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Original Article

High Fatty Diet Effects on Rat Liver

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ABSTRACT

Aim of this study was to investigate effects of high fat diet on rat liver and weight gain. By this purpose 30 Wistar Albino rats were divided into 4 groups. 1. High carbonhydrate diet group for 16 weeks (K16), 2. High fatty diet group for 16 weeks (D16), 3. High carbonhydrate diet group 20 weeks (K20), 4. High fatty diet group 20 weeks (D20). High fatty diet; %60 fat (1/3 canola, 1/3margarine, 1/3 sunflower oil), %20 protein, %20 carbohydrate and high carbohydrate control diet; %69 carbohydrate, %20 protein and %11 margarine was composed. There weren't any significant differences between groups as compared to body weight, liver weight and epididymal fat weight. At the result of biochemical analysis, LDH in 16 weeks high carbohydrate diet and ALT in 20 weeks high carbonhydrate diet was significant higher than high fatty diet groups. In histological research, even though fibrosis, inflamation, steatosis findings observed at portal area of all groups, there wasn't significant statistical differences. Similar to this, a-SMA and TGF-B accumulation in all groups were similar interms of immunohistological investigation. In conclusion; this study showed that comparing to relatively high fatty diet rich with omega-9, high carbonhydrate feeding caused liver injury and anointment and there wasn't significant differences in terms of weight gain. By this way, we think that decreasing of carbonhydrate and increasing amount of olive oil, canola oil and hazelnut oil at which containing omega-9 in diet, liver can be protected.

Key words: *Obesity, rat, liver, high carbohydrate diet, high fatty diet*

Yüksek Yağlı Diyetin Rat Karaciğeri Üzerine Etkileri

ÖZET

Bu çalışmada yüksek yağlı diyetin rat karaciğeri üzerine etkilerinin ve kilo alımındaki rolünün incelenmesi amaçlandı. Bu amaçla 30 adet Wistar Albino rat 4 gruba ayrıldı.1. 16 haftalık yüksek karbonhidratlı diyet grubu (K16), 2. 16 haftalık yüksek yağlı diyet grubu (D16), 20 haftalık yüksek karbonhidratlı diyet grubu (K20), 20 haftalık yüksek yağlı diyet grubu (D20) grupları oluşturuldu. Yüksek yağlı diyet; %60 yağ (1/3 kanola, 1/3 margarin, 1/3 ayçiçek yağı), %20 protein ve %20 karbonhidrattan, yüksek karbonhidratlı standart diyet; %69 karbonhidrat, %20 protein ve %11 yağdan (margarin) oluşturuldu. Vücut ağırlığı, karaciğer ağırlığı ve epididimal yağ ağırlığı karşılaştırıldığında gruplar arasında anlamlı farklılık görülmedi. Biyokimyasal analiz sonucunda 16 haftalık yüksek karbonhidrat grubunda LDH, 20 haftalık yüksek karbonhidrat grubunda ALT anlamlı olarak yüksek bulundu. Histolojik incelemede ise tüm gruplarda portal alanda fibrozis, inflamasyon, steatozis bulguları görülmesine rağmen istatistiksel olarak anlamlı farklılık tespit edilmedi. İmmunohistokimyasal incelemede de tüm gruplarda a-SMA ve TGF-B tutulumu benzer bulundu. Sonuç olarak bu çalışma göreceli olarak omega-9'dan zengin yüksek yağlı diyetle karşılaştırıldığında yüksek karbonhidratlı beslenmenin karaciğer hasarına ve yağlanmaya yol açtığını, kilo alımı açısından anlamlı bir farklılık olmadığını göstermiştir. Bundan yola çıkarak diyetteki karbonhidratları kısıtlayarak ve yüksek oranda omega-9 içeren zeytinyağı, kanola ve fındık yağı miktarı arttırılarak karaciğerin korunabileceğini düşünüyoruz.

Anahtar kelimeler: *Obezite, rat, karaciğer, yüksek karbonhidratlı diyet, yüksek yağlı diyet*

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INTRODUCTION

Obesity is one of the most common and important diseases of today. Approximately, 1.2 billion people are overweight in the world and at least 300 million of them are obese. According to the World Health Organization, obesity is one of the most preventable ten diseases. Obesity is considered as associated with imbalance between energy intake and expenditure. However, studies show that genetic, physiological and behavioral factors play a role in the etiology of obesity (1).

In recent years, so many studies have been conducted about the relationship between diet and obesity. Previously, fats were supposed to play a leading role induce obesity because of including high-calorie, unlike this, some studies have been done about the carbohydrates induce obesity in recent years (1-3). According to Dr. Atkins trigger of obesity on foods are not fat and proteins, but carbohydrates. In Dr. Atkins diet method, daily carbohydrate intake is limited with maximum 20 grams protein and no restrictions are imposed on proteins and fats (1).

Obesity leads to steatosis and steatohepatitis on liver. While a normal liver has oxidative stress-resistant, fatty liver is vulnerable to oxidative stress. Researchers exemplify this with the two pulse hypothesis. In the lubricated liver as a result of obesity, inflammation, and fibrosis are developed and steatohepatitis is show up with effects of oxidative stres, cytokines such as TNF-α, mitochondrial dysfunction, hormones such as adiponectin and leptin (4).

