THE INVESTIGATION OF RELATIONSHIP BETWEEN ORO-GUSTATORY PERCEPTION OF DIETARY LIPIDS AND SPONTANEOUS FAT PREFERENCE WITH MATERNAL NUTRITION STATUS IN OFFSPRING SPRAGUE DAWLEY RATS

by
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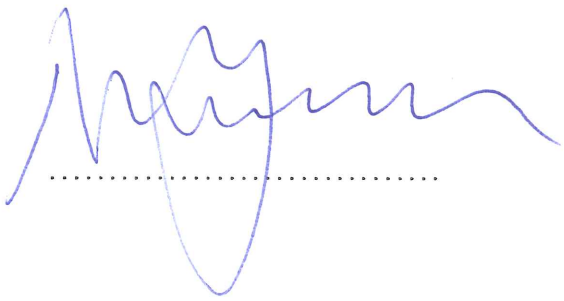
Prof. Dr. Bayram Yılmaz ..... 
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This thesis is dedicated to my dear grandmother Peluze GÜNEY…

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ABSTRACT

THE INVESTIGATION OF RELATIONSHIP BETWEEN ORO-GUSTATORY PERCEPTION OF DIETARY LIPIDS AND SPONTANEOUS FAT PREFERENCE WITH MATERNAL NUTRITION STATUS IN OFFSPRING SPRAGUE DAWLEY RATS

Recent studies have shown that lingual fatty acid receptors CD36 and GPR120 play an important role in the perception of fat taste and the preference for fatty food consumption. However, the relationship between these receptors and maternal nutrition is not known. The aim of this study was to investigate the change in the expression levels of lingual CD36 and GPR120 proteins in rats exposed to different concentrations of fat containing diets during gestation-lactation and maturation periods and to determine the possible effect of exposure of different fat-containing diets to the regulation of these receptors. In our study, rats were fed with low-fat, standard-fat and high-fat diets during the first stage of the study (gestation and lactation) and in the second stage of study (maturation). Daily caloric intake and weekly weight measurements of the rats were followed during the study. Afterwards, the fat perceptions and oily taste preferences of the rats were measured by two-bottle preference tests, blood and tongue tissues were taken and the experiment was terminated. In order to demonstrate the effect of nutritional status on the appetite regulation, the plasma insulin and leptin levels were measured by ELISA and blood glucose and triglyceride levels were measured by a metabolic meter. Expression levels of CD36 and GPR120 receptors on the tongue were determined by immunofluorescence staining and western blot methods. mRNA levels of these proteins were determined by RT-PCR. Our study shows that maternal high fat diet exposure increases the higher fatty solution preference and decreases lingual CD36 and GPR120 expression levels. These findings allow us to identify the dysregulation of the oro-sensorial signalling pathways through the diet in the maternal process and will contribute to the development of new anti-obesity strategies, especially for childhood obesity. The thesis student was supported with 2228-B program within the scope of Tubitak-Bideb 2211 Domestic PhD Scholarship Program. In addition, this project was supported by TUBITAK (117S384).

ÖZET

YAVRU SPRAGUE DAWLEY SIÇANLARDA MATERNAL BESLENME DURUMUYLA YAVRUNUN ORAL YAĞ TADI ALGISI VE YAĞLI BESİN TERCİHİ ARASINDAKİ İLİŞKİNİN İNCELENMESİ

Son dönemde yapılan çalışmalar, yağ tadının algılanması ve yağlı besin tüketme tercihinde dildeki yağ asidi bağlayıcı reseptörlerden CD36 ve GPR120'nin önemli rol oynadığını göstermektedir. Ancak, bu reseptörlerin maternal beslenme ile ilişkisi bilinmemektedir. Araştırmamız, gestasyon-laktasyon ve maturasyon dönemlerinde yağ içeriği bakımından farklı konsantrasyonlardaki diyete maruz kalan sıçanların lingual CD36 ve GPR120 proteinlerinin ekspresyon seviyesindeki değişimi ve yağ içeriği bakımından farklı diyete maruz kalmanın bu reseptörlerin regülasyonuna olası etkisinin tanımlanmasını amaçlamaktadır. Çalışmamızda gestasyon ve laktasyondan oluşan 1. aşama ve maturasyondan oluşan 2. aşama dönemlerinde sıçanlar düşük yağlı, standart yağlı ve yüksek yağlı diyetlere maruz bırakılmıştır. Bu süreçte sıçanların günlük kalori alımı ve haftalık ağırlık ölçümleri takip edilmiştir. Sonrasında sıçanların iki şişe tercih testiyle yağ algıları ve yağlı tat tercihleri ölçülmüş, kanları ve dil dokuları alınarak deney sonlandırılmıştır. Beslenme durumunun iştah regülasyonu üzerindeki etkisinin gösterilmesi için plasma insulin ve leptin seviyeleri ELISA yöntemi ile ölçülmüş, kan glukoz ve trigliserit seviyeleri metabolik ölçüm cihazı ile tespit edilmiştir. Alınan dil dokusundan CD36 ve GPR120 reseptörlerinin ekspresyon seviyeleri immünofloresan boyama ve western blot yöntemleri kullanılarak saptanırken, proteinlere ait mRNA seviyeleri RT-PCR ile belirlenmiştir. Çalışmamız maternal dönemde yüksek yağlı diyete maruziyetin, yüksek yağlı solüsyon tercihini artırdığını. lingual CD36 ve GPR120 ekspresyon seviyesini ise azalttığını göstermektedir. Elde ettiğimiz bu bulgular, maternal dönemde diyet aracılığıyla oro-sensoral sinyal yolaklarında meydana gelen disregülasyonun tanımlanmasına olanak sağlamakta ve özellikle çocukluk dönemi obezitesi için anti-obezitik yeni stratejiler geliştirilmesinin önünü açmaktadır.

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ABCB1	ATP-binding casette B1
AC	Adenylyl cyclase
AEA	N-arachidonoylethanlamine
2-AG	2-Arahidonoyl glycerol
aIC	Agranular insular cortex
AMY	Amygdala
BLA	Basolateral amygdala
BDNF	Brain derived neutrophic factor
BMPs	Bone morphogenetic proteins
BSA	Bovine serum albumin
CALHM1	Calcium homeostasis modulator 1
CB_1	Cannobinid 1 receptor
CCK	Cholecystokinin
cDNA	Complementary DNA
CD36	Cluster of differentiation 36
CGA	Cortical gustatory area
CNCC	Cranial neural crest cell
CRD	Cysteine rich domain
CSF	Cerebrospinal fluid
CTA	Conditioned taste aversion
CVP	Circumvallate papillae
DA	Dopamine
DAG	Diacylglycerol
DAMGO	D-Ala2, N-Me-Phe4-Gly-ol5-enkephalin
DAPI	4',6-Diamidino-2-phenylindole, dihydrochloride
DIO	Diet induced obesity
dIC	Dysgranular insular cortex
DLPFC	Dorsolateral prefrontal cortex
DMPFC	Dorsomedial prefrontal cortex
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

DRK	Delayed rectifying K+
E	Embryonic
EDTA	Ethylene di-amine-tetraacetic acid
EGF	Epithelial growth factor
ENaC	Epithelial sodium channel
ER	Endoplasmic reticulum
FAT	Fatty acid translocase gene
FFAs	Free fatty acids
FFAR1	Free fatty acid receptor 1
FFAR4	Free fatty acid receptor 4
FGF	Fibroblast growth factor
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
GHRP	Growth hormone secretagogue receptor
gIC	Granular insular cortex
G-L	Gestation-lactation
GLP-1	Glucagon like peptide 1
GLP-1R	Glucagon like peptide 1 receptor
Glu	Glutamic acid
GMP	Guanosine-5’-monophophate
GNAT3	Alpha-gustducin
GPCR	G coupled protein receptor
GPR40	G coupled protein receptor 40
GPR120	G coupled protein receptor 120
5-HT	5-Hydroxytryptamine
HFD	High fat diet
IC	Insular cortex
IMP	Inosine-5’-monophophate
IP3	Inositol triphosphate
KCNA	Potassium voltage-gated subfamily A
KCNB	Potassium voltage-gated subfamily B
KCNC	Potassium voltage-gated subfamily C
LCFA	Long chain fatty acids

LFD	Low fat diet
LH	Lateral hypothalamus
MCFA	Medium chain fatty acid
mg	Milligram
ml	Milliliter
MSG	Monosodium glutamate
MUFA	Monounsaturated fatty acids
NAcb	Nucleus accumbens
NE	Norepinephrine
NMDA	N-methyl-D-aspartate
NPY	Neuropeptide Y
NTS	Nucleus of the tractus solitarus
Ob-R	Leptin receptor
OEA	Oleyl ethanolamine
OFC	Orbito-frontal cortex
OLETF	Otsuka Long Evans Tokushima Fatty
OP	Opioids
Otop1	Otopetrin 1
OXT	Oxytocin
OXTR	Oxytocin receptor
PBN	Parabrachial nucleus
PBS	Phosphate buffer saline
PBS-T	Phosphate buffered saline with tween 20
PCR	Polymerase chain reaction
PDE	Phoshodiesterase
PFA	Paraformaldehyde
PFC	Paraformaldehyde
PI3-K	Phosphoinositide-3-kinase
PIP_2	Phosphoinositoldiphosphate
PKD1L3	Polycystin 1 like 3, transient receptor channel
PKD2L1	Polycystin 2 like 1, transient receptor channel
PLC	Phospholipase C
PLC-β2	Phospholipase C- β2

PROP	6-n-Prophythrouracil
PUFA	Polyunsaturated fatty acid
P2X2/P2X3	Purigeneric receptors
PYY	Peptide YY
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction
Shh	Sonic hedgehog signaling
SCFA	Short chain fatty acid
SFA	Saturated fatty acids
SFD	Standart fat diet
SNP	Single nucleotide polymorphism
siRNAs	Small interfering RNAs
Sox2	Sry-related high mobility group box trasncription factor
TAS2Rs/T2Rs	Type 2 bitter taste sensing receptors
TBPT	Two bottle preference test
TBS-T	Tris buffered saline with Tween 20
TMD	7-Trans-membrane domain
T1R2	Type 1 member 2 sweet taste receptor
T1R3	Type 1 member 3 sweet taste receptor
TRCs	Taste receptor cells
TRP	Transient receptor potential
TRPM5	Transient receptor potential type member 5 protein
VEGF-A	Vascular endothelial growth factor-alpha
VFD	Venus flytrap domain
VLCFA	Very long chain fatty acid
VLDL	Very low density lipoprotein
VIP	Vasoactive Intestinal Peptide
VP	Ventral pallidum
VTA	Ventral tegmental area
VPM_{pc}	Parvocellular part of the ventralis posteromedial thalamic nucleus
WHO	World Health Organ

1. INTRODUCTION

Obesity is defined with excess accumulation of adipose tissue by World Health Organization (WHO) and also it gives rise to increased risk for type II diabetes, cardiovascular problems, cancer-related mortality and metabolic syndrome [1]. Although the aetiology of the disease remains unknown, it is undisputed fact that obesity is a result of interaction between environmental and genetic factors. Actually, the main underlying mechanism of obesity is the imbalance between energy intake and energy expenditure. Energy imbalance results in excessive fat accumulation, endocrine changes and low-grade inflammation. Recent studies have claimed that one of the reasons for obesity can be impaired taste function.

There are five basic taste modalities which consist of sweet, sour, salty, bitter and umami and each one also recognizes the quality and nature of foods [2, 3]. Some researchers have claimed that fatty taste perception can be the sixth taste modality and it is well known that the fats, the highest energy in the diet, are more consumed because of their smell, taste, flavour and textural properties. Recent studies have shown that CD36 and GPR120, the fatty acid receptors, play an important role in fatty taste perception and fat preference [4,5]. The mechanisms which regulate these receptors have not been fully elucidated, but it has been found that the high-fat diet disrupts cellular signalling by inducing dysregulation in the expression of these receptors. Thus, it was determined that more fat consumption and obesity emerge due to desensitization of fatty taste.

On the other hand, it is thought that personal fat perception experience starts from the prenatal period. Especially, several animal studies have shown that high-fat diets exposed to the mother during pregnancy and lactation lead to increased hyperphagia and fat consumption preference during adulthood [6,7]. Researchers which focused on the reason of the maternal fatty food preference suspect to increased neuronal activity in the hunger-satiety centers of the brain and opioid and dopaminergic receptor levels in the mesolimbic system [7,8,9,10]. Even though the CD36 and GPR120 receptors are known to be involved in the fatty taste perception, the relationship between maternal nutrition status and CD36 and GPR120 receptors associated choice of consuming fatty food is not yet clear. We hypothesized that the oro-sensorial perception of fatty foods which is related to CD36 and GPR120 expression levels can affect by the maternal nutrition status.

The aim of the study is to investigate the possible effect of exposure to different concentrations of dietary lipids on the level of lingual CD36 and GPR120 protein expressions during gestation-lactation and maturation periods and also, to explain the effect of exposure to different fat-containing diets for the regulation of these receptors.

This study will illuminate high or low fat associated dysregulation of oro-sensorial signalling pathways which are strongly related to maternal food choices during gestation. It is considered that determining the effect of maternal nutrition status on taste sensory disturbances is important in terms of determining the target for, especially, childhood obesity.

2. TASTE PERCEPTION AND GUSTATORY SYSTEM

2.1. TASTE PERCEPTION AS A FACTOR IN THE EATING BEHAVIOR

Eating behaviour is a vital function and sufficient for nutrients and energy. How much we eat and which food we choose are regulated by sensory and metabolic processes of foods. The sensory process is related to a perception of taste and flavour and it is managed by the central gustatory system. This system comprised of taste cells in the lingual papillae, some cranial nerves and associated brain areas [11]. Metabolic processes are related to the cephalic phase of ingestion via satiety hormone responses. The oro-sensory system is effective in the short term food perception and preference while metabolic process generally affects the long term food preference [12].

Spontaneous food consumption is related to individual flavour perception differences. Flavour is defined as a combination of several sensory modalities such as taste, olfaction, oral somatosensations and oral nociception. Flavour perception contributes to appetite, food preference and spontaneous food selection. During the oral process, the characteristic of nutrient originated chemical stimulus is defined as taste. There are five basic taste modalities, sweet, sour, salty, bitter and umami. Each one of these recognizes the quality and nature of foods. For instance, umami taste identifies glutamate amino acid in nutrients, sweet taste means energy-rich foods and salty taste is related to dietary electrolyte balance of foods [2,3]. Another significant factor in specific food selection behaviour is smell. Odors contribute to the determine the food and flavour perception via increased or decreased sensitivity to specific ions and molecules in chemoreceptors. Olfaction closely works together with taste and it also has a role in appetite, food choice and intake and satiation. Odors have a significant role in appetite, food selection and intake which depend on internal (metabolic & personality related) and external (food-related) factors [13]. Olfactory data of the food is carried to the brain only via cranial nerve (I^{st})[14]. The olfactory system has a rapid rate of receptor cell turnover along with the life and rapid adaptation similar to taste sense [15]. The relationship between olfactory signals and eating behaviour is so complex and there are many gaps in the literature between molecular regulatory mechanisms in sense of smell and eating behaviour [13].

Oral somatosensation contributes to identifying the texture, tactile and temperature in consumed foods. The texture is defined as the collective term of sensory information originated from visual, audio and tactile stimuli. Tactile is only related to the sense of touch during mastication [16]. Additionally, oral nociception is defined with food-related unpleasant, sensitive and emotional experiences such as hot or spicy. At the end of the oral processing of food, all of these concepts lead to special eating behaviours such as avoidance or preference.

2.2. AN OVERVIEW TO GUSTATORY SYSTEM

2.2.1. Physiology and Anatomy of the Gustatory System

Mammals sense the taste via various organs including the tongue, soft palate and pharynx. These organs are covered by gustatory epithelia. The taste perception does not occur on the lips, the underside of the tongue, hard palate and inside of cheeks in adults [17]. The major organ of the gustatory system is the tongue. The mammalian tongue is a highly mobile and large muscular organ that found in the oral cavity and connected with the upper pharynx. Anatomically, the main components of the tongue are root, interior surface and dorsum. The tongue is divided into bilateral halves via the presence of a midline fibrous septum. The dorsum is separated into anterior oral and posterior pharyngeal areas via sulcus terminalis which is V-shaped path. The high motion of tongue is associated with a broad attachment of tongue root and connection with a complex group of intrinsic and extrinsic muscles. In addition, the mucosa of the tongue is specialized and it has a functional role in feeding [18].

The gustatory system is responsible for transduction the information of tastants from the tongue to the brain. The chemical stimuli of tastants are detected by taste receptor cells (TRCs) [19]. TRCs are accumulated in taste buds which are found in the lingual papillae. According to shape and localization within the tongue, four types of papillae have defined and these are also known as gustatory papillae except filiform papillae [20].

- **Filiform Papillae***:* They are not gustatory papillae because of having no taste buds like structure. They have appeared as the tube-like epithelial structure at the dorsal surface of the tongue entirely.
- **Fungiform Papillae***:* Their shape likely to the mushroom at the longitudinal section and are innervated by the facial nerve. These are found at the dorsal surface of the tongue.
- **Foliate Papillae***:* They are localized at the posterior lateral surface of the tongue and are innervated by facial and glossopharyngeal nerves.
- **Circumvallate Papillae (CVP)***:* They are found in the 1/3 posterior part of the tongue which localized in front of the sulcus terminalis and also most of the taste buds are present within this area. These structures are identified as dome-shaped and also they are connected with von Ebner's salivary glands which synthesize and release salivary enzymes such as lipase and carbonic anhydrase. They are innervated with glossopharyngeal nerve. The number of CVP shows diversity between species. For instance, the number of CVP on the human tongue vary between 8-12 while rodents have only one CVP.

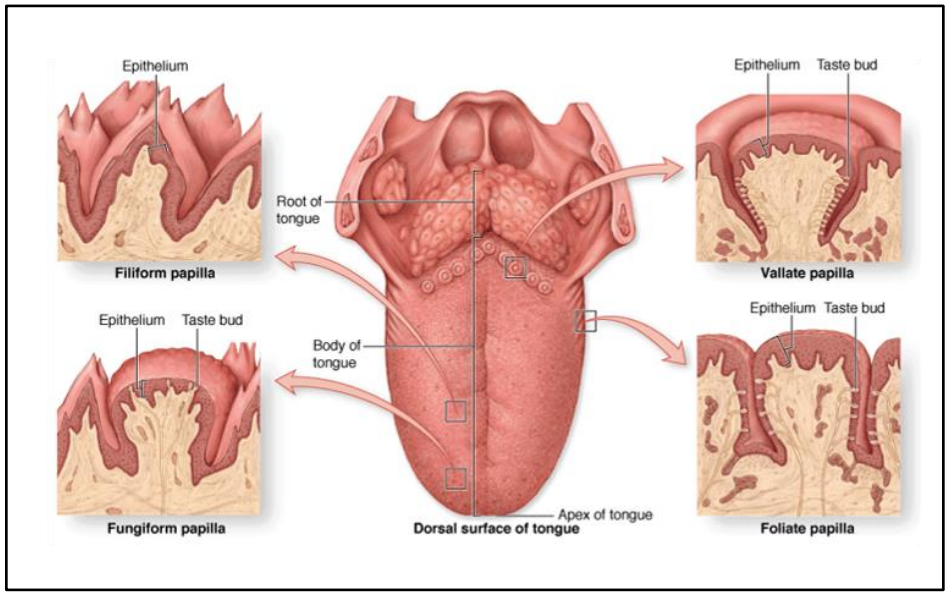


Figure 2.1. The schematic representation of different regions and papillae of the human tongue [21].

Mammalian taste buds are onion or goblet-shaped structure and embedded into lingual epithelia. Goblet shaped bud consists of three major cell types which are supporting cells,

basal cells and gustatory cells and their functions are insulation to taste cells, the precursor of stem cells and TRCs, respectively. Taste buds are distributed on the tongue surface, soft palate, pharynx and epiglottis. Taste buds have been appeared early 7 to 8 weeks of intrauterine life and have been matured after gestation. Each taste bud has include between 50 and 100 neuroepithelial cells. The shape of these cells are small bipolar with no axon and taste receptors are found in microvilli at the apical surface of cells. These microvilli reach to oral cavity via taste pore (Figure 2.2.) [22].

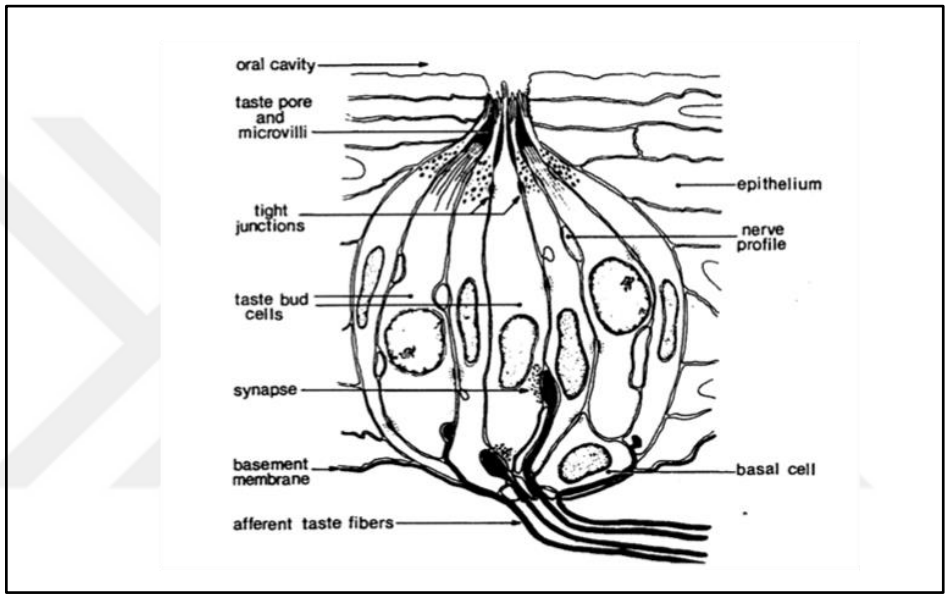


Figure 2.2. The histological structure of a taste bud [23].

Taste buds within papillae have a functional role in the perception of chemical stimuli and taste transduction. During food intake, taste stimuli are released via two basic mechanisms. These are pre-digestion of foods via chewing action as mechanically and oral enzymes released from von Ebner's glands mediated chemical ingestion [17]. The first stage of the gustatory process is triggered by the interaction between each one of tastants which have different taste qualities such as sweet, umami, sour etc. and their special ligands on the TRCs. Four different types of TRCs have been defined and each one has different roles in the gustatory process.

- **Type I TRCs**: These cells are the most abundant subtype of TRCs and having electron-dense cytoplasm. The number of them is between 30 and 35 for each taste bud. Anatomically they are found in the periphery of the taste bud and each one extends 50 μm length of the bud from taste pore to basement membrane.

Throughout its length, each one projects with Type II TRCs, Type III TRCs and nerve fibers due to it possess cytoplasmic lamellae. In addition, these cells play a central role in the regulation of extracellular ionic environment due to the expression of plasma membrane-bound nucleotidase on their surface. This role of them in terminating synaptic transmission, provide glial like supporting function on the taste bud [22, 24]. Therefore, another name of Type I TRCs is glial-like supporting cells. On the other hand, Type I TRCs can be responsible for salty taste perception in mice via expression of epithelial sodium channels (ENaC) on its surface [25, 26, 27]. However, this pathway has not elucidated completely in non-human primates and human.

- **Type II TRCs**: They have various G-coupled protein receptors which provide the perception of sweet, bitter, fat and umami tastes. In this context, a heterodimer of T1R2 and T1R3 responsible for the perception of sweet tastants and artificial sweeteners [28]; heteromeric T1R1/T1R3 related to umami taste [29] and T2R family of receptor proteins associated with the perception of bitter tastants [30]. Lastly, recent studies elucidated that G-coupled protein receptor 120 (GPR120) and a cluster of differentiation (CD36) proteins have a functional role within the perception of long-chain fatty acid (LCFA) [31]. All of these taste modalities have been perceived through gustatory signal transduction. This process not only confined with one taste receptor cell but also required to cell to cell communication via electric coupling gap junction and chemical communication with neurotransmitters [32]. TRCs are electrically active epithelial cells which are likely to neurons because of the transmembrane receptor channels within TRCs can modulate to the depolarization state and also stimulation of signal cascade is resulted with releasing to neurotransmitters. According to the combinatorial model about peripheral taste perception, ATP has a central role in this system. Tastants give rise to ATP release from type II cells via the voltage-gated ATP-permeable ion channel calcium homeostasis modulator 1 (*CALHM1)*. Released ATP activates the purinergic receptors (P_2X/P_2Y) within type II, type III and afferent nerve fibres via non-conventional synapse [33].
- **Type III TRCs**: They are pre-synaptic cells and express various neurotransmitters such as serotonin (5-HT), norepinephrine (NE) and GABA. Releasing of neurotransmitters from type III provides the signal transduction of taste perception

to the brain with gustatory nerves [33]. And also, Type III TRCs responsible for sour taste perception.

- **Type IV TRCs**: They are known as taste cell precursors which are also called as basal cells. Basal cells are differentiated into other types of TRCs every 9 to 15 days, to compensate the damages which are originated from various reasons such as toxic agents, heat and mechanic at the taste bud [17].

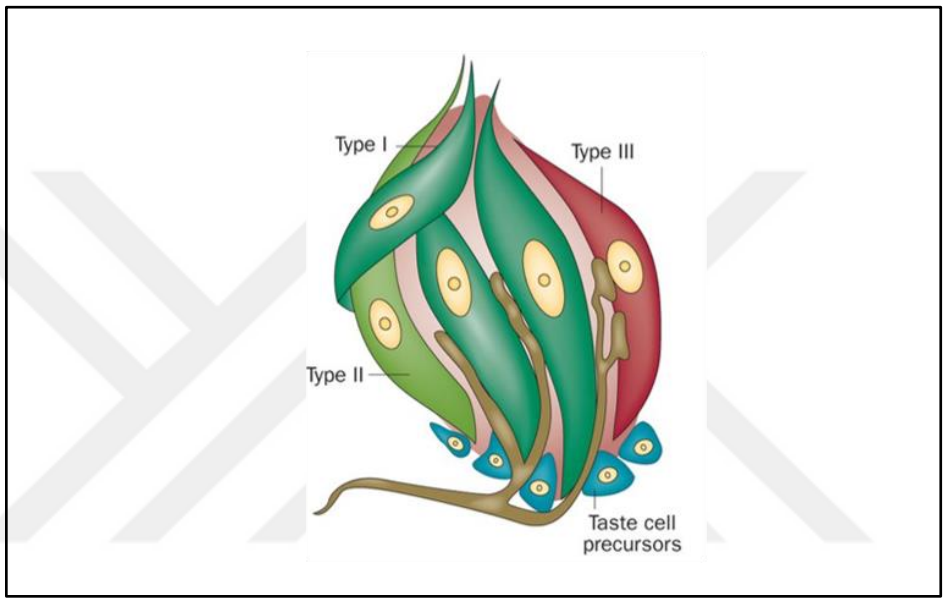


Figure 2.3. The schematic view of TRC [34].

2.2.2. Hormonal Regulation of Taste Perception

Gustatory systems are affected through endocrine and paracrine hormonal modulations. Various hormones such as leptin, insulin, ghrelin etc. bind to their receptors which found in the surface of TRCs and change the palatability of tastants [22]. (Figure 2.4.) Taste perception associated hormones and their functions are summarized in Table 2.1.

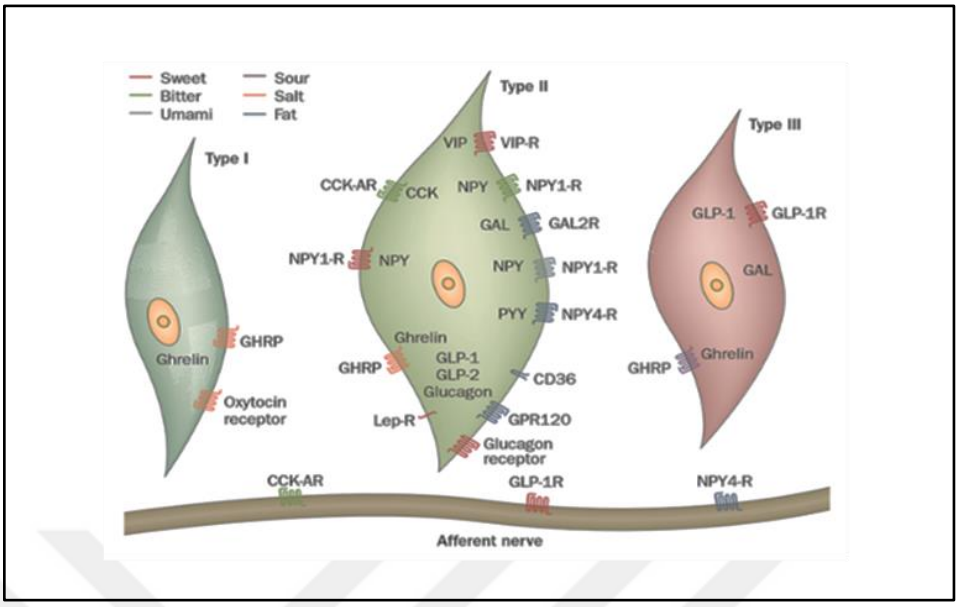


Figure 2.4. The schematic view of hormone receptors which belong to different TRCs [34].

Table 2.1. Hormonal modulation of taste perception.

Hormone	**Primary Production Tissue**	**Receptor**	**Receptor Location in Taste Buds**	**Behavioural Effect**
Leptin	Adipose tissue	*Ob-R*	Type II TRCs	Decreased response to sweet compounds
Insulin	Pancreatic beta cells	*IRS-1* *IRS-2*	Lingual Taste Buds	Enhanced response to sweet tastants
Ghrelin	Stomach	*Ghrp*	Type I TRCs Type II TRCs Type III TRCs Type IV TRCs	Increased response on sour and salty tastants
Glucagon	Pancreatic alpha cells	*Gcgr*	Type II TRCs	Enhance response to sweet tastants
Glucagon-Like Peptide-1	Intestinal L cells	*Glp1r*	Type II TRCs Type III TRCs	Increased sensitivity on sweet taste and decreased sensitivity on umami taste

Cholecystokinin (CCK)	Intestinal I cells	*Cckar*	Type II TRCs	Potential role on the bitter taste
Galanin	Gastrointestinal tract	*Galr2*	Type II TRCs Type III TRCs	Unknown
Oxytocin	Posterior pituitary gland	*Oxtr*	Type I TRCs	Reduced sweet taste perception
Neuropeptide Y (NPY)	Central and peripheral nervous systems	*Npy1r* *Npy2r* *Ppy1r* *Npy5r*	Type II TRCs	Potential role in bitter taste perception
Peptide YY (PYY)	Intestinal L cells	*Npy1r* *Npy2r* *Ppy1r* *Npy5r*	Type II TRCs	Increased sensitivity bitter and fat tastants
Vasoactive Intestinal Peptide (VIP)	Central and peripheral nervous systems & Duodenum	*Vpac1* *Vpac2*	Type II TRCs	Sweet taste preference
Endocannabinoids	Central nervous systemí	*CB1*	Type II TRC	Increased sensitivity to sweet tastants

Leptin: Leptin is primarily synthesized and secreted from adipose tissue and functional as an anti-obesity factor via reducing food intake and regulation of body weight. Leptin hormone binds to specific leptin receptor (Ob-R) in the hypothalamus and this connection causes upregulation of anorexigenic hormones and downregulation of orexigenic hormones. However, excessive releasing of leptin give rise to insensitivity on Ob-R and leptin resistance is associated with obesity [35]. On the other hand, Type II TRCs have been identified as a peripheral target of leptin and the role of leptin in the gustatory system is known as inhibition of sweet taste perception. Kawai et al. demonstrated that leptin administration to the lean mice caused to decreased responses of peripheral taste nerves to sweet tastants and reduced preference to sweet substances. In this study, the function of leptin in sweet taste perception is confirmed in leptin receptor defect db/db mice through increased peripheral neural responses and enhanced sweet tastant consumption [36]. Shigemura et al. examined the relationship between behavioural sweet taste preference and mRNA levels of fungiform and circumvallate papillae in leptin-deficient ob/ob mice, Ob-Rb deficient db/db mice wild type mice. They showed that leptin dependent suppression of sweet taste perception emerges through leptin binding with functional Ob-Rb [37]. On the

other hand, Nakamura et al. demonstrated a relationship between diurnal variation dependent alteration of plasma leptin levels and sweet taste recognition threshold which depend on diurnal variation in humans [38]. Another human study investigated the effect of leptin gene (*LEP*) polymorphisms (G–2548A and A19G) and leptin receptor gene (*LEPR*) polymorphisms (R109K, R223Q, and rs3790439) within sweet preference and obesity. In this study, the LEP A19G and LEPR R109K polymorphisms are found as related to the sweet preference [39].

Insulin: Insulin is synthesized by beta cells of pancreatic islets and plays a central role in the regulation of blood sugar. In the gustatory system, insulin has been identified as a modulator of salty taste perception. Sodium channel ENaC is found on taste cells and provide the apical Na^+ transport into taste cells. Firstly, the role of insulin in the gut is identified by Blazer-Yost et al (1998). They claimed that insulin signalling activation of ENaC receptors and increased ENaC permeability to Na^+ [40]. Baquero and Gilbertson et al. showed that insulin increases amiloride-sensitive Na^+ currents in mouse taste cells and also Na-dependent Na influx takes place through phosphoinositide 3-kinase (PI3-Kinase) in mouse fungiform and vallate TRCs. In the study, insulin administered mice displayed avoidance of low concentration NaCI significantly. Consequently, they claimed that insulin signalling can play a role in maintaining the functional expression of ENaC [41].

Ghrelin: Ghrelin is a small peptide with 28 aa length and it is primarily production location in the stomach. Endogenous ghrelin is a ligand for growth hormone secretagogue receptor (GHRP). Although ghrelin is found in various tissues, ghrelin in hypothalamus and stomach mostly studied. Ghrelin in these tissues provides the stimulation of feeding through the orexigenic effect [42]. Ghrelin and its receptor (GHRP) are expressed in Type I TRCs, Type II TRCs and Type III TRCs. In knock-out mice, studies uncovered that the ghrelin is associated with salty and sour taste perception but not sweet or bitter stimuli. According to the study of Shin et al. (2010), ghrelin receptor null mice display decreased responsivity to NaCI and citric acid tastants [43]. Another study in ghrelin knockout mice demonstrated that ghrelin can be related to fatty taste perception through the regulation of CD36 and GPR120 receptors expression [44].

Glucagon: Glucagon is produced mainly in pancreatic alpha cells. The most known function of glucagon is maintaining glucose homeostasis via triggering glycogen

breakdown and stimulating gluconeogenesis in the liver [45]. Glucagon is coexpressed with its receptor in the subset of TRCs within circumvallate, foliate and fungiform papillae. Glucagon signalling has a functional role in peripheral modulation of sweet taste responsiveness. $Sgr5^{-/-}$ which is genetically lack of mature glucagon animal model had significantly decreased sensitivity to sucrose when compared with wild type mice. Similarly, decreased responsiveness had observed in disruption of glucagon signalling through a pharmacological agent. These results confirmed that glucagon signalling has a modulatory role in the sweet taste sensitivity [46].

Glucagon-Like Peptide-1 (GLP-1): The gut hormone GLP-1 produces in specialized L-enteroendocrine cells located at gastrointestinal tract. GLP-1 has functional in the stimulation of insulin secretion, inhibition of glucagon releasing during the post-prandial period. And also it provides a latency of gastric emptying and enhances satiety signalling [47]. The action of GLP-1 is mediated through glucagon-like peptide 1- receptor (GLP-1R). In the mice circumvallate papillae (CVP), GLP-1 expression is reported in two different classes of TRCs which are serotonergic cells and α-gustducin-expressing/T1R3-expressing cells because of GLP-1 can responsible for different functions of GLP-1 within the taste buds. Firstly, coexpression of GLP-1R with T1R3 can provide a potential function of GLP-1 signalling in the perception of sweet and umami taste. Diminished sweet taste preference has been observed in GLP-1R deficient mice and so it confirmed that the role of GLP-1 is maintaining or increasing to sweet taste sensitivity in α-gustducin-expressing/T1R3-expressing cells [48]. Moreover, it has been observed that GLP-1R knockout mice show critically decreased sensitivity to sweet tastants and increased response to umami tastants [49]**.** Secondly, serotonergic cells primarily consists of Type III cells which are associated with sour taste perception and GLP-1R knockout mice studies showed enhanced sour taste sensitivity. It suggests that GLP-1 signalling can be modulated to sour taste perception [48]. Lastly, Martin et al. have claimed that lipid consumption mediated GLP-1 secretion in taste bud can contribute to the high palatability to fatty tastants through regulation of long-chain fatty acid receptor- GPR120 interaction [49].

Cholecystokinin: Cholecystokinin is primarily produced in the specialized I-enteroendocrine cells which found in the upper small intestine and play role in stimulating the ingestion of fat and protein. Moreover, cholecystokinin responsible for various process in the regulation of food intake such as reducing appetite, stimulating of satiety, inhibition

of orexigenic peptides expression in the hypothalamus, the proliferation of insulin synthesizing beta cells and decreased insulin-induced hyperphagia [50]. In taste cells, CCK expression is co-localized with the CCK-A receptor due to autocrine signalling mechanism. Calcium imaging studies showed that stimulation of the CCK-A receptor through binding with CCK give rise to inhibition of potassium current and elevation of intracellular calcium [51]. Lu et al. have reported that per cent of 60-70 of CCK-responsive cells was sensitive to bitter tastants such as quinine and caffeine [52]. In addition, gut bitter taste receptor signalling involving the efflux activity of ATP-binding cassette B1 (ABCB1) transporter is regulated by CCK release in mouse intestine. ABCB1 transporter provides to various molecules across extra- and intra-cellular membranes [53].

On the other hand, Otsuka Long-Evans Tokushima Fatty (OLETF) rats which has deficient of CCK-1 receptor has preferred more monosodium glutamate (MSG) at the lower concentrations regarding control group. Similarly, excessive sensitivity to sweet tastants such as fructose, sucrose and alanine has been observed in OLETF rats. However, there were not any significant differences in response to NaCl, MgCl2, citric acid, quinine-HCl, and capsaicin between groups [54].

Galanin: Galanin, a neuropeptide with 29-30 amino-acid (aa), is produced mainly in the central nervous system and gastrointestinal system. Galanin is responsible for the regulation of various processes such as food intake, memory, neuroendocrine function, gut secretion and motility. The action of galanin emerges through three G-protein-linked galanin receptor subtypes which are GalR1, GalR2 and GalR3. In the gustatory system, galanin expression is demonstrated in both Type II TRCs and Type III TRCs and also GALR2 expression present in rat taste buds [55].

Oxytocin: Oxytocin (OXT), a neuromodulator hormone, is expressed in the hypothalamus and is released from the posterior pituitary. The most known function of oxytocin is the contraction of the uterus during birth and the releasing of breastmilk after birth. Moreover, oxytocin signalling contributes to the regulation of food intake [56]. In the mice taste buds, OXT expression was not determined locally and but oxytocin receptor (OXTR) expression is demonstrated on the surface of Type I TRCs. In this study, Sinclair et al. (2010) reported that there are not any morphological alteration in taste buds of OXTR knockout mice [57]. Some researchers have claimed that oxytocin signalling can be associated with sweet and

salty food preference due to the postprandial effect of nutrients [22]. Moreover, Sinclair et al. (2015) intraperitoneal injection of OXT in mice cause to reduced sweet taste perception but not any effect on bitter, salty and sour taste perceptions [58].

Neuropeptide Y (NPY): Neuropeptide Y, 36 aa orexigenic peptide, is the most abundant found protein in the mammalian central nervous system. The functions of NPY are stimulation of carbohydrate intake, reduction of times between the meals, elevated motivation to feeding and latency of satiety with improved meal size [59]. Although NPY is known as an orexigenic neuropeptide in the hypothalamus, it has different functions in feeding behaviour depending on receptor isoforms. For instance, NPY-Y1 and NPY-Y5 receptors trigger feeding while NPY-Y2 and NPY-Y4 have a role in appetite inhibition [60]. NPY expression is determined in isolated taste buds of rats using immunocytochemistry and RT-PCR. In addition, it was reported that NPY-positive cells almost coincide with CCK and vasoactive intestinal peptide (VIP) expressing cells. The patch-clamp experiments demonstrated that NPY administration into the isolated taste cells stimulates hyperpolarization via increased K^+ conductance mediated by NPY-Y1 receptor and this action is the antagonistic effect of CCK [61]. In addition, it is claimed that NPY contributes to modulate of excitation on bitter sensitive TRCs and inhibition on sweet sensitive TRCs. However, it has not determined to direct effect of NPY on any taste modalities [51].

Peptide YY (PYY): The gut peptide PYY is produced and released from enteroendocrine L-cells which are found in the distal gastrointestinal tract and also responsible for the regulation of appetite [62]. According to Hurtado et al., four type receptor isoforms of PYY, Y1, Y2, Y4 and Y5 are expressed in several cell types which are epithelial progenitors, keratinocytes, neuronal dendrites and TRCs. Murine and human saliva contain PYY hormone and this hormone act via Y2 receptor. Because of multiple locations are found for PYY receptors, these receptors can contribute to accomplishing of many varieties of tasks such as proliferation, differentiation, motility, taste perception and satiation [63]. Behaviour experiments applied to *PYY* knockout mice demonstrated that PYY signalling modulates sensitivity to bitter tastants and lipid emulsions [64].

Vasoactive Intestinal Polypeptide (VIP): VIP, 28 aa neuroendocrine hormone, has a functional role in various processes such as relaxation of smooth muscle, neuronal

survival, regulation of glycogen metabolism in the cerebral cortex, promotion of electrolyte secretion and protection from oxidant damage [65]. These actions of VIP emerge mediated through *Vpac1* and *Vpac2* receptors. In the taste cells of mice circumvallate papillae, VIP is co-expressed with alpha-gustducin, *T1r1*, *Vpac1* and *Vpac2* receptors. The behavioural effect of VIP on taste preference has been shown using with VIP knockout animal model. As a result, increased sweet taste preference has been observed in VIP knockout mice when compared with wild type mice. In addition, the lack of VIP caused significantly reduced expression of the leptin receptor and increased expression of GLP-1. Both of them can contribute to sweet tastants preference in VIP knockout mice [66].

Endocannabinoids: Endocannabinoids, including anandamide [N-arachidonoylethanolamine (AEA)] and 2-arachidonoyl glycerol (2-AG), have orexigenic effects in the hypothalamus and limbic forebrain which mediated binding with the cannabinoid 1 (CB_1) receptor. The circulating endocannabinoid level depends on the plasma leptin level because of leptin suppresses the endocannabinoid secretion. Therefore, there is an inverse correlation between circulating endocannabinoids levels and plasma leptin levels. Similarly, the function of endocannabinoids in the taste buds is identified as increasing the responses to sweet taste which is opposite to leptin action. Yoshida et al. have reported that cannabinoid CB_1 receptors are found in type II taste cells. Moreover, AEA or 2-AG enhances the preference to the sweet mixture and electrophysiological responses of TRCs to the sweet tastants. This effect is mediated through binding with cannabinoid CB_1 receptors [67, 68, 69]. Niki et al. have demonstrated that the management of sweet taste sensitivities has occurred via leptin and endocannabinoids treatment in mice. They applied the pharmacological antagonists of Ob-Rb and CB_1 into wild type, leptin receptor-deficient db/db, and diet-induced obese (DIO) mice. Their results confirmed that circulating leptin signalling can become more effective modulator for the response to sweet taste than local endocannabinoids in wild type mice. However, the effect of endocannabinoids can be dominant than leptin in response to sweet compounds in leptin receptor-deficient db/db mice because of increased synthesized of endocannabinoids. As expected, in DIO mice the effect of leptin antagonist caused to dimish slowly while the effect of endocannabinoid antagonist caused enhanced during the feeding with high-fat diet (HFD) [70].

2.2.3. Neurobiology of Taste Transduction

The decision of the tastant is delicious or disgusting depends on the evaluation in the taste perception associated brain areas via the central gustatory pathway (Figure 2.5.) [33]. In this pathway, taste message in the TRCs of the taste buds conveys to the first relay nucleus of the tractus solitarus (NTS) in the brain stem through the three cranial nerves. These nerves are facial (VII), glossopharyngeal (IX) and vagus (X) nerves. Anterior fungiform and anterior foliate papillae are innervated with chorda tympani and greater superfacial petrosal branches of facial nerves while remaining foliate papillae and CVP in the posterior part of the tongue are innervated with glossopharyngeal nerve. In addition, taste buds in pharynx and epiglottis synapse with the vagus nerve [71]. Apart from these three nerves, trigeminal nerve (V) carries to the brain about the tactile and texture knowledge of the consumed foods [72].

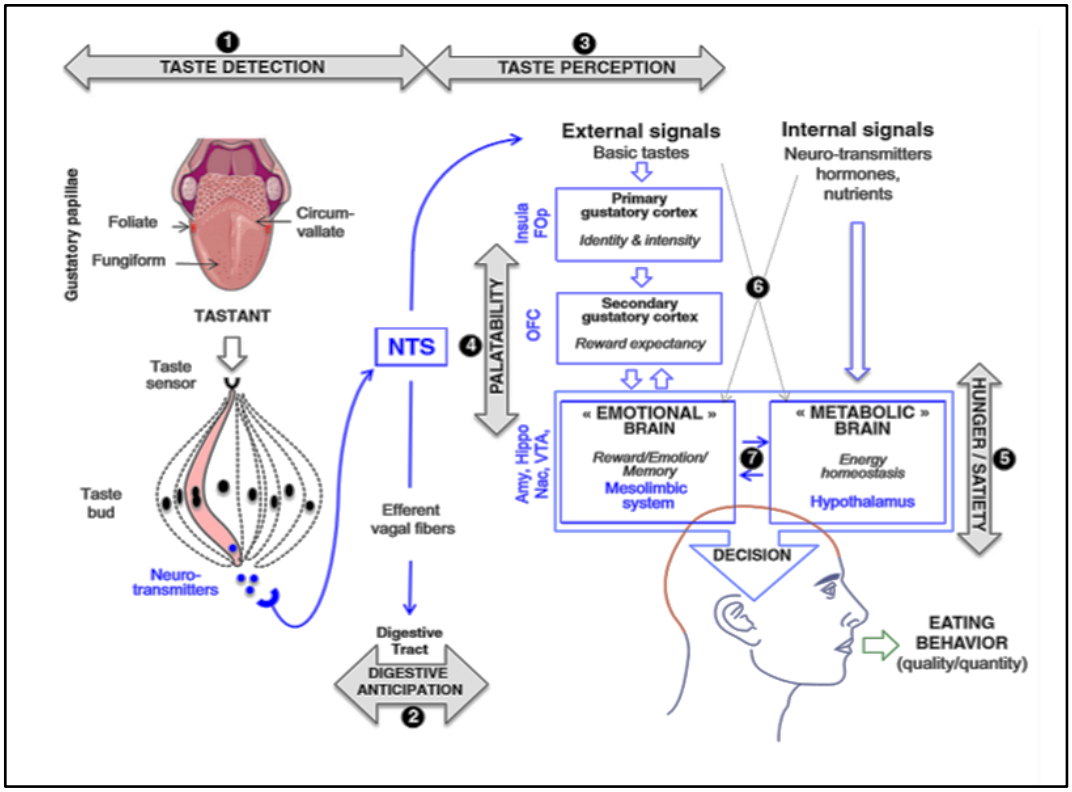


Figure 2.5. Schematic view of the gustatory pathway and its interrelations with the emotional brain and metabolic brain [33].

