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YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF PHYSIOLOGY

The Effect of Dietary Fat Intake During Maternal and Maturation Periods on The Hepatic Oxidative Stress Status in Offspring Sprague Dawley Rats

MASTER THESIS

Oula BENNASER

İstanbul-2020

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated **17.01.2020** and numbered **2020/01-32**

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgement has been made in the text.

Date: 5./1./2020

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BEYAN

Bu tezin kendi çalışmam olduğunu, planlanmasından yazımına kadar hiçbir aşamasında etik dışı davranışımın olmadığını, tezdeki bütün bilgileri akademik ve etik kurallar içinde elde ettiğimi, tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara kaynak gösterdiğimi ve bu kaynakları kaynaklar listesine aldığımı, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını beyan ederim.

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LIST OF SYMBOLS AND ABBREVIATIONS

ACC	Acetyl CoA carboxylase
Apo B	Apolipoprotein B
CAT	Catalase
ChREBP	Carbohydrate response element binding protein
CPT-1	Carnitine palmitoyltransferase I
Cyp7a1	Cholesterol 7-alpha-hydroxylase
DGAT	Diacylglycerol acyltransferase
DNL	De novo lipogenesis
DOHaD	Developmental origins of health and disease
ETS	Electron transmission chain
FAS	Fatty acid synthase
FFA	Free fatty acid
FXR	Farnesoid X Receptor
GPx	Glutathione peroxidase
GSH	Glutathione reductase
GST	Glutathione S-Transferase
HCC	Hepatocellular carcinoma
HFD	High-fat diet
IKK	IκB-kinase
IRS	Insulin receptor substrates
JNK	c-Jun N-terminal kinase
LDL	Low-density lipoprotein
LDL	Low-density lipoprotein
LFD	Low-fat diet
LXR	Liver X receptor α

MAPK	Mitogen-activated protein kinase
MDA	Malonaldehyde
MiR-122	microRNA-122
MRC	Mitochondrial respiratory chain
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OSI	Oxidative stress index
PARs	Predictive adaptive response
PI3K	Phosphatidylinositol 3 kinases
PKB	Protein kinase β
PPARα	Peroxisome proliferator-activated receptor α
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCD1	Stearoyl-CoA-Desaturase-1
SD	Standard deviation
SFD	Standart fat diet
SOD	Superoxide dismutase
SREBPs	Sterol regulatory element-binding proteins
TAG	Triacylglycerole
TAS	Total antioxidant status
TOS	Total oxidant status
VLDL	Very low-density lipoprotein
WAT	White adipose tissue

ABSTRACT

Olla BENNASER. (2019). The Effect Of Dıetary Fat Intake Durıng Maternal And Maturatıon Perıods On The Hepatıc Oxıdatıve Stress Status In Offsprıng Sprague Dawley Rats. Yeditepe University, Institute of Health Science, Department of Physiology, MSc Thesis, İstanbul.

Maternal diet is thought to associated with the determination of obesity, type II diabetes and nonalcoholic fatty liver disease (NAFLD) through oxidative stress and inflammation in the juvenile population. Reactive oxygen species (ROS) contribute to the illness state by harming DNA, lipids, and proteins, driving to a disturbance in cellular homeostasis and an aggregation of harmed molecules. The liver is the controller of vitality body homeostasis and oxidative stress and the liver problems can give rise to numerous metabolic diseases. In this consider, we aimed to determine the impact of different fat-containing diets exposure during maternal and maturation periods on the hepatic oxidative stress in offspring Sprague Dawley rats.

In the first part of the study, mother Sprague-Dawley rats were administered with high-fat diet (HFD), standard fat diet (SFD) and low-fat diet (LFD) during the gestational (21 days) and lactation (21 days) periods. The male pups from three main groups were then administered with HFD, SFD and LFD during the maturation period for 120 days in the second part of the study. A total of 54 male rats in nine different groups were utilized in this study. After the sacrification of experimental groups, a part of liver tissues stored at -80°C in order to determine oxidative stress parameters. In this process, the liver was homogenized and total antioxidant status and total oxidant status in the lysate were determined with commercial kits.

Our study suggests that dietary fat intake during maternal and maturation periods can affect the hepatic oxidative stress status in offspring Sprague Dawley rats.

Key Words: Maternal nutrıtıon, NAFLD, Oxıdatıve stres.

ÖZET

Olla BENNASER (2019). Maternal Ve Maturasyon Dönemlerinde Diyetle alınan Yağın Yavrus Sprague Dawely Sıçanların Karaciğer Oksidatif Stres Durumuna Etkisi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Fizyoloji ABD. Master Tezi. İstanbul.

Maternal diyetin çocuk popülasyonunda oksidatif stres, inflamasyon, obezite, tip II diyabet ve Non-alkolik yağlı karaciğer hastalığı (NAFLD) tanısı ile ilişkili olduğu düşünülmektedir. Reaktif oksijen türleri (ROS), DNA, lipidler ve proteinlere zarar vererek hastalık oluşumuna katkıda bulunur, hücresel homeostazda bir bozulmaya ve hasarlı moleküllerin birikmesine neden olur. Karaciğer, enerji vücut homeostazı ve oksidatif stresin düzenleyicisidir ve karaciğer sorunları, birçok metabolik hastalığa yol açabilir.

Bu çalışmada, maternal ve olgunlaşma dönemlerinde farklı miktarlarda yağ içeren diyetlere maruz kalan Sprague Dawley sıçanların karaciğerlerindeki morfolojik değişimi ve oksidatif stres seviyelerini belirlemeyi amaçladık.

Çalışmanın birinci bölümünde anne Sprague-Dawley sıçanlarına gebelik (21 gün) ve emzirme döneminde (21 gün) yüksek yağlı diyet (HFD), standart yağ diyeti (SFD) ve düşük yağlı diyet (LFD) uygulandı. Çalışmanın ikinci bölümünde üç ana gruptaki erkek yavrulara 120 gün boyunca (olgunlaşma döneminde) HFD, SFD ve LFD uygulandı. Bu çalışmada dokuz farklı grupta toplam 54 erkek sıçan kullanıldı. Deney gruplarının sakrifikasyonundan sonra, karaciğer dokusunun bir kısmı oksidatif stres parametrelerini belirlemek için -80 °C'de saklandı Karaciğer homojenize edildi ve toplam antioksidan durumu ve lizattaki toplam oksidan durumu ticari kitlerle belirlendi.

Çalışmamız maternal ve olgunlaşma dönemlerinde farklı miktarlarda yağ alımının, yavru Sprague Dawley sıçanlarda hepatik oksidatif stres durumunu etkileyebileceğini göstermektedir.

Anahtar Kelimeler: Maternal beslenme, NAFLD, Oksıdatıf STRED.

1. INTRODUCTION AND PURPOSE

Maternal feeding is a very important process which affects the future life and eating habits of the newborn. Feeding with a high-fat or low-fat diet in maternal period may cause several problems such as childhood obesity, type II diabetes, CVS related diseases and other malnutrition complications. According to the Developmental Origins of Health and Disease (DOHaD) hypothesis, the exposures through early life play a basic role which determines the hazard of developing a metabolic disease in adulthood (Skogen and Overland, 2012).

The purpose of the study is to determine the effect and exposure period of exposed diets (HFD-SFD-LFD) which contains different fat concentrations at maternal and maturation periods on liver oxidative stress status.

