 2015). Seaweeds, as dietary components, offer rich reserves of dietary fibers (O'Sullivan et al., 2010), while polysaccharides derived from plants, such as alginates and agaroses, have demonstrated notable health benefits (Mohamed et al., 2012). Consequently, these polysaccharides are now globally recognized and utilized as nutraceuticals in both the food and pharmaceutical industries (Suleria et al., 2015; Wells et al., 2017).

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Spirulina, renowned as one of the most distinguished microalgae species, stands out for its remarkable protein content, exceeding 50% of its dry weight. Alongside its protein richness, *Spirulina* holds significant amounts of vitamins, pigments, fatty acids, and sterols, making it a compelling choice for the food industry. Given the growing interest in functional foods, nutraceuticals, and dietary supplements, *Spirulina*'s utilization in the food sector has experienced a noticeable surge in recent times.

Algae

Algae, commonly known as seaweeds, exhibit diverse characteristics and are classified into two main classes: macroalgae and microalgae. The size disparity between these classes is substantial, with macroalgae having the potential to reach lengths of several meters, while microalgae are typically measured in micrometers. Both categories thrive in various aquatic environments, including freshwater and marine habitats (Rajkumar et al., 2014). Macroalgae, predominantly found in oceanic waters, exhibit a wide distribution, extending from coastal areas to depths of up to 300 meters. These organisms, comprising multicellular structures, belong to the group of photosynthetic eukaryotes and possess relatively simple reproductive mechanisms, devoid of intricate root, stem, or leaf structures (Fleurence & Levine, 2016). The classification of algae extends further, encompassing three primary groups based on their pigmentation and chemical constitution: brown algae (Phaeophyceae), red algae (Rhodophyceae), and green algae (Chlorophyceae) (Xu et al., 2017). Renowned for their rich content of soluble dietary fibers, proteins, minerals, vitamins, antioxidants, phytochemicals, and polyunsaturated fatty acids, algae are recognized for their low-calorie nutritional profile (Mohamed et al., 2012). The compositional diversity of seaweeds varies significantly among species, seasons, habitats, and environmental factors, such as humidity, water temperature, light intensity, and nutrient concentration (Mabeau & Fleurence, 1993; Marinho-Soriano et al., 2006; Marsham et al., 2007).

In-depth investigations through in vivo studies have uncovered the therapeutic potential of red, brown, and green algae, revealing their multifaceted benefits including anti-cancer, anti-obesity, anti-diabetic, anti-hypertensive, anti-hyperlipidemic, antioxidant, anticoagulant, anti-inflammatory, immunomodulatory, antiestrogenic, thyroid-stimulating, neuroprotective, and antiviral properties (Mohamed et al., 2012). While algae are commonly integrated into Asian cuisines, their utilization in Western societies is more localized to coastal regions but has recently attracted growing attention as a valuable dietary source (Macartain et al., 2007).

Microalgae, smaller photosynthetic organisms thriving in both freshwater and marine habitats, are classified based on various characteristics including pigmentation, storage products from photosynthesis, membrane arrangements for photosynthesis, and morphological attributes (Rajkumar et al., 2014). Presently, microalgae are grouped into four primary categories: diatoms (Bacillariophyceae), green algae (Chlorophyceae), blue-green algae (Cyanophyceae), and golden algae (Chrysophyceae) (Khan et al., 2009). Notable microalgae species employed in commercial production encompass *Isochrysis*, *Chaetoceros*, *Chlorella*, *Arthrospira* (*Spirulina*), and *Dunaliella*. Their unique growth requisites, thriving in desolate soils with CO_2 and sunlight in saline water, position microalgae as a potentially sustainable resource for diverse industries such as fuel, food, chemistry, textiles, polymers, and pharmaceuticals (Viegas et al., 2015). Rich in lipids, proteins, and carbohydrates, the protein concentrates derived from microalgae serve various applications in the food, feed, and chemical sectors (Chacón-Lee & González-Mariño, 2010). Considering that microalgae proteins encompass all essential amino acids, they hold immense promise as an alternative and comprehensive protein source (Barbarino & Lourenço, 2005; Becker, 2007; Lourenço et al., 2004; Safi, Liu, et al., 2014; Safi, Ursu, et al., 2014).

Table 1: Different Types of Algaes and Their Nutritional Values (% of dry matter)

Algae	Protein	Carbohydrates	Lipid
Anabaena cylindrica	43-56	25-30	4-7
Aphanizomenon flos-aquae	62	23	3
Vhlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-59	12-17	14-20
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirogyra sp.	6-20	33-64	11-21
Arthrospira maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Spirulina maxima	60-71	13-16	6-7
Synechococcus sp.	63	15	11

(Becker, 2007)

Spirulina, recognized as a prominent microalgae species, represents a filamentous cyanobacterium with distinctive multicellular traits and a robust composition comprising proteins, vitamins, pigments, long-chain polyunsaturated fatty acids, sterols, and an array of bioactive compounds. Its protein content, exceeding 50% of the dry weight (Table 1), positions *Spirulina sp.* as an invaluable source of essential amino acids, including lysine, leucine, isoleucine, tryptophan, and valine (Andrade, 2018). Moreover, *Spirulina* contains phycobiliproteins, specialized pigments integral as light receptors for photosynthesis. These compounds, structured with chromophores connected to cysteine residues of an apoenzyme, fall into three classifications: phycocyanin, allophycocyanin, and phycoerythrin (Santiago-Santos et al., 2004). Phycoerythrins, comprising approximately 15-25% of the microalgae's dry weight (Romay et al., 2003), serve as natural food colorants in non-acidic products like chewing gums, confectionery, and dairy goods (Downham & Collins, 2000). Their blue color makes phycocyanins highly sought-after as natural dyes in food and cosmetics, potentially possessing anti-inflammatory, antioxidant, and anticancer properties (Reddy et al., 2003).

The term "*Spirulina*" encompasses diverse filamentous, multicellular, blue-green microalgae comprising nearly 15 species, classified under the genera *Spirulina* and *Arthrospira*. Specifically, "*Spirulina*" denotes the dried masses of these microalgal species marketed as dietary supplements. Among these species, *Spirulina* (*Arthrospira*) *platensis* remains the most widespread and extensively researched genus, particularly in fields like the food industry and medicine (Beheshtipour et al., 2012). Chemical analyses of *Spirulina* underscore its exceptional composition, serving as an outstanding source of essential macro and micronutrients, such as proteins, vitamins, essential amino acids, dietary minerals, and essential fatty acids. The diverse nutritional profile of *Spirulina* contributes to numerous health advantages encompassing immune modulation, anticancer, antioxidant, antiviral, and antibacterial properties. Additionally, it exhibits beneficial effects in managing various conditions like malnutrition, hyperlipidemia, arthritis, obesity, cardiovascular diseases, diabetes, heavy metal/chemical-induced toxicity, inflammatory allergic reactions, radiation damage, and anemia (Lee et al., 1998; Lorenz & Corporation, 1999). Commercially cultivated for its immune-boosting properties, *Spirulina sp.* also aids in fostering lactic acid bacteria growth within the gastrointestinal system, aiding in the restoration of bodily hormones (Sharma et al., 2007).

Glycans and Glycosylation

Glycosylation, an intricate and widespread post-translational modification in proteins, is characterized by the attachment of carbohydrate-based molecules (glycans) to protein surfaces, a fundamental chemical alteration essential for diverse biological functions (Apweiler et al., 1999; Lehle et al., 2006; Mann & Jensen, 2003; Sears & Wong, 1998; Walsh et al., 2005; Weerapana & Imperiali, 2006). This process exhibits a remarkable structural diversity, presenting variations both in the sites of glycan attachment (macroheterogeneity) and the inherent glycan structures (microheterogeneity). The precise glycosylation patterns of therapeutic glycoproteins are believed to significantly influence their functional attributes (Mamedov & Yusibov, 2011).

Beyond their role in protein stabilization and intercellular communication, glycans have been found to play crucial roles in various biological processes. Studies underscore their significance in preventing pathogen adherence to intestinal epithelial cells, ameliorating rotavirus-induced diarrhea in infants, and reducing leukocyte adhesion to endothelial cells (Bode, 2006). Sialic acid, a vital component of glycans, has also been implicated in infant brain development and the enhancement of learning capabilities (Morgan & Winick, 1980). Through glycosylation, these saccharides interact with diverse biological molecules via *O*-glycosidic or *N*-glycosidic bonds. *N*-linked glycans (*N*-glycans) specifically attach to defined asparagine residues in protein sequences, forming cores composed of two HexNAc and three mannose residues synthesized within the endoplasmic reticulum. On the other hand, *O*-linked glycans (*O*-glycans) bind to hydroxyl groups of serine or threonine amino acids, producing distinct cores (Varki et al., 2017). Various glycan structures are synthesized by incorporating different glycan monomers into *N*- and *O*-linked glycan cores through enzymatic processes involving glycosyl transferase and glycosidase enzymes (Rini et al., 2022).

