

Effect of high-fat diet feeding on leptin receptor expression in white adipose tissue in rats: depot- and sex-related differential response

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Abstract Previous studies have illustrated the importance of leptin receptor (OB-Rb) mediated action on adipocytes in the regulation of body weight. The aim of the present study was to investigate in male and female rats the effects of high-fat (HF) diet feeding on the expression levels of OB-Rb in different depots of white adipose tissue (WAT), and its relation to fatty acid oxidation capacity. Male and female Wistar rats were fed until the age of 6 months with a normal-fat (NF) or non-isocaloric HF-diet (10 and 45% calories from fat, respectively). At this age, the weight of three different fat depots (retroperitoneal, mesenteric and inguinal) and the expression levels of OB-Rb, PPAR α and CPT1 in these depots were measured. HF-diet feeding resulted in an increase in the weight of the different fat depots, the retroperitoneal depot being the one with the greatest increase in both sexes. In this depot, HF-diet feeding resulted in a significant decrease in OB-Rb mRNA levels, more marked in male than in female rats. In the mesenteric depot, the effects of HF-diet feeding on OB-Rb mRNA levels were sex-dependent: they decreased in males rats (associated with a decrease in PPAR α and CPT1 mRNA levels), but increased in female rats. In the inguinal depot, OB-Rb expression was not affected by HF-diet feeding. These results show that a chronic intake of an

HF-diet altered the expression of OB-Rb in WAT in a depot and sex-dependent manner. The decreased expression of OB-Rb in the internal depots of male rats under HF-diet feeding, with the resulting decrease in leptin sensitivity, can help to explain the higher tendency of males to suffer from obesity-linked disorders under HF-diet conditions.

Keywords High-fat diet · Leptin · OB-Rb · Sex-dimorphism · White adipose tissue depots

Introduction

The adipocyte-derived hormone leptin is a key regulator of energy homeostasis and adiposity. It acts directly on the hypothalamic nuclei, along with several other regions of the brain, to suppress food intake and increase energy expenditure [12]. Leptin acts via transmembrane receptors (OB-R), which are members of the cytokine receptor class I and are expressed in the central nervous system and peripheral tissues [23]. The long spliced OB-Rb variant mediates the majority of the myriad effects of leptin via the JAK-STAT signaling pathway [4, 5].

The ability of leptin to regulate food intake, body weight, adiposity and insulin sensitivity has been attributed exclusively to its actions in the hypothalamus [1]. However, recent studies have suggested that leptin can play important physiological roles in an autocrine/paracrine way, acting in different types of cells such as adipocytes, hepatocytes, T-lymphocytes, pancreatic cells, etc. [9, 10, 24, 28]. In fact, the role of leptin on white adipose tissue (WAT) has been reported to be essential in modulating the adipocytes' metabolic function [25], up-regulating fat oxidation [13] and decreasing lipogenesis [27]. In this way, it has been postulated that the peripheral effects of leptin,

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such as in WAT, may also help regulate the entire body weight [7].

The resistance to leptin action on adipose tissue in experimental animals has been related to the development of diet-induced obesity [25]. In human studies, morbid obesity has been associated with a lower OB-Rb mRNA abundance in the subcutaneous adipose depot when compared with lean subjects [20]. In addition, fat tissue-specific leptin resistance has been described to be one of the primary causes of insulin resistance [7]. Thus, peripheral leptin resistance may be a major contributing factor for the development of obesity and its associated disorders.

The distribution and abundance of the leptin receptor seems to depend on the type of adipose depot and differs between lean and obese subjects [20]. In addition to these factors, a sex differential regulation of the leptin receptor in WAT could be expected, as sex steroid hormones are closely involved in the metabolism, accumulation and distribution of adipose tissues [11], and a sex-dependent regulation of the expression of metabolic and fat partitioning-related genes has been previously described by our group [16].

As described above, the leptin paracrine actions, in particular its actions on WAT, are of great importance for the whole body metabolic system, and the study of the factors involved in the regulation of leptin receptor expression in the adipose tissue merits further investigation. The aim of this study was to describe, in a rat model, the effect of a prolonged non-isocaloric HF-diet feeding on the expression levels of the long form of the leptin receptor in three different fat depots (retroperitoneal, mesenteric and inguinal), as well as to study the possible effect of gender on this response. Since the action of leptin on WAT may regulate fatty acid oxidation, peroxisome proliferator activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1) were also analyzed in the different fat depots.