2. GENERAL INFORMATION

2.1. Liver Overview

2.1.1. Anatomy and Histology of Liver

The liver is the biggest inner organ which also functions as a gland. It weighs approximately 1.5 kg and is found in the upper right part of the abdominal cavity above both the stomach and intestines and under the diaphragm. The liver is surrounded by a single layer squamous epithelium (mesothelium) and a visceral peritoneum composed of connective tissue, with the exception of the parts in contact with the diaphragm and abdominal wall. In the lower part of the peritoneum, the Glisson capsule is located, which is a connective tissue rich in collagen and elastic fibers. The Glisson capsule divides the liver into approximately 1 mm in diameter and 1-2 mm in length by 1 million lobules. There are portal areas (Glisson triangle or Kiernan space) in the areas where the hexagonal lobules meet, including the arteries, veins and bile ducts (Racanelli and Rehermann, 2006)

The liver is composed of structures including parenchyma, stroma, sinusoids (sinusoidal capillaries) and disse intervals (perisinusoidal spaces). The parenchyma consisted of hepatocyte plaques separated by sinusoidal capillaries. The stroma, which is in the form of connective tissue, connects with the Glisson capsule. Blood vessels, nerves, lymphatic vessels and bile ducts reside within the stroma. Sinusoids are vascular channels between the hepatocyte layers. Between the hepatocyte and the sinusoidal endothelium is the range of the disse (Racanelli and Rehermann, 2006)

There are different types of cells that compose the liver and these cells have a moreover distinctive embryological beginning: hepatocytes, biliary epithelial cells (cholangiocytes), stellate cells, Kupffer cells, and liver sinusoidal endothelial cells. Every one of these cells sorts capacities in a special manner, but they also cooperate in order to regulate the functions of the liver at numerous levels. Hepatocytes are primary epithelial cells, and they are the abundant cells of the liver. In addition, biliary epithelial cells (cholangiocytes) are the second most prevalent epithelial species and have a more common

epithelial characteristic as they line the bile duct lumen. Stellate cells are a dynamic cell population which can be found in two-state, a dormant or activated state. Upon the dormant state, these cells act as a storage vitamin A within the fat droplets; other functions are not clear during the dormant period. It is known that the stellate cells become active when the liver is damaged. Upon actuation, stellate cells proliferate and dynamically lose vitamin A stores. These cells are also responsible for collagen deposition and organization in the injured liver. However, this mechanism leads to liver scarring then may lead to cirrhosis, which is a critical pathology that contributes to liver disease in the final stages. Kupffer cells are the local population of liver macrophages that identify the pathogenic numerous stimulus presented through the portal circulation. Based on a variety of contributing components, they can achieve pro/anti-inflammatory functions in liver and wound healing. Eventually, liver sinusoidal endothelial cells with special characteristics are a specialized endothelial population. Such cells form strainer plates fenestrated at the sinusoidal lumen. In humans, this structure makes pores extending in estimate from 50-180 nm and in mice 50-280 nm. The role of this arrangement is essential for an exchange of molecules and proteins inside these estimate limits between liver plasma and liver cell sorts while retaining another barrier function (Trefts, 2017).

2.1.2. General Functions of the Liver

The liver is a very important base for various physiological processes. These processes are related macronutrient metabolism, blood volume control, support of the immune system, regulation of growth signalling pathways, homeostasis of cholesterol and lipid and breaking down of xenobiotic substances, which are included in several medications. Apportioning the handling of macronutrients give the vitality required to drive the previously mentioned processes and most of the critical liver functions. The liver also stores glucose within the frame of glycogen, with nourishing and accumulate glucose through the gluconeogenesis pathway, and responding to fasting is very critical. Despite the liver lipids it can also package the excess lipid for secretion and storage in another tissue, such as in adipose. Moreover, the liver is a basic of protein and amino acid metabolizing

organ, because it is responsible for most of the blood-secreted proteins, the processing of amino acids for vitality and elimination of nitrogenous waste from protein dissolution in urea metabolism form. All of these hepatic functions have been consolidated over the course of evolution into one particular organ, which is conserved in all vertebrates. From a developmental point of view, this organ emerges from a complex differentiation system triggered by external signals. cell location indications and a complex hierarchy of transcription factors. Such a mechanism which is entire progress within developing liver is crucial to life. Hepatic failure from various causes (e.g. excess nutrition, viral infection and tumours) could be a worldwide health issue.

2.2. The Overview to Relationship Between Diet and Liver

The high-fat diet (HFD) is the main reason for obesity. Obesity can cause an assortment of a health problem as well as coronary cardiovascular disease, hypertension, stroke, fatty liver disease, diabetes, and also in cancers. Therefore, HFD in these diseases can be responsible for the production of free radical metabolites, and the advancement of systemic aggravation and insulin resistance (Tan et al. 2013). HFD is considered one of the most critical hazard factors associated with the occurrence of non- alcoholic fatty liver disease (NAFLD). When Rodents fed with HFD, it has been observed visceral adiposity, hyperglycemia, dyslipidemia, hyperinsulinemia and hepatic steatosis, these are the findings like to those in human (Cheng et al. 2018).

Obesity/overweight is present in two-thirds of ladies within reproductive age in the United States of America, influencing not only their wellbeing but also their children. (Stang et al. 2016). Obesity can be a result of dysregulation between food intake and energy consumption, generally due to high energy increased or expanded utilization of high fat count calories which leads to increase the white adipose mass (Burgio et al. 2015). In addition, it is a major hazard factor for a liver disorder, for instance, NAFLD (Ahima et al. 2011) Adipocytes react maladaptively to chronic excess energy, deriving to enlarged adipocyte. However, fat swollen adipocytes are resistant to insulin, which reduces the adipokine expression as an anti-inflammatory and anti-insulin sensitizer

adiponectin, and increases of the leptin satiety factor expression. Stressed adipocytes in mice genetically obese or caused by diet and in obese people are exposed to cell death.

The metabolic disorder, insulin resistance and NAFLD are associated with the size and death of fat cells (Machado et al. 2016). Obesity and its common diseases are accompanied by inflammation condition. As in the peripheral tissues, signalling of insulin impairment is associated with inflammation such as central nervous system e.g the hypothalamus and the liver, adipocyte and muscles. The insulin signal can be inhibited by proinflammatory cytokines, TNFα primarily through targeting insulin receptors or insulin receptor substrate (IRS). The stimulation of serine kinases such as c-Jun N-terminal kinase (JNK) and IkB kinase (IKK), by TNFα and other cytokines, phosphorylate IRS proteins. This results in the inactivation of the insulin signalling of the proteins concerned. (De Fante et al. 2016).

2.2.1. Consumption of HFD Caused to Hepatic Dysfunction

The liver is an organ that is sensitive to insulin and important for the regulation of glucose homeostasis. The role of insulin supports the increased glucose uptake in periphery tissues basically skeletal muscles and decreases gluconeogenesis in the liver. Insulin resistance inhibits these typical regulation mechanisms, therefore causes hyperglycemia. In addition, insulin resistance caused by consumption of a HFD, which avoids insulin-mediated inhibition of hepatic gluconeogenesis (Soltis et al. 2017). The liver is the main supporter of in general glycemic control. In fact, insulin-stimulated blood glucose clearance is intervented, partially by the resistance of hepatic gluconeogenesis, and utilization of HFD induces hepatic insulin resistance, which disturbs this process (Pilkis et al, 1992). Previous results have connected changes in liver structure with late stages of liver steatosis that result in non-alcoholic steatohepatitis additionally the progression of liver fibrosis (Ramachandran et al. 2016).

2.2.2. HFD Overfeeding Promotes Non-Detrimental Liver Steatosis

HFD nutrition acts as a demonstration of NAFLD depending on the period of the exposure and the content of the diet. Furthermore, in spite of the fact that long-term HFD causes an additional increase in the weight of the body and related changes such as impaired insulin resistance in mice, the degree of liver steatosis and inflammatory conditions are higher in male mice than in females (Arisqueta et al, 2018). Arisqueta et al. conclude that Takeda G protein-coupled bile acid receptor TGR5 modulates the liver and plasma cholesterol levels in a sex-specific manner in response to HFD. Compared to HFD-fed wild type mice, TGR5 KO female mice nourished with HFD appeared to be critical fat aggregation. In addition to this reaction, other features related to bile homeostasis differentiate female and male TGR5 KO mice (Arisqueta et al, 2018).

