Carbohydrate and fat have different effects on plasma leptin concentrations and adipose tissue leptin production

Kevin EVANS*, Mo L. CLARK† and Keith N. FRAYN†
*Department of Clinical Chemistry, Staffordshire General Hospital, Weston Road, Stafford ST16 3SA, U.K., and †Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE, U.K.

ABSTRACT

Leptin is secreted by adipocytes and plays a role in the regulation of food intake. However, the regulation of leptin production by adipose tissue is unclear. We have investigated whether a mixed meal or a high-fat load given orally, or a pure fat load given intravenously, stimulates adipose tissue leptin production. Six volunteers were studied on two occasions following an overnight fast. On one occasion they consumed tomato soup containing 40 g of triacylglycerol (as Intralipid) and 9.6 g of carbohydrate; on the other occasion Intralipid was infused intravenously over 4 h to give the same fat load. A further eight subjects consumed a mixed meal (containing 37 g of fat and 100 g of carbohydrate) after an overnight fast. Paired blood samples were obtained from an arterialized hand vein and a vein draining subcutaneous adipose tissue at baseline and for 6 h following the meals or the start of the infusion. After both the intravenous and oral fat loads, the arterialized and adipose venous plasma leptin concentrations decreased over 6 h (both $P < 0.001$), as did the leptin veno–arterial difference ($P < 0.01$). Following the mixed meal, there was a slight increase in the arterialized plasma leptin concentration ($P < 0.02$) and a more marked increase in the adipose venous plasma leptin concentration ($P < 0.03$) and in the adipose tissue leptin veno–arterial difference ($P < 0.01$), all peaking at 240 min. We conclude that the increase in plasma leptin concentration observed after meals is not simply a result of an energy load, but is in response to a signal that is not present following a fat load without carbohydrate.

INTRODUCTION

Leptin, the protein product of the ob gene, is a 167-amino-acid protein [1] that is secreted by adipocytes and plays a role in the regulation of food intake by causing satiety effects and promoting weight loss. The main target for leptin action is in the hypothalamus. Neuropeptide Y is a potent stimulator of food intake, and leptin inhibits neuropeptide Y synthesis [2,3] and release, although studies have shown that neuropeptide Y cannot be the sole mediator of leptin’s actions [4]. Leptin may also have direct effects on peripheral tissues, including adipose tissue [5,6]. In addition, leptin seems to have roles in reproduction and neuroendocrine signalling.

Production of leptin by adipose tissue (its major source) has been demonstrated using arteriovenous difference measurements across adipose tissue in humans in vivo [7]. However, the regulation of leptin production by adipose tissue is unclear. Leptin mRNA expression increases following corticosteroid administration [8], insulin administration [9] or eating [10,11] (although this has not been seen in all studies [12]), and decreases with fasting [9,10] (although again this has not been seen in all studies [12]). Similarly, plasma leptin concentrations decrease with fasting [13–15] and isoprenaline infusion [16], and increase with feeding [15] and insulin administration [17], although once again this has not been seen in all studies [18]. Increased adipose tissue leptin production has been reported following a high-carbohydrate meal [16] and a mixed meal [19]. In the case of meals, it is not

Key words: carbohydrate, fat, leptin, postprandial.
Abbreviations: ATBF, adipose tissue blood flow; TAG, triacylglycerol.
Correspondence: Dr Kevin Evans (e-mail kevinevans@doctors.org.uk).
clear whether the signal for leptin secretion is the entry of an energy load or whether there is some specific signal relating to carbohydrate ingestion. Changes in leptin production may be more easily observed using arteriovenous difference measurements than in mixed venous blood. We have investigated whether leptin production is stimulated by a pure fat load given intravenously, a high-fat load given orally or a mixed meal.

Other aspects of these studies have been reported elsewhere [20], and the current results have been presented in abstract form [21,22].

**METHODS**

**Subjects**

For the fat-load studies, six healthy volunteers (four male) were studied on two occasions following an overnight fast (Table 1). On one occasion they consumed tomato soup containing 40 g of triacylglycerol (TAG; Intralipid; Pharmacia Ltd, Milton Keynes, U.K.) and 9.6 g of carbohydrate (Table 2), and on the other occasion Intralipid was infused intravenously over 4 h to give the same fat load. For the mixed meal study, eight healthy volunteers (four male) were studied following an overnight fast (Table 1). They consumed a meal containing 37 g of fat and 100 g of carbohydrate (Table 2). Subjects refrained from strenuous exercise and alcohol for 24 h before each study, and were given instructions to consume a low-fat meal on the evenings before the studies. None of the subjects were smokers. All subjects were normolipidaemic and normoglycaemic. The studies were approved by the Central Oxford Research Ethics Committee, and all subjects gave written informed consent.