In this study, we aimed to investigate effects of relatively high-fat diet rich in omega-9 on the liver. Furthermore, we aimed to reveal that high fat diet or isocaloric high-carbohydrate diet has more active role on weight gain and whether omega fatty acids in canola oil used in high-fat diet content has any protective effect on the liver.

MATERIALS AND METHODS

This experimental study was carried out with the approval of Law No. 2009-20 ethics committee of experimental animals. All rats were provided from Duzce University Experimental Animal Research Center.

30 male Wistar albino rats were divided into 4 groups when they were 28 days old.

1- 16-weeks old high-carbohydrate diet group (K-16 group) (n:7)

2- 16-weeks old high-fat diet group (D-16 group) (n:8)

3- 20-weeks old high-carbohydrate diet group (K-20 group) (n:7)

4- 20-weeks old high-fat diet group (D-20 group) (n:8)

All rats were kept in 12-h light/dark cycle conditions at room temperature (approximately 22°C). The cages where rats are located were maintained regularly. Rats were provided free access to food and water. Weights of rats were measured in every week. The experimental group was fed with high fat diet (60% fat, 20% carbohydrates, 20% protein). Fat in the diet was consisted of 1/3 canola oil, 1/3 sunflower oil and 1/3 margarine. In addition to this, diet included that fiber, ash, NaCl, Ca, P, Na, lysine, methionine, Mn, Zn, and A, D, E, and K vitamins. Isocaloric control group was fed with standard diet (69% carbohydrates, 20% protein, 11% fat). Standard diet contains only margarine as oil. In addition to this, diet included that fiber, ash, NaCl, Ca, P, Na, lysine, methionine, Mn, Zn, and A, D, E and K are vitamins.

Cervical dislocation was performed to the rats by ether anesthesia at the end of the sixteenth and twentieth weeks. Liver, epididymal adipose tissue, and blood samples were taken. After the tissues had been taken, weights were measured with precision scales and then placed in 10% formaldehyde solution. After these tissues had been fixed in formaldehyde solution for 48 hours, they were cut in appropriate size and then were embedded into paraffin block. Sections were stained with hematoxylin eosin and Gomori trichrome. Immunohistochemical staining was performed using by alpha smooth muscle actin (α-SMA)(Biocare Medical, Lot:062410) and transforming growth factor beta (TGF-β)(antibodies, Lot:360350) primary antibodies. Stained sections were examined and photographed with photomicroscope Olympus BX50. Serum levels of glucose, serum albumin, insulin, triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), lactate dehydrogenase (LDH), alanin aminotransferase (ALT), and aspartate transaminase (AST) were measured.

Mann-Whitney U Test was used for statistical evaluation of biochemical data, histological data, rat weights, epydidimal fat and liver weights. Moreover, biochemical evaluation has been analyzed with independent 2-Sample T Test as well.

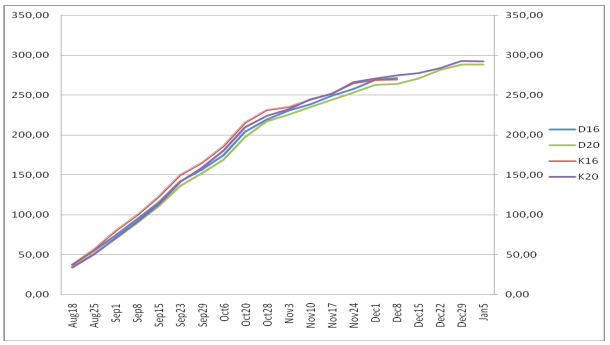

Figure 1. *Weight table of all groups according to dates*

RESULTS

Weights of rats

Weights of total 4 groups (16-weeks old high-carbohydrate diet group, 16-weeks old high-fat diet group, 20-weeks old high-carbohydrate diet group, 20-weeks old high-fat diet group) which are consisting of 30 rats were measured weekly regularly. The obtained data were statistically analyzed by Mann-Whitney-U test. During this assessment 16-weeks high-carbohydrate diet and 16-weeks high-fat diet groups were compared with each other, 20-weeks high-carbohydrate diet and 20-weeks high-fat diet groups were compared with each other (Figure 1). According to these statistical results, no significant difference was found in overall body weight change between16-weeks high-carbohydrate diet group and 16-weeks high-fat diet group (p>0.05). Moreover, according to these statistical results, no significant difference was found in overall body weight change between 20-weeks high-carbohydrate diet group and 20-weeks high-fat diet group (p> 0.05).

Table 1. *Liver and epydidimal fat weight analysis of all groups (Mean±SD)*

Group	*Liver weight(gr)*	*Epididymal fat weight(gr)*
K16	*7,85386±1,605751*	*3,1221±1,29010*
D16	*8,25638±1,710561*	*3,2880±0,96614*
K20	*9,78286±1,595731*	*3,2271±0,94607*
D20	*10,06875±2,189634*	*3,9088±0,90551*

Epididymal fat and liver weights

The obtained data were statistically evaluated by Mann-Whitney-U test. During this assessment 16-weeks high-carbohydrate diet and 16-weeks high-fat diet groups were compared with each other, 20-weeks high-carbohydrate diet and 20-weeks high-fat diet groups were compared with each other. According to these statistical results there was no statistically significant difference between 16-weeks high-carbohydrate diet group and 16-weeks high-fat diet group in terms of both liver weight and epididymal fat weight (p> 0.05). Also, no significant difference was found between 20-weeks high-carbohydrate diet group and 20-weeks high-fat diet group (p >0.05) (Table 1).