Since the liver does not function as a reservoir for fat storage, the stable concentration of hepatic triglycerides is low under physiological conditions. However, in response to feeding and fasting, there is significant trafficking of triglycerides and fatty acids inside and outside the liver. In the feeding condition, the dietary fatty acids are collected in triglycerides after absorption from the small intestine and incorporated into chylomicrons and, these are excreted in lymphocytes and inserted into plasma as chylomicrons rich in triglycerides they provide more than 70% of the fatty acids primarily to fatty tissue, while the remaining back to the liver. On the other hand, in fasting conditions, adipose tissue releases fatty acids and back to the liver, where they are collected into triglycerides, packed to form very-low-density lipoprotein (VLDL) molecules that are excreted into the plasma. Mitochondria in the liver can also oxidise fatty acids. Another disorder is excess carbohydrates, which also make fatty acids inside the liver by de novo lipogenesis. These fatty acids can be converted into other lipid products, like glycerolipids, glycerophospholipids and sterols, which can be packaged into VLDL molecules and excreted into the plasma from the liver (Kawano et al. 2013).

2.3. Non-Alcoholic Fatty Liver Diseases

NAFLD is currently the foremost prevalent liver disease globally and represents about 25% of the adult population (Younossi et al. 2016). Regional prevalence rates are right now highest in the Middle East 32% and American south 30% and least in Africa 13%, but predominant rates are indeed higher in particular in subpopulations such as extremely obese 90% and patients with type II diabetes 76% (Lykkesfeldt et al. 2018). The term NAFLD means that the increased fat within the liver that does not result from alcohol utilization or auxiliary causes as viral diseases, medications, or endocrine dysfunction.

The Characteristic of NAFLD is the intracellular aggregation of lipids, leading to the form of lipid beads inside hepatocytes. This aggregation caused by a dysregulation between lipid formation and oxidation (Bellanti et al. 2017). Despite the etiology of NAFLD is ineffectively understood, a "two-hit hypothesis" was projected in 1998 by Day and James to depict the development of NAFLD and its progression to non-alcoholic steatohepatitis (NASH). On this theory, the first hit comprises hepatic insulin resistance or impairing β-oxidation of fatty acids, both of which contribute to the accumulation of hepatic lipid. The second hit comprises oxidative stress or inflammation, that will exacerbate the existing steatosis and induces to (NASH). Currently, it has been suggested that the first "hit" can occur in utero because of a maternal diet that is great in fat. NASH is the more dangerous form of NAFLD that is characterized histologically through the nearness of hepatocyte ballooning and lobular aggravation. Whereas the predominance of NAFLD is high, as it were the extent of NAFLD patients have NASH. Considers have illustrated that NASH patients have a more propensity to develop progressed liver and cirrhosis and are at expanded risk of hepatocellular carcinoma (HCC) (Wong et al. 2018).

2.3.1. Reasons for NAFLD

Scientifically, the global obesity has increased the elevation in the prevalence of NAFLD which is related to insulin resistance, therefore, NAFLD is categorized as the hepatic manifestation of the metabolic syndrome (Kaswala et al. 2016). Furthermore, the NAFLD pathophysiology and its development as a result of diverse factors, in a multiple-

hit model (Buzzetti et al. 2016; Polyzos et al. 2009), in the same way, several genetic and environmental factors which are (hits) interaction on an individual basis. Moreover, each NAFLD patient has been affected via a combination of diverse lifelong pathogens, which is associated with diagnostic related to therapeutic challenges. Likewise, these fundamental factors contain which are not justified to: multiple determined genetic forms, for instance, phospholipase sector- contains three-gene, transmembrane six super-family organ genes two; epigenetic modifications (Eslam et al., 2018), diet, excessive fat as well as fructose. Additionally, the non-physical activity of the persons (Mahady et al. 2016), obesity belongs to insulin resistance (IR) (Polyzos et al, 2017), lipotoxicity (Mota et al. 2016), oxidative stress endoplasmic reticulum stress (Buzzetti et al. 2016), dysregulation of adipokines (Polyzos et al. 2016), dysbiosis of intestinal microbiota (Koukias et al. 2017), and endocrine disorders (Polyzos et al. 2012), under the combination of several of these factors, which similarly cross each other in the same way, fat remains collected in the beginning in the liver cells leading to simple steatosis (SS). In addition, at any rate, SS remains not administered in a timely manner, therefore, the liver is penetrated via immune cells, as a consequence of adding an inflammatory process, a condition categorized in terms of NASH (Mazzoccoli et al. 2018).

2.3.2. Mechanisms of NAFLD

The pathogenesis of NAFLD can be demonstrated via insulin resistance and free fatty acids (FFAs) liberate from the adipose tissue. Likewise, insulin resistance is associated with obesity that decomposes fatty acid in the adipose tissue via lipolysis. Furthermore, FFAs are taken up into the liver and synthesis fatty acid begins. What's more, mitochondrial and dysfunction down-regulates carnitine palmitoyltransferase I (CPT-1) expression via overexpressing acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) and reduces fatty acid oxidation. Accordingly, as an outcome, fatty acids increase hepatic triglyceride and cholesterol synthesis and lipid droplets. For that reason, the liver secretes triglyceride and cholesterol with apolipoprotein B (Apo B) in the form of the low level of density lipoprotein (VLDL) and raises the circulating low- density lipoprotein

(LDL) (Kim et al. 2016). In addition, based on pathologic mechanisms of NAFLD which are complex and multifactorial, however, they focus on transforming mitochondrial function which might precede the development of NAFLD (Brumbugh et al. 2013). Moreover, there are several types of research that interpret increased intrahepatocellular fat storage in infants born to obese mothers (Rector et al. 2010; Modi et al. 2011). Advanced in life, these offspring are at a greater risk of proceeding to obesity, NAFLD, cardiovascular disease, as well as hepatic carcinoma (Rinella et al. 2016; Houghton et al. 2016).

2.3.3. Mitochondrial Dysfunction

Mitochondria is an important organelle that is necessary for energy generation and is the main site for beta-oxidation of fatty acid (Mantena et al. 2008). Furthermore, the relationship between mitochondrial dysfunction as well as NAFLD has been observed via several advanced researchers (Wei et al. 2008; Begriche et al. 2013). Presently, mitochondrial fatty acid oxidation raises to be adapted to the accumulation of excessive hepatic fat (Sunny et al. 2011). In sequence, it leads to a rise in oxidation products of reactive oxygen species (ROS). Therefore, all over the mitochondrial ROS is detoxified into residual molecules during the activity of the mitochondrial respiratory chain (MRC). On the other hand, raised the level of mitochondrial oxidation can gradually stimulate a vicious circle containing decreasing MRC activity, overproduction of ROS, which belongs to the damage of mitochondrial DNA. Likewise, the unbalanced state that favors ROS production over antioxidant defence is termed as oxidative stress. In the same way, oxidative stress with the mitochondrial ROS has been presented to be excessive to be a part in cell death, inflammation, as well as fibrosis (Orrenius et al. 2007), also, might be a significant role in NASH growth. In addition, in animals schematic causing maternal obesity, mitochondrial dysfunction belongs to oxidative stress in the offspring remained observed, and this was reflected via a decrease in the main components of MRC mitochondrial electron transmission chain complex (ETC) I, II, III as well as IV activity (Bruce et al. 2009), MCR interruption activity (Alfaradhi et al. 2014), reduce liver

Mitochondria DNA copy number (Burgueño et al. 2013) as well as and low level of concentrations of antioxidant enzymes (Zhang et al. 2011).

