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Leptin increases circulating glucose, insulin and glucagon via sympathetic neural activation in fasted mice

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OBJECTIVE: A number of recent studies suggest that leptin has effects on glucose metabolism and pancreatic hormone secretion. Therefore, the effect of leptin administration on circulating glucose, insulin and glucagon in fed and fasted mice was investigated. The potential contribution of the sympathetic nervous system to the effects of leptin was also examined.
DESIGN: Recombinant human or murine leptin was administered intraperitoneally (300 μg/mouse per 12 h over 24 h) to fed or fasted, normal or chemically sympathectomized NMRI mice. Blood samples were collected at baseline and after 24 h.
MEASUREMENTS: Plasma concentrations of glucose, insulin and glucagon.
RESULTS: In the fed state ($n=24$), leptin administration did not affect glucose, insulin or glucagon concentrations after 24 h. Fasting ($n=24$) reduced body weight by 2.2±0.4 g, plasma glucose by 3.7±0.4 mmol/l, plasma insulin by 138±35 pmol/l, and plasma glucagon by 32±7 pg/ml. In fasted mice, human leptin ($n=24$) increased plasma glucose by 1.5±0.2 mmol/l ($P=0.041$), plasma insulin by 95±22 pmol/l ($P=0.018$), and plasma glucagon by 16±3 pg/ml ($P=0.025$), relative to saline-injected control animals. Murine leptin exerted similar stimulating effects on circulating glucose (+1.0±0.2 mmol/l, $P=0.046$), insulin (+58±17 pmol/l, $P=0.038$) and glucagon (+24±9 pg/ml, $P=0.018$) as human leptin in fasted mice ($n=12$) with no significant effect in fed mice ($n=12$). Human leptin did not affect circulating glucose, insulin or glucagon in fasted mice after chemical sympathectomy with 6-hydroxydopamine (40 mg/kg iv 48 h prior to fasting; $n=12$).
CONCLUSION: Leptin increases circulating glucose, insulin and glucagon in 24 h fasted mice by a mechanism requiring intact sympathetic nerves.

Keywords: leptin; insulin secretion; glucagon secretion; glucose; NMRI; mice

Introduction

Leptin is released from the adipocytes in relation to body fat content and recent energy balance.[1–4] Leptin administration inhibits food intake and increases the energy expenditure resulting in reduction in body weight.[1–4] Leptin gene transfection[5] or continuous leptin infusion[6] leads to marked loss of adipose stores. Leptin has also been demonstrated to exhibit metabolic actions. Studies in *ob/ob* mice have demonstrated reductions of circulating glucose and insulin in response to leptin administration that are independent of the reduction in food intake.[7] Circulating leptin concentrations are decreased by fasting[8–10] or energy restriction[11] and increase rapidly after refeeding.[10] The changes of leptin production during positive and negative energy balance may be mediated by insulin-induced alterations of adipocyte glucose uptake and metabolism.[12,13] Administration of leptin during 48 h fasting in mice has been shown to attenuate the fasting-induced changes in glucocorticoids and thyroid hormones.[8] Hence, it is possible that leptin provides the brain with a signal of sufficient peripheral energy reserves, and when these reserves are insufficient, lowered leptin concentrations are a signal that initiates neuroendocrine responses to restore energy balance. The autonomic nervous system may be involved in mediation of these responses; recent studies suggest that leptin activates the sympathetic nervous system.[14–16]

In the present study, we investigated the effects of pharmacological doses of recombinant human and murine leptin on the changes of plasma glucose and the pancreatic hormones, insulin and glucagon during fasting in mice. To determine whether activation of the sympathetic nerves may be involved in the observed effects, we also examined the influence of leptin on glucose, insulin and glucagon responses to fasting in mice treated with 6-hydroxy-dopamine (6-OHDA), inducing selective chemical sympathectomy.[17] Pancreatic islets of mice treated with 6-OHDA are devoid of sympathetic innervation at 48 h after administration of 6-OHDA as evident by

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fluorescent demonstration of catecholamines[18] and immunohistochemical visualization of the noradrenergic nerve marker, tyrosine hydroxylase.[19]

Methods

Animals

NMRI mice (Bomholdtgaard Breeding and Research Center, Ry, Denmark), weighing 24–27 g were used. The animals were fed a standard pellet diet (Lactamin AB, Stockholm, Sweden) and tap water *ad libitum.*

