BAŞKENT UNIVERSITY
HEALTH SCIENCE INSTITUTE
PHYSIOLOGY DEPARTMENT
PHYSIOLOGY MASTER'S PROGRAM

THE EFFECTS OF HIGH DOSE VITAMIN D ON THE SYMPATHETIC SKIN RESPONSE IN RATS

BY

HAIFA MASUD

MASTER'S THESIS

ANKARA- 2021

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THESIS SUPERVISOR
PROF. DR. NAZAN DOLU

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INSTITUTE OF HEALTH SCIENCES

This study, which was prepared by Haifa Masud within the framework of the Department of Physiology Master's Program, was accepted as the Master's Thesis by the following jury.

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Student Name, Surname: Haifa Masud.

Student Number: 21910600

Department: Physiology

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Supervisor's Title /Name, Surname: Prof. Dr. Nazan Dolu

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ABSTRACT

HAIFA MASUD, THE EFFECTS OF HIGH DOSE VITAMIN D ON THE SYMPATHETIC SKIN RESPONSE IN RATS, BAŞKENT UNIVERSITY, INSTITUTE OF HEALTH SCIENCES, MASTER'S THESIS 2021

Vitamin D is a fat-soluble hormone formed predominantly in the skin when exposed to UVB from the sun or absorbed from restricted dietary sources. Vitamin D is a significant regulator of calcium and phosphorus homeostasis and controls many other biological processes, including the immune system, cell growth and division. Electrodermal activity (EDA) measures the skin conductance level (SCL) which innervating of sweat glands. Increased SCL indicate increase sympathetic activity and increase sweat secretion. There is no previous study linked to the effect of vitamin D on electrodermal activity. And the purpose of the present study is to investigate the effect of Vitamin D on sympathetic nerve activity at different high doses using electrodermal activity. This study was carried out with 36 rats. This study was carried out with 36 rats. The rats were divided into 3 groups as sham group receiving tap water by oral gavage, medium dose vitamin group (400IU) and high dose vitamin group (1000IU). A significant difference was found between the medium dose group and the sham group for tonic SCL (p value =0.027). A significant difference was also found for phasic SCL between the medium dose group and the sham group (p value = 0.001) and between the high dose group and the sham group (p value = 0.013). There was no difference when each group's tonic and phasic SCL were compared separately. Our findings showed that sympathetic skin response was increased with vitamin D supplementation, and there was no significant difference between high and medium dose vitamin D.

In conclusion, sweat gland activity and stress response increased with the increase in vitamin D administration, but no dose-dependent effect was observed.

Keywords: Vitamin D, Sympathetic skin response, Skin conductance level, Rat

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

AC	Alternative Current
Ag/AgCl	Silver/Silver Chloride
ANS	Autonomic Nervous System
CNS	Central Nervous System
DC	Direct Current
DBP	Vitamin D Binding Protein
7-DHC	7-dehydrocholesterol
EDA	Electrodermal Activity
FGF-23	Fibroblast Growth Factor 23
NOS	Nitric oxide synthase's
NOx	Nitric oxide
NS-SCR	Nonspecific Skin Conductance Response
25(OH)D	25-hydroxyvitamin D
1,25(OH)2D	1,25 dihydroxyvitamin D
24,25 (OH) 2D	24,25-dihydroxyvitamin D
PNS	Parasympathetic nervous system
PTH	Parathyroid hormone
SC	Skin Conductance
SCF	Skin Conductance Fluctuation Frequency
SCL	Skin Conductance Level
SCR	Skin Conductance Response
SPRs	Skin Potential Responses
SR	Skin Resistance
SSR	Sympathetic Skin Response
VDR	Vitamin D Receptor

1. INTRODUCTION

Vitamin D is a seco-steroid (fat soluble) hormone formed mainly in the skin when exposed to UVB from the sun or absorbed from restricted dietary sources. When 7-dehydrocholesterol (7-DHC) is exposed to UVB radiation, vitamin D is synthesized in the skin and then photolysed into pre-vitamin D3, which is then thermally isomerized into vitamin D3. Vitamin D3 undergoes two consecutive hydroxylation after entering the bloodstream to form 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D (1,25(OH)2D) is the main circulating form of vitamin D, preceded by the hormonal form of vitamin D [1]. This form of vitamin D is inactive, and 1,25 dihydroxy vitamin D is hydroxylated twice, once in the liver and once in the kidney to make the active vitamin D form [2]. Vitamin D can be derived from food sources such as seafood, mushrooms, or fortified foods as long as cutaneous development takes place [1]. Vitamin D is known to have a strong influence on the release and stored of calcium reserves, as well as the calcium ion levels in extracellular fluid. This is achieved by promoting intestinal calcium absorption and secreting calcium from the skeletal tissue by regulating bone turnover. The parathyroid gland plays important role in vitamin D metabolism as the main regulator of calcium. If the parathyroid gland sense low circulating calcium levels, parathyroid hormone (PTH) is released. The rise in circulating 1,25-dihydroxy vitamin D increases the bone to release calcium and phosphate and the small intestine to increase the absorption of these minerals, which feed back to the parathyroid before elevated serum calcium levels are identified by the Chief Cells [3].

Vitamin D promotes several other biological functions, such as the immune system, cell growth and division, besides skeletal health and balance of calcium [2]. Further molecular pathways, consisting of inflammatory signaling and neurotransmitter biosynthesis, have been shown to organize vitamin D in regions of the central nervous system that control cardiovascular function [3].

A Vitamin D deficiency (serum 25-hydroxyvitamin D [25(OH)D] 50 nmol/L or 20 ng/ml) is linked to skeletal problems such fractures and bone loss [14]. It induces childhood rickets and can precipitate and worsen adult osteopenia, and osteoporosis. Vitamin D insufficiency has been linked to high risk of general malignancies, autoimmune disorders, hypertension, depression, and infectious illnesses [4]. Also in large doses, vitamin D is toxic,

and sporadic toxicity reports (hypervitaminosis D) occur. Clinical signs include loss of appetite, nausea, vomiting, polyuria, polydipsia, fatigue, mental status changes and constipation. In metabolic terms, hypervitaminosis D is known as high levels of 25-hydroxyvitamin D in the serum, hypercalcemia or hypercalciuria, or both(25[OH]D). Long-term hypervitaminosis D can cause calcium deposition changes in the soft tissues (especially the kidneys and heart), as well as mortality in severe cases. [5].

Electrodermal activity is an indirect predictor of attention, cognitive effort, or emotional arousal since it displays sympathetic tone, also known as the galvanic skin response or sympathetic skin response. Electrodermal activity is a multisynaptic sympathetic reflex that can be evoked by a number of arousal stimuli produced internally or applied externally. It's used as a measure of psychological processing stimulus properties including meaning, novelty, or emotional significance, as well as effortful processing. A parameter of EDA is skin conductance level (SCL). A high SCL can arise from increased activity of the eccrine sweat gland and sympathetic activity [6].

In general, there are several previous studies related to vitamin D deficiency and few studies have studied the relationship between vitamin D and the function of the autonomic nervous system [7]. Dysautonomia, a disorder of the autonomic nervous system, affects the expression before and after differentiation of the neural cells of genes involved in calcium metabolism, which gives rise to sympathetic and parasympathetic nervous system neurons. Vitamin D is important for the functioning of the sympathetic and parasympathetic nerve systems. Low vitamin D levels may show the many symptoms of migraine, cardiovascular and gastrointestinal problems, and other illnesses [8]. And the possible correlation of low vitamin D levels and autonomic cardiovascular nerve activity in healthy adults has recently been investigated. These investigators demonstrated that low levels of 25(OH)D were correlated with depressed resting autonomic cardiac function [9]. It was also claimed that, vitamin D deficiency reduces the parasympathetic nervous system (PNS) activity. [10] In animal studies, vitamin D deprivation was also related to an increase in renin secretion. [7]. In an *in vitro* research, however, 1,25-dihydroxyvitamin D enhanced tyrosine hydroxylase mRNA, suggesting that vitamin D could activate the rate-limiting step in catecholamine production. [11]. Hence, we will study the connection between vitamin D and sympathetic skin response.

There is no previous study related to the effect of vitamin D on electrodermal activity. The purpose of this study is to see how varied high doses of vitamin D affect electrodermal activity in rats.

2. GENERAL INFORMATION

2.1. Vitamin D:

Vitamin D was discovered in the early 20th century. Nevertheless, in the early 17th century it was reported that Rickets disease is poor, mineralized, and tends to soften and bend bones without being aware of the causal factor. During the twentieth century's industrial revolution, the disease spread to children in industrial centers across the United States and Europe. The researchers experimented with cod liver oil and ultraviolet radiation using a quartz-mercury steam bulb during this time and discovered that diet and an unknown UV light may treat the condition. Professor McCollum and his colleagues were able to attribute the effect in cod liver oil to a chemical called vitamin D in 1922 [12]. The skin's synthesis of vitamin D was not determined until 1978, and vitamin D3 was fully discovered. Vitamin D was quickly identified as a hormone [13]. Vitamin D's main physiological effect is on calcium and phosphorus metabolism, which keeps calcium and phosphorus concentrations in the body within normal ranges [14].

2.1.1. Forms of Vitamin D:

Vitamin D is one of the four fat-soluble vitamins that have been reorganized for important biological functions. the two main forms of which are vitamin D2 (or ergocalciferol) and vitamin D3 (or cholecalciferol) [15]. Vitamin D3 is created in human skin and obtained from animal-derived foods, particularly fish oil, but vitamin D2 is sourced from plants. Only the side chain structure of vitamins D2 and D3 differs “Figure 2.1. The metabolism (i.e. activation) is not affected by differences and both versions act as prohormones. [16,17].

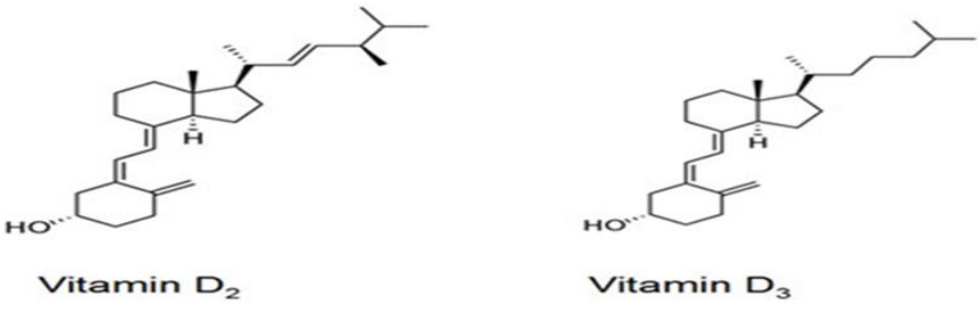

Figure 2. 1. Vitamin D forms (vitamin D2 and vitamin D3) [18]

2.1.2. Sources of Vitamin D:

Vitamin D is received through diet and from the skin upon exposure to sunshine through the synthesis [19]. Dietary and supplementary sources are dietary sources of vitamin D; they include vitamin D in the form of vitamin D3 or D2 (ergocalciferol) [17,20]. The richest sources of vitamin D are fatty fish, egg yolks, supplements, as well as in countries where they're available, fortified products such as margarine, milk, and cereal [20]. Muscle, liver, fat, and kidneys are examples of animal products that provide vitamin D as 25 hydroxyvitamin D (25(OH)D) [21]. Vitamin D is supplied in an endogenous way based on skin exposure at a wave length of 290-315 nm to UVB sunlight [17,22]. The amount of radiation of UV radiation of the equivalent skin length depends on latitude, altitude, air quality, cloud coverage, daylight hours and latitudes of the north and south of the month of the year. Moreover, the effects of UVB on the skin are affected by clothing and the usage of sun screens which absorb UVB, dramatically prevents the vitamin D3 generation by preventing interaction of UVB with 7-dehyrocholesterol. The effectiveness of catenin synthesis in vitamin D depends on the skin condition and age. Intradermal degradation increases after prolonged exposure to UV radiation and depends on the frequency of repeated exposures during each exposure period [23]. Recommended vitamin D intake level “Table 2.1.

Table 2. 1. Recommended vitamin D intake levels of the Institute of Medicine vs. Endocrine Society [145]

Age	Recommended Intake (IU/day)	Upper Limit (IU/day)
National Institute of Medicine		
Children (0–18 years)	400–600	2500 (1–3 years) 3000 (4–8 years) 4000 (13–18 years)
Adults (19–70 years)	600	4000
Older Adults (>70 years)	800	4000
Pregnancy/Lactation	600	4000
The Endocrine Society		
Children (0–18 years)	400–1000	2000–4000
Adults (19–70 years)	1500–2000	10,000
Older Adults (>70 years)	1500–2000	10,000
Pregnancy/Lactation	600–1000 (14–18 years) 1500–2000 (19–50 years)	10,000

2.1.3. Metabolism of Vitamin D:

Vitamin D is a prohormone that requires the metabolism of biologically active products that connect similar nuclear receptors to regulate various physiological processes [24]. Vitamin D is hydroxylated by the liver and released into the bloodstream as 25 (OH) D. The majority of 25 (OH) D transported attached to vitamin D binding protein (DBP) [25,26] It is used as a reservoir to hydroxylate 1,25-dihydroxyvitamin D or 24,25-dihydroxyvitamin D (24,25(OH)2D) into the physiologically active Vitamin D. The 1-hydroxylase (CYP27B1) enzyme converts 25 (OH) D to 1,25 (OH) 2D in the kidney and extrarenal tissues, whereas the 24-hydroxylase (CYP24) enzyme converts 25 (OH) 2D to 24,25 (OH) 2D. The endocrine modulator of calcium and phosphate homeostasis which is produced in the kidney is 1,25 (OH) 2D. The kidneys release and transport Ca^{+2} and P into the bloodstream to organs involved in calcium and phosphorus supply regulation, such as the gut, bone, parathyroid glands, and the kidneys themselves. 1,25 (OH) 2D has a substantially shorter half-life (approximately 4–6 hours) than 25 (OH) D. [27,28]. The 1,25 (OH) 2D generated in extrarenal target tissues is autocrine or paracrine in nature and does not enter the circulatory system in most cases. 1,25 (OH) 2D induces genetic and non-genetic responses in cells of the calcium phosphate homeostasis system and other target tissues via interactions with vitamin D receptors (VDR) [27,29]. Vitamin D and 25 (OH) D have little or no interaction with VDRs [20].

24,25 (OH) 2D is an alternate product of 25 (OH) D hydroxylation in the kidneys and extracellular space. [22]. It circulates in conjunction with DBP at a plasma level that is directly coupled to 25 (OH) D. Its synthesis is usually the initial step in the metabolic pathway that prevents vitamin D intoxication by inactivating and degrading 25 (OH) D. People with vitamin D deficiency, on the other hand, have large levels of 24,25 (OH) 2D. This points to the possibility that this metabolite has a functional role. The effects of 24,25 (OH) 2D support this theory (PTH). However, 1,25 (OH) 2D is thought to be the main metabolite responsible for vitamin D's biological effects. [22,27].

