EFFECTS OF CHRONIC LEPTIN ADMINISTRATION ON GLUCOSE AND LIPID METABOLISM IN LEAN AND OBESE RATS

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ABSTRACT
Leptin is reported to have effects on peripheral tissues that are independent of its central effects on food intake and body weight. In this study we examined the chronic effects of leptin injection on some lipid metabolic parameters, glucose handling by the body tissue and glycogen content in liver and skeletal muscles in lean and obese rats. Rats were divided into 2 groups (20 rats each) one group included lean rats and the other included diet induced obesity (DIO) rats. Ten rats of each group were injected intraperitoneal with leptin in a dose of (7.2 μgm/gm/day) for 2 weeks. The other rats served as control groups. In all rats, body weight non estrified fatty acids (NEFA), triglycerides (TG), glucose tolerance, muscle and liver glycogen were measured. Results revealed that, in lean rats, body weight was decreased, with increased serum non-estrified fatty acids (NEFA), without improvement in glucose tolerance, and without change in liver or muscle glycogen content. In obese rats, body weight decreased, serum insulin level decreased, with increased serum NEFA and triglycerides (TG). Improvement in glucose tolerance with decreased liver and muscle glycogen content was observed. These data indicate that leptin induces a complex metabolic responses with effects on glucose as well as lipid metabolism which are independent of central nervous system. It could be concluded that artificial elevation of serum leptin stimulates lipolysis and inhibits glucose storage as glycogen in the liver and skeletal muscles.
INTRODUCTION

It has been demonstrated that leptin; an adipocyte hormone, acts to decrease food intake and increase energy expenditure (Zhang et al., 1994 and Halaas et al., 1995). The main target of leptin action lies within the central nervous system (Fei et al., 1997). Recently, various effects of leptin on peripheral target organs have been identified. They include suppression of glucose-induced insulin secretion in pancreatic islets (Emilsson et al., 1997); increased partitioning of long chain fatty acids towards oxidation in pancreatic islets (Shimabukoro et al., 1997) and Soleus muscle of normal rats, inhibition of glucose incorporation in soleus muscle of ob/ob mice (Liu et al., 1997) and stimulation of lipolysis (Fruhbeck et al., 1997 and Muoio et al., 1997).

In contrast with growing body of literature concerning the effects of leptin on energy balance, little is known about the effects of leptin on glucose and lipid metabolism in obese rats. Levin et al. (1996) reported that, treatment of ob/ob mouse with leptin reverses the metabolic abnormalities not only by suppression of appetite but also by additional mechanisms. We hypothesized that if leptin is an indicator of body adiposity, then artificial elevations of leptin would result in decreased lipogenesis and increased mobilization of fat stores (i.e. increased lipolysis). The resulting increase in the availability of fatty acids for oxidation would have an indirect effect on glucose metabolism e.g. oxidation and storage as glycogen in skeletal muscles and liver. The aim of this study was to determine the effects of chronic leptin administration on some metabolic parameters of lipid and glucose metabolism including; NEFA, TG, glucose tolerance and muscle and liver glycogen content in both lean and diet-induced obese (DIO) rats.

MATERIALS AND METHODS

Animals and diet

Forty male albino rats of Wistar strain were purchased at weaning they were housed (5/cage) and segregated into 2 groups (20 rats each); one raised on normal chow diet and the other on a high fat-low carbohydrate (70% fat, 10% carbohydrate, and 20% protein) diet for 8 weeks to induce obesity (Mickelsen et al., 1955).

Rats were kept on 12:12 light-dark cycle with the light cycle between
0600 and 1800, and were allowed free access to food and water. Ten rats which exhibiting the greatest body weight gain were selected for the study of leptin treatment. On each day of the treatment schedule, food was withdrawn 0800 hours and returned 2 hours after the intraperitoneal (i.p.) injection of recombinant mouse leptin (Hoechst, Germany) in a dose of 7.2 μg/g body weight daily for 2 weeks (Igel et al., 1997) continuously into lean and obese rats (10 rats/group). Control lean animals and control obese animals (10 rats each) received isotonic saline (as a vehicle) by i.p. injection. Food intake and body weight were observed daily during the treatment period. On day 13 of the treatment, intravenous glucose in a dose 1 gm/kg body weight was administered in 4 rats as previously described by Rizk et al. (1998). On day 14, rats were scarified by decapitation at 1000 hours and trunk blood was collected. Serum were stored at −20°C until assayed. Liver and hind limb skeletal muscles were weighed and collected for glycogen determination according to (Doyle et al., 1993).

