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Research Article

Investigation of Lipid Profile and Liver Enzymes in Rats Fed on Trans Fats Obtained from Cotton Oil

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ABSTRACT

It is known that the intake of trans fats, commonly used in the food industry around the world, has a negative effect on healthy and balanced nutrition. The aim of this study was to determine the biochemical changes in an organism following the consumption of cotton oil (CO) or trans-fat obtained from cotton oil (TF). A total of 18 Wistar Albino male rats were used. The research was performed in SUDAM unit. Rats divided into 3 equal groups: The control (C) group was fed with a standard rat diet, the CO group was given the standard diet added with 5% CO, and the TF group was given the standard diet added with 5% TF (including 30% trans-fat). *Ad libitum* feeding and water were allowed for 8 weeks of the experimental period. Blood samples were collected on day 0 by the supraorbital method and on day 60 by the intracardiac method under anesthesia. Glucose, HbA1c, triglyceride, total cholesterol, HDL- cholesterol, LDL- cholesterol levels, AST, ALT, ALP, GGT, and SOD activities analyses were performed. SPSS (19.0) program was used for the statistical analyses. Trans fat feeding increased LDL-cholesterol and triglyceride levels, decreased HDL-cholesterol level, and caused some changes in the activities of the liver enzymes investigated.

Keywords: Cotton oil, lipid profile, liver enzymes, rat, trans fat

Pamuk Yağından Elde Edilen Trans Yağlarla Beslenen Ratlarda Lipid Profili ve Karaciğer Enzimlerinin Araştırılması

ÖZET

Sunulan çalışmada, trans ve pamuk yağlarının organizmada meydana getirdiği biyokimyasal değişikliklerin belirlenmesi amaçlandı. Çalışmada 18 adet 12 haftalık Wistar Albino cinsi erkek rat kullanıldı. Ratlar 1. Grup kontrol, diğer 2 grup çalışma grubu olarak toplam 3 gruba ayrıldı. Sekiz hafta süresince 1. Gruba standart rat yemi ve su, 2. Gruba pamuk yağlı yem ve su, 3. Gruba trans yağlı yem ve su *ad libitum* olarak verildi. Çalışma gruplarından 0. günde supraorbital ve 60. günde ise intrakardiyak yöntemle anestezi altında kan alımı gerçekleştirildi. Ratlardan alınan kan örneklerinden glikoz, HbA1c, trigliserit, kolesterol, HDL, LDL, AST, ALT, ALP, SOD ve GGT analizleri yapıldı. Analizler için SPSS programı kullanıldı. Sunulan çalışmada trans yağların, LDL ve trigliserit konsantrasyonunu arttırdığı, HDL konsantrasyonunu azalttığı, bazı karaciğer enzim değerlerinde değişmelere neden olduğu görülmüştür. Trigliserit, LDL, ALP analizlerinde istatistiksel açıdan anlamlı sonuçlar elde edilmiştir ($p<0.05$). GGT ve AST analizlerinde ise istatistiksel açıdan anlamlı sonuçlar elde edilememiştir ($p>0.05$).

Anahtar Kelimeler: Pamuk yağı, lipid profili, karaciğer enzimleri, rat, trans yağ

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Introduction

All living organisms need to be fed sufficiently and balanced in order to maintain their vital functions (Çelebi and Karaca, 2006). The nutrients necessary for maintenance and vital activities are stored in the body. The most important of these nutrients; carbohydrates, proteins, and fats. Energy for the digestion of these nutrients is provided by the organism. The organism takes most of this energy from the fats. The fats are an important part of the cell membranes, involve in the absorption of fat-soluble vitamins, and are the source of essential fatty acids in the body (Karaali, 1997; Murray et al., 2003). Depending on the double bonds in their structure, the fatty acids are classified as saturated or unsaturated (Dutton, 1979). Unsaturated fatty acids are either at cis or trans form depending on the location of the hydrogen atoms on the double bonds (Dutton, 1979). Cis fat is found abundantly in nature while trans fat is present in industrial fats. Trans fat forms when cis unsaturated fats are hydrogenated to remove the double bonds and become saturated fats. Presence of saturated or unsaturated fatty acids in the composition of the fats directly affects the cholesterol levels in the blood; saturated fatty acids increase the level while unsaturated fatty acids decrease it.

