Failure of leptin to affect basal and insulin-stimulated glucose metabolism of rat skeletal muscle in vitro

C. Fürnsinn, B. Brunmair, R. Furtmüller, M. Roden, R. Englisch, W. Waldhäusl

Department of Medicine III, Division of Endocrinology and Metabolism, University of Vienna, Vienna, Austria.

Summary Studies on different isolated tissues have provided evidence that leptin may directly modulate cellular glucose handling. The present study was performed to elucidate leptin's action on basal and insulin-stimulated glucose metabolism in native muscle tissue, which under physiological circumstances is the quantitatively most important target tissue of insulin. Isolated rat soleus muscle strips were incubated for 1 h in the absence or presence of leptin (0, 1, 10, or 100 nmol/l) under basal or insulin-stimulated conditions (10 nmol/l). No effects of leptin were found on the rates of ^3H -2-deoxy-glucose transport (basal: control, 314 ± 14 ; 1 nmol/l leptin, 320 ± 17 ; 10 nmol/l leptin, 314 ± 13 ; 100 nmol/l leptin, 322 ± 16 ; insulin-stimulated: control, 690 ± 33 ; 1 nmol/l leptin, 691 ± 29 ; 10 nmol/l leptin, 665 ± 26 ; 100 nmol/l leptin, 664 ± 27 ; $\text{cpm} \cdot \text{mg}^{-1} \cdot \text{h}^{-1}$; NS vs respective control) and on net glucose incorporation into glycogen (basal: control, 1.75 ± 0.18 ; 1 nmol/l leptin, 2.01 ± 0.13 ; 10 nmol/l lep-

tin, 1.92 ± 0.11 ; 100 nmol/l leptin, 1.81 ± 0.13 ; insulin-stimulated: control, 5.98 ± 0.40 ; 1 nmol/l leptin, 5.93 ± 0.30 ; 10 nmol/l leptin, 5.46 ± 0.25 ; 100 nmol/l leptin, 5.85 ± 0.30 ; $\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$; NS vs respective control). In parallel, leptin failed to affect rates of aerobic and anaerobic glycolysis as well as muscle glycogen content. Further experiments revealed that the inability of leptin to directly affect muscle glucose handling prevailed independently of muscle fiber type (soleus and epitrochlearis muscle), of ambient insulin concentrations (0–30 nmol/l), and of leptin exposure time (1 h or 6 h). Thus, our findings fail to support speculations about a physiological role of direct insulin-mimetic or insulin-desensitizing effects of leptin on skeletal muscle tissue. [Diabetologia (1998) 41: 524–529]

Keywords Leptin, insulin, muscle, glucose metabolism, glucose transport, glycogen synthesis, glycolysis.

The peptide leptin is produced and released by adipocytes and exerts regulatory effects on food intake and energy expenditure [1–4]. Elevated circulating serum leptin concentrations prevail in association with increased percentage of body fat and obviously function as a humoral signal to the central nervous system triggering satiety and a decrease in food consumption [1, 5, 6]. Rats chronically treated with leptin exhibit a distinct loss of discernible body fat and markedly decreased triglyceride content of various tissues [7, 8].

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Corresponding author: C. Fürnsinn, Ph.D., Department of Medicine III, Division of Endocrinology and Metabolism, Währinger Gürtel 18–20, A-1090 Vienna, Austria

This effect is related, besides reduced food intake, to increased basal metabolic rate with selective promotion of fat metabolism [2, 3, 7, 8]. Beside the brain, a variety of other tissues express specific receptors for leptin including liver, adipose tissue, skeletal muscle, and pancreatic islets [9–12], and recent evidence suggests that in addition to indirect action via the central nervous system leptin may affect fuel metabolism by direct interaction with various other tissues [7, 11–18].