Dermal vitamin D is generated and reaches the bloodstream and liver, where it is linked to vitamin D protein (DBP). DBP is produced by the liver and is substantially higher in than vitamin D and its metabolites. Dietary 25 (OH) D is absorbed straight into the lymph or portal vein and carried to the liver in the availability of DBP. In general, vitamin D

absorption is continuing in all life [20,30]. When fat intake is minimal, however, it reduces [31].

2.1.4. Regulation of Vitamin D Metabolism:

The balance of 1-hydroxylase and 24-hydroxylase activity is crucial for calcitriol regulation. Serum calcium, calcitriol, and phosphate levels are all tightly regulated by both enzymes. When blood calcium concentrations are low or vitamin D levels are low, the parathyroid gland releases parathyroid hormone (PTH), which induces the manufacture of 1-hydroxylase, resulting in an increase in 1,25(OH)2D activation [32]. PTH also inhibits 24-hydroxylase [33] and can cause produce fibroblast growth factor 23 (FGF-23), which reduces sodium-phosphate transporter expression in the kidney [34]. FGF-23 can also help maintain Vitamin D homeostasis by decreasing 1-hydroxylase expression in the kidneys and promoting 24-hydroxylase, lowering serum calcitriol and, as a result, serum calcium in hyperphosphatemia. [35] "Figure 2.2.

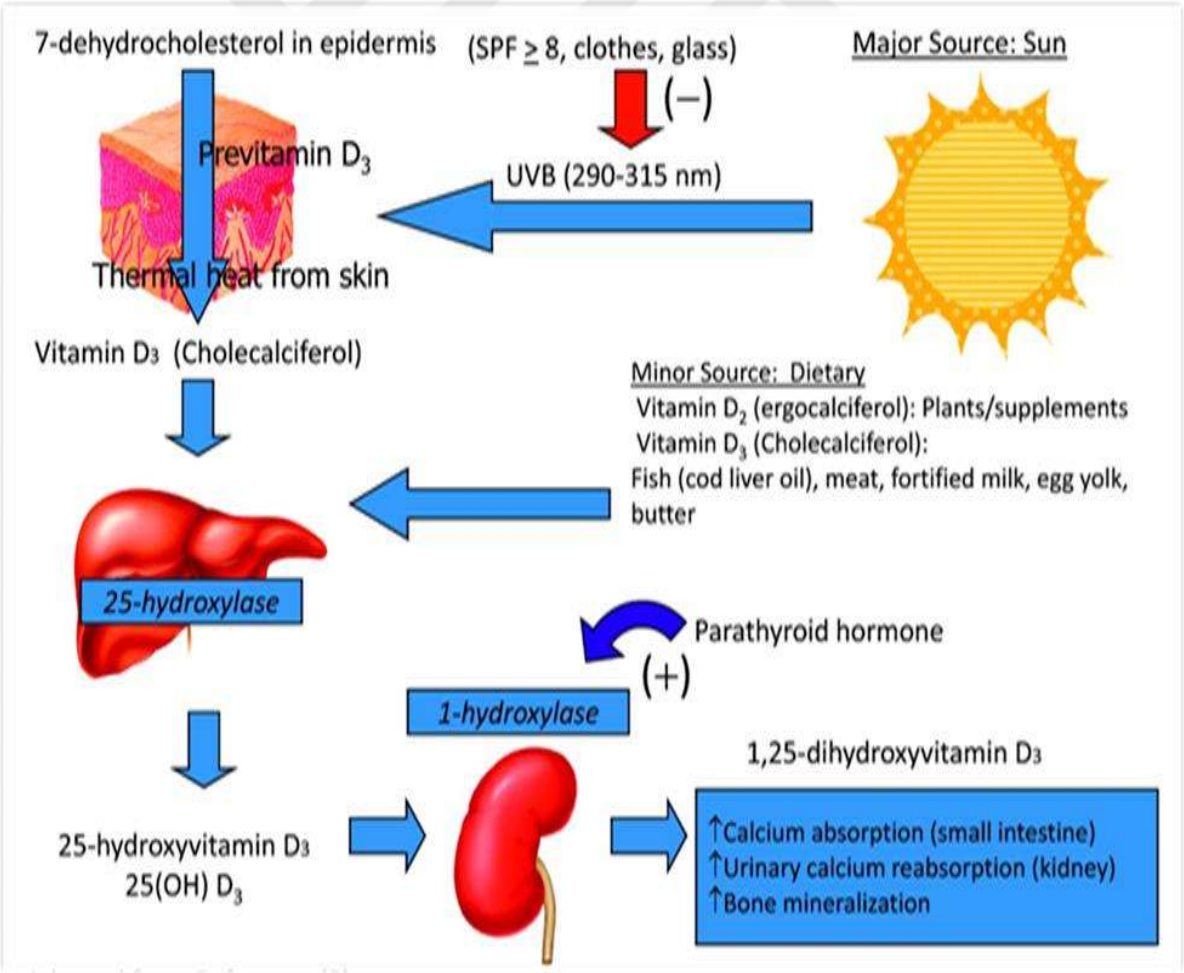

Figure 2. 2. Vitamin D sources and metabolism [36]

2.1.5. Effect of Vitamin D:

Vitamin D is a significant regulator of calcium and phosphorus homeostasis and of the production of a healthy skeleton through its action in the kidney, intestines, bone and parathyroid glands [36]. However, the benefits of vitamin D are not restricted to mineral homeostasis and the maintenance of skeletal health. The presence in other tissues and organs of VDR commits that the physiology of vitamin D reaches well beyond and beyond bone homeostasis [37]. While, VDR is present in the nucleus of several tissues that are not involved in the regulation of the metabolism of calcium and phosphate, it has been clearly identified in tissues also such as epidermal keratinocytes, activated immune system T cells, antigen-presenting cells, macrophages and monocytes, and cytotoxic T cells. In gene array studies made in several cells and tissues, showed that calcitriol controls several hundred genes or as much as 5% of the human genome throughout the body [38].

Vitamin D is known to monitor the growth of parathyroid gland and parathyroid hormones production; it has a major impact on the immune system, and may help suppress some autoimmune disorders and certain cancers [36] Vitamin D plays a role in pancreatic islet beta cells, it has been demonstrated to influence insulin secretion, apoptosis, and gene regulation in pancreatic beta cells in vitro studies[144], and also it has essential function in cardiovascular disease, infection, metabolic syndromes, and all-cause mortality [39].

2.1.6. Vitamin D and Autonomic Nervous System:

In autonomic balance, vitamin D plays a key role and can serve as a core neuroactive substance [8,40]. Vitamin D's neuroprotective properties are linked to its effects on neuromediator production, intracellular calcium homeostasis [40]. In a variety of tissues, vitamin D mediates nongenomic or nontranscriptional stages [41]. Those effects include transmembrane receptors and related secondary messenger systems (G-protein) that activate intracellular pathways via ligand-gated (IP3) [42].

Vitamin D controls and has multi-system implications for the sympathetic and parasympathetic nervous systems. It facilitates serotonin release and functions as an antioxidant that influences the autonomic activities of the brain, cardiac, and gastrointestinal systems [8]. Few research studied the relationship between the autonomous nervous system and vitamin D [9]. Low levels of 1,25 dihydroxy vitamin D were linked to autonomic

dysfunction. Treatment with 25-hydroxyvitamin D, on the other hand, has reduced the autonomic nervous system's adverse response to angiotensin II. [44]. In an in vitro investigation, however, VitD3 might accelerate catecholamine production in the rate-limitation stage [11].

An enhanced renin production was also indicated as an interconnection between vitamin D deficiency and cardiovascular events. Increased renin secretion in animal studies was connected with a vitamin D deficit. However, increased sympathetic activity of the nervous system can lead to the association of vitamin D with renin secretion, as the sympathetic nervous system induces renin secretion. [7].

2.1.7. Vitamin D Deficiency:

Vitamin D deficiency has emerged worldwide as a major public health concern [45,134]. Nearly, 1 billion people have vitamin D deficiency, especially more common among the elderly people [46,47]. This widespread of hypovitaminosis D can be primarily due to decreased sunlight exposure (for example, reduced outdoor activities and air pollution) [48]. A number of studies have found that vitamin D deficiency or insufficiency is related to a variety of chronic and acute illnesses. Also, the inadequate sun exposure, increased usage of sunscreens, obesity, insufficient intakes of vitamin D, gastrointestinal disorders, malabsorption, renal and liver diseases, and other various health conditions are contributing to vitamin D deficiency [49].

Deficiency of vitamin D in infants and children causes rickets, and it cause osteomalacia in adult. Rickets are characterized by failure of ossification in long bone growth plates and cause characteristic bone deformities in older children. Osteomalacia is a deficient osteoid mineralization that is associated with expanded osteoid seams and the presence of looser areas on the trabecular and cortical bone surfaces. Hypocalcemic convulsion and muscle weakness in the limbs, heart, and respiratory systems can be related to with both conditions [50]. Numerous conditions, such as cardiovascular diseases, arterial hypertension, dyslipidemia, type 2 diabetes, cancer, multiple sclerosis, depression, dementia, mental diseases and others, are associated with vitamin D deficiency [51].

2.1.8. Vitamin D Toxicity:

As a fat-soluble vitamin, excess use of vitamin D raise concerns about toxicity. In the United States and Europe, widespread vitamin D fortification of foods and beverages led to a lot of toxicity cases from the 1930s to 1950s [52].

Vitamin D intoxication results from the use of higher formulations than recommended [53], and toxicity can also result from prescription errors without regular monitoring of vitamin D levels [54], this condition frequently seen in patients who require large doses of vitamin D such as osteoporosis, renal osteodystrophy, psoriasis, gastric bypass surgery, celiac disease, or inflammatory bowel disease [55]. Prolonged sun exposure does not cause vitamin D toxicity because of the vitamin D3 turns to photolysis inactive metabolites which have no biological effect [56].

Vitamin D toxicity cause to imbalance of calcium homeostasis and bone metabolism, and results in hypercalcemia, then this condition leads to manifestation of the signs of clinically toxicity [57]. Gastrointestinal disorders including anorexia, diarrhea, constipation, nausea and vomiting are early signs of vitamin D toxicity. Other symptoms that are likely to occur within a few days or weeks are bone pain, drowsiness, continuous headaches, erratic heartbeat, lack of appetite, muscle and joint pain; frequent urination; especially at night, extreme thirst, fatigue, nervousness and itching; kidney stones [58].

2.2. Electrodermal Activity (EDA):

The history of EDA science, which was extensively reviewed by Neumann and Blanton in 1970 date from studies conducted in 1849 in Germany by DuBois-Reymond. The first experiment, which showed a link between the operation of the sweat gland and skin current flow was carried out in Switzerland by Hermann and Luchsinger in the 1878 [59]. In 1888, a French Neurologist F´er´e, found that by passing a low electrical current across two electrodes placed over the surface of the skin and measure response to a stimulus (visual, auditory, etc..), decreases in skin resistance. In 1890 Tarchanoff stated that it was possible for one to measure electrical potential changes between two skin-placed electrodes without adding an external current. Therefore, it is said that F´er´e and Tarchanoff discovered the two fundamental methods in use today to monitor electrodermal activity. The exosomatic method is referred to as the passage of an external current through the skin, while the

recording of the skin response does not require an external current and is therefore named to as the endosomatic method [60].

EDA is an active and passive electrical activity of eccrine sweat glands stimulated by the sympathetic nervous system [61]. The electrodermal signals are a reflection of the activities of eccrine sweats, mainly through the sudomotor neurons, within the autonomic nervous system (ANS) [62].

EDA is a sympathetic reflection of cholinergic sudomotor activity, which causes changes in electrical conduction resistance to the skin. The Sympathetic Skin Response (SSR) assessed by EDA. It was proposed as an easily obtainable sudomotor activity index [63], and as a delicate bodily index, emotion and attention-related arousal [62]. When the sudomotor nerves induce sweat production, the conductivity can measure on the skin surface changes, result from sweat output and changing in ionic permeability of sweat gland membranes [65]. Measurements of EDA are commonly recorded from the palmar sites of the hands or plantar side of feet where the sweat gland density is highest (2,000/cm2) [66].

In the terminological literature, instead of the term EDA, the term electrodermal response, physiogalvanic skin response, peripheral autonomic surface potential or common sympathetic skin response are also used [67].

2.2.1. Physioanatomy of Skin and Sweat Gland:

The human skin is very complex and large organ [68]. It is a selective barrier helps to prevent foreign matter from accessing the body and to selectively promote the transfer of materials from the bloodstream to the body's exterior. It helps to maintain water balance and steady core body temperature [60]. The skin consists of multiple layers that can be easily differentiated “Figure 2.3. These layers are epidermis, dermis, and hypodermis from the surface to the inside, and includes skin derivatives such as nail, sebaceous glands, and some type of sweat glands [59].

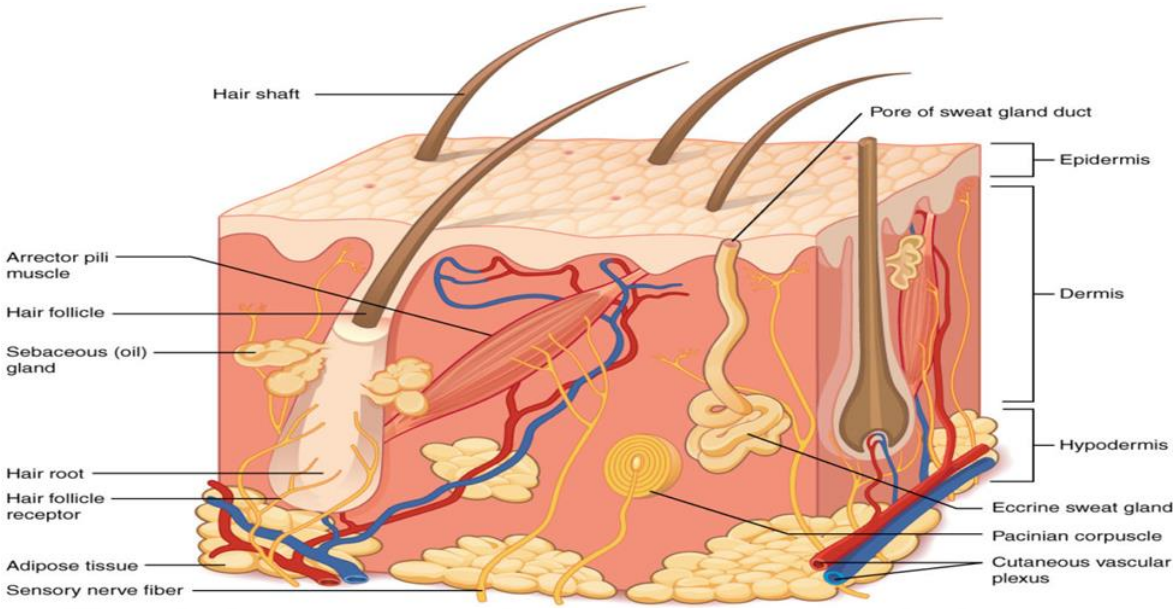

Figure 2. 3. . Structure of skin [69]

2.2.1.1. Epidermis:

Epidermis is the outermost layer. It forms a waterproof, protective wrap over the surface of the body that also acts as an infection barrier and consists of a stratified squamous epithelium with an underlying basal lamina. There are no blood vessels in the epidermis, and cells in the deepest layers are almost entirely fed by diffused oxygen from the surrounding air [59,70]. The major cells type of epidermis are keratinocytes, which are originating of the basal layer, produce keratin, and responsible for creating and secreting lipids to form the epidermal water barrier. Keratinocytes also control calcium absorption by activating cholesterol precursors via UVB light to form vitamin D. Melanocytes produce melanin mainly, which is responsible for the skin's pigment. Langerhans' cells are the first-line skin defenders and play an important role in the presentation of antigens, and Merkel cells perform a sensory function as light touch mechanoreceptors [59,71]

2.2.1.2. Dermis:

At the level of the basement membrane, the dermis is connected to the epidermis and consists of two layers of connective tissue, papillary and reticular layers that fuse together without specific demarcation. Sweat glands, hair, hair follicles, muscles, sensory nerves, and blood vessels are located in the dermis [71]. The dermal blood vessels don't only blood supply for dermis and epidermis. Many of them also play an important role in controlling temperature and therefore the volume of blood flowing through them is regulated to vary the amount heat exchange between these skin surface vessels and the external environment. The

receptor senses pressure, temperature, pain, and other somatosensory inputs at the peripheral end of afferent nerve fibers in the dermis. Efferent nerve endings in the dermis regulate the caliber of the blood vessel, hair erection, and skin exocrine gland secretion [72].