Biochemical Evaluation

Serum insulin, glucose, albumin, triglycerides, total cholesterol, LDL, HDL, LDH, ALT, and AST levels were measured in all groups (Table 2). The data were statistically evaluated using by the Mann-Whitney-U test. Insulin were excluded from statistical analysis for all groups, because of lower than 2µu/ml. According to these statistical results no significant difference was found between 16-weeks high-carbohydrate diet group

Table 2. *Mean and standard deviation of all groups' biochemical data*

	K16	*D16*	*K20*	*D20*
Glucose (mg/dl)	*176,00±23,248*	*161,00±28,127*	*143,00±20,321*	*132,00±20,601*
Albumin (g/dl)	*3,700±0,192*	*3,800±0,106*	*3,600±0,205*	*3,700±0,172*
Triglyceride (mg/dl)	*39,00±8,295*	*49,00±18,605*	*59,00±22,423*	*79,50±28,832*
Cholesterol (mg/dl)	*60,00±11,743*	*68,00±7,566*	*54,00±7,631*	*63,50±12,487*
LDL (mg/dl)	*10,00±3,338*	*10,00±1,976*	*9,00±1,604*	*7,50±2,642*
HDL (mg/dl)	*49,00±9,381*	*53,00±5,490*	*43,00±7,198*	*49,50±9,211*
LDH (U/L)	*1150,00±530,769**	*557,0±393,806*	*713,00±336,990*	*786,50±500,564*
ALT (U/L)	*70,00±20,840*	*49,0±13,831*	*69,00±15,820***	*46,00±7,421*
AST (U/L)	*195,100±106,705*	*113,700±39,465*	*107,800±15,838*	*111,250±15,899*

Table 3. *Statistical analysis of all groups (Mean and standard deviation)*

	Fibrosis	*Steatosis*	*Inflammation*
D16	*32,5±5,34*	*17,5±8,86*	*25 ± 13,09*
K16	*35±6,45*	*15,71±7,86*	*25,71±13,97*
D20	*25±9,25*	*25±14,14*	*19,38±13,74*
K20	*32,86±7,55*	*17,14±12,53*	*16,43±11,80*

and 16-weeks high-fat diet group in terms of glucose, albumin, triglycerides, cholesterol, LDL, HDL, ALT, and AST parameters. Only LDH levels were found significantly higher in 16-weeks high-carbohydrate diet group than 16-weeks high-fat diet group ($p<0.05$). No significant difference was found statistically between 20-weeks high-carbohydrate diet group and 20-weeks high-fat diet groups in terms of glucose, albumin, triglycerides, cholesterol, LDL, HDL, LDH, and AST parameters. Only ALT levels were found significantly higher in high-carbohydrate diet group ($p<0.05$).

No significant difference was found between 16-weeks high carbohydrate group and 20-weeks high carbohydrate group in terms of LDH and ALT levels. Significant difference was found statistically between16-weeks high-carbohydrate diet group and 20-weeks high-carbohydrate group only in terms of glucose level ($p=0.003$). According to this, the mean glucose level was higher in 16-weeks high carbohydrate group. Independent 2-sample T-test was used for this comparison. Statistically significant difference was found between16-weeks high-fat diet group and 20-weeks high-fat diet group in terms of triglycerides and LDL levels. According to this, the mean triglyceride level was higher in 20-weeks high fat diet group ($p=0.036$), the mean LDL level was higher in 16-weeks high fat diet group ($p=0,037$). Independent 2-sample T-test was used for this comparison. LDH and ALT are parameters which indicate hepatocyte damage(5,6). Emergence of these enzymes with elevated levels in high-carbohydrate diet shows that carbohydrates lead to liver damage (Table 3).

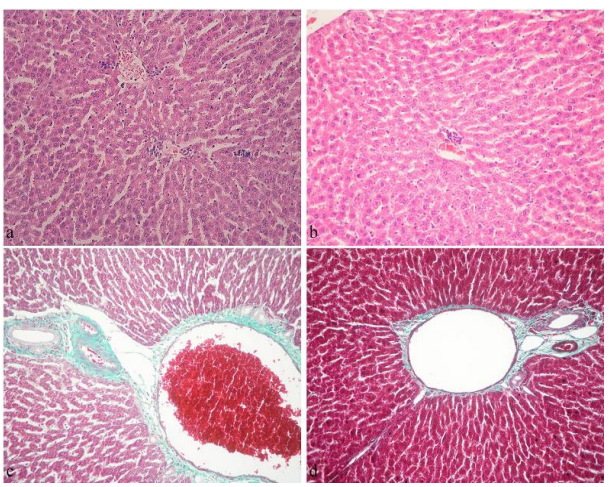

Figure 2. **a.** *Intralobuler inflammation is observed around of V. Centralis of 16-weeks old high-carbohydrate diet group (HE x200). b. Inflammation is observed in 16-weeks old high-fat diet group (HE x200). c. Fibrosis is observed in 16-weeks old high-carbohydrate diet group (Gomori-tricrom x200). d. Increase of collagen fiber is observed in portal area of 16-weeks old high-fat diet group (Gomori-tricrom x200).*

