The effects of taurine supplementation on obesity and browning of white adipose tissue in high-fat diet-fed mice.

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Research Article

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Abstract

Background: In recent years, a new type of adipose tissue (beige adipose tissue) has been mentioned, unlike white adipose tissue (WAT) and brown adipose tissue (BAT). Beige cells are capable of thermogenesis like BAT. In response to various agents, beige cells can develop within WAT through a process called “browning”. Therefore, the prevention of obesity and related diseases by providing WAT browning with new potential agents has been extensively studied in recent years. Taurine has many physiological functions in the body and has beneficial effects on obesity and related metabolic disorders. For this reason, we aimed to investigate whether taurine supplementation has effects on browning of WAT and attenuating obesity.

Methods: Thirty-two male C57BL/6 mice were used for the study. Mice were divided into 4 groups as control, control + taurine, high fat diet (HFD) and HFD + taurine, and fed for 20 weeks. Taurine was given in drinking water (5%). Epididymal WAT samples were obtained from mice and RNA was extracted from these tissues. Expression levels of FLCN, mTOR, TFE3, PGC-1α, PGC1-1β, AMPK, S6K and UCP1 genes were measured by real-time PCR.

Results: Taurine supplementation reduced HFD-induced obesity. No UCP1 expression was detected in any of the groups studied. Any of the gene expressions were not significantly different between HFD and HFD + taurine groups. Reduced PGC-1α and PGC-1β expressions were observed in both HFD and HFD + taurine groups.

Conclusions: Taurine reduced the obesity in HFD fed mice, but had no effect on browning of epididymal WAT in this study.

Introduction

Obesity is defined by a body mass index (BMI) of more than 30 kg/m². Sedentary life and high-calorie diet cause a rapid increase in the prevalence of obesity in developed countries. This is particularly worrisome as it increases numerous obesity-related diseases, including type 2 diabetes, heart disease, insulin resistance, hyperglycemia, dyslipidemia, hypertension, and many types of cancer (Bastien et al. 2014). As a result, the increase in individuals with chronic diseases in the society is the prediction that health expenditures will increase excessively and that future generations will have a shorter lifespan than previous generations. Due to the fact that obesity is considered as one of the most important health problems of the century, it is of great importance to reduce or prevent obesity (Cai et al. 2010; Hammond and Levine 2010).

In recent years, it has been shown that adipose tissue is not only a tissue where energy is stored, but also has very important functions due to various substances secreted from fat cells. There are two types of adipose tissue; white adipose tissue (WAT); It is responsible for the release of free fatty acids (FFA) for the storage of neutral fats and, when necessary, for energy production. Brown adipose tissue (BAT) is capable of thermogenesis (Berry et al. 2013). Different from WAT and BAT, a new adipose tissue type is
also mentioned with the studies carried out in recent years. These adipocytes were named as “beige” adipose tissue as a distinct group (Harms and Seale 2013; Wu et al. 2012).

Targeting energy expenditure is believed to be an attractive approach combating obesity. Thermogenesis improves metabolic homeostasis by dissipating energy in the form of heat. This process is accomplished by activation of UCP1, which is specific to thermogenic adipocytes, including brown and beige adipocytes, and acts as a functional marker for these cells. Promoting thermogenic capacity or acquiring BAT-like properties in WAT may be a therapeutic strategy for the treatment of obesity and its complications (Bartelt and Heeren 2014; Yoneshiro et al. 2013). WAT browning is effective in uptake of glucose from plasma and uptake of lipids. Therefore, the discovery of new molecules that activate WAT browning is expected to contribute to the combat against obesity and diabetes (Mössenböck et al. 2014; Yoneshiro et al. 2013). Thermogenic adipocytes provide improvement of dyslipidemia by taking FFAs from plasma and using them for oxidation. Although inducing the browning process is a very attractive approach in the treatment of obesity and related disorders, further studies are needed to evaluate whether these agents can sustain the browning response in humans as well as their potential long-term side effects (Vargas-Castillo et al. 2017).