Recent studies have demonstrated that trans fatty acids increase cholesterol levels similarly like saturated fatty acids (Sanders et al., 2000; Lichtenstein et al., 2003; Wijendran et al., 2003; Murray et al., 2004). Trans fatty acids raise low density lipoprotein (LDL) levels and reduce high density lipoprotein (HDL) levels. Furthermore, by elevating the LDL/HDL-cholesterol ratio they also increase the risk for heart diseases (Kayahan, 2003; Tasan et al., 2007). They also play critical roles in obesity, cancer, Alzheimer, diabetes, allergy, and fetal development (Innis et al., 1999; Kıralan et al., 2005; Kavanagh et al., 2007). Due to the changes in liver enzymes negative effects of the trans fatty acids are observed in the liver, muscle tissue, bones, and joints such as liver and muscle tissue damage, bone resorption and degeneration (Sharma et al., 2012). Increased intake of trans fatty acids by humans leads to an increase in the number of deaths due to heart attacks. Industrially produced trans fatty acids are effective in increasing this number.

Additionally, the negative effects of trans fatty acids on type 2 diabetes can be observed (Kıralan et al., 2005). Feeding the children during the developmental ages with trans fatty acid containing nutrients have increased the frequency of asthma and allergic disorders (Stender and Dyerberg, 2004). It has been observed that the use of trans fatty acids in pregnant women adversely affects the development of the fetus and increases the number of premature births. Trans fatty acid containing feeding can also be said to increase the probability of Alzheimer's disease in people of middle ages and older (Kıralan et al., 2005).

In this study, the effects of feeding male rats with either cotton oil or trans fat obtained from cotton oil were investigated on blood glucose, HbA1c, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol levels, AST, ALT, ALP, GGT, and SOD activities and live weights.

Material and Methods

For this study, 18 Wistar Albino male rats that 12 weeks old, weighing 350-380 g were used as animal material. Rats were housed in rooms with heat and light adjustment. Feed and water were given as *ad libitum* for 8 weeks of the experimental period. The rats were divided into 3 groups as follows:

1. Group (Control Group, C, n = 6): Standart rat diet

2. Group (Cotton Oil Group, CO, n = 6): Standart rat diet + 5% cotton oil

3. Group (Trans Fat Grovup, TF, n = 6): Standart rat diet + 5% trans fat obtained from cotton oil (contains 30% trans fat)

Preparation of diets:

Cotton oil diet: 95 g standard diet was ground to powder and 5 g cotton oil was added. Then, pellets were made and kept at - 20˚C.

Trans fat diet: 95 g standard diet was ground to powder and 5 g trans fat obtained from cotton oil was added. Added fat contained 30% trans fat in 5 g. Then, pellets were made and kept at - 20˚C

Blood samples were collected on day 0 by the supraorbital method and on day 60 by the intracardiac method under anesthesia. Analysis of glucose, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, AST, ALT, ALP, and GGT was performed in biochemical autoanalyzer (Architect ci 8200 Abbott). HbA1c was measured with Premier HB9210. SOD analysis was performed on the ELISA reader (Biotek ELX800) with the Cayman Chemical branded catalog number 706002.

Data were analyzed with one-way ANOVA and t-test. In comparison between day 0 and day 60 independent t-test was used and Duncan test was employed to detect differences among the study groups. The means and standard errors were given and $P < 0.05$ was considered as significant. All the statistical analyses were performed with the SPSS program.

This study was performed at the Selcuk University Experimental and Medical Center (SUDAM). The ethical approval (2017/28) was obtained from the Ethical Committee of the Faculty of Veterinary Medicine, Selcuk University. It was funded by SUBAP project number 17202049.

Results

The results for biochemical parameters and live weights on day 0 and 60 in the study groups were given in Table 1.