Direct leptin action on isolated pancreatic rat islets includes triglyceride depletion [7] as well as distinct inhibition of glucose-stimulated insulin release [11, 13]. Furthermore, potent inhibition by leptin of insulin's action on lipid and glucose metabolism has been demonstrated in isolated rat adipocytes [14],

and in isolated cells of hepatic origin, acute leptin exposure has been shown to modulate early steps in intracellular insulin signal transduction [15]. With regard to glucose metabolism, however, direct leptin action on the quantitatively most important target tissue of insulin, i.e. skeletal muscle [19], is not yet fully clarified. Under basal conditions *in vivo*, leptin was found to distinctly stimulate whole body glucose turnover and muscle glucose uptake in mice [20], while no evidence for increased glucose turnover was observed in leptin-infused rats [21]. With regard to insulin-stimulated whole body glucose disposal, a stimulatory effect of leptin was seen only in one of two studies [21, 22]. *In vitro*, leptin failed to directly affect glucose metabolism of isolated skeletal muscle from healthy mice [12, 17], but inhibited glycogen synthesis in muscle specimens prepared from leptin-deficient obese *ob/ob* mice [12]. In contrast to native muscle tissue, leptin stimulated glucose transport and glycogen synthesis in cultured C_2C_{12} myotubes suggesting an insulin-mimetic rather than insulin-desensitizing leptin action on myotube glucose metabolism [18].

The true potential of leptin to affect muscle glucose metabolism is therefore not yet clarified and the present study intends to further elucidate the direct action of leptin on skeletal muscle glucose metabolism. Freshly isolated rat muscle specimens were incubated in the absence or presence of leptin, and basal and insulin-stimulated rates of glucose transport, glycogen synthesis, and glycolysis were determined.

Materials and methods

Rats. Male Sprague-Dawley rats purchased from the breeding facilities of the University of Vienna (Himberg, Austria) were used and all experiments were performed according to local law and to the principles of laboratory animal care. Rats were kept at an artificial 12 h light/12 h dark cycle at constant room temperature and were used at fasted body weights of approximately 140 g. Conventional laboratory diet and tap water were provided *ad libitum* until the evening before killing, when only food was withdrawn. Rats were killed by cervical dislocation between 08.30 and 09.30 hours.

Leptin. Recombinant mouse leptin was generously provided by Eli Lilly (Indianapolis, Ind., USA) and biological activity was confirmed by a 41 % decrease in cumulative 24 h food intake after a single intraperitoneal injection of 125 µg leptin in *ob/ob* mice (g food consumed: control, $n = 7$, 5.69 ± 0.37 ; vs leptin, $n = 5$, 3.38 ± 0.54 ; $p < 0.01$ by unpaired Student's *t*-test).

Incubation procedures, short-term exposure. Immediately after animals were killed, epitrochlearis muscles (~20 mg each) or two longitudinal strips of each soleus muscle (~25 mg each) were prepared, weighed, and tied under tension on stainless steel clips as previously described [23]. Muscles were immediately put into 25 ml Erlenmeyer flasks coated with BlueSlick solution (Serva, Heidelberg, Germany), which were placed

Fig. 1A–E. Effects of various leptin concentrations on muscle glucose metabolism. Basal and insulin-stimulated (0 and 10 nmol/l insulin) rates of ^3H -2-deoxy-glucose transport (**A**), glucose incorporation into glycogen (**B**), aerobic glycolysis (CO_2 production; **C**), and anaerobic glycolysis (lactate release; **D**) in isolated soleus muscle strips from Sprague-Dawley rats as exposed to 0, 1, 10, or 100 nmol/l leptin; glycogen content as determined after incubation (**E**); means $\pm$ SEM; $n = 12$ –18 each; NS for presence vs absence of leptin

into a shaking water bath (1 specimen/flask; 37°C ; 130 cycles/min). Each flask contained 3 ml Krebs-Henseleit buffer solution (pH 7.35) supplemented with 1 % (wt/vol) bovine serum albumin and 5.5 mmol/l glucose. An atmosphere of 95 % O_2 :5 % CO_2 was continuously maintained within the flasks.

After preincubation for 30 min (i.e. equilibration period), muscles were immediately transferred to another set of flasks and incubated in 3 ml of identical buffer solution containing additional tracer amounts of D-[U- ^{14}C]glucose or, alternatively, 2-deoxy-D-[2,6- ^3H]glucose plus [U- ^{14}C]sucrose (all from Amersham, Amersham, UK). Where indicated, the incubation