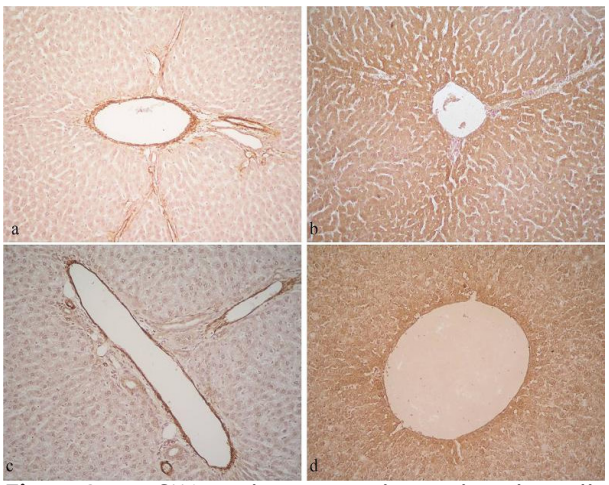

Figure 3.a. α-SMA involvement is observed on the walls of blood vessels of 16-weeks old high-carbohydrate diet group (α – SMA x200). b. TGF-β is observed in hepatocytes especially around of vena centralis of 16-weeks old high-carbohydrate diet group (TGF-β x200). c. α – SMA is observed on the walls of blood vessels of 16-weeks old high-fat diet group (α – SMA x200). d. TGF-β is observed around of vena centralis as intensive in 16-weeks old high-fat diet group (TGF-β x200).

Histological Evaluation

Liver sections of rats in all groups were stained with

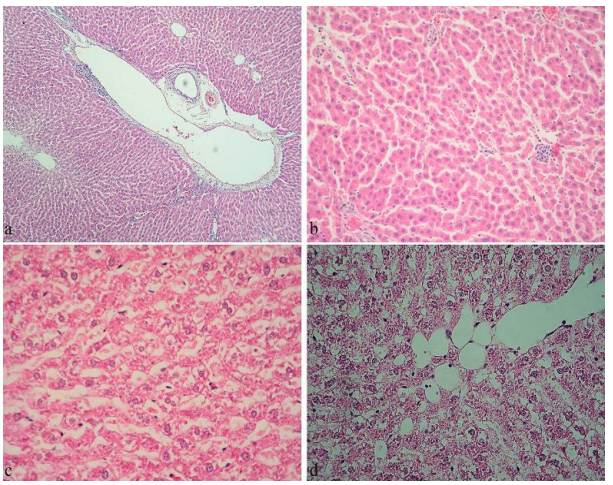

***Figure 4*. a.** *Fibrosis and inflammation is observed in portal area of 20-weeks old high-fat diet group (HEx100). b. Foci of inflammation is observed in 20-weeks old high-carbohydrate diet group (HEx200). c. Steatosis is observed in hepatocytes of 20-weeks old high-carbohydrate diet group (HEx400). d. Steatosis in hepatocydes and dilatation in sinusoids are observed on 20-weeks old high-fat diet group (HE x400).*

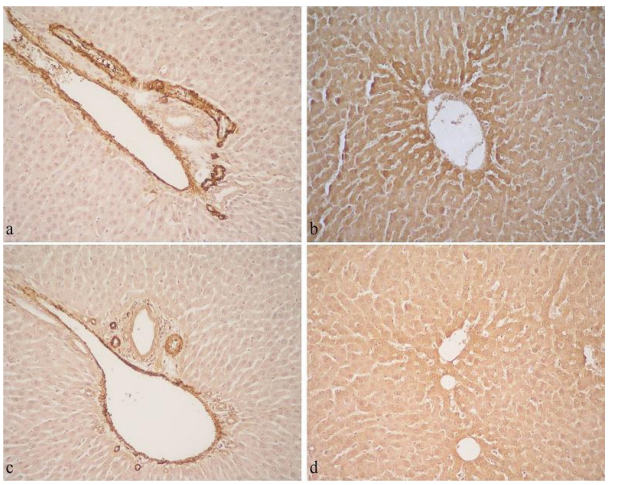

***Figure 5*. a.** *a - SMA is observed on the walls of blood vessels in 20-weeks old high-carbohydrate diet group (a - SMA x200). b. TGF-B is observed around of vena centralis as intensive in 20-weeks old high-carbohydrate diet group (TGF-B x200). c. a - SMA is observed on the walls of blood vessels in 20-weeks old high-fat diet group (a - SMA x200). d. TGF-B is observed in hepatocytes of 20-weeks old high-fat diet group (TGF-B x200).*