2.2.1.3. Hypodermis:

Hypodermis is under the dermis, also known as the subcutaneous fascia. It is the deepest skin layer and includes adipose lobules with some skin appendages such as, hair follicles, sensory nerves, and blood vessels [71]

2.2.1.4. Distribution and Structure of Sweat Glands:

The sweat glands are referred to as exocrine, because sweat glands secrete directly on the skin surface [73,77]. Specific dermal infolding of the epidermis structures the exocrine sweat gland of the skin. The sweat gland spread over much of the body, releases diluted salt solution through a tiny opening, pores of sweat on the surface of the skin, evaporation of this sweat cools the skin and is necessary for temperature control [72]. There are approximately three million sweat glands in the human body, the highest density is located on the palms, sole and legs, head and trunk. [59,72]. There are three type of sweat gland as apocrine, apoeccrine, eccrine sweat glands “Figure 2.4.

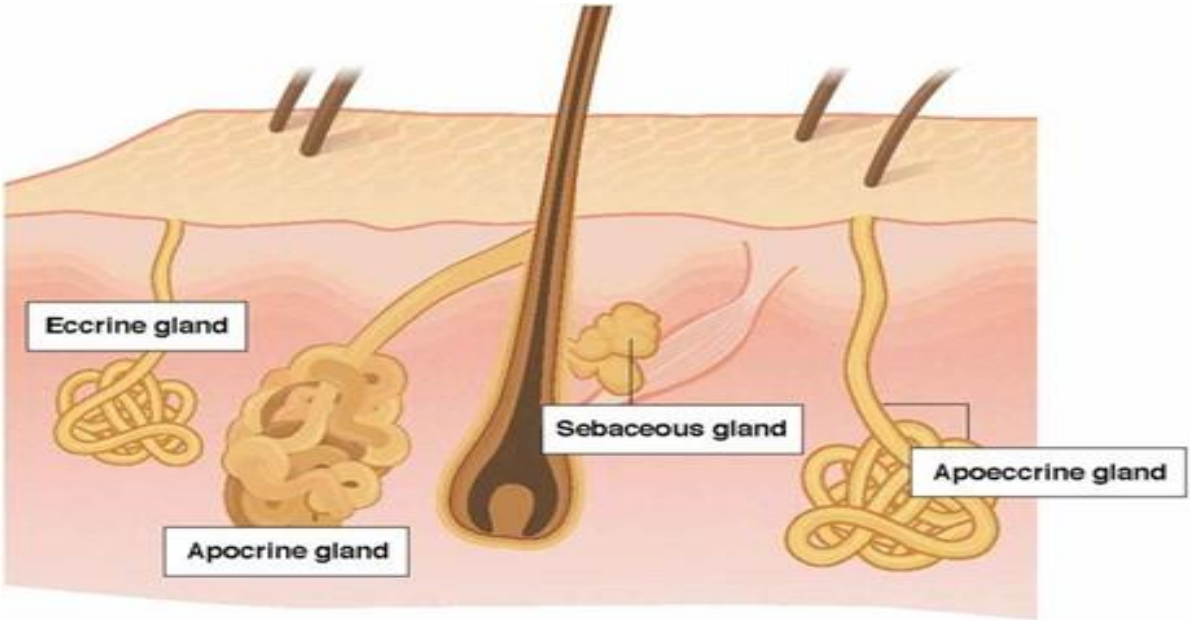

Figure 2. 4. Types of sweat gland [73]

2.2.1.4.1. Apocrine Sweat Glands:

The axilla, breasts, face, scalp, and perineum are the most common sites for apocrine sweat glands [75]. These glands are bigger and open to hair follicles rather than the skin's surface, as opposed to eccrine glands. [76] Furthermore, the apocrine glands' secretory function does not commence until puberty, despite the fact that they are present from birth [75] The apocrine glands produce a viscous, lipid-rich sweat that also contains proteins, carbohydrates, and ammonia. [75,77]. In several animals, the role of apocrine glands is commonly regarded as scent glands involved in pheromone (odor of the body) production. Regardless, this social/sexual function in humans is rudimentary. The apocrine gland's innervation is unknown, however isolated sweat glands have been found to respond similarly to adrenergic and cholinergic stimulation [77].

2.2.1.4.2. Apoeccrine Sweat Glands:

It was identified in 1987 by Sato et al. [77,78]. Apoeccrine glands develop between the ages of approximately 8 and 14 and reach 45 percent of the total axillary glands between 16 and 18 years [77]. They are intermediate in size, and share properties with both eccrine and apocrine glands, as the name implies. Like apocrine glands, the distribution of apoeccrine glands is restricted, as they are found only in the axillary zone. As the distal duct attaches to and empties sweat directly onto the skin surface, apoeccrine glands are resemble to eccrine glands. Also, it releases abundant secretions of salty sweat as eccrine sweat [77]. The role of this secretion is unknown, but as evaporation is inefficient in the axillary region, it is unlikely to play a significant role in thermoregulation. There is still little understanding of the innervation of the apoeccrine gland, but *in vitro* models indicate that the apocrine gland is more responsive to cholinergic stimuli than adrenergic stimuli [77,78]

2.2.1.4.3. Eccrine Sweat Glands:

The first type of sweat gland discovered was eccrine glands, as Purkinje and Wendt originally identified them in 1833 and Breschet and Roussel de Vouzzeme in 1834, until over 100 years later, Schiefferdecker did not name eccrine glands, however, the most numerous type of sweat glands are eccrine sweat glands [79], it is responsible for creating the largest amount of sweat and spread over almost the entire surface of the body except for the lips, the outer ear canal, the clitoris, the minor labia, the glans penis and the nail bed [79,80]. They are found on hairy and non-hairy skin (palms, soles) [81]. The palms and soles

have the largest gland density (250-550 glands/cm2) and respond to both emotional and temperature stimuli [82].

Eccrine glands function early in life, and the overall number of eccrine glands is determined later in life, starting around the age of 2-3 years. [76]. As a result, as the skin expands during childhood, the overall density of sweat glands decreases, and is usually inversely related to body surface area. [75]. Higher sweat gland density, on the other hand, does not always imply a higher rate of sweating. In reality, rather than the total number of active sweat glands, differences in the rate of sweat secretion per gland account for much of the regional and entire-body sweating rate variability within and across people. [83]. The anatomical structure of the eccrine sweat gland consists of a simple tubular epithelium composed of a secretory coil found in the dermis or in the hypodermis and duct that pass through the dermis (called the straight Duct) and then through the epidermis, a spiral (helical) course connects with the epidermis acrosyringium "Figure 2.5. [59,73].

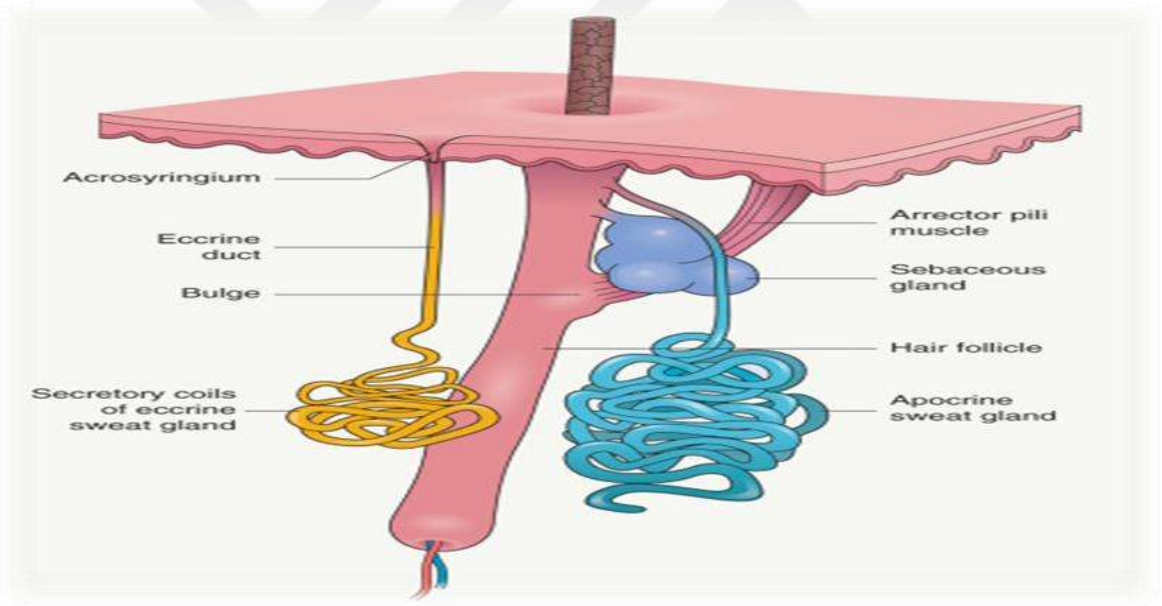

Figure 2. 5. Anatomical structure of eccrine sweat gland [73].

Eccrine sweat consists of primarily water and NaCl, but it also contains a blend of the interstitial fluid and the eccrine gland itself with several other chemicals [73]. Eccrine sweat glands accept sympathetic innervation by cholinergic fibers, that sends impulses in response to changes in core body temperature. The hypothalamus thermoregulatory center mediates the sweat glands with sympathetic innervation. Their neurotransmitter is nicotinic acetylcholine. Acetylcholine, which differs from all other sympathetic postganglionic fibers

that produce norepinephrine, is released into the postganglionic fiber. Induces sweating by cholinergic stimulation of muscarinic receptors [84]. In addition, the eccrine sweat gland is stimulated by adrenalin and noradrenalin because in this gland α1-, β2- and β3-adrenoceptors are expressed. Catecholamine-mediated sweating is caused by stress, including emotional stress [85]. For that reason, it was assumed that EDA was governed by both the sympathetic and parasympathetic branches of the ANS. Human sweat glands are currently recognized to have mostly cholinergic activity from sudomotor fibers coming from the sympathetic chain [86].

Thermoregulation is the primary function of a majority of the sweat glands. However, those found on the palmar and plantar sides are considered to be more concerned with output grabbing, rather than temperature control. Instead of thermal stimuli, these sweat glands respond more to psychological stimuli [60]. Due to the high density of eccrine glands in these regions, this phenomenon is most apparent in hands and feet; however, emotion-evoked sweating includes all eccrine sweat glands [87]. EDA is thus assumed to be a quantitative functional indicator of sudomotor function and, consequently, an objective arousal evaluation [88]

2.2.2. Electrodermal Activity Measurement Method:

EDA includes different phenomena, but typically is referred to as skin conductance (SC) [59,62]. Depending on the human psychological condition, it represents a measure of changes in the electrical properties of the skin [89]. Two methods, endosomatic and exosomatic are used to examine these electrical characteristics. The current is referred to as endosomatic, as only potential differences from the skin itself are reported. Exosomatic recording methods apply either direct current (DC) to the skin, or alternating current (AC). In DC calculation, EDA is directly reported in SC units when voltage is kept constant, whereas skin resistance (SR) units are obtained when current is kept constant [59,89].

2.2.2.1. Exosomatic Direct Current (DC):

The exosomatic DC is most common used method due to their simplicity, the need for just two electrodes and the possibility of tracking both the tonic and phasic skin conductance signals EDA is estimated by passing direct current constant voltage through a pair of silver–silver chloride (Ag/AgCl) electrodes situated on the surface of the skin, and include an electrolyte gel between the metal surface of the electrodes and the skin to ensure maximum

electrical contact and avoid the effects of electrolysis. The use of Ag-AgCl minimizes the polarization of the electrodes and the potential for bias between them [59,60,90]. The concept invoked in measuring skin resistance or conductance is that of the law of Ohm. Specifying that the resistance of the skin (R) is equivalent to the voltage (V) applied between two electrodes situated on the surface of the skin, divided by the current (I) flowing through the skin. It is possible to express this law as R = V/I. It is possible to apply two different measurement methods:

1. **The Constant Current Method:** The current is kept constant, so the voltage between the electrodes, which varies directly with skin resistance, can be determined, and potential changes reflect the change in resistance.
2. **The Constant Voltage Method:** The voltage is kept constant, then the current flow can be determined, that varies directly with the reciprocal skin resistance, skin conductance. This measurement method closely correlates with firing rate of neurons in the skin and skin conductivity [59,60].

2.2.2.2. Alternating Current (AC) Exosomatic Method:

It is not a frequently used method. It changes from the DC measurement because instead of one direct voltage, an alternating voltage source is employed.

2.2.2.3. Endosomatic Method:

It is the method in which the potential difference between areas that contain and not contain sweat glands is recorded. It does not require external electrical current flows through the skin. The only source of electrodermal activity is the skin itself and its interaction with the electrode-electrolyte system. Skin potential responses (SPRs) is recorded by this method. It is an invasive method in which the electrical activity of neurons in the skin is directly measured. There are several type of skin potential responses such as monophasic, biphasic, and polyphasic “Figure 2.6. [59,60].