A wide variety of receptors in NTS is associated with a homeostatic modulator of feeding [73, 74, 75, 76]. At the same time, NTS is the required, the first synapse for mechanosensory, somatosensory and gustatory inputs. The cells with several sensitivity properties in taste responsive-part of rostral NTS have been identified according to electrophysiological recordings of the anaesthetized rats. These cells respond to the many taste modalities such as sweet, sour, salty, bitter and umami, at the same time and many of these cells are also sensitive to touch and /or temperature [77, 78]. Moreover, spike timing in taste-response cells can be responsible for the transmission of an important amount of information in the NTS [79].

There is detailed information about NTS response to taste stimuli in the anaesthetized animal but taste-related NTS response in conscious animals is still unclear. Roussin et al. obtained the electrophysiological recordings from NTS in conscious and freely licking rats and demonstrated that NTS contains some cells among taste-responsive cells called “anti-lick” cells. These cells become silent during licking. Lick-cells modulate the licking pattern by providing the reference point for taste-responsive cells and contribute to the identification of taste quality with other taste-responsive cells. On the other hand, anti-lick cells display a surge in firing rate before and after licking bout which is responsible for predicting the beginning and signalling the end of a lick [80].

In HFD-induced obese rats, impaired neural coding of taste stimuli in the NTS has reported. Actually, obesity cause reduced expression and activation of TRCs and so weakened stimuli from tongue conveys to the brain. However, NTS of obese rats has found a proportionately higher number of taste-responsive cells than non-taste-responsive cells to compensate for weakened taste sensitivity. In this mechanism, non-taste-responsive cells converted into taste-responsive cells in NTS due to obesity. Therefore, NTS can play a role in the adaptive mechanism of taste processing [81].

Taste information is transmitted from NTS to the parabrachial nucleus of the pons (PBN) as the second relay nucleus in rodents and it is associated with neural coding of the taste. The reason for taste-related information conversion from NTS to the PBN is to enable a subset of PBN cells to carry a higher knowledge load at a faster rate [82]. In experiments

performed on conscious rats, it is demonstrated that taste neurons in PBN provide the discrimination of variety of chemical stimuli such as sweet (sucrose), sour (HCl), salty (NaCl), bitter (quinine-HCl), and non-NaCl salts ($MgCl_2$, NH_4Cl, and KCl) [83]. Lesion studies in PBN of rodents have revealed that PBN is a vital component for perception and learning of taste stimuli. Specifically, lesions on PBN cause impaired taste preference, neophobia, failed to acquire conditioned taste aversion (CTA) and inhibition of sodium appetite [84, 85, 86, 87, 88, 89, 90, 91].

In primates and humans, taste message directly is transmitted from NTS to the parvocellular part of the ventralis posteromedial thalamic nucleus (VPMpc), bypassing the PBN [92]. On the other hand, in rodents taste message is sent to VPMpc from PBN [93]. There are many lesion studies try to clarify the role of VPMpc but the function of VPMpc is still unclear [94]. According to some lesion studies in VPMpc, VPMpc had no significant effect on response to preferred and non-preferred tastants [95, 96]. However, Lasiter et al, (1985) have claimed that VPMpc-lesioned animals display decreased taste response [97]. Meanwhile, controversial pieces of evidence have been presented in terms of the effect of VPMpc on CTA. Some studies have showed that VPMpc-lesioned animals have disruption on CTA behavior [89, 97] while other studies have claimed that there is not any relationship between VPMpc and CTA behaviour [95, 98, 99]. In addition, many of studies have pointed to several roles of VPMpc in taste processing which are neophobia, taste discrimination, comparison between novel and familiar tastants, complex gustatory learning tasks and gustatory function [95, 99, 100, 101, 102].

Taste transduction associated neurons in the VPMpc projects to the cortical gustatory area (CGA) in the insular cortex (IC) which is responsible for determining the taste identity and intensity [33]. IC is considered as the primary gustatory cortex and consists of three cytoarchitectural subunits which are granular (dorsal) (gIC), dysgranular (intermediate) (dIC) and agranular (ventral) (aIC) subdivisions [103]. However, very little information is available on the functional organization of insular circuits including taste perception. Nevertheless, extracellular recording in alert rats has demonstrated that generality of taste processing neurons are found in gIC and dIC while neurons in aIC are related gustatory responses. Direct input from VPMpc is transmitted to gIC and dIC and both of them can relay gustatory signals to aIC. Apart from taste, many of signals such as nociceptive,

thermal, visceral, somatosensory, olfactory, visual and auditory signals are processed in IC so, more than one stimuli can activate same neurons in IC [104].

Studies about the taste processing role of IC revealed that decreased of neophobia and impairment of CTA in IC-lesioned animals [105, 106]. Specifically, the role of IC in CTA is to identify the novel and familiar tastes, memory protection of CTA, withdrawal of CTA and disappearance of CTA [107, 108, 109, 110, 111]. Moreover, IC has also functional in taste-odor integration [112]. However, IC has not any direct effect on taste perception and taste discrimination [94].

IC is directly projected with the orbito-frontal cortex (OFC) which is known as the secondary gustatory cortex. Neurons in OFC are responsible for many tasks including maintaining gustatory information in reward memory, decision of eating, encoding reward value and providing the reward expectancy against to the tastant [113, 114, 115]. Various stimuli which are gustatory, somatosensory, visual and olfactory inputs enter to the OFC. Therefore, OFC is considered as a significant place in terms of the decision of food palatability. Interestingly, OFC is associated with sensory-specific satiety in gut-brain interactions. This phenomenon states that if a specific food eaten in order to satiety, it will cause less rewarding without alteration in the taste perception [116]. In non-human primates and human functional magnetic resonance imaging (fMRI) studies supported this sensory-specific satiety changes [117, 118].

Gustatory information is transduced from OFC to the prefrontal cortex (PFC). Researches in non-human primates have demonstrated that neurons in the OFC mostly encodes only the reward degree while neurons in the dorsolateral prefrontal cortex (DLPFC) are responsible for encoding the reward degree and the reward expectancy [119]. Watanabe et al. (2001) claimed that reward data enters the PFC through the OFC passes to the DLPFC [119]. On the other hand, dorsomedial prefrontal cortex (DMPFC) neurons respond to gustatory stimuli [71]. Nevertheless, many behavioural experiments have demonstrated that the PFC is related to different mechanisms in the regulation of feeding including CTA behaviour [120, 121].

PFC is interconnected with reward mechanism associated subcortical areas which are nucleus accumbens (NAcb) of the ventral forebrain and ventral tegmental area (VTA) of the midbrain [122, 123]. NAcb is found in the rostrobasal forebrain and also is the main

component of the ventral striatum. Three essential sub-regions of the NAcb have been identified. The caudal two-third sub territories are known as core and shell while the rostral pole includes the third structure [124]. The shell enters the medial part of the ventral pallidum (VP), the lateral hypothalamus (LH), the VTA, the PBN, and the substancia nigra pars compacta. In addition, the core projects to the dorsolateral part of the VP, the entopeduncular nucleus, and substancia nigra pars compacta [125, 126]. With respect to neurotransmitter inputs, the NAcb receives dopaminergic innervation from VTA [127]. Glutamatergic innervation present from basolateral amygdala (BLA) and the PFC to NAcb [128]. Moreover, opioidergic and GABAergic neurons in NAcb projects to VP in the central gustatory system.

Opioid signalling in NAcb plays a central role in the modulation of feeding behaviours [129]. Microinjection studies which administration of D-Ala2, N-Me-Phe4, Gly-ol5-enkephalin (DAMGO)(μ opioid receptor-(MOP) agonist) into NAcb revealed that elevated opioid level causes to particularly increased consumption of calorie-dense (sucrose, lard) and palatable foods (saccharin, salt) [130, 131, 132]. In addition, when only chow is presented to DAMGO injected rodents, increased consumption of chow has been reported [133]. In the opposite way, Bodnar et al. (1995) have claimed that inhibition of consumption on palatable food observed in opioid antagonists injected rodents [134].

Recent studies have demonstrated that appetitive and aversive stimuli are differentially encoded by the activity of NAc neurons. Núñez-Jaramillo et al. (2012) have suggested that N-methyl-D-aspartate (NMDA) receptors in the NAcb core differ with using taste preference test and CTA model during rewarding appetitive taste learning, aversive taste conditioning and retrieval of appetitive versus aversive taste memory [135].

In reward mechanism, another essential component of emotional brain center is VTA and studies focus on midbrain dopaminergic neurons arising VTA [136]. Using viral-genetic tracing strategies in rodents, Beier et al. (2015) showed that both VTA dopamine (DA) and GABAergic neurons receive from PBN inputs; DA neurons in VTA connect to NAcb [137]. Lesions in VTA can give rise to unresponsiveness to thirst and hunger stimuli. Dopaminergic pathway in the VTA is related to increased the preference of naturally consumed fluids. Lesions of the VTA disrupt the motivation to excessive drinking of

naturally preferred fluid. Moreover, VTA-lesion in both mice and rats have been exhibited decrease palatability to hedonic stimuli and reduce preference to sucrose [138, 139].

Meanwhile, it is well known that neurons in PBN, a gustatory and visceral processing area in the brainstem directly transmitted to the VTA in the midbrain. PBN-VTA pathway is confirmed in several studies but specific taste perception related projection remains unclear [140, 141]. Nevertheless, Boughter et al. (2019) have demonstrated that sweet (sucrose) and quinine (bitter) tastants activate specifically to VTA projection neurons in PBN. They claimed that appetitive and aversive tastes are transduced to a midbrain reward interface through direct projections from the PBN [142].

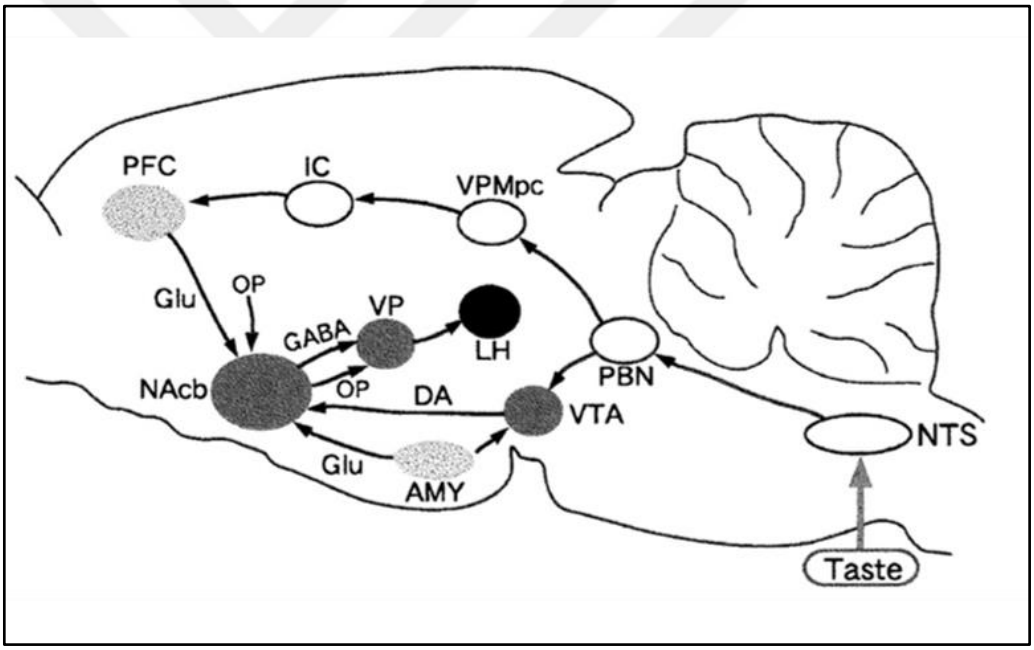


Figure 2.6. Schematic view of the central neural pathway of the gustatory system in rodents. NTS, nucleus of the tractus solitarius; PBN, parabrachial nucleus; VPMpc, parvocellular part of the ventralis posteromedial thalamic nucleus; IC, insular cortex; PFC, prefrontal cortex; _AMY, amygdala; VTA, ventral tegmental area; NAcb, nucleus accumbens; VP, ventral pallidum; LH, lateral hypothalamic area; DA, dopamine; GABA, g-aminobutyric acid; Glu, glutamic acid; OP, opioids [71].

Other centers about the reward mechanism in the central gustatory pathway are amygdala and ventral pallidum (VP). The role of the amygdala is identified as determining of

aversive taste stimuli. Therefore, lesion studies in amygdala considerable focus on CTA behaviour. Results have demonstrated that lesion of the amygdala has differential effects on CTA behaviour which in a range from reduction of the taste aversion to abolition which depends on lesion region [143].

VP is the major target of the NAcb and also VP integrates and processes reward knowledge flowing via the mesocorticolimbic system [144]. Anatomically, VP is found between the NAcb and lateral hypothalamus (LH). Neurons in VP have "hedonic hotspot" for eating motivation and encodes the learned taste aversion.

Finally, the last station of the gustatory pathway is LH which is known as a metabolic brain. Neurons in VP are projected to LH which is a feeding center but interaction between reward and feeding systems has not been elucidated completely. On the other hand, it is well known that orexigenic and anorexigenic signalling mechanisms in LH providing the establishment of energy homeostasis within the body [71].

2.2.4. Developmental Process of Gustatory System

The gustatory system has been conserved along in almost all vertebrates. However, it has been revealed that it can differ between species, even among the individuals in one species when anatomical, histological, cell biological and molecular structure of the taste system is examined in detail. In mammals, the general layout of the taste system and the molecular signalling pathways develop during embryogenesis [17, 145].

2.2.4.1. Embryonic Origin of Tongue Development

The tongue is a complicated organ which is formed the various types of cell populations within the embryo. The mucosa is originated from ectoderm and endoderm of the first and second pharyngeal arches respectively; the musculature derived from the occipital myotomes and paraxial mesoderm and the connective tissue compartments are originated from cranial neural crest cell (CNCC) [18, 146].

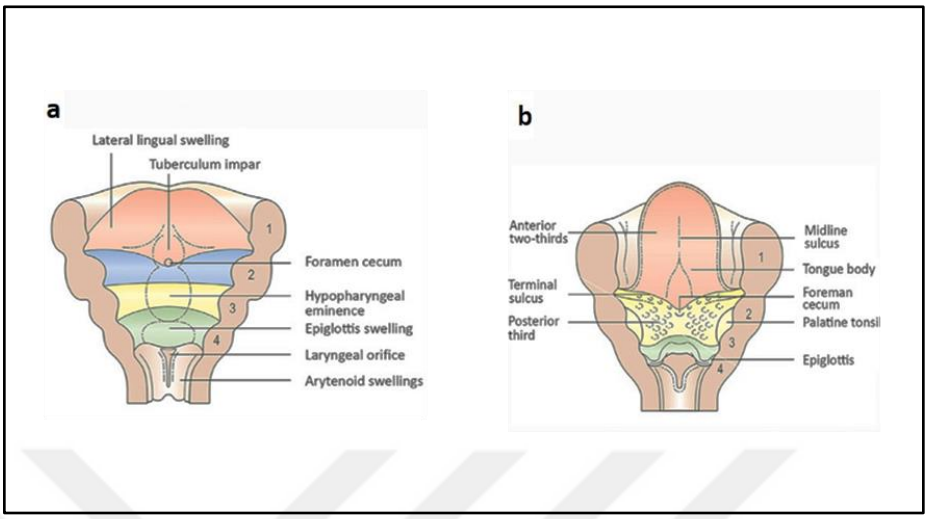


Figure 2.7. Developmental origin of the mammalian tongue. Primordial structures of (a) the mammalian tongue from the first through the fourth branchial arches and (b) differentatiated form of the tongue [18].

The development of tongue starts with a series of primordial structures from the first through the fourth branchial arches. The first arch is constructed by CNCC and mesodermal lineage and it includes three primordial structures which are paired lateral lingual swellings and a tuberculum impar. The first appeared gross structure is Tuberculum impar. The paired lateral lingual swelling is formed subsequently and then, the lateral lingual swellings convert into anterior two-thirds of the tongue which is originated from the first arch of the tongue. Simultaneously, a forward midline swelling which is named as copula or hypopharyngeal eminence displays in the midline of the second, third and fourth arches. The copula is constructed to the posterior third of the tongue. Moreover, the epiglottal swellings are originated from the fourth pharyngeal arch and composed at the most posterior boundary of the tongue. Ultimately it forms to epiglottis of the larynx.

The mucosa of anterior two-thirds of the tongue is originated from pharyngeal the first arch and it is innervated by the trigeminal nerve (cranial nerve V) which is the nerve of the first arch. On the other hand, the mucosa of the posterior third is derived from pharyngeal arch 3rd and it is innervated with glossopharyngeal nerve (cranial nerve IX). Posteriorly located mucosa is derived from pharyngeal arch 4th and it receives the general sensation from the

superior laryngeal branch of the cranial nerve X which is also known as vagus nerve [18, 146].

The taste perception in anterior 2/3 of the tongue is originated from the chorda tympani which is a branch of cranial nerve VII. Actually, cranial nerve VII is also named as facial nerve and it is a pre-trematic nerve to the pharyngeal arch 1st. In addition, taste perception from circumvallate papillae is supplied by glossopharyngeal nerve (cranial nerve IX). Lastly, all the muscles of the tongue, apart from palatoglossus, are originated from the occipital myotomes and innervated by the hypoglossal nerve (cranial nerve XII). Palatoglossus is supplied by the cranial accessory nerve (cranial nerve XI) and it is derived from pharyngeal arch 4th [18, 146].

2.2.4.2. Taste Bud Development in Early Life

It is known that the functional and morphological development of taste cells in humans starts in the first trimester of gestation. The formation of papillae including fungiform, foliate and circumvallate forms approximately at the 10th week of gestation and the synaptogenesis of taste cells elevate during from 8th to 13th weeks. Functional maturation of taste papillae appears in the early phases of the second trimester. The number and distribution of papillae on the tongue is determined during the last trimester. The view of tongue in the last trimester in terms of the distribution of papillae within the tongue surface were similar to childhood and adulthood [145, 147].

On the other hand, the early development and innervation of the tongue in rodents are studied in detail and clearly elucidated. It is known that at embryonic (E) 11.5 day in mice, the tongue rudiment is covered by basal and keratinocytes with a homogeneous epithelial lineage. Subsequently, taste placodes form and sensory nerve fibers reach into the taste placodes at E12.5 day. However, there is no complete innervation into taste placodes until E15.5 day. The formation of papillae morphogenesis starts at E15.5 day. The arrangement of taste placode converted into mushroom-shaped papillae at E18.5 day. Taste bud differentiation completes within a week following of birth [145].

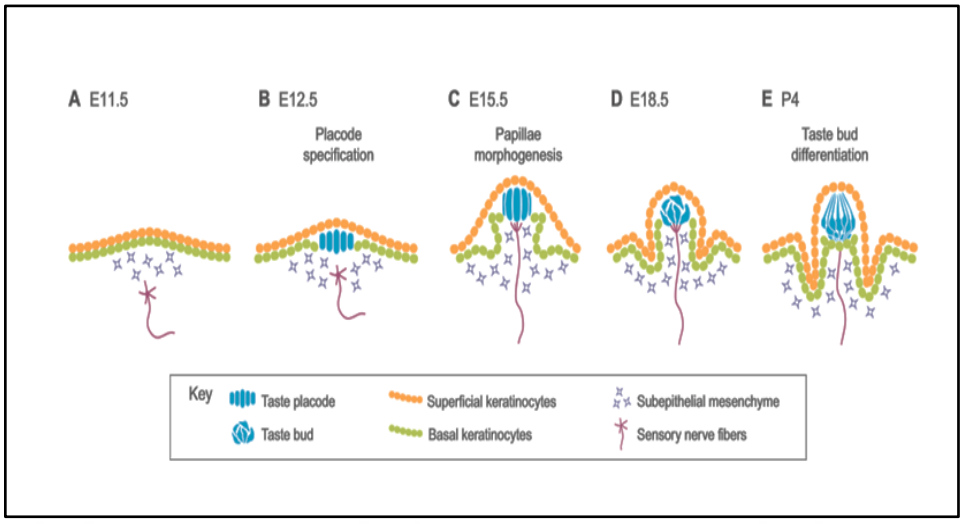


Figure 2.8. Representative view of taste bud and papilla development during the perinatal period in mouse embryos [145].

Embryonic taste bud development is regulated by many signal transduction pathways including Wnt/β catenin, sonic hedgehog (Shh), notch and etc. Detailed information about the function of molecular signalling pathways in the development of taste sense is available in Table 2.2.

Table 2.2. The primary signalling pathways of embryonic taste bud development.

Molecular Signaling Pathways	**Known Physiological Effect**	**References**
Wnt/β Catenin Signaling	Required and sufficient in terms of providing to the primary stimulatory signal in taste placode formation.	[148]
Sonic Hedgehog Signaling (Shh)	*Shh* signalling is downstream of Wnt/ β catenin pathway. It has a functional role as a negative feedback regulation of taste placode formation. Excess signalling of Shh cause to negative stimulation of taste placode formation.	[149]
Fibroblast Growth Factor (FGF) Signaling	FGF signalling has a functional role in various steps of taste bud development. Actually, the FGF signalling pathway regulates the number of posterior taste papillae via controlling progenitor field size. Moreover, it has a functional role in the regulation of anterior versus posterior taste bud as a differential.	[150]
	Before the taste placode differentiation,	

Bone Morphogenetic Proteins (BMPs)	BMPs promotes to taste fate. After the placode specification, BMPs suppresses taste fate in vitro.	[145, 151]
Epithelial Growth Factor (EGF) Signaling	EGF suppresses taste fate in vitro and consistently, blocking of EGF progresses taste fate in vitro	[152]
Sry-Related High Mobility Group Box Transcription Factor (Sox2)	Sox2 is found in downstream of Wnt/β catenin signalling pathway. Activation of Sox2 is required for taste bud formation and but is not sufficient for differentiation of taste bud cells.	[153]
Brain-Derived Neurotrophic Factor (BDNF)	BDNF has a functional role in final phases of axon guidance as a targeting factor. In addition, it is necessary for gustatory neurons to find and innervation of taste epithelium. However, it is known that taste cell formation does not need to gustatory and somatosensory innervation.	[154, 155]
Notch Signaling	It is known that the expression of Notch pathway genes is expressed in taste placodes of mouse embryos. However, to date, any molecular or developmental study could not explain the role of Notch signalling pathway genes in taste bud development. It is only known that Notch signalling controls and regulates cell fate determination and /or differentiation.	[156, 157]

2.2.5. Taste Disorders

When compared with other sensory disorders such as visual or olfactory, taste disorders can be considered as less important. However, taste perception is the essential sensory for life due to it provides us with the discrimination of nutritious food items and potentially toxic agents. Therefore, diagnosis and treatment of taste disorder is important for the quality of life, intake of nutritious food and even surviving [158].

Certain genetic syndromes, craniocerebral injury, infection of the upper respiratory tract, exposition to toxic agents, radiation, certain surgeries (middle ear surgery, tonsillectomy, dental operation) and side effects of drugs and alcohol usage are considered as main reasons of taste disorders in the literature [159].

Taste abnormalities are determined through evaluation of some clinical trials. These are taking of general medical history and complaints, physical examination, regional or spatial taste testing, electro-gustatory recording, some biochemical laboratory tests and craniofacial imaging [160]. These methods can provide the determination of abnormalities in oro-gustatory and central gustatory pathway. Several approaches are available for the treatment of taste disorders and but researches still continue [161].

Typically, taste disorders are divided into two main classes as qualitative and quantitative disorders [162]. In clinical trials, many patients have been determined who suffer from quantitative and qualitative taste disorders [158]. (Figure 2.9.)

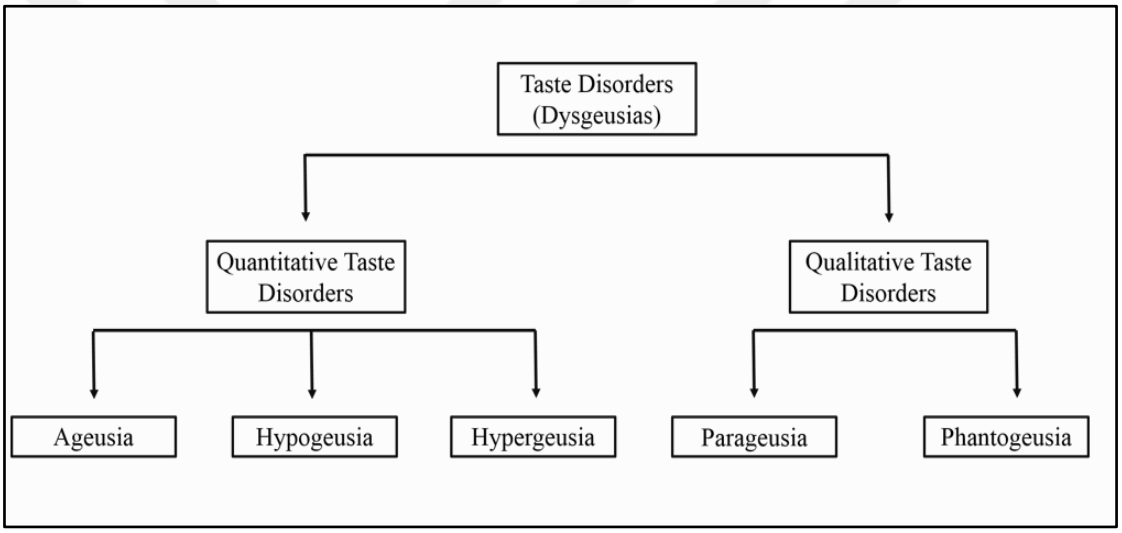


Figure 2.9. The classification of taste disorders.

According to quantitative measurements, taste disorders can be classified into 3 main groups which are ageusia, hypogeusia and hypergeusia.

- Ageusia means complete or severe loss of taste perception. Although the main reasons of ageusia are not identified completely, it is known that the ageusia has been associated with damage in the central gustatory pathway and drug usage such as Clopidogrel. Diagnosis of the disease is rare owing to patients never announce about symptoms of the disease and so disease-related symptoms are not pronounced in the literature [163]. The treatment of the disease mostly depends on the reason of the disease and in most cases, the symptoms of the medication usage associated ageusia can reduce with the termination of the medicine usage [164].

- Hypogeusia is identified as a decreased sense of taste. People who suffer from hypogeusia has been reported decreased sensitivity of taste in foods. Lower socioeconomic level, ageing, certain surgeries, alcohol and drug usage can cause hypogeusia [165]. Specifically, occupational exposition to detrimental toxins and chemicals in lower socioeconomic conditions has also been related to hypogeusia. The diagnosis of hypogeusia is applied with diagnostic tests. Although several methods are offered regard to the treatment of hypogeusia methods are still under searched [166].
- Hypergeusia refers to elevated gustatory sensitivity. The main reason for this abnormality seems as genetic variations [167]. Limited information is found in the literature about hypergeusia.

According to qualitative measurements, taste disorders are classified as parageusia and phantogeusia. This disorders are more annoying and so taken more complaints from patients when compared with other taste disturbances. However, both of them have been frequently observed together and/or very difficult to discriminate from patients medical history [162].

- Parageusia has been defined as an altered perception of taste qualities. Patients with parageusia have been frequently reported that gustatory stimuli differ from their experienced one. In the literature, parageusia has been determined in patients with depression and multiple sclerosis [168, 169].
- Phantogeusia has been described as a perception of taste without a stimulus. Phantogeusia is observed in patients with epilepsy and schizophrenia [170, 171].

2.3. BASIC TASTE MODALITIES AND SIGNAL TRANSDUCTION PATHWAYS

2.3.1. Sweet Taste Perception and Signal Transduction

2.3.1 1. An Overview to Sweet Taste

The sweet taste is accepted as a basic taste and most commonly means the sugar content in foods. Gene
natural and chemical compounds which are sucrose, lactose, glucose, fructose, aldehydes,

ketones, and sugar alcohols have a sweet taste at various level. Nevertheless, non-caloric artificial sweeteners which are saccharin, cyclamate, aspartame, acesulfame-K, neohesperidin, alitame, and sucralose have sweetness at low concentration. Moreover, several amino acids such as alanine, glycine and serine have moderately sweetness [28, 172]. On the other hand, modulation of sweet taste can be managed with some chemical agents such as lactisole, gymnemic acid, miraculin and curculin.

The studies have been continued since the 20th century to understand the chemosensation of sugar at th studies about sweet taste sensation have demonstrated that sac locus, a single principal locus, in mice responsible for influencing the response to several sweet substances and discrimination of sweetness of sweet compounds in mice [173, 174]. In next years, sweet taste receptors have been identified as G protein-coupled taste receptors which are the heterodimeric receptor of type 1 member 3 (T1R3) and a type 1 member 2 (T1R2) subunits. Subsequently, it is revealed that Sac locus encodes the T1R3 [28]. T1R2 and T1R3 are expressed on Type II TRCs and these receptors are activated by sugars, non-caloric artificial sweeteners, D-amino acids and some chemical compounds. [28, 172, 175]. Sweet taste receptors have three compartments which are the venus-flytrap domain (VFD), cysteine-rich domain (CRD) and 7-trans-membrane domain (TMD). The VFD and CRD are located at the extracellular side and TMD is found in the membrane [176]. According to the multipoint attachment theory, the sweet taste perception is mediated with the interaction of multiple binding sites between sweet taste receptor and sweet tastants.

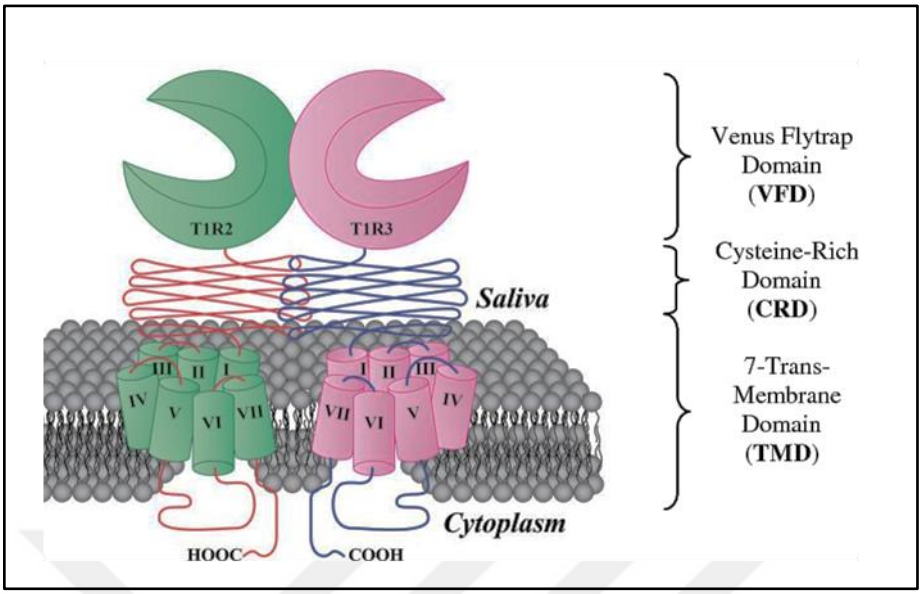


Figure 2.10. The schematic view of sweet taste receptors [176].

2.3.1.2. Sweet Taste Perception Associated Signal Transduction

Basically, sweet taste perception depends on binding of sweet tastants on sweet taste receptor. This interaction leads to conformational alteration of receptor and triggers to activation of G-protein alpha-gustducin. Activated G-protein promotes the activation of specific intracellular secondary messengers [177]. At the same time, the activated G-protein alpha-gustducin causes to release of Gβγ subunits and Gβγ subunits promote the activation of phospholipase-C-β-2 (PCL-β2). Increased level of PCL-β2 causes the elevated concentration of inositol triphosphate (IP3) and IP3 binds to IP3-receptor which is found in endoplasmic reticulum membrane. Releasing of intracellular Ca^{2+} gives rise to the activation of the taste-specific transient receptor potential cation channel subfamily M member 5 protein (TRPM5). Gating of TRPM5 causes an influx of Na^{+} into the cell. Increased intracellular Na^{+} contributes to cell depolarization and neurotransmitter release. Finally, neurotransmitters activate inputs of lingual afferent nerves and sweet taste is sensed with a central gustatory system [178] (Figure 2.11).

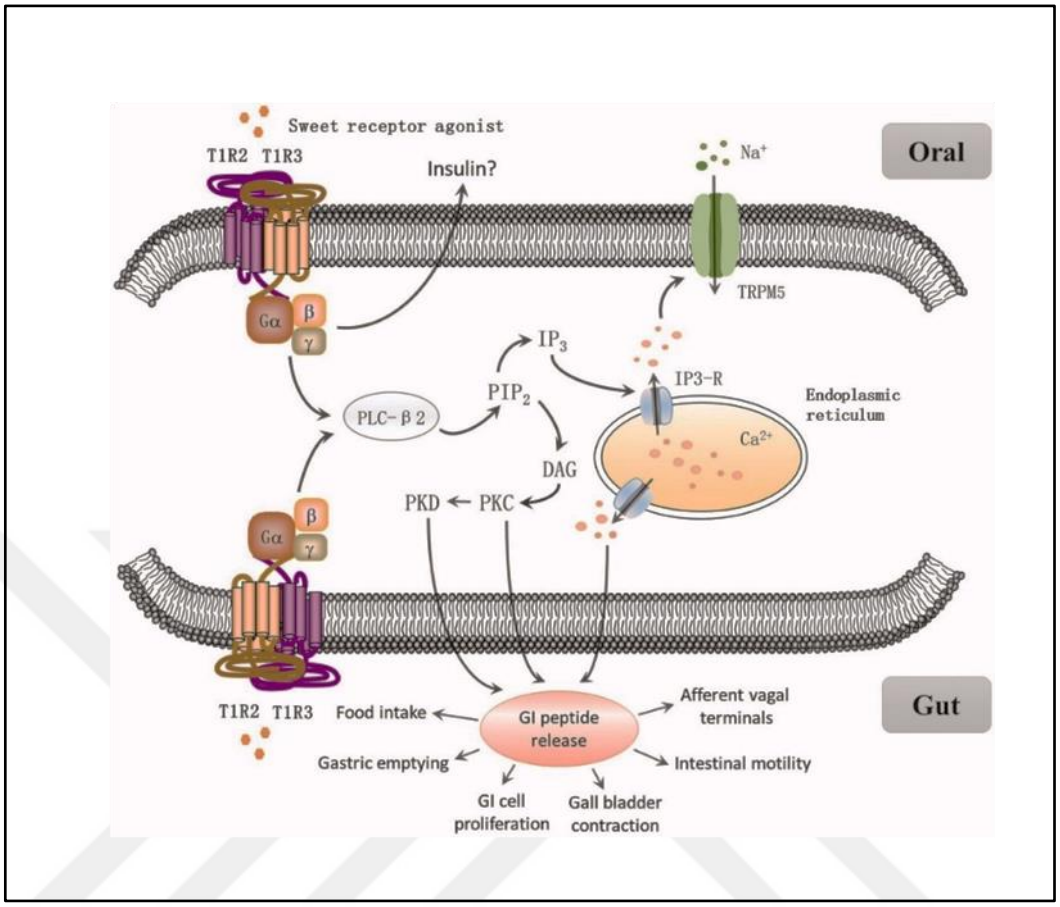


Figure 2.11. The schematic view of sweet taste signal transduction [178].

2.3.1.3. Functions of Sweet Taste Signal Transduction on Extra-Oral Tissues

The location of sweet taste receptor is not only confined with the gustatory system but also there are va peptide releasing provides the regulation of food intake, facilitating of gastric emptying, contraction of the gallbladder, the proliferation of GI cells and intestinal motility and glucose homeostasis [178]. According to a study by Renwick and Molinary, there is no consistent evidence about the relationship between sweet taste receptor activation through low-energy sweeteners and insulin signalling in glucose homeostasis [180]. On the other hand Kyriazis, et al has found that the activation of sweet taste receptor in beta cells plays a key role in the modulation of insulin-release for the postprandial period [181]. Ren et al. have shown that the sweet taste receptors in the brain have an essential role in a glucose-sensing mechanism which is related to body glucose homeostasis and neuronal function [182].

2.3.2. Bitter Taste Perception and Signal Transduction

2.3.2.1. An Overview to Bitter Taste

Bitter taste has special importance in taste sensation when compared the other taste modalities as it provides a determination of the poisonous substances. The bitter taste is innate and triggers behaviours leading to reject [183]. The presence of 680 different bitter compounds has been determined in the literature such as drugs, certain plants, coffee, some alcoholic beverages and etc. Especially, deadly toxins which found in poisonous plants have a bitter taste. The most known bitter compounds are quinine, caffeine and long-chain alkaloids. 6-n-Propylthiouracil (PROP) is used as a bitter taste compound in bitter taste perception associated in vivo studies. [184].

The sense of bitter compounds is perceived by taste receptors, type 2, (TAS2Rs) also known as T2Rs, which is a G-coupled protein receptor [30]. The location of T2Rs is tongue, stomach, intestine, pancreas and also the nasal passage, lungs and heart [179]. 25-30 different types of bitter taste receptors have been identified in the literature [185]. Especially the variations of taste 2 receptor membrane 38 (TAS2R38) bitter taste receptor is the most studied one in the human studies [186].

2.3.2.2. Bitter Taste Perception and Signal Transduction

As bitter taste receptors belong to G-coupled protein receptor family like sweet taste receptors, the bitter taste signalling pathway is quite similar with sweet taste signalling pathway. Briefly, a bitter compound interacts with its G-coupled protein-transducin This interaction activates. phospholipase C (PLC). Activated PLC causes to convert phospho-inositol diphosphate (PIP_2) into inositol triphosphate (IP_3). The increased intracellular concentration of IP_3 leads to the release of Ca^{2++} ions from stores. Finally, Ca^{2++} influx gives rise to secretion of the neurotransmitters [187, 188].

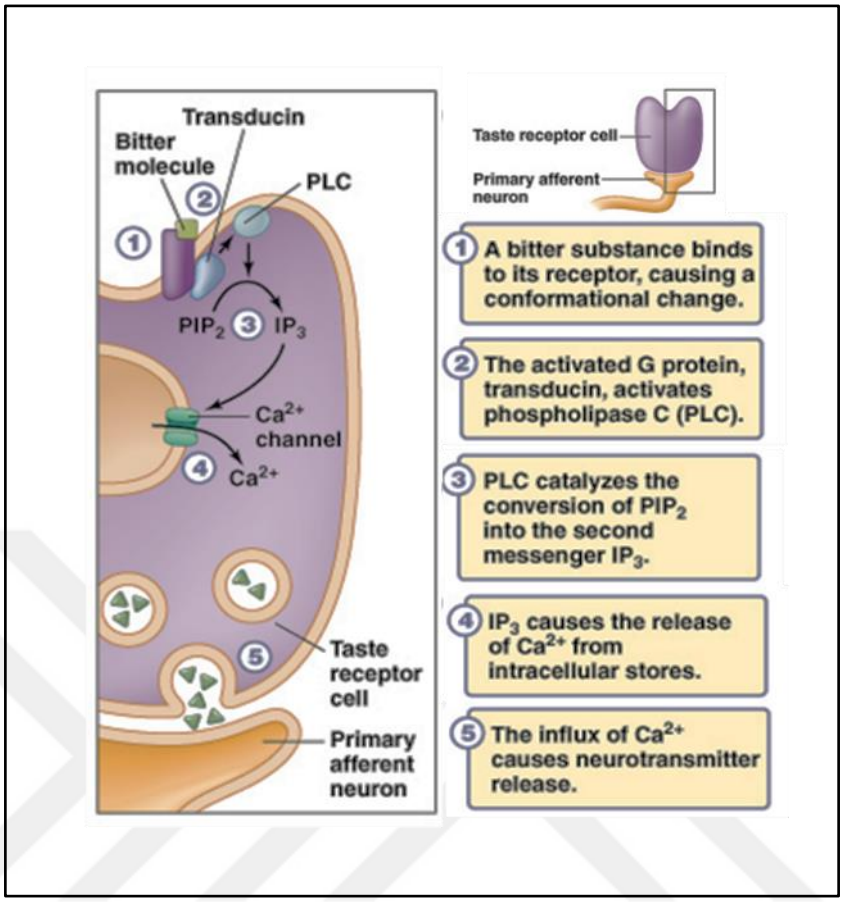


Figure 2.12. The schematic view of bitter taste signal transduction [189].

2.3.2.3. The Other Functions of Bitter Taste Signal Transduction on Extra-Oral Tissues

Bitter taste receptors are found in many extra-oral tissues such as sperm, small intestine, colon, upper and lower airways and lungs. They are responsible for several functions in health and disease as they cause to release various neurotransmitter and hormones [190].

Enteroendocrine cells in small intestine express bitter taste-sensing type 2 receptors (T2R) on the membranes. When bitter compounds bind to the T2R receptors, this binding causes CCK release. The released CCK binds to CCK2 receptors located on enterocytes or intestinal absorptive cells. CCK signalling activates the ATP-binding cassette B1 (ABCB1), transporter which effluxes the toxic substances or unwanted compounds to out of the cell and back into the intestinal lumen [191]. CCK also binds to CCK1 receptors on sensory fibers of the vagus nerve, sending signals to the brain to cease the food intake [192].

In the colon, bitter compounds bind to T2R receptors on epithelial cells and induce the transport of anions and water. This transport causes fluid rushing into the intestine, resulting in diarrhoea that leaks out and cleans the colon [192].

T2R5 bitter taste receptor is found in spermatid and spermatozoa. Ablation of *T2R5* gene leads to a smaller testis [193, 194].

The activation of T2R receptors located on the chemosensory cells of upper airways, nasal passages or trachea, has been initiated by binding the bitter compounds. The stimulation of signal transduction pathway leads the elevated cytoplasmic Ca^{2+} levels which activate the TRPM5 transduction channel. The influx of Na^{+} ions into the cell via TRPM5 channel causes the depolarization of the cell and activates the voltage-gated Ca^{2++} channels. Therefore, extremely increased intracellular Ca^{2++} level contributes to acetylcholine release. The binding of Acetylcholine to its receptor activates nerve fibers and induces protective reflexes such as sneezing [195]. In the lower airway, the smooth muscle cells of the lungs, T2R pathway activation is initiated by the binding of the bitter compound but the final step of the signalling pathway causes hyperpolarization and subsequently relaxation of the muscle cells at [196]. Also in the lungs, T2R receptors on ciliated airway epithelial cells are activated via binding of bitter compounds. This activation triggers to the same G-protein-mediated pathway. Activation of the pathways results in the increase in ciliary beat frequency, which can be suspected to serve to sweep the irritants away from the surface of the cell [197].

2. 3. 3. Salty Taste Perception and Signal Transduction

2.3.3.1. An Overview to Salty Taste

Salty taste perception means the ability of discrimination sodium chloride in the mouth. Because saltine

Salty taste perception receptor, the epithelial sodium channel (ENaC), is a transmembrane protein and has three homologous subunits which are α, β and ϒ. Animal studies have shown that ENaC channel can be blocked with amiloride drug. Researchers suspect that there could be different salty taste receptors apart from ENaC.

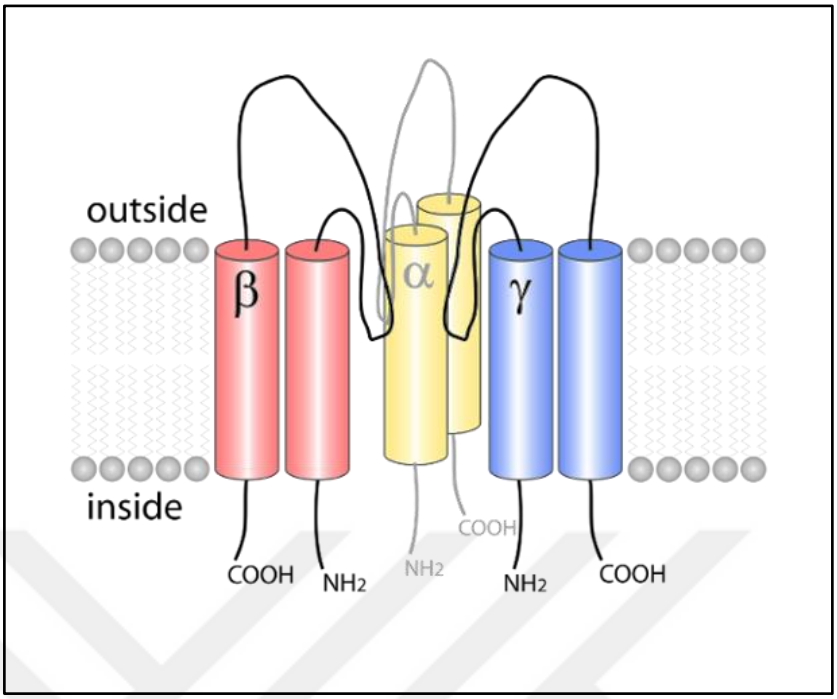


Figure 2. 13. The schematic view of the salty taste receptor [199].

2.3.3.2. Salty Taste Perception and Signal Transduction

Saltiness signal transduction initiates with entering the Na^{+} ions into the taste receptor cell via ENaC channel. The elevated intracellular concentration of Na^{+} cause to depolarization of TRC. Subsequently, the change of intracellular ion status causes to open the voltage-gated Ca^{2++} channels. Extracellular Ca^{2++} influx leads to neurotransmitter release [189].