Fig. 2. Interaction of short-term leptin exposure with skeletal muscle glucose metabolism. Effects of short-term leptin exposure (1 h, 50 nmol/l) on insulin dose-response curves (0, 0.3, 3, and 30 nmol/l insulin); rates of ^3H -2-deoxy-glucose transport (upper graphs) and glucose incorporation into glycogen (lower graphs) in isolated soleus muscle (left, $n = 6$ each) and epitrochlearis muscle (right; $n = 3$ –6 each) from Sprague-Dawley rats; means $\pm$ SEM; NS for presence vs absence of leptin

medium contained recombinant mouse leptin (1, 10, 50, or 100 nmol/l) and/or human insulin (Actrapid; Novo, Bagsvaerd, Denmark; 0.3, 3, 10, or 30 nmol/l). After incubation for 60 min, muscles were quickly removed, blotted, and frozen in liquid nitrogen. Later, muscle strips were lysed in 1 mol/l KOH at 70°C, the lysate was then employed for further analytical procedures as described below.

Incubation procedures, long-term exposure. The preincubation procedure was started as for acute exposure experiments, except that muscles were put into coated 50 ml-flasks containing 20 ml cell culture medium 199 (Sigma, Cat. No. M-4530; pH 7.35; 6 specimens/flask) additionally supplemented with 1% (wt/vol) bovine serum albumin, 5 mmol/l HEPES, 25000 U/l penicillin G, 25 mg/l streptomycin, and where indicated 50 nmol/l leptin. After preincubation for 5 h, muscles were immediately transferred into a set of 25 ml flasks (1 specimen/flask) and incubated for 1 h in 3 ml of identical buffer solution containing 0, 3, or 30 nmol/l insulin. Tracer addition and all further procedures were as described for short-term exposure experiments. Muscle preparations have been demonstrated previously to remain viable and insulin-responsive up to 24 h under these experimental conditions [24].

Analytical procedures. All analytical procedures were performed as described previously [23, 25]. In short, the transport rate of 2-deoxy-D-[2,6- ^3H]glucose, a glucose analogue which does not enter glycolysis and hence accumulates within the cell, was determined employing [^{14}C]sucrose as an extracellular space marker [25]. Net rates of glucose incorporation into glycogen are referred to as glycogen synthesis and were deter-

Fig. 3. Interaction of long-term leptin exposure with skeletal muscle glucose metabolism. Effects of long-term leptin exposure (6 h, 50 nmol/l) on insulin dose-response curves (0, 3, and 30 nmol/l insulin); rates of ^3H -2-deoxy-glucose transport (upper graphs) and glucose incorporation into glycogen (lower graphs) in isolated soleus muscle (left, $n = 6$ –8 each) and epitrochlearis muscle (right; $n = 5$ –10 each) from Sprague-Dawley rats; means $\pm$ SEM; NS for presence vs absence of leptin

mined by measuring conversion of [^{14}C]glucose to [^{14}C]glycogen [23, 25]. Rates of CO_2 production were calculated from conversion of [^{14}C]glucose into $^{14}\text{CO}_2$, which was trapped with solution containing methanol and phenethylamine (1:1) [25]. Rates of lactate release were determined by measuring lactate accumulated in the incubation buffer during the experiment [25]. For the determination of muscle glycogen content, glycogen in the muscle lysate was completely degraded to glucose with amyloglucosidase [25]. Glucose was then measured enzymatically using a commercial kit from Human (Taususstein, Germany).

Statistical analysis. All data are presented as means $\pm$ SEM and leptin action was evaluated by intraindividual comparison of muscle specimens obtained from the same rat. Paired Student's *t*-test was employed for comparison of two groups, while multiple comparisons compared to the same control were performed according to the method of Dunnett [26]. A *p* value less than 0.05 was considered significant.

Results

Acute exposure of freshly isolated soleus muscle strips to leptin as added to the incubation buffer at concentrations of 1, 10, or 100 nmol/l did not exert any significant effect on the determined parameters of basal or insulin-stimulated glucose metabolism, which included rates of ^3H -2-deoxy-glucose trans-

Table 1. Interaction of short-term leptin exposure with skeletal muscle glucose metabolism

