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The Effects of high fat diets with and without N-Acetylcysteine supplementation on the lipin-1 levels of serum and various tissues in rats

[Sıçanlarda N-asetilsistein takviyeli ve takviyesiz yüksek yağlı diyetin serum ve çeşitli doku lipin-1 seviyelerine etkisi]

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ABSTRACT

Objective: Obesity is a public health problem, but the struggle against obesity continues to increase every day. Lipin-1 is an adipokine that modulates lipid metabolism and glucose homeostasis. The aims of the current study were to compare the levels of lipin-1 in serum and tissues (liver, lung, heart, pancreas and kidney) in rat fed with low fat diet (LFD), high fat diet (HFD) and N-acetylcysteine (NAC) supplemented HFD, and to determine the relationships of the lipin-1 levels in serum and tissues with the levels of serum glucose and lipid parameters.

Methods: 18 male Sprague–Dawley rats were divided into three groups: LFD group (n=6), HFD group (n=6) and the NAC-supplemented HFD (HFD+NAC) group (n=6). The feeding period of the diets was 85 days. The lipin-1 levels in serum and tissues were determined by using Rat ELISA kit.

Results: In comparison to serum lipin-1 levels in HFD group were significantly lower, but heart lipin-1 levels were significantly higher (P<0.017). On the other hand, it was determined that serum and tissue lipin-1 levels were not influenced by NAC supplementation. In addition, no other significant difference in lipin-1 levels of the other tissues among the groups was found.

Conclusion: This study showed that HDF affected serum and heart lipin-1 levels, while NAC supplementation did not affect on the lipin-1 levels of serum and of the tissues.

Key Words: N-acetylcysteine, high fat diet, low fat diet, lipin-1, obesity, rat

Conflict of Interest: Authors have no conflict of interest.

ÖZET

Amaç: Obezite, bir halk sağlığı sorunudur. Ancak, obeziteyle mücadele her geçen gün artmaya devam etmektedir. Lipin-1, lipid metabolizması ve glukoz homeostazını düzenleyen bir adipokindir. Bu çalışmanın amacı; düşük yağlı diyet (LFD), yüksek yağlı diyet (HFD) ve N-asetilsistein (NAC)-takviyeli HFD ile beslenen sıçanların serum ve dokularında (pankreas, karaciğer, akciğer, kalp ve böbrek) lipin-1 seviyelerini karşılaştırmak ve bu lipin-1 seviyelerinin serum glukoz ve lipid parametreleri ile olan ilişkisini belirlemekti.

Yöntemler: 18 erkek Sprague-Dawley sıçan üç gruba ayrıldı: LFD grubu (n = 6), HFD grubu (n = 6) ve NAC-takviyeli HFD (HFD + NAC) grubu (n = 6). Diyetler ile beslenme süresi 85 gündü. Serum ve dokularda lipin-1 seviyeleri ELISA kiti kullanılarak belirlendi.

Bulgular: HFD grubunda serum lipin-1 seviyeleri LFD grubundan anlamlı düşük, kalp lipin-1 seviyeleri ise anlamlı yüksek bulundu (P<0.017). Diğer taraftan, NAC takviyesi ile serum ve dokuların lipin-1 düzeylerinin etkilenmediği belirlendi. Ayrıca, gruplar arasında diğer dokuların lipin-1 seviyelerinde anlamlı bir fark bulunamadı.

Sonuç: Bu çalışma, HFD'nin serum ve kalp lipin-1 seviyelerini etkilediğini, NAC takviyesinin lipin-1 seviyelerini etkilemediğini gösterdi.

Anahtar Kelimeler: N-asetilsistein, yüksek yağlı diet, düşük yağlı diet, lipin-1, obezite, sıçan

Çıkar Çatışması: Yazarlar arasında çıkar çatışması yoktur.

Introduction

Obesity is one of the most important public health problems in both developed and developing countries [1]. It is considered as a risk factor for the development of the multiple chronic diseases such as hyperlipidemia, hypertension, insulin resistance and diabetes [2]. It causes oxidative stress by several mechanisms including the following; *1)* The mitochondrial and peroxisomal oxidation of fatty acids can produce reactive oxygen species (ROS) during oxidation reactions; *2)* The over-consumption of oxygen generates free radicals in the mitochondrial respiratory chain. Lipid-rich diets can alter oxygen metabolism and thereby cause the generation of ROS [3].

