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Original Research

Molecular effects of leptin on peroxisome proliferator activated receptor gamma (PPAR-γ) mRNA expression in rat's adipose and liver tissue

A. Abbasi[1,2], A. A. Moghadam[2], Z. Kahrarian[3], R. Abbsavaran[4], K. Yari[5], E. Alizadeh[6,7*]

[1] Department of Biology, Faculty of Basic Science, Islamic Azad University of Science and Research, Tehran, Iran
[2] Department of Clinical Sciences, School of Veterinary Medicine, Razi University, Kermanshah, Iran
[3] Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran
[4] Department of Clinical Sciences, Clinical Science Laboratory, Kermanshah University of Medical Sciences, Kermanshah, Iran
[5] Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[6] Pharmaceutical Sciences Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[7] Nosocomial Infection Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Correspondence to: Alizadeh_elin@yahoo.com

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Abstract: Leptin is a 16-kDa peptide hormone secreted by adipose tissue that participates in the regulation of energy homeostasis. The aim of this study was to determine the effect of leptin injection on mRNA expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) and comparison of PPAR-γ mRNA expression in rat's adipose and liver tissue. Twenty adult male rats were divided into the following groups: Group 1asa control (n=10) that did not receive any treatment. Group 2as a treatment (n=10) that received leptin (30 µg/kg BW) intraperitoneally (ip) for two successive days. Blood samples were taken before and one day after second leptin injection for triglyceride (TG), Free Fatty Acid (FFA), HLD-cholesterol, and LDL-cholesterol measurement. Total RNA was extracted-from the adipose tissue and liver tissues of rats. Adipose and liver tissue cells' cDNA was synthesized to characterize the expression of PPAR-γ. Gene expression of PPAR-γ mRNA was tested by RT- PCR technique. Results show leptin decreases expression of PPAR-γ on rat. Low levels of PPAR-γ mRNA were detected in adipose and liver tissues of treatment rats in comparison to control group. In treatment group, the level of PPAR-γ mRNA in liver tissue was very lower than the adipose tissue. The levels of HDL and FFA in treatment rats were increased whereas serum levels TG, VLDL and LDL were not changed. It is concluded that leptin signal with suppressing of PPAR-γ mRNA expression in rat's adipose and liver tissues can result in lipolysis instead of lipogenesis.

Key words: Leptin; Peroxisome proliferator-activated receptor gamma; Adipose; Liver.

Introduction

Leptin is a 16-kDa peptide hormone secreted by adipose tissue that participates in the regulation of energy homeostasis (1). Leptin mainly acts on the hypothalamus in the brain which it regulates food intake appetite and energy expenditure. However, the direct effects of leptin on adipocytes have been controversial in the cellular level. The expression and secretion of leptin is increased during adipogenesis, adipocyte differentiation and adipocyte development processes. Therefore, the plasma leptin levels have been identified as a factor of obesity (2,3). Many of the studies have shown that leptin has a pleiotropic effect on adiposity, body weight, and glucose homeostasis (4-6). Leptin is transferred and acts on central nerve system, the hypothalamus, where leptin reduces expressions of orexigenic peptides such as neuropeptide Y (NPY) and agouti related protein (AgRP), but induces anorexigenic peptides, including a-melanocyte stimulating hormone (α-MSH), cocaine and amphetamine-related transcripts (CART) and pro-opiomelanocortin (POMC) (7). Although leptin reduces appetite as a circulating signal, obese individuals generally exhibit an unusually high circulating concentration of leptin (5). The leptin deficient animal overeats, resulting in obese phenotype (8). In addition to central nervous tissues, leptin receptors are expressed in peripheral tissues including liver, fat and kidney that implicating leptin's direct functions in adipocytes or other peripheral tissues (9). The leptin receptors are a member of the cytokine family of receptors, that at least five different isoforms of the leptin receptor were identified in mouse (10). Leptin has six types of receptors (Ob-Ra–Ob-Rf, or LepRa-LepRf) that interact with them and receptors are encoded by a single gene, LEPR. (11) Ob-Rb is the only receptor isoform that can signal intra-cellularly via MAPK signal transduction and the Jak-Stat pathways, and is present in hypothalamic nuclei in brain (12).

The peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptorproteins that function as transcription factors regulating the genes expression (13). The PPAR subfamily is composed of three members: PPAR-α, PPAR-β, and PPAR-γ. Most tissues in rodents and humans have all three receptor subtypes, although there is considerable variability in the relative expression. Two known isoforms of PPAR-γ: PPAR-γ1 and PPAR-γ2 but the major isoform is PPAR-γ1, accounting for 85% of PPARs in adipose tissue. The isoforms are generated from the same gene by mRNA splicing and different in their amino terminal

end, with PPAR-γ2 having an additional 30 amino acids (14). In some studies, leptin reduced differentiation of rat adipocytes through the decrease in peroxisome proliferator activated receptor activity (15). PPARs play essential roles in the regulation of cellular development, differentiation, and metabolism (carbohydrate, protein, lipid), and tumor genesis of higher organisms (8,16-18). Peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG), also known as the glitazone receptor, or NR1C3 (nuclear receptor subfamily 1, group C, member 3) is a type II nuclear receptor that in humans is encoded by the PPAR-γ gene (20).

Results suggest that the anti lipogenic effects of leptin may have been partly mediated by suppressing the expression of PPAR-γ (21). Here we test the hypothesis that leptin decreases PPAR-γ expression on rat thatPPAR-γ is a nuclear receptor expressed mainly in adipocytes, which activates the transcription of genes involved in lipid and glucose metabolism (22). RXR-PPAR-γ signaling is a key component in adipogenesis and the function of adipocytes; activation of this heterodimer increases adipose mass in rodents and humans. Thus, inappropriate activation of RXR-PPAR-γ can directly alter adipose tissue homeostasis (23). The aim of this study was to determine the effect of leptin on expression of PPAR-γ and comparison of PPAR-γ mRNA expression in rat's adipose and liver tissue.

Materials and Methods

Twenty adult male rats (weighing 130–140 g; age: 120–130 days) were housed in air-conditioned rooms with controlled lighting and were given free access to laboratory chow and tap water. They were maintained with a 12-h light/12-h dark photoperiod in a humidity and temperature-controlled room (24 °C). The rats were divided into the following groups: Group 1as a control (n = 10) that did not receive any treatment. Group 2as treatment (n=10) that received leptin (30 µg / kg BW) intraperitoneally (ip) for two successive days. The dose of leptin was chose on the base of the previous study in which the male rats were received 300g/kg/day without significantly affecting food or water intake (11).

Biochemical analysis

Blood samples were taken before and one day after the second leptin injection for triglyceride (TG), Free Fatty Acid (FFA), HLD-cholesterol, and LDL-cholesterol. The samples were centrifuged at 4°C for 30 min at 1000g. Serum aliquots were aspirated and frozen at -20°C. Plasma concentrations of TG, FFA, LDL, and HDL were measured by a colorimetric assay using an automatic biochemical analyzer (Hitachi Japan). The rats were killed by decapitation using a guillotine after sedation in a CO_2 chamber at the end of the experiment. Adipose and liver was removed and maintained in liquid nitrogen.

RNA extraction and Reverse Transcription–Polymerase Chain Reaction analysis of PPAR-γ

Total RNA was extractedfrom the adipose tissue and liver tissues using Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's protocol. A hundred milligram of frozen adipose and liver tissues from each sample was homogenized by adding 1 ml of Trizol reagent.

To characterize the expression of PPAR-γ, cDNA of adipose and liver tissue cells was synthesized. To reveal the mechanism of leptin in regulating lipid metabolism, gene expression of PPAR-γ mRNA was tested by RT- PCR technique. A partial PPAR-γ cDNA probe was generated by reverse transcriptase-PCR using total RNA from rat adipose and liver. The reverse transcription of total RNA (1µg) was performed using a reverse transcription kit (Sinagen Co) in a final volume of 20µl containing 1× RT buffer (10mM Tris–HCl, pH 9.0, 50mM KCl and 0.1% Triton X-100), 5mM MgCl2, 1mM each dNTPs, 1µL (200 U) Reverse transcriptase, 0.5 µg oligo (dT) primer and 0.5µL (20 U)RNase inhibitor. The samples were incubated at 42 ◦C for 60 min, inactivated by heating at 99 ◦C for 5 min, and cooled at 4 ◦C for 5min. Master Mix contains: 1/20 synthesized cDNA in a 10µl volume and 0.5µmol/l primers. PCR conditions were as follows: 2 min at 94 ◦C, then 30 cycles of reaction consisting of 30 s at 94 ◦C, 30 s at 62 ◦C and 30 s at 72 ◦C. Amplification reactions contained final concentrations of 1.5mm $MgCl_2$, 50 mm KCl, 400 µm dNTPs, 20 mm Tris-HCl (pH 8.4), 10 pmol of both primers, and 1.5 U of Taq DNA polymerase. The primers for the RT -PCR were following; PPAR-γ For amplification of PPAR-γ cDNA, the revers primer 5'-TGGCAGCAGTGGAAGAATCG-3' and the forward primer 5'-AACCGGAACAAATGCCAGTA-3' were used according to the published rat PPAR-γ, cDNA sequence For GAPDH, used as an internal control, the revers primer was 5'-CACCACCCTGTTGCTGTA-3' and the forward primer was 5'-TATGATGACATCAAGAAGGTGG-3'. After amplification, PCR products were electrophoresed in a 2% agarose gel and bands corresponding to RT-PCR products from PPAR-γ and GAPDH mRNA were detected by ultraviolet light. All results were repeated at least three times from different samples of RNA extracted at different times. Results were quantified by scanning densitometry using image j software (developed by Wayne Rasband, National Institutes of Health, BETHESDA, md; available at http://rsb.info.nih.gov/ij/index.html).