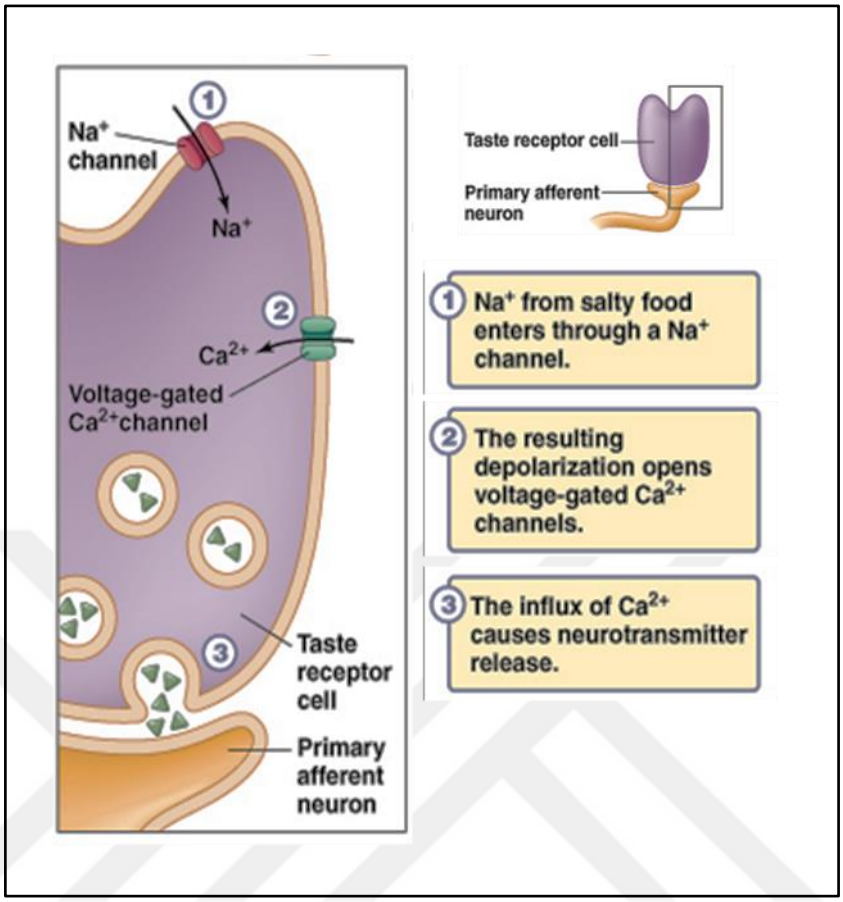


Figure 2.14. The schematic view of salty taste signal transduction [189].

2.3.3.3. The Other Function of Salty Taste Signal Transduction on Extra-Oral Tissues

ENaC channel is working as the rate-limiting step of transepithelial Na^+ reabsorption in many epith abnormalities in ENaC function cause sodium and water imbalances [200, 201]. For instance, the functional mutation of alpha, beta or gamma subunit coding genes of ENaC identified as hereditary salt-losing syndromes/salt-wasting phenotypes. Mutation in the COOH terminus of beta or gamma subunits has been directly linked with hypertension because Na^+ retention which is known as Liddle's syndrome.

According to gene-targeting animal studies, ENaC has a vital role in lung fluid clearance in newborn mice. In this context, disruption of alpha subunit especially causes to completely termination of ENaC-mediated Na+ transport, while knockout of the beta or gamma subunit had less effect in lung fluid clearance. Lastly, disruption of all subunits outcomes salt-wasting syndrome which is similar in humans [202].

2.3.4. Sour Taste Perception and Signal Transduction

2.3 4.1. An Overview to Sour Taste

Sour taste perception means to ability the detection of acidity which presence the amount of H+ ions in
0.7, respectively. The most known sour taste containing foods are lemon, grape, orange, tamarind and melon.

In the early studies, it is supported that sour taste sensation takes place through activation with two tra
taste is not required for expression of these proteins. However, it is known that protons from sour foods can directly pass through the apically located ion channel. Recent studies have shown that the primary protein of proton influx is the proton-elective ion channel otopetrin (Otop1) which can be a novel candidate of sour taste receptor. Transfer of H+ ions into cell stimulate to electrical response and prompt to sour taste perception [205].

2.3.4.2. Sour Taste Perception and Signal Transduction

The first step of sour taste signal transduction pathway is entering of H^+ from acidic foods into TRC through the ion channel. Then, an elevated level of H^+ ions blocks to the K^+ channel and intracellular K concentration increase. The elevated intracellular concentration of Na^+ cause to depolarization of TRC. Subsequently, the change of intracellular ion status opens to voltage-gated Ca^{2++} channels. Extracellular Ca^{2++} influx causes neurotransmitter releasing [206].

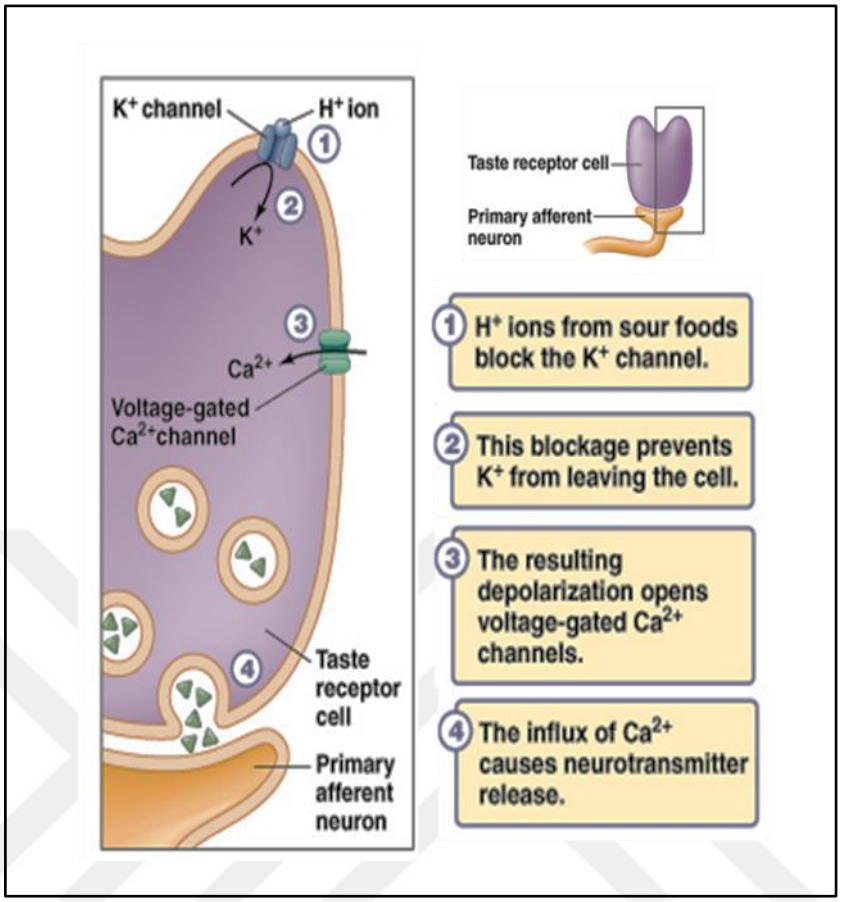


Figure 2.15. The schematic view of sour taste signal transduction [189].

2.3.4.3. The Other Function of Sour Taste Signal Transduction on Extra-Oral Tissues

Sense of sour taste provides the ability of free protons or organic acids and it has a very important role for maintaining body pH homeostasis. In addition, discrimination of sour taste mechanism has been associated with maintaining electrolytic balance in the body [207]. Sour taste receptors, PKD1L3 and PKD2L1 are widely expressed in many of tissues including skeletal muscle, brain, spleen, testis, retina bone marrow, colon, duodenum, gallbladder, spinal cord and kidney. Although deletion of PKD1L2 in animal studies can affect the kidney and retinal abnormalities, disease-associated molecular mechanism studies have been continued [208, 209].

PK2DL1 receptor is found along the spinal cord and it can be related the sense in pH alteration of the cerebrospinal fluid (CSF) [210]. It is suggested that PK2DL1 can be responsible for the perception of bending of the spinal cord during movement. In addition,

PKD2L1 has an essential role for mechanoreception in CSF-contacting neurons and maintenance of spine curvature [211].

2.3.5. Umami Taste Perception and Signal Transduction

2.3.5.1. An Overview to Umami Taste

Umami taste is known as pleasant savoury taste in Japenese word and it is accepted as the fifth taste se is mostly found in meat extract, yeast extract, cheeses, fungi, soy sauce, human breast milk, hydrolyzed vegetable protein, fish and fish products, soups and broths. Mostly, the sense of umami is originated monosodium glutamate (MSG) in meat broths and fermented products. The sodium salt of glutamate, MSG is an industrial substance and it is known as Chinese salt [212].

Mammalian taste buds respond to these various umami compounds via receptors. At last the 20 years, several receptors have been proposed to be responsible for the umami detection in taste buds. These receptors are two different modified forms of glutamate-selective G protein-coupled receptors which are mGluR1 and mGluR4 and the taste bud-expressed heterodimer T1R1+T1R3. These receptors are localized in a small number of cells in anterior and posterior taste buds. The activation of mGluRs starts with the binding of glutamate and certain analogues to the receptor. mGluRs are not sensitive to nucleotides but T1R1+T1R3 complex is sensitive almost all umami compounds such as wide range of amino acids and nucleotides [213].

2.3.5.2. Umami Taste Perception and Signal Transduction

Umami signalling pathway looks like sweet taste perception. Briefly, umami tastants bind to T1R1/T1 stores. Increased Ca^{2++} stimulates the opening of TRPM5 channel which provides Na^{+} influx. Altered ion status causes the depolarization of cell and it results with ATP secretion. On the other hand, Gα gustducin or Gα transducin triggers the activation of phosphodiesterase (PDE) to decrease the intracellular cAMP levels. And also cAMP is reduced by the activation of Gα by inhibition of adenylyl cyclase (AC) rather than by activation of PDE. Although the target of the cAMP is remaining unknown, cAMP has an

antagonized role of umami response. It is demonstrated that it can modify the sensitivity of the phospholipase C (PLC) signalling pathway [214].

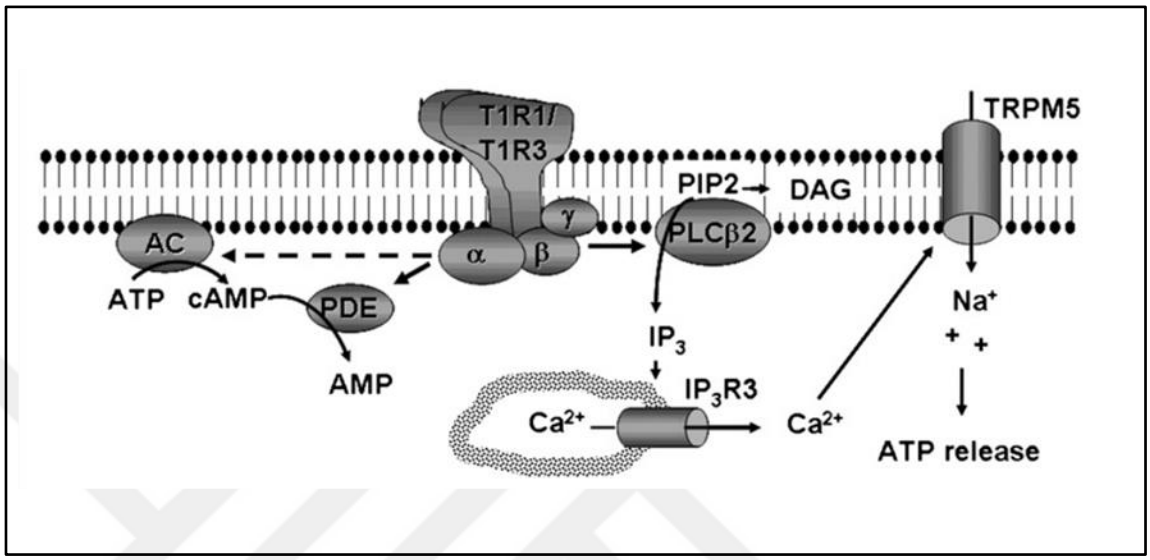


Figure 2.16. The schematic view of bitter taste signal transduction [214].

2.3.5.3. The Other Function of Umami Taste Signal Transduction on Extra-Oral Tissues

When we compared to other taste modalities in terms of expression on extra-oral tissues, there is a lim cAMP levels in spermatozoa. Because of Ca2++ and cAMP control and regulate to the fundamental processes of fertilization and so T1R1 has an essential receptor for maintaining of fertilization [215, 216].

2.4. FATTY TASTE PERCEPTION AS SIXTH TASTE MODALITY

So far the oro-gustatory taste perception of five basic taste modalities associated central pathways is explained. However, the oro-sensorial perception system not only limited to tastes of sour, sweet, salty, bitter and umami but also fat perception could be sixth taste

modality at the recent studies [217, 33]. Dietary fatty acids can be classified in terms of various criteria which including chain length, chain structure, degree of unsaturation, the position of double bonds and etc. Especially, primarily classification of fatty acids based on the absence or presence of double bond within a chain. According to the degree of saturation, fatty acids are divided into two main groups which are saturated fatty acids (SFA) and unsaturated fatty acids. SFA has not any double bonds within the chain and the samples of SFA are lauric acid, myristic acid, stearic acid and palmitic acid. On the other hand, unsaturated fatty acids are evaluated in terms of the number of double bonds as two different groups which are monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). MUFAs have only one double bond within a chain and the most known example of MUFA is oleic acid (omega-9). PUFAs have one more than double bonds within the chain and most known PUFAs are linoleic acid (omega-6) and linolenic acid (omega 3).

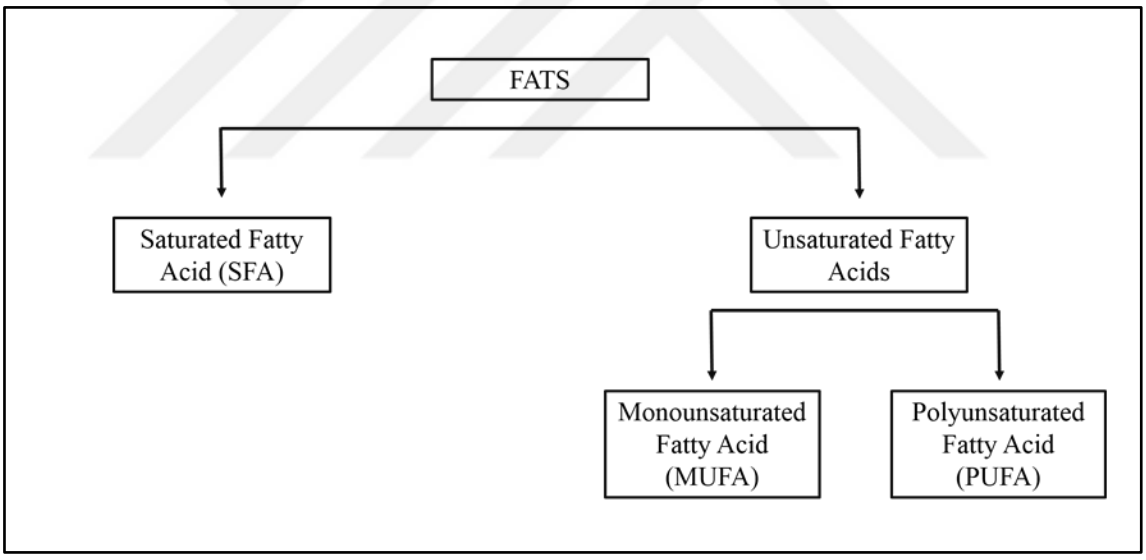


Figure 2.17. Classification of fatty acids according to the degree of saturation within the chain.

Another important classification of dietary fatty acid is based on a number of carbons. According to this system, fatty acids often categorized from short to very long. For instance, short-chain fatty acids (SCFA) have an aliphatic tail of fewer than five carbon number. Medium-chain fatty acids (MCFA) have an aliphatic tail of 6 to 12 carbons, long-

chain fatty acids (LCFA) have an aliphatic tail of 13 to 21 carbons and lastly, very-long-chain fatty acids (VLCFA) are fatty acids with aliphatic tails of 22 or more carbons [218].

On the other hand, numerous studies have suspected to excessive consumption of dietary fat associated with the development of obesity [219]. Because, dietary fat has more energy content than other macronutrients and also obese subjects prefer the fatty taste with various properties of fat such as textural, palatability, visual and olfactory cues [33, 220]. Therefore, recent studies focus on oro-gustatory fat perception and LCFA (C>12) which are released from triglycerides by the lingual lipase, are effective stimuli in order to oro-sensorial detection of lipids [221].

2.4.1. Dietary Fatty Acid Binding Receptors

In this system, various dietary fatty acids binding receptors have recognized which have an affinity to medium-chain fatty acids (MCFA) and/or LCFAs. Especially, a growing number of studies have shown that lingual CD36 and GPR120 have a critical role for fatty taste perception and spontaneous fat preferences in humans and rodents.

Delayed rectifying K^+ (DRK) Channels: DRK channels have a functional role in taste transduction and fatty acids inhibit to the DRK channels in rat TBC. Various subtypes of DRK channels such as potassium voltage-gated subfamily A (KCNA), potassium voltage-gated subfamily B (KCNB) and potassium voltage-gated subfamily C (KCNC) have recognized at TBC of rats. Especially, the KCNA family contributes to chemosensory identification of PUFAs. Interestingly, phenotypic differences appear between obesity-prone and obesity-resistant rodents in terms of fatty acid sensitivity of DRK channel [222]. However, there is no enough evidence about the relationship between spontaneous fat preferences and DRK channels.

GPR40: G-coupled protein receptor-40 (GPR40) is a member of the G-coupled protein receptor family. Mostly expression of lingual GPR40 is present on the taste pore and foliate papillae. Nevertheless, a lower expression of GPR40 was detected on the fungiform papillae. Actually, the expression of GPR40 was found in Type I TRC only [223, 224]. On the other hand, human studies have demonstrated that GPR40 expression is not shown at protein and mRNA levels in fungiform and circumvallate papillae [224, 225]. Consistently,

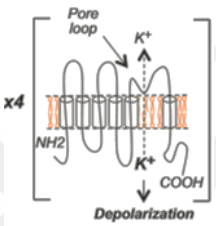
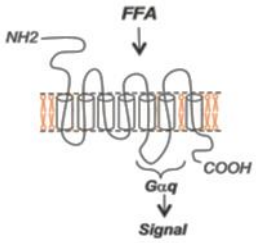
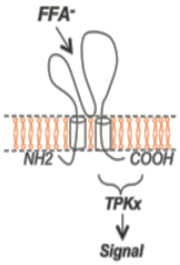
Gilbertson and Khan (2014) have been reported that not expression of GPR40 in rat gustatory papillae [224].

GPR120: G-coupled protein receptor-120 (GPR120) is belong to a family of G-coupled protein receptors. Another name of GPR120 is free fatty acid receptor-4 (FFAR4) and molecular weight of GPR120 is 42 kDa. GPR120 is found in several tissues which are intestine, macrophages, pancreatic islets, liver, bone, skeletal muscle and tongue. Lingual GPR120 is located in fungiform, foliate and circumvallate papillae and it has an affinity to higher concentration (nM) of MCFA and /or LCFA [4, 5]. GPR120 is known to affect the regulation of the expression of CD36 and to influence the preference of fatty foods [226].

CD36: Cluster of differentiation (CD36) is a member of the scavenger receptor family and it is also named as fatty acid translocase gene (FAT). It is found in various tissues including adipose tissue, cardiomyocytes, liver, intestine, colon and tongue. The role of CD36 in the tongue has an affinity to lower concentration (μM) of LCFA and it is located in the circumvallate and foliate papillae generally [226]. In the two-bottle preference test with CD36 knockout mice, the fat preferences of transgenic mice were significantly lower than the control group [227].

Table 2.3. The classification of different dietary fatty acid-binding receptors within the tongue which depend on family type, binding specificity, schematic structure and expression in TBC [33].

Classification Criteria	**DRK**	**GPR40**	**GPR120**	**CD36**

	DCNA5 (KV1.5)	(FFAR1)	(FFAR4)	
Family Type	K^+ channel	G coupled protein receptor	G coupled protein receptor	Scavenger receptor
Binding Specificity	PUFA	MCFA/LCFA	MCFA/LCFA	LCFA
Schematic Structure				
Expression in TBC	Type I, Type II, Type III Rat	Type I?	Type II Rat, Mouse, Human	Type II ?, Type III Rat, Mouse, Human
Oro-sensoral Functional Role	Fat Taste Transduction	Lipid Preferences		Dietary Lipid Perception and Preferences

2.4.2. Physiological Process of Dietary Lipid Perception

The physiological process of perception of fatty taste begins with the breakdown of dietary fats to the LCFAs by the lingual lipase in saliva [228]. Fatty acids in the oral cavity activate the intracellular signalling mechanism by CD36 and GPR120 which are fatty acid-

binding receptors located on the downstream of the taste bud cells. Activation of these receptors depends on the free fatty acids (FFAs) concentration. The calcium release from the endoplasmic reticulum (ER) is triggered and then the decreased calcium storage in the ER stimulates the activation of the Ora1 and Orai1/2 transmembrane proteins which are responsible for uptake of extracellular calcium [229]. The increased intracellular calcium gives rise to modulation of transient receptor potential type M5 (TRPM5) which is known as a Ca^{+2} activated Na^{+} permeable channel and responsible for the depolarization of the TBC [224]. Meanwhile, inhibition of DRK channels with PUFA provides an increase in depolarization level, which is a necessary grade for releasing of ATP via CALHM1. The influx of ATP to the type III receptor causes the release of neurotransmitters such as serotonin, acetylcholine, gamma-aminobutyric acid (GABA), noradrenaline, etc. from type III cells [230, 231]. Serotonin is the major neurotransmitter which is released as a result of intracellular signalling through the lingual CD36 fatty acid receptor and GLP-1 is another neuropeptide which is released into the extracellular space through lingual GPR120 fatty acid receptor signalling [232, 233]. The releasing neurotransmitters are transduced to the nucleus tractus solitarius (NTS) by the facial (VII) and the glossopharyngeal (IX) nerves, which is the termination site of the chemoreceptor afferents on the brain stem. NTS is stimulated by FFAs via the somatosensory system in the non-gustatory epithelium (Figure 2.18).

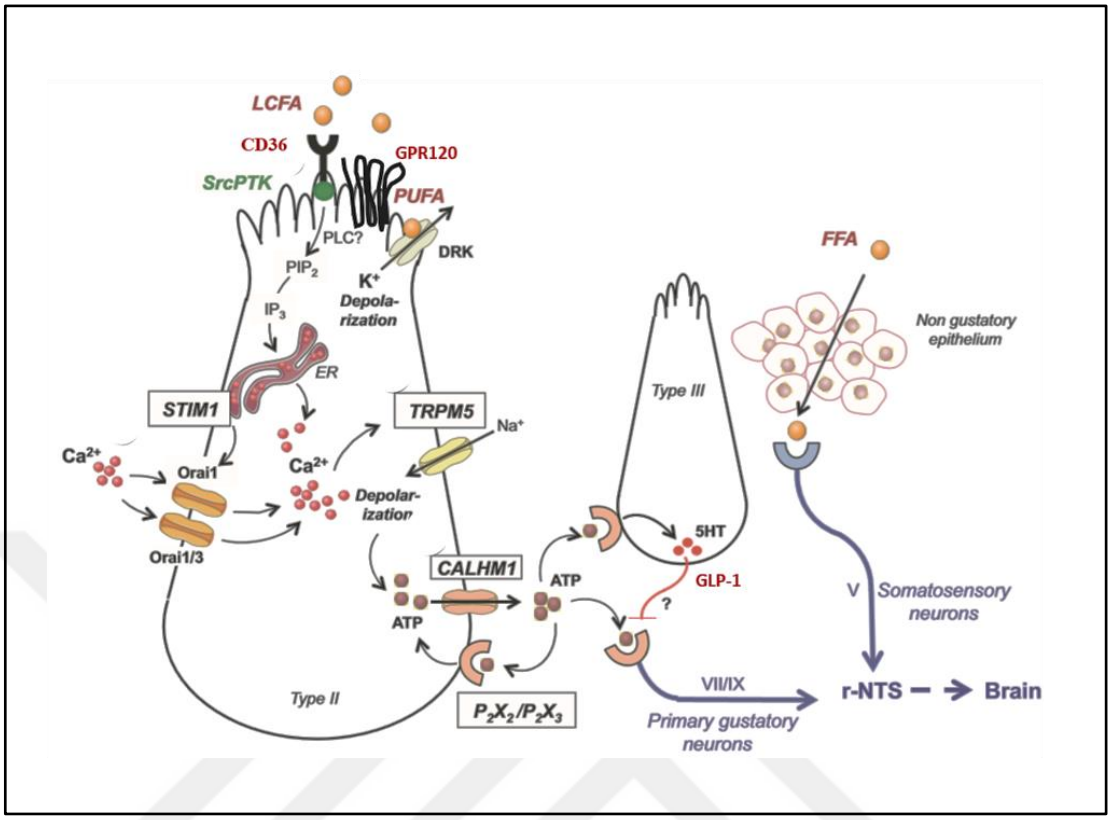


Figure 2.18. Stimulation of NTS with neurotransmitter release of CD36 and GPR120 mediated fatty acid-induced calcium signalling in taste bud cells in the formation of oral fat perception [33].

The perception of fat as a delicious taste, it is necessary to be evaluated by the brain. This process involves (1) the identification of fatty taste in the primary taste cortex of the brain which follows the NTS's neuronal projection, (2) the emotional coding of the taste by the secondary taste cortex, and finally, (3) the opioidergic and dopaminergic system activation in the emotional brain center, respectively [33]. The stimulation of the hunger-satiety center in the hypothalamus requires the digestion of the consumed fat through gastric and pancreatic lipase. At the post-prandial period, the binding of the ingested fatty acids to GPR-120 receptors in the intestinal enteroendocrine cells provides the release of the intestinal hormones such as cholecystokinin (CCK-1), GLP-1 and etc. The secretion of CCK-1 controls the appetite via stimulation of the hypothalamus and stops the food intake [234]. The interaction between the brain's emotional and metabolic compartments is also important for controlling the eating behaviour (Figure 2. 18) [33]

CD36 and GPR120 share a similar functional role and also co-expressed at the same taste receptor cell. To determine the interaction between lingual CD36 and GPR120, Ozdener et al. used the small interfering RNAs (siRNAs) transfection. The complementary mechanism between CD36 and GPR120 has been revealed through the selective downregulation of mRNAs of CD36 and GPR120 via the siRNA targeting [235]. It can be an adaptation mechanism for exposure to the different fatty taste concentrations or have a protective role against the damage for maintaining of fatty taste perception.

2.4.3. Regulatory Mechanisms of The Lingual CD36 And GPR120 Receptors

The regulatory mechanisms of the lingual CD36 and GPR120 receptors have not been elucidated, it has been determined that the expression of CD36 is effected by the diurnal rhythm. For example; CD36 has a higher expression level at hunger and beginning of food intake, then gradually decreases due to increased fatty acid concentration in the oral cavity during a meal. This dynamic regulatory mechanism of CD36 is thought to be post-translationally controlled by the "ubiquitin/proteasome pathway". It has been revealed that GPR120 has a functional role in the regulation of CD36 expression via GLP-1 secretion (Figure 2.19). The expression of GPR120 receptor which is located in the circumvallate papillae has no change between at the hunger and satiety [31].

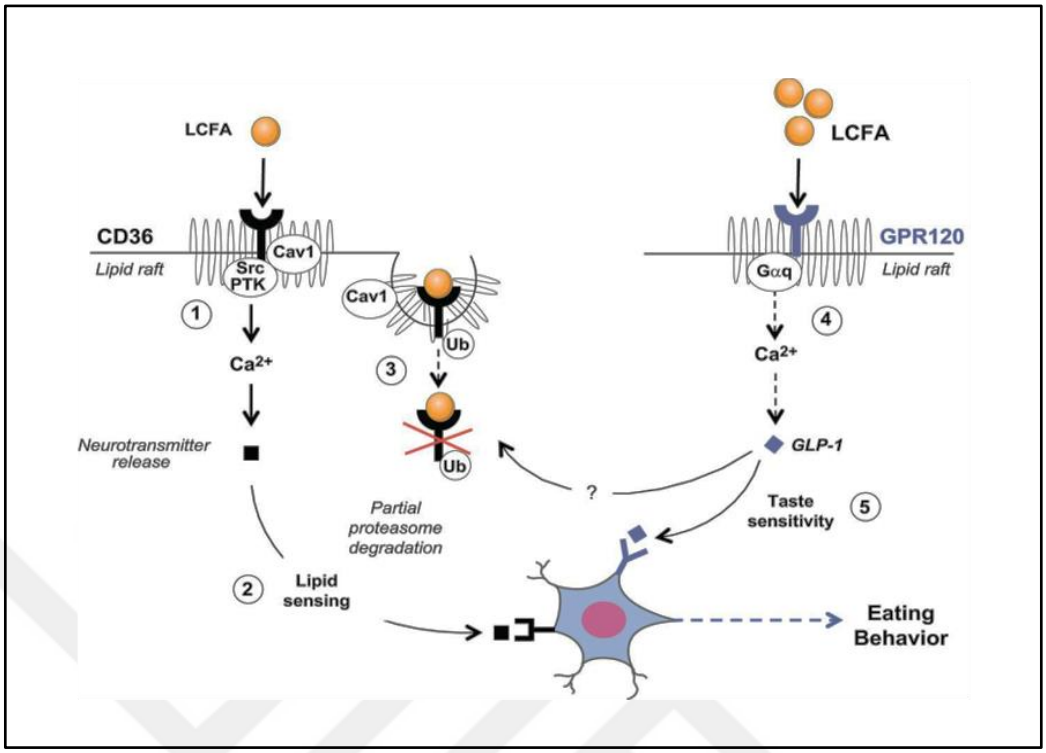


Figure 2.19. CD36 expression is controlled by the ubiquitin/proteasome pathway which is controlled with released GLP-1 from the GPR120 receptor [33].

Animal studies have shown that obesity is caused by the impaired regulation of receptor expressions and non-perceivable dietary fat intake.

The first study has been performed by Zhang et al, the researchers have observed that a decreased expression of CD36 level in circumvallate papillae of high-fat diet-induced obese rat group when compared with the control group [236]. Contrary to, Chevrot et al. (2013) have found that high-fat diet-induced obesity causes the increased expression of CD36 in CVP of the mice, the dysfunction in CD36 mediated Ca^{+2} signalling and insensitivity to dietary lipids. Moreover, they have claimed that there are no statistically significant differences in the CD36 expression level between fasting and satiety in the obese mice [237] (Figure 2.20). The differences between researches may be related to different dietary composition and inflammation status of the animals. On the other hand, it has been determined that rs1761667 polymorphism of CD36 gene leads to a decreased level of lingual CD36 expression and an increased risk of obesity as the individuals have elevated threshold values for fat perception and so increased fat intake in humans [238].

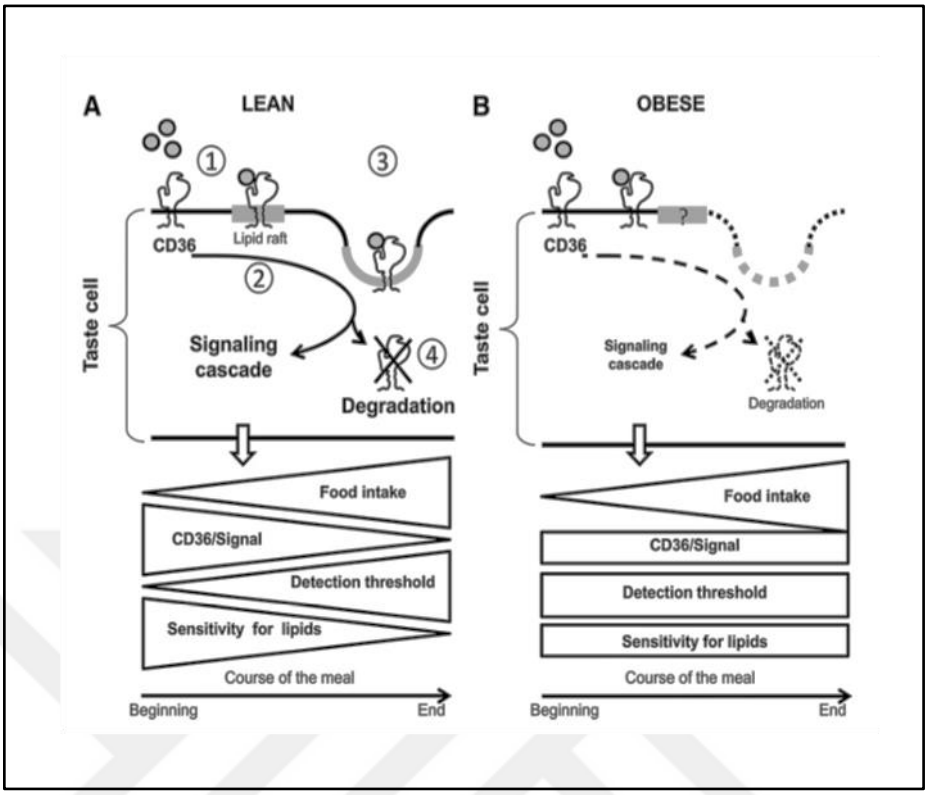


Figure 2.20. Comparison of lingual CD36-mediated fatty acid in obese and control groups during meals [237].

In another study, it was determined that the level of CD36 expression in fungiform papillae in the DIO mice decreased, while the expression and localization of GPR120 increased. To explain the mechanism, it has been claimed that the amplified GPR120 signal causes the increased release of GLP-1, the elevated GLP-1 may be responsible for the reduced CD36 expression. Therefore, it has been declared the lower level of the serotonin release after fat intake. And so that the result of fatty foods consumption cannot be evaluated properly by the emotional center of the brain [235]. This suggests that obesity may be associated with a change in fat perception mechanism due to impairment in the regulation of fatty acid receptors.

2.4.4. The Function of CD36 and GPR120 in Extra-Oral Tissues

Apart from the tongue, CD36 is expressed in various tissues which are brain, liver, stomach, small intestine, pancreas, cardiomyocytes and colon. Therefore, it associated with several functions in each one. For instance, CD36 in brain centers has related to taste perception, cephalic phase of digestion, satiety and energy regulation. On the other hand, the possible physiological effects of CD36 in the small intestine are contributing to chylomicron production and secretion of CCK, secretin, serotonin and oleoyl ethanolamide (OEA). In addition, CD36 in the liver is related to prostaglandin production and very-low-density lipoprotein (VLDL) secretion. Lastly, it was demonstrated that CD36 in the pancreas mediate the impact of LCFAs to stimulation of insulin secretion [239].

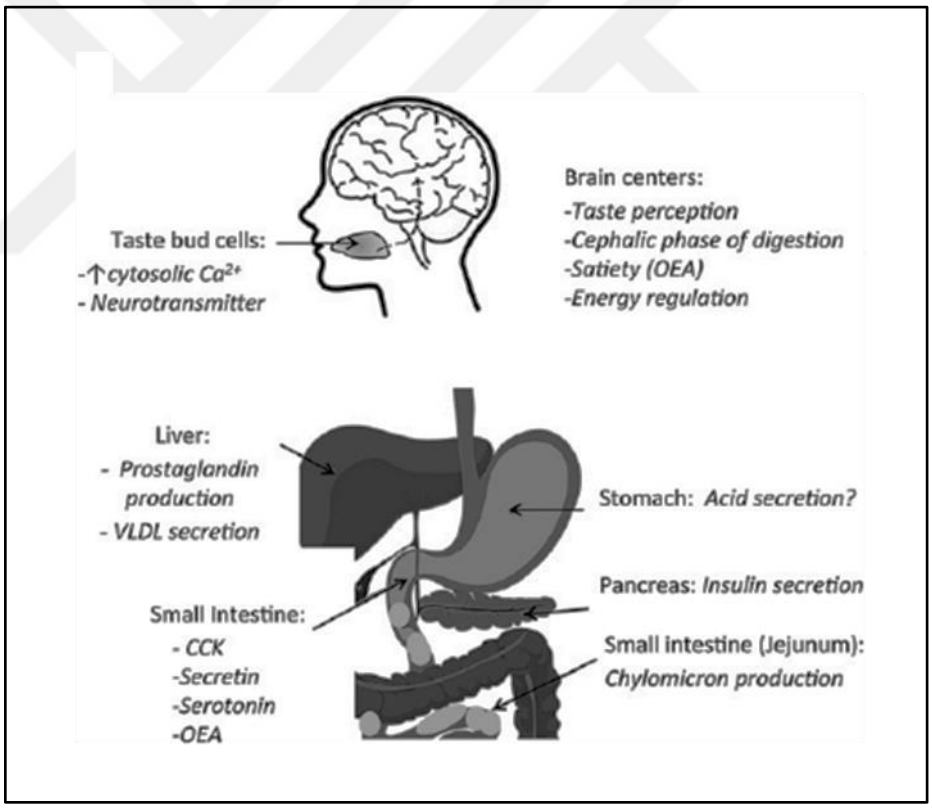


Figure 2.21. The possible function of CD36 in oral and extra-oral tissues [239].

Except for oro-gustatory system, GPR120 which is also known as FFAR4 is found in many tissues including intestine, macrophages, pancreatic islets, liver, bone, skeletal muscle, bone and adipocytes. It is responsible for various tasks in each one. For instance, the role of GPR120 in the intestine is secretion of GLP-1, inhibition of GLP-2 secretion, the

elevation of cytosolic Ca^{2++} and increase the phosphorylation of ERK1/2. In adipose tissue, it provides adipose differentiation, GLUT4 translocation, glucose uptake, vascular endothelial growth factor-alpha (VEGF-A) induced angiogenesis, insulin sensitization and anti-inflammatory effect. GPR120 in macrophages has an important anti-inflammatory role in reducing inflammasome. On the other hand, the known physiological functions of GPR120 in pancreatic islands are protection from lipotoxicity, glucagon secretion and synthesis and blocking the somatostatin secretion. In addition, the physiological roles of GPR120 in skeletal muscle are reducing the inflammation, GLUT4 translocation, improving insulin sensitivity and increasing the glucose uptake. Lastly, GPR120 functions in the liver are cell migration, reducing inflammation and macrophage polarization and also it has multiple tasks in the bone which including reducing inflammation, inhibition of osteoclast development and bone resorption [240].

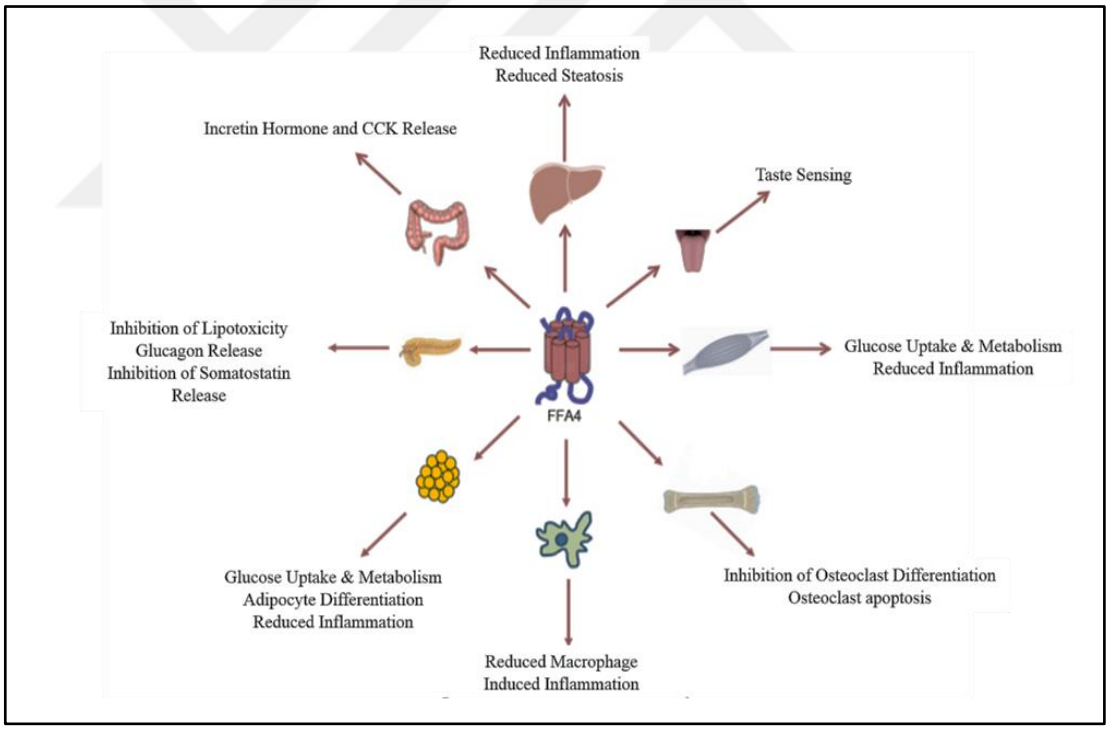


Figure 2.22. The physiological effects of GPR120/FFAR-4 agonists [240].

2.5. TASTE PERCEPTION ASSOCIATED FACTORS

2.5.1. Food Related Factors

Taste perception is a complex process and can be modulated by food-related factors. Some characterist between each other. Especially, odor takes part a significant role for modulation on taste perception such as facilitation or inhibition [241]. On the other hand, except for taste-taste interaction, many food-related factors of taste perception are mentioned in the 2.1 section.

Taste-taste interaction means that when more than one compound including different taste modalities are consumed with together, a number of interactions may occur including enhancement, suppression, both enhancement and suppression or asymmetrical intensity shifts. The perceived taste in the result of interaction depends on the type of taste modality and concentration of taste compounds [242].

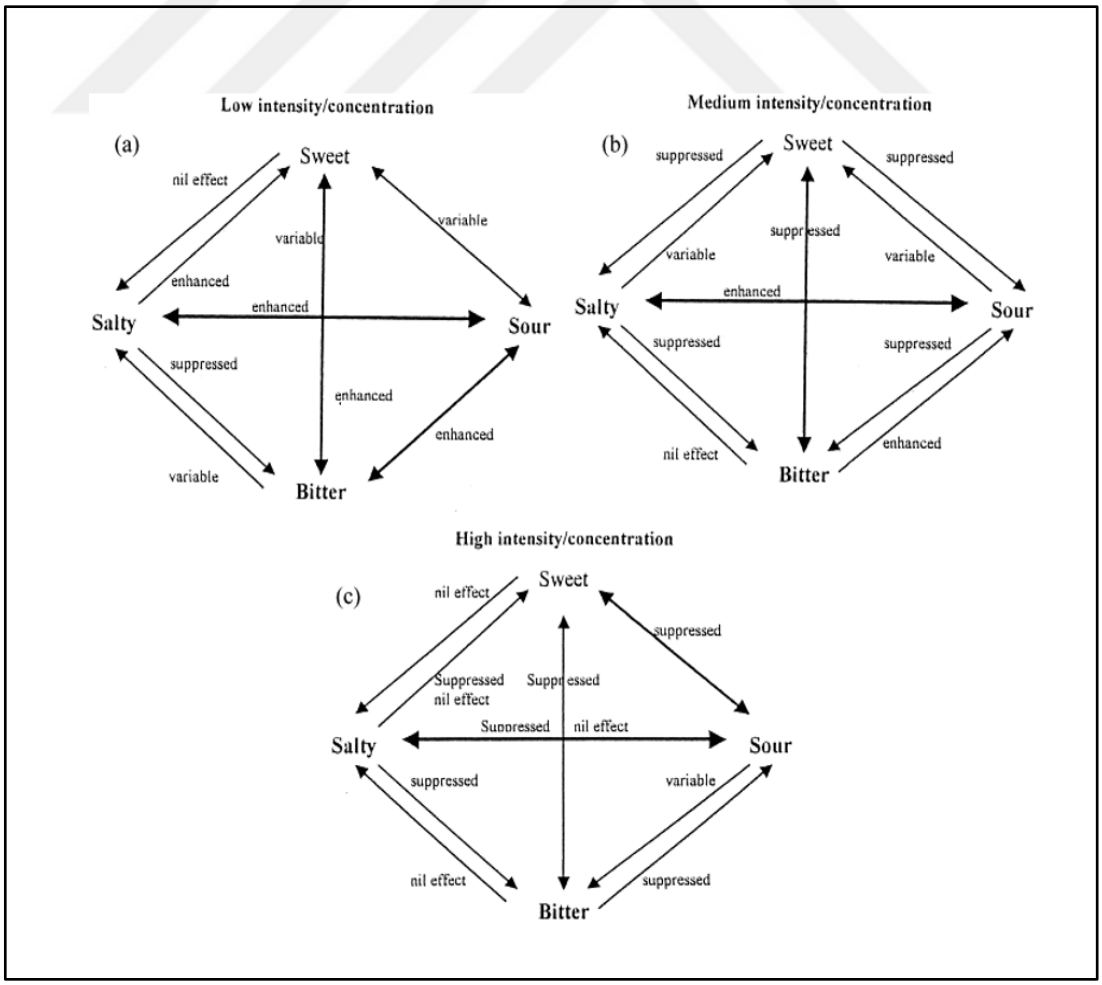


Figure 2.23. Schematic representation of taste-taste interaction [242].

2.5.2. Individual Factors

Except for food-related factors, the perceived taste of the consumed food is affected by a wide range of individual factors including age, gender, hormones, genetic factors, psychological, physiological, cultural and socioeconomic factors [243]. Except for these factors, diurnal rhythm, exercise, smoking, oral and gut microbiota composition can influence to oral taste perception [243, 244, 245, 246]. However, the results of the studies which try to display the exact effects of these factors are limited and controversial and needed to do further studies.

Taste perception related to individual factors is explained in detail below.

Age: The relationship between ageing and change of taste perception is frequently studied in the literature. It is known that in the early years of life, the number of taste buds is higher and the ageing process decreases the number of taste buds. The lost of taste buds is resulted with decreased sensitivity of tastants due to increased threshold of taste perception [247, 248, 249].

Gender: The gender is another important factor for modulation of taste perception. According to numerous physiological and psychological researches, it is known that taste perception in mammalians directly or indirectly associated with the circulating concentration of reproductive hormones. Especially, pregnancy and menstrual cycle have a modulating role in taste sensitivity owing to altered hormonal status. Apart from that, Ammann et al. have demonstrated that there is a statistically significant association between bitter taste sensitivity and gender. In this study, a between disgust sensitivity and PROP (6-n-Prophythiouracil) taster (bitterness) status in males but not in females [250]. In another study, it is found that men more sensitive to sweet, salty and sour tastants whereas women are more responsive to bitterness [251]. On the other hand, in a study with children population, it is found that girls are more sensitive to sweet, bitter and salty tastes than

boys [252]. Although the results of studies present controversial evidence and but it is considered that women are genetically more sensitive to taste compounds than men [253].

Hormones: Many hormones, including insulin, leptin, glucagon, endocannabinoids, cholecystokinin, PYY, oxytocin, galanin, GLP-1, NPY, ghrelin and etc. modulate to the taste perception as suppression or enhancement [22]. In this context, it is a disputed fact that satiety or hunger are key concepts in hormone modulated taste perception. More detailed information presents in part of 2.2.1.1 section.

