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RESEARCH ARTICLE

Determination of hemoglobin A1c (HbA1c), alkaline phosphatase (ALP), aspartate aminotransferase (AST), superoxide dismutase (SOD) values and lipid profiles in rats fed with corn syrup and trans oil added diets

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Mısır şurubu ve trans yağ ilave edilen yemle beslenen ratlarda lipit profilleri ile hemoglobin A1c (HbA1c), alkalen fosfataz (ALP), aspartat aminotransferaz (AST), süperoksit dismutaz (SOD) değerlerinin belirlenmesi

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Öz

Amaç: Son yıllarda mısır şurubu ve trans yağlar gıda üretimi ve tüketiminde önemli bir yere sahiptir. Trans yağlar (TF) ve yüksek fruktozlu mısır şurubunun (HFCS) sağlık üzerinde benzer olumsuz etkilere neden olabileceğinden yola çıkılarak, diyette HFCS ve TF alımını sonucunda organizmada meydana gelen bazı biyokimyasal parametrelerindeki değişiklikleri belirlemek çalışmanın amacını oluşturmaktadır.

Gereç ve Yöntem: *Wistar Albino* erkek sıçanlar (n = 24) 4 eşit gruba ayrıldı. Kontrol grubu (C) 8 hafta boyunca standart fare yemi, 2. grup pamuk yağı, 3. grup trans yağ ve 4. grup trans yağ ve mısır şurubu ile beslendi. Çalışma gruplarından anestezi altında 0. günde supraorbital yöntemle ve 60. günde intrakardiyak yöntemle kan alımı gerçekleştirildi. Glukoz, HbA1c, trigliserit, kolesterol, HDL, LDL, AST, ALT, ALP, SOD ve GGT analizleri yapıldı.

Bulgular: Sunulan çalışmada trans yağların, trigliserit konsantrasyonunu arttırdığı, HDL konsantrasyonunu azalttığı, bazı karaciğer enzim değerlerinde değişmelere neden olduğu görülmüştür. Glukoz ve trigliserit konsantrasyonunda artış, HDL ve GGT analizlerinde azalma istatistiksel olarak anlamlıydı ($p < 0.05$). LDL, AST ve HbA1c analizlerindeki artış ve azalma istatistiksel olarak anlamlı değildi ($p > 0.05$).

Öneri: Gıdalarda bulunan yüksek fruktozlu mısır şurubu ve trans yağ asidi miktarının mümkün olduğunca sınırlandırılması gerektiği ve bu sınırlamaların insan sağlığını olumlu etkileyeceği sonucuna varıldı.

Anahtar kelimeler: Trans yağ, fruktoz, mısır şurubu, rat, lipid değerleri

Abstract

Aim: In recent years, corn syrup and trans fats have an important place in food production and consumption. Since trans fats (TF) and high fructose corn syrup (HFCS) can cause similar negative effects on health, the aim of this study was to determine the changes in some biochemical parameters of an organism following the intake of HFCS and TF in the diet.

Materials and Methods: *Wistar Albino* male rats (n = 24) were divided 4 equal groups. Control group (C) was fed with a standard rat feed, 2nd group with cotton fat, 3rd group with trans fat, and 4th group with trans fat and corn syrup for 8 weeks. Blood samples were collected on day 0 by the supraorbital method and on day 60 by the intracardiac method under anesthesia. Glucose, HbA1c, triglyceride, cholesterol, HDL, LDL, AST, ALT, ALP, SOD, and GGT analyzes were performed.

Results: In the present study, trans fats increased triglyceride concentration, decreased HDL concentration and caused changes in some liver enzyme values. Increase in glucose and triglyceride concentration and decrease in HDL and GGT analyzes were statistically significant ($p < 0.05$). The increase and decrease in LDL, AST and HbA1c analyzes were not statistically significant ($p > 0.05$).

Conclusion: High fructose corn syrup and trans fatty acid found in foods ıt was concluded that the amount should be limited as much as possible, and these limitations will positively affect human health.