There was no statistically significant difference between the study groups and the control group in the glucose levels on day 60 ($P > 0.05$). HbA1C values in the blood samples were found to be statistically significant in CO and TF groups compared to the C group on day 60 ($P < 0.05$). Triglyceride value was significantly lower on day 60 in the CO group compared to C and TF groups ($P < 0.05$). Cholesterol and HDL-cholesterol levels did not differ among the groups on day 60 while the LDL-cholesterol level showed significant differences among the groups both on day 0 and day 60. As GGT, AST and ALT activities were compared among the groups, no statistically significant difference was observed on day 60 ($P > 0.05$). The ALP activity was found to be significantly higher on day 60 in the TF group compared to C and CO groups ($P < 0.05$). Superoxide dismutase activity was higher on day 60 in the TF group ($P < 0.05$). When the live weight values of the control group and the study groups were compared, it was observed that the live weight values of the CO and TF groups were statistically significantly lower on day 60 compared to the control group ($P < 0.05$).

Discussion and Conclusion

In this age of the world, many health problems are caused by irregular, unbalanced and inadequate nutrition. Most of the daily diets include hydrogenated margarine, fast foods, and

Table 1 The results for the parameters investigated on day 0 and 60 of the experimental period (mean ± SE) (n=18)

	DAY	n	CONTROL (C)	n	COTTON OIL (CO)	n	TRANS FAT (TF)
Glucose (mg/dl)	0.	6	192.33 ± 7.82 $^{a.A}$	6	178.33 ± 3.70 $^{a.A}$	6	181.66 ± 9.61 $^{a.A}$
	60.	6	262.83 ± 18.96 $^{a.B}$	6	232.16 ± 26.02 $^{a.A}$	6	235.16 ± 11.49 $^{a.B}$
HbA1c (%)	0.	6	4.41 ± 0.04 $^{a.A}$	6	4.28 ± 0.09 $^{a.A}$	6	4.43 ± 0.05 $^{a.A}$
	60.	6	5.31 ± 0.08 $^{a.B}$	6	4.93 ± 0.14$^{b.B}$	6	4.76 ± 0.08 $^{b.B}$
Triglyceride (mg/dl)	0.	6	117.33 ± 14.87 $^{a.A}$	6	103.33 ± 25.24 $^{a.A}$	6	128.16 ± 17.79 $^{a.A}$
	60.	6	190.66 ± 34.62 $^{a.A}$	6	108.16 ± 14.91$^{b.A}$	6	230.50 ± 27.43 $^{a.B}$
Total Cholesterol (mg/dl)	0.	6	75.16 ± 2.93 $^{a.A}$	6	71.66 ± 3.01 $^{a.A}$	6	74.00 ± 1.86 $^{a.A}$
	60.	6	66.16 ± 3.70 $^{a.A}$	6	69.00 ± 3.18 $^{a.A}$	6	65.50 ± 2.66 $^{a.B}$
HDL-cholesterol (mg/dl)	0.	6	51.50 ± 2.62 $^{a.A}$	6	49.66 ± 3.27 $^{a.A}$	6	54.00 ± 1.50 $^{a.A}$
	60.	6	49.00 ± 2.51 $^{a.A}$	6	53.50 ± 3.28$^{a.A}$	6	46.50 ± 2.44 $^{a.B}$
LDL-cholesterol (mg/dl)	0.	6	16.00 ± 0.89 $^{a.A}$	6	15.16 ± 0.87 $^{ab.\ A}$	6	12.83 ± 0.87 $^{b.A}$
	60.	6	13.00 ± 0.85$^{b.B}$	6	16.00 ± 1.09 $^{a.A}$	6	13.50 ± 0.56 $^{ab.\ A}$
AST (U/L)	0.	6	91.83 ± 3.65 $^{a.A}$	6	85.50 ± 4.95 $^{ab.\ A}$	6	72.66 ± 6.08 $^{b.A}$
	60.	6	131.83 ± 41.37 $^{a.A}$	6	134.83 ± 41.99 $^{a.A}$	6	72.66 ± 7.08 $^{a.A}$
ALT (U/L)	0.	6	48.66 ± 2.67 $^{a.A}$	6	49.66 ± 2.90 $^{a.A}$	6	53.66 ± 4.18 $^{a.A}$
	60.	6	94.66 ± 21.71 $^{a.A}$	6	96.83 ± 39.39 $^{a.A}$	6	54.50 ± 1.74 $^{a.A}$
ALP (U/L)	0.	6	211.00 ± 40.37 $^{a.A}$	6	266.00 ± 24.76 $^{a.A}$	6	291.16 ± 29.84 $^{a.A}$
	60.	6	199.66 ± 28.38 $^{b.A}$	6	284.66 ± 32.03 $^{b.A}$	6	390.33 ± 35.13 $^{a.B}$
GGT (U/L)	0.	6	1.35 ± 0.17 $^{a.A}$	6	1.35 ± 0.17 $^{a.A}$	6	1.48 ± 0.13 $^{a.A}$
	60.	6	0.81 ± 0.29 $^{a.A}$	6	1.21 ± 0.18 $^{a.A}$	6	1.26 ± 0.20 $^{a.A}$
SOD (U/ml)	0.	6	0.081 ± 0.007 $^{a.A}$	6	0.081 ± 0.007 $^{a.A}$	6	0.076 ± 0.003 $^{a.A}$
	60.	6	0.100 ± 0.008 $^{b.B}$	6	0.095 ± 0.009 $^{b.A}$	6	0.227 ± 0.055 $^{a.B}$
Live Weight (g)	0.	6	374.66 ± 10.09 $^{a.A}$	6	353.00 ± 9.86 $^{a.A}$	6	368.50 ± 5.64 $^{a.A}$
	60.	6	490.00 ± 12.20 $^{a.B}$	6	441.66 ± 14.45$^{b.B}$	6	443.66 ± 8.20 $^{b.B}$