Genetic Factors*:* Some people who are super-tasters have a higher number of taste buds owing to genetic traits. They can detect the taste of foods at the lower concentration and so have more sensitivity to the foods than rest of population [254]. Moreover, certain single nucleotide polymorphisms (SNP) in taste receptor genes can determine to sensitivity to the related taste modality. For instance, higher fatty food intake has been considered one of the most common reasons for obesity. Some CD36 polymorphisms (rs1761667 and rs1527483) are associated with the fatty food perception, preferences and development of obesity [255].

Actually, genes and culture may coevolve to determine the variation in dietary habits such as favism in the Mediterranean region [256]. In another example, there is a relationship between insensitivity to bitter taste and the prevalence of malaria in African populations. Because, people who are resident in these regions have an insensitivity to bitter taste and also eating bitter plants can provide some protection against malaria [257].

Health Status*:* Being or not being ill can be criteria for healthy taste perception. Patients complain of “abnormal or bad tastes” related to the illness and treatment periods. This situation results in food aversion, impaired nutritional status and decreased the quality of life. Altered taste sensitivity and taste perception abnormalities have been reported in a wide range of diseases such as obesity, Alzheimer, Parkinson, cancer, chronic renal failure [258, 259, 260]. Especially, there is a well-documented relationship between cancer and the taste response changes which results in weight loss. On the other hand, it is known that cancer patients under chemotherapy have reduced taste response and sensitivity [261].

Psychological Factors*:* Taste perception is modulated by a variety of emotional changes or acute stress. In that context, Noel and Danda used the performed a study by using

competitive sports players to evaluate the effect of day-to-day emotional variation on taste perception and hedonic evaluations. Consequently, they have revealed that positive emotions are paralleled with increased sweet and reduced sour intensities while negative emotions are correlated with heightened sour and decreased sweet tastes. Therefore, they have claimed that emotional manipulations can influence the intensity of taste perception and modulation of taste perception would play a role in emotional eating behaviour [262].

Diet*:* The studies related to how diet affects to taste perception demonstrates mixed and controversial results which are mostly study-specific and difficult to generalize. In some studies, relatively unfavourable foods have been used while others have used more favourable foods. Some studies used unfamiliar foods whereas others used familiar foods [263, 264]. On the other hand, deficiencies of certain vitamins and minerals which including Vitamin A, thiamine, B6, B12, folate, zinc, NaCI and copper can impact to taste sensation. Especially, studies have a focus on zinc deficiencies and supplements [243].

Maternal Food Preferences*:* The ability of perception to postnatal flavours initiates with *in utero* development of the gustatory and olfactory systems. At the end of the gestation, both of these systems are generally similar to the adult-like structures in terms of shape and function. Therefore, it provides an opportunity in order to guess the early sensory learning which is related to postnatal food preferences. More detailed information about the development of taste sense is available in 2.2.3 section. On the other hand, it is known that the development of the gustatory system continues during the postnatal periods. However, there are limited data about the relationship between postnatal taste experience and the food choices in further stages of the postnatal period as a lack of long term studies [14].
Gestation and lactation are known as sensitive or critical periods because they have an important role in the modulation of physiologic stability and behavioural changes in offspring. It is well documented that early taste experiences have modulatory effects on food preferences in adulthood [265, 266]. Amniotic fluid and breast milk can affect oro-gustatory and olfactory development of the newborn [267]. The taste sensation of the newborn is different from older infants and postnatal experiences contribute to the development of taste sensation over time. In this context, fetuses are exposed to some tastants through amniotic fluid. It causes to flavour learning and it also has modulating effects on food preferences in further stages of life. Responsiveness to tastants in newborns

has been obtained from facial expression studies which exhibit to liking or rejection to taste stimuli. According to facial expression studies, it has been obtained that liking for sweet and umami tastants or disliking for sour and bitter stimuli are innately organized but this behaviour can be modified by maternal experiences [268, 269, 270] (Figure 2.24). On the other hand, food experiences such as breast milk and various type of formula in lactation period can influence the taste acceptance and like/dislike status during infancy [269]. Moreover, it was determined that breastfed infants have higher acceptance of new foods and vegetables than formula-fed infants when they introduced to solid infant diets. Consistently, when mothers preferred to the healthy meals throughout the gestation and lactation periods, their infants can develop to healthy eating habits during the childhood and adulthood. This situation suggests that since early life, liking or disliking food can be a learning behaviour which based on biological and sociocultural factors [271, 272].

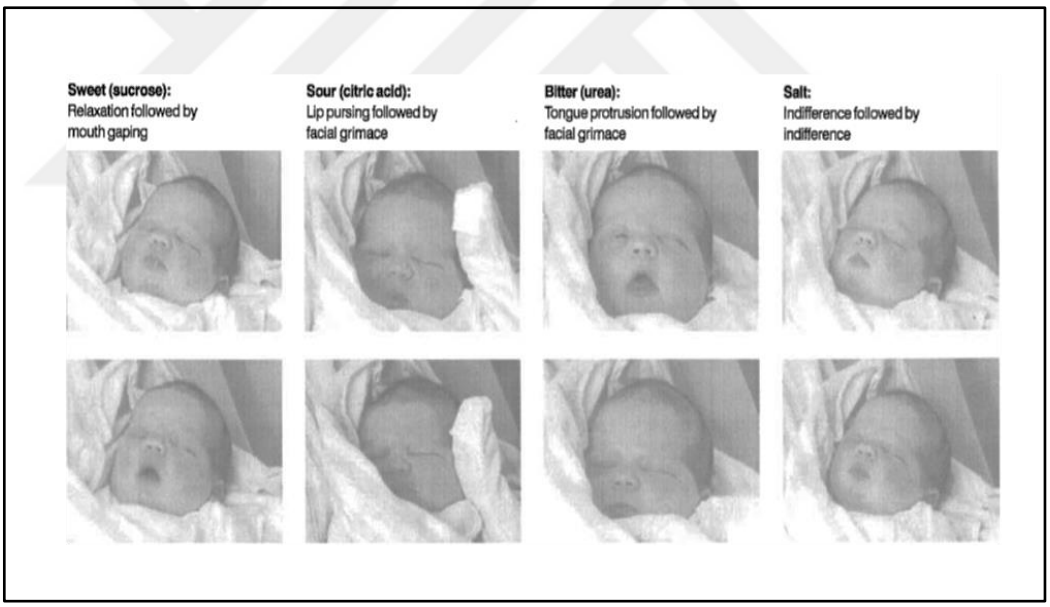


Figure 2.24. Facial expressional response to treatments of some tastants in the 3-day old infant [269].

The maternal nutrient preferences that the individual is exposed to during gestation and lactation affects the metabolic situation of offspring during adulthood [273]. The early life experiences with unhealthy foods and flavours can cause to promote the poor eating habits, which could have an important effect many uncommunicable diseases such as obesity, diabetes and cardiovascular diseases [270]. The animal studies have shown that undernutrition or high-fat diet exposed mothers during this process leads to the

development of the hyperphagia and increases the fatty foods preferences during adulthood (Table 2.4). It has been shown that there is a strong association between maternal high fat diet and increased fat mass of the infant [274, 278].

Table 2.4. Animal studies on increased fat consumption of offspring which are exposed to low or high-fat maternal diet during gestational and lactation.

References	**Developmental Periods**	**Percentage of Dietary Fat**	**Physiological Effects**
[6]	Gestation	High-Fat Chow 50 percent	Increase in fat intake and fat mass in male offspring.
[7]	Gestation and Lactation	High-Fat Chow 60 percent	Increase in fat and sucrose intake in male offspring.
[275]	Gestation and Lactation	Low-Fat Diet 10 percent	Strong preferences for high-fat diet.
[276]	Gestation and Lactation	High-Fat Chow 30 percent	Increase in fat intake and fat mass in male offspring.
[277]	Lactation	High-Fat Chow 55 percent	Increase in fat intake male offspring and increased fat mass in female offspring.

Studies which investigate the effect of a maternal high-fat diet on the food preference have focused on the changes in neuronal activity in the hypothalamus, ventral tegmental area and nucleus accumbens. Especially, the main part of these studies suspects that the decreased expression of the μ-opioid receptor in the ventral tegmental area [7, 8, 9, 10]. The gestation has a significant role in the development of lingual taste perception and food preference [279]. The source of these approaches is related to the perinatal period which involves the embryonic neurogenesis and also the brain which is highly sensitive to environmental stimuli during these periods. This makes us think the maternal diet-induced dysregulation of lingual expressions of the CD36 and GPR120 receptors may also be associated with signal transduction problems in the brain.

Moreover, Salas et al. have shown that the exposition of undernutrition during pregnancy and lactation leads to a significant decrease in the total number of observable taste buds within circumvallate papilla. In this case, the exposed maternal malnutrition leads to damage to the morphological development of the papillae such as reduction of major length, major diameter, total and upper regions in the circumvallate papillae [280].

2.6. HYPOTHESIS OF THE STUDY

Maternal nutrition status can interfere with oro-gustatory signal transduction and food preferences process [280]. On the other hand, it is known that excess fat consumption causes abnormalities in the expression of lingual CD36 and GPR120. It has resulted in taste dysfunction and obesity. However, DIO associated taste dysfunctions can occur every time in life. While no study has been published in the literature to determine the effect of maternal diet on the expression levels of lingual CD36 and GPR120 the fatty acid-binding receptors. Therefore, we hypothesized that maternal fat intake would effect to fatty taste preference through altered the level of lingual CD36 and GPR120 expressions in the circumvallate papillae. Thus, the possible change in the dietary lipid perception and preference of fat consumption in offspring can be explained in terms of the development of the oro-gustatory system.

3. MATERIALS

3.1. INSTRUMENTS

SynergyTM HT Multimod Microplate Spectrophotometer (BioTek Instruments, USA), IDEXX VetTest* Chemistry Analyzer, Floatation (water) Bath (Leica RM2245, Germany), Microtome (Leica RM2245, Germany), Tissue Following Device (Leica 1020, Germany), Parafin Embedding Device (Leica EG1160, Germany), Slide Stainer (LEICA AUTOSTAINER XL, Germany), Confocal Laser Scanning Microscope (Zeiss LSM 700, Germany), Micro-surgery light microscope (Nikon SMZ745T, Japan), PCR-Thermal Cycler (MULTIGENE OPTIMAX) (Labnet International, USA), Gel Electrophoresis System (VWR Power Sources 300, USA), SDS-Gel Electrophoresis System (Invitrogen Life Technologies # Power Ease 300, USA), Trans-Blot Turbo Transfer System (BIO-RAD, USA), Real Time System (Roche, LightCycler96, USA), CO_2 Incubator (Incucell Memmert-55, Netherlands), Centrifuge (Rotina 38R, Hettich Zentrifuges, Germany), Mini Centrifuge (Sigma, USA), Automatic pipettor (Thermo Fisher Scientific, #94410510), Ultrasonic Baths (FALC Instruments, Italy), Light Microscope (Leica CTR6000, Germany), Chemiluminescence Imaging System (BIORAD, USA), -80 °C freezer (New

Brunswick Scientific, USA), -20/ +4 °C refrigerator, Vortex (IKA minishaker, Germany), pH Meter (HANNA Instruments, Italy), Autoclave (Tuttnauer, Germany), Nano-Drop 2000 (Thermo Fisher Scientific, USA).

3.2. EQUIPMENT

VetTest tips, Blood tubes with lithium heparin, Blood tubes with EDTA, Pap-pen hydrophobic pencil (Thermo Fisher Scientific #8877, USA), Curved forceps, Camel haired brush, Scalpel, Slide racks, Clean p*oly-L-lysine* slides (Thermo Fisher Scientific #, USA), Micropipettes 0.2-2 µL, 2-20 µL, 20-200 µL, 100-1000 µL (Thermo Fisher Scientific #FINNIPIPETTE-F2, USA), Serological pipettes 1, 2, 5, 10, 25 mL (Greiner-Bio, USA), Polypropylene centrifuge tubes 0.5, 1, 1.5, 2, 5, 15, 50 mL (Isolab and Axygen, USA), Western membrane (Thermo Fisher Scientific #88518, USA), Bolt 4-12 percent Bis-Tris Plus Gel (Thermo Fisher Scientific #NW0412230X, USA), Filter Paper (Thermo Fisher Scientific #88600, USA), Microtome Blades PATHOCUTTER (Erma Inc, Japan).

3.3. CHEMICALS

Triton-X (Sigma Aldrich #T8787, USA), Tween-20 (Sigma Aldrich #P1379, USA), Tris base (Sigma Aldrich # T1503, USA), Phosphate buffer saline tablets (Thermo Fisher Scientific # 3002, USA), NaCI (Merck #1064041000, USA), Xylene (Sigma Aldrich #16446, USA), Ethanol (Merck Millipore #100983, USA) , percent10 Neutral Formaline (Sigma Aldrich #HT501128, USA), Xanthan gum (Sigma-Aldrich # G1253, USA), Rapeseed oil (Sigma-Aldrich # 83450, USA), Hematoxylin (Merck # HX68297074, USA), Eosin Y Alcoholic Solution (Bio Optica # 05-10003/L, Italy), Donkey serum (Sigma Aldrich #D9663, USA), Glycine (Sigma Aldrich #33226, USA), Methanol (J.T.Baker #8405, England), Skim Milk Powder (Sigma Aldrich #70166, USA), Ethylenediamintetraacetic acid (Sigma Aldrich #03620, USA), Sodium Chloride (ISOLAB #9.690.36.1.000, USA), Tris HCI (Sigma Aldrich #10812846001, USA), Sodium citrate tribasic dihydrate (Sigma Aldrich #71405, USA).

3.4. KITS AND SOLUTIONS

VetTest glucose measurement slide (GLU25, MVM), VetTest triglyceride measurement slide (TRIG12, MVM), Rat Leptin ELISA Kit (Millipore, EZRMI-83K), Rat Insulin ELISA Kit (Millipore, #EZRMI-13K), Presto^{TM} DNA/RNA/Protein Isolation Kit (Geneaid #DRP050, USA), High-capacity cDNA Reverse Transcription Kit (Applied Biosystems # 4368814, USA), QuantiTect SYBR Green PCR Kit (QIAGEN, #204141), Restore Plus Western Blot Stripping Buffer (Thermo Fisher Scientific #46430, USA), PageRuler Prestained Protein Ladder (Thermo Fisher Scientific #26616, USA), NuPAGE MES SDS Running Buffer (Invitrogen #NP0002-02, USA), Pierce Coomassie Plus Assay Kit (Thermo Fisher Scientific, #23236), ECL Chemiluminescence (Thermo Fisher Scientific #34095, USA), Flourished with DAPI (Sigma Aldrich #F6057, USA), Bio Mount HM Mounting Medium (Bio Optica #05-BMHM500, Germany).

3.5. ANTIBODIES

Rabbit polyclonal CD36 antibody (Novus # NB400-144, USA), Rabbit polyclonal GPR120 antibody (Novus # NBP1-00858, USA), Goat Alpha-gustducin (GNAT3) Antibody (Sigma Aldrich #SAB2501807, USA), Dnk pAb to Rb IgG (Alexa Flour 488) (Abcam #ab150073, United Kingdom), Dnk pAb to Goat IgG (Cy3.5) (Abcam #ab6950, United Kingdom), Anti-Rabbit IgG, HRP-linked Antibody (Cell Signaling Technology #7074S, USA).

3.6. PRIMERS

Primer sequences of CD36, GPR120, alpha gustducin and beta-actin are in Table 3.1 and beta-actin was used as a reference.

Table 3.1. Forward and reverse primers of CD36, GPR120, alpha gustducin and beta-actin genes.

Gene	**Product Length**	**Sequence**

CD36	214 bp	F_primer, 5′ GGTGTGCTCAACAGCCTTATC 3′ R_primer, 5′TTATGGCAACCTTGCTTATG 3′
GPR120	191 bp	F_primer, 5′AAGTCAATCGCACCCACTTC 3′ R_primer, 5′GAAGAGGTTGAGCACCAAGC 3′
Alpha Gustducin	253 bp	F_primer, 5′ ATGGAGGGTAGAGTTGCTGAT 3′ R_primer, 5′ CCTAATTGAAAGGCTGATGAC 3′
Beta Actin	124 bp	F_primer, 5′GAGCCACCAATCCACACAGA 3′ R_primer, 5′ CCACCATGTACCCAGGCATT 3′

3.7. ANIMALS

Male and female Sprague Dawley rats were obtained and used from Yeditepe University Experimental Animal Research Center.

3.8. DIETS

Three different fat-containing diets were purchased from Ssniff company (Germany). The ingredients of the experimental diets are available in Table 3.2.

Table 3.2. The ingredients of the standard fat diet (SFD), high-fat diet (HFD) and low-fat diet (LFD).

Ingredients	**Low Fat Diet (LFD) (Ssniff)**	**Standard Fat Diet (SFD) (VRF1-Ssniff)**	**High Fat Diet (HFD) (DIO percent-60 kJ-**

			Ssniff)
Total Calorie (kcal/gr)	3,409	3,345	5,15
Fat (percent kJ)	3	13	60
Protein (percent kJ)	23	23	20
Carbohydrat (percent kJ)	74	64	20
Crude Fat (percent)	1.1	5.0	34.6
Crude Protein (percent) (N x 6.25)	19.0	19.0	24.4
Crude Fiber (percent)	5.5	4.5	6.0
Ash (percent)	6.3	6.5	5.3
Starch	40.3	34.8	0.1
Sucrose	5.6	5.6	9.4
Vitamin A	15,000 IU	25,000 IU	15,000 IU
Vitamin D3	1,500 IU	1,500 IU	1,500 IU
Vitamin E	150 mg	135 mg	135 mg
Vitamin K	20 mg	20 mg	20 mg

Vitamin C	30 mg	87 mg	87 mg
Copper	14 mg	5 mg	15 mg

4. METHODS

This study was performed in Yeditepe University Faculty of Medicine, laboratories at the Department of Physiology, Histology and Embryology and Yeditepe University Experimental Animal Research Center.

4.1. EXPERIMENTAL PROTOCOL

Turkish guidelines for the use and care of laboratory animals were followed and the experimental protocol was applied to the animal research studies in accordance with the Animal Research Ethics Committee of Yeditepe University (Decision No: 575). Animal experiments and euthanasia of Sprague Dawley rats were performed in the Yeditepe University Faculty of Medicine Experimental Research Center. Eighty-four male Sprague

Dawley rats were allowed to access chow *ad libitum* and tap water and accommodated at 22^{o}C temperature and 12 h light: 12 h dark cycle.

The experimental groups were created according to exposure of different amount of fat-containing diets into rats during gestation-lactation and maturation periods. 3 main experimental groups were created and each main group was divided into 3 subgroups. HFD has 60 percent of the total energy from the fat, SFD means to 13 percent of the total energy from the fat and LFD has 3 percent of the total energy from the fat. The experimental design is available in Figure 4.1.

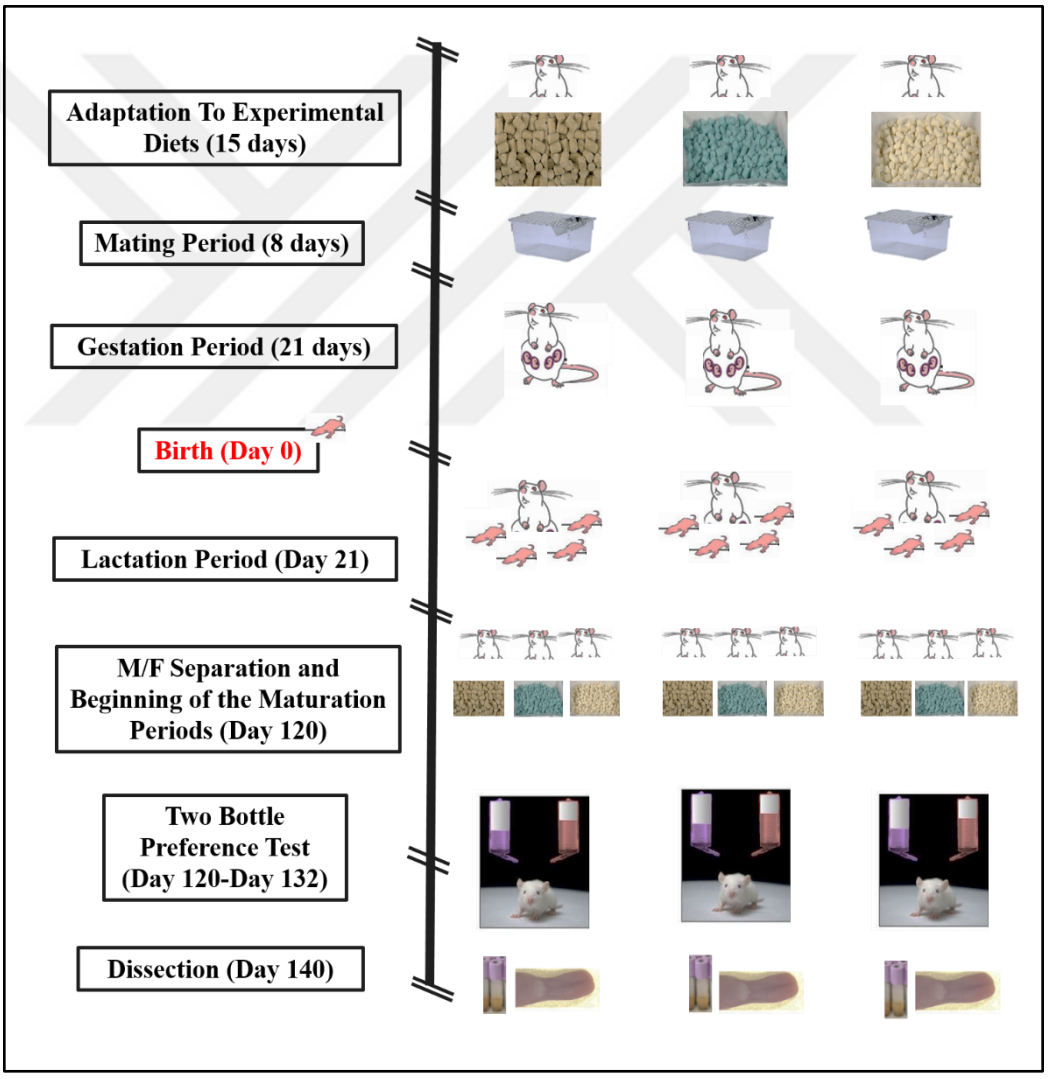


Figure 4.1. Experimental design of the study.

Initially, 250-300 gr female rats were allowed to access the standard fat diet (SFD), high-fat diet (HFD) and low-fat diet (LFD) during two weeks as an adaptation period for the

diets. After food adaptation period one male rat and two female rats were placed together in a cage for the mating period. This period was approximately for 8 days which means two oestrous cycles and at the end of the process, male rats were removed from the cage. During of mating period, vaginal smears were checked to determine the mating day and smear-positive animals were assumed as the first day of gestation. Approximately the 21st day of gestation the offsprings were born and animals continued feeding with same experimental diet in groups during the lactation period. After the lactation period (21st day), the mothers and female pups were taken from cages and remaining animals of each main group separated into three different groups which were feeding with SFD, HFD and LFD for maturation period. At the 120th day, the two-bottle preference test protocol was applied to the male offspring rats. During the experiment, each group was fed with their own diets. In addition, after the two-bottle preference test, rats continued to eat to their own diets in the recovery period which takes one week. At the 140th day, animals anaesthetized with Xylazine (10 mg/kg)-Ketamine (90 mg/kg) mixture intraperitoneally (i.p.). The blood was taken from the abdominal aorta and the experiment was terminated. Tongue tissue was taken and stored at -80 ^{o}C until the analysis.

4.1.1. Experimental Groups

Group 1 (a, b, c): During the gestation and lactation (G-L) periods, mother rats were fed with SFD and then offspring male rats were fed with different concentrations of fat-containing diets (a.SFD, b.HFD, c.LFD) in the maturation period, respectively. These groups are organized to determine the effect of nutrition in the maturation period.

Group 2 (a, b, c): During the G-L periods, mother rats were fed with HFD and then offspring male rats were fed with different concentrations of fat-containing diets (a.SFD, b.HFD, c.LFD) in the maturation period, respectively. These groups are organized to evaluate the effect of a maternal high-fat diet on fatty taste perception and spontaneous fat preference of the offspring.

Grup 3 (a, b, c): During the G-L periods, mother rats were fed with LFD and then offspring male rats were fed with different concentrations of fat-containing diets (a.SFD, b.HFD, c.LFD) in the maturation period, respectively. These groups are organized to

determine the oro-sensorial mechanism of hyperphagia and fatty food consumption preference which is related to maternal fat malnutrition.

Table 4.1. The classification of experimental groups.

Experimental Groups	**G-L PERIODS [21 Days-21 Days]**	**MATURATION PERIOD [100 Days]**
Group 1 (n=6)	**SFD**	sacrified
Group 1a (n=6)	SFD	SFD
Group 1b (n=8)	SFD	HFD
Group 1c (n=8)	SFD	LFD
Group 2 (n=6)	**HFD**	sacrified
Group 2a (n=6)	HFD	SFD
Group 2b (n=8)	HFD	HFD
Group 2c (n=8)	HFD	LFD
Group 3 (n=6)	**LFD**	sacrified
Group 3a (n=6)	LFD	SFD
Group 3b (n=8)	LFD	HFD
Group 3c (n=8)	LFD	LFD

4.2. PARAMETERS

4.2.1. Measurements of Animal Weight, Food and Calorie Intake

All rats were allowed to access the pellets *ad libitum*. The compositions and energy contents of the three diets are available in Table 3.2. Consumption of experimental diets in cages was calculated as the kcal and gr per rat on certain days during G-L periods for the female rats. Before and after two or three days from birth, body weight and food intake were not measured. The amount of fat exposed by mothers during G-L periods was calculated by using the diet contents.

The daily calorie intake of the offspring of male rats during the maturation period was calculated using Table 3.2. In addition, animal weights and food consumption were measured in every two days.

4.2.2. Two Bottle Preferences Test

The two-bottle preference test (TBPT) is a very commonly used test to determine feeding behaviour in animal experiments [12]. In this test, control and test solutions are given to animals at the same time. Before and after the procedure, control and test solutions are weighed and consumption of solutions is calculated thus, the preferred solution can be determined Test solutions can be sweet, umami, bitter, salty or fatty tastants which depend

on the aim of the study. In this study, we used rapeseed oil to determine the fat preference of offsprings.

At the beginning of the test, the animals in each cage were adapted for two bottles by placing two 250 mL of water bottles for 48 hours. The water bottles were replaced each other after 24 hours in order to avoid the side preference. After 48 hours, the freshly prepared weighed and labelled control and test solutions were positioned in the cage for 48 hours. Control solution contains the 0.3 percent xanthan gum in the tap water to mimic the fat texture and emulsify the fat.

The test solutions consisted increasing concentrations of rapeseed oil (0.02 percent, 0.2 percent, 2 percent) containing 0.3 percent xanthine gum in water. Control solution and increasing concentration of rapeseed oil solutions were given into the rats. Each set experiment was separated from each other with one day resting period. In this process, the location of the bottles was determined as randomly. Control and test solutions were replaced every 24 hours in order to avoid side preferences. Percentage of the fat preference for both 24 hours and 48 hours were calculated with the ratio of the fatty liquid consumption to the total liquid consumption [12, 281].

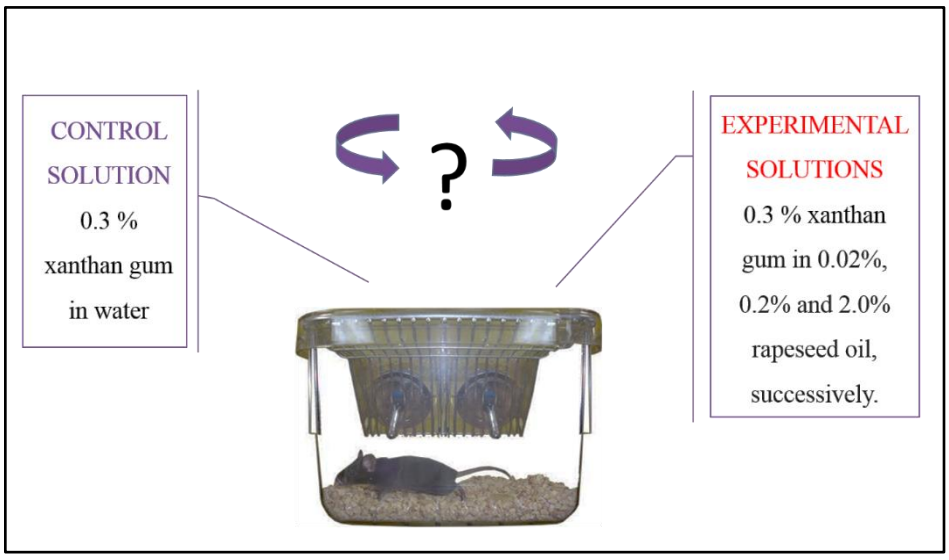


Figure 4.2. Two bottle preference test diagram

4.2.3. Determination of Plasma Insulin and Leptin Levels with ELISA

Blood was taken from Abdominal Aorta to the blood tubes with EDTA. Collected blood was centrifuged at 4500 rpm for 15 minutes at 4°C, plasma was separated then stored at -80°C until assay day. The insulin and leptin levels were determined by using commercially available ELISA kits according to the manufacturer's protocol.

ELISA is based on the detection of the amount of insulin and leptin in the sample by specific antibodies. The plate presented with the commercial kit has been coated with rat-specific polyclonal insulin and leptin antibodies. When the sample is applied to the wells, the insulin or leptin antigens in the sample bind to specific polyclonal antibodies in the wells. When the hormone-specific polyclonal detection antibody is added to the plate, the insulin or leptin in the sample are bound to the antibodies. At the end of the incubation period, the unbound content is removed by washing. When the enzyme solution is added to the plate, it binds to the detection antibody and finally, the specific substrate is added. When the substrate reacts with the enzyme, a coloured compound is formed. Stop solution is added to stop the reaction and absorbance of the coloured compound measured as indicative of the level of insulin and leptin in the sample. The colour intensity is related to the concentration of insulin or leptin in the sample.

According to the guidelines of Elisa kits manufacturer, initially 10X wash solution was diluted with 10 times deionized water and the wells in the plate to were washed 3 times with 300 µL diluted washing solution with the automatic pipettor. 10 µL of Assay Buffer was added to each of the blank and sample wells. 10 µL of Matrix Solution was added to Blank, control and standard wells. 10 µL Rat Insulin or Leptin Standards were put into appropriate wells. In these kits, there were two insulin or leptin Standart to confirm the efficacy and accuracy of the kit. Therefore, 10 µL of QC1 and QC2 were added to the appropriate wells. 10 µL serum or plasma samples were added to the remaining wells which depend on the planned frame. Then 80 µL Detection Antibody was administrated to all wells. In order to get the best results, all additions were completed within 1 hour and the plate was covered with the sealer. To provide the homogenous reaction for all wells, the plate was put into the orbital microtiter plate shaker at 400-500 rpm for 2 hours. At the end of this duration, the sealer was removed from the plate and all solution was poured out of the plate. For washing, 300 µL of diluted washing solution was added to each well. The washing solution was poured out of the plate. The remaining solution was eliminated with the filter paper. 100 µL of enzyme solution was added to each well. The plate was covered

with a sealer and located on medium microtiter plate shakers for 30 minutes at room temperature. The sealer was removed carefully and the solutions were poured from the plate completely. The plate was washed with 300 µL washing solution 6 times per well. 100 µL of substrate solution into each well was added and the plate was covered with the sealer again. The plate was shaken for 5-20 minutes. The blue colour was formed in wells which depends on the increasing concentrations of insulin or leptin. (Figure 4.3).

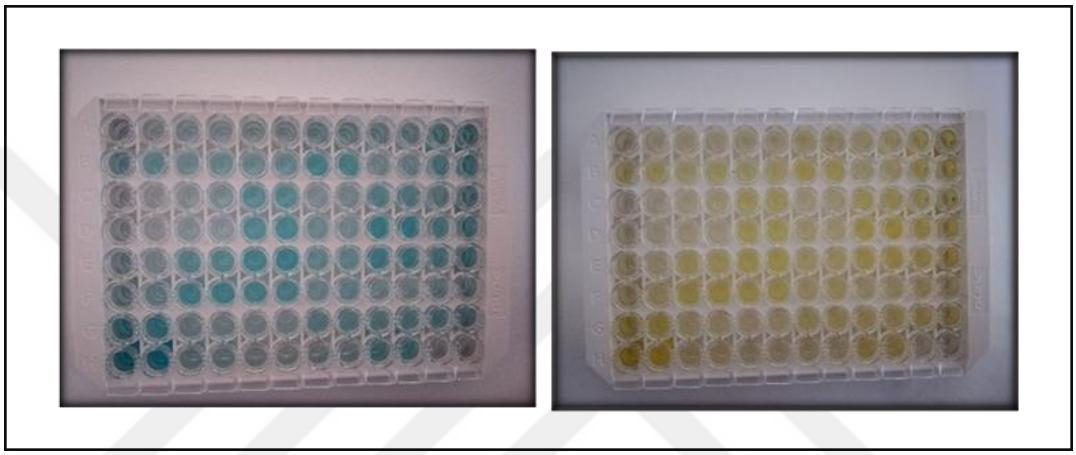


Figure 4.3. After the addition of the substrate solution, the blue colour formation in the plate and then the addition of the stop solution, the blue colour convert to yellow colour.

Then, 100 µL of stop solution was added to all wells and shook gently. Depending on the acidification, the blue colour was turned to the yellow colour (Figure 4.3). After being sure that there are no air bubbles in any well, of the plate was measured with using of the SynergyTM HT Multimode Microplate Spectrophotometer within 5 minutes at 450 nm and 590 nm wavelength. The difference between 450 nm and 590 nm absorbances were saved. The insulin and leptin concentrations of samples were calculated with a standard curve equation. The results are expressed as ng/mL.

4.2.4. Determination of Serum Glucose and Triglyceride Levels in Experimental Groups with IDEXX-VetTest Systems

Blood was taken to the blood tubes containing Lithium Heparin and was centrifuged at 4500 rpm for 15 minutes at 4°C. Immediately, the levels of glucose and triglyceride were determined from each serum sample according to the guidelines of VetTEST IDEXX

System. The VetTest* Chemistry Analyzer can determine 12 different biochemical parameters such as glucose, triglycerides, and etc. from serum, plasma, or urine, approximately six minutes. Through a series of short “beeps” the pipettor is prepared, enough total sample is drawn and then the analysis is started. After taken the sample, the pipettor distributes the sample onto the slide, the sample spread the surface of the slide and the sample is absorbed from the slide. As the sample filters through the layers, colour changes emerged via the occurrence of biochemical reactions. The VetTest analyzer’s optical system determines the intensity of the colours in the surface of the slide and converts into values of the parameter. At the end of all procedure, glucose and triglyceride values of the unknown sample were determined. The results are expressed as mmol/L.

4.2.5. Microtomy of Paraffin-Embedded Blocks

Microtomy inferred that tissue can be sectioned and attached to a surface for further histological examinations. Biological samples often need to be solidified to allow fine sectioning. Paraffin wax embedded blocks of tissues support tissue integrity and provide to be cut very thin sections. Since paraffin is immiscible with water which is the main constituent of tissue, samples need to be dehydrated by progressively more concentrated ethanol baths. This is followed by a clearing agent, usually xylene, to remove the ethanol. Finally, molten paraffin wax infiltrates the sample and replaces with the xylene.

To perform paraffin section microtomy, the tongue was excised and applied tissue following procedure which is given in Table 4.2. This process was performed in tissue following machine except for the fixation. The tongue tissues were embedded into the paraffin and paraffin blocks were waited in room temperature until cutting procedure.

Table 4.2. Tissue following the procedure of rat tongue.

Reagents	**Steps**	**Number of Changes**	**Duration**
Tap Water	Hydration	3 station	3x10 minutes
70 percent Alcohol	Dehydration	1 station	3 hours
80 percent Alcohol	Clearing	1 station	3 hours
90 percent Alcohol	Impregnation	1 station	3 hours
100 percent Alcohol		2 station	2+3 hours
Xylene		2 station	1 +2 hours
Paraffin	Paraffinization	2 station	More than 10 h

Paraffin blocks were diminished as size with a scalpel. To take a serial section, the paraffin-embedded block was placed into related location and serial sections (5 µm) were cut from the tissues with a microtome (Leica RM2245, Germany). Then, sections were put in floatation bath (Leica RM2245, Germany) at 37ºC with using forceps and brush in order to avoid from folding of tissue. After 1-2 minutes, tissues were taken from the water to Poly-L-lysine slides. Slides were dried on the filter paper and then ordered in slide box at room temperature for storage.

4.2.6. Hematoxylin-Eosin (H&E) Staining

The hematoxylin stains the cell nuclei as blue-black, while the eosin stains the cell cytoplasm and connective tissue fibres with intensities of pink and orange through various deep. H&E staining provides the morphological assessments of a huge number of various tissue structures. In this method, initially slides put into 60ºC oven for 15 minutes in order to deparaffinization the cross-section with increased temperature. After deparaffinization, the sections were placed into staining machine (Leica Autostainer XL, Germany). H&E staining was carried out according to the protocol. Slice was covered with mounting medium (Bio Mount, Bio Optica, #05-BMHM500) and then observed under the light microscope (Leica CTR6000, Germany).

Table. 4.3. H&E staining protocol.

Station No	Station	Duration
1	Oven	3 min
2	Xylene	3 min
3	Xylene	3 min
4	Xylene	5 min
5	100 % Alcohol	2 min
6	96 % Alcohol	2 min
7	96 % Alcohol	2 min
8	Tap Water	2 min
9	dH_2O	1 min
10	Hematoxylin	2,1 min
11	Tap Water	1 min
12	Tap Water	1 min
13	Acid Alcohol	1 min

14	Tap Water	1 min
15	Ammoniac	0,1 sec
16	Tap Water	1 min
17	96 % Alcohol	30 sec
18	Eosin Y	4,1 min
19	96 % Alcohol	30 sec
20	96 % Alcohol	30 sec
21	96 % Alcohol	1 min
22	100 % Alcohol	1 min
23	Xylene	1 min
24	Xylene	1 min
25	Xylene	Exit

4.2.7. Immunofluorescent (IFC) Staining

Immunostaining is classically defined as a procedure to detect certain antigens in cellular contexts using specific antibodies. The biological samples can be comprised of tissue and/or cells. Immunofluorescence allows researchers to evaluate whether or not cells in a particular sample express the antigen in question.

In this procedure, slides were reviewed under the microscope to decide which slides will be applied into IFC procedure. The chosen slides were put into an incubator at 37°C overnight. On the next day, the slides were incubated at 60°C for 1 hour for deparaffinization And then the slides were placed in the xylene for 20 minutes, two times. Then, slides were passed through the decreasing concentrations of ethanol baths (100 percent, 90 percent, 80 percent, 70 percent, successively) for dehydration of the tissue sections. Each bath takes 10 minutes. The sections were bathed with distilled H_2O (dH_2O) for 5 minutes, two times and then slides were placed in PBS (phosphate buffer saline) again 5 minutes, two times. And then slides boiled in EDTA (ethylene di-amine-tetraacetic acid) buffer by using a microwave (600 W) for antigen retrieval. This process was started at low watts (300 W) until boiling then, the power was increased about 600 watts for 5 minutes. In order to cool down, slides were placed at room temperature for 20 minutes and then washed with PBS for 5 minutes, three times. Slides were transferred into tris buffered saline with tween 20 (TBS-T) for 5 minutes, three times and then boundaries of sections determined by the hydrophobic pap pen. Sections blocked with 5 percent donkey serum prepared in PBS-T (0.1 percent Triton-X in PBS) at room temperature for 60 minutes on the wet chamber. Then the slides were shaken to remove the blocking solution.

Subsequently, the slides were incubated with anti-rabbit CD36 primary antibody (1:800 diluted) and alpha-gustducin primary antibody (GNAT3), the specific marker for the taste bud cells, (3:1000 diluted) in blocking solution at $+4^0$C overnight on the wet chamber. In this step, each section was incubated with 40 µL primary antibody. Control sections were incubated with PBS. On the next morning, the slides washed with TBS-T for 10 minutes, 3 times. Then incubated with secondary antibody (1: 2000 diluted) for 90 minutes. Each section was incubated with 40 µL secondary antibody in 5 percent donkey serum prepared in PBS-T (0.1 percent Triton-X in PBS). Again, slides were washed with TBS-T for 5 minutes, three times. Finally, the sections covered by mounting medium containing 4', 6-diamidino-2-phenylindole (DAPI). Immunostained sections were observed under the confocal laser scanning microscopy.

Staining specificity was assessed by incubating the slides in the absence of primary antibodies. This procedure was repeated for GPR120 and GNAT3.

4.2.8. Papillae Isolation

For the isolation of the taste papillae on the tongue surface, the epithelium surrounding CVP was peeled with microsurgical scissors and scalpel. In this process, the tongue was pinned on a Sylgard petri dish [282, 283]. Samples were frozen in liquid nitrogen and subsequently stored at -80 ^{0}C for isolation of RNA and protein.

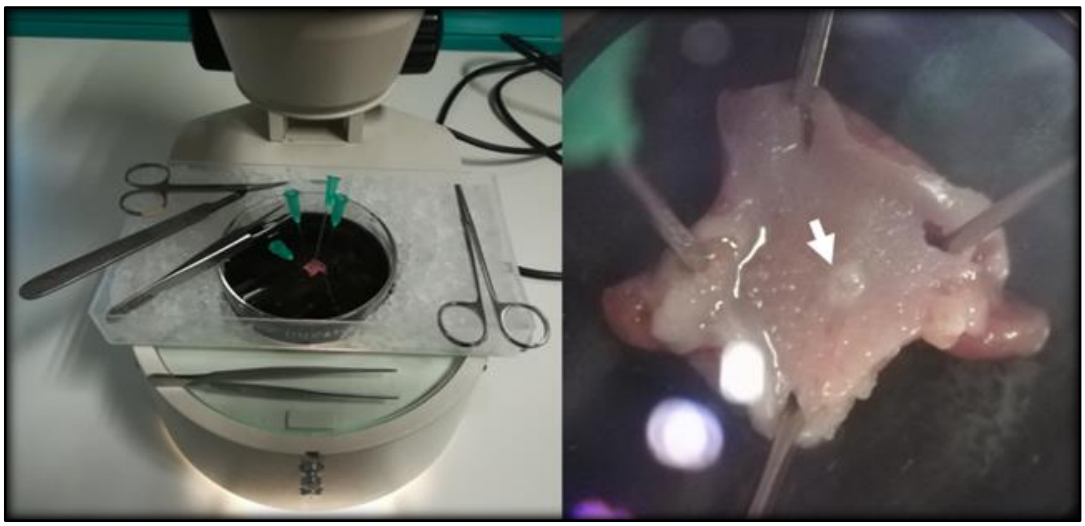


Figure 4.4. CVP isolation. The arrow shows the circumvallate papillae.

4.2.9. Protein and RNA Isolation with Presto™ DNA/RNA/Protein Kit

The instruction manual of Presto™ DNA/RNA/Protein Kit was performed (Figure 4.14). According to the manufacturer's instructions, initially, CVP tissues were transferred to 1.5 mL centrifuge tube which contains 400 µL DR buffer and 4 µL β-mercaptoethanol. Stainless steel beads were added into the tubes and the samples were homogenized with TissueLyser for 20 minutes. The sample lysates were incubated at the room temperature for 5 minutes and then centrifuged at 16,000 x g for 4 minutes. The supernatant was transferred to the GD Column. GD Columns were centrifuged at 16,000 x g for 1 minute. The acquired liquid used for protein and RNA isolation. For DNA isolation the GD column was used. 0.8 mL volume absolute ethanol of flow-through was added into the tube. The acquired liquid was transferred into the RB columns and centrifuged at 16,000 x g for 1 minute. After the centrifugation, RNA binds to RB column and the content of RB column was used for RNA purification while the acquired liquid was used for protein purification. Downstream processing of RNA and protein isolation is present at the below.

- RNA Purification: RB column was placed in a 2 ml collection tube and 400 µL RW1 buffer was added into the RB column. Columns were centrifuged at 16,000 x g for 1 minute and the acquired liquid was collected out of the collection tube. 600 µL RPE buffer was added into the RB columns and centrifuged at 16,000 x g for 1 minute. This step was repeated and columns were centrifuged at 16,000 x g for 4 minutes to dry the column matrix. Finally, clean RNAase free 1.5 mL centrifuge tubes were placed into the RB column and 50 µL RNAase free water was added into the center of the RB column. After the absorption of water, the RB columns were centrifuged at 16,000 x g for 1 minute to obtain the purified RNA. RNA samples were stored at -80 C freezer until the RT-PCR experiments.

- Protein Purification: Four volumes ice-cold acetone and one volume of the acquired liquid were added into the 5 mL centrifuge tubes and incubated on ice for 30 minutes. After the incubation period, tubes were centrifuged at 16,000 x g for 10 minutes and supernatants were discarded from the tubes. Remaining protein pellets were washed with 100 µL of ice-cold 70 percent ethanol. Supernatants were eliminated via the air-dry and 100 µL DV Buffer was added into the tubes for dissolving off the protein pellets. Proteins were stored at -80 C until the determination of protein expressions by western blotting.

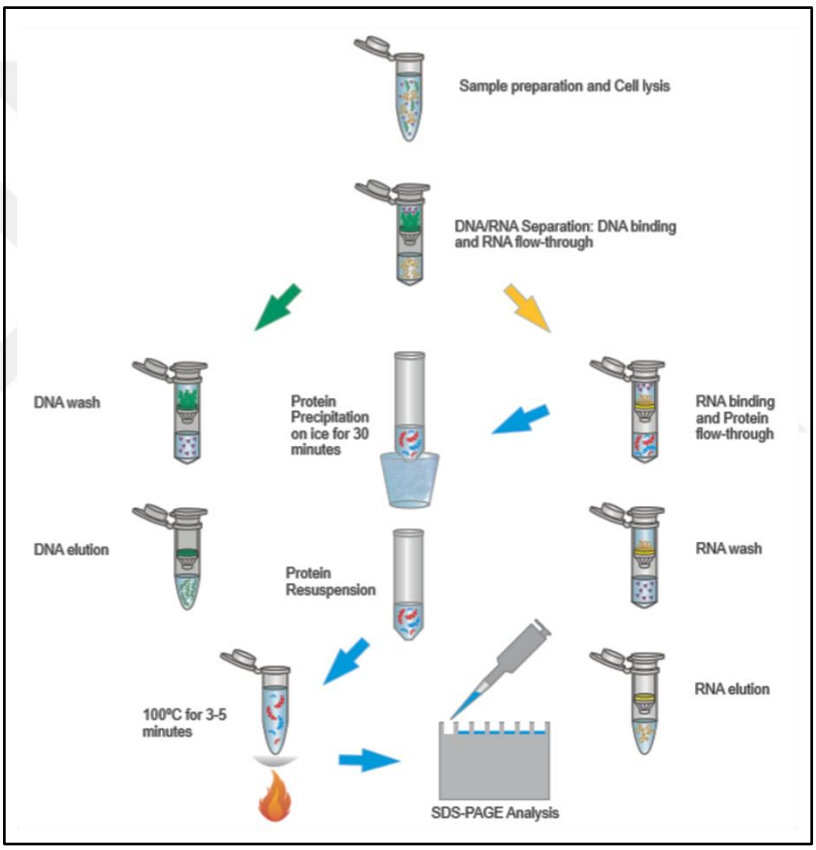


Figure 4.5. Presto DNA/RNA/Protein isolation kit quick protocol diagram [284].