Notably, the excessive branching observed in *N*-glycans has often been correlated with cancer cells and their progression (Varki et al., 2017), prompting focused research on *N*-glycans in cancer therapeutics for potential breakthroughs. Additionally, *N*-glycans intricately regulate crucial proteins in eukaryotic secretory pathways, influencing protein folding and the glycoprotein balance (Helenius, 2001), offering promising avenues for targeted medication development across various diseases (Hebert et al., 2014).

Recent studies investigating *N*-glycosylation in glycoproteins across diverse organisms, including yeast, insects, mammals, and plants, have revealed predominant high-mannose glycans in green microalgal proteins. Some species even exhibit hybrid and complex *N*-glycan structures. However, despite these

findings, there remains a considerable gap in our understanding of the *N*-glycosylation pathway and the specific *N*-glycan structures in green algae (Baïet et al., 2011; Levy-Ontman et al., 2011).

Conclusion

In today's context, an escalating inclination towards healthy dietary habits and mindful consumption has steered individuals toward exploring diverse protein sources, especially those stemming from plant-based origins. It's intriguing to note that in densely populated Asian nations, several types of algae have gained prominence as significant dietary elements, addressing the nutritional requirements of the populace. Within this array of plant-based protein sources, *Spirulina* has emerged as a standout due to its exceptionally high protein content, marking its position as one of the most noteworthy products available. Consequently, there's a burgeoning necessity to expand and intensify ongoing research endeavors aimed at delving deeper into the potential protein functionalities offered by *Spirulina*. This exploration is fundamental in comprehending its role and maximizing its benefits within the broader spectrum of dietary protein sources.

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Chapter 17

A Brief Overview of miRNAs Orchestrating the Pathogenesis of Psoriasis

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Abstract

Psoriasis is a complex chronic inflammatory skin disorder triggered by environmental factors in genetically susceptible individuals. Genomic and immunological studies have guided the development of novel therapies. However, the mechanisms related to the pathophysiology of the chronic inflammatory process, particularly its association with the immune system and epigenetics, have not been fully elucidated. Summarizing miRNA alterations associated with psoriasis in recent studies could represent a groundbreaking revolution in understanding and treating the disease. microRNAs (miRNAs) are vital non-coding RNA molecules playing crucial roles in regulating gene expression within cells, with significant implications for biological processes and diseases. The discovery of miRNAs as variable small regulatory molecules in psoriasis has brought significant advancements. Previous studies have reported critical roles of miRNA alterations associated with the severity of psoriasis. Additionally, the mechanisms of action and functions of miRNA-targeted therapies remain a focal point of ongoing research in psoriasis. This section summarizes the alterations in miRNA molecules involved in psoriasis-related signaling pathways, the discovered miRNA biomarkers, and therapeutic targets, outlining potential directions for future functional studies. Moreover, it may facilitate the generation of new ideas among researchers interested in this field, thereby advancing translational therapies for psoriasis in the clinic and aiding in monitoring therapeutic responses.

Keywords: Psoriasis, miRNA, non-coding RNA, inflammation, immunity, keratinocyte

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Introduction

Psoriasis is a chronic inflammatory skin condition that impacts about 2-3% of the world's population. It is characterized by the abnormal activation of epidermal keratinocytes, irregular neovascularization, and the disruption of immune cell regulation (Pasquali, 2020). It is driven by a coordination of genetic predisposition, environmental triggers, and dysfunctional immune system interactions, particularly abnormal interactions between keratinocytes and T cells. Roughly 125 million people around the world are affected by psoriasis, which has a significant influence on their quality of life and mental health (Lee & Kim, 2023).

Psoriasis is classified into five types: psoriasis vulgaris, inverse, guttate, pustular, and erythrodermic psoriasis. Psoriasis vulgaris, accounting for approximately 80-90% of psoriasis cases, is known as chronic plaque psoriasis commonly spreading on the trunk, extensor surfaces of limbs, and scalp. Clinical diagnostic symptoms involve sharply demarcated, erythematous plaques surrounded by silvery scales, which may coalesce to cover large areas of the skin (Sbidian et al., 2023). Inverse psoriasis affects intertriginous areas with mild erosive erythematous plaques and lesions. Guttate psoriasis commonly affects children or young adults and is often triggered by group A streptococcal tonsil infections. Additionally, around one-third of patients with guttate psoriasis go on to develop plaque psoriasis in adulthood. Pustular psoriasis is characterized by multiple coalescing sterile pustules, which can be localized or generalized. Diffuse pustular psoriasis presents an acute and rapidly progressive course with widespread erythema and subcorneal pustules, usually associated with systemic symptoms (Buja et al., 2023; X. Wu, Ma, Wang, & Qin, 2023).

At the onset of psoriasis, there is an increase in the proliferation of epidermal keratinocytes and abnormal differentiation, along with uncontrolled interactions with immune T cells. This leads to recognized dysregulated interactions between keratinocytes and infiltrating immune cells including neutrophils, mast cells, innate lymphoid cells, and subsets of T cells (Schön & Wilsmann-Theis, 2023). In the disease's progression, cytokines such as interleukins (ILs) (IL-1, IL-17, IL-22, IL-23, etc.), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and transforming growth factor-β (TGF-β) are key players in cell-cell interactions. Psoriasis emerges from a multifactorial pathogenesis involving interactions between various immune cells and keratinocytes, secretion of cytokines like IFN-γ, TNF-α, and some cytokines belonging to the interleukin family (Keskin, Cakir, & Acikgoz, 2023). Due to its complex formation mechanisms and being a recurrent disease, psoriasis lacks a definitive cure to date, and current treatments aim for acute and symptomatic relief. The available options for treating psoriasis include topical treatments, phototherapy, and systemic therapy. Topical

treatments such as vitamin D analogs (calcipotriol) or corticosteroids serve as easy and primary treatments. Secondary treatments include phototherapy (narrowband ultraviolet B radiation (NB-UVB) and ultraviolet A radiation with psoralen (PUVA)) and conventional systemic agents (methotrexate, cyclosporine, and acitretin) (Constantin, Surcel, Munteanu, & Neagu, 2023; Kapoor, Gulati, Rani, & Gupta, 2022). NB-UVB has largely replaced PUVA due to the skin cancer risk associated with cumulative PUVA doses. Methotrexate targets lymphocyte activation through multiple mechanisms, including dihydrofolate reductase inhibition, aminoimidazole carboxamide ribotide transformylase (AICARTase) blockade, and adenosine accumulation (Lee & Kim, 2023). Biologics are monoclonal antibodies or soluble receptors targeting proinflammatory cytokines with therapeutic effects in mild and severe psoriasis. Multiple biologic therapies have been approved for moderate-to-severe chronic conditions, including TNF-α inhibitors adalimumab, etanercept, infliximab, and certolizumab, IL-12/23p40 inhibitor ustekinumab, IL-23p19 inhibitors rizankizumab, guselkumab, and tildrakizumab, and IL-17 inhibitors ixekizumab and secukinumab (Megna et al., 2023; Sbidian et al., 2023). Despite their high efficacy, biologics require regular subcutaneous or intravenous administration. Oral small molecule inhibitors such as apremilast (a phosphodiesterase 4 inhibitor) and dimethyl fumarate have been approved for mild-to-severe psoriasis. Dysfunctions in signaling pathways (mTOR, JAK/STAT, and MAPK) also play roles in the pathogenesis and progression of psoriasis (M. Wu, Dai, & Zeng, 2023). Clinical trials are ongoing for small molecules blocking tyrosine kinase 2 in the Janus kinase signal transducer and activator (JAK-STAT) signaling (Saygılı & Erbaş, 2019).