**Experimental methods**

A 10 cm, 22 gauge Secalon Hydrocath catheter (Ohmeda, Swindon, U.K.) was introduced over a guide wire into a superficial vein on the anterior abdominal wall and threaded towards the groin, so that its tip lay just superior to the inguinal ligament. For the fat-load studies, the catheter was threaded through a superficial vein in the leg, and its tip was placed just above the inguinal ligament. For the mixed meal study, the catheter was inserted into a superficial vein in the arm, and its tip was placed just above the brachial artery. The catheter was connected to a Luer lock and fluid was infused at a rate of 10 ml/min through an infusion pump (Infusor; Braun, Melsungen, Germany). The catheter was connected to a temperature-controlled heating pad (Bed 120; Medigas, Coventry, U.K.) and was kept warm throughout the study.

**Table 1 Subject characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fat-load studies (n = 6)</th>
<th>Mixed-meal study (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.4</td>
<td>54.0–90.5</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.81</td>
<td>1.60–1.89</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>3.6</td>
<td>21.1–26.4</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.1</td>
<td>4.6–5.7</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.3</td>
<td>2.8–5.9</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.3</td>
<td>1.0–1.7</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>0.7</td>
<td>0.2–1.1</td>
</tr>
</tbody>
</table>

**Table 2 Nutrient composition of test meals**

Compositions were determined from manufacturers’ data and food tables [23].

<table>
<thead>
<tr>
<th>Meal</th>
<th>Carbohydrate (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral fat load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried diced onion (4 g)</td>
<td>2.8</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Tomato puree (27 g)</td>
<td>3.5</td>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Drained tinned tomatoes (133 g)</td>
<td>3.3</td>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Intralipid 20% (200 ml)</td>
<td>0</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9.6</td>
<td>40.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Mixed meal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornflakes (30 g)</td>
<td>24.9</td>
<td>0.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Whole milk (130 g)</td>
<td>6.5</td>
<td>5.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Plain chocolate (70 g)</td>
<td>4.5</td>
<td>0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Banana (50 g)</td>
<td>11.6</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Chocolate milkshake</td>
<td>11.3</td>
<td>11.2</td>
<td>5.7</td>
</tr>
<tr>
<td>Total</td>
<td>99.7</td>
<td>37.1</td>
<td>16.2</td>
</tr>
</tbody>
</table>
Postprandial changes in plasma leptin
to the inguinal ligament. As described previously [24],
this provided access to the venous drainage from the
subcutaneous abdominal adipose tissue, uncontaminated
by muscle drainage and with a relatively minor con-
tribution from skin. This adipose tissue depot has been
shown to be representative of whole-body adipose tissue
[25].

A retrograde cannula was placed in a vein draining the
hand, which was warmed in a hot-air box maintained at
60 °C to obtain arterialized blood. The mean oxygen
saturation in the heated hand vein over all the experi-
ments was 95.7%, and did not change with time. The
cannulae were kept patent by a slow infusion of 0.9% (w/v) NaCl.

Adipose tissue blood flow (ATBF) was measured
immediately after each blood sample was taken using the
$^{133}$Xe washout method [26] after injection of 2 MBq of
$^{133}$Xe into the region drained by the subcutaneous
abdominal catheter. The first ATBF measurement
was not taken until at least 30 min later, to allow recovery
from the hyperaemic phase caused by the injection.

Two sets of basal blood samples, 20 min apart, were
taken simultaneously from the arterialized vein and the
abdominal vein. Blood samples were taken over the 6 h
period following the meals or the start of the infusion,
and veno–arterial differences were calculated.

**Analytical methods**

Blood samples were collected into heparinized syringes.
Plasma was separated rapidly from the remaining blood
by centrifugation at 4 °C. Leptin concentrations were
measured in these samples by RIA (Biogenesis, Poole,
Dorset, U.K.). Plasma glucose, insulin and TAG concen-
trations were measured in arterialized samples using
enzymic methods on an IL Monarch centrifugal analyser
(Instrumentation Laboratory, Warrington, U.K.). In the
fat-load studies, glucose was measured in whole blood.
This was converted into plasma values using the Dillon
equation [27]. Plasma insulin was measured using a
double-antibody RIA (Kabi Pharmacia Ltd, Milton
Keynes, U.K.).

**Calculations and statistical methods**

In order to analyse trends with time in leptin veno–
arterial differences, ATBF and leptin production, and to
allow comparison between studies, each subject's results
are expressed as areas under the curve with reference to
baseline arterial concentration (expressed as 100%).
Leptin production was calculated as the product of the
veno–arterial difference and the ATBF.

Repeated-measures ANOVA with SPSS (SPSS UK
Ltd, Chertsey, U.K.) was used to test for the significance
of changes with time, and also to compare oral with
intravenous fat loads.