4.2.10. Western Blotting

4.2.10.1. Protein Quantification Assay

The protein content in the homogenized samples was determined using the Pierce Coomassie Plus Assay Kit - Microplate Protocol at the working range of 20-2000 µg/mL.

According to kit procedure, 2 mg/mL Bovine Serum Albumin (BSA) solution is used as the standard and serial dilutions of 2 mg/mL BSA were prepared with dH_2O. 5 µL of each standard and unknown sample were added into certain wells of the plate. Subsequently, 300 µl of Bradford solution was added into each well, mixed on plate shaker for 30 seconds and incubated at room temperature for 10 minutes. The absorbance of microplate was measured at 595 nm with using SynergyTM HT Multimode Microplate Spectrophotometer (BioTek Instruments, USA). The graph of the standard curve was formed through BSA standard concentrations versus their absorbance values. Through the equation, the protein content of samples was calculated.

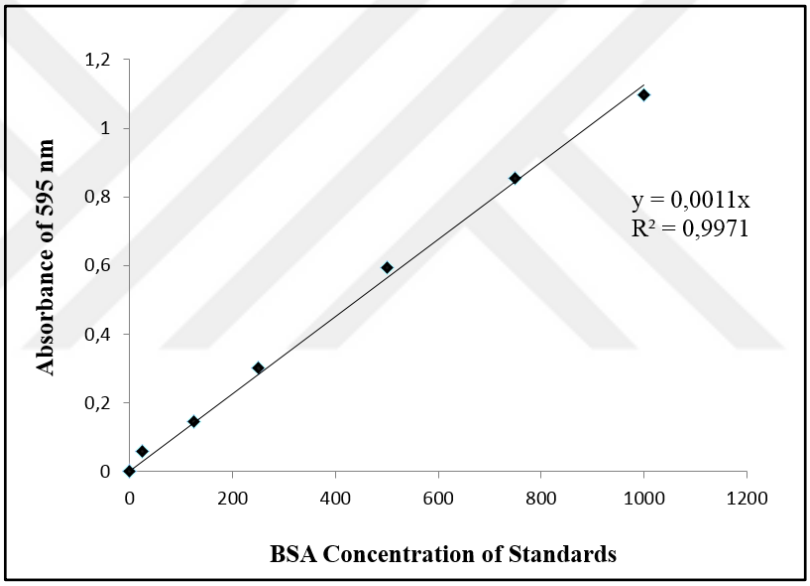


Figure 4.6. A sample from serial dilutions graph of BSA at working range of 0-1 mg/ml BSA.

4.2.10.2. SDS-PAGE Electrophoresis

The loading solution was prepared which consist of 5X loading dye, the samples containing 40 µg total protein and dH_2O in the calculated quantities. For the denaturation of the proteins, samples were mixed with the loading solutions and boiled for 10 minutes at 70°C and spun down. Running buffer (20X) was diluted with dH_2O and transferred to the electrophoresis system. Afterwards, the loading control and the samples were loaded into the 4-12 percent Bis-Tris Plus gel. The loaded samples in the electrophoresis system were

run first at 80V-30mA for 15 minutes and then the process was continued at 120V- 30mA until the samples reach the end of the gel.

4.2.10.3. Immunoblotting

Transfer buffer was prepared with using 3.03 gr Tris Base and 14.4 gr glycine in 200 mL methanol and 800 mL dH_2O. The appropriate size of the membrane was cut and put into methanol for activation of the membrane. The acivated membrane, the filter paper, and the gel were incubated in transfer buffer. Proteins on the gel were transferred to the PVDF membrane. Blotting was performed as semi-dry technique at 22 V for 45 min. After the blotting process, the membrane was incubated with 5 percent skim milk powder prepared in 0.1 percent Tween-20 added Tris Buffer Solution (TBS-T) for with 1 hour. After blocking, the membrane was washed with TBS-T and incubated with the diluted CD36 primary antibody (1:1000, prepared in 5 percent skim milk powder included TBS-T) at +4°C overnight. After the incubation, the membrane washed with TBS-T for 10 minutes, six times and incubated with the peroxidase-conjugated goat anti-rabbit secondary antibody (1:2000) for 2 hours at room temperature. After the secondary antibody incubation, the membrane was washed with TBS-T for 10 minutes, six times and the signal detected by chemiluminescence. The membrane was visualized by BioRad software. Afterwards, strip buffer was prepared and then GPR120 was diluted as 1:1000 in percent5 skim milk powder included TBS-T. The membrane was incubated with the GPR120 at +4°C overnight and after the incubation, the membrane washed with TBS-T for 10 minutes, six times. The membrane was incubated with secondary antibody (1:2000) for 2 hours at room temperature and the membrane was washed with TBS-T for 15 minutes, 3 times. The signal detected by chemiluminescence and membrane was visualized with BioRad software. Stripping process was repeated for Beta-Actin (1:2000) antibody, as an internal reference. The intensity of the CD36, GPR120 and beta-actin proteins bands on the western membrane were determined with usage by Imagelab program. Then, the ratio of CD36 and GPR120 values to beta-actin value were calculated in order to determine expression levels of CD36 and GPR120 proteins.

4.2.11. Real-Time Polymerase Chain Reaction (RT-PCR)

4.2.11.1. Isolated Amount of RNA Measurements

Isolated RNA amount of samples were measured by Thermo Scientific NanoDrop Spectrophotometer. The absorbance at 260 nm and 280 nm was detected and the ratio of these absorbances was used for the evaluation of purity of RNA. If the ratio is approximately 2.0, it is accepted as "pure" RNA.

4.2.11.2. cDNA (Complementary DNA) Synthesize

The components of cDNA kit thawed on ice and the mixture were prepared as 20 μL of each tube for one reaction (Table 4.4). The required volume of components was calculated which depends on the needed number of reactions. Each tube mixed gently. Finally, PCR tubes were placed into the thermal cycling machine. The program of thermal cycling condition is presented below (Table 4.5).

Table 4.4. cDNA (Complementary DNA) synthesize reaction.

Components	Amount/Reaction	Final Concentration
10X Buffer RT	2.0 μL	1X
25X dNTP mix (100 mM)	0.8 μL	1X
10X RT Random primers	2.0 μL	1X

MultiScribe™ Reverse Transcriptase	1.0 µL	
RNase-free water	4.2 µL	
Template RNA	10 µL	
TOTAL VOLUME	20.0 µL	

Table 4.5. Program of the cDNA thermal cycling condition.

Settings	**Step 1**	**Step 2**	**Step 3**	**Step 4**
TEMPERATURE	25°C	37°C	85°C	4°C
TIME	10 min	120 min	5 min	∞

4.2.11.3. Real-Time PCR

The PCR reaction was performed with a reaction mixture containing 10 µL (2x QuantiTect SYBR Green PCR, QIAGEN), 0.6 µL from each primer (0,3 µM final concentration) and 3,8 µL RNase-free water in RT-PCR (Roche, LightCycler96) system. The steps of the PCR programs and the number of repetitions in each step are given below. RT-PCR results were evaluated with $2^{-\Delta Ct}$ method.

Cycling conditions for RT-PCR:

1. step (1 repeat) 94 ^{o}C, 5 minutes
2. step (55 repeat) 95 ^{o}C, 20 seconds
   55 ^{o}C, 45 seconds
   72 ^{o}C, 45 seconds

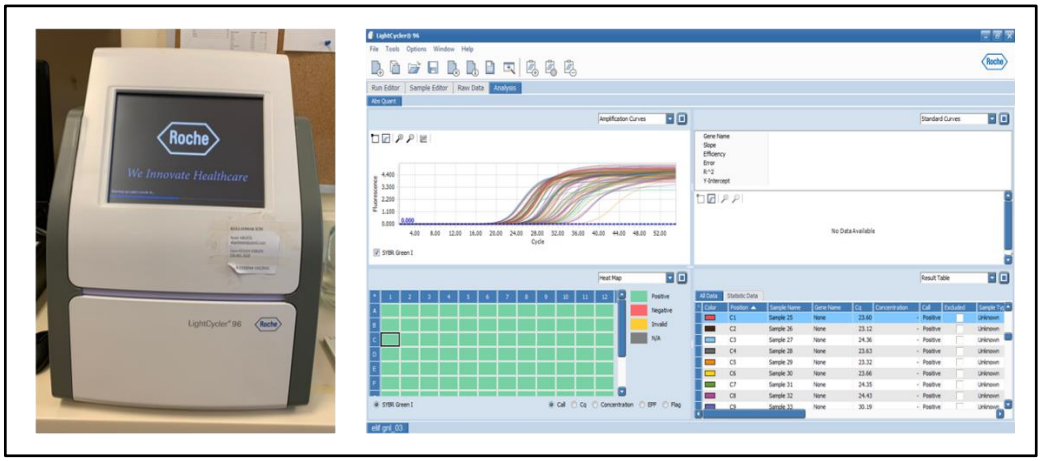


Figure 4.7. RT-PCR (Roche, LightCycler96) system and a sample of RT-PCR result.

4.3. STATISTICAL ANALYSIS

Statistical analyses were performed by the SPSS-21 software program. Kruskal-Wallis Analysis of variance was used to determine the differences between the groups and Mann-Whitney U test was used for the binary comparison of the groups All experimental data were expressed as the mean ± standard deviation and $p<0.05$ was considered as statistically significant.

5. RESULTS

5.1. MEASUREMENTS OF FOOD INTAKE AND ANIMAL WEIGHTS DURING G-L PERIODS

5.1.1. The Food Consumption in Mother Rats During G-L Periods

To evaluate the maternal food consumption, the daily food consumption (gr) of mother rats were followed during gestation and lactation (G-L) periods as mentioned in Section 4.2.1. Cumulative food intake is shown in Figure 5.1. According to cumulative food intake graph, the highest amount of daily food intake was observed in SFD-fed mother rats while the lowest amount of daily food was determined in HFD-fed mother rats.

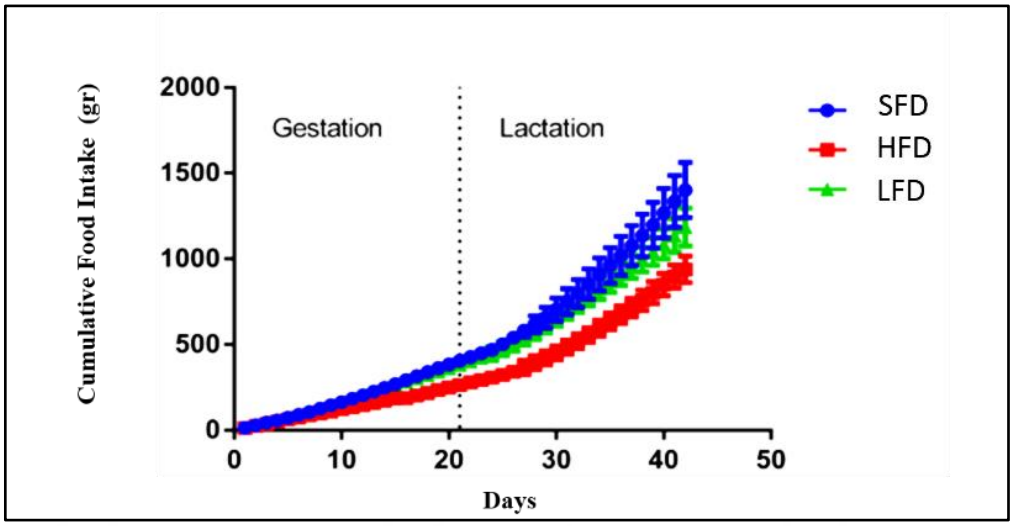


Figure. 5.1. Cumulative food intake (gr) of mother rats during G-L periods. Values are given as the mean of (n=8-10) ± SD.

During the G-L periods, the total pellet consumptions of mother rats were calculated and shown in Table 5.1. It has been observed that the total food intake of mother rats compatible with cumulative food intake graph.

Table 5.1. Total food consumption (gr) of mother rats during G-L periods. Data are given as the mean of (n=8-10) ± standard deviation (SD).

GROUPS	Gestation (gr of pellet)	Lactation (gr of pellet)	G + L (gr of pellet)
Group 1 (SFD)	402,5 ± 27,07	1040,3 ± 157,4	1442,7 ± 163,0
Group 2 (HFD)	280,7 ± 36,6	693,7 ± 65	974,4 ± 88,8
Group 3 (LFD)	392,9 ± 50,3	885,0 ± 144,8	1277,9 ± 179,6

In addition, the statistically significant difference between the groups in terms of total food intake during G-L periods are shown in Figure 5.2. During both gestation and lactation periods, HFD-fed mother rats have significantly diminished food intake when compared to other groups ($p<0.001$). On the other hand, there were no significant differences in food intake between SFD and LFD fed mother rats.

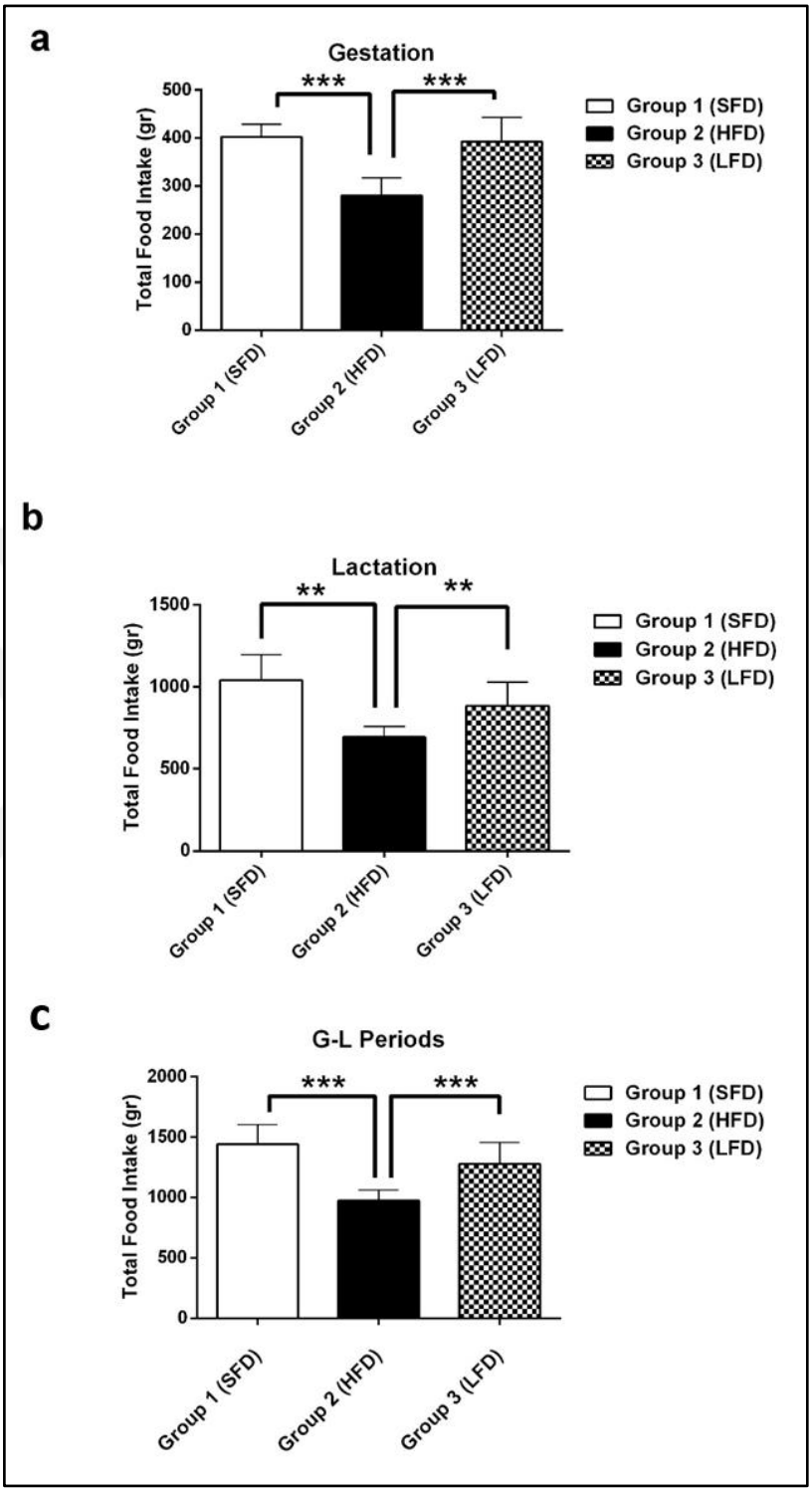


Figure 5.2. Total food intake (gr) of mother rats in (a) gestation, (b) lactation and (c) G-L periods. Data are given as the mean of (n=8-10) ± SD (** $p<0.01$, *** $p<0.001$).

5.1.2. The Calorie Intake in Mother Rats During G-L Periods

In order evaluate the energy intake as calorie (kcal) in G-L periods, the daily average calorie intake of mother rats were calculated by daily food intake and the table of ingredients of experimental diets which is mentioned in Section 4.2.1. Graph of cumulative calorie intake in gestation and lactation periods is shown in Figure 5.3.

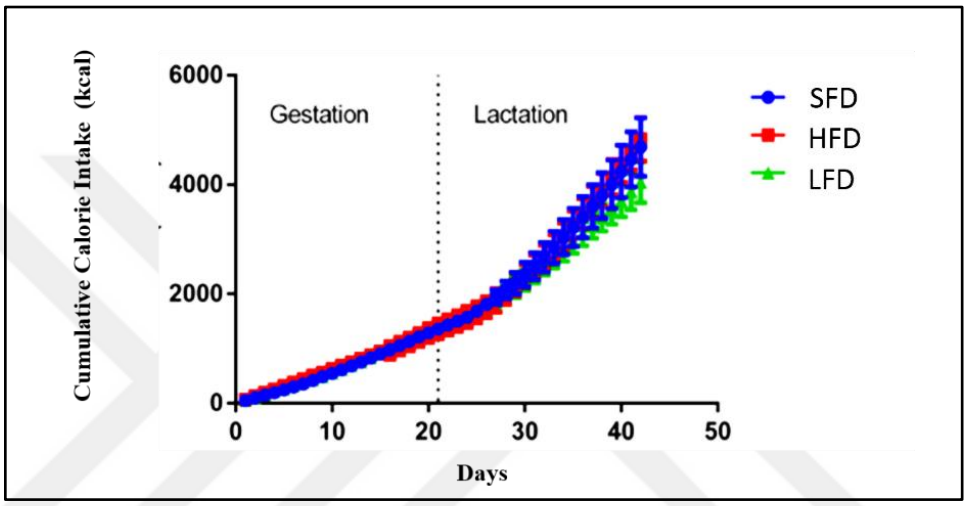


Figure 5.3. Cumulative calorie intake (kcal) of mother rats during G-L periods. Values are present as the mean of (n=8-10) ± SD.

During gestation and lactation, the total energy intake of mother rats was calculated and demonstrated in Table 5.2. It was determined that the energy intake of the mother rats in all groups was very close to each other. Therefore, there were no significant differences in energy intake between the groups as shown in Figure 5.4.

Table 5.2. Total calorie intake (kcal) of mother rats during G-L periods. Data are given as the mean of (n=8-10) ± SD.

GROUPS	Gestation (kcal)	Lactation (kcal)	G + L (kcal)
Group 1 (SFD)	1364,7 ± 137,3	3491,0 ± 567,3	4855,7 ± 606,8
Group 2 (HFD)	1445,4 ± 188,2	3572,5 ± 334,7	5017,9 ± 457,1
Group 3 (LFD)	1339,3 ± 171,5	3016,9 ± 493,6	4356,2 ± 612,2

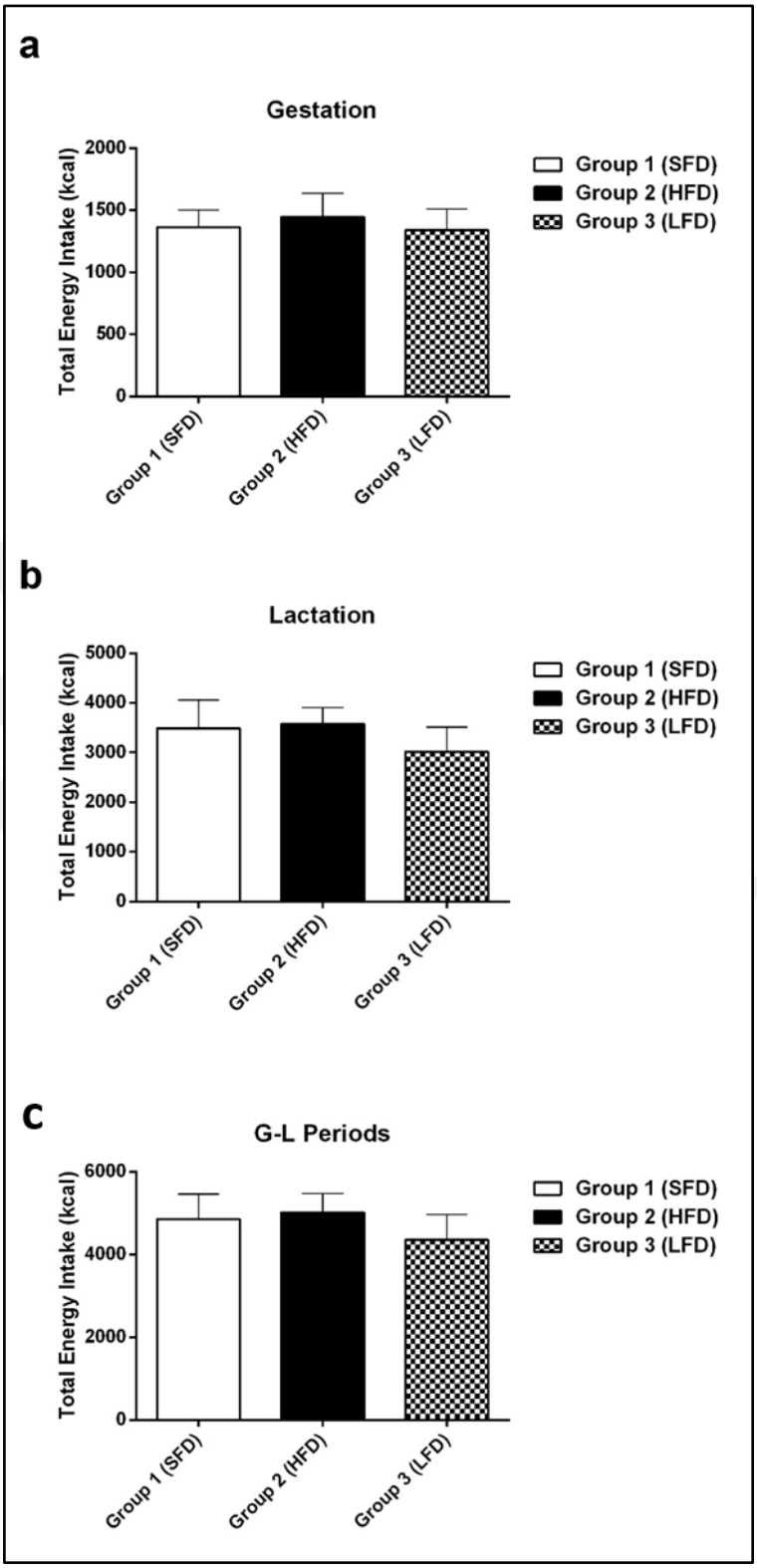


Figure 5.4. Total calorie intake (kcal) of mother rats in (a) gestation, (b) lactation and (c) G-L periods. Data are given as the mean of (n=8-10) ± SD (** $p<0.01$, *** $p<0.001$).

5.1.3. Determination of Macronutrients Intake in Mother Rats During G-L Periods

In order to evaluate the maternal nutrition status, carbohydrate, protein and fat intake of mother rats were determined during G-L periods. In this context, kcal distribution of each consumed macronutrient was calculated through the table of the ingredients of the low fat, standard and high-fat diets which is Table 3.2. The obtained data are presented in Table 5.3. In addition, as the hypothesis of the study covers fat exposure to offsprings in the maternal period, the average of consumed fat by mother rats was calculated for each group. According to the results, the protein intake of all groups was very close to each other. Carbohydrate intake of SFD and LFD-fed mother rats are very close to each other and higher than HFD-fed mother rats due to ingredients of experimental diets. Lastly, it was determined that HFD-fed mother rats were exposed to higher fat content as while LFD-fed mother rats were exposed to a lower amount of fat.

Table 5.3. Energy distribution from each consumed macronutrient in diet and fat intake as gr by mother rats during G-L periods. Data are present as the mean of (n=8-10) ± SD.

Macronutrients	**GROUP 1 (SFD)**	**GROUP 2 (HFD)**	**GROUP 3 (LFD)**
Fat (kcal)	631,2 ± 78,9	3010,7 ± 274,2	130,7 ± 18,4
Carbohydrate (kcal)	3107,7 ± 388,4	1003,6 ± 91,4	3223,6 ± 453,0
Protein (kcal)	1116,8 ± 139,6	1003,6 ± 91,4	1001,9 ± 140,8
Fat (gr)	72,1 ± 8,7	337,1 ± 28,7	14 ± 2

Statistical differences between the groups in terms of fat intake in maternal diet is shown in Figure 5.5. As expected, HFD-fed mother rats have significantly higher fat intake than other groups while LFD-fed mother rats have significantly lower fat intake than other groups ($p<0.001$).

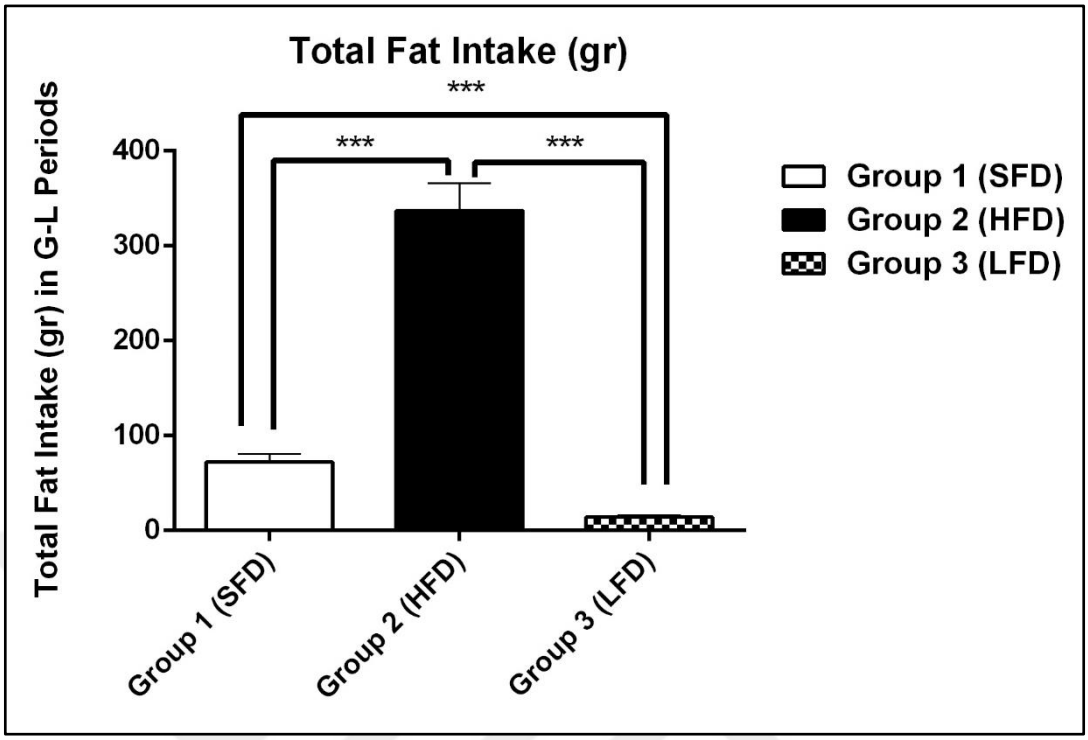


Figure 5.5. Total fat consumption as gr by mother rats during G-L periods. Data are given as the mean of (n=8-10) ±SD (*** $p<0.001$).

5.1.4. The Animal Weights During G-L Periods

As a result of given diets to the mother rats, weight gain of mother rats was evaluated with following the bodyweight during G-L periods. Body weights of mother rats are shown in Figure 5.6. There were no statistically significant differences in body weight between the groups. The highest animal weight for gestation period has been observed in HFD fed mother rats while for lactation period it was observed in SFD fed mother rats. On the other hand, it is determined that LFD-fed mother rats had the lowest body weight.

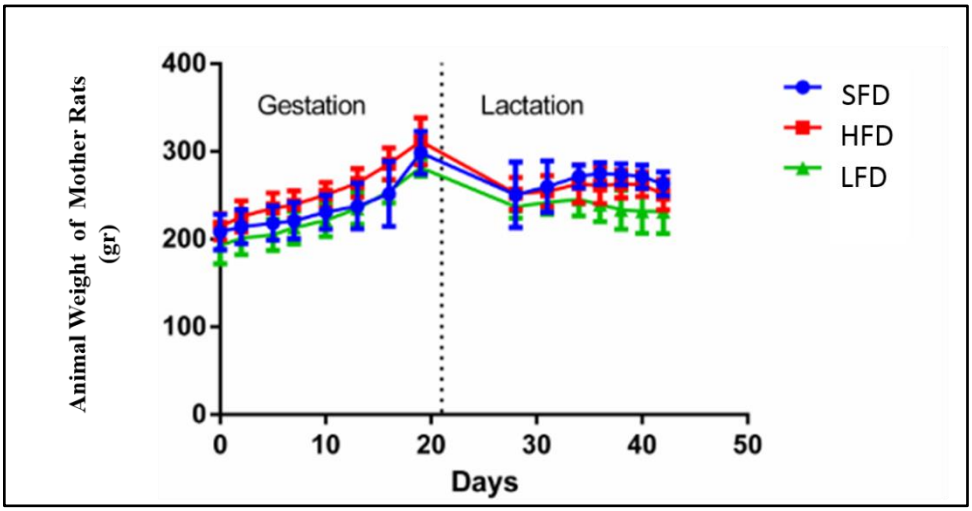


Figure 5.6. The animal weight (gr) of mother rats during G-L periods. Data are given as the mean of (n=8-10) ±SD.

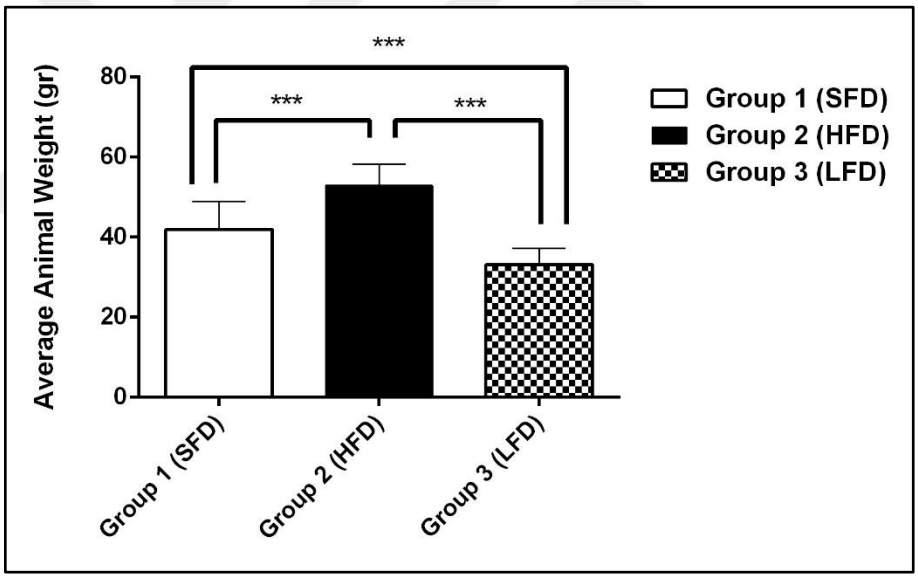


Figure 5.7. At the end of the lactation period, the body weights of pups which were exposed to a different amount of fat-containing diets during G-L periods. Data are given as the mean of (n=22) ±SD (*** $p<0.001$).

Maternal HFD fed group offsprings (Group 2) have higher body weight at the end of the G-L period when compared to other groups The difference was as statistical significant ($p<0.001$). The lowest animal weight has been determined in the offspring of LFD fed mothers ($p<0.001$).

5.2. MEASUREMENTS OF ANIMAL WEIGHT AND FOOD INTAKE IN MALE OFFSPRING RATS DURING MATURATION PERIOD

5.2.1. The Food Consumption in Male Offspring Rats During Maturation Period

In the first part of the study, mother Sprague-Dawley rats were administered with SFD, HFD and LFD during the G-L periods. Then the male pups from every three main groups were fed with HFD, SFD and LFD during the maturation period for 100 days in the second part of the study as mentioned in Section 4.2.1. During the maturation period, daily food intake of male offsprings was followed. The average total food intake was calculated and showed in Table 5.4.

Table 5.4. The total food intake in male offsprings during the maturation period. Data were given as mean of (n=5-8) ± SD.

Groups	Total Food Intake (gr of the pellet)
Group 1a	1999,7 ± 146
Group 1b	1405,7 ± 45,1
Group 1c	1725,3 ± 101,9
Group 2a	2071,6 ± 137,8
Group 2b	1508,9 ± 138,2
Group 2c	1820,2 ± 89,2
Group 3a	1758,7 ± 119,3
Group 3b	1476,9 ± 62,2
Group 3c	1677,8 ± 101,8

During the maturation period, the total food intake of groups are given in Figure 5. 8. Total food intake of Group 1 (a,b,c), Group 2 (a,b,c) and Group 3 (a,b,c) were statistically evaluated in Figure 5.8.(a, b, c), respectively. It has found that the offsprings which were fed with HFD during maturation period, Group 1b, Group 2b and Group 3b, have significantly lower amount of food intake than pups which were fed with SFD and LFD during maturation period ($p<0.01$). In addition, it has been demonstrated that the pups

which fed with SFD during maturation period (Group 1a, 2a, 3a) have a higher amount of food consumption than LFD administrated pups during maturation period which are Group 1c, 2c, 3c ($p<0.05$) (Figure 5.8.(a, c)).

On the other hand, total food intake (gr) of Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c) were statistically evaluated in Figure 5.8. (d, e, f) respectively. It has been found that Group 2a (HFD-SFD) has significantly increased total food intake than Group 3a (LFD-SFD) ($p<0.01$) (Figure 5.8.d). Group 1b (SFD-HFD) have significantly higher than Group 3b (LFD-HFD) ($p<0.05$) (Figure 5.8.e). Lastly, Group 2c (HFD-LFD) has significantly elevated food intake than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) (Figure 5.8.f).

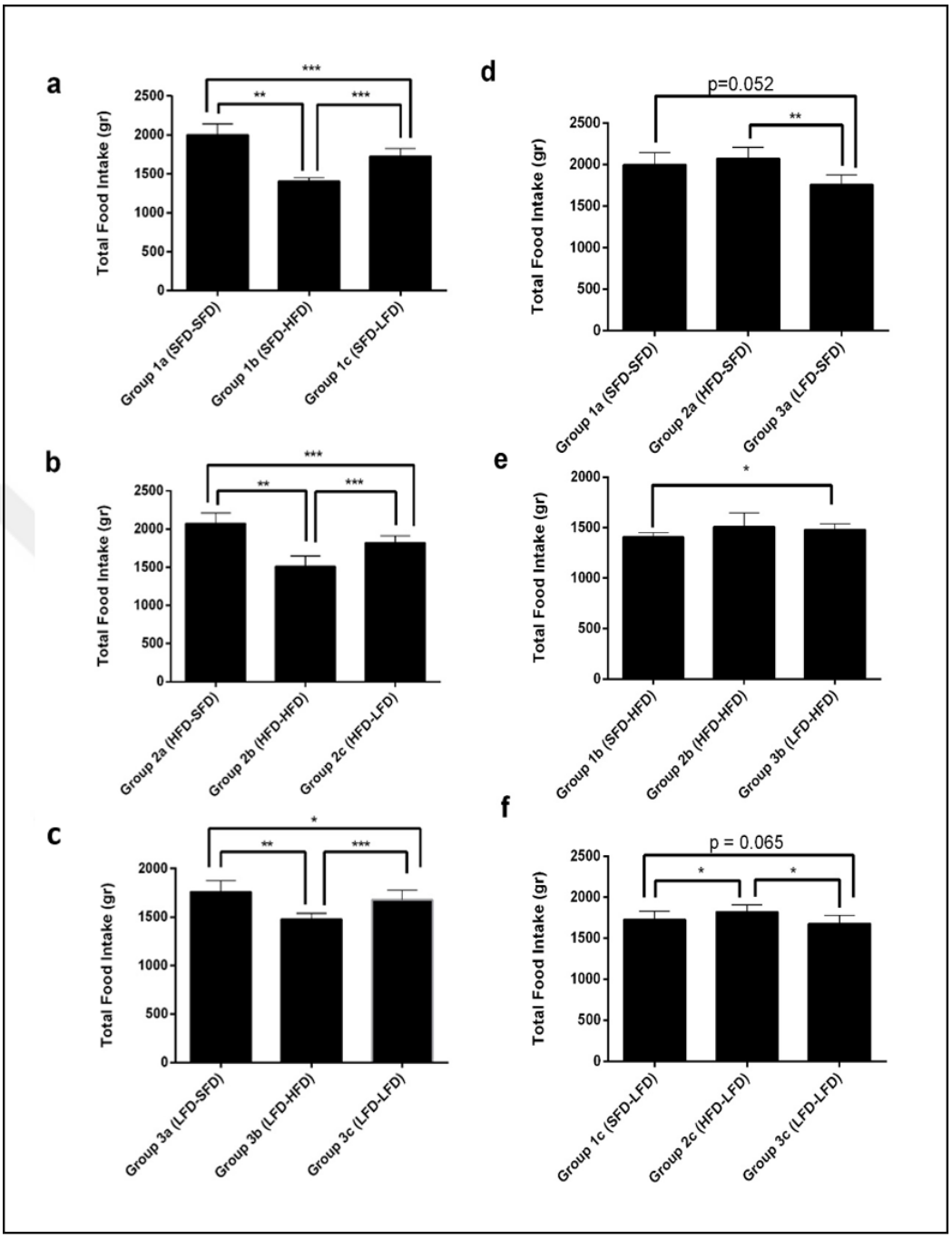


Figure. 5.8. Total food intake (gr) of offspring rats during the maturation period. (a, b, c): The evaluation of total food intake (gr) in in the Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c), respectively. (d, e, f): The evaluation of total food intake (gr) in the Group (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c), respectively. Data are given as mean of (n=5-8) ±SD (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

5.2.2. The Calorie Intake in Male Offspring Rats During Maturation

To investigate the amount of total energy intake of groups, average total energy intake as kcal of male rats were calculated from daily food intake with the usage of the table of ingredients of experimental diets which is mentioned in Section 4.2.1. The total energy intake as kcal of male offspring rats during the maturation period was given in Table 5.5.

Table. 5.5. The total energy intake in male offspring rats during the maturation period. Values are given as mean of (n=5-8) ± SD.

Groups	Total Energy Intake (kcal)
Group 1a	6688,9 ± 488,3
Group 1b	7239,2 ± 232,4
Group 1c	5881,4 ± 347,2
Group 2a	6929,4 ± 460,9
Group 2b	7770,0 ± 711,9
Group 2c	6205,2 ± 304,0
Group 3a	5882,3 ± 398,9
Group 3b	7602,1 ± 320,5
Group 3c	5663,3 ± 361,5

During the maturation period, the total calorie intake of groups were given in Figure 5. 9. It was determined that Group 1c (SFD-LFD) has significantly decreased caloric intake than Group 1a (SFD-SFD) and Group 1b (SFD-HFD) ($p<0.01$) (Figure 5.9.a). Group 2c (HFD-LFD) has significantly reduced calorie intake than Group 2a (HFD-SFD) and Group 2b (HFD-HFD) ($p<0.01$) (Figure 5.9.b). Lastly Group 3b (LFD-HFD) has significantly higher energy intake than Group 3a (LFD-SFD) and Group 3c (LFD-LFD) ($p<0.01$). In addition, Group 3a (LFD-SFD) has higher energy intake than Group 3c (LFD-LFD) ($p<0.01$) (Figure 5.9.c).

On the other hand, It has been found that Group 2a (HFD-SFD) has significantly increased total energy intake than Group 3a (LFD-SFD) ($p<0.01$) (Figure 5.9.d). Group 1b (SFD-HFD) have significantly higher than Group 3b (LFD-HFD) ($p<0.05$) (Figure 5.9.e) Group 2c (HFD-LFD) has significantly increased total energy intake during the maturation period than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) (Figure 5.9.f).

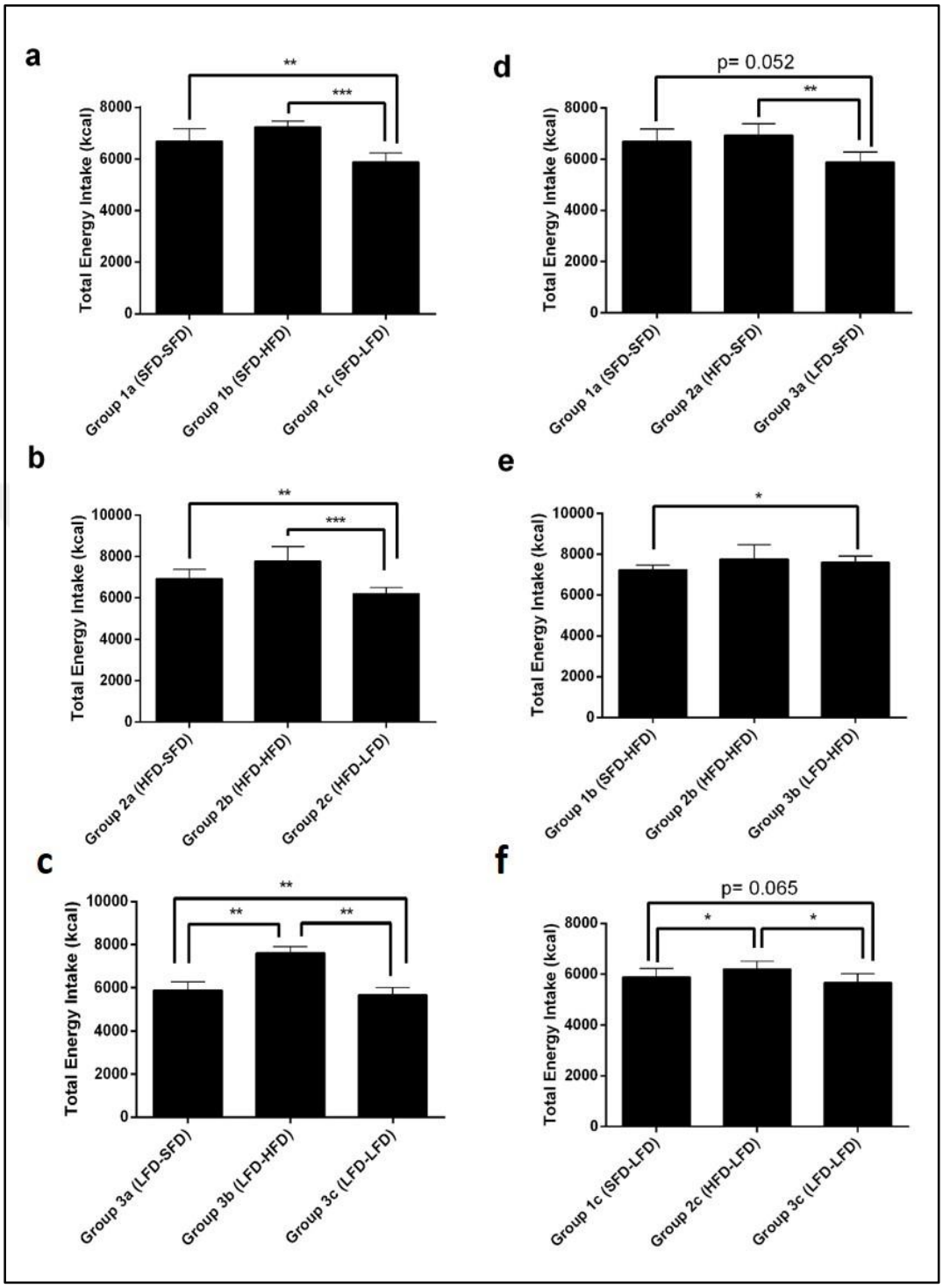


Figure. 5.9. Total energy intake (kcal) in male offspring rats during the maturation period. (a, b, c): The evaluation of the total energy intake in the Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c), respectively. (d, e, f): The evaluation of the total energy intake in the Group (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c), respectively. Data are given as mean of (n=5-8) ± SD (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

5.2.3. Determination of Macronutrients Intake In Male Offspring Rats During Maturation

To investigate the nutrition status during the maturation period, distributions of total calorie intake which comes from each macronutrient of male offspring rats were determined. In this context, kcal distributions of each consumed macronutrient were calculated by using the table of the ingredients of experimental diets which is Table 3.2. The obtained values are presented in Table 5.6. In addition, since the hypothesis of the study is related to fat exposure of offsprings in the maturational period, the total gr of fat consumption was calculated and evaluated as statistically for each group.

Table 5.6. Distributions of total calorie intake which comes from each macronutrient in male offspring rats during the maturation period. Values are present as mean of (n=5-8) ± SD.