For each parameter, differences were indicated in the same line with a, b, c.(ANOVA-Duncan) and in the same column with A, B, C (Paired t test).

shortening. Oils used daily for nutrition contains a high rate of trans fats. The negative effects of trans fats on human health are mentioned in many studies. (Gürcan, 2002; Lichtenstein et al., 2003; Lemaitre et al., 2006). Therefore, this study aimed to investigate some biochemical parameters and live weight gains in the male rats which were fed diets supplemented with cotton oil or trans fat obtained from cotton oil.

There was no statistically significant difference in the glucose levels between groups ($P > 0.05$). When the comparison was made between days within the groups, it was observed that the glucose levels of the TF group on day 60 were statistically higher than day 0 ($P < 0.05$). Kavanagh et al. (2007) evaluated the effects of trans fats on obesity and insulin sensitivity. In contrast to our findings, trans fats were found to have no significant effect on glucose levels compared to the control group. In addition, Destaillats et al. (2005) indicated that decreasing the amount of trans fats in foods had no potential benefit on glucose balance. Huang et al. (2009) showed in a study performed in rats that the trans fat fed rats and control rats had a similar increase in the blood glucose levels. This was thought to be due to the standard rat feed like in the present study. A similar but not significant increase in the blood glucose level between day 0 and day 60 was also observed in this study in the CO group. The present results are in accordance with those reported by Huang et al. (2009) and could indicate that, the standard rat diet could have caused the increase in the blood glucose levels regardless of the fat feeding.

In the present study, CO group rats had significantly lower triglyceride level compared to other groups on day 60; however, TF group rats had significantly higher triglyceride levels on day 60 compared to their day 0 levels ($P < 0.05$). Some earlier studies using trans fat diets observed that triglyceride concentration increased in a similar way as the values presented in the study (Zock and Katan, 1992; Sundram et al., 1997; Rivellese et al., 2003) while others reported no differences in triglyceride levels due to trans fat feeding (Almendingen, 1995; Roos et al., 2003). Moreover, Peter et al. (1992) stated that trans fat taken with foods did not cause major changes in the triglyceride

levels of the blood and the differences could be due to fat supplementation to the feeds.

When the cholesterol levels were examined, there was no statistically significant difference in the cholesterol levels among the groups ($P > 0.05$). There was no statistically significant difference in the LDL-cholesterol levels between groups ($P > 0.05$). The HDL-cholesterol levels did not differ among the groups on day 60 ($P > 0.05$). Earlier studies have demonstrated that trans fat consumption lowers the HDL-cholesterol levels and increases the LDL-cholesterol and total cholesterol levels (Zock and Katan, 1992; Sundram et al., 1997; Rivellese et al., 2003). Murray et al. (2004) reported that nutrients which contain trans fats elevated LDL cholesterol levels, decreased HDL cholesterol levels and increased risk of coronary heart disease. The HDL and LDL-cholesterol values in the study of Murray et al. (2004) are consistent with the results of the present study. Accordingly, it may be considered that changes in cholesterol levels may be observed in a shorter time when the amount of trans fat in nutrients is increased. Also, Peter et al. (1992) stated that trans fat intake decreases HDL/LDL-cholesterol ratio without a significant change in the triglyceride levels. In the present study, the TF group had a decreased HDL/LDL-cholesterol ratio compared to the other groups.