Statistical

Statistical calculations were performed with StatView for Windows, version 5.0 (SAS Institute, Cary, NC). Values before and after treatment were analyzed using paired Student's t test. Data are presented as mean ± SEM. A P value <0.05 was considered statistically significant.

Results

Effect of leptin on serum TG, FFA, VLDL, HDL of treated rats

The effect of leptin injection on some serum biochemical parameters of treatment group were shown in Table 1. The levels of HDL and FFA in treatment rats were increased whereas levels of TG, VLDL and LDL were not changed.

Effect leptin on expression of PPAR-γ

PPAR-γ expression has been shown in Figures 1 and

Table1. TG, FFA, VLDL, and HDL plasma concentration (mg/dl) of treatment rats before and after leptin injection.

Variable	Treatment Group	
	Before	After
FFA	49.72± 0.49[a]	55.42± 0.72[b]
VLDL	15.01± 0.25	15.23± 0.42
HDL	50.49± 1.03[a]	52.45± 1.12[b]
LDL	26.53± 0.59	25.90± 0.46
TG	53.59± 0.37	53.37± 0.19

[a,b]Mean values within the same row sharing a different superscript letter are statistically different at $P < 0.05$.

2.The effect of leptin on PPAR-γ expression in adipose and liver tissue has shown in Figure3. Leptin has decreased the PPAR-γ expression in both adipose and liver tissues of treatment group. Leptin could prevent the lipogenesis effects PPAR-γ in adipose and liver tissue.

As shown in Figure3.PPAR-γ mRNA expression is down regulated by leptin injection. Also, decrease of the PPAR-γ mRNA expression in liver tissue was more than the adipose tissue of the treatment rat group.This picture shows that leptin could increases lipolysis and prevents the development of adipose tissue by decreasing PPAR-γ expression.

Comparison between PPAR-γ expression in adipose adipose and liver

Comparisons of the PPAR-γ mRNA expression in adipose and liver tissues of the treatment and control groups have been shown in figures 4 and 5, respectively.

Decreasing rate of PPAR-γ mRNA expression in liver tissue was more than the adipose tissue. leptin has a direct inhibitor effect on expression of PPAR-γ.leptin has the capability to modify adipose tissue mass by influencing on lipogenesis.

As shown in Fig 4 and Fig 5. Leptin effects on decreasing PPAR-γ mRNA expression in liver tissue were more than the adipose tissue. We propose that the reduction in PPAR-γ expression in both tissues may be one of the factors linking high leptin levels and lipogenesis decrease.

Discussion

In this study, we examined the effect of leptin on PPAR-γ expression and compared the expression level of PPAR-γ in rat's adipose and liver tissue. The present study results show leptin can decrease PPAR-γ expression. Therefore leptin can increase lipolysis and prevents the development of adipose tissue. Also the levels of HDL and FFA in treatment rats were increased whereas levels of TG, VLDL and LDL were not changed.

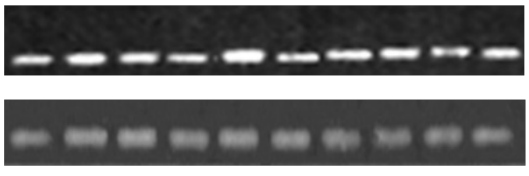

Figure 1.mRNA expression of PPAR-γ in adipose tissue of the control groupsby RT-PCR.

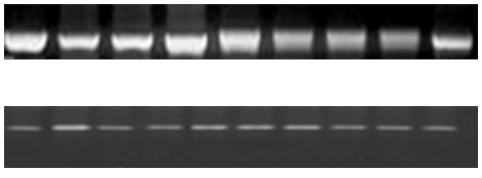

Figure 2.mRNAexpression of PPAR-γ in liver tissue of the control groups by RT-PCR.