Serum analysis

Determination of glucose, non esterified free fatty acids (NEFA), triglycerides (TG) were assayed by a commercial kit (Biomeureue, Frnace). Insulin and leptin hormone were determined by RIA commercial kit (Linco research, USA).

Statistical analysis

All results are given as mean ±SE. All data analysis was performed using prism (Graph Pad, USA) student’s t-test and one way ANOVA by use of the statview IV statistical package.

RESULTS

Effect of leptin on body weight, serum insulin, serum leptin, glucose, NEFA and TG in lean and DIO-rats

The body weights of albino rats fed on a high fat diet were significantly higher by the end of the experiment period (Table 1, P<0.05) than lean control group fed normal chow diet. Injection of leptin for 2 weeks to lean and obese rats reduced body weight by ≈ 28% compared to lean and obese rats receiving the vehicle.

Serum leptin was significantly higher in non-treated obese rats compared to untreated lean rats (Table 1). Injection of leptin increased serum leptin significantly in the treated group.
with about 25 fold increase in lean rats above the basal level in non treated lean rats and up to ≥ 10 fold increase in obese rats above the basal level in leptin untreated obese rats. In the leptin untreated rats, the obese group had leptin levels that were eightfold greater than those of lean, as well as significantly higher insulin, NEFA and TG were also found to be higher in obese than in lean rats (Table 1). Serum insulin levels in lean rats were unaffected by leptin treatment but there was a significant reduction in insulin level in obese rats, though they remained hyperinsulinaemic. Serum glucose was unchanged in both lean and obese rats by leptin injection. Serum NEFA were increased by leptin treatment in both lean and obese rats, while serum TG increased significantly by leptin injection in obese rats only (Table 1).

Effect of leptin on glucose tolerance

In order to determine the effect of leptin injection on glucose handling by the tissues, we performed the I.V. glucose tolerance test. In the leptin untreated rats, obese rats showed a significant impairment in response to the loaded glucose compared to lean control. Administration of leptin for 2 weeks as a therapeutic tool for obesity, improved the tolerance significantly only in obese rats.

Effect of leptin on glycogen content

In lean rats, leptin had no effect on either muscle or liver glycogen. In obese rats significant reduction in glycogen content of muscle and liver were seen (Table 3).
Table 1: Effects of leptin injection (7.2 μg/gm for 2 weeks) on body weight, serum glucose, NEFA, TG and serum leptin levels in lean and DIO-rats. Data represented as mean±SE (10 rats/group).

<table>
<thead>
<tr>
<th>Group</th>
<th>B.W (gm)</th>
<th>Glucose (mM)</th>
<th>NEFA (mM)</th>
<th>TG (mM)</th>
<th>Insulin (ng/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean + Leptin</td>
<td>99.2±5.2</td>
<td>5.1±0.32</td>
<td>1.82±0.2*</td>
<td>4.4±0.2</td>
<td>1.1±0.07</td>
<td>92.2±8.7*</td>
</tr>
<tr>
<td>Lean - Leptin</td>
<td>116.7±4.6*</td>
<td>5.8±0.42</td>
<td>1.13±0.08</td>
<td>4.82±0.18</td>
<td>1.3±0.08</td>
<td>2.5±0.03</td>
</tr>
<tr>
<td>DIO + Leptin</td>
<td>112.5±7.5* &amp; 6.2±0.4</td>
<td>2.85±0.17 †</td>
<td>6.88±0.55</td>
<td>3.2±0.4* &amp; 136.2±9.5* &amp; 16.2±0.74 †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIO - Leptin</td>
<td>154.2±4.9</td>
<td>6.6±0.53</td>
<td>1.88±0.09* &amp; 5.57±0.33* †</td>
<td>4.25±0.62* †</td>
<td>16.2±0.74 †</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean± SE for 10 rats/group.

* : P value is significant (<0.05) between leptin treated and untreated lean and obese rats.
† : P value is significant (<0.05) between leptin untreated lean and DIO rats.
δ : P value is significant (<0.05) between leptin treated lean and leptin treated DIO rats.

Table (2) : Intravenous glucose tolerance test (IVGTT) performed after leptin injection.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Lean-Leptin glucose (mM)</th>
<th>Lean+Leptin glucose (mM)</th>
<th>DIO-Leptin glucose mM</th>
<th>DIO+Leptin glucose (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.1±0.28</td>
<td>3.2±0.36</td>
<td>4.2±0.41</td>
<td>4.1±0.34</td>
</tr>
<tr>
<td>15</td>
<td>5.96±0.34</td>
<td>6.0±0.27</td>
<td>7.59±0.23 †</td>
<td>6.7±0.22* †</td>
</tr>
<tr>
<td>30</td>
<td>7.5±0.16</td>
<td>6.9±0.18</td>
<td>9.29±0.52 †</td>
<td>8.4±0.51* δ</td>
</tr>
<tr>
<td>60</td>
<td>4.77±0.27</td>
<td>5.1±0.37</td>
<td>6.9±0.1 †</td>
<td>5.82±0.23* δ</td>
</tr>
</tbody>
</table>

Data are represented as mean±SE for 4 rats/group.