Materials and methods

Animals and experimental design

The study was performed using 28 male and female Wistar rats fed ad libitum from day 21 (after weaning) until the age of 6 months with a normal-fat (NF) diet (3.8 kcal/g; 20% calories from protein, 70% from carbohydrate and 10% from fat) or an HF-diet (4.7 kcal/g; 20% calories from protein, 35% from carbohydrate and 45% from fat). The NF-diet contained 5.5% calories from soybean oil and 4.5% from lard; the HF-diet contained 5.5% calories from soybean oil and 39.5% from lard. (Ref# D12450B and D12451, respectively, from Research Diets, Inc, NJ, USA).

Rats were single-caged and kept in a room with controlled temperature (22°C) and a 12 h light–dark cycle (light on from 08:00 to 20:00 h).

At the age of 6 months, the animals were killed by decapitation under fed conditions. Three different WAT depots (inguinal, mesenteric and retroperitoneal) were rapidly removed. All samples were immediately frozen in liquid nitrogen and stored at -70°C until RNA analysis.

The animal protocol followed in this study was reviewed and approved by the Bioethical Committee of our university, and guidelines for the use and care of laboratory animals of the university were followed.

RNA extraction

Total RNA was extracted from the different pads of WAT by Tripure Reagent (Roche Diagnostic GmbH, Mannheim, Germany) according to the manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NadroDrop Technologies Wilmington, DE, USA) and its integrity was confirmed using agarose gel electrophoresis.

Real-time quantitative PCR analysis

Real-time polymerase chain reaction (PCR) was used to measure mRNA expression levels of OB-Rb in different pads of WAT. 18S ribosomal RNA was used as invariant control. Each PCR was performed as described before [16].

Statistical analysis

Data are expressed as means \pm SEM ($n = 6-8$). Two-way ANOVA was used to determine the significance of the effect of diet and sex on the parameters measured. Single differences between NF- and HF-diet feeding in both male and female rats were assessed by Student's *t* test. The threshold of significance was defined at $P < 0.05$.

Results

The effects of feeding a non-isocaloric HF-diet on body weight gain and on serum leptin levels have been previously described by our group [17]; the animals consuming ad libitum an HF-diet had greater body weight than the ones consuming NF-diet, in both male and female rats (they were 6.8 and 11% heavier than their NF-diet-fed counterpart, respectively). Serum leptin levels showed a tendency to increase in HF-diet-fed animals compared to NF-diet-fed ones.

The weights of the different fat depots are represented in Fig. 1. The relative weights of all the depots studied were

lower in female than in male rats. HF-diet feeding resulted in a significant increase in the size of fat stores, the retroperitoneal depot being the one with the highest increase in both male and female rats, as previously described [16].

Figure 2 shows OB-Rb, PPAR α and CPT1 mRNA levels in the three different depots studied and the effects of diet and gender. In the retroperitoneal depot, HF-diet feeding resulted in decreased OB-Rb mRNA levels in both male and female rats as compared with the NF-feeding conditions. This effect was greater in male than in female rats (54 vs. 23% decrease, respectively; and only statistically significant in males when analyzed separately; $P < 0.05$ by Student's t test). PPAR α mRNA levels were higher in female than in males rats and no changes were found as an effect of the dietary treatment. The HF-diet feeding tended to decrease the expression levels of CPT1 only in male rats, but this tendency was not statistically significant.

Dietary treatment resulted in a different effect on OB-Rb expression in males and females (interactive effect, sex \times diet; $P < 0.05$ by two-way ANOVA), while expression levels decreased in males under HF-diet, but increased in female rats. PPAR α and CPT1 expression levels were lower in rats fed with the HF-diet than in their NF-diet-fed counterparts. This decrease was more marked and statistically significant only in males when analyzed separately ($P < 0.05$ by Student's t test). Female rats presented with lower mRNA levels of PPAR α than male rats in this depot in both dietary groups.