N-Acetylcysteine ($C_5H_9NO_3S$) (NAC), the rate-limiting substrate in the biosynthesis of glutathione, is a powerful antioxidant and a scavenger of hydroxyl radicals [4,5]. NAC addition to adipocytes inhibits triglyceride accumulation by preventing the increase of ROS during differentiation. This shows the beneficial effect of NAC on the prevention of metabolic changes induced in adipocytes during obesity development [6]. In addition, NAC inhibits lipolysis, which is regulated by ROS, in adipocytes [7].

Lipin-1 is a Mg^{2+}-dependent phosphatidic acid phosphatase (PAP-1) enzyme, catalyzing the dephosphorylation of phosphatidic acid to generate diacylglycerol, which is required for the synthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine [8,9]. It also regulates gene expression through direct protein-protein interactions with DNA-bound transcription factors in liver [10]. It is an adipokine that may link obesity with insulin resistance and diabetes [11]. It has been reported that all forms of lipin mRNA tended to be decreased with obesity [12]. Therefore, lipin-1 has a potential to become a therapeutic target in the treatment of obesity [13].

The aim of the current study was to compare the levels of lipin-1 in serum and in tissues (liver, lung, heart, pancreas and kidney) in rats fed low fat diet (LFD), high fat diet (HFD) and NAC supplemented HFD. In addition, the determination of the relationships of lipin-1 levels in serum and in the tissues with the levels of serum glucose and lipid parameters was aimed.

Materials and Methods

Animals and Experimental Design

18 male Sprague–Dawley rats, aged 6-8 weeks and weighing 150-200 g were obtained from Karadeniz Technical University Surgery Research Center. The animals were kept under standard laboratory conditions (temperature of 20–22°C, humidity of 55–60%, photoperiod of 12:12 hour light: dark cycle). Initially, all rats were fed LFD composed of 65% carbohydrate, 30% protein, and 5% fat, by energy (Research Diet #D12450B (3.85 kcal/g) fed *ad libitum*) for 15 days. Then they were weighed (261-329 g) *and were divided into three groups* (with *equal weight* distributions) each containing 6 rats:

Group I (LFD): Fed a low-fat diet explained above (Research Diet #D12450B, fed *ad libitum*)

Group II (HFD): Fed a high-fat diet composed of 45% fat, 35% carbohydrate and 20% protein, by energy (Research Diet # D12451, (4.73 kcal/g) fed *ad libitum*)

Group III (HFD+NAC): Fed a high-fat diet (Research Diet # D12451, fed *ad libitum*) and received 2 g/L NAC (Amresco, 98% purity) in its drinking water.

After feeding with the diets for 85 days, all the rats were again weighed. They were sacrificed under ketamine anesthesia in sterile conditions. Blood was taken from aorta. Each tissue sample was removed, harvested under dry ice conditions and immediately stored in microcentrifuge tubes at −80 °C until the tests were performed. The study protocol was reviewed and approved by Karadeniz Technical University Ethics Committee for Animal Research (Protocol No.2010/39 and 2012/37). The experimental design is shown in Fig. 1.

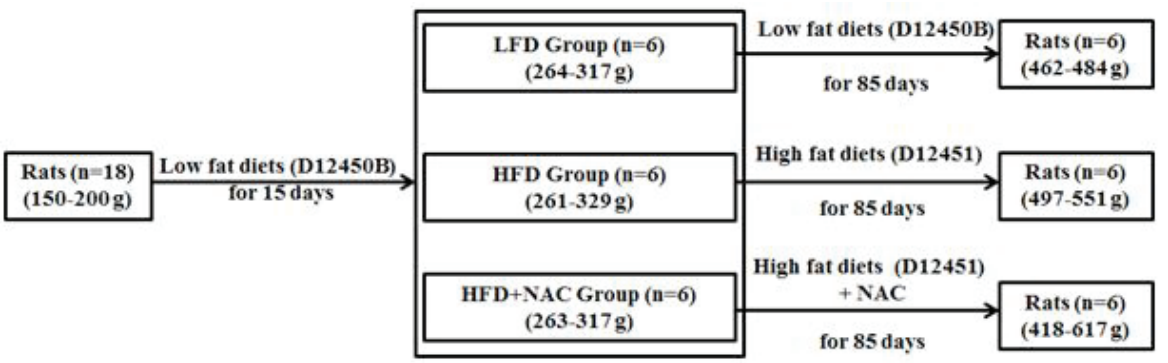

Figure 1. Experimental design of the present study.
HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol. LFD, low fat diet; HFD, high fat diet; HFD+NAC, High fat diet + N-acetylcysteine.

