The Relationship between High-Fat Diet and Fibronectin Type-III Domain-Containing Protein 5 mRNA Expression

Yüksek Yağlı Diyet ve Fibronektin Tip III Alan İçeren Protein 5 mRNA Ekspresyonu Arasındaki İlişki

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Abstract

Aim: Fibronectin type-III domain-containing protein 5 (FNDC5) is abundant in both muscle and brown adipose tissues. It has been suggested that due to their various effects FNDC5 and irisin (fragment of FNDC5) might be used as a therapeutic molecule in the treatment of obesity-related metabolic diseases. Due to the recent discovery of irisin/FNDC5, there are yet a limited number of studies in the literature. In this study, we aimed to investigate the relationship between a high-fat diet and the FNDC5 mRNA expression in the muscle and brown adipose tissues, associated with metabolic diseases.

Materials and Methods: Eighteen male Sprague-Dawley rats aged 1.5 to 2 months were randomized into three groups of six rats each. Each group was fed its group-specific diet for 85 days. At the end of the feeding period, measurements of FNDC5 mRNA expression were performed in collected specimens of muscle and brown adipose tissues.

Results: It was observed that the FNDC5 mRNA levels in the brown adipose tissue were higher for the obese group in comparison to the control group (p=0.016) and to the antioxidant group (p=0.010), relatively. In the muscle tissue, however, FNDC5 mRNA expression levels were measured to be lower, compared to the controls (p=0.006).

Discussion and Conclusion: Our study indicated that a high-fat diet caused a change in FNDC5 mRNA expression levels in the brown adipose tissue, but not in the muscle tissue, and thus might help regulate FNDC5 mRNA expression in the brown adipose tissue.

Keywords: brown adipose tissue; FNDC5; high-fat diet; obesity

Öz

Amaç: Hem kahverengi yağ dokusunda hem de kas dokusunda fibronektin tip III alan içeren protein 5 (FNDC5) bol bulunur. FNDC5 ve FNDC5'in fragmenti olan irisinin çeşitli etkilerinden dolayı obezite ile ilişkili metabolik hastalıkların tedavisinde terapötik molekül olarak kullanılabileceği ifade edilmektedir. Membran proteini olan FNDC5 ve irisin yakın zamanda keşfedildiği için literatürde henüz sınırlı sayıda çalışma bulunmaktadır. Bu çalışmanın amacı kas ve kahverengi yağ dokusunda yüksek yağlı diyet ve metabolik hastalıklarla ilişkili FNDC5 mRNA ekspresyonu arasındaki ilişkiyi incelemektir.

Gereç ve Yöntemler: Çalışmada 18 adet 1,5–2 aylık erkek Sprague-Dawley sıçan her birinde 6 sıçan olacak şekilde rastgele üç gruba ayrıldı. Sıçanlar gruplarına özgü yemle 85 gün beslendi. Beslenme sürecinin bitiminde kas ve kahverengi yağ dokuları toplandı ve bu dokularda FNDC5 mRNA ekspresyon ölçümleri yapıldı.

Bulgular: Kontrol grubu ile obez grubun FNDC5 mRNA seviyeleri karşılaştırıldığında, kahverengi yağ dokusunda bu seviye obez grupta kontrol grubundakinden daha yüksekti (p=0,016). Yine aynı dokuda obez grubun FNDC5 mRNA seviyesinin antioksidan grubununkinden daha yüksek olduğu tespit edildi (p=0,010). Kas dokusunda ise antioksidan grubunda kontrol grubuna göre daha düşük FNDC5 mRNA ekspresyonu ölçüldü (p=0.006).

Tartışma ve Sonuç: Çalışmamızdan yüksek yağlı diyetin kahverengi yağ dokusundaki FNDC5 mRNA ekspresyonunu değiştirdiği fakat kas dokusunda değiştirmediği, bu nedenle yüksek yağlı diyetin kahverengi yağ dokusunda FNDC5 mRNA ekspresyonunun düzenlenmesinde etkili olabileceği anlaşılmaktadır.

Anahtar Sözcükler: kahverengi yağ dokusu; FNDC 5, yüksek yağlı diyet; obezite

Geliş Tarihi /*Received* : 03.11.2017
Kabul Tarihi /*Accepted*: 02.01.2018

DOI: 10.21673/anadoluklin.3419294

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INTRODUCTION

High-fat foods increase adipose tissue size and induce obesity (1). In industrialized countries, obesity is the most prevalent nutritional condition that leads to serious ailments such as certain cancers, diabetes mellitus type II, and cardiovascular diseases (2,3).