2.3.4. Insulin Resistance

Insulin failure to stimulate glucose transport into its target cells and plays a pivotal role in metabolic syndrome which is termed as a critical node amongst metabolic syndrome and insulin resistance of NAFLD, as well as, is harmful to the liver for the reason that it similarly promotes NAFLD. Furthermore, insulin is a pleiotropic hormone that regulates several cell functions containing stimulation of glucose transfer, energy balance, cell growth and regulation of gene expression. In the same way, insulin initiates its function through connecting to the insulin receptor which leading to dimerization and autophosphorylation of this insulin receptor. Moreover, in turn, recruits and phosphorylates the insulin receptor substrate (IRS), IRS2 being the most represented isoform within the liver. Additionally, this representative modulates two diverse signalling pathways which are: firstly, is phosphatidylinositol 3-kinase (PI3K)-protein kinase B (PKB/AKT) pathway, which promotes the insulin metabolic actions; also, secondly, is the mitogen-activated protein kinase (MAPK) pathway, which regulates the genes expression involved in cell culture growth and differentiation. In addition, alteration of these signalling pathways via numerous factors might so cause insulin resistance (Mendez-Sanchez et al. 2018).

2.3.5. Oxidative Stress

In the scientific world, oxidative stress is termed as the imbalance between the production of reactive oxygen and nitrogen species (ROS/RNS) generated in the aerobic metabolism and their elimination via antioxidant defence, which contains enzymes, for instance, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), as well as non-enzyme particles of electron receptors, for instance, glutathione (GSH) and vitamin C/E, which might be triggered in the liver via various factors, for instance, obesity, virus, alcohol, drugs, as well as other toxins (Li et al. 2015). Furthermore, ROS are produced in the cells via cellular metabolic activities, for instance, cell survival, stressor

responses and inflammation (Zuo et al. 2015; He et al. 2015). In fact, ROS is necessary at a certain level in the body to perform its vital physiological functions. Moreover, the production of ROS is a natural part of aerobic life, which is responsible for the manifestation of cellular functions containing signal transduction pathways, defence against invading microorganisms and gene expression to promote growth or death (Arisqueta et al. 2018). In the case of diabetes, oxidative stress impairs the absorption of glucose in muscle and fat and reduces insulin secretion from pancreatic β cells. Increased oxidative stress also underlies the pathophysiology of hypertension, and atherosclerosis (Furukawa et al. 2017). At the same point, oxidative stress plays a crucial role in the pathogenesis of multiple diseases. Consequently, the development growth of NASH is highly associated with increased hepatic lipogenesis and decreased fatty acid oxidation. In addition, oxidative stress and pro-inflammatory mediators accelerate the deterioration of hepatic steatosis to NASH via overproducing reactive oxygen species (ROS) (Levonen et al. 2014). As a consequence, the oxidation of excessive fatty acids generates lipid peroxides (Paradies et al. 2014). In the same way, these toxic metabolites activate the lysosomal cell death pathway and stimulate cells to cytotoxicity (Wang et al. 2014). Likewise, these mechanisms finally lead to cellular apoptosis through collaborating with hepatic inflammation, ROS, GSH and adipose tissue-derived endotoxins (Kim et al. 2016). Additionally, this raises the possibility of hepatic fibrosis which is associated with cirrhosis via activating hepatic stellate cells. On the other hand, these symptoms can be decreased as activating hepatic anti-oxidative enzymes, for instance, superoxide dismutase (SOD), glutathione peroxidase (GPx) and CAT (Kim et al. 2016). Furthermore, an antioxidant enzyme, for instance, CAT, SOD, as well as GSH-Px and non-enzymatic electron receptors, for instance, GSH remained affected the usage as indicators to observe the level of oxidative stress. Therefore, both enzymatic and non-enzymatic antioxidant framework which is necessary for cellular response in order to deal with oxidative stress based on physiological condition (Arisqueta et al. 2018). In addition, the advanced antioxidant framework has been developed in mammals to maintain the redox homeostasis in the liver. In addition, once the ROS amount remains excessive, the homeostasis will be disturbed, leading to oxidative stress, which

plays a crucial role in liver diseases and conventional chronic as well as degenerative disorders (Arisqueta et al. 2018).

2.3.6. Hepatic Lipid Accumulation

In fact, the main requirement of NAFLD is the accumulation of lipids. Therefore, the accumulation of fatty acid takes place once fatty acid uptake and synthesis exceed the oxidative capacity of hepatic cells. Furthermore, during a human study, Donnelly et al. have documented that utilizing stable isotopes, that the dominant source of fat accumulated in the liver arises from serum-free fatty acids belongs to this is closely followed via de novo lipogenesis (Donnelly et al. 2005). In addition, lipolysis in white adipose tissue (WAT) remains a specific contributor to serum-free fatty acid concentrations (Fabbrini et al. 2010).

2.3.7. De Novo Lipogenesis

The liver synthesizes fatty acids “de novo lipogenesis, DNL” in the duration of a complex cytosolic polymerization that acetyl-CoA is turned to be malonyl-CoA via acetyl-CoA carboxylase (ACC), also undergoes through numerous cycles of metabolic reactions to form one palmitate molecule. Furthermore, the rate of DNL is structured via the fatty acid synthase (FAS) complex, ACC 1 as well as 2, diacylglycerol acyltransferase (DGAT) 1 as well as 2, stearoyl-CoA desaturase 1 (SCD1), and several nuclear transcription factors: Sterol Regulatory Element-Binding Proteins (SREBPs), Carbohydrate Responsive Element-Binding protein (ChREBP), liver X receptor α (LXRα), farnesoid X receptor (FXR), as well as peroxisome proliferator-activated receptors (PPARs). Moreover, hepatic DNL remains synchronized independently via insulin and glucose, through the activation of SREBP-1c which is related to ChREBP, which are transcriptionally activating almost all genes involved in DNL. In addition, INFO based on several types of research has been presented in mouse models which are reported that hepatic overexpression of SREBP-1c or hyperinsulinemia stimulates lipogenesis as well as cause hepatic steatosis (Asrih et al. 2015).

2.3.8. Lipotoxicity

In the experimental scientific world, Lipotoxicity remains the deleterious effects of lipid accumulation in non-adipose tissue, for instance, the liver which is termed to be associated with insulin resistance, hepatic steatosis, cardiomyopathy, nephropathy, as well as endothelial dysfunction (Mendez-Sanchez et al. 2018; Yazıcı and Sezer 2017). Additionally, to reduce fatty acid clearance from circulation, enlarged fatty tissue mass releases a lot of free fatty acid (FFA) to circulation rise the ultimate plasma FFA levels in obesity. Furthermore, once plasma FFA levels are rising, the anti-lipolytic action of insulin is inhibited. In this way, the rate of FFA release into the circulation will further rise (Engin AB, 2017). Moreover, the presence of fat in non-adipose tissues depends not only on lipotoxicity, however, also for the reason that maintaining homeostasis of lipid and metabolism of fat drops. Similarly, in fasting, the lipolysis process of adipose tissue is necessary for the energy requirements of non-adipose tissues (Engin AB, 2017). Whatever, improper accumulation of excess lipids in non-adipose tissues is related to a chronic inflammatory response that is characterized via abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signalling pathways. As an outcome, FFA-mediated lipotoxicity causes cellular stresses and inflammation via weakening normal cell signalling that may cause apoptotic cell death (Engin AB, 2017). Additionally, obesity causes a chronic rise of circulating fatty acids that can reach toxic levels within non-adipose tissues. In addition, fatty acids might enter harmful pathways after the over-accumulation of lipids in non-adipose tissues throughout overnutrition (Unger RH, 2002).