Determinations of the Levels of Glucose and Lipid Parameters

Blood samples were taken from aorta, and the sera were separated by low speed centrifugation and stored at −80 °C until the tests were performed. The levels of glucose, triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured by Roche Cobas 8000 autoanalyzer (Roche, Modular, Switzerland).

Determination of Lipin-1 Levels

The lipin-1 levels in serum and tissues were determined by using Rat Lipin1 ELISA kit (Eastbiopharm, Hangzhou, and Cat. No: CK-E90289). Serum samples were used directly. But, 50 mg tissue samples were homogenized in 1 mL of ice cold homogenization buffer composed of 10 mM phosphate-buffered saline (pH 7.4), 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, 1% Triton X-100 and 1 U of proteinase inhibitors (proteinase inhibitor cocktail; Sigma) by a homogenizer (Ika T-18 Basic Ultra Turrax Homogenizer, USA) for 2 min at 13500 rpm on ice. The homogenates were centrifuged at 10000 × *g* for 10 min, and the supernatants were used.

Statistical Analysis

Data were expressed as median (minimum-maximum). Comparisons among groups and among tissues were done using nonparametric Kruskal-Wallis test and then the Mann-Whitney U-test with Bonferroni correction was used. *P*-values <0.017 and <0.005 were considered to be statistically significant, respectively. The correlations were evaluated by using Spearman's rank test and *P*-values less than 0.05 were considered to be statistically significant.

Results

After a feeding period of 85 days, the weights and glucose levels of rats in both the HFD and the HFD+NAC groups were significantly higher than the LFD group (*P<0.017*). But the levels of the lipid parameters did not show any significant difference among the groups. NAC supplementation did not show any significant effect on the weight, glucose and lipid parameters (*P>0.017*) (Table 1).

Decreased serum lipin-1 levels were obtained both in HFD and NAC-supplemented HFD rats with respect to control LFD group (*P<0.017*). However no significant difference was obtained between HFD group and HFD+NAC group (Table 1).

When we compared the lipin-1 levels among tissue types in LFD group; the higher to lower levels were as follows: Kidney > Lung > Heart ≈ Liver > Pancreas. These results showed that lipin-1 levels in this group were the highest in kidney (not statistically significant) but the lowest in pancreas (statistically significant). On the other hand, lipin-1 levels in pancreas, liver, lung and kidney did not show any significant difference among the groups. Only, heart lipin-1 levels in HFD feeding rats were significantly higher than LFD feeding rats (*P<0.017*) (Table 2).

A borderline significant negative correlation (*r= -0.771; P=0.072*) was obtained between serum glucose and lipin-1 levels in HFD feeding rats. In addition, it was found that there was a borderline significant positive correlation (*r= 0.943; P= 0.05*) between serum TC and HDL-C in this group.

Discussion

High-energy diets induce obesity [14]. In the current study, after feeding with the diets for 85 days all the

Table 1. The weights and the levels of serum glucose and lipid parameters of the rats in the LFD, HFD and HFD+NAC groups.

	LFD Group	HFD Group	HFD+NAC Group
Initial weight (g)	280 (264- 317)	278 (261-329)	282 (263-317)
Final weight (g)	460 (462-484)	514 (497-551)*	514 (418-617)*
Glucose (mg/dL)	129 (121-140)	149 (133-167)*	155 (143-181)*
TG (mg/dL)	171 (118-253)	185 (111-264)	139 (101-218)
TC (mg/dL)	79 (71-94)	78 (72-88)	88 (68-96)
HDL-C (mg/dL)	42 (25-45)	27 (26-45)	47 (33-52)
LDL-C (mg/dL)	13 (5-14)	12 (10-16)	16 (9-22)
Lipin-1 (μg/mL)	4.32 (2.49-6.01)	1.91 (1.07-2.96)*	2.01 (1.08-2.77)*

TG, Triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; LFD, low fat diet; HFD, high fat diet; HFD+NAC, High fat diet + N-acetylcysteine.
Data were expressed as median (minimum-maximum).
* Statistically significant with respect to LFD group (*P<0.017*).