In the inguinal depot, OB-Rb mRNA expression levels were double in female than in male rats, but no significant changes were found in association with HF-diet feeding. HF-diet feeding increased PPAR α mRNA levels in both male and female rats, but this increase was greater in male rats. CPT1 mRNA expression levels increased as an effect

of HF-diet feeding in male rats, whereas no diet effect was found in female rats.

Discussion

Adipocytes play an important role as regulators of whole body metabolism [3, 21]. In these cells, the coordinated regulation between lipid synthesis and oxidation determines fat mass. The two opposing biochemical processes, lipolysis and lipid synthesis, are controlled by different enzymes, which are differentially regulated by hormones. Leptin can act as an autocrine or paracrine signal to change the rate of synthesis and degradation of lipids [6, 22]. Therefore, changes at the level of the leptin receptor in the adipose tissue can be of great importance in the development of obesity and other metabolic disorders. In this work, we show that a chronic intake of a non-isocaloric HF-diet altered the expression levels of OB-Rb in WAT, and this response was depot- and gender-dependent. In addition, this response may determine changes in the expression of some genes involved in fatty acid oxidation.

The effects of an HF-diet inducing central leptin resistance have been previously described [15], and this response seems to be dependent on gender [17]. Moreover, leptin resistance can be regarded as a main factor in the initiation and development of diet-induced obesity [19]; however, it seems that this effect may not only rely on the central level, but also on the peripheral level, in which the adipose tissue can be of great importance [14]. In this sense, Wang et al. have previously shown that HF-diet feeding in male rats induced a decline in OB-Rb mRNA in the adipose tissue (the authors do not specify the type of fat depot analyzed) [25]. Here we found that the effect of HF-diet feeding was dependent on the gender and the depot studied. OB-Rb mRNA levels decreased in the retroperitoneal depot in both male and female rats; in the mesenteric depot, they decreased in males and increased in female rats; whereas the levels remained unchanged in the inguinal depot in both sexes. Depot-related differential responses to HF-diet feeding on the expression of metabolic-related genes have been previously described [16]. These differences are of interest considering the known relationship between fat-depot localization and function [26]. In this sense, we found site-specific differences in the expression pattern of genes involved in lipid metabolism, showing that lipogenesis and lipolysis-related genes were highly expressed in the retroperitoneal depot, while genes related with mitochondrial fatty acid oxidation had a predominant expression in the inguinal and mesenteric depots (our unpublished data).

Changes in the expression of OB-Rb in the different depots can partially account for changes in the weight of

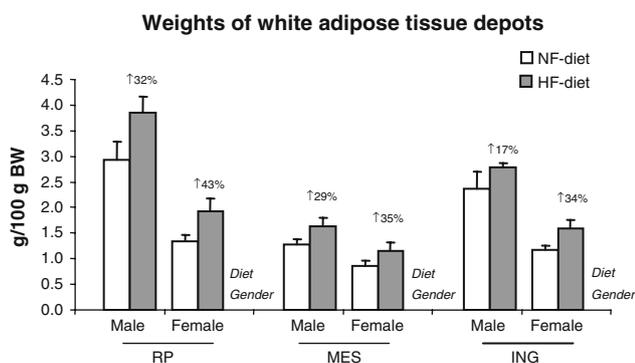


Fig. 1 Weight of retroperitoneal (RP), mesenteric (MES) and inguinal (ING) white adipose depots of 6-month-old rats fed after weaning with a normal-fat (NF) or a high-fat (HF) non-isocaloric diet. Results are expressed as mean \pm SEM of six to eight animals per group. Diet, gender effect of diet or gender by two-way ANOVA ($P < 0.05$)

