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THE RESPONSE OF SKELETAL MUSCLE TO LEPTIN

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1. ABSTRACT

There is now compelling evidence that, in addition to signaling to the central nervous system (CNS), leptin also exerts its metabolic effects acting directly on peripheral tissues. It has been demonstrated by *in vivo* and *in vitro* studies, that leptin increases glucose and fatty acid metabolism in skeletal muscle. These direct leptin effects are supported by the presence of the long form of the leptin receptor, considered to be capable of performing intracellular signaling, in peripheral tissues, including skeletal muscle. The exposure of soleus muscle to supra-physiological leptin concentrations stimulate the activity of both the pyruvate-dehydrogenase (PDH) complex and Krebs cycle. This could be due to a direct stimulation of PDH and krebs cycle by leptin or a consequence of an indirect effect of this hormone activating the mitochondrial uncoupling process. In addition, in soleus and extensor digitorum longus (EDL) muscles, leptin and insulin had opposite effects on lipid metabolism, with leptin favoring lipid oxidation and insulin favoring lipid storage as triglycerides (TG). The leptin effects on free fatty acid (FFA) oxidation were more pronounced in soleus than in EDL. The differences in response of soleus compared with that of EDL was probably due to differences in fiber type composition and metabolic characteristics.

It has been demonstrated that leptin reduces the TG content of skeletal. When tissue TG content is severely depleted by hyperleptinemia in normal rats, there is a dramatic increase in insulin sensitivity. This lipopenic effect of leptin may protect from the development of insulin resistance and diabetes in animals. In humans, obesity is also associated with an increase in insulin resistance and the development of Type II diabetes, however, contrary to rats and mice, there is abundance of leptin, indicating a state of resistance to this hormone in humans. Future studies are necessary to investigate the reasons why lean subjects seem to respond properly to endogenous leptin while obese ones don't. The understanding of the putative direct leptin signaling pathway in skeletal muscle could be an important step towards the utilization of leptin or a leptin receptor agonist as therapeutic tools to treat obesity and its related metabolic disorders.

2. INTRODUCTION

Skeletal muscle represents approximately 40% of total body mass in non obese subjects and can account for up to 30% of resting metabolic rate (1). Additionally, muscle tissue has been identified as the primary site of insulin-stimulated glucose disposal at euglycemia (2,3) and fatty acid oxidation (4). Diseases such as obesity, type 2 diabetes, and atherosclerosis can be very much influenced by muscle metabolism (4,5). The understanding of the physiopathology of these diseases have improved due to the utilization of animal models of study. The *ob/ob* mouse is a model of obesity with multiple metabolic abnormalities resembling those of type 2 diabetes mellitus in humans (6). Recently, with the cloning of the ob gene (7) and the purification of its encoded protein, it was demonstrated that the phenotypic characteristics of *ob/ob* mice are determined by the lack of an active form of the hormone leptin (8-10). In fact, exogenous administration of recombinant leptin to *ob/ob* mice reverses the genetic phenotype, i.e. dramatically reducing body weight and improving metabolic defects inherent in these mice (8-11).

Leptin is an adipocyte-derived hormone that represents an "adipostat" signal reflecting energy stores (12). Although the primary effects of leptin appear to be