Table 2. Lipin-1 levels of the tissues (μg/gwt) in LFD, HFD and HFD+NAC groups.

	LFD Group	HFD Group	HFD+NAC Group
Pancreas	28 (14-43)	32 (18-46)	30 (13-36)
Liver	132 (109-146)$^{\alpha}$	129 (113-152)$^{\alpha}$	142 (117-168)$^{\alpha}$
Heart	130 (119-171)$^{\alpha}$	174 (152-188)$^{*, \alpha}$	146 (118-176)$^{\alpha}$
Lung	187 (148-194)$^{\alpha, \beta}$	177 (137-189)$^{\alpha}$	177 (162-179)$^{\alpha}$
Kidney	250 (228-288)$^{\alpha, \beta, \gamma}$	216 (185-251)$^{\alpha, \beta}$	231 (177-282)$^{\alpha, \beta}$

LFD, low fat diet; HFD, high fat diet; HFD+NAC, High fat diet + N-acetylcysteine.
Data were expressed as median (minimum-maximum).
* was used to compare the lipin-1 levels of the groups but $^{\alpha, \beta,}$ and $^{\gamma}$ were used to compare the tissue lipin-1 levels.
* Statistically significant difference with respect to the LFD group ($P = 0.004$).
Statistically significant ($P<0.005$) difference with respect to $^{\alpha}$ pancreas, $^{\beta}$ liver and $^{\gamma}$ heart.

rats gained weight (Table 1). LFD (5% fat) group gained weight because of the high carbohydrate (65%) content. But rats fed HFD (45% fat) gained more weight than rats fed LFD because of the higher total energy consumption (4.73 kcal/g and 3.85 kcal/g, respectively). The weights of HFD+NAC group rats were very similar to the weights of HFD group rats. This means that NAC supplementation to HFD did not show any significant effect on the weight. There are conflicting results in the literatures regarding the effect of NAC on weight. Omar *et al.* [15] reported that the treatment of diabetic rabbits with NAC caused an increase in body weight. Another study also found a significant increase in the final bodyweight of the rats fed a low protein diet (LP) +NAC compared to the rats fed LP diet [16]. On the contrary, it was reported that NAC administration to rats reduced body weight and visceral fat in rats [6], and it was suggested that NAC may be a useful anti-obesity drug and food supplement [17]. Our finding did not support these findings.

Glucose levels were found to be higher in the HFD and the HFD+NAC groups than in the LFD group. But, the levels of lipid parameters (TG, TC, HDL-C and LDL-C) did not show any significant difference among groups (Table 1). Winzell *et al.* [18] determined that circulating glucose increased after one week on HFD and remained elevated during 12-month study period in mouse. High fat feeding induces insulin resistance by impairing glucose metabolism and causes an increase in fat oxidation [19]. In contrast to our study, one study said that plasma level of cholesterol and TG in HFD group significantly increased [20]. However, another study on hypertriglyceridemic men found that TG and cholesterol concentrations decreased after both LFD and HFD, but did not show any significant difference between LFD and HFD [21]. In addition, they did not find significant differences in HDL-C and LDL-C after low and high fat diets. Similarly, Garcia-Diaz *et al.* [22] did not find any significant change in TG levels after feeding high fat cafeteria diet (59.2% of energy as lipids) for 65 days in male Wistar rats. In the present study a borderline significant positive correlation ($r= 0.943; P= 0.05$) between serum TC and HDL-C was found in the HFD group (data not shown). This finding supports the well-known idea that HDL is the main rat-cholesterol carrier [23]. According to Shimizu *et al.* [24], NAC treatment caused serum cholesterol levels to decrease. NAC decreased the levels of cholesterol probably by regulating cholesterogenesis [25]. Lin and Yin [26] found that HFD significantly increased hepatic TG and cholesterol contents but NAC significantly decreased hepatic lipid levels. On contrast to the above studies, Panamonta and Chunlertrith [27] did not find any significant difference in serum glucose, TG, HDL-C and LDL-C levels in metabolic syndrome patients with NAC treatment.