Groups	Carbohydrate (kcal)	Fat (kcal)	Protein (kcal)
Group 1a	4280,9 ± 312,5	869,6 ± 63,5	1538,4 ± 112,3
Group 1b	1447,8 ± 46,5	4343,5 ± 139,5	1447,8 ± 46,5
Group 1c	4352,2 ± 256,9	176,4 ± 10,4	1352,7 ± 79,9
Group 2a	4434,8 ± 1,3	901 ± 0,3	1593,8 ± 0,5
Group 2b	1554,1 ± 142,4	4662,4 ± 427,1	1554,1 ± 142,4
Group 2c	4591,9 ± 225	186,1 ± 9,1	1427,2 ± 69,9
Group 3a	3765 ± 255,3	765 ± 51,9	1353,1 ± 91,8
Group 3b	1520,4 ± 64,1	4561,2 ± 192,3	1520,4 ± 64,1
Group 3c	4197,6 ± 280,9	170,2 ± 11,4	1304,6 ± 87,3

During the maturation period, the total fat intake of groups are given in Figure 5.10. Total fat intake (gr) of Group 1 (a, b, c), Group 2 (a, b, c) and Group 3 (a, b, c) were statistically evaluated in Figure 5.10. (a, b, c) respectively. In each of three series (Groups 1, Groups 2 and Groups 3), it has found that the pups which were fed with HFD during maturation period, Group 1b, Group 2b and Group 3b, have higher amount of fat intake than pups of Group a and Group c which were fed with SFD and LFD during maturation period ($p<0.01$). In addition, it has been determined that the pups which fed with SFD during

maturation period (Groups 1a, 2a, 3a) have a higher amount of fat consumption as gr than LFD-administrated pups during maturation period which is Group 1c, 2c, 3c ($p<0.01$) (Figure 5.10.(a, b, c)).

On the other hand, the total fat intake of Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c) were statistically evaluated in Figure 5.10. (d, e, f) respectively. It has been found that Group 2a (HFD-SFD) has significantly increased amount of total fat intake than Group 3a (LFD-SFD) ($p<0.01$) (Figure 5.10.d). Group 1b (SFD-HFD) have significantly higher than Group 2b (HFD-HFD) and Group 3b (LFD-HFD) ($p<0.001$) (Figure 5.10.e). Group 2c (HFD-LFD) has significantly elevated total fat intake than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) during the maturation period (Figure 5.10.f).

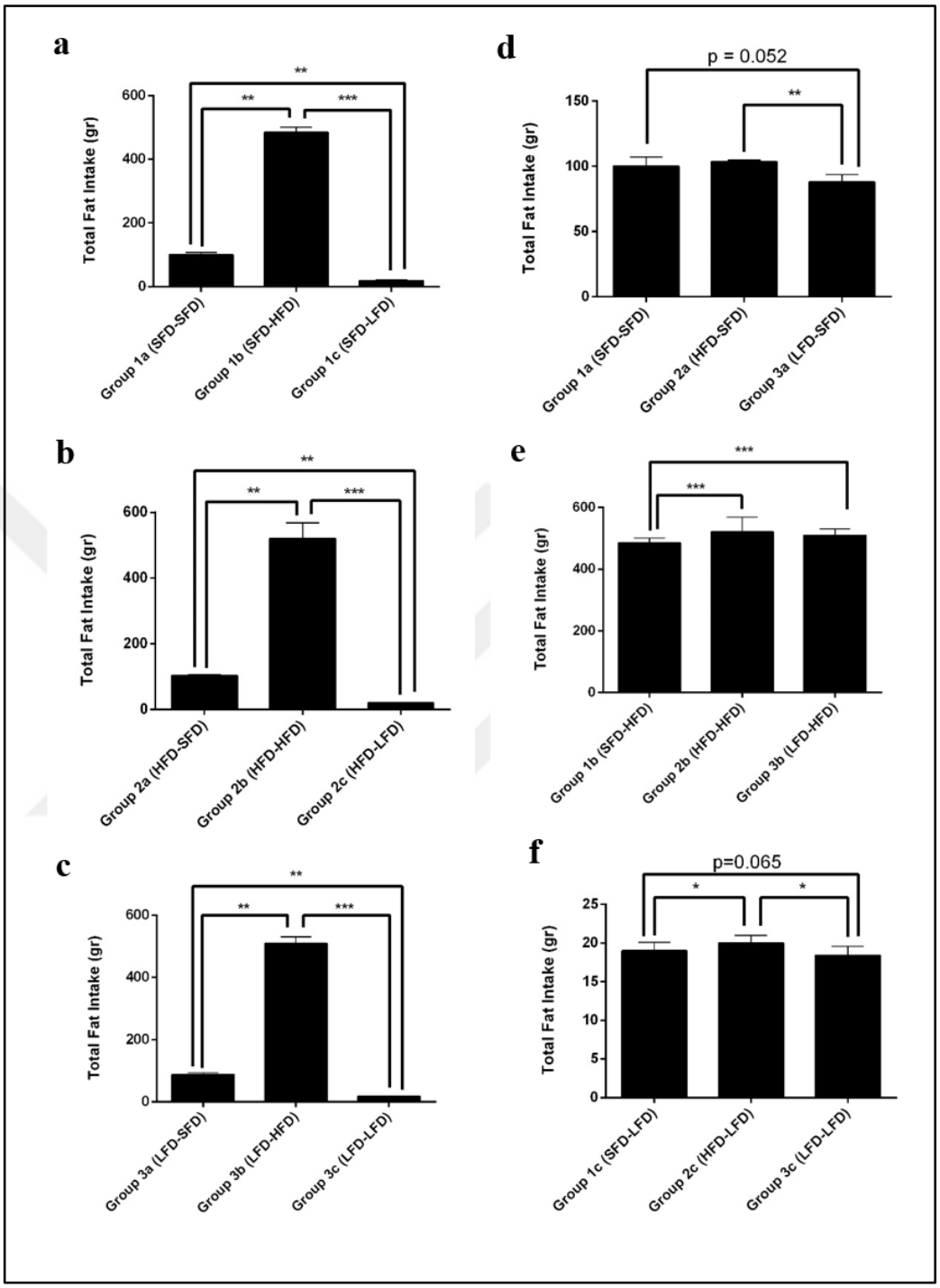


Figure. 5.10. Total fat intake (gr) in male offspring rats during the maturation period. (a, b, c): The evaluation of the total fat intake (gr) in the series of Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c), respectively. (d, e, f): The evaluation of the total fat intake (gr) in the series of Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c), respectively. Data are given as mean of (n=5-8) ± standard deviation (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

5.2.4. The Offspring Animal Weights During Maturation

Both in the beginning and end of the maturation period, bodyweight of male Sprague Dawley male offspring rats were measured which is mentioned in Section 4.2.1. Values were given in Table 5.7.

Table 5.7. Body weight of male Sprague Dawley offspring rats in the beginning and end of the maturation period. Data are given as mean of (n=5-8) ± SD.

Groups	Body Weight at the Beginning of Maturation Period	Body Weight at the End of Maturation Period
Group 1a	41,8 ± 5,6	373,1 ± 38,6
Group 1b	44,4 ± 1,6	440,7 ± 21,2
Group 1c	39,7 ± 10,4	389,3 ± 33,6
Group 2a	53,2 ± 1,5	398,5 ± 29,3
Group 2b	52,2 ± 6,6	461,9 ± 50,1
Group 2c	53,3 ± 6,4	388,2 ± 34
Group 3a	34,0 ± 4,9	322,5 ± 30,9
Group 3b	33,3 ± 2,5	439,9 ± 32,3
Group 3c	32,5 ± 4,7	347,9 ± 33,1

At the beginning of the maturation period, the average body weight of experimental groups were given in Figure 5. 11. It was determined that Group 1b (SFD-HFD) had significantly higher body weight than Group 1c (SFD-LFD) ($p<0.05$) (Figure 5.11.a). In the serie of Group 2 (a, b, c), body weights of offsprings had nearly much the same at beginning of the maturation period (Figure 5.11.b). Similarly, Group 3a, 3b and 3c had nearly much the same average animal weight at beginning of the maturation period (Figure 5.11.c).

On the other hand, it has been found that in the series of Groups (1a, 2a, 3a), bodyweight had nearly much the same at the beginning of the maturation period (Figure 5.11.d). Body weight of Group 2b (HFD-HFD) had significantly higher than Group 1b (SFD-HFD) and Group 3b (LFD-HFD) ($p<0.01$) (Figure 5.11.e). Lastly, Group 2c (HFD-LFD) had

significantly higher body weight than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) at the beginning of the maturation period (Figure 5.11.f).

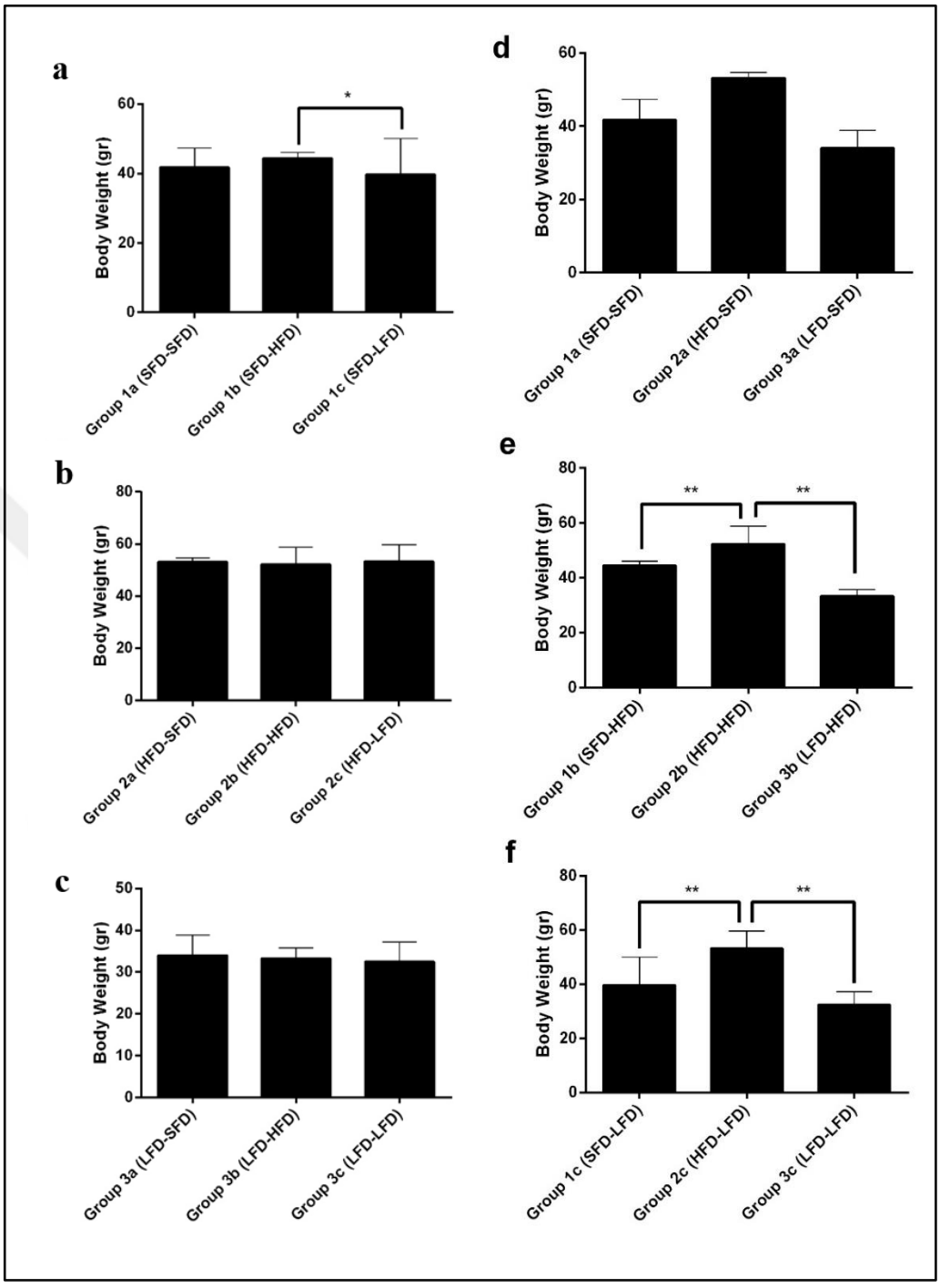


Figure. 5.11. Body weight (gr) in male offspring rats at the beginning of the maturation period. (a, b, c): The evaluation of the body weight (gr) at the beginning of the maturation period in the Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c), respectively. (d, e, f): The evaluation of the body weight (gr) at the beginning of the maturation period in the Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c), respectively. Data are given as mean of (n=5-8) ± SD (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

At the end of the maturation period, the average body weight of experimental groups are given in Figure 5. 12. Group 1b (SFD-HFD) has significantly higher body weight than Group 1a (SFD-SFD) and Group 1c (SFD-LFD) ($p<0.05$) (Figure 5.12.a). Group 2b (HFD-HFD) has significantly elevated body weight than Group 2a (HFD-SFD) and Group 2c (HFD-LFD) ($p<0.05$)(Figure 5.12.b). Similarly, Group 3b (LFD-HFD) has significantly higher body weight than Group 3a (LFD-SFD) and Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.12.c).

On the other hand, to figure out the effect of different percentages of fat-containing diets administration during the maternal period on the bodyweight of offspring rats, Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c) were statistically evaluated in Figure 5.12.(d, e, f). There were no significant differences between the groups.

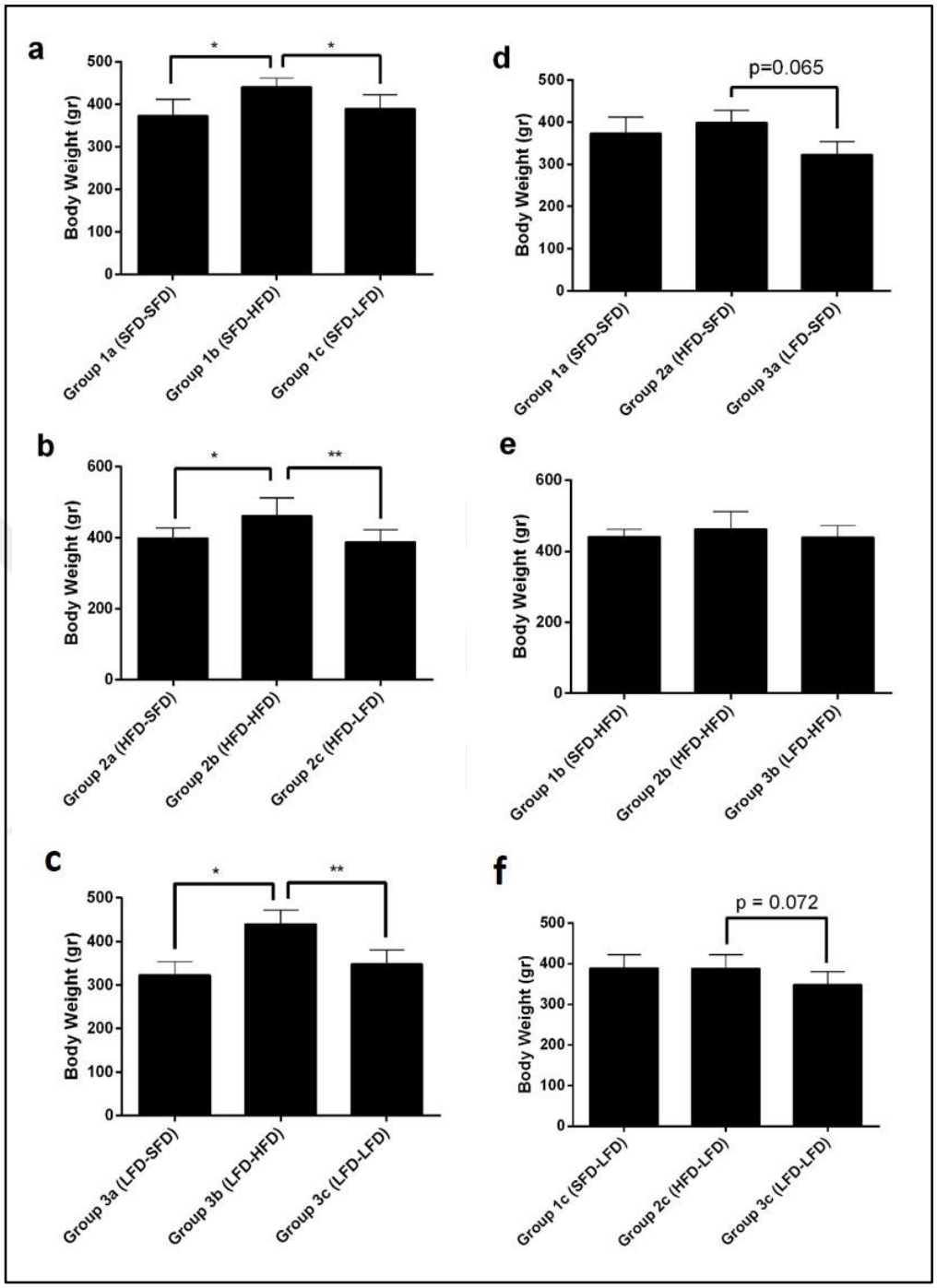


Figure. 5.12. Animal weight (gr) in male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the body weight (gr) at the end of the maturation period in the Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c), respectively. (d, e, f): The evaluation of the body weight (gr) at the end of the maturation period in the Groups (1a, 2a, 3a), Groups (1b, 2b, 3b) and Groups (1c, 2c, 3c), respectively. Data are given as mean of (n=5-8) ± SD (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

5.3. TWO BOTTLE PREFERENCE TEST (TBPT)

In order to determine the effect of different amount of fat-containing diets during maternal and maturational periods on fatty solution preference in male pups, TBPT has been applied which is mentioned as Section 4. 2. 2. In the first part of the test, 0.02 percent rapeseed oil in 0.3 percent xanthan gum and 0.3 percent xanthan gum as control solution are presented to the rats. At the end of the 24 hours and 48 hours, the percentage of preference of 0.02 rapeseed oil containing solution has been calculated and presented in Table 5.8. Expectedly, standard deviation of the values was so higher in 24 hours TBPT due to side preference effect. To eliminate this effect, the statistical significance of 48 hours TBPT has been evaluated and it has been presented in Figure 5.13. Nevertheless, there were not any significant differences between groups in terms of preference of 0.02 percent rapeseed oil.

Table 5.8. The percentage ratio of 0.02 percent rapeseed oil solution consumption to total solution consumption for 24 hours and 48 hours at the TBPT. Data are given as mean of (n=5-8) ± SD.

Groups	24h TBPT Percentage of 0.02 Percent Rapeseed Oil Solution Preference	48h TBPT Percentage of 0.02 Percent Rapeseed Oil Solution Preference
Group 1a	55.9 ± 13.6	61.8 ± 9.7
Group 1b	55.2 ± 8.0	56.2 ± 8.0
Group 1c	45.6 ± 15.7	48.3 ± 9.2
Group 2a	60.0 ± 16.3	60.0 ± 8.0
Group 2b	57.4 ± 15.9	49.0 ± 13.4
Group 2c	46.6 ± 19.6	46.6 ± 11.4
Group 3a	68.4 ± 11.2	63.3 ± 10.7
Group 3b	47.4 ± 22.8	49.0 ± 11.3
Group 3c	54.5 ± 21.1	54.1 ± 3.2

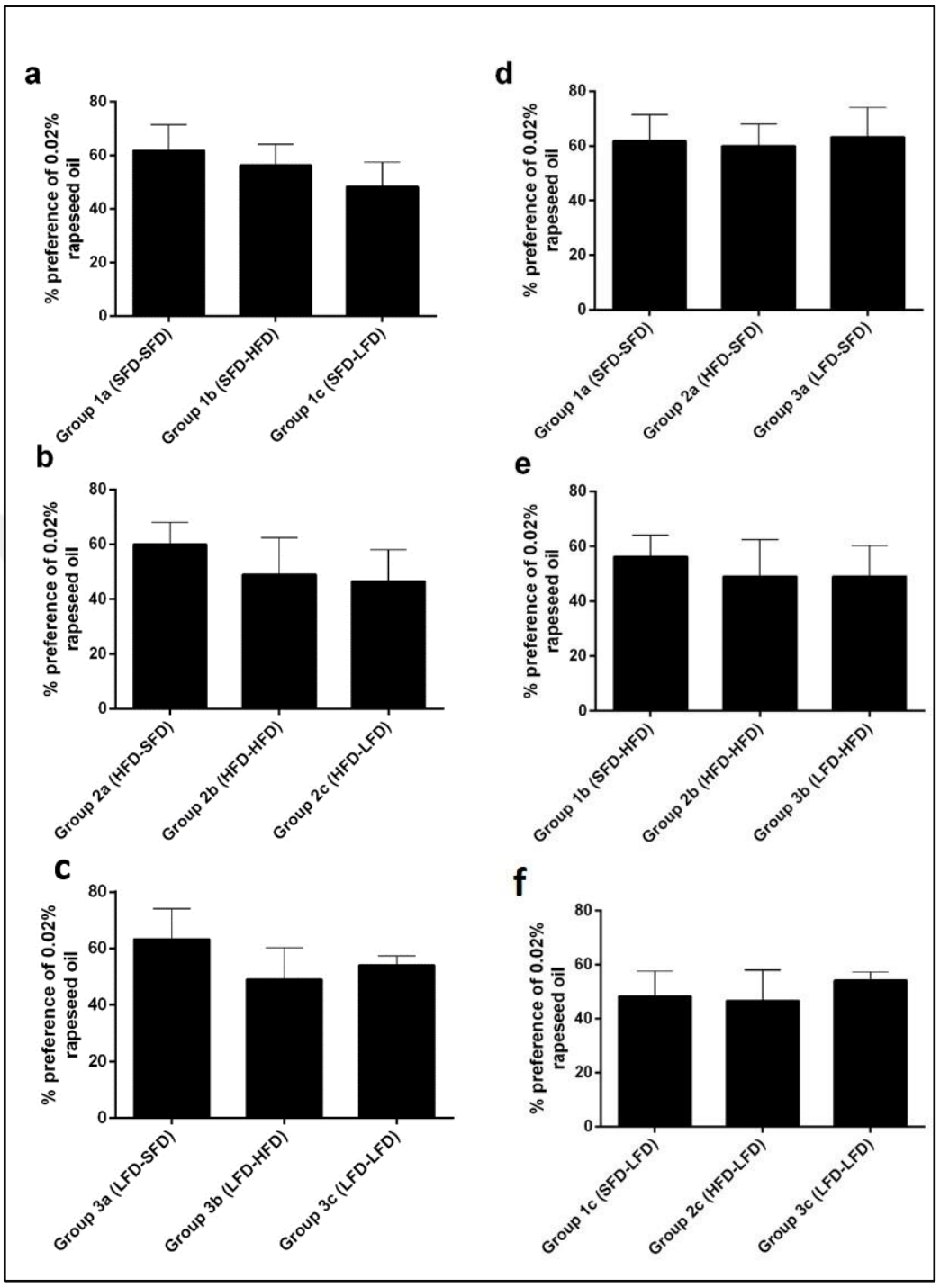


Figure 5.13. Average percentage preference of 0.02 percent rapeseed oil solution. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the preference of 0.02 percent rapeseed oil solution. (d, e, f): The evaluation of different amount of fat-containing diets administration during G-L periods on the preference of 0.02 percent rapeseed oil. Data are given as mean of (n=5-8) ± SD.

In the second part of the preference test, 0.2 percent rapeseed oil in 0.3 percent xanthan gum and 0.3 percent xanthan gum as control solution are subjected to the male offspring rats. At the end of both 24h and 48h, the percentage ratio of 0.2 percent rapeseed oil solution consumption to total solution consumption was calculated and presented in Table 5.9.

Table 5.9. The percentage ratio of 0.2 percent rapeseed oil solution consumption to total solution consumption for 24 hours and 48 hours at the TBPT. Data represents as mean ± SD (n=5-8).

Groups	**24h TBPT Percentage of 0.2 Percent Rapeseed Oil Solution Preference**	**48h TBPT Percentage of 0.2 Percent Rapeseed Oil Solution Preference**
Group 1a	57.1 ± 13.7	58.1 ± 16.1
Group 1b	62.6 ± 20.7	60.1 ± 13.9
Group 1c	72.4 ± 17.3	65.4 ± 13.8
Group 2a	47.2 ± 18.3	54.1 ± 6.4
Group 2b	63.5 ± 6.4	57.4 ± 9.9
Group 2c	58.5 ± 19.6	55.7± 3.2
Group 3a	72.5 ± 16.6	60.4 ± 9.9
Group 3b	39.2 ± 24.1	42.2 ± 7.5
Group 3c	59.6 ± 23.7	55.5 ± 6.2

For 48 hours, TBPT results were evaluated and presented in Figure 5.14. According to results, Group 3b (LFD-HFD) had significantly lower preference to 0.2 percent rapeseed oil solution when compared to Group 3a (LFD-SFD) and Group 3c (LFD-LFD) ($p<0.05$)(Figure 5.14.c). Moreover, Group 1b (SFD-HFD) and Group 2b (HFD-HFD) had significantly higher preference to 0.2 percent rapeseed oil than Group 3b (LFD-HFD) ($p<0.05$)(Figure 5.14.e). Apart from Group 3b, there were no significant differences in percent preference of 0.2 percent rapeseed oil between experimental groups.

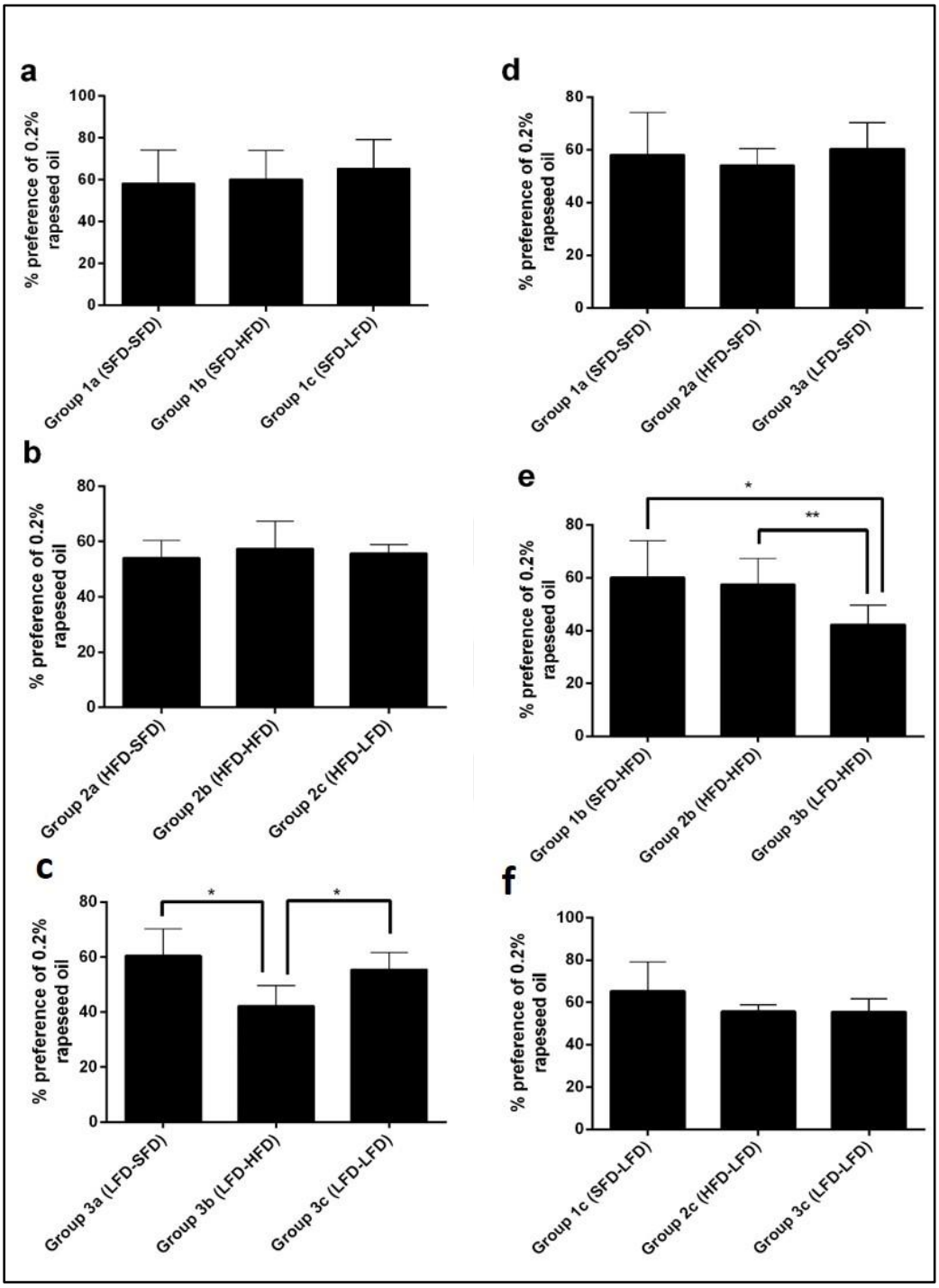


Figure. 5.14. Average percentage preference of 0.2 percent rapeseed oil. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the preference of 0.2 percent rapeseed oil solution. (d, e, f): The evaluation of different amount of fat-containing diets administration during G-L periods on the preference of 0.2 percent rapeseed oil. Data is given as mean of (n=5-8) ± SD (* $p < 0.05$, ** $p < 0.01$).

In the last session of the preference test, 2 percent rapeseed oil in 0.3 percent xanthan gum and 0.3 percent xanthan gum as control solution are presented to the male pups. At the end of both 24h and 48h, the percentage ratio of 2 percent rapeseed oil solution consumption to total solution consumption was calculated and presented in Table 5.10.

Table 5.10. At the TBPT, the percentage preference ratio of 2 percent rapeseed oil solution consumption to total solution consumption for 24 hours and 48 hours. Data is present as mean (n=5-8) ± SD.

Groups	24h TBPT Percentage of 2 Percent Rapeseed Oil Solution Preference	48h TBPT Percentage of 2 Percent Rapeseed Oil Solution Preference
Group 1a	66.2 ± 18	62.3 ± 15.5
Group 1b	72.4 ± 8.2	67.0 ± 14.4
Group 1c	80.2 ± 7.8	79.2 ± 2.3
Group 2a	83.3 ± 9.4	82.2 ± 7.8
Group 2b	85.3 ± 8.2	84.3 ± 4.6
Group 2c	76.9 ± 11.8	80.3 ± 4.8
Group 3a	71.7 ± 13.1	71.0 ± 11.7
Group 3b	57.3 ± 20.4	57.3 ± 12.8
Group 3c	78.0 ± 7.2	70.8 ± 12.1

At the end of the 48h, percentage of preference of 2 percent rapeseed oil solution in male pups has been shown in Figure 5.15. Group 1a (SFD-SFD) has significantly higher preference to 2 percent rapeseed oil solution than Group 1c (SFD-LFD) (Figure 5.15.a). Maternal HFD administrated rats (Group 2a, Group 2b and Group 2c) have higher preference to 2 percent rapeseed oil solution and there were no any significant difference between them (Figure 5.15.b). In addition, the lower preference to 2 percent rapeseed oil solution was determined in Group 3b (LFD-HFD), but it was not statistically significant (Figure 5.15.c).

On the other hand, Group 2a (HFD-SFD) has significantly higher percentage on the preference of 2 percent rapeseed oil solution than Group 1a (SFD-SFD) ($p<0.05$) (Figure

5.15.d). Group 2b (HFD-HFD) has very significantly higher percentage on the preference of 2 percent rapeseed oil solution compared to Group 1b (SFD-HFD) and Group 3b (LFD-HFD) ($p<0.05$) (Figure 5.15.e). Lastly, Group 2c (HFD-LFD) has significantly higher percentage on the preference of the 2 percent rapeseed oil solution than Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.15.f).

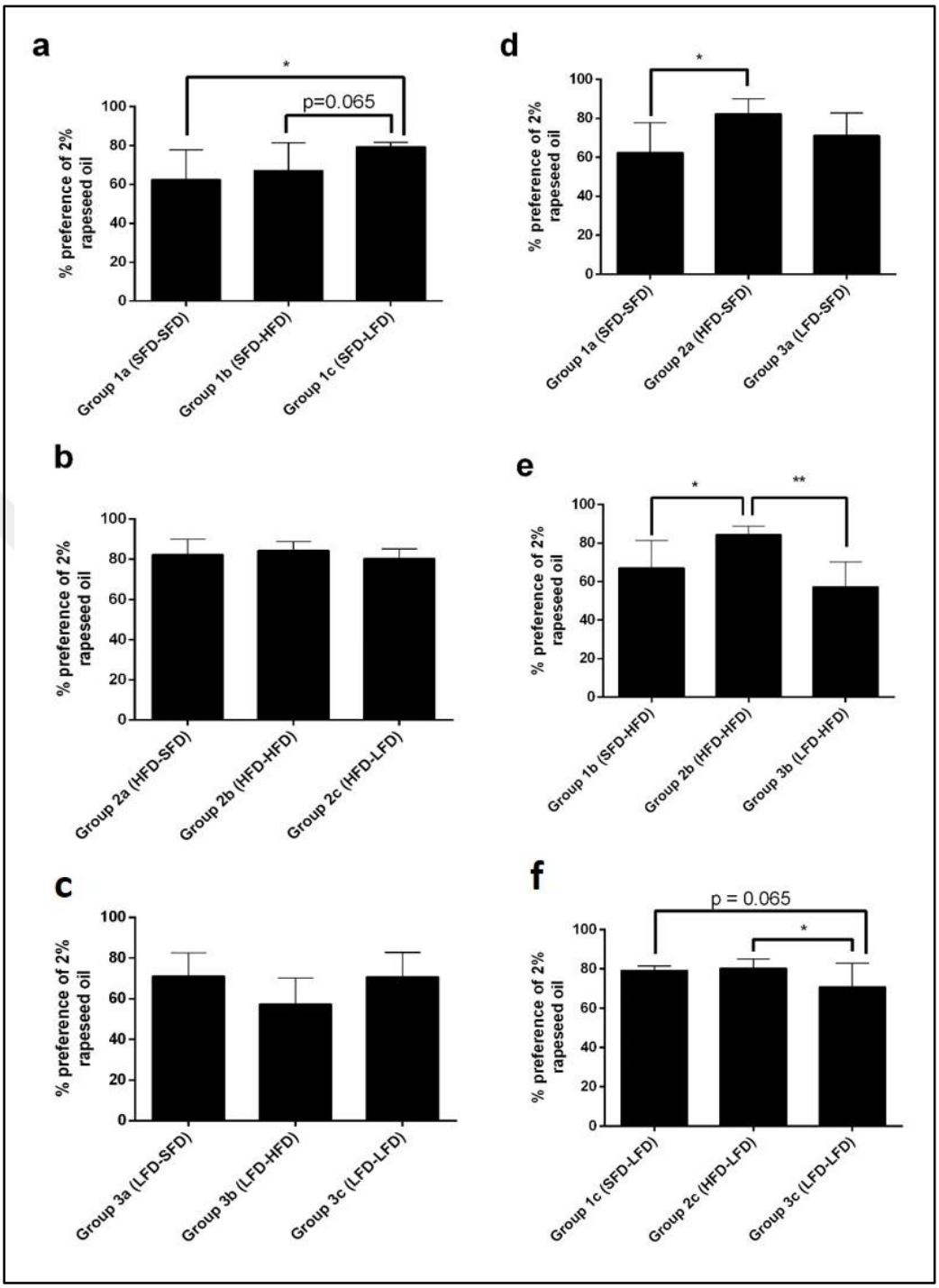


Figure. 5.15. Average percentage preference of 2 percent rapeseed oil. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the preference of 2 percent rapeseed oil solution. (d, e, f): The evaluation of different amount of fat-containing diets administration during G-L periods on the preference of 2 percent rapeseed oil. Data is given as mean of (n=5-8) ± SD (* $p < 0.05$, ** $p < 0.01$).

5.4. DETERMINATION OF PLASMA INSULIN AND LEPTIN CONCENTRATIONS WITH ELISA

Plasma insulin and leptin concentrations of all experimental groups were determined with ELISA which is mentioned in Section 4.2.3. Values are shown in Table 5.11.

Table 5.11. Plasma insulin and leptin concentrations of male offspring rats in all experimental groups. Data are given as mean ± SD (n=5-8).

Groups	Insulin (ng/mL)	Leptin (ng/mL)
Group 1 (SFD)	1.9 ± 0.9	1.5 ± 0.7
Group 1a (SFD-SFD)	1.6 ± 0.5	9.5 ± 1.9
Group 1b (SFD-HFD)	2.4 ± 0.6	18.6 ± 3.6
Group 1c (SFD-LFD)	3.3 ± 0.6	9.5 ± 1.8
Group 2 (HFD)	1.9 ± 0.8	6.4 ± 1.9
Group 2a (HFD-SFD)	3.4 ± 0.6	14.9 ± 4.8
Group 2b (HFD-HFD)	2.6 ± 0.4	26.6 ± 8.7
Group 2c (HFD-LFD)	2.6 ± 0.4	8.6 ± 1.7
Group 3 (LFD)	2.4 ± 1.4	1.4 ± 0.2
Group 3a (LFD-SFD)	1.6 ± 0.1	7.6 ± 2
Group 3b (LFD-HFD)	2.8 ± 0.2	17.7 ± 1.2
Group 3c (LFD-LFD)	3.7 ± 0.8	6.9 ± 1.4

5.4.1. Plasma Insulin Concentrations in Male Offspring Sprague Dawley Rats

Plasma insulin concentrations (ng/mL) of pups which were dissected at the end lactation period was evaluated and given in Figure 5.16. There were no statistically significant differences in plasma insulin concentration between the groups.

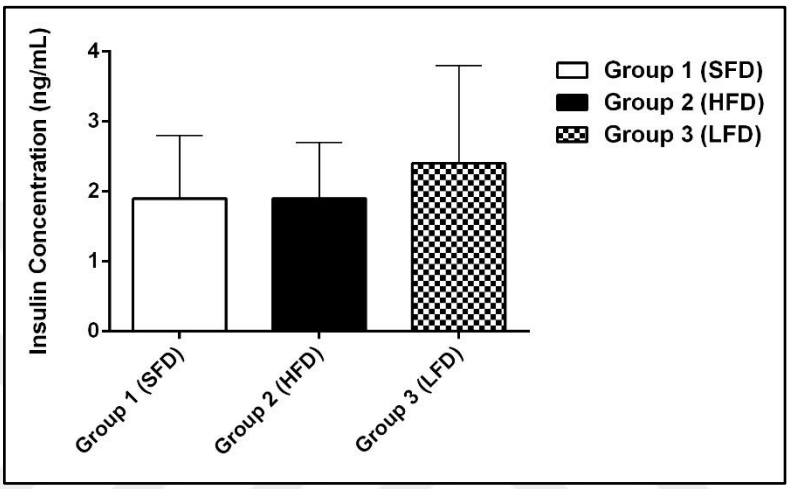


Figure 5.16. Plasma insulin concentrations of the offspring rats at the end of the G-L period. Data are given as mean of (n=4-6) ± SD.

The plasma insulin concentrations of pups which were dissected at the end of the maturation period are shown in Figure 5.17. It was determined that both in Groups 1 and Groups 3 series, offsprings which fed with LFD during maturation period have significantly higher plasma insulin concentration than offsprings which fed with SFD during maturation period ($p<0.05$) (Figure 5.17.(a, c)). In contrast, offsprings in Group 2a (HFD-SFD) have higher plasma insulin concentration when compared to Group 2b (HFD-HFD) and Group 2c (HFD-LFD) and but it was not statistically significant (Figure 5.17.b).

On the other hand, pups of Group 2a (HFD-SFD) has significantly higher plasma insulin concentration than Group 1a (SFD-SFD) ($p<0.05$) (Figure 5.17.d). In the pups of the 1b, 2b and 3b groups, plasma insulin levels were close to each other and so it was no statistically significant differences between them (Figure 5.17.e). Lastly, pups in the Group 2c (HFD-LFD) have significantly higher plasma insulin concentration than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.17.f).

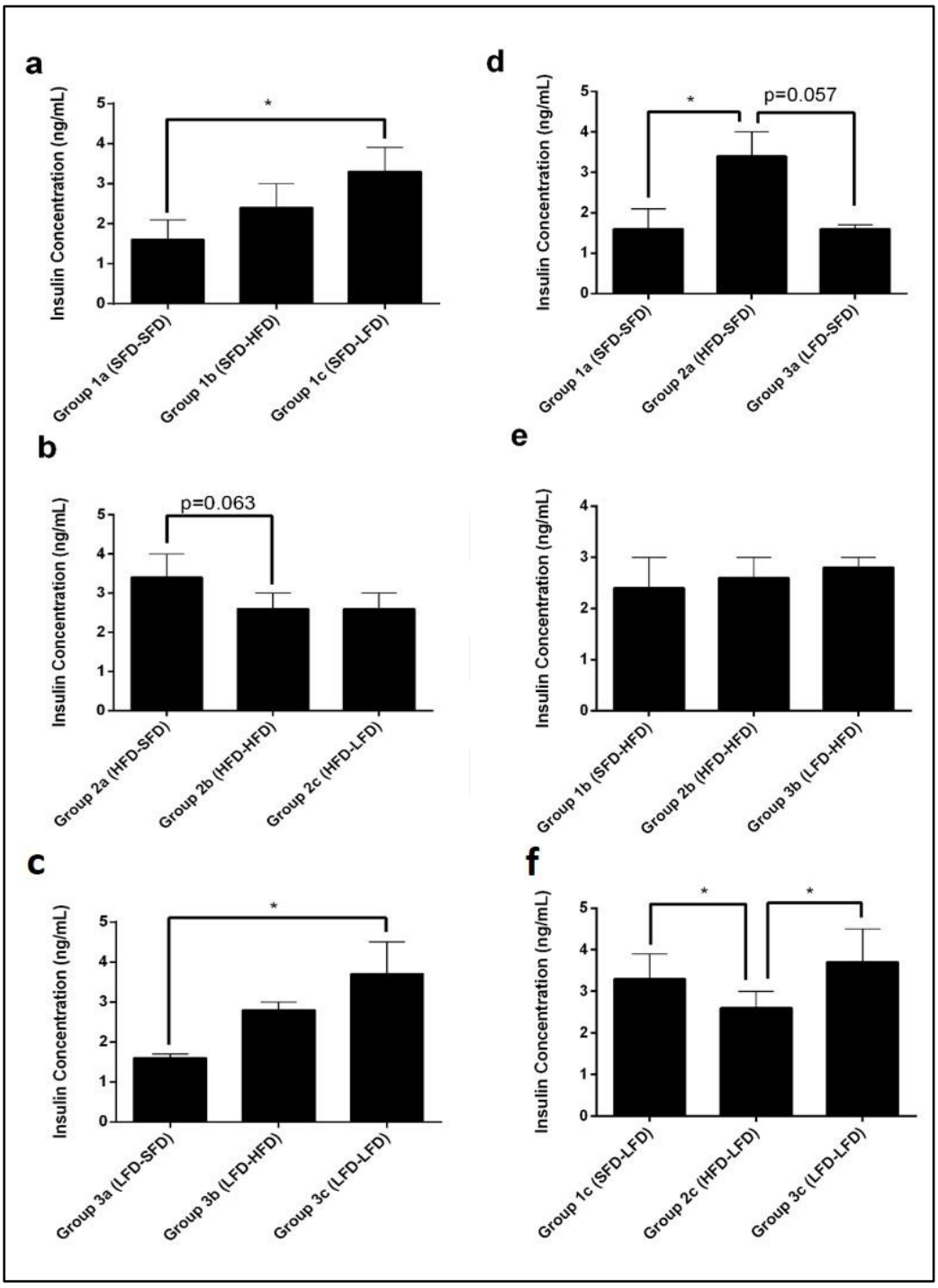


Figure 5.17. Plasma insulin concentrations of the offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the plasma insulin concentration of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the plasma insulin concentration of the offspring rats . Data is given as mean of (n=3-5) ± SD (* $p< 0.05$, ** $p< 0.01$).

5.4.2. Plasma Leptin Concentrations in Male Offspring Sprague Dawley Rats

In groups which are dissected at the end of the lactation period, plasma leptin concentrations are given in Figure 5.18. Group 2 (HFD) offsprings have significantly higher plasma leptin concentration than pups of Group 1 (SFD) and Group 3 (LFD) ($p<0.05$).

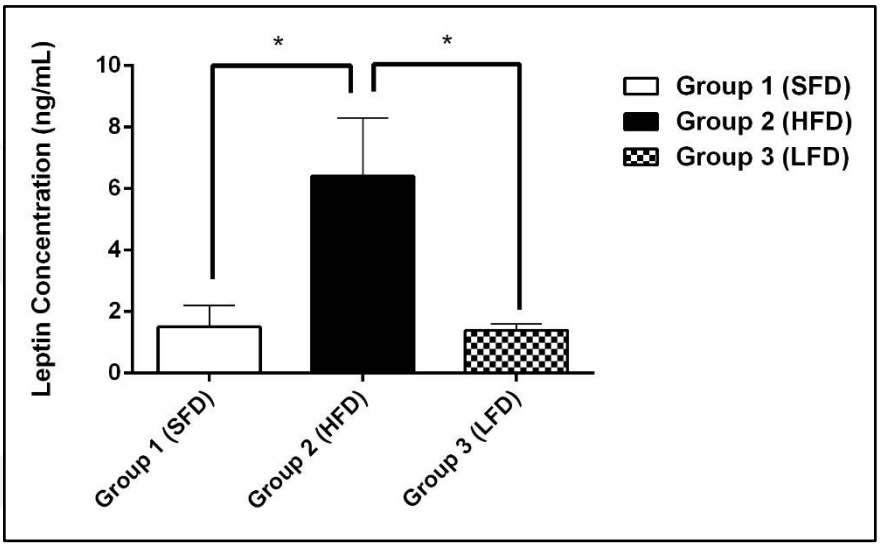


Figure 5.18. Plasma leptin concentrations of the offspring rats at the end of the G-L periods. Data are given as mean of (n=4-6) ± SD) (* $p< 0.05$).

At the end of the maturation period, the plasma leptin concentrations of offspring rats are present in Figure 5.19. The offsprings which were fed with HFD during maturation period (Group 1b, Group 2b and Group 3b) have significantly higher plasma leptin concentration than pups which were fed with SFD and LFD during maturation period ($p<0.05$) (Figure 5.19.(a, b, c)).

On the other hand, it was no significant differences in leptin concentrations between 1a, 2a and 3a groups (Figure 5.19.d). However, Group 2b (HFD-HFD) has significantly higher plasma leptin level than Group 1b (SFD-HFD) and Group 3b (LFD-HFD) ($p<0.05$) (Figure 5.19.e). Finally, it was showed that offsprings of Group 1c (SFD-LFD) have significantly higher plasma leptin concentration than Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.19.f).