2.4. Maternal Nutrition and Liver

Historically, maternal nutrition has been termed as a key determinant for offspring well-being, which is associated with pregnancy is a significant time duration that can influence the growth as well as the development of offspring. Furthermore, the developmental origins of healthcare belong to disease (DOHaD) hypothesis propose that exposures through early life play a critical role in determining the health risk of developing metabolic diseases in adulthood (Skogen and Overland, 2012). What's more, Oliveira et al.

have reported that maternal HFD increased hepatic triglycerides, free cholesterol and the total value of lipid content in the same direction to decreased cholesterol ester as well as increase the level of the liver weight of adolescent rat offspring, that might dispose to later NAFLD (Oliveira et al. 2016). On the other hand, the nutritional environment in the duration of preconception, gestation and early life are essential for perfect offspring development which is concerned with long-term well-being. In the last century, Barker and Osmond have documented that infant mortality is associated with later-life ischemic heart disease, suggesting that poor conditions throughout childhood raise the risk of adult cardiovascular disease (Barker and Osmond, 1986). In addition, several types of research based on famine events like the Dutch Hunger Winter which is concerned with the Great Chinese Famine have exposed that the associations amongst poor early life nutrition as well as later life disease are not only limited to cardiovascular disease, however, also involve obesity and the metabolic syndrome (Wang et al. 2010; Roseboom et al. 2006). On the other hand, evaluating the risk of developmental programming on later health consequences is specific to diverse contexts which are linked with outcomes. Additionally, the "thrifty phenotype" in terms of predictive adaptive response (PARS) hypotheses have been recommended to clarify this phenomenon (Hales and Barker, 1992; Gluckman and Hanson, 2004). In addition, these scientific theories argue that fetal malnutrition leads to metabolic adaptations that make the most of the availability of limited nutrients, thereby increasing the chances of survival possibility in persistent adverse conditions after the birth. On the other hand, these adaptations have raised the risk of metabolic disorders when exposed to an enriched postpartum nutrient environment. Stimulatingly, a variety of studies on diabetic pregnancies, for instance, gestational diabetes, sort 2 diabetes as well as maternal obesity; which is has been indicated that excess calories intake in the time duration of the early life which have similar effects on offspring long-term health outcomes (Whitaker RC, 2004; Pettitt et al. 1983).

To sum up, the human body weight, as well as the nutritional status of the pregnant woman, are an immediate practical concern for the management of gestation and childbirth for the reason that excessive weight and diabetes are related to negative peripartum

outcomes. In addition, prenatal requirement factors can have a great long-term impact after the childbirth. Furthermore, the impact of fetal undernutrition on future ill health has been well recognized and established from epidemiological researches and from relevant animal models. Moreover, it appears that the supply of excess nutrients to the fetus might raise the risk of lifetime obesity and the onset of metabolic syndrome. Likewise, given the prevalence of obesity, excess gestational weight gain and diabetes in pregnancy in women conditions of fetal over-nutrition can induce permanent changes in energy homeostasis. In addition, animal researches have proved that excess in utero nutrient supply can induce changes in body composition, insulin signalling as well as mitochondrial activity in muscles and increased the level blood pressure in the offspring (Li et al. 2015).

Finally, the liver constitutes a fundamental organ in lipid metabolism, as a central regulator of lipid homeostasis. As well, the liver is responsible for coordinating the synthesis of new fatty acids, their export and subsequent redistribution to other tissues, as well as their utilization as an energy substrate (Younossi et al. 2016). Furthermore, the liver regulates hepatic cholesterol levels through several pathways. Likewise, one of these pathways involves catabolism of cholesterol to bile acids and excretion from the body through cholesterol 7 alpha-hydroxylase (Cyp7a1). Additionally, Cyp7a1 is organized via the oxysterol-binding transcription factor Lxrα. Along with transcriptional regulation of Cyp7a1 via transcription factor Lxrα, Cyp7a1 is similarly regulated via microRNA-122 (miR-122) a small RNA that destabilizes Cyp7a1 mRNA as a consequence, decreasing Cyp7a1 translation and bile acid synthesis (Zinkhan et al. 2018). In addition, miRNAs are a class of tiny nucleotides (18 to 25 nucleotides) non- coding RNA molecules that have been recognized as genetic organizations in the transmission of maternal obesity into metabolic outcomes in the offspring (Nicholas et al. 2016; Benatti et al.2014).

2.5. Hypothesis of the Study

Feeding with a high-fat diet in gestation-lactation periods and also after birth in the maturation period may induce oxidative stress in the liver. This feeding-related increased oxidative stress may lead to liver problems, or hepatic dysfunction related disorders in adult life such as NAFLD -or NASH. These functional and morphological distruptions may be prevented by exposed diets, in other words, by changing feeding behaviour.

3. MATERIALS AND METHODS

3.1. Experimental Procedure

In the first part of the study, mother Sprague-Dawley rats were administered with high-fat diet (HFD), standard fat diet (SFD) and low-fat diet (LFD) during the gestational (21 days) and lactation (21 days) periods. Then, the male pups from three main groups were administered with HFD, SFD and LFD during the maturation period for 120 days in the second part of the study. The macronutrient percentages of experimental diets are given in Table 3.1.

Table 3.1. The Macronutrient Percentages of Experimental Diets

Facts of Diets	Standard Fat Diet (SFD)	High Fat Diet (HFD)	Low Fat Diet (LFD)
Total Calorie (kcal/gr)	3,3	5,2	3,4
Fat (% kJ)	13	60	3
Protein(%kJ)	23	20	23
Carbohydrat(%kJ)	64	20	74

A total of 54 male rats in nine groups were utilized in this study. After the scarification of experimental groups, a part of liver tissues stored at -80 °C in order to determine the oxidative stress parameters. Experimental groups are available in Table 3.2.

Table 3.2 Experimental groups

Groups	MATERNAL PERIOD [GESTATION & LACTATION] [21 days-21 days]	MATURATION PERIOD [100 Days]
Group 1 (n=6)	SFD	SFD
Group 2 (n=6)	SFD	HFD
Group 3 (n=6)	SFD	LFD
Group 4 (n=6)	HFD	SFD
Group 5 (n=6)	HFD	HFD
Group 6 (n=6)	HFD	LFD
Group 7 (n=6)	LFD	SFD
Group 8 (n=6)	LFD	HFD
Group 9 (n=6)	LFD	LFD

3.2. PARAMETERS

3.2.1. Determination of Liver Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Oxidative Stress Index (OSI)

Liver oxidative stress status has been determined with commercial total oxidant status (TOS) and total antioxidant status (TAS) (Rel Assay, Turkey) kits. Oxidative stress index is calculated with the formulation given in the kit procedure.

3.2.1.1. Homogenization of Liver Tissues

1 gr of frozen liver tissues from all experimental groups are weighed and transferred to tubes. 140 mmol KCI is prepared as a working solution in deionized water. 9 mL working solution is added to the tubes for per gr of liver tissue. Tissues were disrupted with

a homogenizer (Dia X 900 Heidolph, Germany). Then, tubes were centrifuged at 3000 rpm for 5 minutes. Supernatants were taken into new tubes and were used as a sample for TAS and TOS assays.