Experiments

In the first experimental series, a baseline blood sample (250 µl) was taken from the retrobulbar intraorbital capillary plexus. The mice were then either fasted on grids or had free access to their normal food for 24 h. Two intraperitoneal injections of either saline or recombinant human or murine leptin (Amgen Inc., Thousand Oaks, CA) were given at a dose of 300 µg per mouse (volume 250 µl/mouse). The first injection was administered immediately after the initial blood sample was collected and the second was given 12 h later. After 24 h, a second blood sample was taken. Body weight was determined before the initial and final blood sample. In a second experimental series, mice were injected intravenously *via* the tail vein with 6-OHDA hydrochloride (40 mg/kg dissolved in 10 mg/ml ascorbic acid; Sigma Chemical Co, St Louis, Mo, USA; volume 250 µl) or vehicle. Two days later, the mice were subjected to the protocol as described above.

Analysis

Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labelled human insulin as tracer and rat insulin as standard (Linco Research, St. Charles, MO, USA). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficient of variation is less than 3%. Plasma glucagon was determined radioimmunochemically with the use of a guinea pig antiglucagon antibody specific for pancreatic glucagon, ^{125}I-labelled-glucagon as tracer, and glucagon standard (Linco). Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 7.5 pg/ml and the coefficient of variation is less than 9%. Plasma glucose was determined with the glucose oxidase method.

Statistics

Data are presented as means ± sem. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons for the differences between the different groups of mice within each experimental series were performed by ANOVA with Bonferroni *post-hoc* analysis.

Results

Effects of human leptin in fed and fasted normal mice

During 24 h fasting ($n = 24$), body weight, plasma glucose, plasma insulin and plasma glucagon were all reduced, whereas in the fed controls ($n = 24$), these parameters did not change significantly during the 24 h study period. In the fed state, leptin administration ($n = 24$) did not significantly alter circulating levels of glucose, insulin or glucagon. In contrast, in fasting animals, leptin administration ($n = 24$) increased circulating levels of glucose, insulin and glucagon. Figure 1 illustrates the reduction in the parameters in fasting animals with or without administration of leptin. Human leptin attenuated the fasting-induced reductions of glucose (by 49%), insulin (by 74%), and glucagon (by 97%). (see Table 1, Figure 1.)

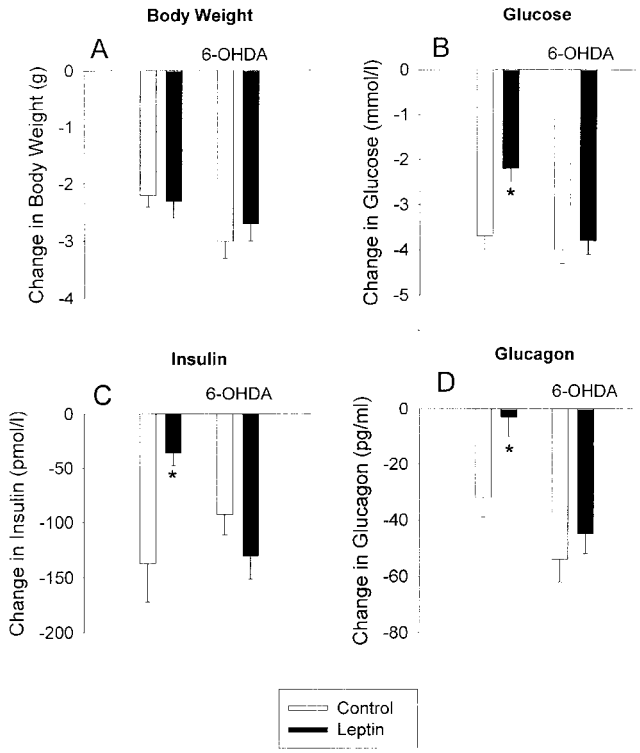

Figure 1 Changes in body weight (A) and plasma levels of glucose (B), insulin (C) and glucagon (D) after 24 h fasting in normal or chemically sympathectomized mice without or with administration of recombinant human leptin (2×300 µg/mouse). Chemical sympathectomy was induced by 6-OHDA (40 mg/kg) given 48 h before the experiments. Means ± SEM are shown. Asterisks indicate probability level of random difference between the groups. $^*P < 0.05$. There were 24 animals in each group of normal mice and 12 animals in each group of sympathectomized mice.