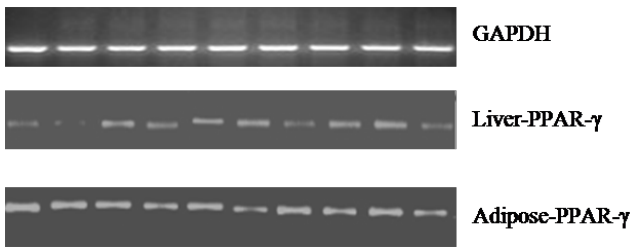

Figure3. PPAR-γ mRNA expression is down regulated by leptin injection.

Figure 4. Comparison of the PPAR-γ mRNA expression in adipose and liver tissues of the treatment group by RT-PCR method.

Figure 5. Comparison of the PPAR-γ mRNA expression in adipose and liver tissues of the control groups by RT- PCR method.

The PPARs family of nuclear receptors is comprised of three distinct isoforms (α, β, γ) with specific and different tissue distribution. PPAR-α is expressed predominantly in tissues that undergo peroxisomal proliferation such as liver, heart, and kidney and is activated by several hypolipidemic drugs and fatty acids, resulting in induction of the expression of enzymes involved in peroxisomal fatty acid β oxidation (17). PPAR-γ is expressed ubiquitously by fatty acids, and has no known specific ligand(s) (20). Although it has been suggested that PPAR-γ could play an important role in the adipogenic effect of overfeeding (18), no clear physiologic function has been described (24). PPAR-γ is a relatively new member of the PPARs family that was recently suggested thathave a key role in the regulation of gene expression in adipose tissue. Thus, PPAR-γ has been implicated in mediating the expression of fat-specific genes and in activating the program of adipocyte differentiation (25). Activation of PPAR-γ could constitute an important part of the molecular mechanism behind the adipogenic effect of overfeeding. In over expression studies, it has most recently been shown that PPAR-γ has the greatest adipogenic action, that PPAR-α also has some ability to stimulate adipogenesis (26). Adipogenesis is a complex process of differentiation of preadipocytes, involving alteration of cytoskeletal and extracellular matrix proteins, and assembly of the lipid-synthesizing machinery (27). RXR-PPAR-γ signaling is

a key component in adipogenesis and the function of adipocytes activation of this heterodimer increases adipose mass in rodents and humans. Thus, inappropriate activation of RXR-PPAR-γ can directly alter adipose tissue homeostasis (23).

It has been reported that leptin sufficiently increases lipolysis and decreases the activities of lipogenic enzymes in mature adipocytes (28). The present study results show leptin can increases lipolysis and prevents the development of adipose tissue by decreasing PPAR-γ expression. In this study PPAR-γ mRNA is suppressed by leptin injection. It seems that one of the effects of leptin in inducing lypolisis is due to decreased expression of adipogenic transcription factors, including PPAR-γ expression. This is in agreement with the results that leptin-induced STAT1 that might directly bind to the PPAR-γ promoters resulting in the inhibition of PPAR-γ transcription during adipogenesis. Leptin signaling is known to be mediated through JAK/STATs, PI-3 kinase, and ERK1/2 MAP kinase pathways. It has reported that anti-adipogenic effects of leptin involve JAK/STAT1 and/or ERK1/2 MAP kinase pathways with little involvement of PI-3 kinase and STAT3 pathways (29). Therefore, leptin-induced STAT1 activation might directly regulate the expression of PPAR-γ. Consistent with the notion that PPAR-γ is known to be induced during adipocyte differentiation and to have critical roles in the maintenance of adipocyte differentiation (30). The previous studies had shown that the role of ERK/MAPK pathway is important in adipocyte differentiation (21). Leptin stimulated the prolonged phosphorylation of ERK1/2, and such a sustained activation of ERK1/2 pathway may lead to inhibit PPAR-γ expression (29). On the other hand leptin-induced STAT1 might directly bind to the PPAR-γ promoters resulting in the inhibition of PPAR-γ transcription duringadipogenesis.

Injection of leptin increased FFA and HDL levels. The increase of the FFA level likely is due to suppress of expression of PPAR-γ. PPAR-γ is a nuclear receptor expressed mainly in adipocytes, which activates the transcription of genes involved in lipid and glucose metabolism (4,9, 31-34).

The mRNA expression of PPAR-γ in treatment group in both tissues was lower than the control group. Leptin effects on decreasing PPAR-γ mRNA expression in liver tissue were more than the adipose tissue. Also PPAR-γ mRNA expression is down regulated by leptin injection. Previous studies showed that leptin has a direct inhibitor effect on expression of PPAR-γ (9). However, further studies required to examine and confirm the function mechanism of leptin on expression of PPAR-γ on rat's.

It is concluded that injection of leptin not only can change expression level of PPAR-γ in adipose and liver tissues but also can change some serum lipids profile. However, further studies required to examine exact mechanism of leptin on PPAR-γ expression on rats.

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