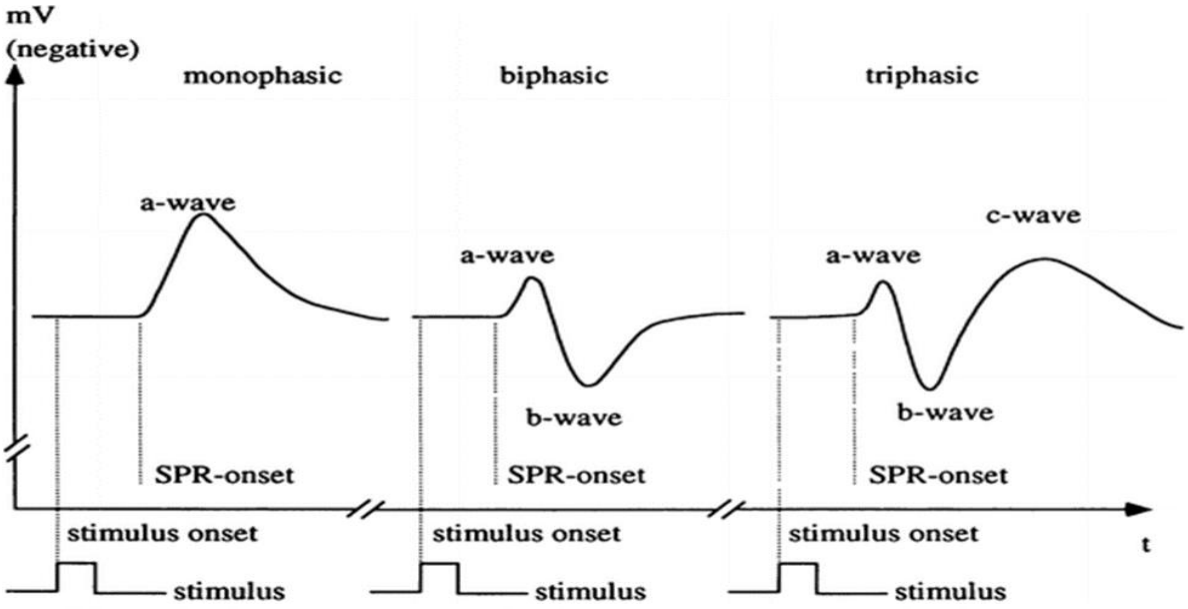

Figure 2. 6. Different types of SPRs: mono-, bi-, and triphasic forms. Higher values mean greater negativity of the active with respect to the passive recording site [59]

2.2.3. Biophysical Basis of Electrodermal Activity:

When biological tissues such as skin and sweat glands are applied to an external current, they behave like resistor and capacitor-built electrical networks. The skin which is well nourished with blood and interstitial fluids has good changeable electrical conductivity. The resistance of this layer is dependent on the electrolyte level rather than on its humidity. Sweat gland ducts serve as conductivity short circuits via the stratum corneum. This is critical for electrodermal recording in the palmar and plantar regions, which have a high sweat gland density “Figure 2.7. [59,60].

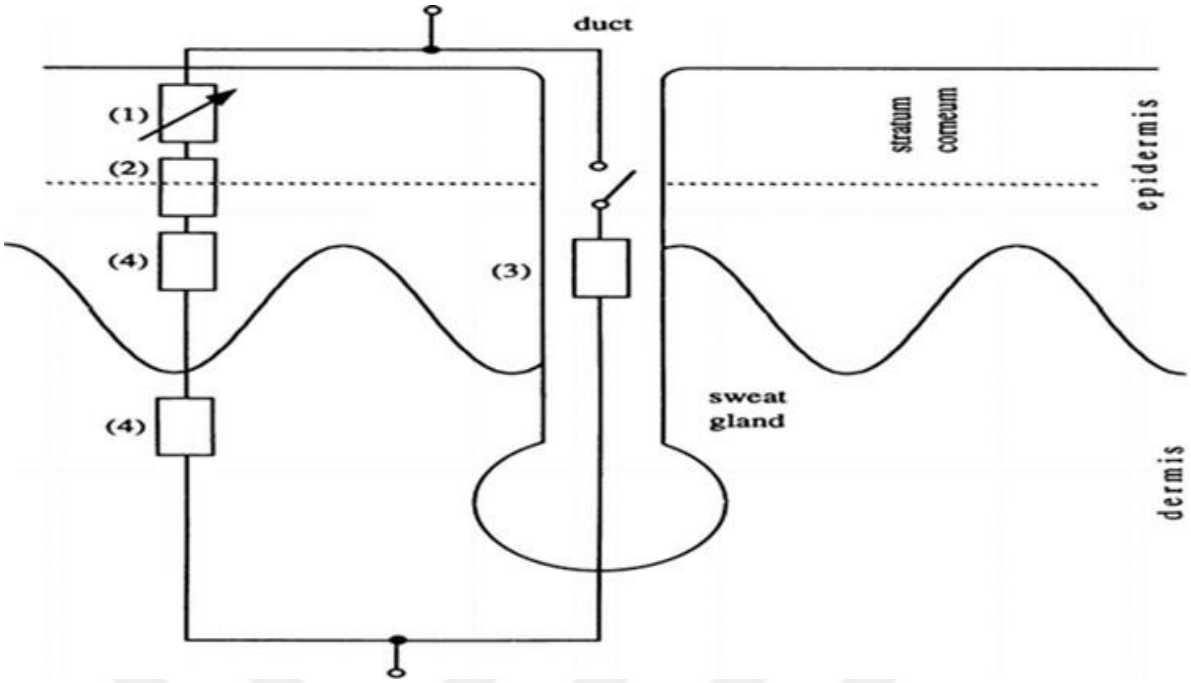

Figure 2. 7. Schematic illustration of resistive pathways through the skin and the sweat gland [59].

As shown in Figure 2.7, Sweat gland system can be explained as serial- and parallel-connected resistors.

When a current is administered to the skin from the outside, the cell membranes act as capacitor structures Since they are selectively permeable to ions related to electric current. In recording of electrodermal activity Ohm's low which is a physical principle is used for measurement of change in resistance. According to this low change in current when current is passed at constant voltage between the electrodes used during the electrodermal measurement. Since conductivity in electricity is defined as opposite to of resistance R = 1/G, G=1/R; where R equals resistance and G equals conduction. It is proportional to changes in conductivity [90].

2.2.4. EDA Parameters:

EDA are divided into two as tonic and phasic parameters, which are called differently according to the method. Tonic parameters, their activities with continuity over time, phasic parameter describe the short-term activity that occurs in response to stimulus. Phasic responses are also caused by changes in sweat gland activity caused by autonomic stimuli [59,60,95].

2.2.4.1. Tonic Activities:

- These are the electrical activity of the skin recorded without an exogenous stimulus
- Skin conductance level (SCL) can vary widely between different subjects and in different psychological states within the same subject, the standard range is between 2 μS and 20 μS [60].
- Fluctuations in electrical activity observed without the delivery of an exogenous stimulus. Responses can, however, be elicited by deep breaths and motions of the body.
- Skin conductance fluctuation frequency (SCF) typically is between 1 and 3/min also called Nonspecific Skin Conductance Response (NS-SCR) [60,62].

2.2.4.2. Phasic Activities:

It is the recording of the electrical change that develops in response to an exogenous stimulus, typically, the size of an elicited skin conductance response (SCR) ranges between 0.1 and 1.0 Ms [60,59].

Since skin conductivity was recorded in this study, the parameters suitable for the method are described below:

1.**Skin Conductance Level (SCL):**

Activity of sweat glands at rest. Its unit ohm, SCL is the average value of the total amount of skin conductivity over time and the measurement range is generally between 2-20 ohm.

2.**Skin Conductance Response (SCR):**

The stimuli used in EDA studies are applied in series, the response to stimulus sequence needs to be defined for the change in conductivity to be accepted as the response. Most of researchers accepted the change in conductivity that occurs between 1-5 second following each stimulus as an answer, conductivity changes occurring outside the specified time interval are considered as nonspecific fluctuation, not as response “Figure2.8. [59,60].

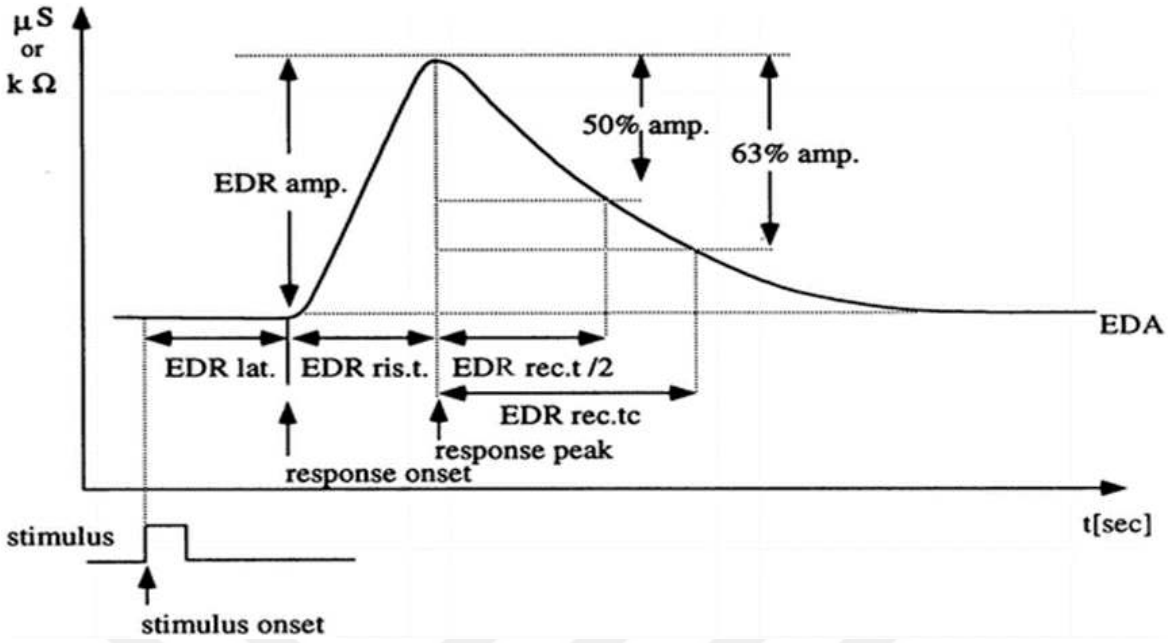

Figure 2. 8. DC recorded exosomatic EDR (Electrodermal response) and the parameters to be obtained from it [59].

2.2.5. Effect of Stimulus on EDA:

As a result of the repetition of different stimuli in the measurement of electrodermal activity, may cause a decrease in the electrodermal response or the amplitude response. In other words, the reaction severity to repetitive stimuli gradually decreases. This situation is called habituation [60,96]

2.2.6. Control of the EDA:

It is thought that the centers related to attention, information and perception function are responsible for EDA control. Studies performed by stimulating or damaging different parts of central nervous system (CNS) have revealed the importance the CNS in the formation and control of EDA. The neural control of the eccrine sweat glands which are the source of electrodermal activity can be examined in two parts at the spinal and supraspinal levels [59,62].

2.2.6.1. Spinal Control:

Some of the autonomic nervous system neurons located in the grey matter of the medulla spinalis perform spinal control of eccrine sweat glands. The fiber that innervates the eccrine sweat glands called sudomotor fibers and they are axons of neurons that secrete

acetylcholine from the postganglionic end but anatomically belong to the sympathetic subdivision of the autonomic nervous system. The cell bodies of the sudomotor fiber are located between C8-L12 in the intermediolateral nucleus of the spinal cord, the efferent nerve fiber originating from preoptic sweating center of the hypothalamus descend through the ipsilateral brainstem and medulla and synapse with these cell bodies in the intermediolateral nucleus [59,81,91]. The preganglionic sudomotor neurons migrate to the postganglionic neurons, along with sympathetic nerve fibers. Nonmyelinated class C nerve fibers that terminate around sweat glands make up postganglionic nerve fibers. The sympathetic cholinergic fibers that surround the sweat glands have a transmission speed of 1.2 to 1.4 m/s. [59,91] At the level of the spinal cord, preganglionic sudomotor neurons are in anatomical relationship with vasomotor and pilomotor sympathetic fibers; the only way to distinguish human sudomotor from vasoconstrictor fibers in the periphery, but not at the level of the spinal cord, is via the stimulation and blocking procedures, where sudomotor efferent cannot be distinguished from other sympathetic fibers. [59,97]

2.2.6.2. Supraspinal Control:

The hypothalamus is not the only source of sweat gland activity in the central nervous system. At the same time, the limbic system, particularly the amygdala and hippocampus, is undergoing changes. It has an effect on the hypothalamus regions' thermoregulatory function. As result, it is thought that the hypothalamic area which provides temperature regulation play role in the emergence of EDA, there are three different pathways that control the sudomotor fibers in the spinal cord:

I. Ipsilateral Limbic- Hypothalamic Electrodermal Source (EDA 1)

Limbic – hypothalamic originated ipsilateral efferent pathway, it is subsystem that controls EDA as result of both thermoregulatory and emotional changes. A strong relationship was found between prefrontal area measurements and left temporal cortex and spontaneous or evoked EDA response. Fiber originating from the premotor cortical area (Brodmann area 6) have an important contribution to the stimulation of EDA responses. These fibers are the source of electrodermal changes accompanying motor movements. Thermoregulatory hypothalamic activity is controlled by the limbic system primarily the amygdala and hippocampus, fibers originating mainly from the hippocampus and amygdala directly affect the hypothalamic sudomotor area [59,98,99]. In cortical injuries with frontal

lesion, especially from the right hemisphere to the anterior lobe, it has been observed that the skin conductivity response to physiological current signals decreased [92,99].

II. Contralateral Premotor and Basal Ganglia (EDA 2)

The contralateral efferent pathway originating from the premotor and basal ganglia. The premotor – basal ganglia source that enables the formation of electrodermal components accompanying specific motor movements is called EDA-2. This second path is crossed in the medulla before reaching the sympathetic pathway "Figure 2.9. Primary and associative frontal motor areas control the sweating response to a stimulus that requires motor response and feels dangerous [59,64].

