A fat-enriched, glucose-enriched diet markedly attenuates adiponectin mRNA levels in rat epididymal adipose tissue

Ebrahim K. NADERALI*, Debora ESTADELLA†, Milagros ROCHA‡, Lucy C. PICKAVANCE*, Sameer FATANI*, Raphael G. P. DENIS* and Gareth WILLIAMS*

*Neuroendocrine and Obesity Biology Unit, Department of Medicine, University of Liverpool, Daulby Street, Liverpool L69 3GA, U.K., †Department of Physiology, Federal São Paulo University, São Paulo, Brazil, and ‡Department of Animal Biology II, Faculty of Biological Sciences, Complutense University, 28040 Madrid, Spain

ABSTRACT

Adiponectin levels are decreased in subjects with obesity, diabetes and coronary artery disease. In the present study, we have investigated whether the decrease in the levels and mRNA expression of adiponectin is due to obesity or to the diet itself. Wistar rats were either fed standard laboratory chow throughout (controls) or given a fat-enriched, glucose-enriched diet (diet-fed) for 2 days or 16 weeks. After 2 days of diet feeding, total body weight, fat pad masses and the plasma levels of glucose, insulin and leptin were all comparable between the two groups, while plasma NEFA (non-esterified fatty acid) and triacylglycerol levels were increased in the diet-fed animals (P < 0.01 for both). There was a marked (P < 0.01) decrease in plasma adiponectin levels. After 16 weeks of diet feeding, diet-fed rats had significantly higher body weight, fat pad mass and plasma levels of leptin, adiponectin, NEFA and triacylglycerol (P < 0.001 for all) compared with chow-fed controls, whereas plasma levels of glucose and insulin were similar in the two groups. After 2 days of diet feeding, there were no significant changes in Ob mRNA levels in epididymal fat, whereas there was a marked decrease in adiponectin mRNA levels. After 16 weeks of diet feeding, rats had significantly increased levels of Ob mRNA, but decreased adiponectin mRNA levels, in epididymal fat compared with the chow-fed group (P < 0.001 for both). These findings suggest that obesity per se is not a factor in the decreased adiponectin levels observed in obese subjects. We propose that the lipid profile of the plasma and/or the constituents of the diet consumed by rats may contribute to adiponectin levels more than obesity per se.

INTRODUCTION

Obesity is strongly associated with increased cardiovascular morbidity and mortality [1,2]. It is now well accepted that adipose tissue is not simply an energy storage organ, but also functions as an endocrine organ, producing a variety of biologically active molecules, which can exert metabolic and vascular actions. These molecules include leptin [3], tumour necrosis factor-α [4], plasminogen activator inhibitor type 1 [5] and adiponectin [6–8].

The adipocyte-derived hormone adiponectin [6–8] has been shown to play important roles in insulin sensitivity and determining the levels of circulating plasma lipids, and its experimental administration decreases plasma glucose and NEFA (non-esterified fatty acid) levels [9,10], while increasing fatty acid utilization in muscle [11]. Recent reports point to anti-atherogenic properties of

Key words: adiponectin mRNA, diet, obesity, plasma adiponectin level.
Abbreviations: NEFA, non-esterified fatty acid(s).
Correspondence: Dr E. K. Naderali (e-mail naderali@liverpool.ac.uk).
adiponectin. Adiponectin inhibits monocyte adhesion to endothelial cells and lipid accumulation in human monocyte-derived macrophages *in vitro* [12,13]. Since adiponectin is mainly synthesized by and released from white adipose tissue, it may be expected that its expression in adipocytes would increase in conditions such as obesity. However, circulating levels of adiponectin are paradoxically decreased in human obese subjects [14] and in those with the metabolic syndrome [15], and its plasma levels inversely predict cardiovascular events [16].

We have shown previously that a fat-enriched, glucose-enriched diet induces marked metabolic and vascular malfunctions in the presence or absence of obesity in animal models [17,18]. In the present study, we aimed to investigate the effects of diet and diet-induced obesity on adiponectin production in Wistar rats.

**MATERIALS AND METHODS**

**Animals**

In experiment 1 (short-term diet feeding), adult male Wistar rats (*n* = 12) were randomly assigned to a control group (*n* = 6) or a diet-fed group (*n* = 6). In experiment 2 (long-term diet feeding), adult male Wistar rats (*n* = 16) were randomly assigned to a control group (*n* = 8) or a diet-fed group (*n* = 8). All had free access to water and were housed individually under controlled environmental conditions (19–22 °C; 30–40 % humidity) and a 12 h light/dark cycle (lights on at 07:00 hours). Control groups were fed on standard pelleted laboratory chow (CRM Biosure, Cambridge, U.K.), which provides 60 % of energy as carbohydrate, 30 % as protein and 10 % as fat (by weight). ‘Diet-fed’ groups had free access to a fat-of-energy as carbohydrate, 30 % as protein and 10 % as fat (by weight). The total energy content of the chow diet was 13.1 kJ/g, whereas that of the palatable diet was 10.1 kJ/g [18]. Animals in experiment 1 were studied for 2 days, while animals in experiment 2 were studied for 16 weeks.