The IL-23/IL-17A axis is a significant triggering factor in disease development, and biological molecules blocking IL-17A or IL-23 have shown efficacy in clinical treatment, though disease recurrence after a certain period indicates the complexity of psoriasis and suggests that current understanding of its mechanisms is far from complete (Takeshita et al., 2017). Recent advancements in psoriasis research indicate its multifactorial nature and the inadequacy of traditional research focused on a unified signaling axis or biomarkers. Moreover, there is yet no definitive biomarker with diagnostic and prognostic value for psoriasis. However, emerging evidence from new molecular techniques, particularly the regulation of target gene expression mediated by microRNAs (miRNAs), has revealed the significant role of the epigenetic factor in the development and progression of psoriasis (Aslani et al., 2024; Xiuli & Honglin, 2021). These findings demonstrate that miRNAs are abnormally expressed in skin lesions and plasma as a result of psoriatic inflammation, with

various miRNAs and their target genes contributing to the disease's development and progression through different signal transduction pathways. Thus, miRNAs are garnering significant attention for research in clinical applications to elucidate the pathophysiological mechanisms of psoriasis.

microRNAs (miRNAs) biogenesis and regulation

miRNAs are small, non-coding RNA molecules that regulate gene expression by binding to messenger RNA (mRNA) molecules and inhibiting their translation into proteins. Approximately 5000-10,000 miRNAs have been identified in mammals since their initial discovery in *Caenorhabditis elegans* in 1993 (Sonkoly et al., 2007; Tiucă et al., 2023; Wahid, Shehzad, Khan, & Kim, 2010). miRNAs constitute 1-5% of all genes in the human genome. It has been demonstrated that about 20-60% of protein-coding genes are regulated by miRNAs. Comprising approximately 22 nucleotides, miRNAs modulate the genetic information of the cell, affecting protein production (Danielsen, Olsen, Wilsgaard, & Furberg, 2013; García–Rodríguez et al., 2017). miRNAs play crucial roles in the normal development, differentiation, growth, apoptosis, and various biological processes of cells. The biogenesis of miRNAs involves distinct stages. miRNAs are encoded within DNA by specific genes (Ha & Kim, 2014; Ivey & Srivastava, 2015). These genes are first transcribed into "primary miRNA" (pri-miRNA) by RNA polymerase II or III. In the nucleus, pri-miRNA is cleaved by an RNA-severing enzyme called Drosha, creating an intermediate structure known as "precursor miRNA" (pre-miRNA) of approximately 70 nucleotides in length. The pre-miRNA is then transported to the cytoplasm via nuclear pores. In the cytoplasm, pre-miRNA is processed by another RNA-severing enzyme called Dicer, converting it into a mature miRNA of about 22 nucleotides in length. The mature miRNA is then loaded into the RNA-induced silencing complex (RISC). The miRNA loaded into RISC specifically binds to the 3' untranslated region (UTR) of target mRNA molecules. This binding leads to a decrease in the stability of the target mRNA or inhibition of its translation, thus reducing or preventing protein production (Friedman, Farh, Burge, & Bartel, 2009; Sbidian et al., 2023; Wahid et al., 2010; Zibert et al., 2010). The fate of the bound mRNA depends on the degree of complementarity with the miRNA; it is either degraded or its translation is inhibited. These processes constitute the fundamental basis for the ability of miRNAs to fine-tune gene expression in cells. Through these regulations, miRNAs help cells adapt to various situations, contribute to the development of diseases, or protect against them (Ha & Kim, 2014; Tétreault & De Guire, 2013; Zibert et al., 2010).

Roles of miRNAs in psoriasis

The use of microRNAs (miRNAs) in the treatment and research of psoriasis has been evolving since the altered miRNA expressions in psoriatic skin were first described in 2007 (El-Komy, Amin, El-Hawary, Saadi, & Shaker, 2020; Treiber, Treiber, & Meister, 2019). Over 250 miRNAs have been discovered to be differentially expressed in psoriatic skin or blood tissue since then. These miRNAs play a crucial role in regulating keratinocyte proliferation, differentiation, apoptosis, and cytokine production, as well as T cell activation and function. This knowledge emphasizes their potential in understanding the pathogenesis of psoriasis and creating advanced therapeutic approaches. They also influence the activation and functions of immune cells, directing cutaneous inflammation and disease symptoms (Pradyuth et al., 2020; Raharja, Mahil, & Barker, 2021; S. C. Yang et al., 2022). Therefore, understanding the altered expressions of miRNAs can reveal underlying mechanisms of the disease and offer new avenues for the development of more effective treatment methods.

The most significant miRNA in psoriasis may vary depending on the context and specific mechanisms of the disease, but miRNAs such as miR-146a, miR-203, and miR-125b are often emphasized due to their roles in inflammation, immune function, and keratinocyte regulation in psoriatic disease (Antonatos, Asmenoudi, Panoutsopoulou, & Vasilopoulos, 2023; Sbidian et al., 2023; Tiucă et al., 2023; Tomar, Gorantla, & Singhvi, 2023). Each plays a critical role in the pathogenesis and progression of psoriasis, making them key targets for research and potential therapeutic intervention. Some of the most studied miRNAs in psoriasis research include miR-146a, miR-203, miR-125b, miR-21, and miR-31. These miRNAs are often highlighted for their significant roles in regulating inflammation, immune response, and skin cell proliferation and differentiation, which are key aspects of psoriasis pathology (Krishnan & Kõks, 2023; Madaan et al., 2023).

miR-146a is involved in regulating immune response and inflammation (Diotallevi et al., 2023); miR-203 in skin differentiation, proliferation, and formation of psoriatic lesions (Mostafa et al., 2022); miR-125b in inflammatory pathways and immune responses (K. Yan et al., 2023); miR-21 in inflammatory processes and T-cell regulation (Meisgen et al., 2012); and miR-31 in keratinocyte proliferation and the pathogenesis of psoriatic lesions (Borska et al., 2017). These miRNAs are frequently targeted due to their regulatory roles in immune functions, keratinocyte behavior, and inflammatory responses in psoriasis.

In psoriatic plaques, a reduction in the expression levels of certain miRNAs (miRNAs) has been observed. This decrease in miRNAs can play significant roles

in the pathogenesis of psoriasis, as these RNA molecules regulate gene expression, affecting skin inflammation, keratinocyte functions, and immune system responses (Timis & Orasan, 2018). However, detailed and updated scientific research is necessary to identify which specific miRNAs are decreased in psoriatic plaques. Reduced miRNA levels can disrupt the normal functions of keratinocytes, the primary type of cells in the skin's outer layer, contributing to the formation of characteristic psoriatic skin lesions. The decrease in certain miRNAs may lead to the overactivation of immune system cells and inflammation, as well as the disruption of inflammatory processes, exacerbating psoriatic inflammation and symptoms such as erythema (Pradyuth et al., 2020; Smith et al., 2020; C. Yang et al., 2020).

The initial microRNA (miRNA) discovered in individuals with psoriasis is miR-203. Research has revealed that miR-203 can suppress the expression of SOCS3 by directly connecting to the 3'-UTR of SOCS3. The function of SOCS3 is to degrade the mRNA structure, which then activates the JAK2/STAT3 signaling pathway, which is crucial for the progression of psoriasis. The absence of SOCS-3 results in continuous activation of STAT3 in response to IL-6, a cytokine produced in psoriasis lesions. This suggests that miR-203 suppresses SOCS-3 in psoriatic lesions, causing persistent activation of STAT3. The activation of STAT3 in keratinocytes, which is already present, can cause psoriasis to develop on its own. One potential consequence of this activation is that miR-203 may be upregulated, which could have important implications for the development of psoriasis. Specifically, miR-203 may prevent the upregulation of SOCS3 in response to cytokines, which could play a role in the development of psoriasis (Annese, Tamma, De Giorgis, & Ribatti, 2020; Pradyuth et al., 2020).

One example of a miRNA variant is miR-155, which holds significant importance across a range of physiological and pathological processes, such as hematopoietic differentiation, immunity, inflammation, cancer, and cardiovascular disease (Friedman et al., 2009). The relationship between the levels of miR-155 in peripheral blood mononuclear cells and the psoriasis area and severity index (PASI) score of patients has been discovered to be positively correlated (Sonkoly et al., 2007).

miR-223 and miR-143 have been observed to be increased in psoriasis patients and display a positive correlation with the PASI score. Moreover, certain miRNAs have been discovered to be decreased in psoriasis tissues, and they contribute to the development of psoriasis (Bantwal, Shetty, Girisha, & Noronha, 2023; Ganguly, Laha, Senapati, Chatterjee, & Chatterjee, 2023).

The expression of miR-320 is reduced in psoriasis tissues, and it may play a role in the development of psoriasis by controlling STAT3 and SAPK/JNK signaling pathways. Additionally, it has been found that miR-205-5p is decreased in psoriasis skin tissue and may negatively regulate the Wnt/β-catenin signaling pathway, potentially reducing psoriasis symptoms triggered by imiquimod (IMQ) (Lowes, Bowcock, & Krueger, 2007). The expression of miR-194-5p has been observed to decrease significantly in both cell models and patients diagnosed with psoriasis. Furthermore, this decrease in miR-194-5p expression has been implicated in the progression of psoriasis by enabling the proliferation, migration, and increased production of keratinocytes in response to IL-22 (Faraoni, Antonetti, Cardone, & Bonmassar, 2009).