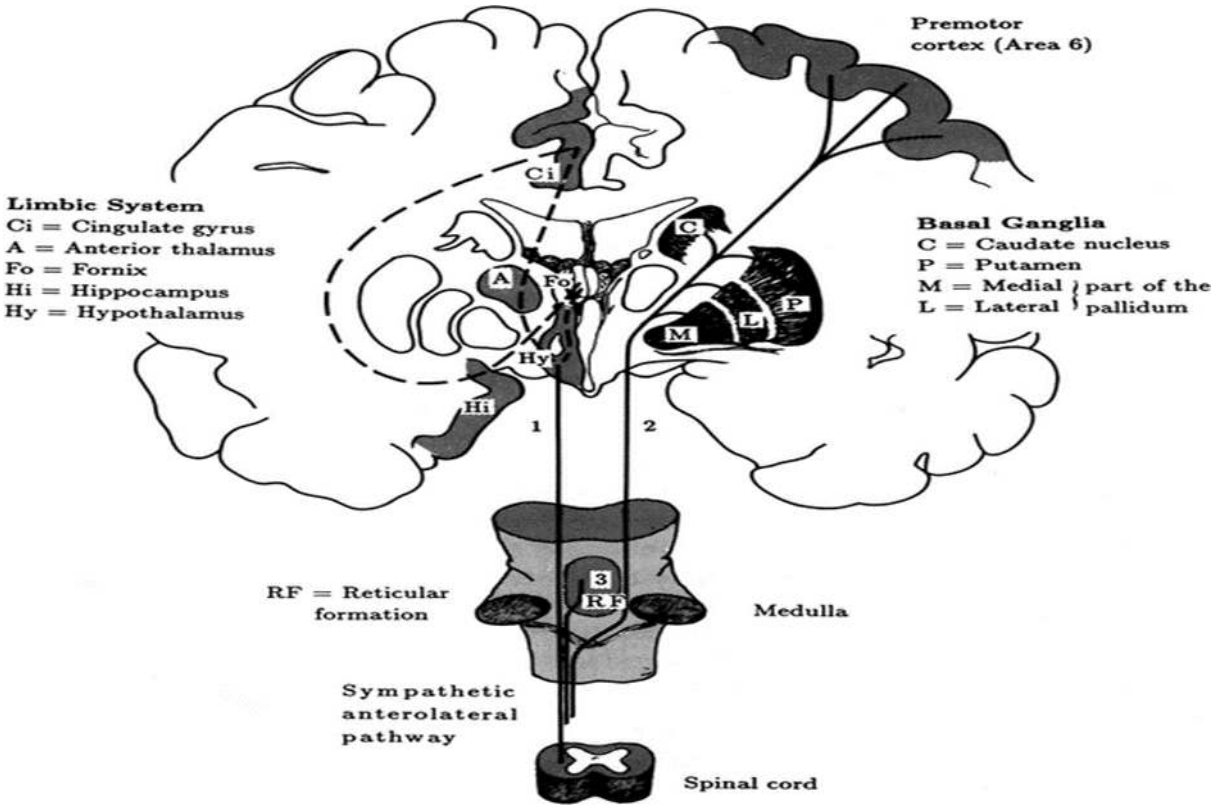

Figure 2. 9. Central nervous system elicitation of EDA in humans: 1: ipsilateral influences from the limbic system via hypothalamic thermoregulatory areas (EDA1); 2: contralateral influences from premotor cortical and basal ganglia areas (EDA2); 3: reticular influences [59]

III. Bilateral Reticular Modulator Source (EDA 3):

Bilateral reticular modulator – induced pathway is subsystem that controls inhibitory effect and mediates EDA changes seen in alertness changes. Thus, it is stated that reticular formation can also scald the EDA or have modulatory effect on EDA [59,60]. the reticular formation occupies the medulla and midventral part of midbrain. It is basically anatomical

area, complex multi-junction road with separate function of fibers and various neural deposits. There are cell bodies and fibers of serotonergic, noradrenergic, and adrenergic systems for EDA. In this region area related to the regulation of heart rate, blood pressure and respiration. There are numerous areas some of descending fibers here inhibit conduction in sensory pathway in the spinal cord [59,100]. EDA provided by reticular formation activation probably increased muscle tone with all movements, and EDA which is related to hypothalamic activity is likely to result from the relevant thermoregulatory sweating, and EDA which is related to amygdala activation, is also likely a reflection of the emotional process [60].

2.2.7 Factor Affecting Electrodermal Activity:

2.2.7.1. Physiological Factors:

I. **Thermoregulatory System**: It is part of homeostatic mechanisms that arise against physical or pathological trauma in the hypothalamus and is not related to cognitive function.

II. **Sudomotor Vasomotor System**: Sudomotor region are rich in arteriovenous anastomoses and arterioles. Sympathetic vasoconstrictor innervation of arterioles also affects eccrine sweating. Mental-emotional stimuli activate sudomotor and vasomotor events and vasomotor events.

2.2.7.2. Hormonal Factors:

Intradermal injection of adrenaline and noradrenaline causes local sweating, this effect is blocked by phentolamine, not atropine. Circulating catecholamines reduce eccrine sweating. Antidiuretic hormone decreases sweat secretion by increasing the permeability of sweat gland ducts to water. Aldosterone affects sodium absorption from sweat gland ducts. Progesterone reduces palmar eccrine sweating [59,90,100].

2.2.7.3. Factors of Measurement System:

I. **Electrode Area:** Since there is a direct relationship between skin conductivity measurements and the electrode area. It is evaluated with specific conductivity unit in mho / cm2 [59,93,94].

II. **Electrolyte**: The contact medium of the electrodes should be isotonic, close to the ion concentration of sweat liquid that is 0.05M is suitable for the silver/silver chloride (Ag/AgCl) electrode [59,60,62,95].

III. **Electrode:** It is recorded with bipolar system. In this system, it is based on the principle of recording two active electrodes located in two similar areas. Thus, the potential difference between two electrodes seen in the unipolar system disappears with bipolar system [59,62,95].

IV. **Polarization**: It is the development of an electromotive force in the opposite direction to applied current and affect the measurements. Electrodes where a metal comes into contact with one of its salts are used to minimize polarization [59].

V. **Registration Zones**: The recording of conductivity is normally taken from hand's palms and feet. The choice of locations is decided by the experimental assignment. Possible sites for electrodermal recording are palms and volar surfaces of the distal and medial phalanges. It has been found that the activity and number of sweat glands are higher in these regions and These areas are usually absent of scarring "Figure2.10. [59,93,94,95].

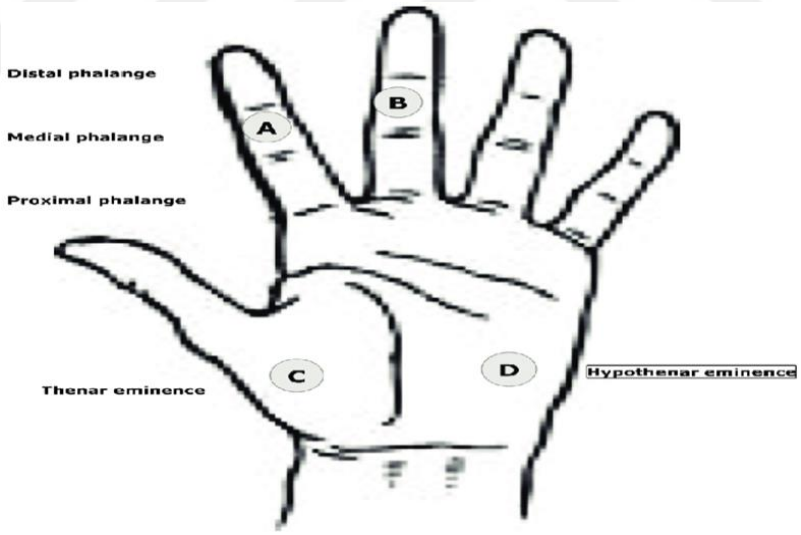

Figure 2. 10. Electrode sites in electrodermal measurement [59]

2.2.7. 4. Environmental Factors:

I. **Environmental Temperature**: Typically, as the room temperature rises, the skin conductance rises as well. There has been several research on the effects of body temperature, room temperature, and even season on skin conductance. They report different, if not diametrically opposed, outcomes. As a result, it's a good idea to keep

a monitor on the air temp during the measurements to ensure a more accurate comparison of the data. [90].

II. **Environmental Humidity:** It has reported that there is a negative relationship between skin conductivity and moist content.

III. **Time of Measurement**: It has been reported that the maximum value of skin conductivity is the middle of day [59,60,95,89].

2.2.7.5. Personal Factors:

I. **Age:** In adolescence, skin conductivity increases two times compared to puberty, while skin conductivity gradually decreases with aging.

II. **Gender:** In adolescence skin conductivity of girls is lower than the boys, this difference disappears with aging.

III. **Race:** Blacks have lower skin conductivity than whites.

IV. **Psychological Condition:** The psychological state of the person affects the skin conductivity [59,60,95].

2.2.8. Clinical Use of Electrodermal Activity:

2.2.8.1. Use of Sympathetic Skin Response in Diagnosis of Autonomic Functional Disorder:

The autonomous nervous system is a complicated system that affects each organ and system. So, the diagnosis of autonomic nervous system disease is more difficult. For this reason, sympathetic skin response has been used in diagnosis of some autonomic functional diseases. Sympathetic skin response is used clinically, in diagnosis of autonomic diseases such as sympathetic dystrophy, in diagnosis of thin unmyelinated fiber lesions in uremic neuropathy and diabetic neuropathies, by examining the measurements of prolongation of latency and decrease in amplitude [67,101].

Abnormal sympathetic skin response has also been found in familial amyloid neuropathy [102], alcoholic neuropathy [103,104], and lepromatose neuropathy [105,106]. There was no sympathetic skin response in acute and chronic inflammatory neuropathy with autonomic dysfunction [107,108]. Abnormal sympathetic skin response was seen in HIV-

positive patients whose disease was in the early asymptomatic period [109], and in the carpal tunnel syndrome, which the response is characterized by low amplitude [110,111].

2.2.8.2. Sympathetic Skin Response in Central Nervous System Disease:

Sympathetic skin response abnormalities have been observed in more than 50% of patients with multiple scleroses in clinical studies. This has been considered as a sign of lesion of the central sympathetic pathways [112,113]. A decrease in the amplitude of the sympathetic skin response and prolonged latency have been observed in patients with Parkinson's disease [114]. In a study conducted in patient with Parkinson's, the auditory stimulus for activation of the EDA-1 pathway, and the patellar tendon reflex for activation of the EDA-2 pathway. Sympathetic skin response was measured using the auditory stimulus from normal and Parkinson's patients. Although there was no important difference between skin response latencies of both groups, a significant difference was found between the induced sympathetic skin response latencies obtained by patellar tendon reflex [115]. In Alzheimer's disease they investigate the effect of Vinpocetine on autonomic dysfunction and sympathetic activity and discovered that vinpocetine increased sympathetic skin response but was ineffective in treating Alzheimer's autonomic function [116].

In amyotrophic lateral sclerosis, sympathetic skin response is not found in 40% of 25 patients, and prolongation of latency length and decrease in amplitude was observed [117]. Abnormal sympathetic skin response has also been found in patients with cervical myelopathy [118], syringomyelia [119], Wilson's disease [120], Duchenne's muscular dystrophy and another dystrophy [121]. Abnormal sympathetic skin response also presents in most patients with Shy Drager syndrome, sporadic Olivo-Ponto-Cerebellar Atrophy (OPCA) and straitonigral degeneration, but in patients with familial OPCA, it was normal [122].

2.2.8.3. Electrodermal Activity Studies in Other Disease:

Failure to observe skin conductivity response or lack of habituation is one of the psychophysiological symptoms frequently encountered in schizophrenic patients. It is stated that this situation is caused by the dysfunction of the central catecholamine noradrenaline system and that is effective in the pathogenesis of schizophrenia [123]. EDA is also used as the response of the autonomic nervous system to examine attention-related processes, habituation, and orienting response in humans [124]. In a study conducted among patients

with hyperthyroidism who were found to have no significant psychiatric disorder, it was shown that the function change in the hypothalamic-pituitary-thyroid axis caused abnormal EDA and SCR, and in non-medicated patients, they observed lower onset latency and length of skin conductance response and a higher degree of skin conductance than healthy controls [125]. Also, other study stated that the increase in EDA during seizures in epileptic people is an indicator of the participation of the central nervous system in this event [126]. The increase in skin conductivity observed in the case of fibromyalgia has been interpreted as the increase in cholinergic activity of the peripheral sympathetic nervous system and a decrease in the activity of its adrenergic component [127]. Regarding to a study conducted in terms of bilateral electrodermal activity responses, it was observed that the SCR recorded from the contralateral hand was higher than the other hand compared to the half of the brain used preferentially. However, it has been stated that there isn't single cortical mechanism on asymmetry in the electrodermal system [128]. EDA is used in the diagnosis of sympathetic impairment in hypothyroid patient [129], hyperthyroid [130], diabetic patient [131], scleroderma, erectile dysfunction, autoimmune vitiligo, rheumatoid arthritis, Behcet disease, primary autoimmune hypothyroidism, depression and psychosis [67].

3- MATERIALS AND METHODS

3.1. Experimental Animal:

A total of 36 male Wistar rats, 8 weeks old age, were used. Animal were obtained from Başkent University Experimental Research Center. They were housed in standard cages and allowed *ad libitum* access to feed and water.

All animal experiments carried out in Başkent University, Faculty of Medicine, Department of Physiology, Animal Experiment Laboratory. The experiments were conducted after the approval of the Animal Experiments Ethics Committee of Başkent University (20/18).

3.2. Experimental Groups:

Rats were randomly divided into 3 groups in equal numbers (n = 12)

- Sham group (group 1) [n=12]: Each rat was given normal drinking water by oral gavage.
- Medium dose Vitamin D (group 2) [n=12]: Each rat was given vitamin D by oral gavage at the doses of 400 International Unit [IU]/ day. [146]
- High dose Vitamin D (group 3) [n=12]: Each rat was given vitamin D by oral gavage at the doses of 1000 IU / day.

Recommended levels of vitamins D3 to be added per kg diet for rats is 300IU [147].

Vitamin which we used it in experiment Devit-3 oral drops, each 15 ml drop contains 50,000 IU of vitamin D3 (Deva Holding A.Ş, Turkey). The active ingredient cholecalciferol (vitamin D3) is produced from sheep's wool fat.

The substances were administered to the groups once a day for 8 weeks. At end of this period EDA records were taken. “Figure 3.1 shows the schedule of study.