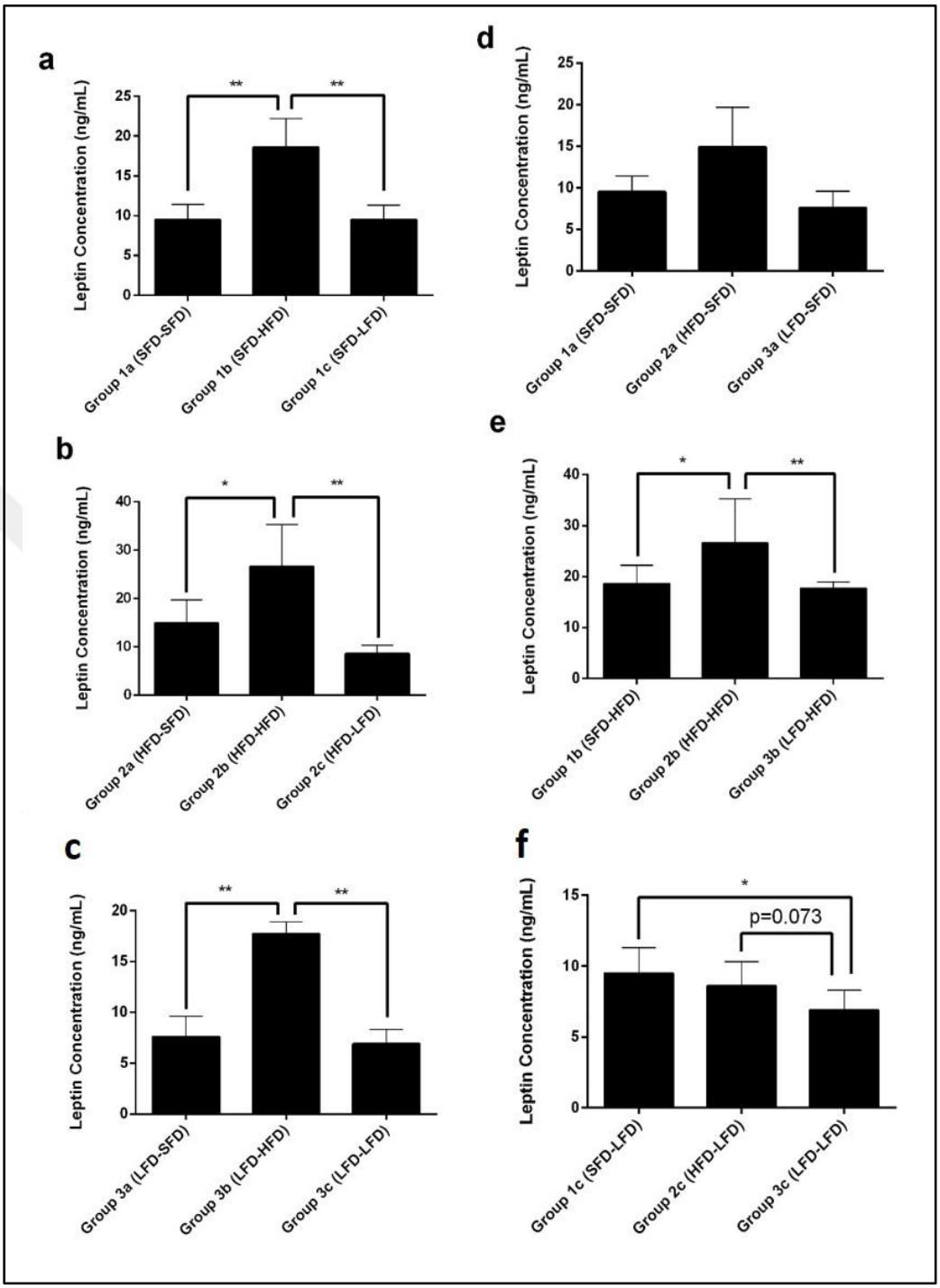


Figure 5.19. Plasma leptin concentrations in the offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the plasma leptin concentration of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the plasma leptin concentration of the offspring rats . Data is given as mean of (n=4-8) ± SD (* $p< 0.05$, ** $p< 0.01$).

5.5. DETERMINATION OF SERUM GLUCOSE AND TRIGLYCERIDE LEVELS WITH IDEXX-VETTEST SYSTEMS

Serum glucose and triglyceride levels of male offspring rats for all experimental groups were determined with IDEXX-VetTest Systems which is mentioned in Section 4.2.4. Results were given in Table 5.12.

Table 5.12. Serum glucose and triglyceride levels of male offspring rats in all experimental groups. Data are given as mean of (n=5-8) ± SD.

Groups	Glucose (mmol/L)	Triglyceride (mmol/L)
Group 1 (SFD)	9.5 ± 0.8	0.9 ± 0.4
Group 1a (SFD-SFD)	17.8 ± 1.0	0.7 ± 0.04
Group 1b (SFD-HFD)	12.6 ± 1.4	0.5 ± 0.1
Group 1c (SFD-LFD)	12.8 ± 1.6	0.7 ± 0.2
Group 2 (HFD)	10.8 ± 1.2	0.3 ± 0.1
Group 2a (HFD-SFD)	16.3 ± 1.6	0.8 ± 0.4
Group 2b (HFD-HFD)	14.4 ± 1.4	0.9 ± 0.2
Group 2c (HFD-LFD)	16.5 ± 1.2	0.5 ± 0.1
Group 3 (LFD)	10.8 ± 1.2	1.6 ± 0.4
Group 3a (LFD-SFD)	14.7 ± 1.6	0.5 ± 0.09
Group 3b (LFD-HFD)	13.7 ± 1.0	0.7 ± 0.09
Group 3c (LFD-LFD)	10.8 ± 1.1	0.5 ± 0.1

5.5.1. Serum Glucose Levels in Male Offspring Sprague Dawley Rats

Serum glucose levels of pups which were dissected at the end lactation period was evaluated and given in Figure 5.20. There were no statistically significant differences in serum glucose levels between the groups.

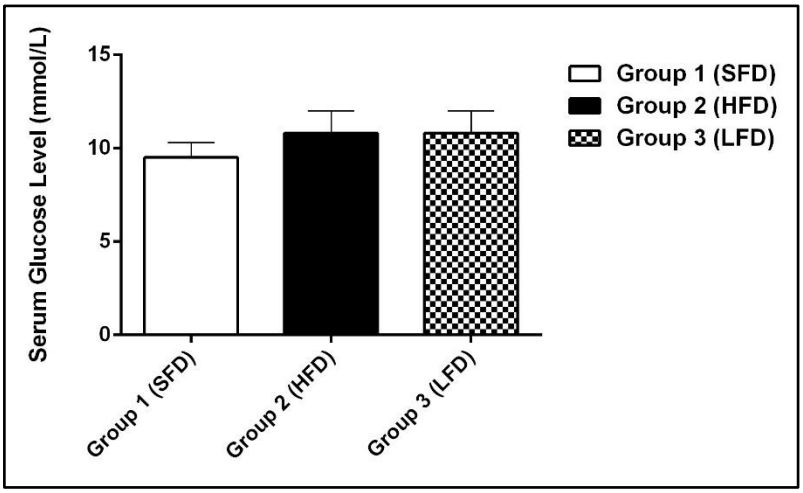


Figure 5.20. Serum glucose levels in the offspring rats at the end of the G-L periods. Data is given as mean of (n=6) ± SD.

At the end of the maturation period, the serum glucose levels of groups are present in Figure 5.21. It was determined that offsprings of Group 1a (SFD-SFD) have significantly higher serum glucose level than Group 1b (SFD-HFD) and Group 1c (SFD-LFD) ($p<0.01$) (Figure 5.21.a). Offsprings in Group 2b (HFD-HFD) has significantly lower serum glucose level than Group 2a (HFD-SFD) and Group 2c (HFD-LFD) ($p<0.05$) (Figure 5.21.b). Lastly, Group 3c (LFD-LFD) has significantly lower serum glucose level than Group 3a (LFD-SFD) and Group 3b (LFD-HFD) ($p<0.01$) (Figure 5.21.c).

On the other hand, it has been found that pups of Group 1a (SFD-SFD) has significantly higher serum glucose level than Group 3a (LFD-SFD) ($p<0.05$) (Figure 5.21.d). Among the pups of the 1b, 2b and 3b groups, serum glucose levels of Group 2b (HFD-HFD) have significantly higher than Group 1b (SFD-HFD) ($p<0.05$)(Figure 5.21.e). Finally, offsprings in the Group 2c (HFD-LFD) have significantly higher serum glucose level than Group 1c (SFD-LFD) and Group 3c (LFD-LFD) ($p<0.01$). In addition, Group 1c (SFD-LFD) has significantly higher serum glucose level than Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.21.f).

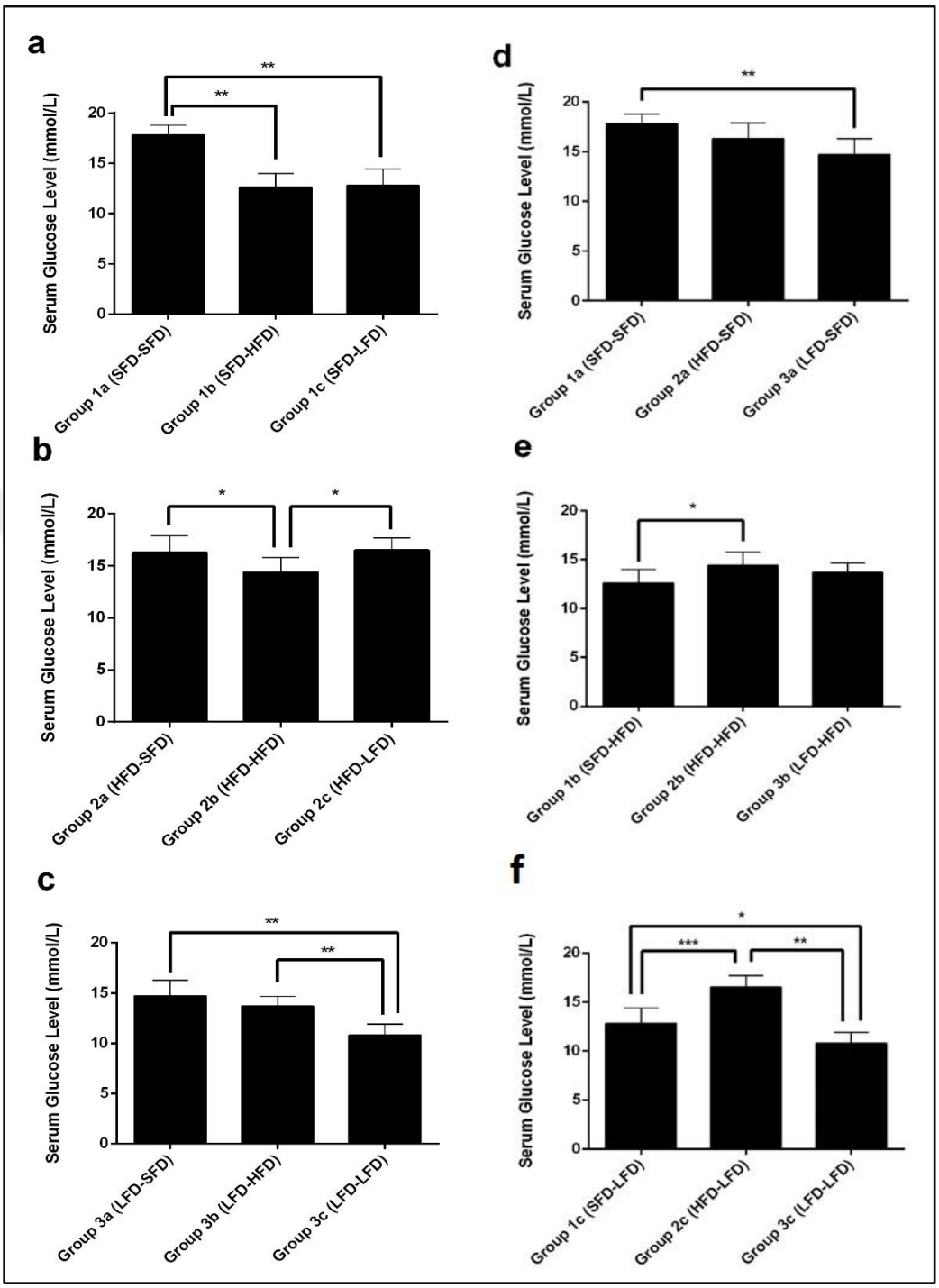


Figure 5.21. Serum glucose levels in the offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the serum glucose level of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the serum glucose level of the offspring rats . Data is given as mean of (n=5-8) ± SD (* $p< 0.05$, ** $p< 0.01$, *** $p<0.001$).

5.5.2. Serum Triglyceride Levels in Male Offspring Sprague Dawley Rats

Serum triglyceride levels of offsprings which were dissected at the end of the G-L period, are given in Figure 5.22. Offsprings of Group 2 (HFD) have significantly lower serum triglyceride level than offsprings of Group 1 (SFD) and Group 3 (LFD) ($p<0.05$).

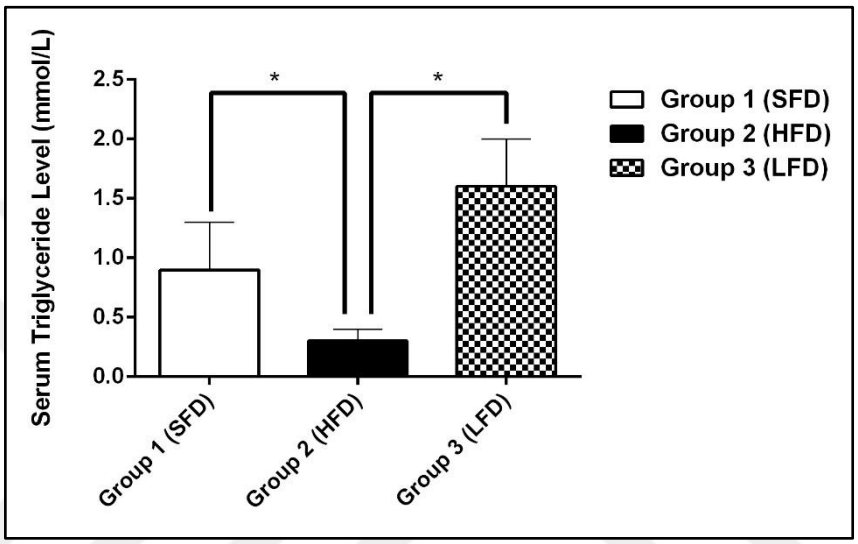


Figure 5.22. Serum triglyceride levels of the offspring rats at the end of the G-L periods. Data is given as mean of (n=4) ± SD (* $p< 0.05$).

At the end of the maturation period, the serum triglyceride levels of groups are present in Figure 5.23. It was determined that Group 1a (SFD-SFD) has significantly higher serum triglyceride level than Group 1b (SFD-HFD) ($p<0.05$) (Figure 5.23.a). Group 2b (HFD-HFD) has significantly increased serum triglyceride level than Group 2c (HFD-LFD) ($p<0.01$) (Figure 5.23.b). Finally, in the series of Groups 3 (a,b,c), Group 3b (LFD-HFD) has higher triglyceride level than other groups and but it was not significantly different (Figure 5.23.c).

Moreover, Group 1a (SFD-SFD) has significantly higher triglyceride level than Group 3a (LFD-SFD) ($p<0.05$) (Figure 5.23.d). Serum triglyceride level of offsprings in Group 1b (SFD-HFD) have significantly lower than Group 2b (HFD-HFD) ($p<0.05$)(Figure 5.23.e). Lastly, the offsprings of Group 1c, 2c and 3c had nearly much the same serum triglyceride level (Figure 5.23.f).

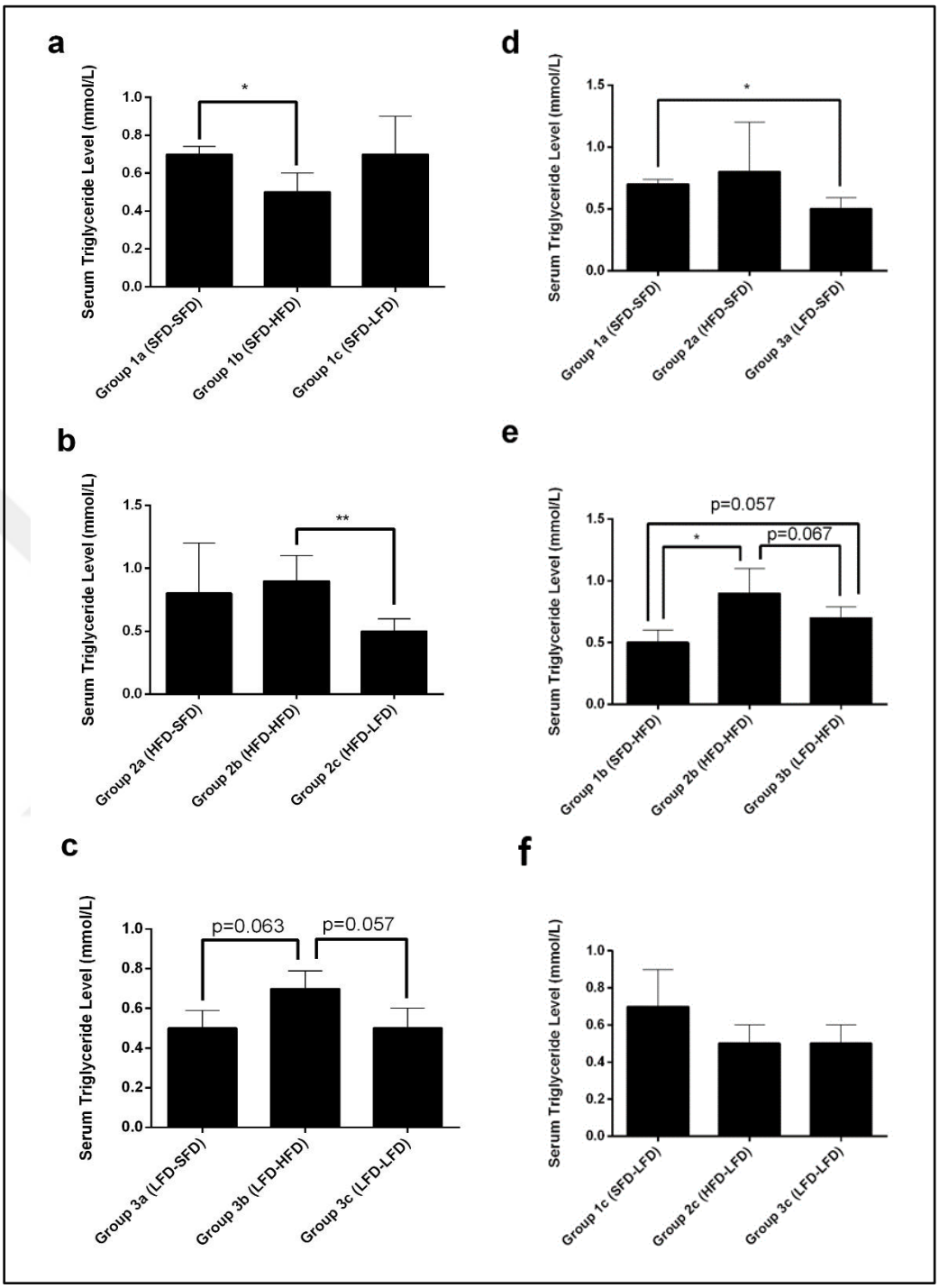


Figure 5.23. Serum triglyceride levels in the male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the serum triglyceride level of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the serum triglyceride level of the offspring rats. Data is given as mean of (n=4-8) ± SD (* $p < 0.05$, ** $p<0.01$)

5.6. H&E AND IFC STAINING OF CVP IN EXPERIMENTAL GROUPS

5.6.1. H&E and IFC Staining of CVP in Male Offspring Sprague Dawley Rats at the End of the G-L Periods

After the CVP was determined by H&E staining, the localization and expression of the CD36, GPR120 and alpha-gustducin (GNAT3) proteins on the CVP were demonstrated by single and double immunofluorescence staining. At the end of the G-L periods, CD36 and GPR120 protein expressions on CVP were observed to be close to each other in all experimental groups (Figure 5.24 & Figure 5.25).

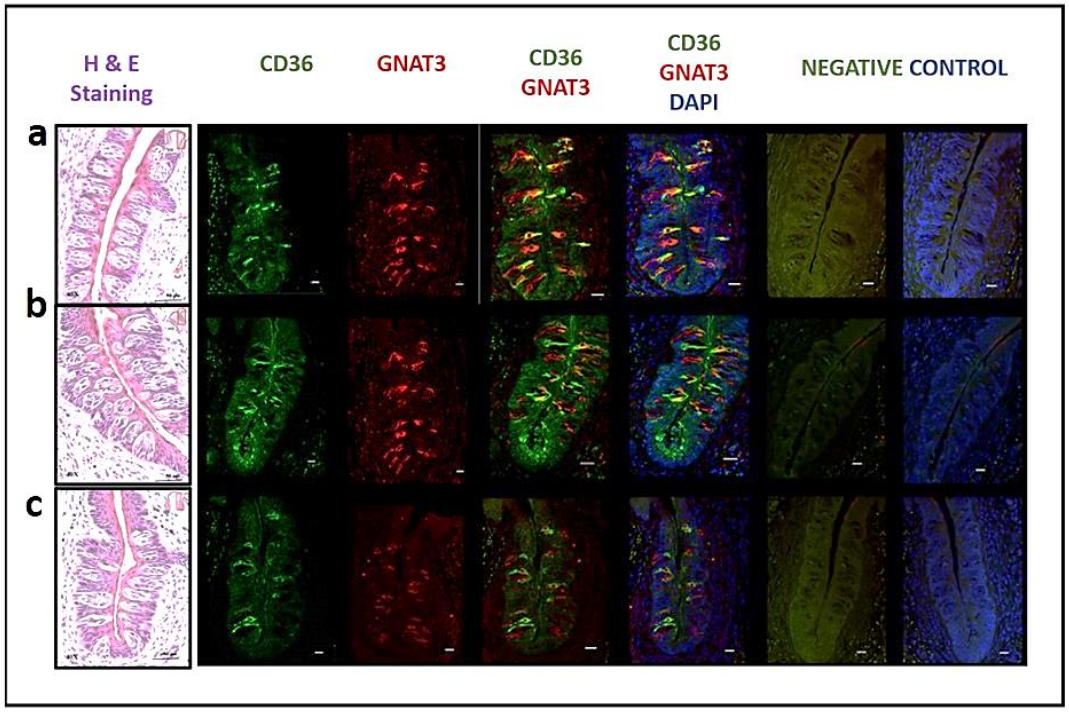


Figure 5.24. (a) H& E staining, CD36, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with SFD during G-L periods. (b) H& E staining, CD36, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with HFD during G-L periods. (c) H& E staining, CD36, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with LFD during G-L periods. 20X objective, scale bar 20 μm.

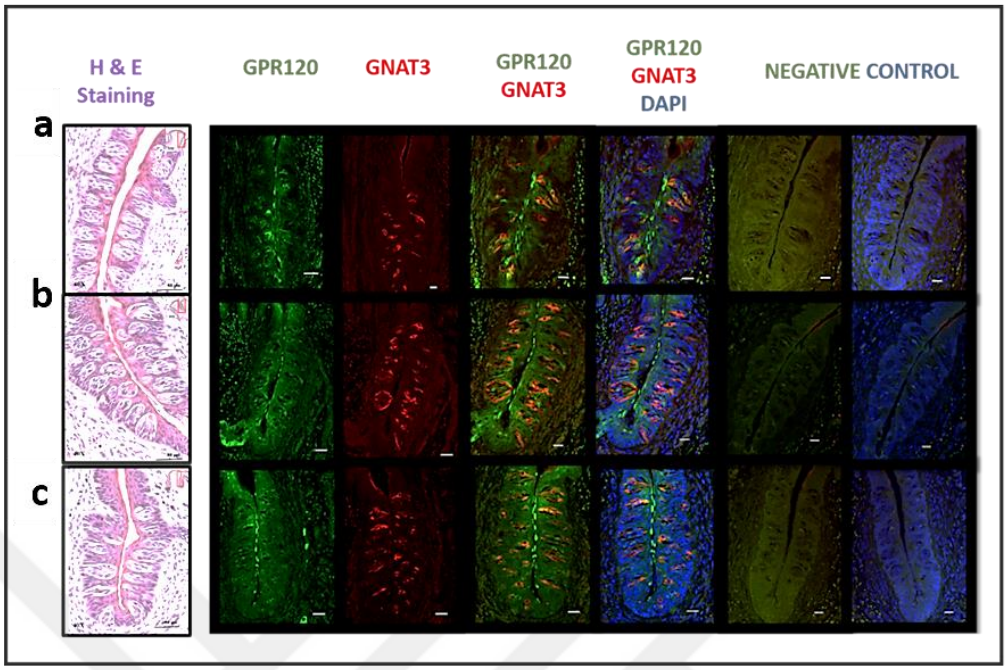


Figure 5.25. (a) H& E staining, GPR120, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with SFD during G-L periods. (b) H& E staining, GPR120, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with HFD during G-L periods. (c) H& E staining, GPR120, GNAT3 and DAPI immunofluorescence staining of CVP in offsprings which mother fed with LFD during G-L periods. Images were taken at 20X objective and scale bar represents 20 µm.

5.6.2. H&E Staining and Immunofluorescence Staining of CVP in Male Offspring Sprague Dawley Rats at the End of the Maturation Period

To investigate the effect of exposure to the different amount of fat containing diets during maternal period on the expression of CD36, series of the Groups a, Groups b and Groups c were observational evaluated from Figure 5.26. It has been found that Group 3a (LFD-SFD) and Group 3c (LFD-LFD) have higher CD36 expression level when compared with maternal SFD exposed groups. On the other hand, pups of Group 2a (HFD-SFD) and Group 2b (HFD-HFD), have decreased expression in CD36 on the CVP when compared with maternal SFD exposed groups.

Lastly, to figure out of the effect of the different amount of fat containing diets during maturation period on the CD36 expression, series of the Groups (1a, 1b, 1c), Groups (2a, 2b, 2c) and Groups (3a, 3b, 3c) were observationally evaluated. However, apart from the decreased expression on the Group 3b (LFD-HFD) than Group 3a (LFD-SFD) and Group 3c (LFD-LFD), no difference was observed generally between the subgroups. (Figure 5.26).

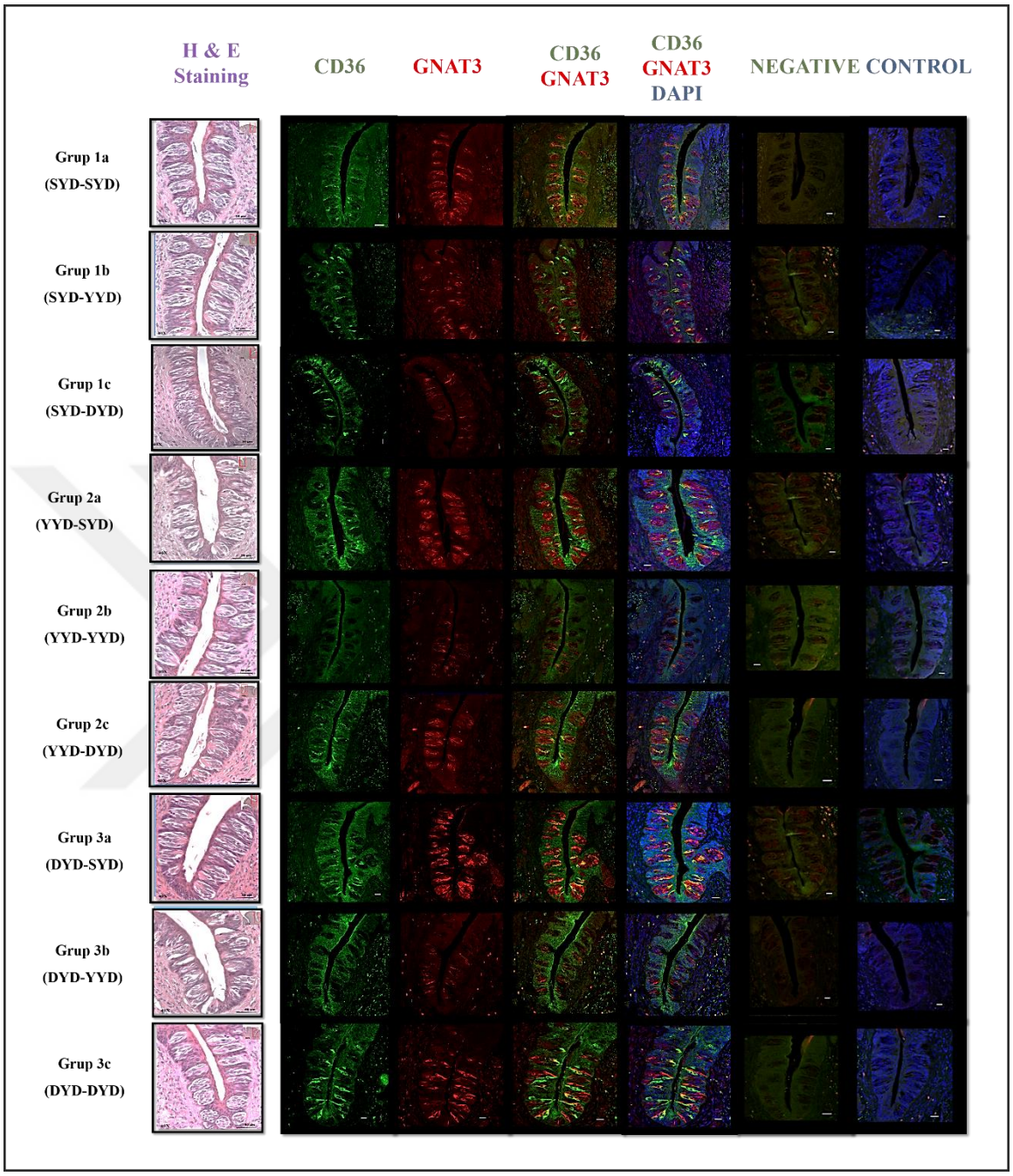


Figure 5.26. After the determination of CVP by H&E staining, demonstration of the localization and expression levels of CD36, GNAT3 and DAPI proteins in CVP of the sacrificed experimental groups at the end of the maturation period by single and double immunofluorescence staining. Images were taken at 20X objective and the scale bar represents 20 µm.

H&E staining and immunofluorescence staining results of GPR120 and GNAT3 proteins localized on the CVP of tongue tissues of sacrificed animals at the end of the maturation period has been also given in Figure 5. 27. When GPR120 immunopositive signals have evaluated observative, it has found that GPR120 positive signal increased in maternal LFD exposed experimental groups (Groups 3a, 3b, 3c) compared to SFD and HFD exposed groups during G-L periods. On the other hand, it has not determined any apparent differences in immunopositive GPR120 signalling between the subgroups in which the effect of exposed diets during the maturation was evaluated. In addition, excessively elevated immune positive GNAT3 signalling has noticed at the Group 2a (HFD-SFD) and Group 3a (LFD-SFD) when compared with other experimental groups (Figure 5.27).

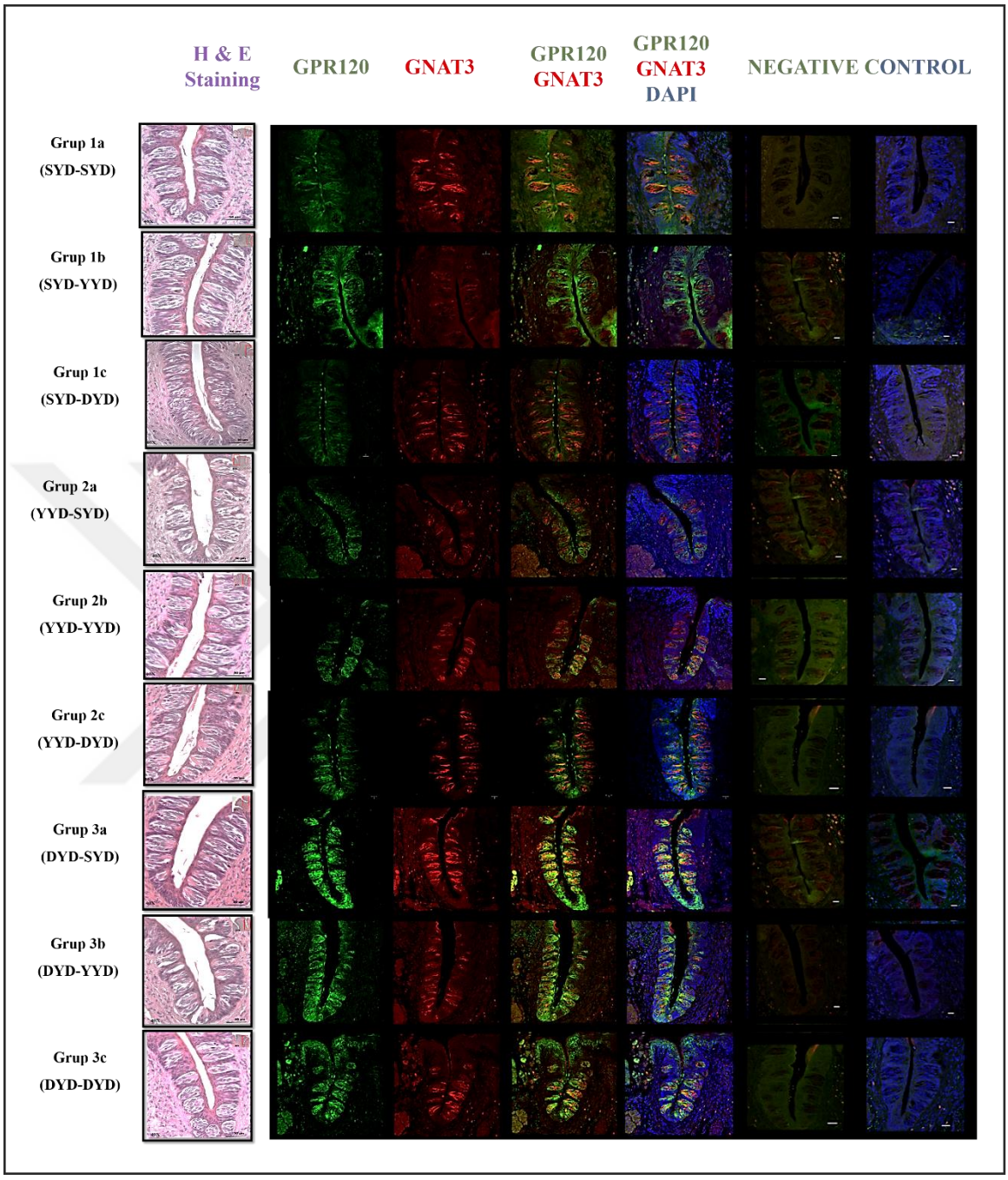


Figure 5.27. After the determination of CVP by H&E staining, demonstration of the localization and expression levels of GPR120, GNAT3 and DAPI proteins in CVP of the sacrificed experimental groups at the end of the maturation period by single and double immunofluorescence staining. Images were taken at 20X objective and scale bar represents 20 µm.

5.7. WESTERN BLOTTING

5.7.1. Determination of Expression Levels of CD36 and GPR120 Proteins in CVP of Male Offspring Sprague Dawley Rats at the End of the G-L Periods

The effect of exposure to the different amount of fat containing diets during maturation period on the expression levels of CD36, GPR120 and beta-actin proteins in CVP of the experimental groups were determined by western blotting as shown in Figure 5.28.

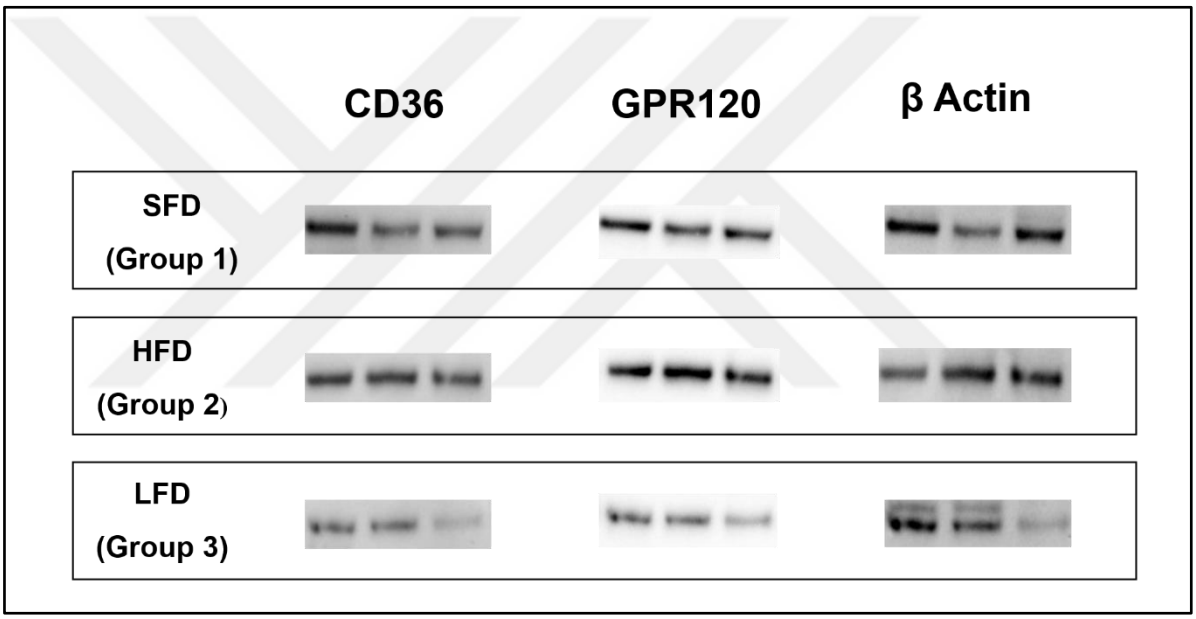


Figure 5.28. Representative image of bands of CD36, GPR120 and beta-actin proteins in CVP of male offspring rats at the end of the G-L periods.

The expression levels of CD36 and GPR120 proteins are present in Table 5.13 and statistical significance between groups were evaluated in Figure 5.29. There was not any significant different between the groups in terms of expression levels of CD36 and GPR120 proteins in the CVP (Figure 5.29).

Table 5.13. According to western blotting experiments, the expression levels of CD36/β Actin and GPR120/ β Actin in CVP of male offspring rats at the end of the G-L periods. Data are given as mean of (n=3-4) ± SD.

GROUPS	CD36 / β Actin	GPR120 / β Actin
Group 1 (SFD)	0,99 ± 0,28	0,81 ± 0,14
Group 2 (HFD)	1,25 ± 0,25	1,01 ± 0,21
Group 3 (LFD)	1,06 ± 0,19	1,22 ± 0,38

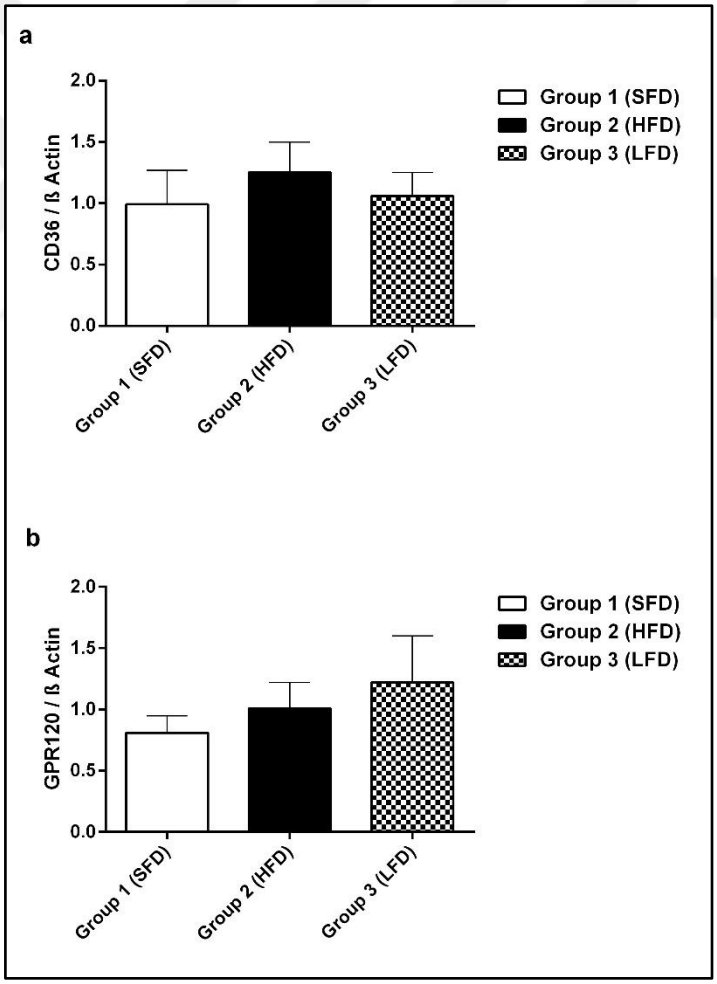


Figure 5.29. According to beta-actin, normalized expression levels of (a) CD36 and (b) GPR120 in CVP of male offspring rats at the end of the maternal period. Data are given as mean of (n=3-4) ± SD.

5.7.2. Determination of Expression Levels of CD36 and GPR120 Proteins in CVP of Male Offspring Sprague Dawley Rats at the End of the Maturation Periods

Western blot bands of CD36, GPR120 and beta-actin proteins of the sacrificed experimental animals at the end of the maturation period are shown in Figure 5.30. The calculated values of CD36/β actin and GPR120/β actin are presented in Table 5.14.

Table 5.14. According to western blotting experiments, the expression levels of CD36, GPR120 and beta-actin proteins in CVP of male offspring rats at the end of the maturation period. Data are given as mean of (n=4-6) ± SD.

Groups	CD36 / β Actin	GPR120 / β Actin
Group 1a (SFD-SFD)	0,5 ± 0,1	1,13 ± 0,19
Group 1b (SFD-HFD)	0,7 ± 0,3	1,63 ± 0,63
Group 1c (SFD-LFD)	0,3 ± 0,1	0,69 ± 0,1
Group 2a (HFD-SFD)	0,38 ± 0,07	1,03 ± 0,11
Group 2b (HFD-HFD)	0,1 ± 0,01	0,7 ± 0,25
Group 2c (HFD-LFD)	0,43 ± 0,08	1,34 ± 0,49
Group 3a (LFD-SFD)	1,08 ± 0,3	5,05 ± 0,45
Group 3b (LFD-HFD)	1,57 ± 0,21	4,97 ± 1,4
Group 3c (LFD-LFD)	1,39 ± 0,18	4,8 ± 1,1

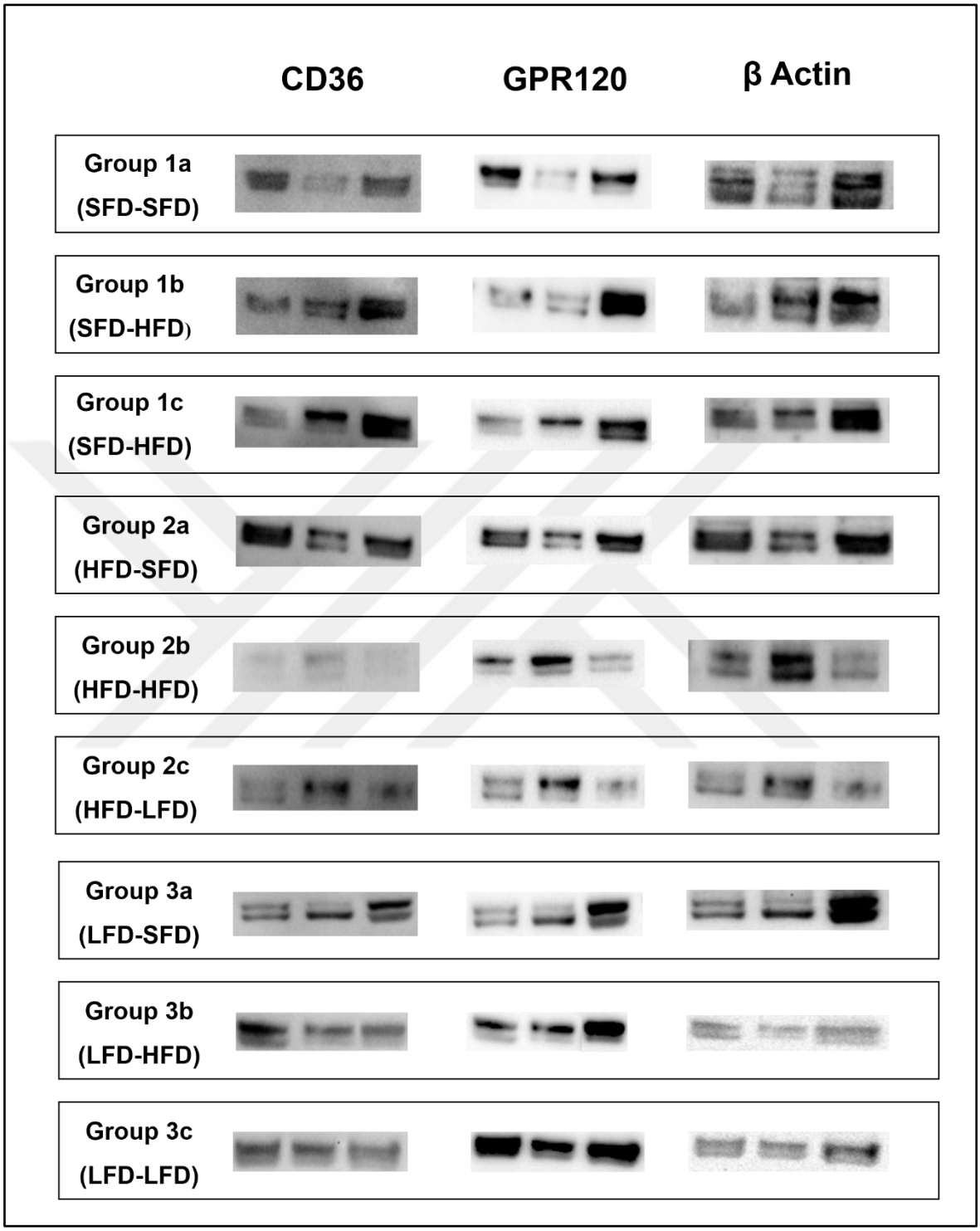


Figure 5.30. Representative image of bands of CD36, GPR120 and β actin proteins in CVP of male offspring rats at the end of the maturation period.

After the maturation period, the lingual CD36 expression levels of experimental groups are evaluated and shown in Figure 5.31. In the offsprings of Group 1c (SFD-LFD) has significantly decreased CD36 expression level in CVP than offsprings of Group 1a (SFD-SFD) and Group 1b (SFD-HFD) ($p<0.05$) (Figure 5.31.a). Additionally, Group 2b (HFD-HFD) has significantly lower CD36 expression level than Group 2a (HFD-SFD) and Group 2c (HFD-LFD) ($p<0.05$) (Figure 5.31.b).