Taurine is a sulfur-containing, non-standard amino acid that is abundant in seafood (Murakami 2015). In animals, dietary taurine has been suggested to be protective against hypertension, liver damage and hypercholesterolemia (Xu et al. 2008). Taurine supplementation has been observed to reduce body mass and abdominal fat stores and increase glucose tolerance and insulin sensitivity in obese mice (Tsuboyama-Kasaoka et al. 2006). It has been observed that taurine has beneficial effects on serum lipids in overweight and obese individuals (Zhang et al. 2004). Considering all these studies, it has been suggested that dietary taurine may be beneficial in preventing obesity and its complications.

Traditional methods such as calorie restriction and exercise or pharmacological interventions seem to be insufficient in terms of preventing/reducing obesity. In recent years, Browning of WAT with various agents and by this means preventing obesity and related diseases has been a field which has been studied intensively. Due to the high content of UCP1-expressed mitochondria, it is thought that weight loss can be achieved by increasing energy expenditure in BAT or beige adipose tissue. Many studies have shown that taurine supplementation reduces obesity and related complications. According to the common view, the conversion of WAT to beige adipose tissue occurs with the activation of the standard pathway, the AMPK/mTORC1/S6K/PGC-1α signaling pathway (Liu et al. 2016). However, it has recently been suggested that the browning program can be induced through the FLCN/mTORC1/TFE3/PGC-1β signaling pathway as an alternative to the AMPK/mTORC1/S6K/PGC-1α signaling pathway (Wada et al. 2016). In light of all this information, our aim in this study was to investigate whether taurine supplementation influences on reducing obesity and inducing WAT browning.

**Methods**

**Animals**
Animals to be used in the study were obtained from Bilkent University, Faculty of Science, Department of Molecular Biology and Genetics, Experimental Animals Research Unit. Thirty-two male 8-week-old C57BL/6 mice were used in the study. The feeding of the mice was carried out in Sivas Cumhuriyet University Faculty of Medicine, Animal Laboratory. Prior to the experiment, mice were fed with control-adaptation food (CA190-10, Altromin; Germany) for 2 weeks under the same conditions to adapt to their new environment and the diet to be used in the experiment. After a 2-week of adaptation period, the experiment was started with 10-week-old mice. Mice were divided into 4 different groups according to their diet, with 8 mice in each group as following: Control, control + 5% taurine (T), HFD and HFD + 5% taurine, and fed for 20 weeks. To produce diet-induced obesity, mice in HFD groups were fed with high-fat diet (C1090-60, Altromin; Germany). For feeding of mice of control groups, control-adaptation food (CA190-10, Altromin; Germany) was used. Taurine (Taurine ≥ % 99, Sigma-Aldrich, ABD) was given in drinking water (5% w/v). The mice were fed *ad libitum*. Mice were housed at 22 ± 2 °C, and kept on a 12 hour light/dark cycles. Weekly average weights were determined by measuring the weight of the mice in each group at the end of each week. Similarly, the average weekly feed and water consumption of mice in each group was measured.

**Removal of epididymal white adipose tissue (eWAT) from mice**

At the end of the experiment, mice in each group were euthanized. eWAT consisting of fat surrounding the epididymis, a tissue in which fat is abundant in mice and WAT browning has been shown in previous studies, was preferred for the study. For this purpose, ~ 100 mg of eWAT was obtain from the each of mice. Tissues were immediately taken into RNA stabilizing solution (RNAlater RNA Stabilization Reagent, Qiagen; Germany) and were stored at -20 °C.