The rats were killed by CO₂ inhalation, and the epididymal and perirenal fat pads and the gastrocnemius muscle were dissected and weighed. Blood was collected by cardiac puncture into cold heparinized tubes. The plasma was immediately separated by centrifugation before being frozen at −40 °C for later measurements of blood analytes. The plasma glucose concentration was determined using a glucose oxidase method (Boehringer Mannheim), whereas NEFA and triacylglycerol levels were measured using commercial diagnostic kits (Roche Diagnostic and Sigma Diagnostic respectively). Insulin, leptin and adiponectin concentrations were measured using RIA kits (Pharmacia/Upjohn Diagnostics and Linco Research, Biogenesis).

All animal procedures accorded with University of Liverpool guidelines and current U.K. legislation.

**RNA isolation and Northern blotting**

Adiponectin and leptin mRNA levels in epididymal adipose tissues were measured by Northern blotting using oligonucleotide probes in conjunction with a chemiluminescence procedure [19]. Total RNA was extracted from each epididymal fat depot using Trizol® (Gibco) according to the manufacturer’s instructions. The RNA concentration was determined from the absorbance at 260 nm. Aliquots of 20 µg of RNA were fractionated by size on a 1 % (w/v) agarose/formaldehyde gel, blotted on to a positively charged membrane (Roche) overnight and then cross-linked under UV light. The membrane was pre-hybridized in Easyhyb® solution (Roche) at 42 °C for 1 h and hybridized in the same solution with a digoxigenin end-labelled 30-mer antisense oligonucleotide probe for rat adiponectin or leptin at 42 °C overnight. The sequence of the antisense oligonucleotide probe employed for adiponectin was 5′-GTTCAGTGGAATTAGCCAGTGCTGCGTCA-3′; the sequences of the oligonucleotides for leptin and 18 S rRNA were as described previously [20]. Following post-hybridization washes, the membrane was incubated with an antibody against digoxigenin (Fab fragment; Roche) for 30 min and then with the chemiluminescence substrate CDP-Star (0.25 mM; Tropix) for 10 min at room temperature. Signals were collected by exposure of the membrane to X-ray film for 15–20 min at room temperature.

The blots were stripped and re-probed sequentially with digoxigenin end-labelled oligonucleotides for leptin mRNA and 18 S rRNA. Autoradiographs were quantified by densitometry with image-analysis software (NIH Image). The abundance of a specific mRNA was expressed as the ratio of the target mRNA/18 S rRNA signals.

**Data interpretation and statistical analysis**

Normal distribution of data was tested using the Shapiro Wilk W test. Statistical significance was tested using Student’s *t* test or repeated-measures ANOVA with the Bonferroni correction, as appropriate. Results were considered statistically significant at the *P* < 0.05 level.

**RESULTS**

**Metabolic data**

In experiment 1, after 2 days of diet feeding, there were no significant differences in body weight, epididymal and perirenal fat pad masses or gastrocnemius mass, or plasma levels of glucose, insulin or leptin between the two groups. Nonetheless, the diet-fed group had significantly
Table 1  Physiological and metabolic characteristics of rats fed over the short term or the long term on either standard chow or a fat-enriched, glucose-enriched diet

Data are means ± S.E.M. for n = 6 in experiment 1 and n = 8 in experiment 2. NS, not significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1 (2 days of diet feeding)</th>
<th>Experiment 2 (16 weeks of diet feeding)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chow-fed</td>
<td>Diet-fed</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>171 ± 2</td>
<td>172 ± 3</td>
</tr>
<tr>
<td>Final</td>
<td>176 ± 3</td>
<td>177 ± 2</td>
</tr>
<tr>
<td>Epididymal fat-pad mass (g)</td>
<td>0.75 ± 0.03</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>Perirenal fat-pad mass (g)</td>
<td>2.17 ± 0.05</td>
<td>2.16 ± 0.08</td>
</tr>
<tr>
<td>Gastrocnemius muscle mass (g)</td>
<td>1.60 ± 0.04</td>
<td>1.61 ± 0.03</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>10.10 ± 0.65</td>
<td>10.25 ± 0.85</td>
</tr>
<tr>
<td>Plasma insulin (µ-units/ml)</td>
<td>7.36 ± 0.05</td>
<td>7.39 ± 0.90</td>
</tr>
<tr>
<td>Plasma leptin (ng/ml)</td>
<td>1.60 ± 0.48</td>
<td>1.63 ± 0.37</td>
</tr>
<tr>
<td>Plasma triacylglycerols (mM)</td>
<td>0.58 ± 0.03</td>
<td>0.78 ± 0.07</td>
</tr>
<tr>
<td>Plasma NEFA (mM)</td>
<td>0.24 ± 0.03</td>
<td>0.49 ± 0.05</td>
</tr>
<tr>
<td>Plasma adiponectin (µg/ml)</td>
<td>42.3 ± 4.2</td>
<td>30.5 ± 2.5</td>
</tr>
</tbody>
</table>