Insulin (nmol/l)		0		0.3		3		30	
Leptin (nmol/l)		0	50	0	50	0	50	0	50
<i>Soleus muscle</i>									
CO ₂ Release, (nmol glucose · g ⁻¹ · h ⁻¹)	<i>n</i>	6 344 ± 17	6 347 ± 48	6 359 ± 34	6 325 ± 27	6 261 ± 20	6 302 ± 18	6 350 ± 37	6 311 ± 10
Lactate Release, (μmol · g ⁻¹ · h ⁻¹)	<i>n</i>	6 8.0 ± 0.8	6 6.3 ± 0.1	6 7.0 ± 0.5	6 6.8 ± 0.5	6 9.3 ± 0.5	6 9.7 ± 0.6	6 11.3 ± 0.7	6 12.1 ± 1.0
Glycogen Content, (μmol glucosyl units · g ⁻¹)	<i>n</i>	6 9.8 ± 0.3	6 10.2 ± 0.5	6 10.3 ± 0.7	6 10.1 ± 0.6	6 12.2 ± 0.9	6 11.6 ± 0.4	6 11.9 ± 1.0	6 12.6 ± 1.3
<i>Epitrochlearis muscle</i>									
CO ₂ Release, (nmol glucose · g ⁻¹ · h ⁻¹)	<i>n</i>	5 582 ± 100	5 413 ± 42	5 529 ± 82	5 584 ± 139	5 892 ± 212	5 734 ± 234	5 1068 ± 163	5 950 ± 256
Lactate Release, (μmol · g ⁻¹ · h ⁻¹)	<i>n</i>	6 9.7 ± 1.7	6 9.9 ± 0.9	6 10.6 ± 0.7	6 11.4 ± 1.7	6 13.5 ± 1.4	6 13.5 ± 1.7	3 16.1 ± 2.5	3 15.8 ± 2.6
Glycogen Content, (μmol glucosyl units · g ⁻¹)	<i>n</i>	5 10.8 ± 0.8	5 11.2 ± 0.9	6 12.0 ± 1.2	6 12.0 ± 0.6	5 12.4 ± 1.6	5 11.0 ± 1.1	6 12.4 ± 0.5	6 13.1 ± 1.3

Effects of short-term leptin exposure (1 h, 50 nmol/l) on insulin dose-response curves (0, 0.3, 3, and 30 nmol/l insulin); rates of CO₂ production and lactate release in isolated soleus muscle

and epitrochlearis muscle from Sprague-Dawley rats; glycogen content as determined after incubation; means ± SEM; NS for presence vs absence of leptin

port, glycogen synthesis, aerobic glycolysis (i.e. CO₂ production), anaerobic glycolysis (i.e. lactate release), and glycogen content as determined after the experiment (Fig. 1).

Likewise, 50 nmol/l leptin failed to affect glucose metabolism in isolated soleus muscle as well as in isolated epitrochlearis muscle exposed to insulin concentrations ranging from 0.3 to 30 nmol/l (Fig. 2, Table 1) or exposed to leptin for a prolonged time period of 6 h (Fig. 3, Table 2).

Discussion

Exposure to leptin in vitro has been shown to decrease the phosphorylation/activation of the insulin receptor subunit-β and of insulin receptor substrate-1 in isolated cells of hepatic origin as well as in rat-1 fibroblasts overexpressing the human insulin receptor [15, 16] and more recently, a very distinct potential of leptin to inhibit the metabolic effects of insulin was reported for isolated adipocytes [14]. Based on these findings, a role for leptin in the natural development of obesity-associated insulin resistance was hypothesized [14–16, 18].

The quantitatively most important target tissue for insulin with regard to glucose metabolism, however, is skeletal muscle [19] and the present study was performed to elucidate the potential role of direct interaction of leptin with skeletal muscle glucose handling. Muscle preparations employed in this study re-

sponded well to insulin, but no effects of short- or long-term leptin exposure were observed on the rates of basal and insulin-stimulated 2-deoxy-glucose transport, glycogen synthesis, CO₂ production, and lactate release. Our experiments were designed to cover basal and partially as well as maximally insulin-stimulated conditions, but leptin neither influenced insulin-independent glucose metabolism nor did it affect insulin sensitivity or insulin responsiveness. Because leptin's potential to modulate glucose metabolism may depend on muscle fiber type, we further tested leptin action on muscle specimens of both soleus muscle (red muscle, mainly type I fibers) and epitrochlearis muscle (white muscle, mainly type II fibers) [27]. A leptin concentration of 50 nmol/l, which has been found to blunt insulin's stimulatory effect on 2-deoxy-glucose transport in adipocytes with a 40 % decrease observed after 30 min and nearly complete abolition after 5 h [14], lacked any influence on muscle glucose metabolism independent of muscle fiber type and prevailing insulin concentration.