hematoxylin-eosin, Gomori trichrome. When the sections examined, fibrosis in portal area (Figure 2c, 2d), steatosis in hepatocytes (Figure 4c, 4d), and lobular inflammation in the portal area (Figure 2a, 2b, 4a, 4b) were observed in all groups. Hepatocyte nuclei and sinusoids observed in the normal structure, perisinusoidal fibrosis wasn't observed. Sections were stained with α-SMA primary antibodies to evaluate the hepatic fibrosis as immunohistochemically and were stained with TGF-β primary antibodies to determine hepatocyte damage. α-SMA was observed on the walls of blood vessels (Figure 3a, 3c, 5a, 5c). Especially, TGF- β was intensively observed around of vena centralis in hepatocytes (Figure 3b, 3d, 5b, 5d). Fibrosis, steatosis, and inflammation were scored for the statistical analysis. Rating: The rating has been done as unavailable /not exist:0, minimal: ±, focal: +, slightly:++, moderately +++, intensive (heavy) degree ++++ and 0:0, ±:5 and each + have been scored as 10 (7). Mann-Whitney-U test was statistically used for the assessment of significant differences between the groups. 16-weeks high-carbohydrate diet group was compared with 16-weeks high-fat diet group; 20-weeks high-carbohydrate diet group was compared with 20-weeks high-fat diet group (Figure 6); 16-weeks high-fat diet group was compared with 20-weeks high-fat diet group and 16-weeks high-carbohydrate diet group was compared with 20-weeks high-carbohydrate diet group. No significant difference was found statistically in these comparisons ($p > 0.05$).

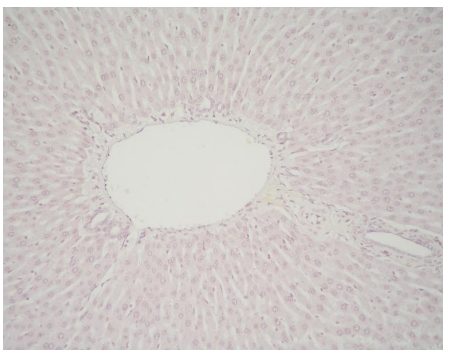

***Figure 6*. a.** *20-weeks old high-fat diet group negative control (x200).*

DISCUSSION

In this study, we aimed to investigate the role of a high-fat diet on weight gain and effects on liver. Therefore, 16-weeks old and 20-weeks old rat groups were arranged for comparison with high-fat diet including %60 fat (1/3canola, 1/3 margarine and 1/3 sunflower oil) and standard rat diet including high-carbohydrate. At the end of the experiment body weight, liver weight and epididymal fat weight were measured. Glucose, albumin, insulin, triglycerides, total cholesterol, HDL, LDL, VLDL, LDH, ALT, and AST levels were examined in cardiac blood. Liver tissues were examined histologically.

Although there was no significant difference between the groups compared with body weight, liver weight and epididymal fat weight, LDH in 16-weeks high carbohydrate group and ALT in 20-weeks high-carbohydrate group was found significantly higher.

In spite of detecting fibrosis, inflammation, steatosis findings in portal area histological examination of all groups, no significant differences were detected statistically. α-SMA and TGF-β were similar in immunohistochemical examination.

However, Zeng-Jie Xu et al.(8), noticed that the high fat diet (HFD) fed rats were observed as significantly increased in point of body weight, epididymal fat weight and liver index (Liver indeks(LI): liver weight / body weight X 100%) comparing to the control group rats. Although, serum ALT, serum free fatty acid (FFA) and serum TNF-α concentrations were higher in HFD-fed rats, there is no significant difference between the two groups in serum triglyceride (TG) levels.

In the livers of rats fed a normal diet, both macroscopic and microscopic abnormality weren't observed, while in rats fed with HFD diet, especially from 24-weeks to 48-weeks severe steatosis, from 16-weeks hepatic fibrosis findings were observed. Immunohistochemical examination of liver sections of rats fed with HFD from 12-weeks, the α-SMA and TGF-β increased expression were observed(8).

Similarly, Altunkaynak (9) compared high fat diet group (fat 30%, carbohydrate 50-52%, protein 18-20%, vitamin and minerals 1-2%; 210 kcal/100gr/day) with Standard diet control group (fat 7-10%, carbohydrate 68-70%, protein 18-20%, vitamin and minerals 1-2%; 210 kcal/100gr/day). As a result of this, dilatation of sinusoids, central veins and the branches of the portal vein, mononuclear cell infiltration and fibrosis were observed in high-fat diet groups (9). However, some studies carried out high-fat diet induced hyperglycemia and insulin resistance, also damaged the liver (10-11).

Kucera et al. (12) fed rats with ad libitum a Standard diet (10% kcal fat), a medium – fat gelled diet (MFGD) (35% kcal fat) and a high - fat gelled diet (HFGD) (71% kcal fat). Compared to Standard diet, there were no significant differences in serum biochemical parameters, except lower concentrations of triacylglycerols in HFGD and MFGD groups. This study showed that rats fed with HFGD developed comparable simple steatosis without signs of progression to non-alcoholic steatohepatitis (12).

Omagari et al. (15) compared high fat diet (HFD) with low fat standard diet(LFD). HFD included 45% fat (6%of soybean oil and 39% of lard, kcal) and LFD included 10% fat (6%of soybean oil and 4% of lard, kcal). No significant differences were found between the groups in body weight, epididymal fat weight, serum insulin, glucose, total cholesterol, triglyceride, leptin, adiponectin, ALT and AST levels. According to NASH Clinical Research Network scoring system, no significant differeneces were found histopatological examination among the three groups in frequency of steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis.

The lack of a statistically significant difference between high-fat diet and the control diet in biochemical and histopathological examination, suggest that oleic acid which has been in high fat diet content of soybean oil (13) and lard (14) would have protective effect (15). In another study, it was found that soybean oil decrease lipogenesis and liver fat density (16).