Quellet et al. suggested a reverse relationship between the brown adipose tissue (BAT) activity and body mass index (4). They also determined that BAT oxidative metabolism significantly contributed to energy consumption. These findings have aroused an interest in the therapeutic potential of BAT for weight loss (4). BAT protects the body against both obesity and developing insulin resistance. In addition, BAT helps maintain the body temperature by thermogenesis (5).

The sequence of fibronectin type-III domain-containing protein 5 (FNDC5) was first identified in two different studies with mice in 2002 (6,7). In 2012, Boström et al. identified the secreted part of FNDC5, irisin (8). In humans and mice, irisin is a 112-amino acid polypeptide fragment cleaved from FNDC5 (8).

It was shown that the uncoupling protein 1 (UCP1) mRNA levels were significantly increased in the experiment group using FNDC5 at a concentration of 20 nM (8). It was determined that the BAT marker genes were robustly stimulated in primary human adipocyte culture after FNDC5 treatment (9). Moreover, in primary human adipocyte culture, FNDC5 treatment enhanced the basal and uncoupled oxygen consumption levels. These cells were able to react to beta-adrenergic stimulation, an important property of brown adipocyte functionality (9). Pontus et al. determined that FNDC5 stimulated the browning of the inguinal fat layer and thermogenesis by increasing UCP1 levels (8). It is suggested that FNDC5 and irisin (fragment of FNDC5) might be used as a therapeutic molecule in the treatment of diseases such as diabetes and obesity (10–12).

The FNDC5 mRNA expression levels were analyzed in forty-seven different human tissues. While most abundant in the muscle tissue, FNDC5 was found in different levels in the other forty-six tissues (13). However, the FNDC5 mRNA expression levels were not analyzed in BAT that originates from the same stem cells as the muscle tissue. Given the recent discovery of FNDC5/irisin (6-8), the relevant studies in the literature are limited (8,12,13).

In the present study, we investigated the relationship between a high-fat diet and the FNDC5 mRNA expression in the muscle and brown adipose tissues. We also investigated the potential effects of N-acetylcysteine (NAC) as an antioxidant on the FNDC5 mRNA expression.

MATERIALS AND METHODS

Animals and Experimental Design

This study was performed with the permission of the Karadeniz Technical University Animal Experimentation Ethics Committee (protocol no. 2014/7).

The rats used in the experiment were provided from the Karadeniz Technical University Surgical Application and Research Center, and they were fed in the feedyard and maintained in a 12/12 hour light/darkness cycle. Eighteen male Sprague-Dawley rats, each of which was 1.5 to 2 months old and weighed 150 to 200 g, were used. The number of the animals in the groups was determined in accordance with the ethics committee directions. All of the animal feed (composition of which is shown in Table 1) was bought from *Research Diets*. Initially, all of the rats were fed a low-fat standard diet for two weeks, and then each rat was weighed. Then the rats were randomized into three groups, each of which consisted of six rats. For eighty-five days, the control group was fed a low-fat standard diet, the obese group a high-fat diet, and the antioxidant group a high-fat diet and 2 g/L of NAC. Each rat was given *ad libitum* access to feed and water.

At the end of the 85-day feeding period, the rats were weighed and sacrificed under anesthesia (80 mg/kg ketamine). The psoas muscle tissue and interscapular BAT were obtained and frozen on dry CO_2. The specimens were immediately placed into Eppendorf containers and then stored at -80 °C until the analysis.

FNDC5 mRNA Expression

RNA isolation was carried out by using TRIzol reagent (Invitrogen). For cDNA synthesis, the Roche transcriptor first strand cDNA synthesis kit (Cat. No: 04 896 866 001) was used. SYBR green dye was used to specify the expression of FNDC5 and glyceraldehyde

Table 1. Low-fat standard diet and high-fat diet elements

	Low-fat standard diet		High-fat diet	
Product	**g%**	**kcal %**	**g%**	**kcal %**
Fat	4.3	10	24	45
Protein	19.2	20	24	20
Carbohydrate	67.3	70	41	35
Total	-	100	-	100
kcal/g	3.89	-	4.73	-