Keywords: Trans fat, fructose, corn syrup, rat, lipid values

Introduction

Fats are the source of energy at high levels, an important part of the cell membranes, involve in the absorption of fat-soluble vitamins, and the source of essential fatty acids in the body (Karaali 1997, Mayes et al 2004, Yiğit 2007). Unsaturated fatty acids are either at cis or trans form depending on the location of the hydrogen (H) atoms on the double bonds; in cis form, H atoms are on the same side of the carbon chain and in trans form in the opposite side (Dutton 1979).

It is commonly known that trans fats elevate cholesterol (Murray et al 2014), decrease HDL cholesterol, and increase LDL cholesterol levels leading to increases in the LDL / HDL ratio and the risk of heart disease (Kayahan 2003). Intake of trans fats could be related to the development of certain cancers (Innis et el 1999). Earlier studies have shown that trans fatty acids may trigger the development of allergy and asthma in children and also type II diabetes (Taşan et al 2007). Furthermore, the daily intake of trans fats weaken the immune system, increases the risk of diabetes and promotes the production of free radicals. Although trans fats have negative effects on human health (Taşan and Dağlıoğlu 2005), they have an important place in the food industry due to their flavor and extended shelf life.

Sugar (naturally found in fruits and vegetables), sucrose and HFCS are used to increase flavor in processed foods (Cozma and Sievenpiper 2014). As the share of the processed food rises in the market, intake of HFCS by humans also increases. HFCS is preferred by the manufacturers because of the late crystallization in foods, prolonged shelf life, increased sweetness, and low cost (Akar 2011).

Chemically produced HFCS is classified into three groups depending on the amount of fructose it contains; 42%, 55%, and 90%. The first one is used in canned foods, bakery products, sauces, and soups; the second one is used in soda and fruit juices; the third one is used in small quantities in light products as a sweetener (Parker et al 2010). HFCS is also used to increase color brightness in products such as ketchup and jam (Palmer 1982). Fructose, due to its flavor enhancer property and direct cell passage, causes a delay in the feeling of satiety. Thus, indirectly, the increase in the amount of food consumption leads to health problems such as metabolic disorders, obesity, and diabetes (Gaby 2005, Angelopoulos et al 2009). Studies have shown that hyperinflammation, hyperglycemia, and hypertension are associated with the consumption of HFCS (Aytekin 2009). Due to its different form, fructose in HFCS escapes crebs cycle which generates energy and instead transforms into triglyceride in the liver and fat in the body (Morell and Nagel 2009). Excess consumption of fructose causes increased fatty acid level in the blood after metabolism in the liver, which triggers insulin resistance and accelerates the formation of fat storage and thus fatness (Elliott et al 2002, Stanhope and Havel 2008). Furthermore, high levels of fructose intake are also known to induce high blood pressure and kidney stones (Korkmaz 2008, Stanhope and Havel 2008, Knight et al 2010).

The aim of this study was to investigate glucose, HbA1c, triglyceride, cholesterol, HDL, LDL, AST, ALT, ALP, SOD and GGT levels in rats which were fed trans fat and HFCS.

Material and Methods

Rats (n = 24, 12 weeks old, male) were allocated into 4 groups. The trans fat obtained from the manufacturer was used as a reference and based on the literature and the decision by the ethics committee, the rats were fed for a 8-week period. The control (C, n = 6) group was fed with a standard rat diet; the cotton oil (CO, n = 6) group was given the standard diet + 5% CO; the trans fat (TF, n = 6) (5 g trans fat obtained from cotton oil) group was given the standard diet + 5% TF (contains 30% trans fat); and the corn syrup (CS, n = 6) group was fed with standard diet + 5% TF (including 30% trans fat) + 15% HFCS in water. *Ad libidum* feed 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, triglyceride, cholesterol, HDL, LDL, AST, ALT, ALP, GGT (Biochemical Autoanalyzer ARCHITECT Ci 8200 ABBOT), HbA1c (Biochemical Autoanalyzer PREMİER HB9210) and SOD (Elisa reader, Biotek ELX800) were performed. 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. SPSS (19.0) program was used for the statistical analyses.