It is stated that when the intake of industrially produced trans fats causes an increase of 2% in the energy levels, the risk of developing heart disease rises by 55% (Stender and Dyerberg, 2004). On the other hand, replacing the source for this energy from trans fats to unsaturated fats decreases the rate of coroner diseases about 53% (Kitao and Hattori, 1983). Feldman (1999) demonstrated that an increase in the total and LDL cholesterol values results in an increase in the risk of developing cardiovascular disease and elevation in the HDL cholesterol causes a decrease in that risk. Mersink and Katan (1990) stated that the intake of trans fats was associated with an increase in the plasma lipid levels. In addition, replacing cis-unsaturated fatty acids in foods with trans fats caused an increase in total and LDL cholesterol levels. Based on the information given above, it is understood that the long period of trans fat feeding as in the current study could increase the risk of developing cardiovascular diseases and that the replacement of trans fats in the diets with unsaturated fats would reduce the incidence of coronary diseases.

In the present study, liver function tests were performed and AST and ALT activities were found be not affected by the experimental feedings although TF group had a numerically, but not significantly, lower values ($P > 0.05$). However, ALP activities were significantly higher in the rats of the TF group ($P < 0.05$). Gama glutamyl transpeptidase activities were also unaffected and based on the report by Ersoy (2012), no changes in the GGT activities could be related to the location of the liver damage. It is thought that GGT activities may significantly change with longer duration of feeding the rats with trans fats.

When HbA1C and body weight values were compared between groups on day 60, HbA1C and body weight values of CO and TF groups were found to be statistically lower than the control group ($P < 0.05$). Kavanagh et al. (2007) fed monkeys for 6 years with trans fats containing diets which caused a 7.2% increase in the body weight while feeding with a monounsaturated fat diet resulted in a 1.8% increase. In addition, a study with women (Stender and Dyerberg, 2004) showed that the increase in the risk of obesity leads to an increase in the likelihood of type 2 diabetes in humans. More studies (Stender and Dyerberg, 2004; Kıralan et al., 2005) indicate that trans fats facilitate the development of type II diabetes. Kırılan et al. (2005) reported that trans fats caused an increase in insulin resistance by causing changes in the ion structure of the cell wall. In another study (FDA, 2003), it was stated that trans fats caused undesirable effects in diabetic patients by reducing the response of red blood cells to insulin, and a positive correlation between the development of type 2 diabetes and trans fat intake in obese women has been observed. Ghafoorunissa (2008) stated that the increase in the intake of linoleic acid (poliunsturated omega 6) adversely affects insulin resistance and therefore trans fat intake should be reduced.

In recent studies, trans fats are reported to be associated with the development of some types of cancers (Innis et al., 1999), type 2 diabetes, and they trigger asthma and allergies in children (Tasan and Daglioglu, 2005; Tasan et al., 2007). Therefore, developed countries impose restrictions on the amount of trans fats in nutrients. FDA and WHO inform the public about reducing the consumption of trans-fat-containing foods. Countries such as the USA, Netherlands, and Canada require that the labels on the food products should include information about their trans fat contents (FDA, 2003; Stender and Dyerberg, 2004).

In Turkey, efforts are made to decrease the use and consumption of trans fats. Restrictions are considered to limit the use of industrial trans fatty acids in food products. Specifically, the use of alternative methods for the hydrogenation process is suggested in the production of margarine and shortenings. New regulations have been applied for the preservation and storage of foods. By providing public awareness by the public and non-governmental organizations, it is aimed to reduce trans fatty acid consumption.

In conclusion, the presence of trans fats in foods causes significant changes in biochemical blood parameters, which leads to the metabolic disorders and therefore the use of trans fats should be taken into consideration in order for a healthy life.

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