**RNA isolation and gene expression analysis**

RNA extracted from 35 mg eWAT using the (RNeasy Lipid Tissue Mini Kit, Qiagen; Germany (with QIAzol)) was used for RNA isolation. Isolation was performed according to the kit's protocol. Complementary DNA (cDNA) was synthesized from these RNA with (RT2 First Strand cDNA synthesis kit, Qiagen; Germany). In our study, primers whose temperature profiles were optimized by the manufacturer were used to generate a gene-specific PCR product. Since the primers were lyophilized, 1.1 mL of TE buffer (pH 8.0) (containing 10 mM Tris-Cl and 1 mM EDTA) was added to each primer (QuantiTect Primer Assay, Qiagen; Germany). Expression levels of FLCN, mTOR, TFE3, PGC-1α, PGC-1β, AMPK, S6K and UCP1 genes were determined with real-time PCR device (Rotor-Gene Q, Qiagen; Germany) and quantified by relative quantification using $2^{-\Delta\Delta Ct}$ method with RT2 Profiler PCR Array Data Analysis v3.5 program (https://dataanalysis2.qiagen.com)

**Statistical Analysis**

"Statistical Package for the Social Sciences" (IBM SPSS Version 22; IBM, USA) statistical software and GraphPad software (GraphPad 9 Software Inc.; San Diego, CA, USA) were used for the statistical analysis of the findings of our study. In statistical tests, the error level was considered as 0.05. Data were shown
as mean ± SD. “Shapiro-Wilk” test was used to evaluate the conformity of the data with normal distribution. It was observed that the data in all groups showed normal distribution. Between the two groups, the mean daily food and water consumption per mouse at the end of each week were analyzed using the "Student's T-test". Paired sample T-test was used in dependent groups to analyze the difference between the mean weight of each group measured on prior the study and the average weight between end of the experiment. One-way analysis of variance (ANOVA) was used to determine the differences between groups. “Tukey test” was used as post hoc analysis for paired comparisons of the groups. The significance test of the difference between the two means, the "Student's T-test", was used to calculate the fold-change ($2^{\Delta \Delta Ct}$) between the two groups using the data ($C_t$ value) of the expressions of the genes in our study.

Results

Body Mass Changes of Mice

Figure 1 (A) shows the graphs of weekly mass change of the mice and Fig. 1 (B) shows comparisons of the groups. We observed that the mice in the control group had an average mouse weight of 22.58 g at the beginning of the study and reached 29.01 g at the end of the study. The average mass change was 6.43 g and there was a 28.47% mass increase compared to the initial week. Figure 1 (B). In control + T group, while the average weight per mouse was measured as 23.56 g at the beginning of the study, it was observed that it reached 28.03 g weight at the end of the study. The mean body mass increase compared to the initial week was 4.47 g (18.97%). On the other hand, we observed that the mice in the HFD groups gained more weight because they were fed a high-fat diet. In HFD group, while the study started with average 23.43 g of per mouse weight, the mice reached a mass of 41.95 g at the end of the study. The mass of the mice increased on average by about 18.52 g (79.04%). In HFD + T group, the initial mass was 24.58 g, but the mice at the end of the study measured 36.32 g. The mean mass change in the group was 11.74 g (47.76%). This change was less than the change (18.52 g) in the HFD-fed group.

Figure 1(B) shows the comparison of mean mass changes between groups at the end of 20 weeks of feeding versus baseline body mass. The difference in mean mass change between mice in the control group and those in the control + T group was not statistically significant (p > 0.05). However, when the control group and HFD group were compared, the difference between the mass changes was found to be significant (p < 0.001). Similarly, it was found that the mean body mass change was statistically significantly different between HFD and HFD + T group and the control + T and HFD + T group (p < 0.05).