Results

In glucose levels, there were significant differences observed between the C group and the experimental groups ($p < 0.05$). A significant difference was detected between TF and CS group ($p < 0.05$). No significant difference was found in the HbA1c values among the groups ($p > 0.05$). In our study, no significant difference was observed between body weight values ($p > 0.05$).

Triglyceride values differed between C and CO groups ($p < 0.05$). Cholesterol and HDL levels decreased in the TF group compared to the C group ($p < 0.05$). LDL levels were not different either among the groups or within the groups. AST levels did not differ statistically among the groups ($p > 0.05$), however, the CS group had higher AST levels numerically. ALT levels in CO, TF, and CS groups were found to be lower compared to the C group ($p < 0.05$). ALP levels did not show any difference ($p > 0.05$). SOD values did not display any

Table 1. Biochemical parameter measure in the C, CO, TF, and CS group

	DAY	Control (C)	Cotton Oil (CO)	Trans Fat (TF)	Trans Fat and Corn Syrup (CS)
Glucose (mg/dl)	0.	188.50 ± 9.88 a.A	177.66 ± 9.90 a.A	190.00 ± 11.39 a.A	207.66 ± 9.92 a.A
	60.	232.83 ± 11.71 b.B	192.66 ± 12.85 c.A	174.00 ± 4.58 c.A	281.66 ± 14.52 a.B
HbA1c (%)	0.	4.35 ± 0.07 a.A	4.33 ± 0.13 a.A	4.26 ± 0.06 a.A	4.33 ± 0.04 a.A
	60.	5.11±0.13 a.B	5.01±0.09 a.B	4.83±0.06 a.B	5.05±0.08 a.B
Triglyceride (mg/dl)	0.	123.83 ± 15.54 a.A	114.66 ± 32.55 a.A	77.66 ± 6.89 a.A	86.00 ± 8.01 a.A
	60.	215.50 ± 16.53 a.B	136.00 ± 20.20 b.A	184.33 ± 24.47 ab.B	175.00 ± 17.06 ab.B
Cholesterol (mg/dl)	0.	79.50 ± 5.30 a.A	67.16 ± 2.32 a.A	69.33 ± 4.97 a.A	70.33 ± 3.26 a.A
	60.	73.66 ± 5.84 a.A	66.50 ± 3.92 ab.A	58.16 ± 3.73 b.B	62.00 ± 2.56 ab.A
HDL (mg/dl)	0.	56.83 ± 3.96 a.A	45.83 ± 1.30 b.A	53.33 ± 3.87 ab.A	52.66 ± 1.81 ab.A
	60.	57.50 ± 5.80 a.A	49.83 ± 2.86 ab.A	44.33 ± 2.88 b.B	49.00 ± 1.57 ab.A
LDL (mg/dl)	0.	15.83 ± 0.94 a.A	14.66 ± 1.02 a.A	12.66 ± 1.42 a.A	13.16 ± 1.53 a.A
	60.	14.83 ± 2.22 a.A	15.33 ± 1.02 a.A	11.66 ± 1.14 a.A	11.00 ± 0.51 a.A
AST (U/L)	0.	92.66 ± 6.30 bc.A	109.00 ± 10.90 ab.A	123.16 ± 13.69 a.A	76.00 ± 4.81 c.A
	60.	120.50 ± 25.12 a.A	88.83 ± 8.22 a.B	89.33 ± 5.85 a.A	143.16 ± 51.25 a.A
ALT (U/L)	0.	44.16 ± 2.65 a.B	52.16 ± 3.19 a.A	49.83 ± 2.41 a.A	45.33 ± 1.45 a.A
	60.	80.16 ± 13.54 a.A	48.16 ± 1.55 b.A	52.33 ± 2.40 b.A	45.50 ± 7.33 b.A
ALP (U/L)	0.	249.33 ± 48.90 a.A	183.33 ± 18.36 a.A	172.16 ± 17.56 a.A	190.33 ± 12.52 a.A
	60.	266.33 ± 30.90 a.A	309.16 ± 55.19 a.A	355.83 ± 66.53 a.B	260.00 ± 20.12 a.A
SOD (U/ml)	0.	0.084 ± 0.004 a.A	0.072 ± 0.005 a.A	0.074 ± 0.002 a.A	0.072 ± 0.001 a.A
	60.	0.137 ± 0.025 a.A	0.246 ± 0.125 a.A	0.205 ± 0.024 a.B	0.138 ± 0.118 a.B
GGT (U/L)	0.	1.48 ± 0.24 a.A	1.35 ± 0.17 a.A	1.75 ± 0.13 a.A	1.35 ± 0.17 a.A
	60.	1.34 ± 0.26 a.A	1.35 ± 0.17 a.A	1.08 ± 0.26 ab.A	0.42 ± 0.27 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).