The miR-146 family comprises of miR-146a and miR-146b, which are encoded by genes situated on chromosomes 5 and 10, respectively. According to a study, miR-146a exhibits overexpression in lesioned skin and peripheral blood mononuclear cells (PBMCs) of individuals suffering from psoriasis (García–Rodríguez et al., 2017). According to Srivastava et al., miR-146a has a protective effect against early onset psoriasis. When the genetic makeup of miR-146a was altered, it resulted in increased skin inflammation in miR-146a -/- mice in response to imiquimod stimulation. This overexpression led to a decrease in neutrophil infiltration and an increase in erythema, epidermal thickness, and scaling (Srivastava et al., 2017). miR-146b has been reported to potentially assist miR-146a in suppressing the inflammatory response in psoriasis (S. C. Yang et al., 2022).

miR-21 is responsible for T-cell activation and inhibition of apoptosis. It also appears to be effective in keratinocyte proliferation and inflammation (IL-1β, CCL5, and CXCL10) (Shen et al., 2022). In vitro, LncRNA MEG3 is regulated by and directly targets CASP8 to regulate keratinocyte proliferation (He et al., 2021). Additionally, it stimulates cell growth by controlling the activity of the AKT/PI3K and TGFβ signaling pathways (Meisgen et al., 2012; Xia et al., 2012). In terms of its role in inflammation, UVB exposure has promoted the upregulation of miR-21-3p in keratinocytes, leading to the production of proinflammatory cytokines IL-6 and IL-1β, and chemokines CCL5 and CXCL10 in keratinocytes (Jia et al., 2019). The level of miR-21 expression is elevated in both TH1 and TH2 T cell subsets following stimulation with anti-CD3 and anti-CD28, implying that this microRNA plays a role in T cell activation regardless of the cell type. Additionally, it exhibits an anti-apoptotic function in activated T cells (C. Yang et al., 2020).

miR-489-3p negatively regulates TLR4 expression at the post-transcriptional level, inhibiting the TLR4/NF-κB pathway and consequently preventing the proliferation of keratinocytes and the secretion of inflammatory cytokines. These results highlight miR-489-3p as a promising therapeutic target for psoriasis (Degueurce et al., 2016).

miR-17-92 is a group of microRNAs that control cell growth and immune responses in psoriasis. The specific microRNA, miR-17-3p, was examined by Li et al. (2022) for its influence on keratinocyte division and the release of pro-inflammatory cytokines, and its connection to psoriasis (Li et al., 2022). The research initially revealed that miR-17-3p was increased in psoriatic skin lesions, and bioinformatic analysis suggested that CTR9 might be a target gene of miR-17-3p. The group confirmed that suppressing CTR9 is partly responsible for the effects of miR-17-3p in keratinocytes. These results indicate that miR-17-3p partially regulates keratinocyte proliferation and proinflammatory cytokine secretion by targeting CTR9, leading to the inactivation of downstream STAT3 protein, suggesting miR-17-3p as a potential therapeutic target for psoriasis.(Ye, Wang, & Zhou, 2021).

The Frizzled (FZD) family comprises atypical G protein-coupled receptors that serve as receptors in the wingless associated integration site (Wnt)/β-catenin pathways and other signaling pathways, playing a crucial role in cell proliferation. Activation of Wnt occurs when its ligand binds to the FZD receptor protein. By analyzing FZD expression in lesioned skin of psoriasis patients, miR-125a has been found to be closely associated with immunity and inflammation. Additionally, miR-99a has been reported to be down-regulated in psoriatic lesions by targeting FZD5 and FZD8 (Li et al., 2022; S. C. Yang et al., 2022).

Yan et al. found that miR-145-5p is down-regulated in psoriatic lesions and plays a significant role in suppressing epidermal keratinocyte proliferation and IL-17A-induced secretion of chemokines including CCL2, CCL7, CCL20, CXCL1, CXCL2, CXCL5, CXCL8, and CXCL10. MiR-145-5p positively regulates NF-κB and STAT-3 signaling by targeting mixed lineage kinase 3 (MLK3). This study reveals that down-regulation of miR-145-5p both promotes epidermal thickening and exacerbates skin inflammation in psoriasis (Kasprzak, 2020; J. J. Yan et al., 2019).

According to Tang et al., miR-187 alleviated symptoms in both cytokine-activated HaCaT cell line and lesioned skin of psoriasis patients. To enhance miR-187 levels, they administered miR-187 agomir intradermally to imiquimod-treated psoriasiform mice. Consequently, they observed that overexpression of miR-187 resulted in a reduction in acanthosis and inflammation in mice by inhibiting hyperproliferation through targeting CD276, a B7-CD28 family

member immune checkpoint molecule. Additionally, miR-193b-3p, another anti-inflammatory miRNA, has been utilized to treat psoriasis (Tang et al., 2019).

The roles and effects of miRNAs in the development of psoriasis, a persistent immune-related inflammatory skin condition, can be grouped into five main categories.

I. Regulation of keratinocyte functions: miRNAs play a role in regulating keratinocyte proliferation and differentiation. In psoriasis, irregular miRNA expression significantly affects the proliferation and/or differentiation processes of these cells, contributing to the formation of the characteristic thickened, scaly plaques of psoriatic lesions (Tembhre, Imran, & Jaiswal, 2023).

II. Mediation of immune dysfunction: Psoriasis is characterized by immune dysregulation involving T cells, dermal dendritic cells, Langerhans cells, and other immune cells. miRNAs influence the function of these immune cells, affecting the overall immune response in psoriasis and contributing to the inflammatory aspect of the disease (Tarannum, Priya, Pandey, & Jain, 2023).

III. Inflammation and cytokine production: miRNAs play a role in regulating cytokine and chemokine production in keratinocytes. Cytokines and chemokines are signaling proteins that mediate and regulate immunity, inflammation, and hematopoiesis. In psoriasis, the dysregulation of these proteins leads to an increased inflammatory response, a hallmark of the disease (Madaan et al., 2023; Man, Orăsan, Hoteiuc, Olănescu-Vaida-Voevod, & Mocan, 2023).

IV. Psoriasis potential biomarkers: The potential use of circulating microRNAs (miRNAs) in the blood of psoriasis patients as biomarkers for diagnosis, prognosis, and monitoring therapy responses is promising. Specific miRNA profiles in psoriatic skin, blood, and hair samples can potentially reflect the severity of the disease and help assess the effectiveness of treatments (Haschka et al., 2023; Jiang et al., 2023).

V. Development of new therapeutics: Given their regulatory role in psoriasis pathogenesis, targeting specific miRNAs could be a novel therapeutic approach. Therapies could involve the use of miRNA antagonists, miRNA mimics, or the topical delivery of exogenous miRNAs to modulate the disease process (Krishnan & Kõks, 2023; Laha, Senapati, Chatterjee, & Chatterjee, 2023).

miRNAs play a vital role in the development of psoriasis due to their ability to control keratinocyte functions, regulate immune responses, and influence cytokine production. Additionally, they may serve as potential biomarkers and therapeutic targets. Despite their potential, further research is necessary to fully understand their functions and to effectively apply this knowledge in clinical practice.

Conclusion

Using specific miRNA profiles can aid in the diagnosis of diseases and tracking their progression. The levels of miRNAs in various bodily fluids, such as tissues, blood, and others, can indicate the presence of psoriasis and serve as a measure of the response to treatment. Since miRNAs regulate a vast network of genes and biological pathways, their universality is crucial for understanding complex biological processes in cells and organisms. As a result, miRNAs have emerged as crucial molecular tools for comprehending biological processes, pathological conditions, diagnosing, and treating diseases. Consequently, investigating miRNA profiles in inflammatory skin diseases is a significant area of innovative and translational research in molecular biology, immunogenetics, cellular therapeutics, and preclinical applications.

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Chapter 18

Emotional Eating and Theories

Gökhan DEGE[1]

1- INTRODUCTION

Every individual needs a certain number of calories per day to survive; this is related to biological hunger. Factors such as gender, age, height, weight and muscle mass, as well as metabolic factors, influence this calorie requirement. The calorie intake of individuals with biological hunger usually does not exceed the daily requirement. Individuals without eating disorders or emotional eating behaviors usually eat on a regular basis at certain times of the day or when they are hungry. Biological hunger is also called homeostatic hunger today (Ayyıldız et al., 2021).

The act of eating occurs physiologically as a result of symptoms such as feeling hungry, stomach rumbling and noise. However, the desire to eat can also occur outside of physiological hunger. Consuming delicious foods only for pleasure without feeling the need for energy is called hedonic eating (Köse et al., 2015). In this case, eating is considered as a pleasurable activity by stimulating pleasure zones in the body. Another concept that affects the state of eating and drinking by stimulating the urge to eat other than hunger-fullness signals is emotional eating. Emotional eating occurs with the desire to eat caused by negative or positive emotional states such as loneliness, sadness, anger, happiness, anxiety (Litwin et al., 2017).