Food and water consumption of mice

The daily food and water consumption for per mouse is shown in (Fig. 1C and D). Daily food consumption of mice in control group was measured as 2.59 ± 0.08 g. The food consumption of the mice in the control + T group was similarly measured as 2.62 ± 0.1 g. No statistically significant difference was found between the two groups (p > 0.05). For water consumption, the mice in the control + T group
consumed more water than that of the control group (control: 2.93 ± 0.16 mL; control + T: 4.06 ± 0.57 mL). This difference was statistically significant (p < 0.001). While the daily food consumption of the mice in the HFD group was 2.66 ± 0.33 g, it was observed that the mice in the HFD + T group consumed 2.81 ± 0.38 g of feed. There was no significant difference between the two groups in terms of food consumption (p = 0.22). Furthermore, the mice in the HFD group consumed 2.46 ± 0.27 mL and the mice in the HFD + T group consumed 3.13 ± 0.22 mL water. The difference between the groups was significant (p < 0.001). There was no significant difference in terms of daily food consumption between the Control and HFD groups (p > 0.05). In addition, the control group consumed more water than that of HFD group (Control: 2.93 ± 0.16 mL; HFD: 2.46 ± 0.27 mL). This difference was found significant (p < 0.001). When the mean daily food consumption of the mice in the HFD + T group and the mice in the control + T group were compared, no statistically significant difference was found (p > 0.05). It was found that the mice in the control + T group consumed significantly more water than the HFD + T group (Control + T: 4.06 ± 0.57 mL; HFD + T: 3.13 ± 0.22 mL; p < 0.001).

**WAT browning related genes in epididymal white adipose tissue**

We analyzed the expression of several WAT browning related genes in eWAT. Figure 2 shows that relative expression levels of we studied. We did not detect any expression in the UCP1 gene. Therefore, no UCP-1 gene was included in any group comparison. Relative gene expressions of control + T group compared to control group are given in the Fig. 2 (A). Although FLCN (-1.92) and PGC-1β (1.72) gene expression levels in the control + T group were found to be significantly different from the control group, this change was not statistically significant. No significant difference was found between the two groups in other genes. The statistical analysis of gene expressions in the HFD group and the HFD + T group is presented in the Fig. 2 (B). Although PGC-1α (1.53) and S6K (1.79) gene expression levels in the HFD + T group were found to be highly different compared to the HFD group, this change was not statistically significant. In other genes, there was no significant difference between the two groups. Figure 2 (C) shows the relative gene expression levels in HFD group compared to control group. In mice fed HFD, 6.42-fold (p = 0.012) expression in the PGC-1α gene and 2.16-fold (p = 0.049) expression in the PGC-1β gene were found compared to the control group. Although FLCN gene expression decreased 2.26-fold, this rate was not statistically significant (p = 0.12). Similarly, we found that the expressions of the mTOR, TFE3, AMPK and S6K genes in the HFD group to be lower than that of control group. However, even though it is very close to the statistical significance limit, p values were found to be > 0.05. Lastly, relative gene expression levels in the HFD + T group compared to control + T group are shown in Fig. 1 (D). The expression of PGC-1α, PGC-1β and AMPK genes was statistically significantly decreased in the HFD + T fed group compared to control + T group. (3.77-fold down-regulation in PGC-1α gene, 2.99-fold down regulation in PGC-1β gene, and 2.44-fold down regulation in AMPK gene, p < 0.05)

**Discussion**
Currently, pharmacological treatment for obesity is very limited and many drugs are often withdrawn because of serious side effects. Basically, the approach to mechanisms in the treatment of obesity focuses on two options: 1) central effect, (i.e., reducing food intake) 2) environmental effect, inhibition of lipid absorption etc. (Tseng et al. 2010). However, since there is inflammation in the adipose tissue and its function is impaired, there is no approved drug that is directly effective in obesity treatment. At the same time, impaired adipose tissue as an endocrine organ secretes several molecules that affect other organs and contribute to the development of abnormalities related to carbohydrate metabolism (Vargas-Castillo et al. 2017).