3-phosphate dehydrogenase (GAPDH) genes. The RT-PCR procedure was performed with the Roche Light Cycler 480 II devices in accordance with the Roche kit (DNA master SYBR green I qRT-PCR Cat. No: 12 239 264 001) procedure. 5' CGAGAAGATGGCCTCTA-AGAAC sequence was used as a forward primer for FNDC5. As for 5' TGTTATTGGGCTCGTTGTCC 3' sequence, it was used as the reverse primer for FNDC5 (iontek). One hundred and seventy-six base pairs in length amplicons were produced with these primers. GAPDH was used as a reference gene. 5' AGA TGG TGA AGG TCG GTG TG 3' sequence was used as a forward primer for GAPDH, and 5'CAT TCT CAG CCT TGA CTG TGC 3' sequence as the reverse primer for GAPDH (Integrated DNA technologies). With GAPDH primers, 189 base pairs in length amplicons were produced. The results were calculated with relative quantification.

Statistical Analysis

The results were expressed in median (minimum–maximum). The nonparametric Kruskal-Wallis test was used for the comparisons among the groups, and then the Mann-Whitney U-test was performed. The statistical analyses were carried out by using the SPSS 16.0 software. $p<0.05$ was considered statistically significant.

RESULTS

The rats had *ad libitum* access to feed for 85 days in the Surgical Application and Research Center. At the end of the nutrition, the final weights of the rats were measured, and the FNDC5 mRNA expression levels in BAT and the muscle tissue were quantified. The results are shown in Table 2.

While in the control group the FNDC5 mRNA expression level was lower in BAT compared to the muscle tissue, it was almost the same in BAT and the muscle tissue in the obese and antioxidant groups (Figure 1).

DISCUSSION AND CONCLUSION

FNDC5 indicates effects such as decrease in insulin resistance and obesity (8), increase in oxygen consumption (9), UCP1 (8), and browning in subcutaneous adipose tissue (SAT) (8). Accordingly, it is stated that FNDC5 may be used as a therapeutic molecule for obesity-related metabolic diseases (10–12).

Concerning the effects on SAT and the visceral adipose tissue (VAT) of male Sprague-Dawley rats, Arturo et al. stated that short-time endurance exercise training stimulated FNDC5 secretion (14) whereas Pontus et al. expressed that FNDC5 mRNA was not stimulated in SAT by acute exercise (8). In Arturo's study, FNDC5/irisin was barely expressed and excreted FNDC5/irisin in secretomes of BAT according to white adipose tissue under the *ad libitum* condition (14). We observed different findings in BAT in our study: While FNDC5 mRNA expression was found in BAT at a level of 37% with respect to the muscle tissues of the low-fat standard diet-fed rats, it was found in BAT at 95% with respect to the muscle tissues of the high-fat diet-fed rats. However, Arturo's study was

Table 2. FNDC5 mRNA expression levels in BAT and the muscle tissue

Parameters	Control group	Obese group	Antioxidant group
Final weight (g)	450 (399–484)	517 (497–551)	514 (418–607)
FNDC5 mRNA levels in BAT (arbitrary unit)	0.25 (0.16–0.65)	0.68 (0.4–1.0)[a]	0.37 (0.25–0.56)[b]
FNDC5 mRNA levels in the muscle tissue (arbitrary unit)	0.67 (0.48–1)[c]	0.72 (0.32–1.08)[c]	0.37 (0.32–0.53)

[a] Statistically significant with respect to the control group $p<0.05$

[b] Statistically significant with respect to the obese group $p<0.05$

[c] Statistically significant with respect to the antioxidant group $p<0.05$

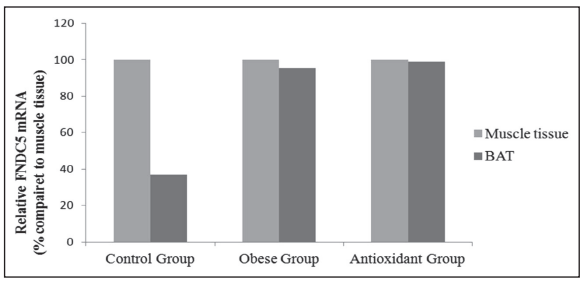

Figure 1. Relative FNDC5 mRNA expression in BAT and the muscle tissue
Fibronectin type-III domain-containing protein 5 (FNDC5), brown adipose tissue (BAT)

conducted in the BAT secretomes while ours in BAT. This might explain the different findings in the two studies.