Various alternative dietary methods are recommended for different illnesses (Çağıran and Yılmaz, 2022).Eating behavior is formed as a result of the interaction of psychological, physiological, genetic and social factors that affect the timing of the need for food, food preference and the amount to be taken (Grimm & Steinle, 2011). Although this behavior differs from individual to individual with the influence of different cultures and personality traits, it can be affected by the current mental state of the individual, sociological characteristics, experiences, external factors and the individual's appetite. Eating behavior is shaped by the characteristics of the society in which the individual is born and over time, it turns into negative eating habits such as unconscious eating, fast eating, and skipping meals (Dege & Yildirim, 2023; Ünal Aslan et al., 2022).

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Apart from the biological hunger felt by emptying the stomach during the day, there are different known types of hunger. These hunger types can be divided into three as biological hunger (homeostatic hunger), hedonic hunger (sensory hunger) and emotional hunger (Çolak & Aktaç, 2019).

Hedonic hunger is a type of hunger that occurs in the case of enjoying food. The individual often turns to delicious foods and uses food to reward himself (Gündüz et al., 2020). In hedonic hunger, more calorie intake than necessary can be seen (Coşkunsu, 2021). Individuals may also start to gain weight when they start taking more calories than the average daily calorie intake (Alpcan & Arıkan Durmaz, 2015; Yau & Potenza, 2013). Emotional hunger is another type of hunger that can lead to excess calorie intake, as it creates the need to eat even when full. Emotional eating behavior is directly related to emotional hunger. Emotional hunger can be defined as a state of hunger that occurs in response to emotional triggers (Braden et al., 2018; Yildiz et al., 2020)

Emotional eating, as defined by Bekker et al. (2004), is an eating behavior that occurs not only because of hunger, meal time or social obligation, but also in response to specific emotions (Senturk et al., 2023). At the same time, Bekker et al. (2004) stated that emotional eating can also be considered as an avoidance coping strategy due to the avoidance of feeling and expression of negative emotions. Emotional eating (i.e., eating in response to negative emotions), especially overeating, is based on the assumption that it makes individuals feel better and functions to distract them from negative emotions. Individuals who experience NDE experience a constant feeling of hunger, often without being fully aware of what they are feeling. However, they may resort to emotional eating behavior to eliminate various negative situations (Öge, 2020).

Emotional hunger is rooted in a variety of causes, which often overlap with the causes of emotional eating. Although it is related to psychological factors, it is also known that emotional hunger has a spiritual and spiritual dimension. In this regard, there are various studies and experiments showing that especially mindfulness meditations have a reducing effect on emotional eating (Epel et al., 2019; Katterman et al., 2014). Eating behavior is not only a physical act, but also important for an individual's psychology (Arslantaş et al., 2019). In emotional hunger, which is related to psychology, the individual wants to eat to cope with negative emotions even though they do not physically need to eat (Bilgen, 2018). The eating behavior exhibited during emotional hunger is called emotional eating behavior (EIB). The term emotional eating, which is "emotional eating" in English, is also referred to as "stress eating" and "emotional overeating" in English sources (Bjørklund et al., 2018). Another definition of NDE is

"overeating during dysphoric moods such as feeling lonely, depressed or anxious" (De Lauzon-Guillain et al., 2006, p. 133).

Therefore, emotional eating can be defined as an eating impulse or tendency that occurs independently of hunger when faced with negative emotions. This may be caused by intense feelings of dissatisfaction in the individual. The resulting cycle of excessive and high-calorie eating can lead to weight gain and the development of obesity (Çağıran and Yılmaz, 2023). The higher feelings of dissatisfaction that arise as weight is gained can create a vicious cycle of overeating and weight gain. In a study on the causes of eating in response to negative emotions (Van, 2018), it was reported that conditions such as excessive calorie restriction, poor interoceptive awareness, alexithymia (inability to express emotion), emotion dysregulation, or disruption of the hypothalamic-pituitary-adrenal (HPA) axis in dieters may contribute to this behavior.

When emotional eating continues with sedentary behavior, individuals may face the risk of weight gain and obesity (Koenders et al., 2011; Yılmaz, 2021). In addition, obese individuals' responses to appetite may be weaker to emotional eating behavior than normal weight individuals. Bruch (Bruch, 1964) defines emotional eating as eliminating the desire to eat by matching the feeling of physiological hunger with the negative feeling brought on by the negative emotion, and not being able to distinguish it from the symptoms of real hunger.

In a study of 1562 working individuals, Koenders et al. (Koenders et al., 2011) found that high levels of emotional eating were directly correlated with weight gain and obesity, but exercise had a consistent effect on Body Mass Index (BMI), and phase angles (Yılmaz and Kuzuner, 2023). Conversely, extremely intense physical activity was more likely to trigger emotional eating. Negative emotions can lead overweight and obese individuals to overeat. Exercise and sporting activities can prevent excess weight gain, but real success cannot be achieved without treating the underlying causes of emotional eating.

Emotional eating behavior is a behavior that an individual exhibits in order to reduce and control the negative psychological state even if he/she is not hungry (Duman, 2005; Van Strein, et al., 2005). Emotional eating behavior includes an individual's tendency to overeat in response to negative emotions (Pinaguy et al., 2003). Individuals who exhibit emotional eating behavior generally tend to prefer sugary, fatty and high-calorie foods (Frayn & Knäuper, 2018).

Emotional eating behavior has serious psychological and physical effects on an individual's health (Yildirim & Yildiz, 2022). Emotional, cognitive and psychological characteristics can influence an individual's eating habits and weight control. This behavior is often associated with eating disorders, weight gain and depression. Emotional eating behavior is often seen in obese or

overweight individuals, but this is not always the case. It can also be seen in individuals who are underweight or have normal body measurements (Özkan & Bilici, 2018; Tan & Chow, 2014).

Emotional eating behavior is eating behavior that occurs during the struggle with negative emotions or is performed to control emotions (Ganley, 1989; Konttinen, 2012; Ehring et al., 2010; Güngör et al., 2020). In other words, emotional eating behavior refers to changes in food intake under the influence of negative emotions (Geliebter & Aversa, 2003). Emotional eating behavior is a physiological behavior that occurs due to the negative emotions that the individual is experiencing at that moment and tends to overeat. This situation, which usually develops to cope with or suppress negative emotions, may become a habit (Taş & Kabaran, 2020).

Emotional eating behavior, first proposed by Bayles and Ebaugh (1950), is a reaction to negative emotions such as anger, loneliness, depression and anxiety and refers to eating more or less than normal depending on the current emotional state of the individual (Bekker et al., 2004; De Lauzon-Guillain et al., 2006; Fleurbaix Ville Sante Study Group, 2004)."

Individuals with emotional eating behavior state that they prefer food to cope with negative emotions and reduce the severity of these negative emotions in the body by overeating (Sevinçer et al., 2013). In a study conducted with obese individuals (Lemmens et al., 2011), it was observed that individuals consume higher calorie, carbohydrate and fatty foods under stress.

Some researchers believe that 'comforting' foods high in sugar and fat, which are thought to provide pleasure, affect the functioning of the HPA axis and induce a reverse stress response and consequently improve mood (Tomiyama et al., 2011). Obese individuals have impairments in the HPA axis and glucose metabolism. When individuals maintain a dietary profile rich in fat and simple carbohydrates, they become prone to insulin resistance and abdominal adiposity (Michopoulos et al., 2015, Sinha 2018), and have a higher risk of diabetes and cardiovascular diseases (Frayn et al., 2018). Emotional states of psychological origin, especially depression, stress, and anxiety, can trigger binge eating behavior in some individuals and are characterized by decreased appetite in some individuals (Serin et al., 2018). It is reported that negative emotions increase emotional overeating behavior and especially food choices are high-calorie unhealthy junk foods, while positive emotions lead to healthier choices with less emotional eating (Evers et al., 2013, Levitan et al., 2010). Stress in particular is thought to have a widespread effect on emotional eating (Flaskerud 2015).

Emotional eating, triggered by psychological factors such as anger, distress, or stress, often occurs during non-hungry moments. While mainly associated with

mental states, the choice of beverages, like natural mineral water, plays a role in overall well-being (Serin & Şanlıer, 2018). Natural mineral water, rich in essential minerals, may contribute to a balanced diet compared to alternatives like tap or purified water (Çağıran et al., 2023). Choosing such options consciously aligns with fostering healthier habits alongside addressing emotional triggers for overeating (Simmons & Limbers, 2019; Sevinçer & Konuk, 2013).