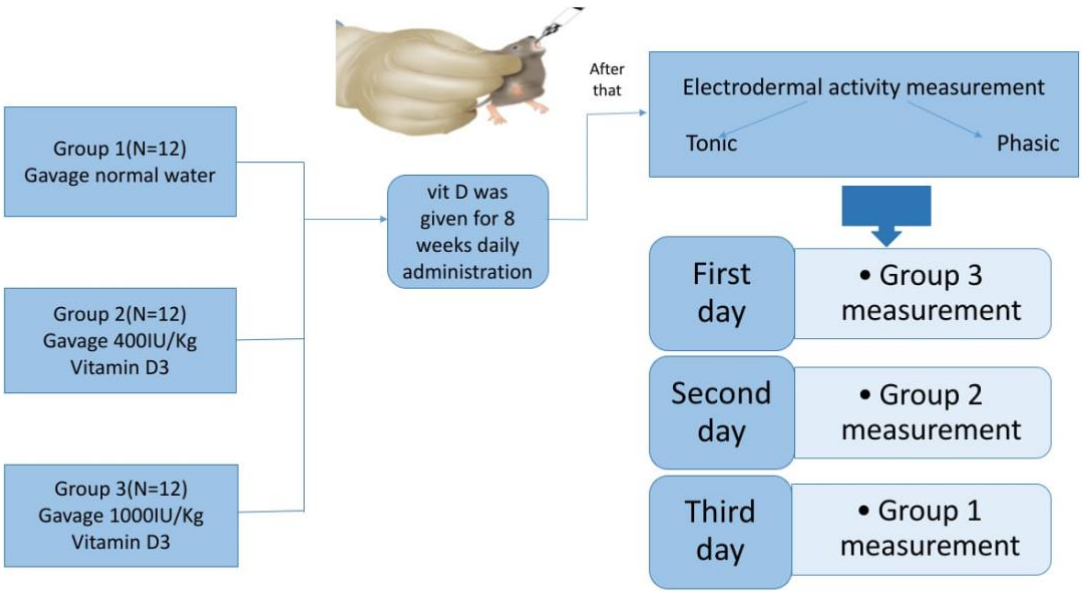

Figure 3. 1. Schedule of the study

3.3. EDA Recording:

EDA recorded immediately after completing the period of giving the vitamin D [8 weeks] in 3 days consecutively starting with high dose group in first day then medium dose group in second day and sham group in third day. The rats were placed in the immobilizer, excluding the tail and hind limbs. The plantar surfaces of the back of two extremities of the rat were wiped with 70% alcohol and 0.8 cm diameter Silver /Silver Chloride (Ag/AgCl) were placed. Electrodes were connected to MP30 system with GSR (Galvanic Skin Response) transducer. In order to increase the conductivity by decreasing the resistance between the skin and electrodes. After the electrodes were placed, the animal allowed to calm down in the room where it was kept dark and silent for 5 minutes, then a 2-minute tonic recording was taken without any sound stimulation. At end of the tonic recording, a 10-minute phasic recording was taken in which 15 sound stimuli were randomly given at intervals varying between 30-80 seconds without pausing. A stimulus generator (sound amplifier) was used for sound stimuli that generate electrodermal responses. The sounds of 1000Hz frequency and 90 dB intensity generated from the stimulus generator were played to the animals with two speakers installed in the experimental room “Figure 3.2.

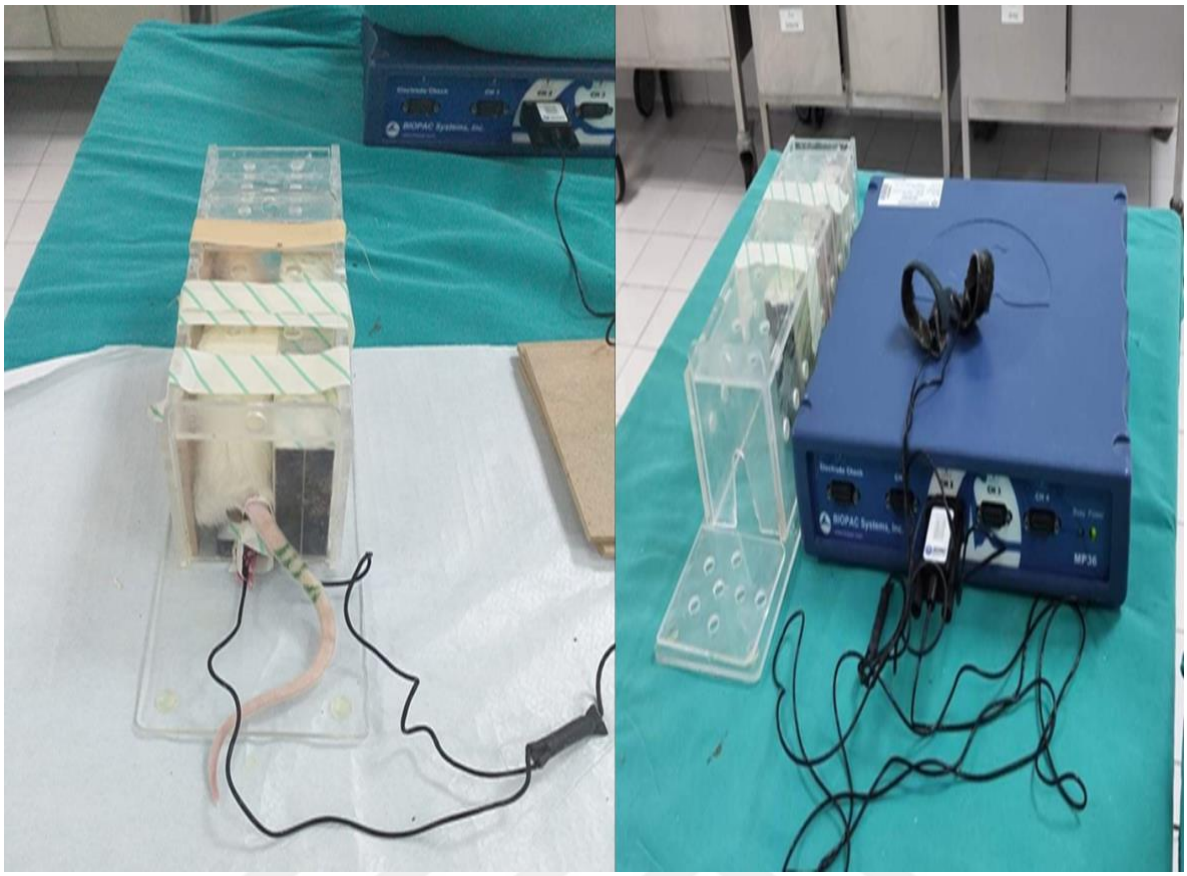

Figure 3. 2. The immobilizer and electrodermal electrodes of EDA.

Skin conductivity signals recorded with the MP30 system were passed through an Analog / Digital converter, and the digital signals were stored in computer environment for data analysis “Figure 3.3.

Figure 3. 3. EDA Apparatus

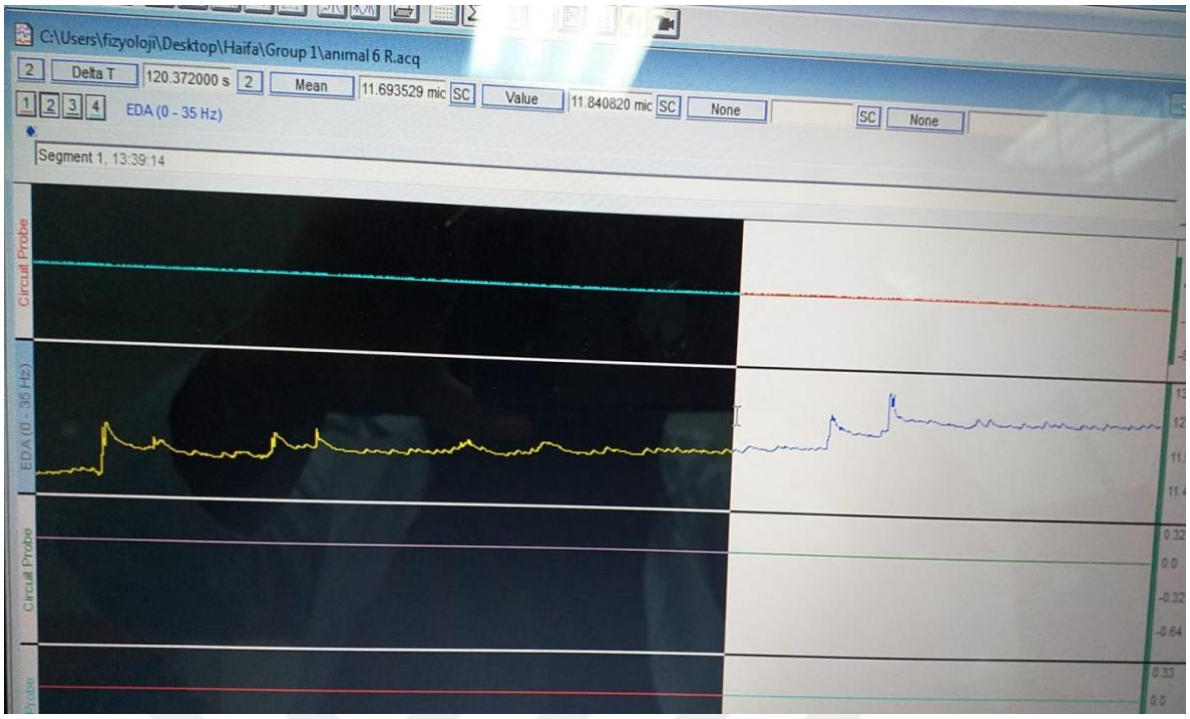

Figure 3. 4. Sample of electrodermal analysis

Just before the recordings, the skin conductivity unit and the MP30 software system were calibrated. For this purpose, the calibration buttons on the conductivity unit were pressed and the values that should be read were entered in the appropriate places in the software. Skin conductance levels (SCL) were recorded with EDA measurements. An increase in SCR indicates an increase in sympathetic activity and anxiety.

The experiment was carried out in two stages:

- Tonic EDA: It recorded in first 2 minute (120 second) without any stimulus, it was selected with the cursor and the mean of electrodermal response was calculated
- Phasic EDA: It was made immediately after tonic EDA recording for 10 minutes with stimulus.

After the recording process was completed, the animal was taken out of the immobilizer and returned to its cage. Immobilizer and electrodes were cleaned with alcohol and clean water after each animal.

3.4. Data Analysis of EDA:

Skin conductance levels (SCL) were analyzed with software of MP30 system on the registered PC computer from EDA recordings. SCL was analyzed with a cursor of our

system and the mean of electrodermal response was calculated. Analyses of skin conductance is evaluated as micromho (μmho) “Figure 3.4.

3.5. Statistical Evaluation:

Mean values (±the standard errors of the mean) of tonic SCL and phasic SCL were calculated. These data were analyzed with analysis of variance (ANOVA), with GROUP as main factors. Homogeneity of variance was confirmed by the Welch test. When appropriate, posthoc comparisons were made with the Scheffe test. Tonic and phasic SCL were compared separately for each group with paired sample t test. The $p<0.05$ level was accepted as significant.

4.RESULTS

One rat in the high dose group died before EDA could be recorded. therefore, 12 animals in the other groups and 11 animals in the high-dose group were recorded.

The tonic SCL of three groups are shown in “Table 4.1. There was significant difference among groups [F (2,33) = 4.374, p-value =0.021]. It should be noted that the post-hoc analysis revealed statistical differences at the tonic SCL between the medium dose group and the sham group (p value =0.027), but there was not significant difference between other groups.

The phasic SCL of three groups are shown in Table 4.1. There was significant difference among groups [F (2,33) = 11.013, p-value =0.001]. It should be noted that the post-hoc analysis revealed statistical differences at the phasic SCL between the medium dose group and the sham group (p value =0.001), and between the high dose group and the sham group (p value =0.013), but there was not significant difference between the high dose group and medium dose group.

Table 4. 1. Means and standard error (Mean ± SE) of Tonic and phasic SCL values of the groups

SCL **(мohm)**	Sham group (n=12) Mean ± SE	Medium dose group (n=12) Mean ± SE	High dose group (n=11) Mean ± SE
Tonic SCL	8,43 ± 0,80	14,78 ± 1,72	14,93 ± 0,68
Phasic SCL	8,30 ± 0,87	18,37 ± 1,33	14,99 ± 0,63

When we compared the tonic and phasic skin conductivity levels of each group within the group, no significant difference was found (p>0.05)

5- DISCUSSION

Vitamin D is a neuroactive hormone that regulates the nervous systems (sympathetic and parasympathetic) and has a wide range of health benefits [8]. Also, vitamin D is essential for autonomic equilibrium and has the potential to function as a central neuroactive substance. And it is a steroid hormone that helps in the absorption of calcium and phosphate [132].

Within our research, we looked into the effect of two doses (400IU, 1000IU) of vitamin D on sympathetic skin response. We observed that sympathetic skin response increased when high doses of vitamin D administrated for 8 weeks to rats.

Electrodermal indices can be used to determine eccrine sweat gland function and how it varies with changes in psychological state. Sympathetic fibers innervate the eccrine sweat glands, and palmar (or plantar) glands react to psychological rather than thermoregulatory stimuli in normal ambient temperatures [133].

There have been very few studies on the effect of vitamin D on the sympathetic nervous system. Previously, study looked into whether changes in autonomic nervous system activity could explain the link between serum vitamin D and arterial stiffness. This suggests that vitamin D may influence catecholamine production and/or secretion in the adrenal medulla, which may play a role in the correlation between blood vitamin D and cardiovascular risk. [7] In contrast, 1,25-dihydroxyvitamin D enhanced tyrosine hydroxylase mRNA in an in vitro investigation, implying that vitamin D could accelerate the rate-limiting step in catecholamine production [11]. Vitamin D deficiency has also been linked to an increase in renin production, which has been hypothesized as a mechanism linking vitamin D deficiency and cardiovascular events. Vitamin D deficiency has been associated with increased renin secretion in animal studies [148].

In the literature, there were no studies whether vitamin D has an effect on electrodermal activity, which is accepted as an indicator for sympathetic nerve activity, or not.

We know that endocrine effects of vitamin D are primarily involved in calcium homeostasis in the bloodstream. Vitamin D and calcium are often used in the same sentence because they work closely together, the most important function of vitamin D is to regulate calcium levels in the bloodstream by enabling calcium and phosphate absorption from the

intestine or calcium absorption from the bones [134]. And the calcium is needed for sweat stimulation, and it is possible that this ion plays a role in sweat hypotonicity regulation [135].

Low vitamin D levels may explain migraine headaches, cardiac and gastrointestinal problems, and oxidative stress in dysautonomia patients. The relationship between vitamin D insufficiency and autonomic dysfunction is little researched and understood. vitamin D is a neuroactive hormone that regulates the sympathetic and parasympathetic nerve systems and maintains autonomic homeostasis. [8].

Sweat production is triggered when nonmyelinated class C sympathetic postganglionic fibers release acetylcholine [136], which binds to muscarinic (subtype 3) receptors on the clear cell's basolateral membrane causes intracellular Ca storage to be released and extracellular Ca to be pumped into the cytoplasm [73]. So, the Ca2+ ion which vitamin D plays important role in its regulation appears to be an important regulatory ion and second messenger in the sweat gland's clear cells (secretory cells) sweat expulsion response. [149] In response to adrenergic stimulation, eccrine glands can also release sweat but are far less significant than the cholinergic stimulus [137].

Other neuromodulators, including as vasoactive intestinal peptide, calcitonin gene-related peptide, and nitric oxide(NOx), have also been identified to have small roles in eccrine sweating neural activation [73,138]. Endothelial cells, osteoblasts, microglial cells, macrophages, and astrocytes are all affected by the active vitamin D (1,25-dihydroxyvitamin D [1,25(OH)2D]), which regulates NOx generation and/or the expression of inducible nitric oxide synthase (iNOS) [139]. and vitamin D's sweat gland enhancing effect may be mediated by nitric oxide.