Table 1 Body weight and plasma levels of glucose, insulin and glucagon before and after 24 h in mice subjected to fasting or continuous feeding with or without administration of recombinant human leptin (300 μg i.p.) administered after the initial blood sampling and after 12 h

	Fed mice given saline (n = 24)	*Fed mice given human leptin (n = 24)*	*Fasted mice given saline (n = 24)*	*Fasted mice given human leptin (n = 24)*
Body weight (g)				
Initial	27.5 ± 0.8	27.8 ± 1.1	27.3 ± 0.9	26.9 ± 1.0
After 24 h	27.6 ± 0.7	27.0 ± 1.1	25.1 ± 0.6**	24.6 ± 0.9**
Plasma glucose (mmol/l)				
Initial	7.8 ± 0.2	8.2 ± 0.2	8.0 ± 0.3	8.0 ± 0.2
After 24 h	8.1 ± 0.2	8.5 ± 0.2	4.3 ± 0.3**	5.8 ± 0.3**[a]
Plasma insulin (pmol/l)				
Initial	151 ± 21	204 ± 37	214 ± 39	208 ± 13
After 24 h	213 ± 31	196 ± 36	76 ± 16**	171 ± 11**[a]
Plasma glucagon (pg/ml)				
Initial	54 ± 5	54 ± 6	63 ± 7	50 ± 6
After 24 h	70 ± 6	73 ± 9	31 ± 3*	47 ± 4[a]

Asterisks indicate probability level of random difference between the initial sample and that taken at 24 h by means of ANOVA with Bonferroni *post-hoc* analysis, *$P < 0.05$, **$P < 0.01$. [a]indicates probability level of random difference between fasted animals given saline *vs* fasted animals given human leptin of $P < 0.05$.
Means ± SEM are shown.

Effects of murine leptin in fed and fasted normal mice
Similar to human leptin, murine leptin did not affect plasma glucose, insulin or glucagon after 24 h in the fed state ($n = 12$), whereas administration of murine leptin during the fasting period ($n = 12$) increased circulating concentrations of glucose, insulin and glucagon relative to saline-treated control mice ($n = 12$). Therefore, murine leptin attenuated the fasting-induced reductions of glucose (by 25%), insulin (by 56%), and glucagon (by 82%) (see Table 2).

Fed and fasted chemically sympathectomized mice
In chemically sympathectomized mice, body weight as well as plasma glucose, insulin and glucagon were reduced by 24 h fasting ($n = 12$), whereas in continuously fed mice, these parameters were unchanged ($n = 12$). Administration of human leptin did not significantly affect glucose, insulin, or glucagon in sympathectomized mice either under fasting ($n = 12$) or fed ($n = 12$) conditions. Figure 1 illustrates the reduction in the parameters in sympathectomized fasting animals with or without administration of leptin. It is seen that human leptin did not affect the fasting-induced reduction in glucose, insulin or glucagon (see also Table 3).

Discussion

Leptin is produced in the adipocyte in relation to the degree of adiposity and recent energy balance. Leptin acts in the brain to inhibit food intake and increase energy expenditure, and thus may provide a negative feedback signal to restrict energy storage.[1,20] Leptin is likely to be of importance in times of insufficient energy reserves, such as fasting or starvation.[4,8] During fasting, circulating leptin concentrations

Table 2 Body weight and plasma levels of glucose, insulin and glucagon before and after 24 h in mice subjected to fasting or continuous feeding with or without administration of recombinant murine leptin (300 μg i.p.) administered after the initial blood sampling and after 12 h

	Fed mice given saline (n = 12)	*Fed mice given murine leptin (n = 12)*	*Fasted mice given saline (n = 12)*	*Fasted mice given murine leptin (n = 12)*
Body weight (g)				
Initial	29.1 ± 0.4	29.5 ± 0.3	28.2 ± 0.9	28.5 ± 1.0
After 24 h	28.9 ± 0.3	28.9 ± 0.3	24.9 ± 0.8**	25.0 ± 0.7**
Plasma glucose (mmol/l)				
Initial	8.9 ± 0.2	8.7 ± 0.2	7.7 ± 0.4	7.9 ± 0.2
After 24 h	9.1 ± 0.3	9.1 ± 0.2	4.6 ± 0.4**	5.6 ± 0.2**
Plasma insulin (pmol/l)				
Initial	201 ± 23	248 ± 59	193 ± 28	196 ± 38
After 24 h	233 ± 33	194 ± 21	106 ± 26**	162 ± 26[a]
Plasma glucagon (pg/ml)				
Initial	86 ± 5	79 ± 5	65 ± 11	70 ± 12
After 24 h	83 ± 5	88 ± 4	42 ± 5*	66 ± 10[a]