*: P<0.05 is significant between leptin treated and leptin untreated groups.
† : P<0.05 is significant between leptin untreated lean and leptin untreated obeses rats.
δ : P<0.05 is significant between leptin treated lean and leptin treated obese rats.
Table (3) : Effect of chronic leptin injection (7.2 μg/gm/day for 2 weeks) on skeletal muscles and liver glycogen content in lean and DIO-rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycogen content (μmol/g wet wt) in the muscle</th>
<th>Glycogen content (μmol/g wet wt) in the liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leal-Leptin</td>
<td>22.5±0.7</td>
<td>208.9±8.1</td>
</tr>
<tr>
<td>Lean +Leptin</td>
<td>22.8±0.9</td>
<td>200.3±7.6</td>
</tr>
<tr>
<td>DIO-Leptin</td>
<td>23.2±0.8</td>
<td>221.4±10.3</td>
</tr>
<tr>
<td>DIO +Leptin</td>
<td>16.8±0.9*</td>
<td>173.3±2.6*</td>
</tr>
</tbody>
</table>

Values are mean±SE for (10 rats/group).

*; P is significant (<0.05) between leptin treated and leptin untreated obese and lean rats.

**DISCUSSION**

This study demonstrated that chronic leptin treatment had significant effect on glucose and lipid metabolism in both lean and diet-induced obesity rats. In lean rats, administration of leptin led to increase in lipolysis as evidenced by marked elevation of serum NEFA and body weight reduction. Chronic injection of lean rats with leptin didn’t enhance glucose handling by tissues and the glycogen content was unchanged in both skeletal muscles and the liver. In obese rats, injection of leptin produced remarkable reduction of body weight and enhancement of lipolysis with increased serum NEFA and serum TG). On the other hand no effect on glucose level was found.

The increased lipolysis is consistent with the hypothesis that leptin modulates energy homeostasis by directing lipid away from storage to oxidation, as suggested by in vitro studies (Muller et al., 1997). Our results are consistent with another study by Bryson et al., (1996) that demonstrated increased serum NEFA after chronic administration of leptin which indicated increased lipid oxidation. The increased serum TG level may be explained by the mobilizing effect of leptin on intracellular TG (Dobbin et al., 1998).

The improvement in glucose toler-
ance after administration of leptin to obese rats (Table 2) may be due to; improvement of insulin sensitivity after reduction of the body weight and adiposity.

Our study have also shown reduction in glycogen stores in obese but not lean rats. The preservation of hepatic glycogen content in lean rats may be explained by; First, a reduction in Pyruvate dehydrogenase complex activity (PDHCa) (Bryson et al., 1999), resulting in reduction in glucose oxidation and lowering of the demand for glycogen breakdown. Second, the availability of glucose may allow glycogen synthesis which may compensate for any leptin - induced increase in glycogenolysis. This would result in no net change in glycogen content or in plasma glucose level. Third, the increased NEFA, with increased fatty acid oxidation may inhibit glucose oxidation by way of the glucose/fatty acid cycle (Randle et al., 1963). On the other hand, in obese rats Bryson et al. (1999) demonstrated no inhibitory effect for leptin on elevated PDHCa, and therefore glucose could be still oxidized, with no sparing (as in lean rats) to allow glycogen synthesis to compensate for any increase in glycogenolysis. The reduction of glycogen content in the muscles of obese rats may be explained by, glycogenolysis was not matched by an increase in glycogen synthesis, possibly due to fall in insulin actions in skeletal muscles. The decreased insulin action could be related to the hyperleptinaemia. The increased endogenous leptin in obese rats could be responsible for both decrease in plasma insulin and insulin resistance (Table 1). However, several conflicting reports about the effects of leptin on glycogen stores were studied. Acute leptin treatment decrease hepatic content of glycogen in ob/ob (Harris et al., 1998) but not lean rats (Lee et al., 1996). Acute leptin treatment decreased hepatic glycogen in lean mice while increasing muscle glycogen content (Bryson et al., 1996), and whole body glycogen synthesis in rats is reported to be unchanged by leptin infusions (Roduit and Thorens, 1997). In vitro, studies also showed variable effects on glycogen synthesis in skeletal muscles (Berti et al., 1997) and Furnsinn et al., 1998) and adipocytes (Mercer et al., 1998). These mixed reports show that leptin effects on glucose metabolism are dependent on both dose and duration of the leptin injection and the degree of insulin resis-
stance in the animal model studied, thus making comparative studies so difficult.