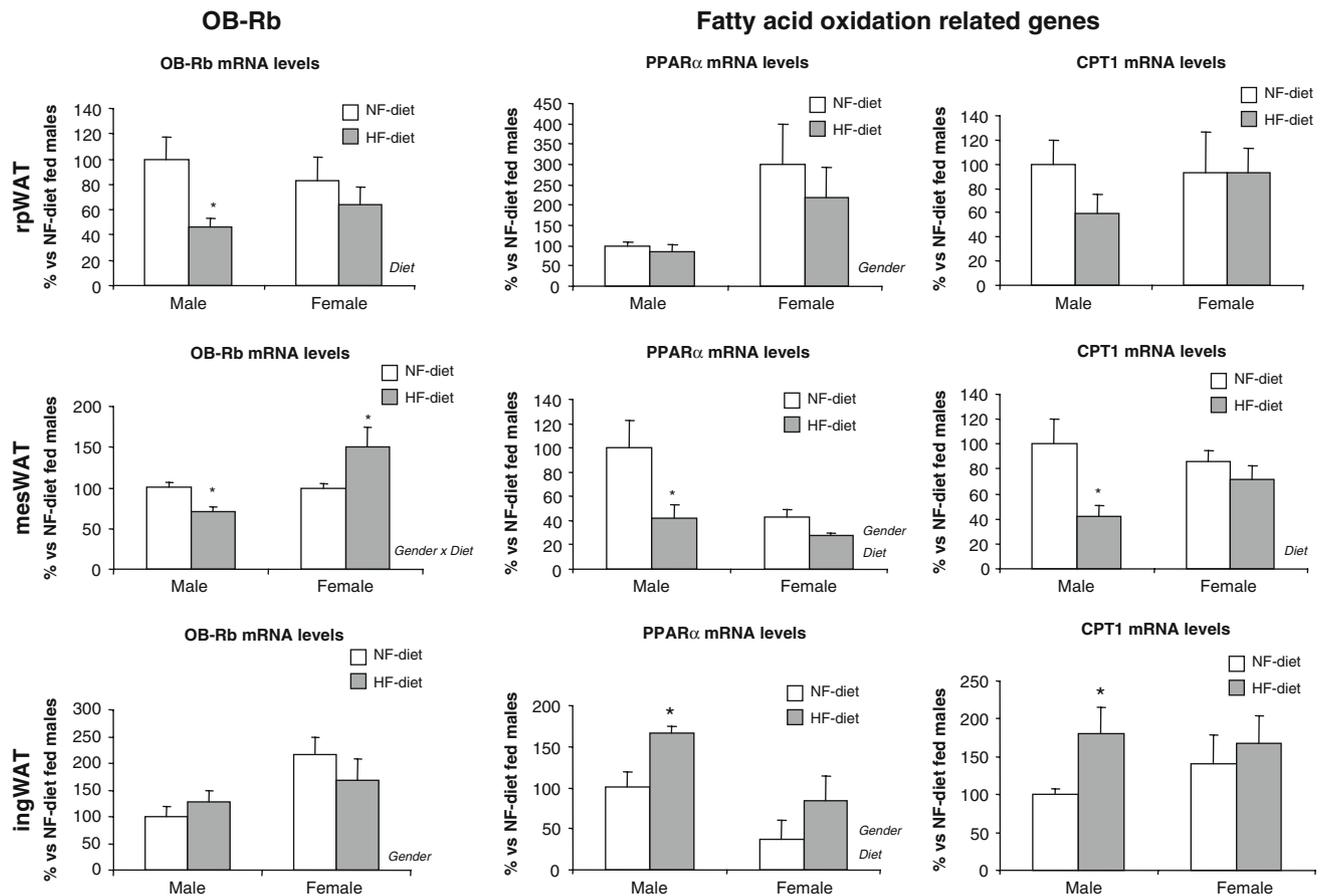


Fig. 2 Long-form of the leptin receptor (OB-Rb), peroxisome proliferator activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1) mRNA levels in retroperitoneal (rp), mesenteric (mes) and inguinal (ing) white adipose tissue (WAT) of 6-month-old rats that were fed after weaning with a normal-fat (NF)

or a high-fat (HF) diet. Results are mean \pm SEM of six to eight animals per group. *Diet*, *gender* effect of diet or gender by two-way ANOVA ($P < 0.05$). *Diet* \times *diet* interaction between gender and diet by two-way ANOVA ($P < 0.05$). Asterisks indicate different from their respective NF-diet-fed control ($P < 0.05$, Student's *t* test)