Asterisks indicate probability level of random difference between the initial sample and that taken at 24 h means of ANOVA with Bonferroni *post-hoc* analysis, *$P < 0.05$, **$P < 0.01$. [a]indicates probability level of random difference between fasted animals given saline *vs* fasted animals given human leptin of $P < 0.05$.
Means ± SEM are shown.

Table 3 Body weight and plasma levels of glucose, insulin and glucagon before and after 24 h in chemically sympathectomized mice subjected to fasting or continuous feeding with or without administration of human leptin (300 μg i.p.) administered after the first blood sampling and after 12 h. The mice had been chemically sympathectomized 48 h before the initial blood sampling by means of i.v. administration of 6-hydroxydopamine (40 mg/kg)

	Fed mice given saline (n = 12)	*Fed mice given human leptin (n = 12)*	*Fasted mice given saline (n = 12)*	*Fasted mice given human leptin (n = 12)*
Body weight (g)				
Initial	29.5 ± 0.5	30.5 ± 0.7	30.5 ± 0.5	30.2 ± 0.3
After 24 h	29.6 ± 0.6	30.5 ± 0.6	27.5 ± 0.6**	27.5 ± 0.3**
Plasma glucose (mmol/l)				
Initial	7.5 ± 0.3	7.5 ± 0.2	7.9 ± 0.2	7.6 ± 0.3
After 24 h	8.2 ± 0.5	7.4 ± 0.1	3.9 ± 0.2**	3.8 ± 0.3***
Plasma insulin (pmol/l)				
Initial	199 ± 19	184 ± 23	174 ± 21	201 ± 23
After 24 h	201 ± 11	176 ± 19	81 ± 20**	71 ± 21**
Plasma glucagon (pg/ml)				
Initial	65 ± 8	74 ± 10	65 ± 10	60 ± 8
After 24 h	72 ± 10	63 ± 11	11 ± 12**	15 ± 13**

Asterisks indicate probability level of random difference between the initial sample and that taken at 24 h by means of ANOVA with Bonferroni *post-hoc* analysis, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.
Mean ± SEM are shown.

fall[8,9] and a series of neuroendocrine responses are initiated, including stimulation of glucocorticoid secretion and inhibition of thyroid hormone secretion.[8] Therefore, one function of leptin might be to provide the brain with information of sufficient peripheral energy stores and recent energy balance, whereas low leptin concentrations provide a signal of decreasing food intake and energy stores. The most pronounced effects of exogenously administered leptin would therefore by anticipated in the fasted state.

In the previous study by Ahima *et al*,[8] leptin was found to attenuate the effects of fasting on glucocorticoid, thyroid and reproductive hormones in mice, but did not influence the fasting-induced reductions of circulating glucose and insulin. In that study, leptin was administered at a lower dose (2 × 1 μg/g daily) than in the present study, in which, an approximately tenfold higher dose (2 × 10 μg/g) of leptin increased circulating glucose, insulin and glucagon during fasting. In contrast, concentrations of glucose, insulin and glucagon were unaffected by this dose of leptin in the fed state. These results suggest that plasma levels of glucose and two islet hormones are more readily increased by leptin during fasting than in the fed state, and that pharmacological doses appear to be required in order to observe these effects.

A previous study in normal mice has shown that leptin at high doses slightly reduces circulating glucose without altering plasma insulin,[21] and another study has shown that leptin increases glucose turnover without altering the basal concentrations of glucose or insulin.[22] In markedly glucose intolerant *ob/ob* mice, leptin reduces plasma glucose and insulin.[7] Elevations of plasma leptin in rats resulting from either 48 h continuous subcutaneous infusion of a low dose of leptin[23] or 14 d after transfecting the livers of rats with an adenovirus expressing the rat leptin cDNA[24] lowered glucose and insulin concentrations. Other studies have shown no change in circulating glucose but markedly reduced plasma insulin after infusing leptin in rats for 3 h[14] or after marked elevation of leptin for 28 d.[5] Together, these results are consistent with an effect of leptin to increase insulin sensitivity, as has recently been demonstrated in rats by using euglycaemic, hyperinsulinaemic clamp techniques.[6,25] Our results differ from these findings in that no changes of plasma glucose or insulin were observed after leptin administration in the fed state; and in the fasted state, leptin increased circulating glucose and insulin relative to saline-injected animals. Therefore, in the present study, pharmacological doses of leptin do not appear to enhance insulin sensitivity, particularly during fasting. Recent results from a similar experiment with high dose leptin administration in rhesus monkeys have shown that leptin increased fasting plasma glucose without altering circulating insulin concentrations.[26]