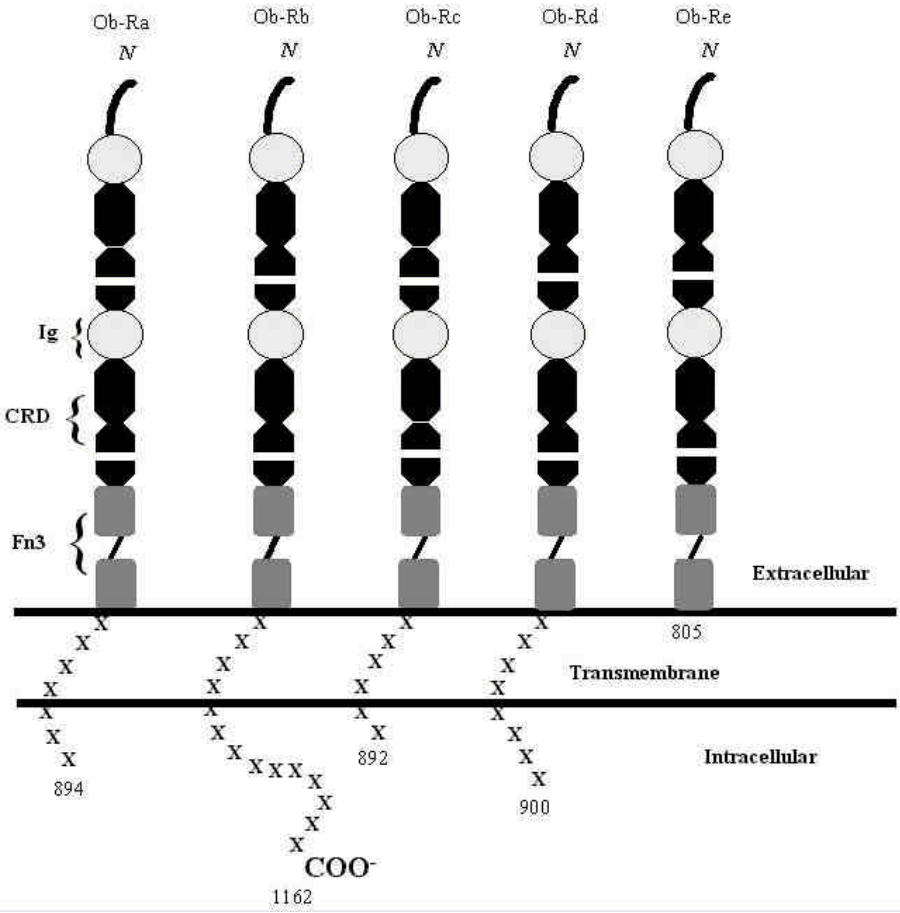

Figure 1. Leptin receptor isoforms. There are at least five different isoforms of the leptin receptor Ob-R(a-e) in mouse. All share identical extracellular ligand-bind domains but they differ at the C terminus. Four of the five have transmembrane domains but only Ob-Rb encodes all protein motifs capable of activating the Jak-Stat signal transduction pathway. The remaining isoform, Ob-Re, is truncated before the membrane-spanning domain and is secreted. Ig = Imunoglobulin domain; CRD = Citokine receptor domain; Fn3 = Fibronectin III domain.

mediated by the long form of the leptin receptor (Ob-Rb) expressed at high levels in the hypothalamus (13,14), the Ob-Rb isoform has also been detected in non-neuronal tissues (15-19), including skeletal muscle (19,20). Peripheral injection of recombinant leptin in *ob/ob* mice has been shown to reduce fat mass, food intake, hyperglycemia, and hyperinsulinemia and increase oxygen consumption and energy expenditure (8-11). Importantly, the effects on hyperglycemia, hyperinsulinemia and oxygen consumption cannot be fully explained by decreased food intake, since the lowest dosages of leptin administered to *ob/ob* mice normalized blood glucose and body temperature, but failed to reduce food intake (8-10). Furthermore, pair feeding studies (21) have pointed out that food intake does not completely account for adiposity reduction after leptin infusion, suggesting a significant metabolic regulation role for this hormone. These observations raise the possibility that leptin could exert its effects acting directly on peripheral tissues. On this review we will focus on the direct effects of leptin on skeletal muscle and its implications to glucose and fatty acid metabolism.

3. THE EXPRESSION OF LEPTIN RECEPTORS IN SKELETAL MUSCLE

The receptor for leptin was first cloned from a mouse choroid plexus cDNA expression library (13) following identification of [^{125}I] leptin binding sites in this tissue. The receptor presents considerable homology with the gp130 subunit of the IL-6 receptor and has been considered as a member of the extended class I cytokine-receptor family (13) including interleukin-6 (IL-6), the granulocyte-colony stimulating factor (G-CSF) and the leukemia-inhibitory factor (LIF) (13,22). However, the leptin receptor oligomerizes with itself but not with its closely related cytokine signal transducer gp130 (23). At the cellular level Ob-R, has been found to activate Janus Kinase (JAK) and to function as a signal transducer and activator of transcription (STAT) pathways (24).

There are at least five splice variants of the Ob-R(a-e) (25) (Figure 1). Except for the Ob-Rb, all other isoforms are considered short, possessing a truncated intracellular domain (13,19) and are considered not capable

of intracellular signaling. However, there has recently been demonstrated that a short form of the Ob-R (Ob-Ra) is capable of performing signal transduction (expression of immediate early genes, *c-fos*, *c-jun* and *jun-B*) in CHO cells added with leptin (26). Most tissues, including skeletal muscle, express 10 to 30 times more mRNA encoding the short form than the long form. Only the hypothalamus expresses more long than short form [2-fold] (19). Both the short and long Ob-R isoforms are expressed in predominantly slow-twitch or fast twitch muscles, which have metabolic diverse characteristics (27). In addition, no qualitative differences have been observed in the pattern of response ob Ob-R mRNA expression of mainly oxidative or glycolytic muscles in the pre-prandial and postprandial state (28).