Taurine is an amino acid abundant in the human body. Taurine plays a variety of physiological roles in the body and has been investigated as a beneficial molecule for reducing metabolic dysfunctions such as dyslipidemia, insulin resistance, and hyperglycemia, which are mostly associated with obesity (Lambert et al. 2015; Kim et al. 2012). Therefore, the anti-obesity effect of taurine has attracted great interest by many researchers as a potentially safe agent for weight loss in the global age of obesity (Murakami 2017). It has been reported that obese people have lower amounts of taurine in their bodies (Rosa et al. 2014). Studies have shown the effects of taurine supplementation on adipose tissue. These effects can be listed as; reducing WAT storage (Tsuboyama-Kasaoka et al. 2006; Nardelli et al. 2011; Batista et al. 2013), increasing BAT (Cao et al. 2016), increasing PKA activity and β-adrenergic-induced lipolysis (Pina-Zentella et al. 2012), increasing PGC-1α (Tsuboyama-Kasaoka et al. 2006; Cao et al. 2016) and UCP1 expression (Guo et al. 2019), reducing proinflammatory molecules and increasing anti-inflammatory molecules (Caetano et al., 2017;). In particular, animal studies have shown that taurine effectively reduces or delays obesity in HFD-fed mice (Lin et al., 2013; Batista et al., 2013). Taurine along with exercise improved genes related to lipid metabolism (De Carvalho et al. 2021a) and improves inflammatory markers in plasma of obese women (De Carvalho et al. 2021b). A randomized clinical trial showed that taurine supplementation along with a weight-loss diet may be more effective in improving lipid profile and metabolic risk factors (Haidari et al 2020). These observations show that taurine deficiency in the body can indirectly cause metabolic dysfunctions such as obesity and dyslipidemia, and that taurine is a beneficial amino acid with very important effects. Therefore, it is even more important to clarify the molecular mechanism by which taurine inhibits metabolic dysfunction.

The primary aim of our study is to investigate whether taurine supplementation influences on reducing body mass in our experimental conditions. For this purpose, both HFD and HFD + T groups were fed for 20 weeks. The mean body weight of the HFD + T group was 36.32 ± 5.14 g, and the mean body weight of the mice in the HFD group was 41.95 ± 7.50 g. We examined whether these weight gains were related to food consumption, and we found the food consumption amounts of the HFD and HFD + T groups to be similar. Although their food consumption was similar, the increase in body weight was less in the HFD + T group compared to the HFD group. Although there was no difference in food consumption in taurine-supplemented groups, water consumption was higher in taurine-supplemented groups than in taurine-free groups. There are many studies that indicate that taurine supplementation increases renal excretory function. To maintain taurine homeostasis in the body, the renal taurine transporter co-transporter (Na+-Cl- taurine co-transporter) is tightly regulated. Excess taurine intake in the diet decreases the expression
of the renal taurine transporter co-transporter, resulting in excess taurine in the urine. When renal co-transporter activity decreases, less taurine is taken up into tubule cells, followed by less sodium transport into tubule cells (Mozaffari and Schaffer 2002). Therefore, taurine supplementation is likely to lead to taurineuria by increasing natriuresis and diuresis. It can be thought that diuresis may have increased due to excessive consumption of taurine in the taurine supplemented groups in our study, and increased diuresis may also increase water consumption in the taurine supplemented groups.

Another aim of our study was to examine whether taurine supplementation has any effect on inducing the WAT browning program and the possible pathways of this effect. For this purpose, expression levels of AMPK, mTOR, S6K, PGC-1α, FLCN, TFE3, PGC-1β and UCP1 genes were determined in eWAT obtained from mice in each group. These studied genes are those reported to be involved in the browning pathways of WAT. Many studies have reported that the browning of WAT occurs through proteins encoded by the AMPK/mTORC1/S6K/PGC-1α genes. In a study published in 2016, it was reported that WAT browning can also occur via an alternative pathway other than this pathway. According to the aforementioned study, it is stated that the interactions of the proteins encoded by the FLCN, mTORC1, TFE3 and PGC-1β genes can cause browning (Wada et al. 2016). In this study, we investigated the expression levels of genes that are effective in both pathways to determine both the effect of taurine on browning and through which pathway it exerts this effect.