A major finding in the present study was that leptin did not significantly affect circulating glucose, insulin and glucagon in the 24 h fasted mice that were pretreated with 6-OHDA. Although in this study, we did not perform tissue catecholamine measurements or histology for sympathetic nerves in the mice, 6-OHDA at the same dose level (40 mg/kg) produces marked reductions of tissue noradrenaline and decreases the number of tyrosine hydroxylase containing nerves.[18,19] Furthermore, 6-OHDA is known to produce a selective chemical sympathectomy at the level of post-ganglionic nerve terminals and does not affect the adrenal medulla.[17,18,19] Therefore, our results suggest that intact sympathetic innervation is required for the gluco-regulatory actions of leptin during fasting. In addition, circulating adrenaline could contribute to the failure of leptin to increase plasma insulin in fasted 6-OHDA treated animals,

because leptin[15,27] and fasting[28] can both increase adrenal medullary activity as might a compensatory increase after 6-OHDA.[29] These would all tend to increase circulating adrenaline, which is known to inhibit insulin secretion.[30]

It has previously been reported that leptin activates the activity of the sympathetic nerves, determined as increased noradrenaline turnover in brown adipose tissue[16] and increased firing rates of sympathetic nerves in several tissues.[14,15] Such a stimulatory action of leptin on sympathetic nerve activity could explain the increases of glucose and glucagon we observed during fasting, since sympathetic nerve activation stimulates hepatic glucose production and glucagon secretion.[30,31] Similarly, it has recently been reported that pharmacological α- and β-adrenergic blockade prevents the increases of circulating glucose, lactate, and glucagon induced by leptin in rhesus monkeys,[26] demonstrating a role for the sympathetic nervous system in mediating the metabolic effects of leptin administration. However, such an action would not explain the increase of circulating insulin, since sympathetic nerve activation is known to inhibit insulin secretion.[30] It is possible that the leptin-induced increase of plasma insulin observed in the present study occurs as an indirect response to sympathetic activation, perhaps in conjunction with adrenaline released from the adrenal medulla.[32,33] This would stimulate glucagon secretion and increase hepatic glucose production,[35] resulting in increased circulating glucose and glucagon, which in turn stimulate insulin secretion.

A direct stimulatory action of leptin could also contribute to the increased hepatic glucose production and insulin secretion since leptin receptors are expressed in the liver and on pancreatic β-cells.[36,37] However, such a direct effect is unlikely since the changes of glucose and pancreatic hormones were prevented by sympathectomy with 6-OHDA. Furthermore, this latter explanation is also less likely with regard to the observed increased plasma insulin, because most reports have shown an inhibitory influence of leptin on pancreatic insulin secretion.[38–41]

The reduction in circulating glucagon after 24 h fasting is consistent with the reduction in circulating glucose, since glucagon contributes to glucose production during fasting.[35] The observed reduction of plasma glucagon after 24 h fasting is, however, at variance with a previous study in which we observed a 22% increase in plasma glucagon after overnight fasting in mice in conjunction with similar level of glycaemia as in the present study.[42] The glucagon response to fasting is not well understood because both increased,[43] decreased[44,45] and unaffected[46] levels have been reported in humans. Whether these discrepancies in glucagon responses to energy restriction result from different lengths of fasting, different antibodies for the determination of glucagon (some antibodies cross react with immunoglobulins,[47]) or other conditions, deserves to be studied in more detail.

Conclusion

Our present results demonstrate that pharmacological doses of leptin increase circulating glucose, insulin and glucagon in fasted, but not in fed, mice. These effects are prevented by chemical sympathectomy induced by administration of 6-OHDA, suggesting that changes in circulating glucose, insulin and glucagon induced by leptin administration during fasting require an intact sympathetic nervous system.

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