Lipin-1 modulates lipid metabolism and glucose homeostasis [28]. It acts as PAP-1 to generate diacylglycerol, which is important in TG synthesis and acts as a transcriptional regulator [10,29]. In the present study, the decreased levels of serum lipin-1 were observed in both HFD and HFD+NAC groups with respect to LFD group ($P<0.017$). Furthermore, serum lipin-1 levels were negatively correlated with serum glucose levels in rats fed with HFD (a borderline significance $r= -0.771; P=0.072$). Chen *et al.* [11] reported that lipin-1 levels were significantly decreased in rats with HFD, and lipin1 expression was negatively correlated with obesity and insulin resistance. On the other hand, lipin-1 deficiency impairs adipocyte differentiation and causes lipodystrophy in mice, whereas the overexpression of lipin1 in adipose tissue promotes obesity by enhancing lipogenic gene expression [29]. In the current study, NAC supplementation to HFD did not affect serum lipin-1 levels. No significant difference was observed between diet-induced obese rats with and without NAC supplementation.

The lipin-1 levels of the heart tissues were higher in HFD group than in both LFD group (significant) and HFD+NAC group (not significant) (Table 2). Heart requires constant and substantial energy supply for

continuous pumping and fatty acid oxidation supplies 50% of the energy for a normal adult heart [30]. Lipin-1 promotes the expression of genes (e.g. peroxisome proliferator-activated receptor α (*PPARα*) encoding fatty acid oxidation enzymes [10]. HFD feeding results in overexpression of PPAR-α, which promotes lipid utilization as an energy substrate for mitochondrial oxidation [31]. In addition, HFD increases oxidative stress and mitochondrial damage [32]. Oxidative stress may cause the degradation of phospholipids (in particular cardiolipin) by oxidation, resulting in mitochondrial dysfunction [33] which is a major contributor in heart failure [34]. The substrate of lipin-1, phosphatidate, is converted to various phospholipids including cardiolipin, a critical component of mitochondrial membranes [35]. It was reported that NAC reduces the oxidative stress and prevents the metabolic shifting in cardiac tissue, enhancing fatty acid oxidation and reducing anaerobic metabolism in high-sucrose fed conditions [36]. Considering the above information, increased lipin-1 levels with HFD may cause mitochondrial dysfunction in heart, and decreased lipin-1 levels with NAC may have protective effect for heart function.

The lipin-1 levels in pancreas, liver, lung and kidney did not show any significant difference among study groups. When we compared the lipin-1 levels among tissue types, the higher to lower levels order in LFD group were as follows: Kidney > Lung > Heart ≈ Liver > Pancreas. Some studies reported that the highest level of lipin-1 expression occurs in white/brown adipose tissue, skeletal muscle, cardiac muscle and testis, with low expression in the liver, kidney, brain, Schwann cells of peripheral nerves and pancreatic beta cells [11,37]. In liver, lipin-1 interacts with the PPARα and its coactivator (PPARγ coactivator 1α (PGC-1α) to enhance the expression of genes involved in fatty acid oxidation [10]. PAP-1 activity is essential in lung for *de novo* synthesis of phospholipids for cellular membranes and 1, 2-dipalmitoyl-*sn*-3-phosphatidylcholine for pulmonary surfactant by type II cells [38].

To the best of our knowledge, the present study is the first study comparing the levels of lipin-1 in kidney, liver, lung, heart and pancreas by ELISA method. In addition, this is the first study to investigate the effect of NAC supplementation to HFD on lipin-1 levels of these tissues. However, there are some study limitations: Firstly, tissue TG levels could be analyzed to compare the tissue lipin-1 levels, but this had not been designed for this study. Secondly, the number of the rats (n=6) for each group was small. Thirdly, the time of HFD could be longer, and thereby a significant effect could be caught if there was.

In conclusion, this study showed that HDF affected serum and heart lipin-1 levels. But NAC supplementation did not affect on the serum and tissue levels of lipin-1. These findings need to be supported with new investigations.

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Conflict of interest: Authors have no conflict of interest.

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