On the other hand, CD36 expression levels of in Group 2a (HFD-HFD) significantly lower than Group 3a ($p<0.05$) (Figure 5.31.d). Similarly, CD36 expression levels of offsprings in Group 2b (HFD-HFD) significantly lower than Group 1b (SFD-HFD) and Group 3b (LFD-HFD) ($p<0.05$). Additionally, offsprings of Group 3b (LFD-HFD) have significantly higher CD36 expression levels than Group 1b (SFD-HFD) ($p<0.05$) (Figure 5.31.e). Finally, offsprings of Group 3c (LFD-LFD) have significantly higher expression levels of CD36 in CVP when compared with Group 1c (SFD-LFD) and Group 2c (HFD-LFD) ($p<0.05$) (Figure 5.31.f)

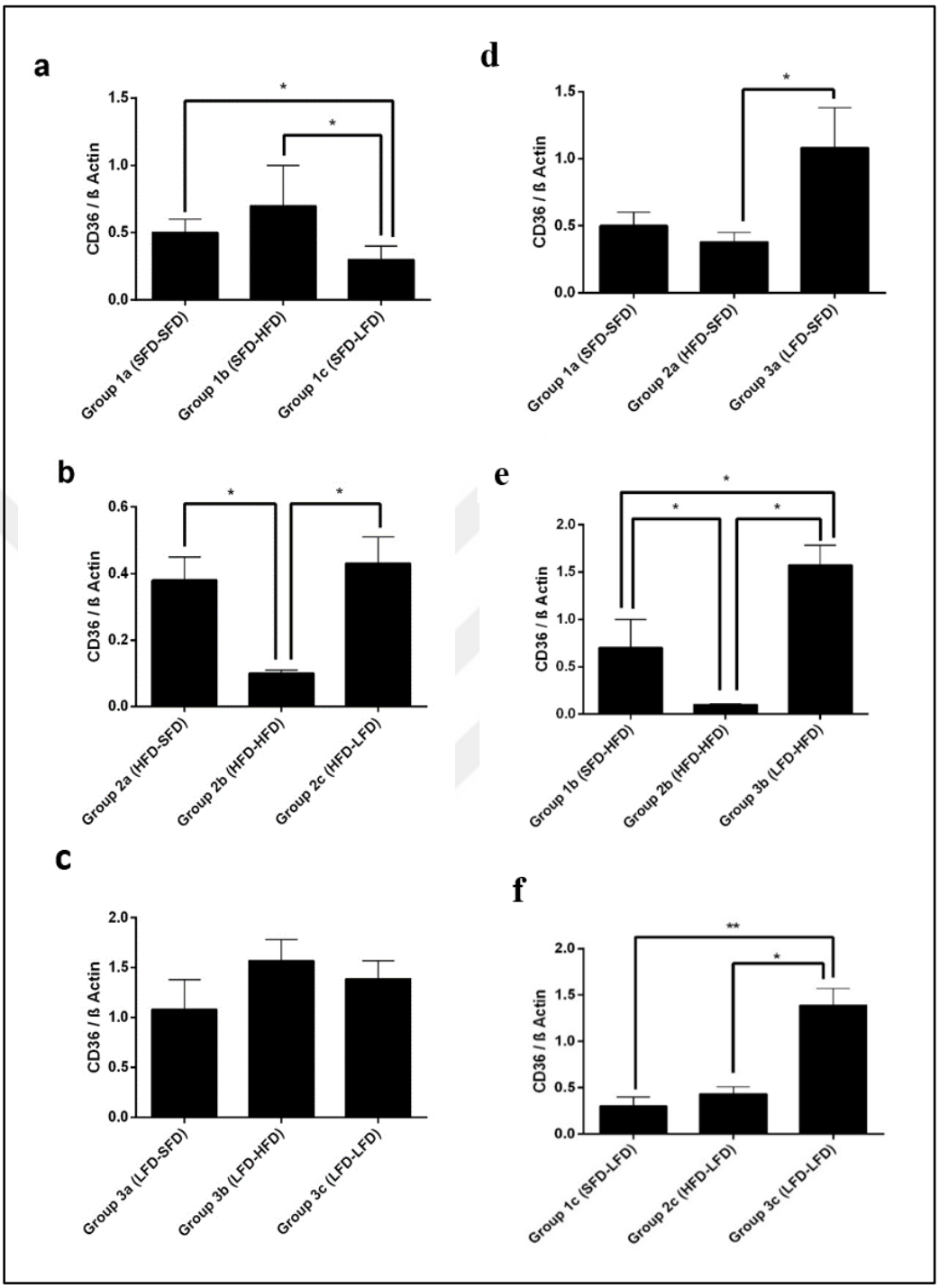


Figure 5.31. CD36/β Actin expression levels in CVP of male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the CD36 expression level of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the CD36 expression level of the offspring rats. Data is given as mean of (n=3-6) ± SD (* $p < 0.05$).

After the maturation period, the GPR120 expression levels in CVP of experimental groups are evaluated and shown in Figure 5.32. It was found that offsprings in Group 1b (SFD-HFD) have significantly higher GPR120 expression level than Group 1c (SFD-LFD) ($p<0.05$) (Figure 5.32.a). However, there were not any statistical significant relationship in the series of Groups 2(a,b,c) and Groups 3(a,b,c) (Figure 5.32.b) (Figure 5.32.c).

On the other hand, the offsprings in Group 2a (HFD-HFD) have significantly lower GPR120 level in CVP than Group 3a (LFD-SFD) ($p<0.05$) (Figure 5.32.d). GPR120 expression levels of Group 2b (HFD-HFD) has significantly lower CD36 protein expression level than Group 1b (SFD-HFD) and Group 3b (LFD-HFD) ($p<0.05$). Additionally, the GPR120 expression levels of pups in the Group 3b (LFD-HFD) were significantly higher than Group 1b (SFD-HFD) ($p<0.05$) (Figure 5.32.e). Finally, it was found that the expression levels of GPR120 in CVP of Group 3c (LFD-LFD) pups was significantly increased than Group 1c (SFD-LFD) and Group 2c (HFD-LFD) ($p<0.05$). And also, Group 2c (HFD-LFD) has significantly higher lingual GPR120 expression level than Group 1c (SFD-LFD) ($p<0.05$) (Figure 5.32.f)

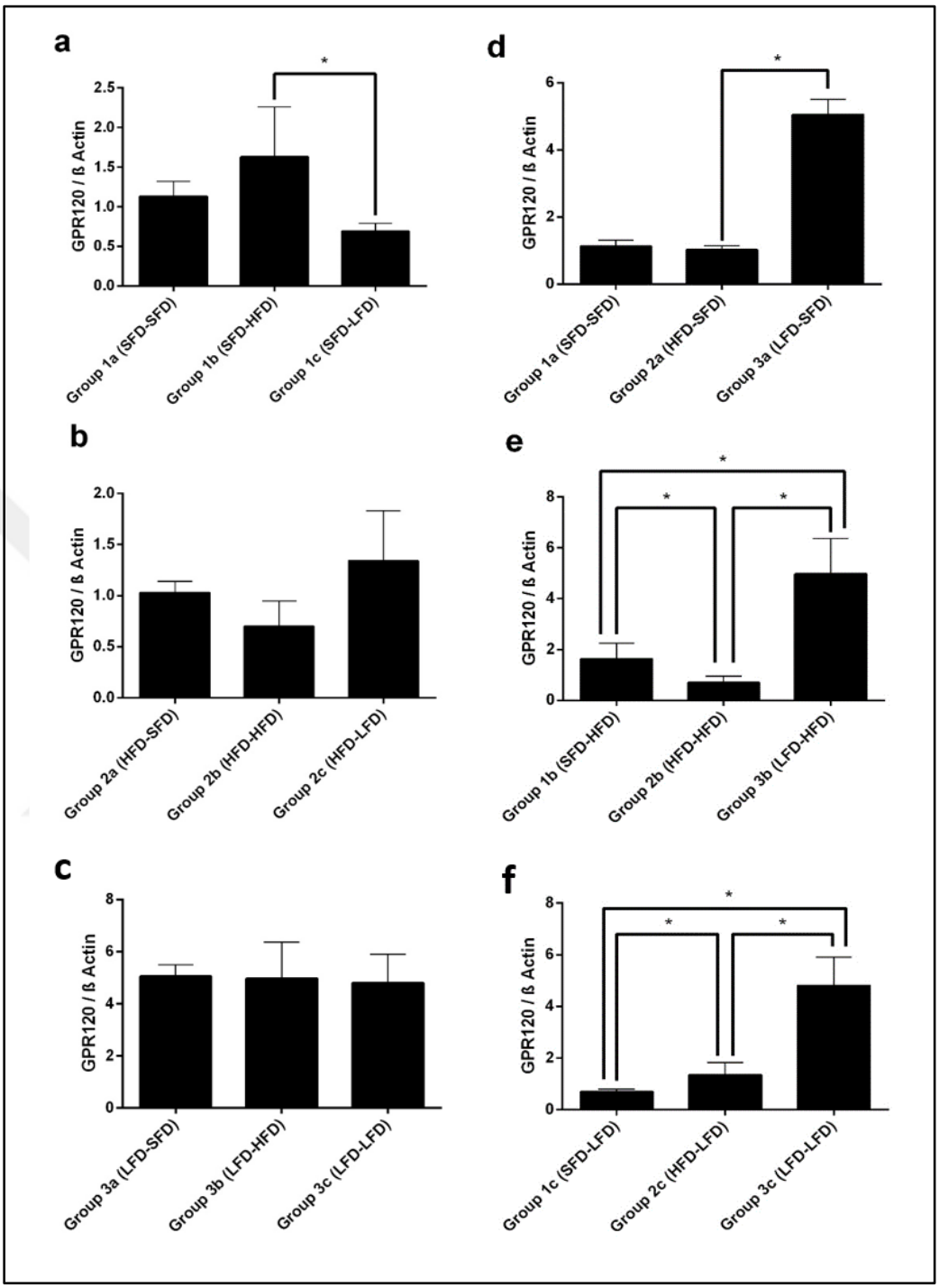


Figure 5.32. GPR120/β Actin expression levels in CVP of male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the GPR120 expression level of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the GPR120 expression level of the offspring rats. Data is given as mean of (n=3-6) ± SD (* $p < 0.05$).

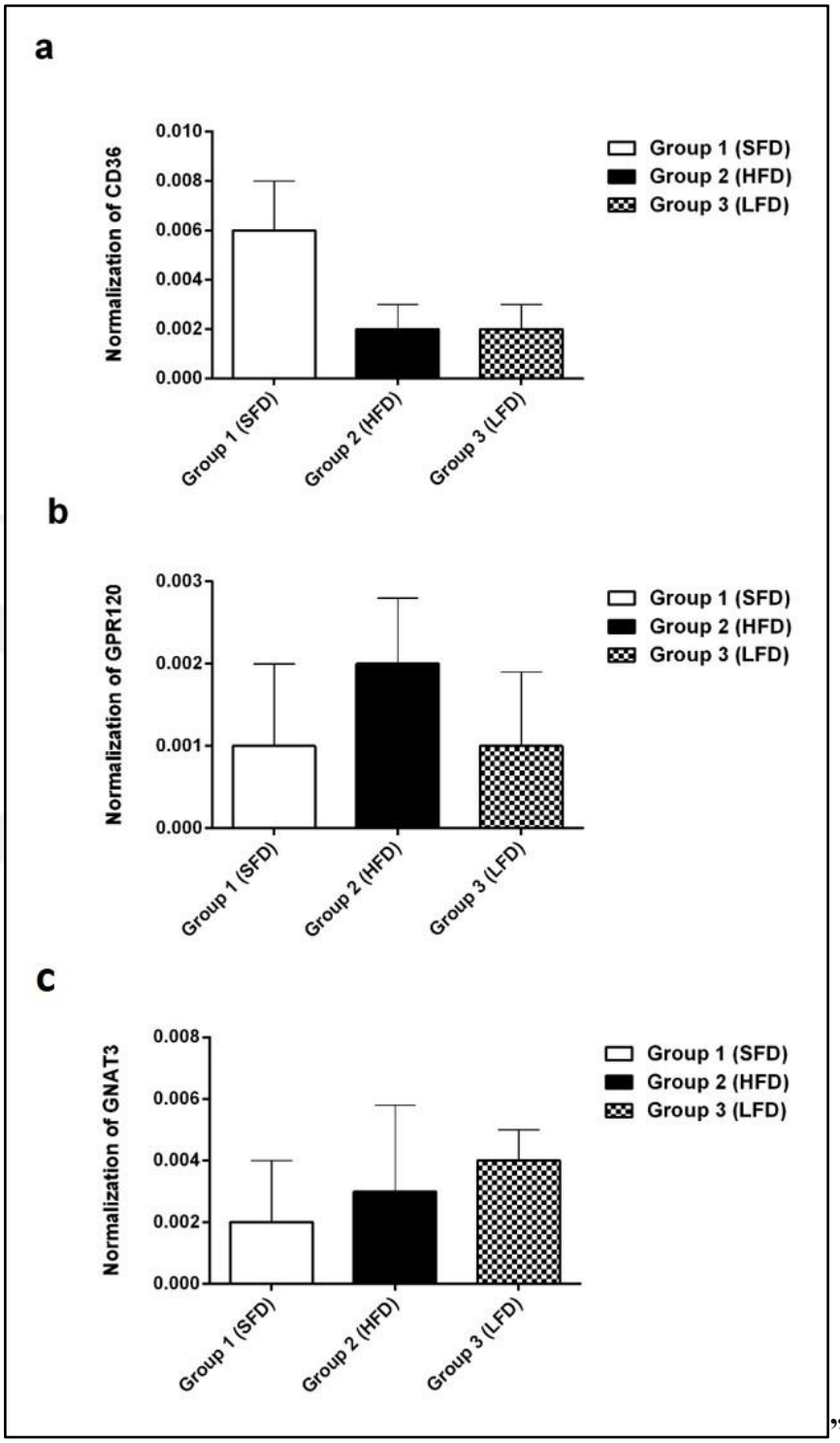
5.8. RT PCR

5.8.1. Determination of mRNA Levels of CD36, GPR120 and GNAT3 in CVP of Male Offspring Sprague Dawley Rats at the End of the G-L Periods

According to beta-actin Ct values, normalized mRNA values of CD36, GPR120 and GNAT3 in CVP for pups of Group 1 (SFD), Group 2 (HFD) and Group 3 (LFD) which are dissected at the end of the lactation period were given in Table 5.15. In addition, the statistical evaluation between groups was performed and showed in Figure 5.33. There were not any significant differences in mRNA levels of CD36, GPR120 and GNAT3 between groups.

Table 5.15. According to β Actin Ct values, the normalized mRNA levels of CD36, GPR120 and GNAT3 to in CVP of male offspring rats at the end of the G-L periods. Data are given as mean of (n=3-4) ± SD.

GROUPS	Normalization of CD36 mRNA level	Normalization of GPR120 mRNA level	Normalization of GNAT3 mRNA level
Group 1 (SFD)	0,006 ±0,002	0,001 ±0,001	0,002 ±0,002
Group 2 (HFD)	0,002 ±0,001	0,002 ±0,0008	0,003 ±0,0028
Group 3 (LFD)	0,002 ±0,001	0,001 ±0,0009	0,004 ±0,001



”

Figure 5.33 Normalized values of (a) CD36, (b) GPR120 and (c) GNAT3 mRNA levels of Group 1 (SFD), Group 2 (HFD) and Group 3 (LFD). Data are given as mean of (n=3-4) ± SD.

5.8.2. Determination of mRNA Levels of CD36, GPR120 and GNAT3 in CVP of Male Offspring Sprague Dawley Rats at the End of the Maturation Periods

According to beta-actin Ct values, normalized mRNA values of CD36, GPR120 and GNAT3 in CVP of experimental groups at the end of the maturation period were given in Table 5.16.

Table 5.16. According to β Actin Ct values, the normalized mRNA levels of CD36, GPR120 and GNAT3 to in CVP of male offspring rats at the end of the maturation period. Data are given as mean of (n=3-6) ± SD.

GROUPS	Normalization of CD36 mRNA level	Normalization of GPR120 mRNA level	Normalization of GNAT3 mRNA level
Group 1a (SFD-SFD)	0,014 ± 0,012	0,18 ± 0,02	0,03 ± 0,01
Group 1b (SFD-HFD)	0,001 ± 0,0005	0,06 ± 0,02	0,01 ± 0,004
Group 1c (SFD-LFD)	0,001 ± 0,0001	0,19 ± 0,07	0,003 ± 0,002
Group 2a (HFD-SFD)	0,006 ± 0,003	0,98 ± 0,08	0,45 ± 0,1
Group 2b (HFD-HFD)	0,007 ± 0,002	0,27 ± 0,08	0,05 ± 0,01
Group 2c (HFD-LFD)	0,003 ± 0,001	0,28 ± 0,04	0,03 ± 0,02
Group 3a (LFD-SFD)	0,006 ± 0,001	2,21 ± 1,37	0,26 ± 0,12

Group 3b (LFD-HFD)	0,180 ± 0,163	0,14 ± 0,05	0,03 ± 0,001
Group 3c (LFD-LFD)	0,002 ± 0,001	0,1 ± 0,02	0,004 ± 0,003

According to beta-actin Ct values, normalized mRNA values of CD36 in the CVP of of the experimental groups were statistically evaluated and shown in Figure 5.34. The offsprings of Group 2b (HFD-HFD) have significantly higher lingual CD36 mRNA level than Group 2c (HFD-LFD) ($p<0.05$) (Figure 5.34.b). Offsprings of Group 3b (LFD-HFD) have significantly higher CD36 mRNA level compared to Group 3a (LFD-SFD) and Group 3c (LFD-LFD) ($p<0.05$). In addition, lingual CD36 mRNA levels of Group 3a (LFD-SFD) has significantly higher than Group 3c (LFD-LFD) (Figure 5.34.c).

Moreover, among the groups of 1b, 2b and 3b, CD36 mRNA levels of Group 1b (SFD-HFD) has significantly lower than Group 2b (HFD-HFD) and Group 3b (LFD-HFD) ($p<0.05$). And also, CD36 mRNA levels of Group 2b (HFD-HFD) were significantly decreased compared to Group 3b (LFD-HFD) ($p<0.05$) (Figure 5.34.e). Lastly, CD36 mRNA levels of Group 1c has signifcantly decreased mRNA levels than Group 2c and Group 3c (Figure 5.34.f).

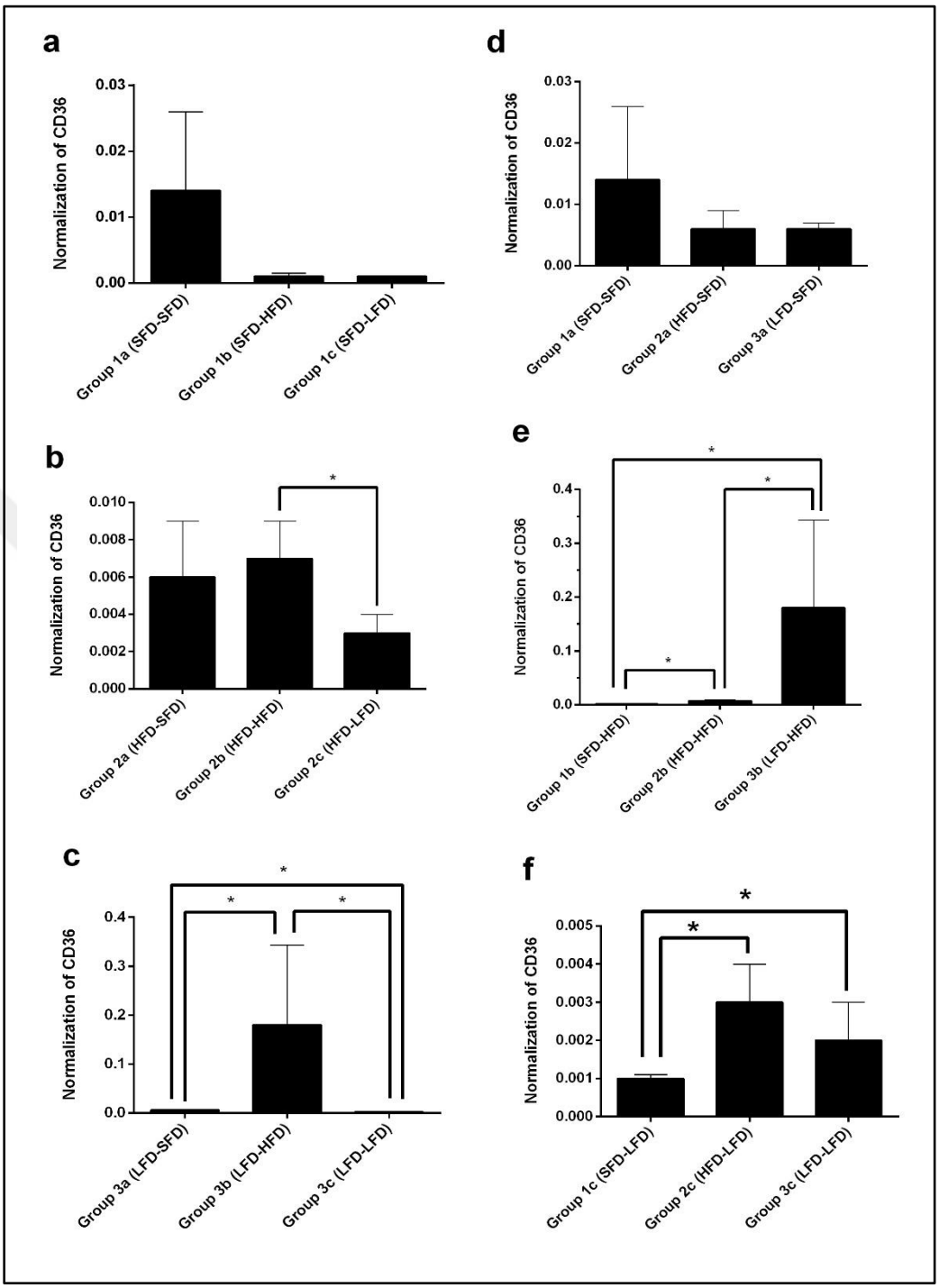


Figure 5.34. Normalized values of CD36 mRNA levels in CVP of male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the CD36 mRNA levels of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the CD36 mRNA level of the offspring rats. Data is given as mean of (n=3-6) ± SD (* $p < 0.05$)

According to beta-actin Ct values, normalized mRNA values of GPR120 in the CVP of of the experimental groups were statistically evaluated and shown in Figure 5.35. It was determined that the offsprings of Group 2a (HFD-SFD) have significantly higher GPR120 mRNA level than Group 2b (HFD-HFD) ($p<0.05$) (Figure 5.35.b). In addition, GPR120 mRNA levels in the CVP of Group 3a (LFD-SFD) were significantly higher than Group 3b (LFD-HFD) and Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.35.c). In addition, it was found that GPR120 mRNA levels of Group 1b (SFD-HFD) were significantly lower than Group 2b (HFD-HFD) ($p<0.05$) (Figure 5.35.e).

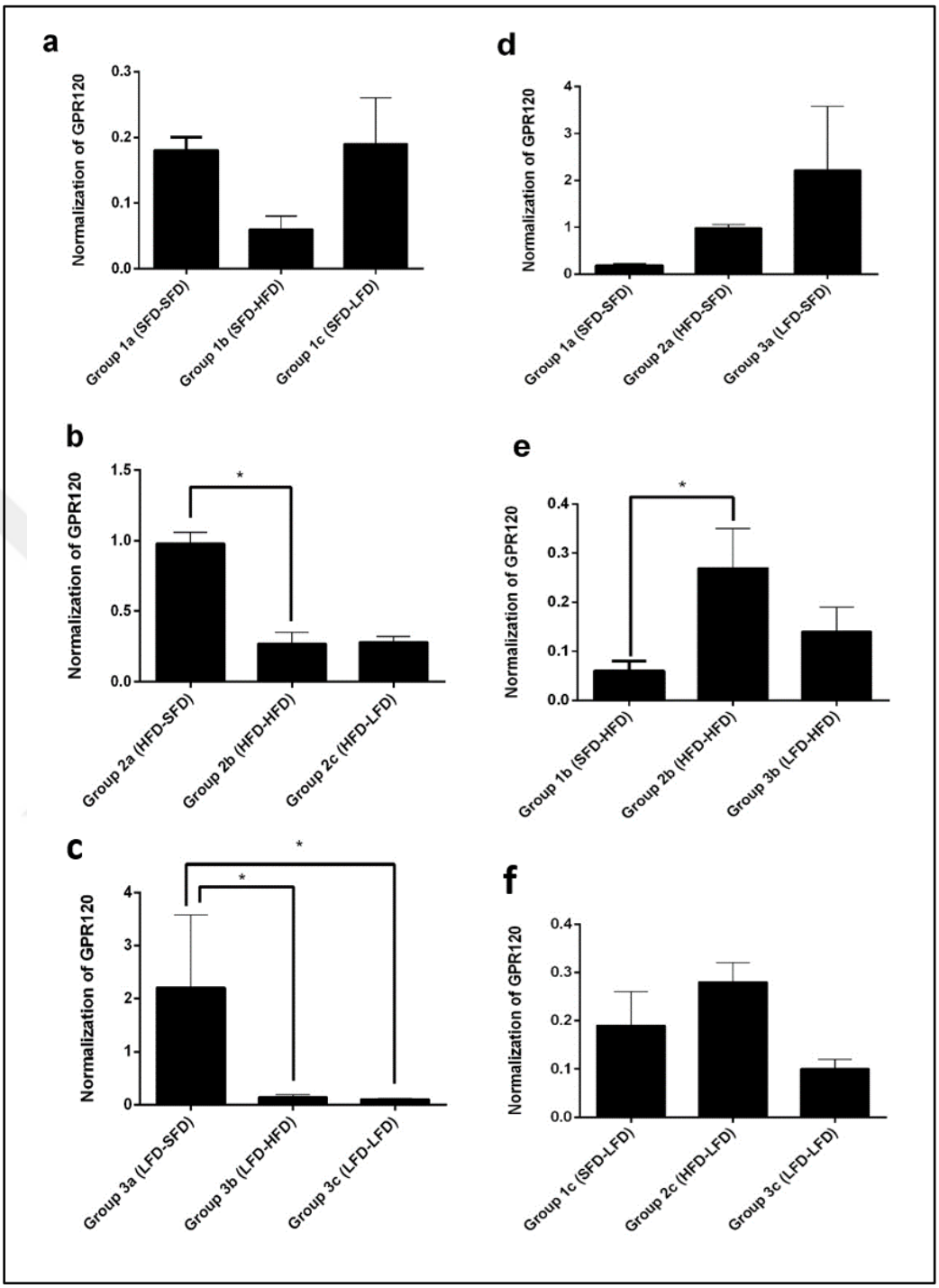


Figure 5.35. Normalized values of GPR120 mRNA levels in CVP of male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the GPR120 mRNA levels of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the GPR120 mRNA level of the offspring rats. Data is given as mean of (n=3-6) ± SD (* $p < 0.05$).

According to beta-actin Ct values, normalized mRNA values of GNAT3 as taste bud marker in the CVP of of the experimental groups were statistically evaluated and shown in Figure 5.36. It was determined that the offsprings of Group 2a (HFD-SFD) have significantly higher GNAT3 mRNA levels in CVP than Group 2b (HFD-HFD) and Group 2c (HFD-LFD) ($p<0.05$) (Figure 5.36.b). Similarly, GNAT3 mRNA levels in offsprings of Group 3a (LFD-SFD) were significantly higher than Group 3b (LFD-HFD) and Group 3c (LFD-LFD) ($p<0.05$) (Figure 5.36.c).

On the other hand, it was revealed that the GNAT3 mRNA levels in CVP of Group 3a (LFD-SFD) were significantly higher than Group 1a (SFD-SFD) ($p<0.05$) (Figure 5.36.d). Additionally, offsprings of Group 1b (SFD-HFD) have significantly lower GNAT3 mRNA levels in CVP than Group 2b (HFD-HFD)($p<0.05$) (Figure 5.36.e). Lastly, Group 2c (HFD-LFD) have significantly higher GNAT3 mRNA level in CVP when compared with Group 1c (SFD-LFD) and Group 3c (LFD-LFD) ($p<0.05$)(Figure 5.36.f).

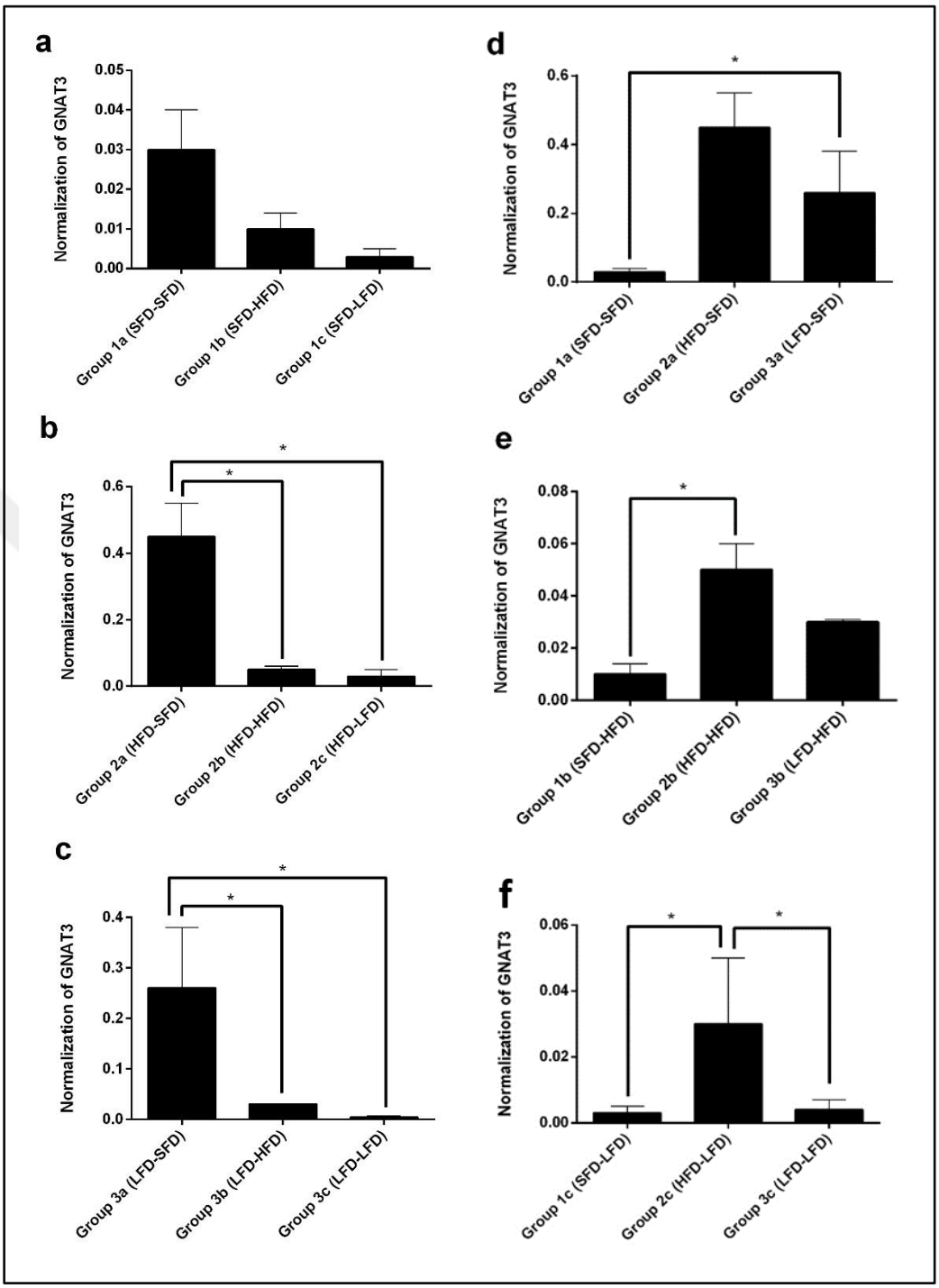


Figure 5.36. Normalized values of GNAT3 mRNA levels in CVP of male offspring rats at the end of the maturation period. (a, b, c): The evaluation of the effect of different amount of fat-containing diets administration during the maturation period on the GNAT3 mRNA levels of the offspring rats. (d, e, f): The evaluation of different amount of fat-containing diets administration during maternal period on the GNAT3 mRNA level of the offspring rats. Data is given as mean of (n=3-6) ± SD (* $p < 0.05$).

6. DISCUSSION

Fats as a macronutrient have the highest energy content in the diet and mostly preferred by obese individuals because of its smell, taste, flavour and textural properties. Lingual fatty acid receptors CD36 and GPR120 play an important role in the preference and perception of fatty taste but the regulation mechanisms of these receptors are not clear [4, 5]. It is known that obesity develops due to more consumption of fatty foods because of the desensitization of fatty taste. The exposed diet affetcs the preference and perception of fatty taste. HFD-induced obese rats have some dysregulations in oro-sensorial fatty taste perception system [236]. Many animal studies show that feeding the animals with HFD during maternal period leads to an increase in eating behaviour, hyperphagia and more fat consumption during adulthood [6, 7]. On the other hand, it is shown that individual experience in taste perception starts in the prenatal period [145, 154, 268, 269, 270, 271]. The researchers who focus the reason of the maternal obesity suspect that the increased neuronal activity in the hunger-satiety centers of the brain and opioid and dopaminergic receptor levels in the mesolimbic system [7, 8, 9, 10]. However, the relationship between maternal nutrition status and expression levels of lingual CD36/GPR120 receptors in the perception /preference of fatty taste is not yet clear. Our study aims to clarify the relationship between oro-gustatory fat perception mechanism and maternal nutrition status in offspring Sprague Dawley rats.

In our study, mother rats were exposed to special diets which contain different concentrations of fat during G-L periods. Weight measurements, food consumption and energy intake of the mothers were followed during these periods. Our findings revealed that food consumption was significantly lower in HFD-fed mother rats but there were no statistically significant differences between the groups for body weight and energy intake. The reason for different food consumptions and energy intake between groups is related to energy content of the diets. Our results are consistent with the fact that energy intake is parallel with the weight gain. On the other hand, according to our results the weight of maternal-HFD exposed male pups were significantly higher than maternal- LFD exposed group at the end of the G-L period. This demonstrates that the primary effect of exposed diet during maternal period dominantly affects the offsprings' body weight. On the other hand, how much fat the animals have been exposed to during G-L periods was calculated, evaluated and significant differences were determined between the groups. This confirms

that the experimental maternal fat exposure model which constitutes the first stage of our study was performed succesfully.

Our study has reported that serum glucose levels were close to each other at the end of G-L periods. Consistently, plasma insulin levels of offsprings' were parallel with glucose levels at the end of G-L periods. In addition, we have found that plasma leptin levels were significantly higher in the HFD exposed pups (Group 2) during the G-L (maternal) period when compared to the other groups. This suggests that increased body weight in Group 2 may be related to elevated plasma leptin levels.

Male offsprings were exposed to three different fat concentrations (SFD, HFD, LFD) containing diets during the maturation period. The animal body weights and energy intake were measured. As expected the weight gain and energy intake were significantly higher in the group 1b, 2b and 3b which were fed with HFD during the maturation period when compared to LFD and SFD fed pups during the maturation period. The calculated results of fat exposure during the maturation period show that our experimental model was done successfully.

Two-bottle preference tests for 48 h were performed to determine the fatty taste preferences at the end of the maturation period. In the first stage of this test, the test solution (0.02 percent rapeseed oil in 0.3 percent xanthan gum) and the control solution (0.3 percent xanthan gum) were given to the animals and there was no statistically significant difference between the groups. This situation could be explained by the fact that the oil concentration of the given solution was too low for detection by the gustatory system. In the second stage of TBPT, the test solution (0.2 percent rapeseed oil in 0.3 percent xanthan gum) and the control solution (0.3 percent xanthan gum) was given to the animals during 48 hours. At the end of the test, it was found that Group 3b (LFD-HFD) has significantly decreased oil preference for in both maternal subgroups (Group 1b, 2b, 3b) and maturation subgroups (Group 3a, 3b, 3c). In the last stage of TBPT, the test solution (2 percent rapeseed oil in 0.3 percent xanthan gum) and the control solution (0.3 percent xanthan gum) was given to animals. It was determined that the maternal HFD-fed subgroups (2a, 2b, 2c) have higher preferences for 2 percent rapeseed oil solution compared to maternal SFD and LFD-fed groups. In addition, the preferences in Group 2a, 2b and 2c were similar. This finding indicates that HFD exposure in the maternal period

leads to an elevated preference for a higher concentration of oily solution independent with the presented fat concentration during the maturation period. Consistently, many of animal studies have shown that HFD exposure during G-L preriods leads to the development of the hyperphagia and increased the fatty foods preferences during adulthood of the offspring. [6, 276, 277]. On the other hand, we found that Group 1b (SFD-HFD) and Group 3b (LFD-HFD) have lower preferences for 2 percent rapeseed oil. This can be related to the amount of fat in diet. Feeding the animals with HFD may be enough. But there was a tendency to increased fatty solution preference in Group 2b (HFD-HFD).This can be related to the impaired fatty taste perception system because of high fat. Lastly, Group 1c (SFD-LFD) has a higher preference to 2 percent rapeseed oil solution when compared to Group 1a (SFD-SFD). Similarly, Nakashima and Sato have demonstrated that feeding the mother rats with LFD (percent10 kcal,fat originated) during G-L periods causes a strong preference for fatty solution in offsprings [275]. These LFD-fed animals were exposed to fat restriction during maturation period, the increased preferences could be related to compensating the fat deficiency in the body with metabolic signalling or oro-sensorial regulations.

In the literature, Chevrot et al.performed the 12h-TBPT protocol to DIO mice and control groups. Consequently, they reported that the obese mice did not display a preference or an avoidance in consumption percentage of 0.02 percent rapeseed oil solution (~ 50 percent). On the other hand, obese mice less preferred the 2 percent rapeseed oil solution when compared to the control group. Chevrot et al. explained this situation with abnormalities occurring in reward centers in obesity [238]. In our study, the similar result with Chevrot et al. was obtained in Group 3b (LFD-HFD). However, low-fat taste preferences in this experimental group thought us the question "the perception of fatty taste in animals is impaired or if the amount of fat in their diet is sufficient?" The answer to this question can be explained by western blot and immunofluorescence results within CVP, the start point of the oro-gustatory system.

After the TBPT, blood and tongue tissues of the rats were taken and the experimental protocol was terminated. According to the blood biochemistry results, plasma leptin concentration increased in HFD fed animals during maturation when compared to SFD and LFD exposed subgroups. On the other hand, plasma insulin concentration increased in LFD exposed experimental groups (groups 1c, 3c). In addition, it was determined that

maternal high fat exposure (Group 2a) causes higher plasma insulin levels in offsprings. As similar with our results,feeding the mothers with HFD during G-L periods was caused the increased adipose mass and development of leptin and insulin resistance in offsprings [285, 286, 287]. In addition, the higher preference of fatty taste in maternal HFD exposed groups could be associated with elevated insulin and leptin levels due to alteration in hunger-satiety centers.

Lingual fatty acid-binding receptors CD36 and GPR120 expressions were determined at the end of G-L (maternal) period. CD36 and GPR120 protein levels in CVP in G-L groups were similar according to western blotting and IFC experiments. Consistently, mRNA levels of CD36, GPR120 and GNAT3 in these groups were similar with protein expression levels. We have not revealed any oro-sensorial differences in terms of lingual fatty acid-binding receptors. However, at the end of the maturation period, we found that Group 1c (SFD-LFD) has a decreased expression of GPR120 when compared to Group 1b (SFD-HFD), Group 2c (HFD-LFD) and Group 3c (LFD-LFD). Moreover, Group 1c (SFD-LFD) has lower expression in CD36 when compared to Group 1b (SFD-HFD), Group 1a (SFD-SFD) and Group 3c (LFD-LFD). These results indicate that significantly increased preference to 2 percent rapeseed oil solution in Group 1c could be related to decreased levels of CD36 and GPR120 protein expressions. According to RT-PCR results, mRNA levels of CD36 and GPR120 in Group 1c were not significantly different when compared to other groups. This indicates that the post-transcriptional or translational regulations of these proteins could be effective in protein expression levels.

In our study, it was revealed that the protein expression levels of CD36 and GPR120 receptors decreased in CVP of maternal HFD exposed groups (Groups 2a, 2b, 2c) when compared to other groups. On the other hand, CD36 and GPR120 expression levels increased in the maternal LFD fed animals (Groups 3a, 3b, 3c). In the literature, Zhang et al. have found that CD36 expression level in the CVP was lower in the HFD-induced obese rats compared to control group [236]. This suggests that the maternal HFD exposure in our experimental model could be cause to dysregulation in the oro-sensorial system. In contrast to the study by Treesukkosol et al. have claimed that there was no major effect of maternal diet on the intake of corn oil emulsions according to brief access test and maternal HFD induced differences in the diet preference also is not related to the alteration in the oro-sensory component of the taste stimulation [9]. They have suspected the changes in satiety

signals or absorptive systems. The differences of our results and the study by Treesukkosol et al maybe be originated from the performing the different behavioural tests and different experimental procedure.

In the literature, Chevrot et al. have been reported that there was no statistically significant difference in CD36 protein expression level between obese,HFD-fed mice and control group. They also found that the expression of CD36 changes with hunger-satiety status in the control group while CD36 expression did not change in obese mice [238]. In our study, it has been revealed that CD36 protein expression level increased in Group 3b (LFD-HFD), whereas the expression level of the same protein decreased in Group 2b (HFD-HFD). Differences between groups may be related to maternal regulation of oro-sensorial fatty taste perception system. In addition, it has been claimed that the differences in the expression levels of related receptors may be due to the inflammatory process and the different dietary contents.

Protein expression data obtained by western blott technique and confirmed by immunofluorescence staining. Only one contradiction was detected between CD36 protein expression level and immunofluorescence staining. Although the increased CD36 protein expression was determined by western blotting in Group 3b (LFD-HFD). the same increase was not observed for CD36 in IF experiments. Although the CD36 signal in the epithelial and connective tissue around the papilla of Group 3b tongue tissue increased, CD36 signal increase was not observed in the taste bud. This suggests that the decreased in percent2 rapeseed oil solution preference in Group 3b (LFD-HFD) can be related to oro-sensory system dysregulations.

In our study, mRNA levels of these receptors were determined by RT-PCR to elucidate the transcriptional or translational controls of protein expressions.It was determined that the mRNA level of GNAT3, also known as the marker of taste perception signal, decreased in experimental groups fed with HFD (Groups 1b, 2b, 3b) and LFD (Groups 3c and 1c) during maturation compared to other groups. Consistently, immun-positive signaling in GNAT3 of these groups were lower when compared to others.

Lastly, It was determined that CD36 and GPR120 mRNA levels did not completely match with the protein levels. This is due to the fact that the macronutrient contents of the applied

diets are different and these different macronutrients cause a difference in transcriptional and/or translational mechanisms due to inflammation, insulin and leptin resistances.

7. CONCLUSION AND FUTURE ASPECTS

In this study, we have reported that diets administrations which have different fat concentrations during both maternal and maturation periods are effective in fatty taste preference of offsprings. We have considered that this effect is associated with regulation of oro-sensory signalling pathways. Especially, we have determined that the maternal HFD administration is associated with higher percentage of oily solution preference, regardless of the fat ratio in the exposed diets during the maturation period. In maternal HFD-induced rats groups' CVP, IFC staining and western blot studies confirmed that decreased expression levels of CD36 and GPR120 receptors compared to other groups. These findings indicate that maternal nutrition status can related to dysregulation in oro-sensory signalling pathways and contribute to development of childhood obesity. However, only determination in the expression levels of these proteins is not sufficient to evaluate the taste perception process. Oro-sensorial fatty taste perception system includes various proteins associated calcium-dependent signalling pathway. At the end of the pathway, neurotransmitters released and these are stimulated to central gustatory pathway in the brain via nerves. In further studies, it should be shown that the maternal nutrition status associated alteration in the fatty taste perception by using calcium imaging studies. In this way, it will provide evidence precisely for maternal HFD-induced abnormalities in oro-sensorial fatty taste signalling pathways.

This study defines the primary role of maternal nutrition in regulating the expression of lingual CD36 and GPR120 receptors whose effect on fatty taste preference in later life of the offspring. This is the first study in the literature showing that the oro-sensorial system-mediated fatty nutrient choice in adulthood is closely associated with maternal nutritional status. Determining the effect of maternal nutritional status on taste perception abnormalities in development process is very important in terms of determining the target for new anti-obesity interventions. However, this study only addresses the relationship between the expression levels of fatty acid-binding receptors in the tongue and the diet

which is administered in various stage of development. However, the lingual CD36 and GPR120 receptors can be affected by many factors such as hormones, hunger, meal timing etc. and the regulation mechanisms of these proteins have not yet been fully described in the literature. Future studies to clearly identify the genetic and epigenetic mechanisms of these receptors which are associated with maternal nutrition status are particularly important in developing new strategies for the treatment of childhood obesity.

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APPENDIX A: ETHICAL APPROVAL FORM

YEDİTEPE ÜNİVERSİTESİ

T.C. YEDİTEPE ÜNİVERSİTESİ, DENEY HAYVANLARI ETİK KURULU

(YÜDHEK)

ETİK KURUL KARARI

Toplantı Tarihi	Karar No	İlgi	Proje Yürütücüsü
23.12.2016	575	07.12.2016	Yrd.Doç.Dr. Burcu GEMİCİ BAŞOL

'Sprague Dawley Sıçanlarda Maternal Beslenme Durumuyla Yavrunun Yağ Tadı Algısı ve Yağlı Besin Tercihi Arasındaki İlişkinin İncelenmesi' adlı bilimsel çalışma etik kurulumuzda görüşülmüş olup, çalışmanın etik kurallara uygun olduğuna oy birliğiyle karar verilmiştir.

Etik Onay Geçerlilik Süresi: 3 Yıl	Hayvan Türü ve cinsiyeti: Rat ♂	Hayvan Sayısı: 84

GÖREVİ	ADI SOYADI	İMZA
Başkan	Prof. Dr. Bayram YILMAZ	KATILMADI
Başkan Yardımcısı	Prof. Dr. Erdem YEŞİLADA	
Raportör	Vet. Hekim Engin SÜMER	
Üye	Prof. Dr. M. Ece GENÇ	KATILMADI
Üye	Doç. Dr. Rukset ATTAR	
Üye	Doç. Dr. Soner DOĞAN	
Üye	Doç. Dr. Ediz DENİZ	
Üye	Prof. Dr. Gamze TORUN KÖSE	KATILMADI
Üye	Yrd. Doç. Dr. Aylin YABA UÇAR	
Üye	Hakan GÖKSEL	
Üye	Ahmet ŞENKARDEŞLER	