The increased lipolysis and decreased glycogen content found in this study after chronic in vivo leptin treatment might suggest that leptin has a deleterious effect on insulin sensitivity. However, despite improved i.v. glucose tolerance in treated obese rats, the association between hyperleptinaemia and insulin resistance remain unresolved (Ranganathan et al., 1998), as leptin had been shown to have both insulin-like and anti-insulin action. A rise in leptin may be part of the process that makes these rats insulin resistant in attempt to limit the size of the energy stores.

If leptin was increasing insulin resistance, then an increase in hyperinsulinaemia could be expected and not the reduction in plasma insulin seen in our study and improvement in glucose tolerance. This effect is consistent with in vitro studies that have shown that the inhibitory effect of leptin on the pancreatic islets is an important modulator of insulin secretion (Emilsson et al., 1997 and Muoio et al., 1997).

Several factors may have contributed to the difference in the response of the lean and obese rats to leptin in this study. First, there was a greater percent increase above basal endogenous serum leptin levels in lean rats compared to the obese rats after leptin injection (25 fold higher in lean) and (10 fold higher in obese rats). This suggests greater suppression of endogenous leptin secretion in obese rats or increased clearance in obese rats. This may explain the decrease in some metabolic response in obese rats because they receive less of an increasing circulating leptin. Because normal endogenous leptin levels are already elevated in obese rats, leptin resistance may further diminish the response to increase in leptin levels.

Second, may be that obese rats are insulin resistant, and this state combined with the leptin induced reduction in hyperinsulinaemia may have resulted in an inability to compensate for leptin effects, e.g., increased glycogen synthesis to balance leptin’s effect on glycogenolysis.

The reduction of body weight after chronic intraperitoneal injection of leptin in both lean and obese rats is consistent with the role of leptin on body weight. This may be due to the central
action of leptin on hypothalamic centers controlling food intake and body weight (Halaas, 1995) but also can be explained by the increased lipolysis shown in our study (increased NEFA) in serum with increased lipid oxidation.

Conclusion:
This study shows that chronic artificial elevations of circulating leptin stimulates lipolysis and inhibits glucose storage as glycogen. These results are consistent with the role of leptin in the regulation of fat store by modulating glucose and lipid metabolism. Thereby, leptin reduces body weight not only through its central effects but also through its effects on peripheral tissues.

REFERENCES


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تأثير هرمون اللبتيتين على أيض الجلوكوز والدهون في الفئران السمينة والتحييفة

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أجريت هذه الدراسة لدراسة تأثير هرمون اللبتيتين على أيض الجلوكوز والدهون في أنسجة عضلات وكبد الفئران التحية والسمنة، وذلك بتقديم نحو 100 فئران، وتم تقسيمها إلى مجموعتين، ومجموعتي هرمون اللبتيتين بجرعة 2.5 ميكروجرام/جم من الوزن لمدة 15 يوماً، والمجموعتان المجتذبتان. (تعدهما سمنية والأخريات نحيفة) لم يتم إعطاؤها هرمون اللبتيتين (مجموعتان ضابطتان لمجموعتي السابقتين).

ويمكن تلخيص نتائج البحث كالتالي:

إعطاء هرمون اللبتيتين للفئران النحيفة أدى لانعدام متابعة (نقص) ذات دلالة إحصائية في وزن الجسم، دون تغير في أيض الجلوكوز أو كمية الجليكيرين المختزنة في الكبد أو العضلات.

إعطاء هرمون اللبتيتين للفئران السمينة أدى لانعدام متابعة ذو دلالة إحصائية في وزن الجسم ومستوى الأنسولين في الدم مع تكييف في أيض الدهون، كما أخذت تقني في كمية السكر في الأنسجة وعلي الجانب الآخر حدث تدقق في كمية الجليكيرين المختزنة في الكبد والعضلات.

وعلى ضوء هذه النتائج يمكن مدي التأثير المعنيد لهرمون اللبتيتين على أيض الدهون والسكر، هذا التأثير المعنيد لهرمون اللبتيتين يقترح إيجاد إشارات جديدة من الأنسجة الطرية تفسر عمله بعيداً عن الجهاز العصبي المركزى.