In some studies about high – fat diet, corn oil was used as the content of the high-fat diet and the negative effects such as liver steatosis, inflammation were observed (17,18). Corn oil includes 24.1% oleic acid, 61.9% linoleic acid, 11% palmitic acid, 2% stearic acid and linolenic acid 0.07% (19) and linoleic acid (omega-6) has inflammatory, thrombotic, mitogenic and hyperalgesic effects (20). These negative effects may depend on amount of omega - 6 fatty acids.

Omega-3 rich fish oil was found more protective in a study of lard and fish oil rich diet (21).

ALT levels, insulin resistance and IL-1ß levels were found higher in another study of high trans-fat diet (22).

Parthasaraty et al. (24), fed rabbits either a newly developed variant sunflower oil, containing more than 80% oleic acid and only 8% linoleic acid, or conventional sunfloweroil, containing only 20% oleic acid and 67% linoleic acid. LDL isolated from the plasma of animals fed with the variant sunflower oil was highly enriched in oleic acid and very low in linoleic acid. These oleate-rich LDL particles were significantly resistant to oxidative modification. These results show that diets sufficiently enriched in oleic acid, in addition to their LDL-lowering effect, may slow the progression of atherosclerosis by generating LDL that is highly resistant to oxidative modification (23).

Berry et al. (25), conducted a study which was randomly assigned to 24-weeks crossover study of monounsaturated fatty acid (MUFA) vs polyunsaturated fatty acid (PUFA) diets (50% carbohydrate, 32% fat, 18% protein) fed alternately during two 12-week periods. Total plasma cholesterol (TC) decreased significantly by 10% and 16% on the MUFA and PUFA diets, respectively. Low-density-lipoprotein cholesterol (LDL-C) decreased with an additional significant effect between periods in both groups.

Concentrations of high density lipoprotein cholesterol did not change significantly. There was significantly higher tendency toward lipid peroxidation on the PUFA diet, and lower to oxidative stress of LDL susceptibility on the MUFA diet(24).

Riveros et al. (26), suggested that high-oleic acid provided higher protection against lipid oxidation (25). Nakbi et al. (27) concluded that protective effect of oleic acid against oxidative damage (26). These findings clarifies that how a high-fat diet protects the liver in our study.

Gomez-Lechona et al. (28), designed a study to define an experimental model of hepatocellular steatosis, and found that a high proportion of palmitic acid (oleate/palmitate, 0:3 ratio) might represent a cellular model of steatosis (27).

In another study, Ricchi et al.(29) found that palmitic acid impaired insulin signalling. Despite the higher amount of fat resulting from incubation of the two fatty acids combined, the apoptosis rate and impaired insulin signalling were found lower than in cells treated with palmitic acid alone, indicating a protective effect of oleic acid (28).

Hussein et al.(30) aimed to evaluate the effects of different types of dietary fats on the hepatic lipid content and oxidative stress parameters in rat liver with experimental non-alcholic fatty liver disease. A total of 32 rats were divided into five groups. The rats in the control group were on chow diet, rats on methionine choline-deficient diet (MCDD), rats on MCDD enriched with olive oil, rats on MCDD with fish oil, rats on MCDD with butter fat. According to this study, olive oil decreases the accumulation of triglyceride in the liver of rats (29).

As mentioned in literature, depending on reduce to oxidative stress on LDL, Omega-9 decrease tissue damage. Only Ricchi et al.(29) found that oleic acid is protective against apoptosis, but more steatogenic than palmitic acid. One of the reason we couldn't find any significant differences between control group and high fat diet group, would be protective properties of omega-9 fatty acid.

Romestaing et al. (31), fed 21 day-old rats for 14-weeks, with either coconut oil or butter, and they were compared with rats fed with a standard diet or a methionine choline-deficient (MCD) diet, a nonphysiological model of nonalcoholic steatohepatitis (NASH). Long term high saturated fat feeding led to increased "peripheral" fat storage and brown adipose tissue thermogenesis but did not induce hepatic steatosis and NASH (30). The reason of this, compared to the standard diet rich in coconut oil and butter diet, would be having higher rate of oleic acid.

Some studies have stated that high-carbohydrate diet increases hepatic fatty acid synthesis(31), and on the contrary, some studies reported that a high-fat diet increases hepatic lipid level (8,9).

Berke et al. (32) fed obese and non-obese rats either a high fat diet or high carbohydrate diet. The rats fed with high fat diet in first 6-weeks, afterwards some of them fed with high carbohydrate diet until 10-weeks. Carbohydrate feeding resulted in increased hepatic fatty acid synthesis regardless of the earlier feeding of the fat diet, but the obese rats were more responsive to the carbohydrate diet than the non-obese rats (31). This study support our findings in case of carbohydrate diet increases hepatic fatty acid synthesis.

We couldn't find any significant differences between high-carbohydrate diet and high-fat diet both in terms of weight gain and liver damage in this study. In the mentioned studies, while any histopatologic finding concerning steatohepatitis wasn't found in high-carbohydrate diet groups, we found steatosis, fibrosis, and inflamation findings involved in steatohepatis in both high-carbohydrate diet groups and high-fat diet group but couldn't find any statistically significant differences between these two groups. We found that only LDH levels in high-carbohydrate diet group at 16-weeks and ALT levels in high-carbohydrate diet group at 20-weeks was significantly higher in biochemical evaluation. The primary reason of this, a high-fat diet rich in omega-9 fatty acids and the relative protective effect on the liver also the second reason diet rich in carbohydrates have adverse effects on the liver.