4. *IN VITRO* EFFECTS OF LEPTIN ON SKELETAL MUSCLE METABOLISM

The dramatic effects of leptin on glucose and fatty acid metabolism in mice (8-10,30) and rats (29,31) and the fact that leptin receptors are present in peripheral tissues, have suggested that leptin could exert direct effects independently of signaling to the CNS. Considering that skeletal muscle is quantitatively the most important site for insulin-stimulated glucose disposal and for fatty acid oxidation, a direct effect of leptin on this tissue could play a central role in insulin resistance and obesity. Indeed, it has been demonstrated that leptin *per se* acutely raises basal glucose uptake in isolated rat (32,33) and mice (34) soleus muscles. Furthermore, leptin increases basal and insulin-stimulated glucose decarboxylation in rat muscles (33). Utilizing [1-^{14}C]- and [2-^{14}C]-pyruvate it was also found that leptin stimulates the activity of both the PDH complex and Krebs cycle in soleus muscles (33). This could be due to a direct stimulation of PDH and krebs cycle by leptin or a consequence of an indirect effect of this hormone activating the mitochondrial uncoupling process. Mitochondria uncoupling reduces the intracellular ATP/ADP ratio, which activates PDH and the enzymes of the krebs cycle (35). It has been demonstrated that daily intraperitoneal injections of leptin increase energy expenditure, metabolic rate and body temperature in mice (8,9). Until recently, the thermogenic effect of leptin was thought to be confined to brown adipose tissue, the major site of uncoupling protein (UCP-1) expression. However, the recent discovery of UCP-2 (36), a far more ubiquitously expressed protein, and UCP-3 (37), predominantly expressed in skeletal muscle, raises the possibility that other tissues may also contribute to thermogenesis. It has been reported (30,38) that hyperleptinemia up-regulates UCP-2 expression in fat tissue (epididimal, retroperitoneal and subcutaneous). In fact, the expression of UCP-2 was increased up to 2-fold in adipose tissue (white and brown) of leptin treated mice (30). It has been demonstrated that leptin up-regulates the expression of UCP-3 in skeletal muscle, while no effect was observed for UCP-2, in skeletal muscle of *ob/ob* mice treated with intraperitoneal injections of leptin (39). In addition, the activity of the UCP can be highly regulated by purine nucleotides and fatty acids (37,40). Based on this findings, and considering the high increment in glucose and pyruvate decarboxylation previously demonstrated in leptin-incubated soleus muscle (33), we can speculate that leptin may up-regulate the uncoupling activity of UCP-3 in skeletal muscle, since the 1-hour-incubation experiment was no long enough to cause significant alterations in UCP-3 expression.

Besides glucose metabolism, evidence has also been provided that leptin directly increases the conversion of [^{14}C]-oleate into $^{14}CO_2$ and opposes the lipogenic effects of insulin in muscles isolated from mice (41,42). In intact soleus and EDL muscles, leptin and insulin had opposite effects on lipid metabolism, with leptin favoring lipid oxidation and insulin favoring lipid storage in TG. Hormone-induced alterations in muscle lipid metabolism were more pronounced in soleus than in EDL. The differences in response of soleus compared with that of EDL was probably due to differences in fiber type composition and metabolic characteristics (41). Compared with EDL, soleus is highly oxidative and has higher activities of oxidative enzymes, more mitochondria, and a greater capacity to store and use lipid fuels (43).

The mechanism by which leptin affects glucose and fatty acid metabolism is skeletal muscle remains to be determined. However, there seems to be a crosstalk between the leptin and the insulin intracellular signaling pathways at the level of PI3-kinase (phosphatidylinositol-3 kinase) (44). According to data obtained from C_2C_{12} myotubes, leptin activates JAK-2 which induces tyrosine phosphorylation of IRS-2 (insulin receptor substrate) leading to activation of PI3-kinase and this is accompanied by an increase in glucose uptake (44,45). C_2C_{12} myotubes express predominantly the glucose transporter isoform GLUT-1 (glucose transporter) and it has been assumed that both the insulin and leptin effects on glucose uptake are mediated by the translocation of GLUT-1 in these cells (44). In skeletal muscle, the insulin effect on glucose uptake is mainly mediated by GLUT-4 translocation and a central role of PI3-kinase both in the signaling to GLUT-4 translocation and glycogen synthesis has been demonstrated in this tissue (46,47). Leptin-treated mice presented an increase of up to 2-fold in GLUT-4 expression, suggesting that glucose uptake and insulin sensitivity may be enhanced by chronic exposure to leptin (30). However, it is important to notice that the results of *in vitro* studies were obtained with soleus muscles being incubated in the presence of leptin for up to 1 hour (32-34). This period of time does not seem to be sufficient to induce a significant increase in GLUT-4 expression, so it is more likely that under acute *in vitro* conditions GLUT-1 and possibly GLUT-4 translocation were altered by leptin so that basal glucose uptake was facilitated in soleus muscles (32-34).