3.2.1.2. Measurement of TAS Level on the Liver

Principle of the assay: Measurement of various antioxidant molecules independently can be time-consuming, labour-intensive, expensive, Complex techniques required and additive to their antioxidant impact. Therefore, the total antioxidant potential of a sample is measured which is known as total antioxidant status (TAS). In the sample, antioxidants decrease radical dark blue-green coloured ABTS to the colourless reduced form of ABTS. The change in sample absorbance at 660 nm is correlated with the total sample antioxidant level. The assay is calibrated with a stable antioxidant standard solution which is traditionally defined as Trolox Equivalent which is a vitamin E analogue.

Method: In the first stage; 18 µl of samples, standards and dH_2O were put into certain wells in 96 well plates. 300 µl of Reactive 1 (R-1) solution was added to whole wells and mixed by pipetting. The absorbance in the 660 nm of the plate was measured with Microplate Spectrophotometer (BioTek Instruments, USA) [First measurement: A1]. Then, 45 µl of Reactive 2 (R-2) solution per well is added and mixed with Reactive 1 in the plate. The plate has waited for 10 minutes at room temperature. After the incubation, the absorbance in the 660 nm of the plate was measured again [Second measurement: A2]. ΔAbs was calculated for each well with A2 minus from A1. The formulation of TAS is available below.

TAS (mmol/L) = [ΔAbs H_2O-ΔAbs Sample] / [ΔAbs H_2O-ΔAbs Sample]

3.2.1.3. Measurement of TOS Level on the Liver

Principle: The sample includes oxidants that oxidize the ferrous ion– chelator complex to ferric ion. The reaction to oxidation is prolonged by enhancer molecules, which are abundant in the reaction medium. The ferric ion in acidic solution gives a colour complex with the chromogenic molecule. The strength of the colour is correlated with the

total oxidizing ions in the sample, the colour variations can be spectrophotometrically assessed, This test has been calibrated with H_2O_2 and the results are communicated as mM H2O2 comparable per liter (µmol H2O2 Equiv. /L) (Erel, 2005).

Method: 45 µl of samples and standards were put into certain wells in 96 well plates. 300 µl of Reactive 1 (R-1) solution was added to all wells and mixed with pipetting. The absorbance in the 530 nm of the plate was measured with Microplate Spectrophotometer (BioTek Instruments, USA) [First measurement: A1]. Then, 15 µl of Reactive 2 (R-2) solution per well is added and mixed with Reactive 1 in the plate. The plate has waited for 10 minutes at room temperature. After the incubation, the absorbance in the 660 nm of the plate was measured again [Second measurement: A2]. ΔAbs was calculated for each well with A2 minus from A1. The formulation of TOS is available below.

TOS (µmol/L) = [ΔAbs Sample] / [ΔAbs Standard] * 10

3.2.1.4. Determination of OSI Level on the Liver

Oxidative stress index is considered as a new index of oxidative stress status and its equation is given below. According to the formulation, OSI is calculated with TOS divided by TAS. However, the units of TAS and TOS values should be the same in the equation.

OSI = [(TOS, µmol H_2C_2 equivalent/L)/(TAS, , µmol Trolcx equivalent/L)] x 100

4. RESULTS

4.1. Liver TAS, TOS and OSI Levels

4.1.1. Total Antioxidant Status of Liver Tissues

As shown in table 4.1 total antioxidant status of the liver (TAS) was measured 1.5 ± 0.2 mmol/L in SFD-SFD group. The TAS level significantly decreased when the SFD fed animals continue feeding with HFD in maturation period (1.2 ± 0.1 mmol/L, $p<0.05$,). There no significant difference in TAS level was observed when the SFD animals continue feeding SFD or LFD in maturation periods. (Figure 4.1)

When HFD-SFD group compared to HFD-HFD group statistically significant decrease was observed in HFD-HFD group. No significant difference in TAS level was observed in TAS level when the HFD animals continue feeding SFD or LFD in maturation periods.

No significant difference was found in TAS level when the LFD animals continue feeding SFD, or HFD or LFD in maturation periods. As shown in figure 4.2 feeding type in the maternal period did not cause any difference in TAS levels in the liver.

Table 4. 1. TAS, TOS and OSI values of livers of experimental groups (means ± standard deviation)

Groups	TAS (mmol/L)	TOS (µmol/L)	OSI
Group 1 (SFD-SFD)	1.5 ± 0.2	46.8 ± 4.7	179.2 ± 39.2
Group 2 (SFD-HFD)	1.2 ± 0.1	35.1 ± 9.6	289.2 ± 69.2
Group 3 (SFD-LFD)	1.5 ± 0.2	29.0 ± 6.4	205.6 ± 65.8
Group 4 (HFD-SFD)	1.4 ± 0.2	30.4 ± 5.0	219.4 ± 17.3
Group 5 (HFD-HFD)	0.9 ± 0.2	35.4 ± 7.3	403.9 ± 144.4
Group 6 (HFD-LFD)	1.4 ± 0.1	33.3 ± 6.1	242.3 ± 49.9
Group 7 (LFD-SFD)	1.3 ± 0.2	32.6 ± 5.2	255.5 ± 17.2
Group 8 (LFD-HFD)	1.2 ± 0.3	52.2 ± 13.0	459.0 ± 113.6
Group 9 (LFD-LFD)	1.3 ± 0.2	42.8 ± 5.5	340.1 ± 74.2

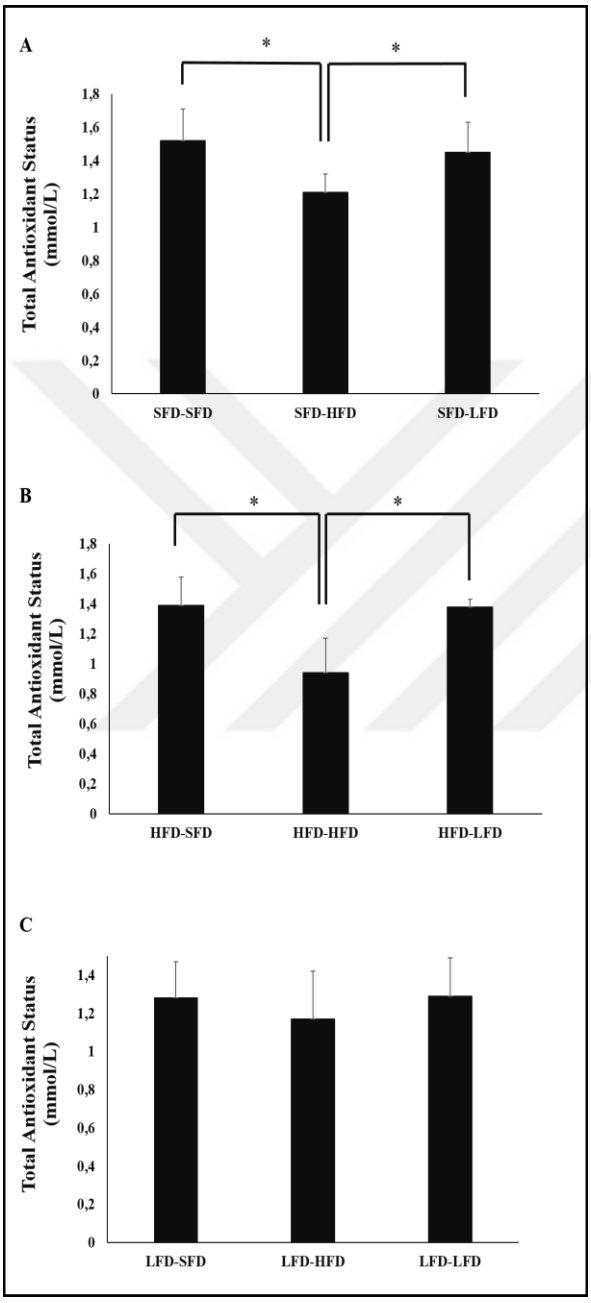

Figure 4.1. The effect of SFD, HFD and LFD during the maturation period on TAS of liver A. B. C. (mean ± standard deviation) (n=5)