Comparisons of both control and control + T, and HFD and HFD + T groups showed that there was no significant difference in expression levels of the genes we examined. In our experimental conditions, it was observed that taurine did not have any effect on the expression of these genes. In a study published in 2019, while our study in progress, Guo et al. (2019) administered taurine (daily 150 mg/kg for per mouse) intraperitoneally to C57BL/6 mice fed with HFD from the last 5 weeks of the feeding period (14 weeks) at +4 °C. They observed that five weeks of taurine supplementation protected mice against weight gain. They observed that UCP1 and PGC-1α gene expressions were induced in BAT, iWAT and eWAT with taurine supplementation, mostly in iWAT. As a result, researchers reported that taurine has anti-obesity properties and induces WAT browning, especially in iWAT. After the study of Guo et al., in another study, Kim et al (2020) conducted a study with ICR mice. They fed the mice for 28 weeks. Although UCP-1 expression was found to be high in brown adipose tissue, they detected it at very low levels in inguinal WAT (iWAT). Taurine supplementation was done as 2% in drinking water. Kim et al. (2020) showed a decrease in fat mass similar to our study in mice fed HFD with taurine supplementation. However, they found at minimal level of UCP1 expression in iWAT. In addition, they found that UCP1 expression decreased in HFD + Taurine group compared to HFD group. This result is the opposite of the results of Guo et al. (2019). The authors attributed this difference, in part, to differences in the genetic background based on diet-induced obesity (DIO) between C57BL/6 and ICR mice. In our study, the mRNA level of the UCP1 gene, which is the most important browning marker, could not be analyzed eWAT since it was not detectable in any group. This shows that WAT browning did not occur at all or was at a minimal level that could not be detected in all groups.
In some previous studies, it was reported that UCP1 gene expression was not detected in WAT (García-Ruiz et al. 2015; Castrejón-Tellez et al. 2016). Various studies have shown that eWAT is less sensitive to browning agents compared to iWAT (Wang et al. 2015; Seale et al. 2011; Zhang et al. 2014; Guo et al. 2019). In study of Guo et al. (2020) the existence of UCP1 gene expression in both iWAT and eWAT may be because of they kept the mice at + 4 °C during the study. In their study, it is possible that cold-induced (adaptive) thermogenesis was induced by feeding at + 4 °C. Because it is known that cold exposure is the most powerful stimulus of UCP1 gene expression (Chouchani et al. 2019). In our study, mice were fed at approximately room temperature (22 ± 2 °C). The reason why we could not detect UCP1 gene expression in any group may be the ambient temperature in which the mice were fed. In addition, the studies of Guo et al. (2019) and our study differ from each other in terms of the way taurine was administered to mice. While they administered taurine intraperitoneally, we gave it dissolved in drinking water. Therefore, although taurine reached the tissue directly in their study, in our study, factors related to the absorption of taurine may have affected the amount of taurine reaching the tissue, since it was absorbed from the gastrointestinal tract and reached through the blood. Furthermore, Kim et al. (2020) used a different mouse model (ICR) than ours (C57BL/6). Therefore, as the authors point out, the genetic characteristics of the two mice are markedly different, and this may be the reason for the discrepancy between the results of the studies. In our study, although, we investigated the expression of the genes in eWAT, they did it in iWAT. In addition, they administered a different dose (2%) of taurine than we did (5%). Therefore, these effects may also be dose and adipose tissue type dependent.