High-carbohydrate diet leads to hyperglycemia, and hyperglycemia leads to fast insulin secretion, hyperinsulinemia, insulin and leptin resistance (2,3). As a result of this, fat deposition in the hepatocytes, the increase in lipid peroxidation, the emergence of free oxygen radicals, oxidative stress and mitochondrial dysfunction occur (2).

Oboh et al. (33) found that high-carbohydrate, low-fat (HCLF) diet led to hypertriglyceridemia, as an indicator of hepatocellular injury, reduced serum protein and also increased AST, ALT, urea levels (32).

Lomba et al. (34) compared with high-sucrose (HS) diet and isocaloric control diet. HS diet increased adiposity, decreased plasma total cholesterol and HDL levels. Although there weren't any significant differences in terms of serum glucose, insulin, adiponectin, free fatty acids and liver malondialdehyde levels, slight increase was observed in serum and liver triglyceride levels.

These results show that high sucrose diets would induce mitochondrial dysfunction in adipose tissue due to excessive weight gain and metabolic deterioration (33).

It was found in other studies (34-38), high-carbohydrate diet caused to increase of adipose tissue and hepatic steatosis (39).

Also, it was determined that high-carbohydrate diet increased hepatic fat synthesis, caused to the accumulation of triglyceride in the liver, rise of ALT, AST levels, and caused mitochondrial dysfunction in adipose tissue (31-38).

This study has showed that LDH get higher in 16-weeks high-carbohydrate diet group, ALT get higher in 20-weeks high-carbohydrate diet group, carbohydrates cause to hepatocyte damage and relatively high-fat diet rich in oleic acid is protective. Also high-fat diet rich in omega-9 has protective effect on the liver and it does not cause weight gain compared with high-carbohydrate diet and high-carbohydrate diet leading more liver damage. As a result of this, in order to prevent weight gain and protect the liver, we suggest low carbohydrate diet rich in oleic acid.

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REFERENCES

1. *Wilborn C, Beckham J, Campbell B, at al. Obesity: Prevalence, Theories, Medical Consequences, Management, and Research Directions. J Int Soc Sports Nutrit 2005; 2(2): 4-31.*
2. *Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 2002 May 8;287(18):2414-23.*
3. *McAuley KA, Hopkins CM, Smith KJ, McLay RT, Williams SM, Taylor RW, Mann JI. Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. Diabetologia (2005) 48: 8-16.*
4. *Dowman J. K, Tomlinson J.W.and Newsome P.N. Pathogenesis of non-alcoholic fatty liver disease. Q J Med 2010; 103:71-83.*
5. *Murray RK, Granner DK, Mayes PA, Rodwell VW. Harper's Illustrated Biochemistry (26th Edition) Published by McGraw-Hill, 2003 pp:57.*
6. *Smith C, Marks A, Lieberman M. Mark's Basic Medical Biochemistry: A Clinical Approach 2nd Edition pp:858-859.*
7. *Baybutt CR, Molteni A. Dietary β-carotene protects lung and liver parenchyma of rats treated with monocrotaline. Toxicology 1999; 137: 69-80.*
8. *Xu JZ, Fan JG, Ding XD, Qjao L, Wang GL. Characterization of High- Fat, Diet Induced, Non-alcoholic Steatohepatitis with Fibrosis in Rats. Dig Dis Sci.2009.*
9. *Altunkaynak Z. Effects of High Fat Diet Induced Obesity on Female Rat Livers (A Histochemical Study). Eur J Gen Med 2005;2(3): 100-9.*
10. *Jornayvaz FR, Jurczak MJ, Lee HY, at al. A high-fat, ketogenic diet causes hepatic insulin resistance in mice, despite increasing energy expenditure and preventing weight gain. Am J Physiol Endocrinol Metab 2010; 299(5):*

808-15.

11. Tanoue S, Uto H, Kumamoto R, at al. Liver regeneration after partial hepatectomy in rat is more impaired in a steatotic liver induced by dietary fructose compared to dietary fat. Biochem Biophys Res Commun 2011; 407(1): 163-8.

12. Kucera O, Garnol T, Lotkova H, at al. The effect of rat strain, diet composition and feding period on the development of a nutritional model of non-alcoholic fatty liver disease in rats. Physiol Res 2011; 60(2): 317-28.

13. Hwang J, Jun HS, Shim E. Rates of Change in Fatty Acid Composition When Dietary Soybean Oil Is Switched to Olive Oil. Journal of Health Science 2010; 56(3): 275-86.

14. Hammer CT, Wills ED. The role of Lipid Components of the Diet in the Regulation of the Fatty Acid Composition of the Rat Liver Endoplasmic Reticulum and Lipid Peroxidation. Biochem J 1978; 174: 585-93.

15. Omagari K, Kato S, Tsuneyama K, at al. Effect of a Long-Term High-Fat Diet and Switching from a High-Fat to Low-Fat, Standart Diet on Hepatic Fat Accumulation in Sprague-Dawley Rats. Dig Dis Sci 2008; 53: 3206-12.

16. Reis SRL, Feres NH, Souza LMI at al. Soybean diet reduces liver fat in recovered rats of protein restriction in early life. 18th European Congress on Obesity Proceedings Book, pp:145. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.