Table 2. Live weight in the C, CO, TF, and CS group

	DAY	Control (C)	Cotton Oil (CO)	Trans Fat (TF)	Trans Fat and Corn Syrup (CS)
Live weight (g)	0.	358.33 ± 7.08 ab.A	373.16 ± 6.58 a.A	344.50 ± 6.87 b.A	354.33 ± 5.34 ab.A
	60.	498.66 ± 24.87 a.B	464.33 ± 16.18 a.B	452.16 ± 10.16 a.B	455.33 ± 11.31 a.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).

changes among the groups ($p > 0.05$). GGT level was significantly lower in the CS group than the other groups ($p < 0.05$). The results are given in tables.

Discussion

The leading sources of health problems nowadays are irregular, inadequate and unbalanced nutrition. Refined products, edible margarine, ready-to-eat foods (hamburgers, chips, fast food, pizza, etc.) and fructose from corn starch are the main reasons for the nutrition-related health problems. Industrial foods that contain hydrogenated fats have trans fats (Kahraman and Küplülü 2011). Trans fatty acids are naturally found in trace amounts in the fat in the milk and meat of ruminant animals (Taşan ve Dağlıoğlu 2005). Naturally occurring trans fats do not pose a danger to the living organism. Refined sugars in foods cause negative effects on health and may result in excessive caloric intake as a result of consuming more sugar than the other nutrients which are required for health such as essential fatty acids, vitamins, minerals, etc. (Gaby 2005). In the last 35 years, it has been stated that there is a relationship between the increase in obesity and the consumption of YFMŞ in the USA. (Bray 2008).

In this study, glucose, HbA1c, triglyceride, cholesterol, HDL, LDL, AST, ALT, ALP, SOD and GGT levels were investigated in rats which were fed trans fat and HFCS. These parameters were selected because trans fat and corn syrup had more prominent effects on these blood values.

In studies conducted with a high fructose diet, it was concluded that it may cause hypertension, cardiovascular diseases, insulin resistance, glucose tolerance, obesity and type 2 diabetes (Ross et al 2009). In rat fed with trans fat and corn syrup (CS group), blood glucose levels demonstrated significant differences ($p < 0.05$). However, HbA1c levels did not differ among the groups ($p > 0.05$). This may be due to the 60 day period of the trials, which was propably not enough to induce important changes in HbA1c levels. Aksoy et al (2016) also compared HbA1c levels in rat fed with control diet and corn syrup and did not found any significant changes. In the presented study, an increase was observed in the glucose values of the control group. In a study, it was stated that standard rat feed may cause an increase in glucose level (Huang et al 2009). In another study conducted with mice fed with 15% YFMŞ-55 for 30 weeks, it was shown that the glucose values of mice increased significantly compared to the control group (Collino et al 2013). It has been stated that 55% YFMŞ (in proportion to sucrose) causes statistically an increase in body weight (Light et al 2009). Bocarsly et al (2010) reported in their study that YFMŞ caused an increase in the body weight of rats. Contrary to this studys, in our study, no significant increase was observed in the body weight of the group in which 15% corn syrup was added to their water compared to the control group.