When an individual feels angry, he or she thinks he or she can find peace by chewing a food quickly. In the same way, they may calm themselves down by eating sweets. Unfortunately, these effects are short-lived. In the long run, emotional eating behavior can lead to increased feelings of regret and guilt and loss of self-esteem. Even though these individuals know that their problems cannot be solved by eating, they resort to this method to calm down. As a result of this behavior, overweight problems may arise and it may become more difficult to watch what they eat and lose excess weight (Türk, 2018; Seven, 2013).

Emotional eating behavior varies by gender and is more common when women cope with situations such as stress, fear, tension, and anxiety (İnalkaç & Arslantaş, 2018). This may be due to the fact that women are more prone to negative emotions such as depression and anxiety (Deveci et al., 2017). In addition, it has been reported that individuals tend to eat unhealthy and irregular diets and consume sugary and fatty foods more frequently when they are stressed and anxious (Michels et al., 2012).

Understanding the psychological aspects of emotional eating, it's noteworthy to mention the role of the placebo effect in shaping dietary habits. The placebo effect, a phenomenon where individuals experience improvements in symptoms due to their beliefs and expectations rather than the actual properties of a treatment, could also influence one's approach to emotional eating. For instance, individuals may perceive certain foods as mood-enhancing or stress-relieving placebos, impacting their emotional eating patterns (Atay and Fırat, 2023). In emotional eating behavior that occurs in response to stress and anxiety, the individual may increase food intake by consuming favorite foods and gain weight (Deroost & Cserjesi, 2018; Baños & Cebolla). Exploring the interplay between psychological factors, like the placebo effect, and dietary choices can provide valuable insights into developing effective strategies for managing emotional eating tendencies.

There are studies showing that emotional eating occurs in different emotional states between men and women. According to these studies, while women exhibit emotional eating behavior in situations such as stress, anxiety, and sadness, men tend to show this behavior more when they are surprised (Nguyen-Rodriguez et al., 2009). However, some studies reveal that women show emotional eating

behaviors such as eating disorders more (Akfırat & Kılçık, 2021; Levitan & Davis, 2010; Yücel, 2009). In this case, concerns about weight gain may also be effective. For example, although ovarian hormones, which can lead to eating disorders and overeating behaviors and increase during menstruation in women, do not directly affect emotional change; weight gain and edema formation in the body during menstruation may cause an increase in stress hormone in women and accordingly, an increase in emotional eating behavior may be seen in women (Carr-Nangle et al., 1994; Serin & Şanlıer, 2018).

NDE has been mentioned together with binge eating disorder in some sources (Bekker et al., 2004). Although it is a similar concept to binge eating disorder, they are different concepts in terms of the amount of food consumed and the frequency of symptoms. It is possible to say that the main difference is in the amount of food consumed. By definition, binge eating refers to eating until extremely uncomfortably full, while emotional eating may involve lower calorie consumption or irregular meals (Saljoughian, 2021).

Theories on Emotional Eating

1. The intrinsic-extrinsic obesity theory

Schacter's externality theory of obesity (Schachter, 1971; Schachter & Rodin, 2014) is a theory developed to better explain the psychosomatic theory. Unlike the psychosomatic theory, this theory attributes eating behavior to external factors rather than internal ones. According to Schacter's theory, obese people may be more reactive to external eating cues and less sensitive to internal hunger and satiety signals than thin people (Boutelle et al., 2014). They may resort to overeating because they are attracted by the smell and appearance of food, but otherwise they do not think about food and do not live food-oriented lives (Serin & Şanlıer, 2018). In addition, in this theory, it is argued that obese individuals being more sensitive may be due to their personal characteristics (Tanrıverdi, 2020).

This theory was developed by Macht and Simons (2011). According to this theory, obese and fat individuals have a low level of response to physical stimuli, that is, it is difficult for them to perceive the feeling of hunger with their internal impulses. The individual reacts to external stimuli such as the smell, taste and appearance of food and exhibits emotional eating behavior (Bilgen, 2018; Masheb & Grilo, 2006). Individuals may be particularly affected by the external appearance of food. They may also feel appetite even if they are not hungry (Schachter, S., 1968; Schachter, 1971)

In the extrinsic theory, external stimuli replace internal hunger drives. The sensitivity of the individual to the external influence causes the individual to be

vulnerable to foods that look good and tasty instead of physical hunger and leads the individual to overeat (Tüzen, 2019).

2. Psychosomatic Theory

When we think about the exchange of love and nutrients through breast milk between the mother and the baby, where human beings experience their first emotions and bodily interaction, the relationship of this theory with Emotional Eating Behavior (EOB) is linked to the first periods when human beings open their eyes to life. Since it emphasizes the relationship between physical and mental health, "eating disorders are a good example of psychosomatic sensitivities" (Yücel, 2009, p. 39). This theory links obesity to internal factors (Tanrıverdi, 2020). At the same time, the digestive system is associated with most psychosomatic diseases between eating and emotions. The term "emotional eating" is used in psychosomatic theory to refer to the act of eating in response to various negative emotions such as anxiety, depression, anger and loneliness (Faith et al., 1997). Emotional eaters may have difficulty distinguishing whether their hunger is emotional or physiological (İnalkaç & Arslantaş, 2018). In addition, according to this theory, due to the lack of introseptive awareness in individuals with NDE, individuals cannot distinguish whether they are full or hungry and cannot understand whether they have another physical illness (Tanrıverdi, 2020).

Excessive emotional food consumption may be associated with a lack of unconditional love during childhood, which is related to the connection the child establishes with his/her mother during the first feeding process (Çevik & Ünal, 1989). This bond between mother and child may influence eating habits in later periods, linking food consumption to their mothers. These children may often seek help from their mothers to carry out daily activities, such as eating and dressing, and may be dependent on their mothers for a long period of time. Children who have negative experiences and do not receive enough love may turn to food to overcome these difficulties and may continue this tendency into adulthood. For this reason, it has been suggested that obese children who overeat are more emotional children. These children may tend to become angry more quickly and may turn to food to escape the effects of disappointment, failure and traumatic events (Tanrıverdi, 2020).

Psychosomatic theory is shaped by a focus on the relationship between eating behavior in infancy and calming. This theory suggests that feeding is linked to the infant's ability to satisfy hunger through the urge to suck, or to reduce restlessness or stress. According to the theory, even if the baby does not feel hungry, feeding helps the baby learn the importance of coping with negative

emotions. In this way, the baby tends to engage in emotional eating behavior when restless or crying, and this behavior becomes a habit over time (Sevinçer & Konuk, 2013). Since this feeling is embedded in the subconscious, it may occur from time to time throughout life.

According to the psychosomatic theory, individuals who exhibit emotional eating behavior are not aware of their emotions and the consequences of these emotions; they have difficulty understanding that they are neither hungry nor full. This theory argues that emotions, not feelings of hunger or fullness, control emotional eating behavior (Spoor et al., 2007).

The leading views of psychosomatic theory belong to Kaplan and Kaplan and Brunch (Moreno et al. 2011). According to Kaplan and Kaplan, even if the individual is not hungry, he/she exhibits emotional eating behavior in order to reduce negative emotions, situations and problems. According to this theory, eating affects neurotransmitters and serotonin absorption in the brain, which leads to the emergence of a pleasurable behavior. However, obesity can often be seen as a result of overeating due to emotional eating (Kaplan and Kaplan 1957, Ruderman 1983).

Brunch (1964) associated emotional eating behavior with the misrecognition of hunger and stated that individuals who exhibit emotional eating behavior have both instinctive and learned aspects of hunger. He states that children who are fed by their mothers to calm them down during their childhood and who are fed in this way cannot identify internal stimuli such as hunger-satiety. According to Brunch (1964), when children become adults, they tend to eat when they feel uncomfortable and restless, and this can lead to excessive and rapid weight gain (Brunch 1964)."

3. Theory of constraint

Herman and Mack proposed the theory that diets that individuals follow for weight control may cause a tendency to overeat and emotional eating from time to time by creating a sense of restriction (Demirdöğen et al., 2021). Herman and Mack (1975) concluded that individual differences in eating behavior may be a critical determinant of relative deprivation rather than obesity, and a scale was developed in this context. The "Revised Restraint Scale," revised by Herman and Polivy (1980), argues that the less calories an individual tries to take in and the more they restrict themselves, the more the body can increase eating behavior by switching to physiological defense. A study conducted by Polivy et al. (2005) supported Herman and Mack's theory and showed that individuals deprived of chocolate consumed more chocolate than other group members. Restricted individuals craved these foods more than those who were not restricted.

Similarly, in another study, it was observed that restricted individuals tended to eat more in situations of fear and anxiety (Heatherton et al., 1991).

In another study, 71% of women increased their food intake while dieting (Zellner et al., 2006). In conclusion, it seems that deprivation causes cravings and overeating, and this is especially observed in restricted eaters.