these depots under non-isocaloric HF-diet feeding. Interestingly, the retroperitoneal depot was the one that suffered the greatest increase in weight in both sexes as an effect of HF-diet, and this can be associated with the decrease in OB-Rb mRNA expression levels. Leptin has a direct action on WAT by up-regulating fat oxidation [13] and decreasing lipogenesis [27]. However, we did not find any significant effect of the HF-diet feeding on the expression of the two fat-oxidation related genes studied (PPAR α and CPT1). The retroperitoneal is a depot with a low capacity of mitochondrial fatty acid oxidation compared with other depots (our unpublished data), so possible changes in the expression levels of these two genes may have little impact on the amount of fat stored in this depot. It is plausible that the diet-induced leptin resistance in this depot may result in alterations in the regulation of other genes (such as lipogenesis-related genes), which could explain the great increase in the weight of this fat depot associated with HF-diet feeding.

The mesenteric depot showed a clear sex-dimorphic response to HF-diet feeding concerning OB-Rb expression. In male rats, a decrease in leptin sensitivity (as evidenced by lower expression of this receptor) was associated with a decrease in fatty acid oxidation capacity (as evidenced by a decrease in PPAR α and CPT1 mRNA expression levels). Conversely, in female rats, OB-Rb mRNA levels increased under HF-diet, and this allowed the maintenance of fatty acid oxidation capacity (as suggested by the maintenance of PPAR α and CPT1 mRNA expression levels). Adipocyte-selective reduction of the OB-Rb has been associated with obesity and type 2 diabetes [7]. We have previously described that male rats fed with an HF-diet have increased serum insulin levels and obesity-linked disorders, such as fat accumulation in the liver, while female rats did not present, at least to this extent, these metabolic complications [16]. OB-Rb abundance in the different depots may be determinant for an adequate response to an HF-diet. Thus, although these results are only based on mRNA

levels, the decrease in OB-Rb levels occurring in the internal depots studied, retroperitoneal and mesenteric, in male rats may contribute to increased peripheral leptin resistance in these internal depots and hence accelerate the metabolic impact of HF-diet feeding. Unlike male rats, the results of OB-Rb mRNA expression levels in these internal depots in female rats did not evidence increased leptin resistance under HF-diet feeding, in accordance with our previously published results [16].

In the inguinal depot, we did not find significant changes concerning OB-Rb expression levels as an effect of HF-diet; however, a slight tendency to higher levels was found in male rats. In these animals, we also found increased PPAR α and CPT1 mRNA levels. The increased fatty acid oxidation capacity of this fat depot in HF-diet-fed male rats, together with the maintenance of the OB-Rb mRNA levels, may contribute to explain the relatively lower increase in the size of this fat depot compared with the other depots studied.

The mechanisms that determine this sexual dimorphism in leptin receptor under HF-diet conditions and why these effects are tissue-dependent are currently unknown. It can be speculated that steroid hormones, particularly estradiol and testosterone, which are known to regulate leptin action at the central and also at the peripheral level could play a role. In particular, testosterone has been found to reduce OB-Rb expression in Leydig cells [8], while estradiol administration to ovariectomized rats increases OB-Rb protein levels in the skeletal muscle and also affects its levels in the adipose tissue depending on the dose and the time of action [2]. Thus, changes in steroid hormone levels and/or in the relative abundance of their receptors in visceral and subcutaneous fat could account, at least in part, for this sexual dimorphism under HF-diet feeding, which seems to affect particularly the mesenteric depot. In fact, the visceral adipose tissue has been shown to be especially sensitive to changes in steroid hormones. Female rats with hormone imbalance (mimicking postmenopausal women) display early visceral obesity and age-related mimicry of metabolic syndrome [18], emphasizing the role of steroid hormones in fat metabolism and distribution.

In conclusion, these results show that changes in the OB-Rb expression levels in WAT, associated with HF-diet feeding, depend on the anatomical localization and the gender of the animals. The decreased expression of OB-Rb in internal depots in male rats under HF-diet feeding, with the resulting decrease in leptin sensitivity, may help to explain the obesity-related metabolic complications associated with visceral fat deposition. It is suggested that peripheral OB-Rb expression may have a strong impact on metabolism and may help to explain the depot-dependent response to HF-diet feeding.

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Conflict of interest statement The authors declare no conflict of interest.

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