In all experimental groups compared to the C group and day 0 values, triglyceride levels increased significantly ($p < 0.05$). Earlier clinical studies reported similar increases due to the intake of fats and trans fats (Peter et al 1992, Sundram et al 1997, Lichtenstein et al 2003, Rivellese et al 2003, Wijendran et al 2003, Rasmussen and Rivellese 2006) or no changes in the blood triglyceride levels (Almendingen et al 1995, Roos et al 2003). In the presented study, LDL cholesterol decreased in the TF and CS groups compared to the C group, but not significantly ($p > 0.05$). In addition, Peter et al (1992) suggested that trans fats would not cause a significant change in triglyceride concentration and that fats in standard feeds could cause a difference in triglyceride concentration. Linchtenstein et al (2003) suggested that by using trans fatty acids instead of saturated fatty acids, HDL cholesterol concentration decreased, triglyceride level increased and total cholesterol / HDL cholesterol ratio decreased. According to Peter et al (1992) diets that contain trans fats result in an increase in LDL level, a decrease in HDL level and HDL/LDL ratio while no changes could be seen in triglyceride levels. Zock and Katan (1997) stated that trans fats decreased HDL levels, but increased LDL and lipoprotein levels. In this study, HDL cholesterol levels were lower in the TF group that the level observed in C group ($p < 0.05$). Zock and Katan (1997) that trans fatty acids reduce HDL levels, they stated that it increased LDL and lipoprotein a levels. In the presented study, there was a decrease in LDL cholesterol in the groups given trans fat and trans fat + corn syrup compared to the control, but this decrease was not statistically significant ($p > 0.05$). There was a significant decrease in HDL and cholesterol concentrations in the trans fat group compared to the control group ($p < 0.05$), ıt was determined that the triglyceride level increased significantly in the cotton oil group ($p < 0.05$).

Long term or short term feeding with high fructose causes an elevation in triglyceride levels. A 3-week feeding of rats with high fructose increases plasma triglyceride levels (Sharabi et al 2007); HFCS addition to the diet for 6-7 months formed hypertriglyceridemia (Bocarsly et al 2010). In this study, HFCS given group (CS group) had higher, but not significant levels of triglyceride ($p > 0.05$).

ALT and AST play an important role in diagnosing heart and liver disorders caused by various toxicities and infections and are used as markers of cell damage (Nelson and Cox 2005). In a study performed in male rats, an increase in ALT concentration was reported by administering 15% HFCS solution 3 days a week (Figlewicz et al 2009). In a study with high fat/high fructose diet, hepatic steatosis and an increase in ALT and AST levels were observed (Collino et al 2010). In this study, ALT levels were significantly different in the experimental groups compared to the control group ($p < 0.05$). AST levels increased in the CS group while decreasing in the TF group and these changes not significant compared to the C group ($p > 0.05$). SOD and GGT are parameters used to determine if there is liver and biliary tract damage. According to Aslankoç et al (2019) a decrease in SOD level causes an increase in free radical formation. An increase was observed in SOD concentration, but was not significant when compared to the C group ($p > 0.05$). It is thought that a significant difference will be obtained if the working period is kept longer. GGT, one of the important liver function tests; ıt has been reported to be elevated in many diseases such as liver cancer, diabetes, and obesity (Altunkaş et al 2014). In one study, mother rats were fed with YFMŞ and it was reported that fatty liver was observed in fetuses as a result of the study (Malo et al 2013). In our study, an increase in GGT concentration was not observed; GGT levels were lower in the CS group than the other groups and statistically significant ($p < 0.05$).

Long term feeding of this type is thought to cause insulin resistance, obesity and type 2 diabetes in rats. It is suggested that the increase in cholesterol and LDL values can be achieved after a long-term feeding with the increased trans-fat ratio in the diets and the cardiovascular diseases may be observed with the increase in these values. Based on the chan-

ges in liver function tests, it is expected that skeletal muscle and myocardial diseases can occur in the trans-fat + corn syrup (CS) group and the likelihood of pathological changes in the liver is high in the TF group.

Conclusion

In conclusion, nutrition with diets that combine high fructose corn syrup and trans fats may cause significant changes in some blood values and metabolic disorders. Therefore, it was concluded that the consumption of high fructose corn syrup and trans fat should be limited, and this limitation would positively affect human health.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

The presented study was conducted with the approval and permission of the Selcuk University Faculty of Veterinary Medicine Ethics Committee with the decision number 2017-27 dated 25.07.2017.

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