Moreover, previous research has shown that nitric oxide (NO) and/or oxidative stress in the brain are critical for sympathetic nervous system modulation. [150], according to research, NO may act as a messenger molecule in both parasympathetic postganglionic neurons and preganglionic sympathetic neurons [151].

Sweat secretion is unquestionably dependent on Ca2+. The eccrine glands' first response to cholinergic input is a significant rise in cytosolic Ca2+. This is performed through two mechanisms: extracellular interstitial fluid influx and intracellular Ca2+ storage release. When Ca2+ was removed from the culture medium of isolated monkey eccrine glands, muscarinic sweating was absolutely suppressed (140,141), and in humans, local

Ca2+ chelation resulted in a considerable reduction in cholinergic sensitivity [142]. The role of vitamin D in calcium mechanism is unquestionable.

6-CONCLUSION AND RECOMMENDATIONS

Our results suggest sympathetic skin response increased with high doses vitamin D supplementation and there is no certain difference between high and medium dose of vitamin D. In this context, it was observed that the increase in vitamin D administration increased sweat gland activity and stress response may be due to vitamin D increases cholinergic activity by increasing calcium metabolism which plays major role in sweat secretion.

More research is required to show the mechanisms by which vitamin D increases eccrine sweat gland activity and sympathetic skin response.

Based on the previous results and conclusions, this study concluded the following recommendations:

- It necessitates conducting research that focuses on the study of vitamins, drugs and hormones, especially vitamin D on experimental animals.
- It is recommended to increase the period of time for experiments more than two months to get more results.
- In future studies, it is preferable to increase the number of experimental mice to obtain more evidence and comparisons.
- Trying to use doses higher than 1000 IU of the vitamin D on experimental animals to know the effects and changes.

REFERENCES

1. Desai NS, Tukvadze N, Frediani JK, Kipiani M, Sanikidze E, Nichols MM, et al. Effects of sunlight and diet on vitamin D status of pulmonary tuberculosis patients in Tbilisi, Georgia. Nutrition. 2012;28(4):362-366.

2. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. Nutrients. 2015;7(4):3011-3021.

3. Mann MC, Hollenberg MD, Hanley DA, Ahmed SB. Vitamin D, the autonomic nervous system, and cardiovascular risk. Physiol Rep. 2015;3(4):e12349.

4. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87(4):1080S-6S.

5. Blank S, Scanlon KS, Sinks TH, Lett S, Falk H. An outbreak of hypervitaminosis D associated with the overfortification of milk from a home-delivery dairy. Am J Public Health. 1995;85(5):656-659.

6. Dolu N, Keloglan S, Bitiktas S, Cug S. The effects of the enriched environment on sympathetic skin response in pentylenetetrazol-kindled rats. Biomed Environ Sci. 2013;26(5):394-397.

7. Burt MG, Mangelsdorf BL, Stranks SN, Mangoni AA. Relationship between vitamin D status and autonomic nervous system activity. Nutrients. 2016;8(9):565.

8. Wadhwania R. Is vitamin D deficiency implicated in autonomic dysfunction?. J Pediatr Neurosci. 2017;12(2):119-123.

9. Mann MC, Exner DV, Hemmelgarn BR, Sola DY, Turin TC, Ellis L, et al. Vitamin D levels are associated with cardiac autonomic activity in healthy humans. Nutrients. 2013;5(6):2114-2127.

10. Lopez J, Campa A, Lewis JE, Huffman FG, Liuzzi JP, Li T, et al. Assessing the relationship between vitamin D status and impairments in cognitive and physical performance in older adults using a dual task physical performance test. J Prev Alzheimers Dis. 2017;4(1):29-36.

11. Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK. Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. Brain Res Mol Brain Res. 1996;36(1):193-196.

12. Rajakumar K. Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics. 2003;112(2):e132-e135.

13. Deluca HF. History of the discovery of vitamin D and its active metabolites. Bonekey Rep. 2014;3:479.

14. Holick MF. Vitamin D: physiology, molecular biology, and clinical applications. New York: Springer Science & Business Media;2010.

15.William J. Stackhouse. Vitamin D: biochemistry, nutrition and roles. 1st ed. New York: Nova Science; 2010.

16. Gil Á, Plaza-Diaz J, Mesa MD. Vitamin D: classic and novel actions. Ann Nutr Metab. 2018;72(2):87-95.

17. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. Dietary reference intakes for calcium and vitamin D. Washington (DC): National Academies Press (US); 2011.

18. Ledesma-Amaro R, Santos MA, Jiménez A, Revuelta JL. Microbial production of vitamins. In Microbial production of food ingredients, enzymes and nutraceuticals. Woodhead Publishing. 2013; p. 571-594.

19. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004;80(6 Suppl):1678S-88S.

20. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. Am J Clin Nutr. 2008;88(2):500S-506S.

21. Ovesen L, Brot C, Jakobsen J. Food contents and biological activity of 25-hydroxyvitamin D: a vitamin D metabolite to be reckoned with?. Ann Nutr Metab. 2003;47(3-4):107-113.

22. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008;88(2):491S-499S.

23. Webb AR. Who, what, where and when-influences on cutaneous vitamin D synthesis. Prog Biophys Mol Biol. 2006;92(1):17-25.

24. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29(6):726-776.

25. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res. 2014;55(1):13-31.

26. Lund B, Sørensen OH, Lund B, Bishop JE, Norman AW. Vitamin D metabolism in hypoparathyroidism. J Clin Endocrinol Metab. 1980;51(3):606-610.

27. Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ. Vitamin D: metabolism. Endocrinol Metab Clin North Am. 2010;39(2):243-253.

28. Brandi L, Egfjord M, Olgaard K. Pharmacokinetics of 1,25(OH)(2)D(3) and 1alpha(OH)D(3) in normal and uraemic men. Nephrol Dial Transplant. 2002;17(5):829-842.

29. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008;88(2):491S-499S.

30. Sitrin MD, Pollack KL, Bolt MJ, Rosenberg IH. Comparison of vitamin D and 25-hydroxyvitamin D absorption in the rat. Am J Physiol. 1982;242(4):326-332.

31. Dawson-Hughes B, Harris SS, Lichtenstein AH, Dolnikowski G, Palermo NJ, Rasmussen H. Dietary fat increases vitamin D-3 absorption. J Acad Nutr Diet. 2015;115(2):225-230.

32. Christakos S. In search of regulatory circuits that control the biological activity of vitamin D. J Biol Chem. 2017;292(42):17559-17560.

33. Nishimura A, Shinki T, Jin CH, et al. Regulation of messenger ribonucleic acid expression of 1 alpha,25-dihydroxyvitamin D3-24-hydroxylase in rat osteoblasts. Endocrinology. 1994;134(4):1794-1799.

34. Brown RB, Razzaque MS. Dysregulation of phosphate metabolism and conditions associated with phosphate toxicity. Bonekey Rep. 2015;4:705.

35. Perwad F, Portale AA. Vitamin D metabolism in the kidney: regulation by phosphorus and fibroblast growth factor 23. Molecular and cellular endocrinology. 2011;347(1-2):17-24.

36. Taylor SN, Hollis BW, Wagner CL. Vitamin D needs of preterm infants. NeoReviews. 2009; 10(12):590-599.

37. Pedrosa MA, Castro ML. Papel da vitamina D na função neuro-muscular [Role of vitamin D in the neuro-muscular function]. Arq Bras Endocrinol Metabol. 2005;49(4):495-502.

38. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80(6 Suppl):1689S-96S.

39. Zella LA, Shevde NK, Hollis BW, Cooke NE, Pike JW. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. Endocrinology. 2008;149(7):3656-3667.

40. Kim D. The role of vitamin D in thyroid diseases. Int J Mol Sci. 2017;18(9):1949.

41. Wrzosek M, Łukaszkiewicz J, Wrzosek M, Jakubczyk A, Matsumoto H, Piątkiewicz P, et al. Vitamin D and the central nervous system. Pharmacol Rep. 2013;65(2):271-278.

42. Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor?. Arch Biochem Biophys. 2012;523(1):123-133.

43. Boland RL. VDR activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol. 2011;347(1-2):11-16.

44. Mann MC, Exner DV, Hemmelgarn BR, et al. Vitamin D supplementation is associated with improved modulation of cardiac autonomic tone in healthy humans. Int J Cardiol. 2014;172(2):506-508.

45. Tuyen le D, Hien VT, Binh PT, Yamamoto S. Calcium and Vitamin D Deficiency in Vietnamese: Recommendations for an Intervention Strategy. J Nutr Sci Vitaminol (Tokyo). 2016;62(1):1-5.

46. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-281.

47. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. J Clin Endocrinol Metab. 2009;94(4):1244-1250.

48. Chen S, Sun Y, Agrawal DK. Vitamin D deficiency and essential hypertension. J Am Soc Hypertens. 2015;9(11):885-901.

49. Khayyatzadeh SS, Bagherniya M, Abdollahi Z, Ferns GA, Ghayour-Mobarhan M. What is the best solution to manage vitamin D deficiency? IUBMB Life. 2019;71(9):1190-1191.

50. Prentice A. Vitamin D deficiency: a global perspective. Nutr Rev. 2008;66(10 Suppl 2):153-164.

51. Vranić L, Mikolašević I, Milić S. Vitamin D deficiency: consequence or cause of obesity? Medicina. 2019;55(9):541.

52. Holick MF. Vitamin D deficiency: what a pain it is. Mayo Clin Proc. 2003;78(12):1457-1459.

53. Kaur P, Mishra SK, Mithal A. Vitamin D toxicity resulting from overzealous correction of vitamin D deficiency. Clin Endocrinol (Oxf). 2015;83(3):327-331.

54. Taylor PN, Davies JS. A review of the growing risk of vitamin D toxicity from inappropriate practice. Br J Clin Pharmacol. 2018;84(6):1121-1127.

55. Marcinowska-Suchowierska E, Kupisz-Urbańska M, Łukaszkiewicz J, Płudowski P, Jones G. Vitamin D toxicity-a clinical perspective. Front Endocrinol (Lausanne). 2018;9:550.

56. Holick MF. Vitamin D is not as toxic as was once thought: A historical and an up-to-date perspective. Mayo Clin Proc. 2015;90(5):561-564.

57. Ozkan B, Hatun S, Bereket A. Vitamin D intoxication. Turk J Pediatr. 2012;54(2):93-98.

58. Schwalfenberg G. Not enough vitamin D: health consequences for Canadians. Can Fam Physician. 2007;53(5):841-854.

59. Boucsein W. Electrodermal activity. 2nd ed. Germany: Springer Science & Business Media;2012.

60. Cacioppo JT, Tassinary LG, Berntson G. Handbook of psychophysiology. 3rd ed. Cambridge: university press;2007.

61. Naveteur J, Sequeira-Martinho H. Reliability of bilateral differences in electrodermal activity. Biol Psychol. 1991;31(1):47-56.

62. Fowles DC, Christie MJ, Edelberg R, Grings WW, Lykken DT, Venables PH. Committee report. Publication recommendations for electrodermal measurements. Psychophysiology. 1981;18(3):232-239.

63. Knezevic W, Bajada S. Peripheral autonomic surface potential. A quantitative technique for recording sympathetic conduction in man. J Neurol Sci. 1985;67(2):239-251.

64. Schliack H, Schiffter R. Neurophysiology and pathophysiology of sweat secretion. In normal and pathological physiology of the skin II. Berlin, Heidelberg: Springer;1979:394-458.

65. Edelberg, R. Electrical activity of the skin: Its measurement and uses in psychophysiology. Handbook of psychophysiology. 1972; 367-418.

66. Kappeler-Setz C, Gravenhorst F, Schumm J, Arnrich B, Tröster, G. (2013). Towards long term monitoring of electrodermal activity in daily life. Personal and ubiquitous computing. 2013;17(2):261-271.

67. Kucera P, Goldenberg Z, Kurca E. Sympathetic skin response: review of the method and its clinical use. Bratisl Lek Listy. 2004;105(3):108-116.

68. Serup J, Jemec GB, Grove GL. Handbook of non-invasive methods and the skin. 2nd ed. Boca Raton: CRC press;2006.

69. Millás A, Lago J, Vasquez-Pinto L, Massaguer P, Maria-Engler SS. Approaches to the development of 3d bioprinted skin models: the case of natura cosmetics. International Journal of Advances in Medical Biotechnology-IJAMB. 2019;2(1):03-13.

70. Stücker M, Struk A, Altmeyer P, Herde M, Baumgärtl H, Lübbers DW. The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis. J Physiol. 2002;538(Pt 3):985-994.

71. Yousef H, Alhajj M, Sharma S. Anatomy, skin (integument), epidermis. Treasure Island: StatPearls;2017.

72.Serwood L. Human. Physiology from Cell to Systems. 9th ed. Boston: Cengage Learning;2019.

73. Baker LB. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. Temperature (Austin). 2019;6(3):211-259.

74. HIBBS RG. Electron microscopy of human apocrine sweat glands. J Invest Dermatol. 1962;38:77-84.

75. Montagna W, Prota G, Kenney Jr. The structure and function of skin.3rd ed. London: Elsevier;2012.

76. Weiner JS, Hellmann K. The sweat glands. Biological Reviews. 1960; 35(2):141-186.

77. Sato K, Leidal R, Sato F. Morphology and development of an apoeccrine sweat gland in human axillae. Am J Physiol. 1987;252(1 Pt 2):R166-R180.

78. Sato K, Sato F. Sweat secretion by human axillary apoeccrine sweat gland in vitro. Am J Physiol. 1987;252(1 Pt 2):R181-R187.

79. Li H, Chen L, Zhang M, Tang S, Fu X. Three-dimensional culture and identification of human eccrine sweat glands in matrigel basement membrane matrix. Cell Tissue Res. 2013;354(3):897-902.

80. Baker LB, Wolfe AS. Physiological mechanisms determining eccrine sweat composition. Eur J Appl Physiol. 2020;120(4):719-752.

81. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. J Am Acad Dermatol. 1989;20(4):537-563.

82. Taylor NA, Machado-Moreira CA. Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extrem Physiol Med. 2013;2(1):4.

83. Sato K, Dobson RL. Regional and individual variations in the function of the human eccrine sweat gland. J Invest Dermatol. 1970;54(6):443-449.

84. Hodge BD, Sanvictores T, Brodell RT. Anatomy, Skin Sweat Glands. In: StatPearls. Treasure Island (FL): StatPearls Publishing;2020.

85. Hu Y, Converse C, Lyons MC, Hsu WH. Neural control of sweat secretion: a review. Br J Dermatol. 2018;178(6):1246-1256.

86. Wallin BG. Sympathetic nerve activity underlying electrodermal and cardiovascular reactions in man. Psychophysiology. 1981;18(4):470-476.

87. Shields SA, MacDowell KA, Fairchild SB, Campbell ML. Is mediation of sweating cholinergic, adrenergic, or both? A comment on the literature. Psychophysiology. 1987;24(3):312–319.