**RESULTS**

**Fat-load studies**

The glucose, insulin and TAG results for the fat-load
studies have been presented previously [20], and are given
again here for comparison with the mixed meal results.
Glucose concentrations were slightly higher following
the oral fat load than the infusion (Figure 1A; $P < 0.001$
for glucose time by study interaction). Insulin concen-
trations showed a very small rise to peak at 9 m-unit/l at 60 min after the oral fat load, and decreased gradually following the infusion (Figure 1B; \(P < 0.001\) for time effect; \(P = 0.03\) for time by study interaction). Plasma TAG concentrations increased, as expected, with both the oral and intravenous fat loads (Figure 2; \(P < 0.001\)), with a greater increase following the intravenous fat load (\(P < 0.001\) for time by study interaction).

Following both the intravenous and oral fat loads, arterialized plasma leptin concentrations decreased over the subsequent 6 h (Figure 3; \(P < 0.001\) for time effect), with no difference between the oral and intravenous fat loads. The adipose venous plasma leptin concentrations also decreased during the study (Figure 3; \(P < 0.001\) for time effect), again with no difference between oral and intravenous fat loads. The leptin veno–arterial difference decreased with time (Figure 4A; \(P < 0.01\)). There appeared to be an initial increase in ATBF, although this did not reach statistical significance (Figure 4B). When overall leptin production was considered, however, this showed a marked increase (Figure 4C; \(P = 0.04\)).

DISCUSSION

Our results show that the increase in plasma leptin concentration observed after meals is not simply a result of an energy load, but is in response to a signal that is not present following a fat load without carbohydrate. A previous study [16] showed a significant increase in arterial and adipose venous plasma leptin concentrations over 5 h after a high-carbohydrate meal, while leptin
concentrations fell with continued fasting. The decreases in plasma leptin concentrations (by 31% in arterial samples and 36% in adipose venous samples) were similar to those observed in the present study (25% in arterialized and 18% in adipose venous samples following oral fat load, and 31% in both arterialized and adipose venous samples following intravenous fat infusion). Dallongeville et al. [15] reported a 29% decrease in plasma leptin concentrations after an 8 h fast. The same study showed increases of 18–26% in plasma leptin concentrations 6–8 h after a mixed meal. In another study, there was no change in arterial or adipose venous leptin concentrations following a mixed meal, but the veno–arterial leptin difference had increased by 30 min postprandially, and returned gradually to baseline after 60–120 min [19]. The increase in plasma leptin concentration seen between 0 and 120 min after oral Intralipid is in agreement with the findings of Astrup et al. [19]. In that study, the increase in leptin production paralleled the increase in glucose and insulin secretion, but occurred before increases in the plasma TAG concentration, suggesting that leptin secretion is stimulated by dietary carbohydrate. Another study showed that circulating glucose and insulin both appeared to have a stimulatory effect on leptin production [28]. However, both insulin and glucose have an effect on glucose uptake by cells, and glucose uptake may be the single factor by which circulating glucose and insulin concentrations modulate leptin secretion [28]. A previous study found decreases in leptin production by adipose tissue of 37% and 40% during and following intravenous Intralipid infusion respectively, although this did not reach statistical significance [29]. In contrast, leptin mRNA expression in subcutaneous fat has been shown to increase during a 5 h Intralipid and heparin infusion, although plasma leptin concentrations did not change [30]. The increases in plasma leptin concentrations observed in the present study after the mixed meal were in contrast with a continued decrease following a fat load. Also, they were the result of a single meal, and similar effects may accumulate over a longer period to cause a substantial change.

From our results, we conclude that the increase in plasma leptin concentrations observed after meals is not simply a result of an energy load, but is in response to a signal that is not present following a fat load without carbohydrate. This is consistent with a number of previous reports demonstrating that carbohydrate-rich foods have a higher satiety effect than fat-rich foods [31,32]. It is also consistent with the findings that serum leptin concentrations are significantly related to short-term changes in dietary carbohydrate intake, but not to changes in dietary fat, protein or total energy intake [33], and that the decrease in plasma leptin concentrations with fasting could be prevented with a euglycaemic clamp [13].

ACKNOWLEDGMENTS

This study was partially supported by the Wellcome Trust.

REFERENCES


Frayn, K. N., Coppack, S. W. and Humphreys, S. M. (1993) Subcutaneous adipose tissue metabolism studied by local catheterization. Int. J. Obesity 17 (Suppl. 3), S18–S21


Dillon, R. S. (1965) Importance of the hematocrit in interpretation of blood sugar. Diabetes 14, 672–674


Received 14 July 2000/27 November 2000; accepted 1 February 2001