On the other hand, some *in vivo* (48,49) and *in vitro* (50-52) studies have provided evidence against a direct effect of leptin on glucose metabolism in skeletal muscle. Leptin-stimulated glucose uptake was decreased in denervated leg in both EDL and soleus relative to the intact muscle (49). However, glucose uptake in denervated soleus muscle after leptin treatment, although lower than that of intact leg, was greater than in PBS-treated controls.

According to the authors of the study, this residual increase in glucose uptake by denervated soleus could be the result of an increase in triiodothyronine (T3) levels in the leptin-treated group (49). Furthermore, no effects of short-term (50,51) and long-term (52) leptin exposure were observed on the rates of basal and insulin-stimulated 2-deoxy-glucose transport, glycogen synthesis, CO_2 production, and lactate release by incubated skeletal muscle. Differences in the experimental approach used to study the effects of leptin could underlie the diverse findings. Intact soleus muscle from recently weaned Wistar rats as well as slices of soleus muscle from adult Sprague-Dawley rats and C_2C_{12} myotubes have been studied. Age related differences in the response to leptin treatment were observed (53). Species and strains of the animals, muscle extraction procedure and source of leptin have also to be taken into account. In addition, native muscle tissue more closely resemble the physiological condition than cell culture (27).

5. THE LIPOPENIC EFFECT OF LEPTIN ON SKELETAL MUSCLE

Obesity has long been associated with insulin resistance, and free fatty acids (FFA) are widely viewed as the main etiologic factor (54). The prevailing concept is based on the Randle glucose-fatty acid cycle (55,56) and implicates a direct inhibitory effect of FFA from the plasma upon glycolysis, glucose oxidation and glucose uptake in target tissues as skeletal muscle (57-62). Originally, it was believed that FFA from the plasma were responsible for the insulin resistance of obesity; however, the intracellular amount of FFA acid, rather than the rapidly changing levels in plasma may exert a more important influence. It has been demonstrated that the plasma FFA taken up by skeletal muscle cells are first esterified to TG, stored within skeletal muscle cells, and subsequently retrieved and oxidized (63). This concept of a TG pool in skeletal muscle may apply to other tissues as well (54).

It's been argued that when this putative intracellular TG pool exceeds a certain amount, it alters function within muscle cells and somehow causes insulin resistance in animals and humans (4,55,56). Recently, it was demonstrated that hyperleptinemic male Wistar rats, induced by adenovirus gene transfer, present a profound reduction in muscle TG content (64). The TG content of skeletal muscle extracted from hiperleptinemic rats was 8% of the free feeding control rats and 25% of pairfed controls (64). When tissue TG content is severely depleted by hyperleptinemia in normal rats, there is a dramatic increase in insulin sensitivity (31,64,65). Whether this lipopenic effects of leptin is due to a direct effect of the hormone or mediated by the CNS is still under debate. However, it has demonstrated in rats that the transport of leptin across the blood-brain barrier is saturated at plasma leptin levels between 4 and 15 ng/ml (66). Treatment of the high-fat-fed rats (60% fat for 8 days) with adenovirus coding for leptin raises plasma leptin levels up to 60 ng/ml without changing CART mRNA (cocaine- and amphetamine-regulated transcript) or food intake, indicating that leptin action on hypothalamus had not been increased. Nevertheless, their body fat declined 36%, suggesting that an extra-hypothalamic mechanism was responsible (66). This is in agreement with in vitro studies showing that skeletal muscle increases its rate of glucose (33) and fatty acid (41,42) oxidatioin in the presence of supra-physiological leptin concentrations (100 to 10000 ng/ml). The leptin concentration used is comparable to the relative concentration of insulin required to elicit a maximal response in incubated skeletal muscle (41). Concentrations closer to the physiological range (5-10 ng/ml) have been reported to be sufficient to provoke direct effects on glucose and fatty acid metabolism in isolated adipocytes (67,68) and hepatocytes (69), but compared with perfused muscle or cultured cells, the muscle fiber architecture may not permit *in vitro* optimal exposure of receptors to hormones.