In Arturo's study, the obese rats were found to show an important increment of FNDC5 secretion in both SAT and VAT, compared to the control group. In addition, it is stated that nutritional status influences FNDC5 secretion in SAT and VAT (14). In our study, we reached findings in BAT in line with those of Arturo et al. We found that in diet-induced obesity FNDC5 mRNA expression was significantly increased in BAT, but not in the muscle tissue. Our study indicated that FNDC5 mRNA expression in BAT was changed by feeding a high-fat diet. It was determined that high-fat diet increased peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC1-α) in BAT (15). As PGC1-α stimulates FNDC5 (8), PGC1-α may increase FNDC5 via PGC1-α—FNDC5 pathway in BAT. High-fat diet may be a regulatory factor (via high-fat diet—PGC1-α—FNDC5 pathway) in the regulation of FNDC5 mRNA expression in BAT.

In PGC1-α transgenic mice, Boström et al. showed that PGC1-α overexpression stimulated FNDC5 mRNA expression in muscles. They also showed that exercise activated FNDC5 mRNA expression in muscles. In addition, they stated that FNDC5/irisin substantially increased in plasma after endurance exercise in humans and mice (8). Our study indicated that a high-fat diet caused no change in FNDC5 expression in the muscle tissue.

Our study showed that NAC decreased FNDC5 mRNA expression in both BAT and muscle tissue. Exercise training increases FNDC5 via reactive oxygen species-PGC1-α signal pathway (16,8). Since NAC is an antioxidant molecule, it may decrease reactive oxygen species in this signal pathway and minimize FNDC5 mRNA expression.

In conclusion, our study indicated that FNDC5 mRNA expression was changed in BAT by feeding a high-fat diet, but not in the muscle tissue. In addition, we determined that NAC significantly decreased FNDC5 mRNA expression both in BAT and muscle tissue. Our findings suggest that a high-fat diet might be an effective factor in the regulation of FNDC5 mRNA expression in BAT.

Acknowledgements

This study was partly supported by the Scientific and Technological Research Council of Turkey (TUBİTAK).

REFERENCES

1. Wang CY, Liao JK. A mouse model of diet-induced obesity and insulin resistance. Methods Mol Biol. 2013;821;421–33.
2. Kopelman PG. Obesity as a medical problem. Nature. 2000;404;635–43.
3. Mitchell N, Catenacci V, Wyatt H R, Hill JO. Obesity: overview of an epidemic. Psychiatr Clin of North Am. 2011;34;717–32.
4. Ouellet V, Labbe SM, Blondin DP, Phoenix S, Guerin B, Haman F, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest. 2012;122;545–52.
5. Kozak LP. Brown fat and the myth of diet-induced thermogenesis. Cell Metab. 2010;11;263–67.
6. Teufel A, Malik N, Mukhopadhyay M, Westphal H. Frcp1 and Frcp2, two novel fibronectin type III repeat containing genes. Gene. 2002;297;79–83.
7. Ferrer-Martinez A, Ruiz-Lozano P, Chien KR. Mouse PeP: a novel peroxisomal protein linked to myoblast differentiation and development. Dev Dyn. 2002;224;154–67.
8. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012;481;463–8.
9. Lee P, Linderman JD, Smith S, Brychta RJ, Wang J, Idelson C, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab. 2014;19;302–9.

10. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab. 2013;98;e769–e778.

11. Raschke S, Elsen M, Gassenhuber H, Sommerfeld M, Schwahn U, Brockmann B, et al. Evidence against a beneficial effect of irisin in humans. PLoS One. 2013;8;e73680.

12. Elsen M, Raschke S, Eckel J. Browning of white fat: does irisin play a role in humans? J Endocrinol. 2014;222;R25–R38.

13. Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism. 2012;1725–38.

14. Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Crujeiras AB, et al. FNDC5/Irisin is not only a myokine but also an adipokine. PloS One 2013;8;e60563.

15. Betz MJ, Bielohuby M, Mauracher B, Abplanalp W, Muller HH, Pieper K, et al. Isoenergetic feeding of low carbohydrate-high fat diets does not increase brown adipose tissue thermogenic capacity in rats. PLoS One 2012;e38997.

16. Lira VA, Benton CR, Yan Z, Bonen. A PGC-1 regulation by exercise training and its influences on muscle function and insulin sensitivity. Am J Pyhsiol Endocrinol Metab. 2010;299;e145–e161.