88. Illigens BM, Gibbons CH. Sweat testing to evaluate autonomic function. Clin Auton Res. 2009;19(2):79-87.

89. Pabst O, Tronstad C, Grimnes S, Fowles D, Martinsen ØG. Comparison between the AC and DC measurement of electrodermal activity. Psychophysiology. 2017;54(3):374-385.

90.Geršak G. Electrodermal activity-a beginner's guide. Elektrotehniski Vestnik. 2020;87(4):175-182.

91.Roy JC, Boucsein W, Fowles DC, Gruzelier JH. Progress in electrodermal research 1st edition. Vol. 249, New York. Plenum Press. 1993;249:173-186.

92. Zahn TP, Grafman J, Tranel D. Frontal lobe lesions and electrodermal activity: effects of significance. Neuropsychologia. 1999;37(11):1227-1241.

93. Schmidt S, Walach H. Electrodermal activity (EDA)--state-of-the-art measurements and techniques for parapsychological purposes. Journal of Parapsychology. 2000; 64(2):139.

94. Lykken DT, Venables PH. Direct measurement of skin conductance: a proposal for standardization. Psychophysiology. 1971;8(5):656-672.

95. Boucsein W, Fowles DC, Grimnes S, Ben-Shakhar G, roth WT, Dawson ME, et al. Publication recommendations for electrodermal measurements. Psychophysiology. 2012;49(8):1017-1034.

96. Raszewa M, Hausmanowa-Petrusewicz I, Błaszczyk M, Jabłońska S. Sympathetic skin response in scleroderma. Electromyogr Clin Neurophysiol. 1991;31(8):467-472.

97. Sequeira H, Roy JC. Cortical and hypothalamo-limbic control of electrodermal responses. In Progress in electrodermal research. Springer, Boston, MA. 1993;249: 93-114.

98. Grapperon J, Pignol AC, Vion-Dury J. La mesure de la réaction électrodermale [The measurement of electrodermal activity]. L'Encephale, 2012;38(2):149–155.

99. Mangina CA, Beuzeron-Mangina JH. Direct electrical stimulation of specific human brain structures and bilateral electrodermal activity. Int J Psychophysiol. 1996;22(1-2):1-8.

100. Roy JC, Sequeira H, Delerm B. Neural control of electrodermal activity: Spinal and reticular mechanisms. In Progress in electrodermal research. Springer, Boston, MA.1993;249:73-92

101. Shahani BT, Halperin JJ, Boulu P, Cohen J. Sympathetic skin response--a method of assessing unmyelinated axon dysfunction in peripheral neuropathies. J Neurol Neurosurg Psychiatry. 1984;47(5):536-542.

102. Montagna P, Salvi F, Liguori R. Sympathetic skin response in familial amyloid polyneuropathy. Muscle Nerve. 1988;11(2):183-184.

103. Miralles R, Espadaler JM, Navarro X, Rubiés-Prat J. Autonomic neuropathy in chronic alcoholism: evaluation of cardiovascular, pupillary and sympathetic skin responses. Eur Neurol. 1995;35(5):287-292.

104. Tugnoli V, Eleopra R, De Grandis D. Hyperhidrosis and sympathetic skin response in chronic alcoholic patients. Clin Auton Res. 1999;9(1):17-22.

105. Brown TR, Kovindha A, Wathanadilokkol U, Smith T, Kraft GH. Abnormalities of the sympathetic skin response in lepromatous leprosy. Muscle Nerve. 1996;19(10):1357-1358.

106. Tekatas A, Koca SS, Tekatas DD, Aksu F, Dogru Y, Pamuk ON. R-R interval variation and sympathetic skin response in systemic lupus erythematosus. Clin Rheumatol. 2014;33(1):65-70.

107. Taly AB, Arunodaya GR, Rao S. Sympathetic skin response in Guillain-Barré syndrome. Clin Auton Res. 1995;5(4):215-219.

108. Lyu RK, Tang LM, Wu YR, Chen ST. Cardiovascular autonomic function and sympathetic skin response in chronic inflammatory demyelinating polyradiculoneuropathy. Muscle Nerve. 2002;26(5):669-672.

109. Malessa R, Ohrmann P, Agelink MW, Brockmeyer NH, Diener HC. HIV-1-assoziierte autonome Dysfunktion (HIVAD) [HIV-1 associated autonomic dysfunction (HIVAD)]. Der Nervenarzt. 1996;67(2):147–154.

110. Caccia MR, Galimberti V, Valla PL, Salvaggio A, Dezuanni E, Mangoni A. Peripheral autonomic involvement in the carpal tunnel syndrome. Acta Neurol Scand. 1993;88(1):47-50.

111. Reddeppa S, Bulusu K, Chand PR, Jacob PC, Kalappurakkal J, Tharakan J. The sympathetic skin response in carpal tunnel syndrome. Auton Neurosci. 2000;84(3):119-121.

112. Yokota T, Matsunaga T, Okiyama R, Hirose K, Tanabe H, Furukawa T, et al. Sympathetic skin response in patients with multiple sclerosis compared with patients with spinal cord transection and normal controls. Brain. 1991;114 (Pt 3):1381-1394.

113. Linden D, Diehl RR, Kretzschmar A, Berlit P. Autonomic evaluation by means of standard tests and power spectral analysis in multiple sclerosis. Muscle Nerve. 1997;20(7):809-814.

114. Ke JQ, Shao SM, Zheng YY, Fu FW, Zheng GQ, Liu CF. Sympathetic skin response and heart rate variability in predicting autonomic disorders in patients with Parkinson disease. Medicine (Baltimore). 2017;96(18):e6523.

115. Esen F, Celebi G, Ertekin C, Colakoglu Z. Electrodermal activity in patients with Parkinson's disease. Clin Auton Res. 1997;7(1):35-40.

116. Dolu N, Gündüz S, Kara AY, Acer H, Tasan SN, Khan A. Is there treatment effect of vinpocetine on autonomic dysfunction in rats with Alzheimer. Asian J Pharm Clinical Research. 2017;10 (2):89-91

117. Dettmers C, Fatepour D, Faust H, Jerusalem F. Sympathetic skin response abnormalities in amyotrophic lateral sclerosis. Muscle Nerve. 1993;16(9):930-934.

118. Nair KP, Taly AB, Arunodaya GR, Rao S, Murali T. Sympathetic skin response in myelopathies. Clin Auton Res. 1998;8(4):207-211.

119. Masur H, Schulte-Oversohl U, Papke K, Oberwittler C, Vollmer J. Involvement of the autonomic nervous system in patients with syringomyelia--a study with the sympathetic skin response. Electromyogr Clin Neurophysiol. 1996;36(1):43-47.

120. Chu EC, Chu NS, Huang CC. Autonomic involvement in Wilson's disease: a study of sympathetic skin response and RR interval variation. J Neurol Sci. 1997;149(2):131-137.

121. Marcello N, Baratti M, Ortaggio F, Vescovini E, Zanoni P, Tugnoli V, et al. Sympathetic skin response in patients with Duchenne muscular dystrophy. Electromyogr Clin Neurophysiol. 1995;35(7):387-395.

122. Yokota T, Hayashi M, Tanabe H, Tsukagoshi H. Sympathetic skin response in patients with cerebellar degeneration. Arch Neurol. 1993;50(4):422-427.

123. Yamamoto K, Arai H, Nakayama S. Skin conductance response after 6-hydroxydopamine lesion of central noradrenaline system in cats. Biol Psychiatry. 1990;28(2):151-160.

124. Barry RJ, Moroney R, Orlebeke JF, de Vries J. HGM1: a research-oriented portable heart rate and galvanic skin response monitor. Int J Psychophysiol. 1991;11(2):161-165.

125. Dolu N, Süer C, Ozesmi C, Keleştimur F, Eşel E. Electrodermal activity in nonmedicated hyperthyroid patients having no depressive symptoms. Biol Psychiatry. 1997;42(11):1024-1029.

126. Weitkunat R, Bührer M, Sparrer B. Cortical initiation of phasic electrodermal activity. Int J Psychophysiol. 1990;9(3):303-314.

127. Qiao ZG, Vaerøy H, Mørkrid L. Electrodermal and microcirculatory activity in patients with fibromyalgia during baseline, acoustic stimulation and cold pressor tests. J Rheumatol. 1991;18(9):1383-1389.

128. Schulter G, Papousek I. Bilateral electrodermal activity: relationships to state and trait characteristics of hemisphere asymmetry. Int J Psychophysiol. 1998;31(1):1-12.

129. Dolu N, Süer C, Ozesmi C, Keleştimur F, Ozcan Y. Electrodermal activity in hypothyroid patients and healthy subjects. Thyroid. 1999;9(8):787-790.

130. Dolu N, Özesmi CD, Suer C, Keleştimur F. Electrodermal responding in hyperthyroid patients. Indian J Physiol Pharmacol. 1999;43(1):79-83.

131. Dolu N, Ozesmi C, Comu N, Süer C, Gölgeli A. Effect of hyperglycemia on electrodermal activity in diabetic rats. Int J Neurosci. 2006;116(6):715-729.

132. Chaudhari SA, Sacerdote A, Bahtiyar G. 1-α hydroxylation defect in postural orthostatic tachycardia syndrome: remission with calcitriol supplementation. BMJ Case Rep. 2012;2012:bcr0220125730.

133. Christie MJ. Electrodermal activity in the 1980s: a review. J R Soc Med. 1981;74(8):616-622.

134. Mostafa WZ, Hegazy RA. Vitamin D and the skin: Focus on a complex relationship: A review. J Adv Res. 2015;6(6):793-804.

135. Prompt CA, Quinton PM. Functions of calcium in sweat secretion. Nature. 1978;272(5649):171-172.

136. SATO, KENZO. The mechanism of eccrine sweat secretion. Perspectives in exercise science and sports medicine. Exerc Heat Thermoregul, 1993, 6:85-118.

137. Sato K. Stimulation of pentose cycle in the eccrine sweat gland by adrenergic drugs. Am J Physiol. 1973;224(5):1149-1154.

138. Shibasaki M, Crandall CG. Mechanisms and controllers of eccrine sweating in humans. Front Biosci (Schol Ed). 2010;2:685-696.

139. Al-Daghri NM, Bukhari I, Yakout SM, Sabico S, Khattak MNK, Aziz I, et al. Associations of serum nitric oxide with vitamin D and other metabolic factors in apparently healthy adolescents. Biomed Res Int. 2018;2018:1489132.

140. Cui CY, Schlessinger D. Eccrine sweat gland development and sweat secretion. Exp Dermatol. 2015;24(9):644-650.

141. Sato K, Sato F. Role of calcium in cholinergic and adrenergic mechanisms of eccrine sweat secretion. Am J Physiol. 1981;241(3):C113-C120.

142. Metzler-Wilson K, Sammons DL, Ossim MA, Metzger NR, Jurovcik AJ, Krause BA, et al. Extracellular calcium chelation and attenuation of calcium entry decrease in vivo cholinergic-induced eccrine sweating sensitivity in humans. Exp Physiol. 2014;99(2):393-402.

143. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. Eur J Clin Nutr. 2020;74(11):1498-1513.

144. Bornstedt ME, Gjerlaugsen N, Pepaj M, Bredahl MKL, Thorsby PM. Vitamin D increases glucose stimulated insulin secretion from insulin producing beta Cells (INS1E). Int J Endocrinol Metab. 2019;17(1):e74255

145. Ogan D, Pritchett K. Vitamin D and the athlete: risks, recommendations, and benefits. Nutrients. 2013;5(6):1856-1868.

146. Šimoliūnas E, Rinkūnaitė I, Bukelskienė Ž, Bukelskienė V. Bioavailability of different vitamin D oral supplements in laboratory animal model. Medicina (Kaunas). 2019;55(6):265.

147. Clarke HE, Coates ME, Eva JK, Ford DJ, Milner CK., O'Donoghue P N, et al. Dietary standards for laboratory animals: report of the Laboratory Animals Centre Diets Advisory Committee. Lab Anim. 1977;11(1):1-28.

148. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-238.

149. Metzler-Wilson K, Wilson TE. Impact of calcium regulation on eccrine sweating and sweating disorders: the view from cells to glands to intact human skin. Exp Physiol. 2016;101(3):345-346.

150. Kishi T. Regulation of the sympathetic nervous system by nitric oxide and oxidative stress in the rostral ventrolateral medulla: 2012 Academic Conference Award from the Japanese Society of Hypertension. Hypertens Res. 2013;36(10):845-851.

151. Ceccatelli S, Lundberg JM, Zhang X, Åman K, Hökfelt T. Immunohistochemical demonstration of nitric oxide synthase in the peripheral autonomic nervous system. Brain research. 1994; 656(2): 381-395.

APPENDIX 1: PROJECT APPROVAL

1993
BAŞKENT ÜNİVERSİTESİ
Tıp ve Sağlık Bilimleri Araştırma Kurulu

Sayı : 94603339-604.01.02/ 33346
Konu : Proje Onayı

18/11/2020

FİZYOLOJİ ANABİLİM DALINA

Fizyoloji Anabilim Dalında görev yapmakta olan Prof. Dr. Nazan Dolu'nun danışmanlığında Sağlık Bilimleri Enstitüsü / Fizyoloji Tezli Yüksek Lisans Programı öğrencisi Haifa S. Muftah Masud'un sorumluluğunda yürütülecek olan DA20/20 nolu "The effect of high dose vitamin D on the sympathetic skin response in rats" başlıklı araştırma projesi Kurulumuz ve Hayvan Deneyleri Yerel Etik Kurulumun 02/11/2020 tarih ve 20/18 sayılı kararı ile uygun görülmüştür. Projenin başlama tarihi ile çalışmanın sunulduğu kongre ve yayınlandığı dergi konusunda Kurulumuza bilgi verilmesini rica ederim.

e-imzalıdır

Not: Çalışma bildiri ve/veya makale haline geldiğinde "Gereç ve Yöntem" bölümüne aşağıdaki ifadelerden uygun olanının eklenmesi gerekmektedir.

— Bu çalışma Başkent Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu tarafından onaylanmış (Proje no:...) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

— This study was approved by Baskent University Ethical Committee for Experimental Resarch on Animals (Project no:...) and supported by Baskent University Research Fund.

DAĞITIM
Sağlık Bilimleri Enstitüsü Müdürlüğüne
Fizyoloji Anabilim Dalına

Bu belge 5070 sayılı Elektronik İmza Kanununun 6. Maddesi gereğince güvenli elektronik imza ile imzalanmıştır.

APPENDIX 2: ETHICAL APPROVAL

1993
BAŞKENT UNIVERSITY

LOCAL ETHICS COMMITTEE FOR ANIMAL EXPERIMENTS DECISION		
SESSION NO	**DECISION NO**	**DATE OF DECISION**
09	20/18	02/11/2020

Project DA20/20 no entitled "The effect of high dose vitamin D on the sympathetic skin response in rats" pending to be conducted by Nazan Dolu with the Department of Physiology has been reviewed and unanimously approved by the Local Ethics Committee for Animal Experiments.