6. HUMAN STUDIES

Exogenous leptin administration results in a loss of body fat in animals (8-10,21), and very few humans who have a genetic deficiency of leptin exhibit extreme obesity (70). In humans, serum leptin levels and body fat are positively correlated (71). Leptin concentrations in the cerebrospinal fluid increase with body fat (72-74) but are generally 2 orders of magnitude lower than serum concentrations. The higher serum leptin concentration in obese subjects may suggest that exogenous leptin administration would be ineffective in decreasing adiposity (72). To the best of the authors' knowledge, so far only one human trial has been carried out and the results recently published show very modest changes in body weight (-7.1 kg) after 6 months of subcutaneous leptin injection in obese subjects (75). However, it is worth noting that the group receiving the highest amount of leptin (0,30 mg/kg per day) elicited a progressive and constant reduction in body weight throughout the 24-week-treatment period, and reached serum leptin concentrations as high as 667.0 ng/ml, while the placebo group did not go higher than 25 ng/ml (75). Based on these results, and also on information from *in vivo* (30,64-66) and *in vitro* (32,33,41,42) animal studies, we can speculate that under this extremely high leptin concentrations, direct effects of the hormone on skeletal muscle take place and become responsible for the increased energy expenditure that provoked this progressive body fat reduction in obese humans injected with leptin. Unfortunately, no data was provided regarding the response of different peripheral tissues to the leptin treatment and further human studies are necessary to answer questions regarding the direct effects of supra-physiological leptin concentrations on skeletal muscle. This may not be relevant to the physiology of leptin but is important for the pharmacological approach of obesity and other related metabolic disorders.

7. PERSPECTIVE

Skeletal muscle plays an important role in FFA and TG clearance as well in glucose and whole body fat metabolism. In intact rat and mouse muscle, leptin and insulin exert opposite metabolic effects, with leptin favoring substrate oxidation and inhibiting insulin-stimulated glucose and FFA storage in TG (Figure 2).

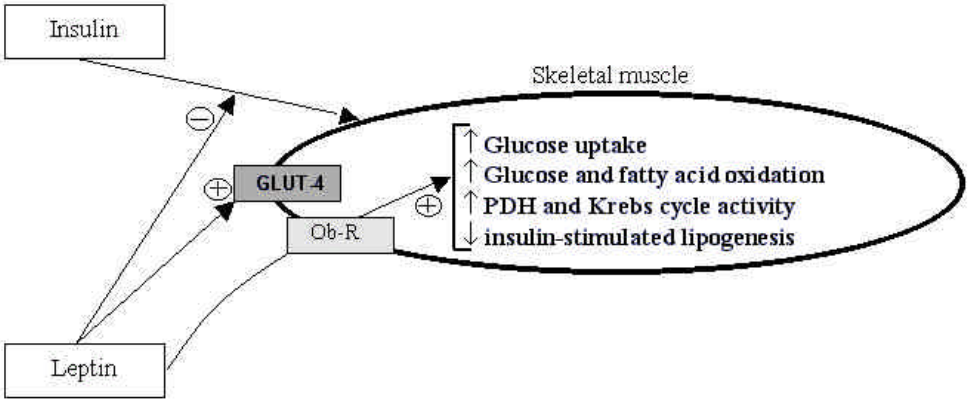

Figure 2. Schematic representation of the interaction of leptin and insulin and its effects on skeletal muscle metabolism. ↑ increase, ↓ decrease, + stimulation, – inhibition. Ob-R, leptin receptor; PDH, pyruvate-dehydrogenase complex; GLUT-4, glucose transporter.

Therefore, skeletal muscle could be considered critical in mediating the effects of leptin on fuel homeostasis, weight loss and obesity. Both obesity and type II diabetes are associated with decreased fatty acid oxidation and increased concentrations of muscle triacylglycerol and diacylglycerol (2-5,76). However, while a leptin-induced lipopenic effect is clearly observed in animal models of obesity and diabetes, in humans a similar effect is yet to be demonstrated. In fact, in obese humans, high levels of circulating leptin have been reported (77,78) indicating a state of central and/or peripheral resistance to this hormone (72-74). Future studies are necessary to investigate the reasons why lean subjects seem to respond properly to endogenous leptin while obese ones don't. The understanding of the direct leptin signaling pathway in skeletal muscle could be an important step towards the utilization of leptin or a leptin receptor agonist as therapeutic tools to treat obesity and its related metabolic disorders.

8. ACKNOWLEDGMENT

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