It must be emphasized that the absence of direct leptin action on isolated native muscle does not exclude an indirect effect of leptin on muscle glucose handling in vivo as potentially mediated via leptin interaction with the central nervous system [1, 6, 20], liver [15, 21], adipose tissue [14], or pancreas [7, 11, 13]. Recently, a nervous mode of action was indicated in that leptin-stimulated glucose uptake into skeletal muscle was found to be abolished by muscle denerva-

Table 2. Interaction of long-term leptin exposure with skeletal muscle glucose metabolism

Insulin (nmol/l)		0		3		30	
Leptin (nmol/l)		0		50		0	
<i>Soleus muscle</i>							
CO ₂ Release, (nmol glucose · g ⁻¹ · h ⁻¹)	<i>n</i>	8 693 ± 62	8 644 ± 64	6 969 ± 117	6 793 ± 44	6 1081 ± 71	6 1012 ± 45
Lactate Release, (μmol · g ⁻¹ · h ⁻¹)	<i>n</i>	6 11.6 ± 0.8	6 10.9 ± 0.6	6 13.8 ± 0.9	6 15.2 ± 1.0	6 13.9 ± 1.1	6 13.0 ± 0.5
Glycogen Content, (μmol glucosyl units · g ⁻¹)	<i>n</i>	8 11.0 ± 0.7	8 10.5 ± 0.5	6 12.1 ± 0.6	6 10.7 ± 0.4	6 13.7 ± 1.1	6 12.4 ± 1.5
<i>Epitrochlearis muscle</i>							
CO ₂ Release, (nmol glucose · g ⁻¹ · h ⁻¹)	<i>n</i>	4 1166 ± 139	4 1085 ± 82	6 1190 ± 151	6 1136 ± 189	10 1216 ± 101	10 1343 ± 102
Lactate Release, (μmol · g ⁻¹ · h ⁻¹)	<i>n</i>	8 12.4 ± 1.1	8 13.2 ± 1.1	6 13.9 ± 1.1	6 13.0 ± 0.5	5 15.0 ± 1.6	5 14.0 ± 1.4
Glycogen Content, (μmol glucosyl units · g ⁻¹)	<i>n</i>	5 8.0 ± 1.6	5 7.1 ± 1.1	6 11.9 ± 0.6	6 13.7 ± 2.0	10 10.3 ± 1.2	10 11.0 ± 1.0

Effects of long-term leptin exposure (6 h, 50 nmol/l) on insulin dose-response curves (0, 3, and 30 nmol/l insulin); rates of CO₂ production and lactate release in isolated soleus muscle and

epitrochlearis muscle from Sprague-Dawley rats; glycogen content as determined after incubation; means ± SEM; NS for presence vs absence of leptin

tion [20]. Taken together, our results fail to provide any evidence for direct effects of leptin on healthy rat muscle glucose handling, in line with the recently reported lack of leptin-dependent modulation of glucose metabolism in isolated mouse soleus and extensor digitorum longus muscles [12, 17].

Contrary to our observations in native muscle tissue, exposure of C₂C₁₂ myotubes to a leptin concentration of only 1 ng/ml (approximately 0.06 nmol/l) for 1 h insulin-independently stimulated the rates of glucose transport (84 % of the insulin effect) and glycogen synthesis (38 % of the insulin effect), while no effect was seen in the presence of insulin [18]. Failure to document any insulin-mimetic leptin action in isolated rat soleus or epitrochlearis muscles therefore suggests major differences in the interaction of leptin with C₂C₁₂ myotubes compared to native muscle tissue, whereby the experimental approach used in our study is more closely related to the physiological situation than myotube culture.

In conclusion, leptin exposure for 1 h and for 6 h failed to directly modulate rates of basal and insulin-stimulated glucose metabolism in isolated rat skeletal muscles of different fiber types. Therefore, if they take place under physiological conditions, short-term leptin-dependent changes in muscle glucose handling must depend on an indirect mode of action involving the central nervous system, liver, pancreatic islets, and/or adipose tissue.

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