17. Torres DDO, Santos ACO, Silva AKS, at al. Effect of Maternal Diet Rich in Omega-6 and Omega-9 Fatty Acids on the Liver of LDL Receptor-Deficient Mouse Offspring. Birth Defects Research (Part B). 2010; 89: 164-170.

18. Lieber CS, Leo MA, Mak KM, at al. Model of nonalcoholic steatohepatitis. American J Clin Nutr 2004; 79(3): 502-9.

19. Zou Y, Li J, Lu C, at al. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sciences 2006; 79(11): 1100-7.

20. White PJ, Linda M, Duvick P, Duvick S. Improving the fatty acid composition of corn oil by using germplasm introgression. Lipid Technology 2006; 19(2): 35-8.

21. Simopoulos AP. Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. Food Reviews International Vol. 20, No. 1, 2004, pp. 77-90.

22. Lionetti L, Mollica MP, Donizzetti I at al. Mitochondrial morphology and functions are differently affected by high fat diet rich in lard or in fish oil in the development of hepatic injury. 18th European Congress on Obesity Proceedings Book, pp:50. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.

23. Koppe SW, Elias M, Moseley RH, Gren RM. Trans fat feding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet. Am J Physiol Gastrointest Liver Physiol 2009; 297(2): 378-84.

24. Parasarathy S, Khoo JC, Miller E, Barnett J, Witztum JL, Steinberg D. Low density lipoprotein rich in oleic acid is protected against oxidative modification: Implications for dietary prevention of atherosclerosis. Proc Natl Acad Sci 1990; 87:3894-8.

25. Berry EM, Eisenberg S, Haratz D, at al. Effect of diets rich in monounsaturated fatty acids on plasma lipoproteins-the Jerusalem Nutrition Study: high MUFAs vs high PUFAs. Am J Clin Nutr 1991; 53: 899-907.

26. Riveros CG, Mestrallet MG, Gayol MF, Quiroga PR, Nepote V, Grosso NR. Effect of storage on chemical and sensory profiles of peanut pastes prepared with high-oleic and normal peanuts. J Sci Food Agric 2010; 90(15): 2694-9.

27. Nakbi A, Tayeb W, Girssa A, at al. Effect of olive oil and its fractions on oxidative stres and the liver`s fatty acid composition in 2,4-Dichlorophenoxyacetic acid-treated rats. Nutr Metab 2010; 7:80.

28. Gomez-Lechon MJ, Donato MT, Martinez-Romero A, Jimenez N, Castell JV, O'Connor JE. A human hepatocellular in vitro model to investigate steatosis. Chem Biol Interact 2007; 165(2): 106-16.

29. Ricchi M, Odoardi MR, Carulli L, at al. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. J Gastroenterol Hepatol 2009; 830-40.

30. Hussein O, Grosovski M, Lasri E, Svalb S, Ravid U, Assy N. Monounsaturated fat decreases hepatic lipid content in non-alcoholic fatty liver disease in rats. World J Gastroenterol 2007; 13(3): 361-8.

31. Romestaing C, Piquet MA, Bedu E at al. Long term highly satured fat diet does not induce NASH in Wistar rats. Nutr Metab 2007; 4:4.

32. Berke BM, Kaplan ML. Effect of high fat and high carbohydrate diets on development of hepatic and adipose lipogenesis in fa/fa and non-fa/fa rats. J Nutr 1983; 113(4): 820-34.

33. Oboh HA, Omofoma CO, Olumese FE, Eiya B. Effects of High Carbohydrate Low Fat Nigerian-Like Diet on Biochemical Indices in Rabbits. Pakistan J Nutr 2007; 6(4): 399-403.

34. Lomba A, Milagro FI, Garcia-Diaz DF, Campion J, Marzo F, Martinez JA. A high-sucrose isocaloric pair-fed model induces obesity and impairs NDUFB6 gene function in rat adipose tissue. J Nutrigenet Nutrigenomics 2009; 2(6): 267-72.

35. Haubert NJ, Padovan GJ, Zucoloto S, Vannucchi H, Marchini JS. Experimental induction of steatosis in different tissues after the ingestion of a carbohydrate-rich diet: effect on the liver, on the heart and on indicators of oxidation. Arg Gastroenterol 2010; 47(4): 388-92.

36. Waddell M, Fallon HJ. The effect of high-carbohydrate diets on liver triglyceride formation in the rat. J Clin Invest 1973; 52: 2725-31.

37. Nseir W, Nassar F, Assy N. Soft drinks consumption and nonalcoholic fatty liver disease. World J Gastroenterol 2010; 16(21): 2579-88.

38. Tapply L, Schneiter Ph, Bortolotti M, Le KA. Hepatic lipotoxicity: modulation by nutrients. 18th European Congress on Obesity Proceedings Book, pp:10. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.

39. Crescenzo R, Bianco F, Falcone I, at al. Dietary fructose-induced obesity and insulin resistance: is there a role for altered hepatic mitochondrial energetics. 18th European Congress on Obesity Proceedings Book, pp:149. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.

40. Fuente-Martin E, Garcia-Caceres C, Diaz F, at al. Interaction between neonatal over-nutrition and a subsequent sucrose-enriched diet on adipose tissue acquisition. 18th European Congress on Obesity Proceedings Book, pp:47. 18th European Congress on Obesity, Istanbul, Turkey, 25-28 May 2011.