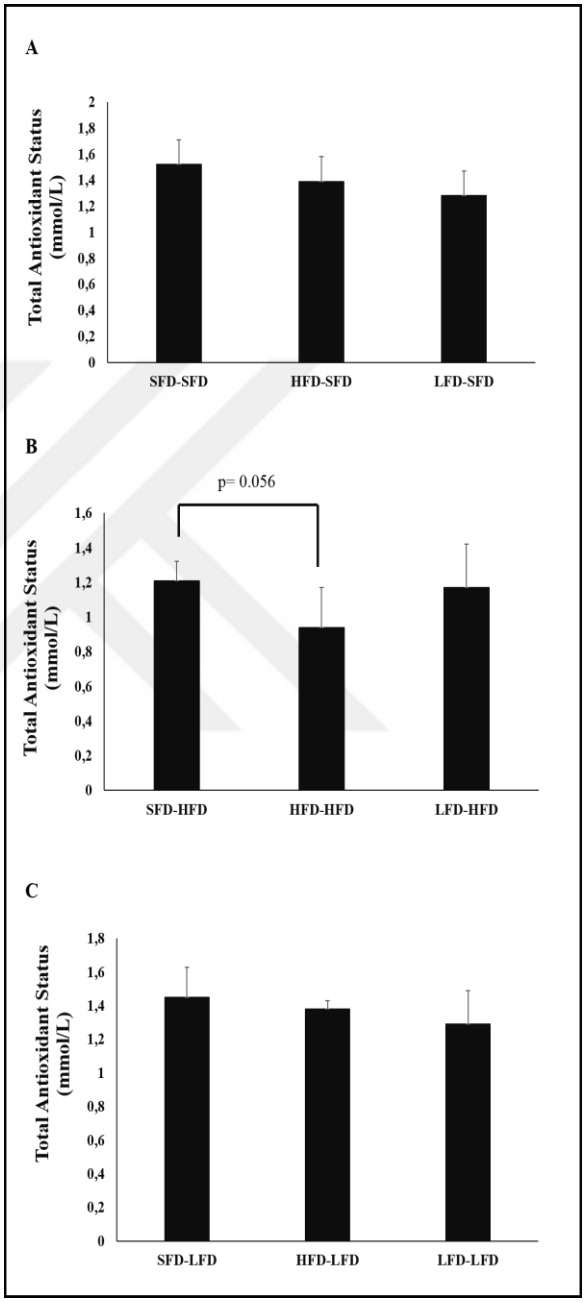

Figure 4.2. The effect of SFD, HFD and LFD during the maternal period on TAS of liver A. B. C. (mean ± standard deviation) (n=5) (*p<0.05).

4.1.2. Total Oxidant Status of Liver Tissues

As shown in table 4.1 total liver oxidant status (TOS) in the SFD-SFD category was 46.8 ± 4.7 μmol / L. No significant difference was found in the TOS level when the SFD or HFD animals continue feeding SFD or LFD in maturation periods (Figure 4.3 A-B). Feeding the animals with HFD or LFD in maturation period caused to increase in the oxidant status in the LDF group (Figure 4.3 C). The highest TOS was determined when the animals fed with LFD in maternal and HFD in maturation periods (figure 4.4.B). And if the animals were

fed with LFD in entire life, their TOS level was higher than SFD-LFD group (Figure 4.4.C).

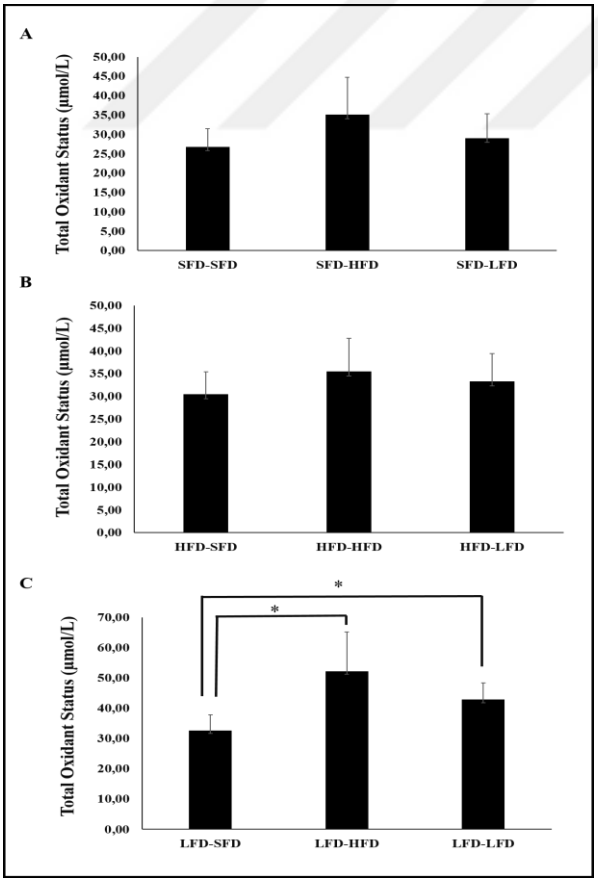

Figure 4.3. The effect of SFD, HFD and LFD during the on TOS of liver A. B. C. (mean ± SD) (n=5) (*p<0.05).

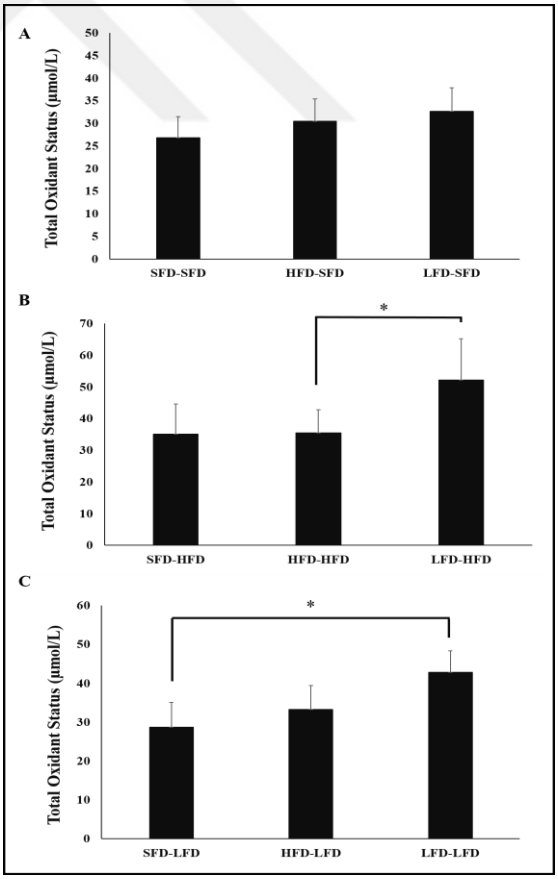

Figure 4.4. The effect of SFD, HFD and LFD during the maternal period on TAS of the liver in A. B. C. (mean ± SD) (n=5) (*p<0.05).

4.1.3. Oxidative Stress Index of Liver Tissues

As shown in table 4.1 total oxidative stress index of the liver (OSI) was calculated 179.2 ± 39.2 in SFD-SFD group. If the animals continued feeding with HFD, the OSI increased significantly (289.2 ± 69.2, $p<0.05$, Figure 4.5.A). Feeding the animals with HFD in maturation period caused to increase in the OSI in HDF group (Figure 4.5 B). The lowest OSI was determined in SFD-SFD group and the highest OSI was determined in LFD-HFD group (459.0 ± 113.6).