Figure 1  Effects of chow and fat-enriched, glucose-enriched diets on levels of Ob mRNA (upper panels) and adiponectin mRNA (lower panels) in rat epididymal fat pads

The left panels are representative Northern blots, and the right panels are quantitative analysis of the area under the curve (AUC) intensity of Northern blotting. C, control; diet, fat-enriched, glucose-enriched diet. Data are presented as means ± S.E.M. for n = 8 in each group for the long-term experiment (16 weeks) and n = 6 in each group for the short-term experiment (2 days).

Higher levels of NEFA (P < 0.01) and triacylglycerols (P < 0.05) than the chow-fed control group, whereas plasma levels of adiponectin were significantly (P < 0.01) lower than in the chow-fed counterparts (Table 1).

In experiment 2, after 16 weeks of diet feeding, there were significant increases in body weight (P < 0.0001), epididymal and perirenal fat masses (P < 0.0001 for both), but not gastrocnemius mass, compared with the chow-fed control group (Table 1). In addition, the diet-fed rats had significantly higher plasma levels of leptin (P < 0.0001), adiponectin (P < 0.0001), NEFA (P < 0.01) and triacylglycerols (P < 0.01), while glucose and insulin concentrations were comparable between the two groups (Table 1).

Adiponectin and leptin mRNA levels in epididymal adipose tissue

Ob mRNA levels

Northern blotting of epididymal adipose tissue obtained from rats in experiment 1 (diet feeding for only 2 days), indicated that Ob mRNA levels were comparable between the two groups (P = 0.9466; Figure 1). However, Ob
mRNA levels in animals that were diet-fed for 16 weeks were significantly ($P < 0.0001$) higher than in their chow-fed counterparts (Figure 1). Statistical analysis showed that older animals had significantly higher Ob mRNA levels in the epididymal adipose tissue than their younger counterparts. Thus chow-fed animals in experiment 2 had significantly ($P = 0.0093$) higher Ob mRNA levels than the chow-fed group in experiment 1. Likewise, diet-fed animals in experiment 2 had significantly ($P < 0.0001$) higher Ob mRNA levels than diet-fed animals in experiment 1 (Figure 1).

**Adiponectin mRNA levels**

There was a marked and significant ($P = 0.0005$) decrease in adiponectin mRNA levels in epididymal fat from 16-week diet-fed rats compared with their chow-fed control counterparts (Figure 1). Adiponectin mRNA levels were also markedly ($P < 0.0466$) attenuated in rats that were diet-fed for 2 days (Figure 1). Statistical analysis indicated a significant ($P = 0.0006$) increase in adiponectin mRNA levels in chow-fed animals in experiment 2 compared with chow-fed animals in experiment 1. However, there were no significant differences in adiponectin mRNA levels between diet-fed animals in experiments 1 and 2 (Figure 1).

**DISCUSSION**

The exact physiological role of adiponectin is not fully clear as yet. Experimental studies have indicated potential anti-atherogenic and anti-inflammatory properties for adiponectin [13,21,22]. Adiponectin inhibits tumour necrosis factor $\alpha$ production and has direct effects on vascular adhesion molecules [13,21,22], and thus it could act to prevent the development of vascular disease.

Hypoadiponectinaemia in obesity and Type II diabetes has been suggested to be involved in the development of insulin resistance and hyperinsulinaemia [15,23,24]. Adiponectin gene expression is dysregulated and markedly decreased in ob/ob mice [7] and in obese subjects with Type II diabetes [25], suggesting that adiponectin may have a role in the pathogenesis of obesity and Type II diabetes. Adiponectin mRNA levels and its plasma concentration are reduced in obese subjects, but the mechanism underlying the decrease in adiponectin production in obesity remains unknown. Matsubara et al. [26] have postulated that the hyperleptinaemia and/or the excess of adipose tissue in obesity might cause 'leptin resistance', resulting in a subsequent decline in adiponectin production. These hypotheses suggest a negative feedback mechanism for adiponectin production within adipose tissue.