In our study, when compared to the control group, PGC-1α mRNA level in the HFD group down-regulated 6.42 times compared to the control group. Similarly, there was a 2.16-fold decrease in PGC-1β mRNA level. No significant difference was found in other genes. It was determined that the expression of PGC-1α, PGC-1β and AMPK genes was significantly decreased in the HFD + T group compared to the control + T group. The expression decreased 3.77 times in the PGC-1α gene, 2.99 times in the PGC-1β gene, and 2.44 times in the AMPK gene. In previous studies, it was reported that the expression of PGC-1α and AMPK decreased in animals fed with HFD compared to animals fed with control food (Barroso et al. 2018; Lindholm et al. 2013). In our study, we observed that HFD decreased PGC-1α, PGC-1β and AMPK expressions in adipose tissue, and taurine supplementation had no effect on the expression of these genes. It is suggested that overfeeding and obesity reduce the expression of mitochondrial and metabolic genes through decreased PGC-1α expression, reducing the rate of oxidative phosphorylation and lipid oxidation, thus causing insulin resistance and type 2 diabetes (Liang and Ward 2006; Patti et al. 2003). On the other hand, Kim et al. (2020) found lower level of PGC-1α expression and found no different PGC-1α expression between HFD and HFD + T groups in iWAT. Furthermore, Guo et al. (2019) found increased PGC-1α expression both iWAT and eWAT in HFD + Taurine group to HFD. Therefore, further studies are needed to understand the reason for the differences in PGC-1α expressions in these studies.

In conclusion, we found that taurine supplementation had significant effects on reducing body mass. Therefore, we can say that taurine is an amino acid with anti-obesity properties. In our study, no effect of taurine on WAT browning was detected. More studies are needed in different experimental conditions to consider the effects of taurine on WAT browning.
Declarations

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Author Contributions The experiment was designed by G.B and H.O. G.B performed the experiments. G.B and H.O participated in data analysis. All authors read and approved the final manuscript.

Conflict of interests The authors declare that there are no conflicts of interest.

Ethical Approval Ethics committee approval was obtained from Sivas Cumhuriyet University Animal Experiments Local Ethics Committee with the number of 25.05.2017-43.

References


**Figures**

![Figure 1](image1)

**Figure 1**

**Taurine supplementation decreases body weight in high-fat diet (HFD)-mice.** 10-week-old mice were divided into 4 different groups according to their diet, with 8 mice in each group as following: Control, control + 5% taurine, HFD and HFD + 5% taurine, and fed for 20 weeks. To produce diet-induced obesity, mice in HFD groups were fed with high-fat diet. For feeding of mice of control groups, control-adaptation food was used. Taurine was given in drinking water (5% w/v). (A) Weekly average weights of mice. (B) Body weight change (%) of mice in the groups. (C) Average food consumption of mice in the groups. (D) Average drinking water consumption of mice in the groups. Between the two groups, the mean daily food (C) and water consumption (D) per mouse at the end of the experiment were analyzed using the One-way analysis of variance (ANOVA). "Tukey test" was used as post hoc analysis for comparisons of the groups.
Paired sample T-test was used for the calculation of average body weight change (B). The error level was considered as 0.05. Data were shown as mean ± SD *p < 0.05; **p < 0.001.

Figure 2

Relative gene expression between groups of mRNAs from epididymal adipose tissues of mice.

We did not detect any expression in the UCP1 gene. Therefore, no UCP-1 gene was included in any group comparison. Relative gene expressions of control + T group compared to control group are given in the Figure 2 (A). Relative gene expressions in the the HFD + T group compared to HFD group are presented in the Figure 2 (B). Figure 2 (C) shows the relative gene expression levels in HFD group compared to control groups. In mice fed HFD, 6.42-fold (p = 0.012) expression in the PGC-1α gene and 2.16-fold (p = 0.049) expression in the PGC-1β gene were found compared to the control group. Relative gene expression levels in the HFD + T group compared to control + T group are shown in Figure 2 (D). The expression of PGC-1α, PGC-1β and AMPK genes was statistically significantly decreased in the HFD + T fed group compared to control + T group. (3.77-fold down-regulation in PGC-1α gene, 2.99-fold down regulation in PGC-1β gene,
and 2.44-fold down regulation in AMPK gene). Relative expression levels of FLCN, mTOR, TFE3, PGC-1α, PGC-1β, AMPK, S6K and UCP1 genes were quantified by relative quantification using $2^{-\Delta\Delta\text{Ct}}$ method. Data were shown as mean ± SD. *$p < 0.05$. 