Feeding animals with LFD in maternal period caused a significant increase in OSI when compared with both SFD and HFD fed animals ($p<0.05$)

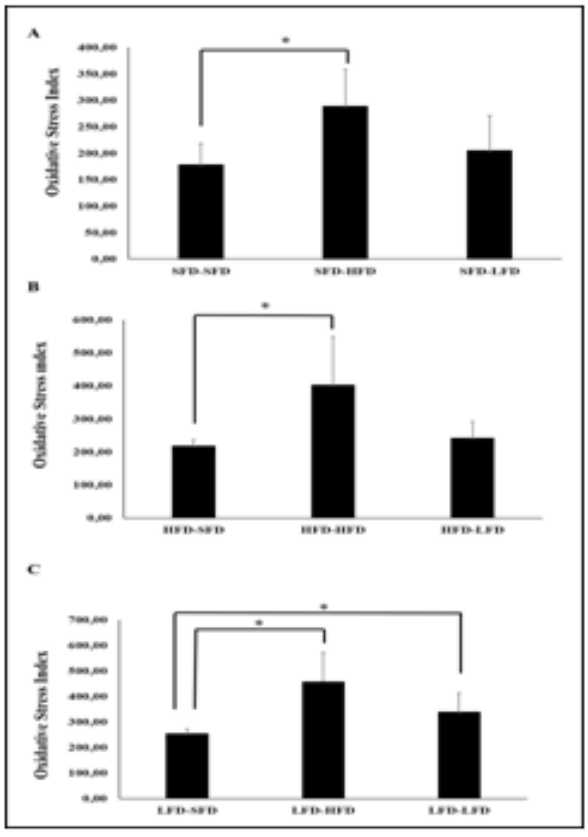

Figure 4.5. The effect of SFD, HFD and LFD during the maturation period on OSI of liver. A. B. C. (mean ± SD) (n=5) (* $p<0.05$).

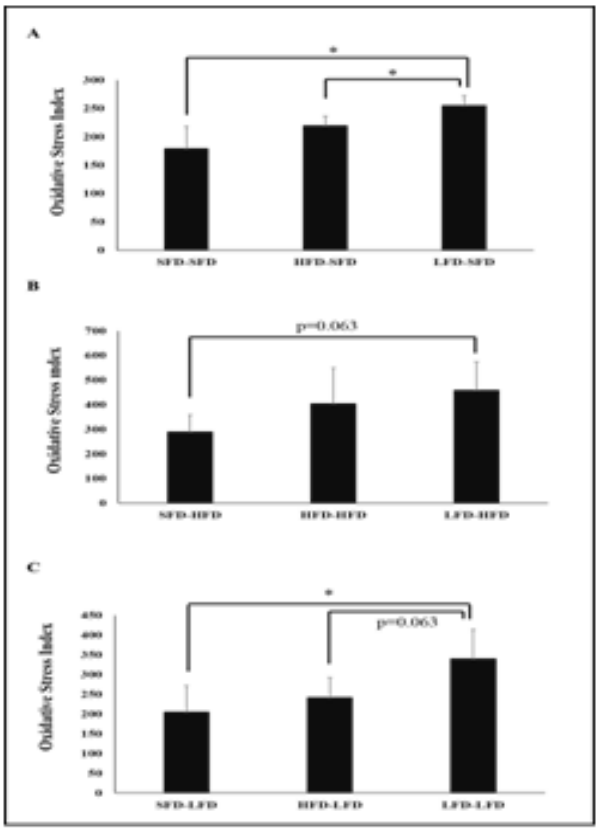

Figure 4.6. The effect of SFD, HFD and LFD during the maternal period on OSI of the liver. A. B. C. (mean ± standard deviation) (n=5) (* $p<0.05$).

5. 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. But consumption High-Fat diet or exposure HFD in maternal period is thought to be associated with the diagnosis of obesity, diabetes type II, cardiovascular diseases and (NAFLD) in adult life of the offspring. All these pathologies are mostly related to impaired metabolism. HFD induced oxidative stress is the key factor for pathophysiological changes in the body. Since ROS damages DNA, lipids and proteins, causing disturbance and aggregation of damaged molecules in cellular homeostasis. ROS are produced as a by-product in normal aerobic metabolism; however, if the level under stress is increased, it may cause basic health hazard (A. Rahal et al., 2014). The mitochondrion is the predominant organelle in the production of ROS cells. It produces adenosine triphosphate (ATP) through a series of processes for oxidative phosphorylation, have been one or two-electron reductions instead of four-electron reductions of oxygen occurred, which eventually leads to the formation of H_2O_2 or $O_2^{\cdot}$, and conversion to other ROS (M.Inoue et al,2003).

Oxidative damage occurs as a result of increased production of ROS and decreased antioxidant defence, and HFD magnifies the ROS overproduction and a decrease in antioxidant capacity. In our research, we pointed to decide the effect of different fat-containing diets exposure during maternal and maturation periods on the hepatic oxidative stress in offspring Sprague Dawley rats. So, mother rats were exposed to special diets which contain different concentrations of fat during G-L periods. After birth, male offsprings were exposed to three different fat concentrations (SFD, HFD, LFD) containing diets during the maturation period.

According to our results, if the HFD or SFD fed animals in maternal period continue feeding with HFD in maturation period their TAS decreased. Feeding with HDF caused to decrease TAS level.

In maternal period, LFD fed all animals if continued feeding with HFD in the maturation period, their oxidant status increased significantly. This can be the effect of feeding with HFD or in other words, excess fat intake increases the B-oxidation of free fatty acids, that causes excessive electron stream utilizing cytochrome-c oxidase and enhances ROS production. The increasing tendency has been observed for SFD or HFD fed animals in maternal periods, but it was not statistically significant. If animals start early life with LFD instead of HFD and continue feeding with HFD, their TOS level was greater than HFD-HFD animals. This is the effect of feeding with high carbohydrate. Feeding with high carbohydrate in early life increases the oxidant status. If the animals start early life with a feeding LFD and continue with LFD which means the animals fed with high carbohydrate entire life has greater TOS levels ($p<0,05$). If you change feeding the animals from SFD to HFD in maturation period OSI increases ($p<0,05$). The animals fed with LFD entire life have a greater level of OSI when compared to LFD-SFD animals ($p<0,05$). If the animal starts early life with LFD and then continue with HFD has greater OSI index when compared to LFD-SFD animals.

6. CONCLUSION

Maternal and maturational nutrition is a very important process for entire life. Feeding with high-fat diet in any time period of life maternal or maturation period affects the oxidative status in liver tissue feeding with HFD causes to increase the oxidant status and also decreases the antioxidant status. Feeding with a low-fat diet is as dangerous as HFD because high carbohydrate causes high oxidative stress like HFD. The best option of feeding in regard to OSI start early life with a standard diet and continue with it.

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APPENDIX 1. Curriculum Vitae

Personal Informations

Name	Oula	**Surname**	Bennaser
Place of Birth	libya	**Date of Birth**	23 June 1979
Nationality	libyan	**TR ID Number**	99197714524
E-mail	bennaserolla@yahoo.com	**Phone number**	0 551 167 24 42

Education

Degree	Department	The name of the Institution Graduated From	Graduation year
Master	Departmen of physiology	**Yeditepe University**	
University	Laboratory medicine	**Omar Almuktar University**	2001\2002
High school	Scientific Section	**Alzahuf Alakhdar School**	1997\1998

Languages	Grades
Arabic	Mother tongue
English	Proficincy (Turkey 2017)

Work Experience

Position	Institute	Duration (Year)
Assistant doctor and medical laboratory technician in microbiology and barasitology and blood banking	Medical Technology Faculty	2003 -2013

Computer Skills

Program	Level
Microsoft office	Good

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	