In the present study, 16 weeks of diet feeding of rats resulted in pronounced obesity, signified by a 2-fold increase in fat pad masses and a $>3$-fold increase in plasma leptin concentration. Northern blotting of epididymal adipose tissues from obese rats showed that Ob mRNA levels were markedly increased; in contrast, there was a marked decrease in adiponectin mRNA levels, indicating a pronounced down-regulation of adiponectin mRNA expression in dietary-obese rats. Despite this, dietary-obese rats had significantly higher plasma levels of adiponectin than their chow-fed counterparts. This finding is in contrast with a recently published report, where inverse relationships between plasma adiponectin and leptin levels in non-diabetic normal-weight and obese humans [26] were shown. Taken together, our findings and reports by others [7,15,23–27] suggest that, in dietary-obese rats, a decrease in adiponectin mRNA levels does not translate to a parallel decrease in plasma adiponectin concentration. Consequently, the notion that either hyperleptinaemia and/or an excess of adipose tissue may attenuate adiponectin production does not give an accurate picture of the changes in the adiponectin cycle in dietary-induced obese rats [27]. Moreover, an unexpected higher level of plasma adiponectin in the face of decreased adiponectin mRNA levels in dietary-obese rats underlines the possible existence of post-transcriptional control mechanism(s) for circulating adiponectin levels in obese rats. This may involve decreased metabolism and/or excretion of circulating adiponectin proteins under non-physiological conditions such as obesity and essential hypertension [28]. Mallamaci et al. [28] reported that hypertensive men had significantly higher plasma adiponectin levels than their body-weight-matched normotensive counterparts. This finding led these authors to speculate that higher levels of adiponectin in disease states such as essential hypertension may be the expression of a counter-regulatory response aimed at mitigating endothelial damage and cardiovascular risk associated with high arterial pressure [13,21,22]. However, this hypothesis remains to be investigated further.

In rats fed for only 2 days on the fat-enriched, glucose-enriched diet, which increased only terminal plasma levels of NEFA and triacylglycerols without having any noticeable effects on total body weight, fat pad masses and/or leptin levels, there was a significant reduction in adiponectin mRNA, whereas no changes were seen in Ob mRNA levels. Interestingly, unlike long-term diet-fed rats, short-term diet-fed rats had significantly lower concentrations of adiponectin than their chow-fed counterparts. Hence our data, in agreement with others [27], rule out any significant association between total body weight, fat pad mass and plasma leptin levels and the attenuation in adiponectin production, at least in rats. Collectively, our data point to a role for lipids, in particular triacylglycerols and NEFA, in regulating adiponectin expression and secretion. Similar findings have also been reported in human subjects, indicating a negative correlation between plasma adiponectin and...
triacylglycerol levels, independent of body mass index [24,29], underlining a possible role for triacylglycerols in adiponectin production and/or metabolism. Thus it is possible that high levels of triacylglycerol somehow dysregulate adiponectin mRNA expression, while other factors may be involved in its metabolism. This hypothesis is further strengthened by the fact that the insulin-sensitizing agent rosiglitazone, which increases adipose tissue mass while reducing plasma lipid levels [30], markedly increases plasma adiponectin levels [9,31], suggesting a positive effect of a decrease in plasma lipids on adiponectin production. Nonetheless, we cannot rule out the possibility that the improvement in insulin sensitivity caused by these agents has a positive effect on adiponectin production.

Another very recent study has reported a small decrease in adiponectin mRNA expression in rat white adipose tissues 3 h after a high-sucrose meal [32]. Unlike our present study, the decrease in adiponectin mRNA was not statistically significant, which may have been due to the shorter time scale of the study (3 h, compared with 2 days and 16 weeks in our study); nonetheless, this result underlines a possible role for the diet and its constituents in regulating adiponectin expression.

We therefore conclude that, firstly, adiponectin mRNA levels in rat epididymal adipose tissue are not dependent on total body weight. Secondly, either plasma triacylglycerol and NEFA levels or the composition of adipose tissue, thereby dysregulating adiponectin production and/or its clearance.

ACKNOWLEDGMENTS

E. K. N. is a British Heart Foundation Research Fellow. D. E. was supported by the Brazilian agency CAPES. Probes for Ob and adiponectin mRNAs were generously donated by Professor P. Trayhurn (Neuroendocrine and Obesity Biology Unit, University of Liverpool, U.K.).

REFERENCES

19 Trayhurn, P. and Duncan, J. S. (1994) Rapid chemiluminescent detection of the mRNA for uncoupling protein in brown adipose tissue by northern hybridization with a 32-mer oligonucleotide endlabelled with digoxigenin. Int. J. Obesity Relat. Metab. Disord. 18, 449–452