This theory, developed by Herman and Mack (1975), refers to the individual's resistance to food cravings, self-restraint and abstinence. Emotional eating behavior, although cognitive, develops for the relief of cravings and causes the individual to worry about his/her eating behavior and to express that he/she is constantly engaged in eating behavior. This complaint causes the individual to focus on eating behavior, binge eating and excessive weight gain (Ünal, 2018; Sevinçer & Konuk, 2013).

According to this theory, the individual disrupts his/her diet and shows excessive, binge eating behavior and feels anxiety and regret after consuming food (Herman & Polivy, 1980). Although such individuals restrict themselves in terms of food intake for a certain period of time, due to the weakening of their self-control, they tend to stop the diet suddenly and eat more than usual (Gözegir, 2020).

4. Escape theory

According to this theory, the individual, who is afraid of facing the things he/she experiences in his/her inner world, often turns to eating food that gives him/her pleasure to escape from painful situations (Tanrıverdi, 2020). It is a preferred method to get away from disruptions and problems in life. Individuals can do this not only through eating behavior but also through smoking and alcohol consumption (Beşirli, 2007). According to this theory, it is noticeable that individuals with emotional eating behavior avoid awareness (Serin & Şanlıer, 2018). Emotional eaters avoid environments and situations that will increase their awareness as long as a problem occurs in their lives. The basis of the avoidance of awareness may lie in the fact that the individual sets goals that are difficult to achieve (Tanrıverdi, 2020).

According to the Escape Theory, individuals show emotional eating behavior in order to distract, distract and avoid negative emotions and environments that they see as a threat to themselves. According to this theory, individuals exhibit emotional eating behavior because they want to get away from or avoid the negative situations and emotions they feel (Heatherton & Baumeister 1991; Özdemir, 2015; Serin & Şanlıer 2018). Escape theory is also expressed as a strategy to combat emotional eating behavior. Individuals exhibit emotional eating behavior to get away from negative emotions, stress and pressure.

Emotional eating behavior is a problematic behavior that develops to meet the psychological needs of the individual rather than physiological needs. As a result of individuals exhibiting avoidance behavior, intensive diet programs and binge eating are considered problematic behaviors (Spoor et al., 2007). Instead of avoidance behaviors, it is more beneficial to tend to solve problems by cooperating. Pessimism and negative expectations lead to the continuation of avoidance behavior. Avoidance behavior usually occurs in parent-child conflict about eating. Conditions such as neglect, anxiety, personality disorders can cause avoidance behavior in individuals (Morrison, 2017).

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Chapter 19

Magnesium and Physiological Effects

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The physiological functions of magnesium were essentially ignored until recently. The development of new technologies to measure the intracellular free concentration of magnesium, which is biologically very important, has subsequently increased interest in the biochemical, molecular, physiological and pharmacological functions of magnesium. The absorption and subsequent metabolism of a particular nutrient in the intestine depends to some extent on the presence of other nutrients. Magnesium is the most abundant cation in intracellular fluid and is essential for maintaining normal cellular physiology and metabolism. It acts as a cofactor of many enzymes and regulates ion channels and energy production. Mg is a mineral that is essential for many processes in the body, including muscle function and sleep. Magnesium can affect sleep deprivation and muscle contraction in different ways, depending on the level of magnesium in the body and the amount of physical activity. Mg, which is involved in neuronal processes from presynaptic to postsynaptic events, plays an essential role in the excitability of the peripheral and central nervous systems (CNS). The mechanism of magnesium entry and exit into the cell, its transport within the cell, its absorption from the intestines, its excretion from the kidneys, and the effect of various hormones on these processes are still unclear. Magnesium deficiency is not a rare condition. Especially in the Western world, magnesium intake has decreased over the years. Experimental animal models and human studies have shown that changes in peripheral magnesium concentration are associated with various behavioral disorders (schizophrenia, anxiety, aggression, and stress) and sleep organization (daytime falling asleep, sleepiness, snoring, and sleep duration), eclampsia, hypertension, atherosclerosis, heart diseases. It shows that it is associated with and plays a role in their pathophysiology.

Physiological Mechanisms of Magnesium

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Magnesium (Mg) is the most abundant mineral in the body, after potassium, calcium, and sodium. Magnesium is an essential mineral used as a cofactor in more than 300 biochemical reactions required to maintain homeostasis in the human body. The biological functions of Mg are broad and diverse and include the production of nucleic acids, all adenosine triphosphate (ATP) reactions, and modulation of any activity mediated by intracellular calcium concentration fluxes (e.g., insulin release muscle contraction) (1).

1. Participation in Energy Metabolism

Within the cell, all magnesium is in the nucleus, mitochondria, and sarcoplasmic reticulum. While the majority (about 4-5 mmol/L) is found in the cytosol in complex with adenosine triphosphate (Mg21-ATP) and other phosphometabolites, a small part (0.5-1.2 mmol/L) is found free. Magnesium is important for cellular energy production because magnesium-dependent oxidative phosphorylation produces ATP, and ATP-dependent reactions require magnesium to hydrolyze and transfer phosphate groups. Therefore, magnesium has a crucial role in various metabolic pathways, including DNA synthesis and transcription, glycolysis, protein synthesis, intracellular signaling, and membrane potential determination. The functions of magnesium in energy metabolism support the critical role of ATP in cellular energy transfer (2, 3).

Phospholipid Metabolism: Phospholipids are the basic building blocks of cell membranes and play an essential role in forming biological membranes. Cell membranes regulate the passage of various molecules inside and outside the cell and maintain the integrity of the cell. Magnesium helps phospholipids to organize correctly in cell membranes, increasing the stability of membranes. Phospholipids are important for cellular signal transduction. Cellular signaling molecules interact with phospholipids in the cell membrane to trigger various cellular responses. Phospholipase enzymes hydrolyze phospholipids in cell membranes to produce free fatty acids and other components (7). Magnesium can affect cellular signal transduction and lipid metabolism by regulating the activity of phospholipase enzymes. This affects the structure and function of the cell membrane. These roles of magnesium indicate that phospholipid metabolism is linked to fundamental processes such as cellular integrity, energy production, and signal transduction. Therefore, adequate magnesium intake is important for healthy cell membranes and regular maintenance of cellular functions (8).

Glycolysis Reactions: Magnesium is involved in this process by regulating the activity of glycolytic enzymes. Magnesium acts as a cofactor of various enzymes during glycolysis reactions and regulates the process (9). Glycolysis is an

essential metabolic pathway in which glucose is broken down in the cell to produce energy. At the beginning of glycolysis, hexokinase is phosphorylated by the enzyme hexokinase to allow glucose to enter the cell and start glycolysis. During this reaction, magnesium contributes to the initiation of glycolysis by supporting the catalytic activity of hexokinase. The enzyme phosphofructokinase (PFK), which acts as a regulator and rate control point in glycolysis, regulates the rate of glycolysis by regulating phosphofructokinase activity. In this process, magnesium acts as a cofactor of this enzyme and supports the catalytic activity of phosphofructokinase (10). In the last step of glycolysis, the pyruvate kinase enzyme takes part in the reaction in which phosphoenolpyruvate is converted to pyruvate. Magnesium increases the catalytic activity of pyruvate kinase during this reaction and ensures the formation of pyruvate. Another critical enzyme involved in glycolysis is glucose-6-phosphate isomerase. This enzyme isomerizes glucose-6-phosphate to fructose-6-phosphate (11).

Magnesium can act as a cofactor in this isomerization reaction. These reactions are involved in energy transportation and storage processes. In addition, magnesium affects insulin activity, a hormone that regulates the use of glucose in the cell and energy production (12). Magnesium supports the role of insulin in glucose metabolism by affecting insulin receptor activity (13). Therefore, an adequate level of magnesium is vital for cellular energy production.

2. Structural Roles of Magnesium

Magnesium plays a vital role in many structural roles in the body.

Bone and Dental Health: Magnesium is one of the building blocks of bones and teeth, along with minerals such as calcium and phosphorus. It protects bone health by supporting bone mineral density. Magnesium regulates the passage of calcium in and out of cells. This is important for controlling cellular calcium levels, nerve conduction, muscle contraction, and maintaining homeostasis in many cellular processes (14).

Cell Membranes, Protein, and Nucleic Acid Stability: Magnesium maintains the structural stability of cell membranes, proteins and nucleic acids (DNA and RNA). Stabilization of cell membranes maintains the integrity of the cell by regulating the passage of various molecules inside and outside the cell. DNA and RNA stability is essential for correct genetic material replication and maintaining the ordered structure of proteins inside the cell. Magnesium acts as a cofactor in the polymerization reactions of nucleic acids during cell division, DNA, and RNA synthesis. In particular, it ensures the stability of the DNA double helix. This is essential for the correct and efficient production of genetic material. Magnesium

is a structural component of ribosomes and ribonucleic acid (RNA). This is important for regulating and supporting protein synthesis processes within the cell (15, 16).

Enzymatic Activity: Magnesium is used as a cofactor by many enzymes. This involves the participation of magnesium in biochemical reactions by regulating the catalytic activity of enzymes. Magnesium's roles in enzymatic activities are critical in regulating biochemical reactions and maintaining cellular function. These roles of magnesium in enzymatic activities are;

1. It is critical in regulating energy transfer reactions and cellular functions by stabilizing nucleotide triphosphates such as ATP. Participates in the transfer of phosphate groups during the catalytic activities of many enzymes.
2. Magnesium's role in phosphorylation reactions contributes to energy transfer processes.
3. The activity of DNA and RNA polymerases is influenced by magnesium during the synthesis of DNA and RNA chains. Magnesium promotes nucleic acid synthesis by interacting with the substrates of these enzymes.
4. Participates in RNA degradation processes by regulating the activity of ribonuclease enzymes involved in RNA degradation (16).

3. Protein Synthesis and Structure

Magnesium ensures the stability of ribosomes during protein synthesis in the cell. Since ribosomes are structures composed of RNA and protein components involved in protein synthesis, magnesium stabilizes these structures and supports the proper functioning of ribosomes (17). Transferring amino acids to ribosomes ensures the correct folding and stability of RNA (tRNA), which facilitates the transport of the correct amino acid to the ribosome and regulates protein synthesis. Regulates the catalytic activity of aminoacyl-tRNA synthetase enzymes, ensuring that the correct amino acid pairs with the proper tRNA. It facilitates ATP binding to ribosomes and supports protein synthesis by utilizing the energy generated during ATP hydrolysis (18). It regulates the protein synthesis process by regulating the activity of GTP-bound proteins during protein synthesis. In addition to these functions, it also contributes to cellular protein functions by regulating the activity of many enzymes (19, 20).

4. Effect on Second Messengers and İons

Magnesium's effects on second messenger systems and the regulation of ions suggest that it plays a vital role in fundamental processes such as cellular signal transduction, energy transfer, and maintenance of cellular homeostasis.

Magnesium is essential in cellular signal transduction. It controls nerve conduction and muscle contraction by regulating the interactions of intracellular and extracellular signaling molecules. Magnesium is a cofactor in the intracellular stabilization and hydrolysis reactions of ATP and GTP, which are important in synthesizing second messenger molecules and energy transfer. It can affect cAMP production by regulating the activity of the enzyme adenylate cyclase, which acts as a secondary messenger in regulating many processes within the cell (21).

Magnesium contributes to the control of cellular calcium levels by affecting the activity of calcium channels in the cell membrane. It can affect the balance of calcium and sodium within the cell by regulating the activity of the Natrium-Calcium exchanger (NCX) pump in the cell membrane. It also regulates calcium homeostasis by storing calcium inside the cell or pumping it out of the cell by affecting the activity of intracellular calcium pumps (22, 23).

5. Muscle Functions and Magnesium

Magnesium is the essential mineral involved in muscle contraction and relaxation. Muscle contraction is a process in which nerve impulses are transmitted to muscle fibers, followed by contraction of the muscle fibers. Magnesium, specifically, affects the communication between the nervous system and muscle cells by regulating the passage of nerve impulses to muscle fibers. A nerve impulse initiates this communication and triggers muscle contraction. During muscle contraction, metabolic by-products such as lactic acid can accumulate in muscle cells. Magnesium may help maintain muscle performance and muscle contraction by reducing the adverse effects of this acidic environment (24). It acts as a cofactor in the energy transfer of ATP molecules in muscle fibers, which provide the necessary energy during contraction. Magnesium acts on the mechanisms that initiate and stop muscle contraction by providing a controlled release of calcium inside the cell. There are various calcium binding sites in muscle (e.g. myosin troponin and calmodulin) that are involved in the regulation of muscle contraction and some enzymatic processes. Since many of these proteins also bind to magnesium, it is important to consider the effect of high concentrations of free magnesium in muscle on these sites. The main effect of magnesium is to greatly reduce the binding rate of calcium by binding to these sites that bind both magnesium and calcium (in a relaxed muscle, it is primarily bound by Mg). In smooth muscle, calcium enters the cell through two different calcium channels, activating the myofilaments and initiating contraction. Calcium channel blockers or nitro compounds inhibit these channels. Magnesium relaxes muscles by inhibiting both of these calcium channels (25).

6. Cardiovascular System and Magnesium

Magnesium affects cardiac conduction and contraction mainly by regulating ion channels. Magnesium regulates neuronal excitation, intracardiac conduction, and myocardial contraction by regulating many ion transporters in the heart tissue, including calcium and potassium channels. Magnesium-modulated potassium and calcium channels and sodium-potassium ATPase pumps of cardiac myocytes and pacemaker cells influence cardiac excitation and autorhythmicity. Thus, magnesium plays a role in regulating the membrane potential of cardiac myocytes and pacemaker cells (26).

Magnesium also regulates vascular muscle tone, calcification, atherogenesis, thrombosis, proliferation, and migration of endothelial and vascular smooth muscle cells. Increasing evidence supports the beneficial effects of reduced dietary magnesium intake on the risk of cardiovascular disease and the beneficial effects of magnesium in the prevention and treatment of these diseases (27).

The kidneys are the primary regulator of magnesium homeostasis. Kidney diseases can cause both magnesium deficiency and its excessive increase. With this effect, it can also increase the risk of cardiovascular disease. Mild to moderate magnesium deficiency has been shown to cause abnormal cardiac excitation, atherosclerosis, coronary artery disease, arrhythmias, and congestive heart failure. In contrast, severe levels of deficiency have been associated with ventricular arrhythmias and sudden cardiac death. On the other hand, studies with magnesium supplementation have also reported that magnesium overload causes adverse effects. Therefore, there is no definite recommendation for routine magnesium supplementation except in cases where hypomagnesemia is proven or suspected to cause cardiac arrhythmias (28-31).

7. Immune System System and Magnesium

Minerals play a role in various functions in our body, such as creating building materials for bones, affecting muscle and nerve functions, and regulating the body's fluid-electrolyte balance. They are also components of hormones, enzymes, and other biologically active compounds. Some minerals also play essential roles in the optimal functioning of the immune system. This relates to both the innate defense system and the adaptive immune response. Accordingly, minerals can affect susceptibility to infections and also have effects on the development of chronic diseases (32).

Mg has multiple roles in the regulation of immunological functions, particularly in terms of activation and function of immune system cells. It is essential to have adequate magnesium levels for the immune system cells to function properly.

The acute phase response of Mg is demonstrated by the responses of macrophages to stocks. Mg supplementation in monocytes causes a decrease in nuclear factor kappa-light chain stimulator of activated B cell translocation by reducing cytokine production after toll-like receptor (TLR) stimulation (33).

Increased phagocytosis and peripheral neutrophilia associated with oxidative stress were observed in Mg-deficient rats. It has been reported that systemic IL-6 levels increased, the release of acute phase proteins increased, and thiobarbituric acid (TBARS), an oxidant marker, increased in rats fed a Mg-reduced diet. In parallel, it has been reported that there is a decrease in superoxide dismutase (SOD) and catalase activity and glutathione synthesis (34). Even a short-term magnesium deficiency has been shown to cause the release of various pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). There is also evidence that the microbiota is also affected by systemic Mg deficiency. In this deficiency, the barrier function of the intestinal wall is impaired while the concentration of bifidobacteria in the intestine decreases, resulting in increased expression of TNF-α and IL-6 in the intestine and liver (35).

8. Nervous System and Magnesium

Magnesium is a mineral that regulates communication between nerve cells and mainly affects the glutamatergic nervous system. Glutamate is one of the primary excitatory neurotransmitters in the central nervous system and plays a vital role in learning, memory, neural plasticity, and many other neural functions. Its interaction with the magnesium N-methyl-d-aspartate (NMDA) receptor is one of its most fundamental neurological functions. NMDA receptors are an ion channel that allows glutamate to enter the cell through the synaptic cleft and strengthens connections between nerve cells. Magnesium prevents overstimulation by blocking NMDA receptors. Therefore, magnesium prevents overstimulation of nerve cells by limiting the binding of glutamate to NMDA receptors and the entry of excessive amounts of calcium ions into the cell (36). Increased glutamatergic neurotransmission at low magnesium levels can lead to excitotoxicity, oxidative stress, and neuronal cell death. Abnormal glutamatergic neurotransmission has been associated with many neurological and psychiatric disorders, including migraine, chronic pain, epilepsy, parkinson, and stroke, as well as anxiety and depression (37).

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