THE EFFECTS OF WEIGHT CYCLING ON SERUM LEPTIN LEVELS AND LIPOGENIC ENZYME ACTIVITIES IN ADIPOSE TISSUE

Department of Biochemistry, Medical University of Gdansk, Poland

Weight cycling is one of the widely used weight reduction strategies; however, the adverse effects of this method include regaining significant amounts of weight. The molecular mechanisms underlying weight gain following cycles of dietary deprivation and refeeding are still poorly understood. One of the possibilities is that repeated loss and gain of weight may promote fat deposition in adipose tissue. To test this hypothesis we investigated serum leptin levels and lipogenic enzyme activities in white adipose tissue (WAT) of male Wistar rats during 12 days of ad libitum feeding following multiple cycles of alternating food deprivation and refeeding. Rats subjected to eight cycles of food deprivation and refeeding (MFR group) showed significantly decreased circulating leptin levels when compared with control rats (nearly 50% decrease in leptin levels, P < 0.01). Throughout 12 days of ad libitum feeding, serum leptin levels increased modestly but remained significantly (24%, P < 0.05) lower than control levels. Fatty acid synthase (FAS) and malic enzyme (ME) activities (chosen as representatives of enzymes directly involved in fatty acid synthesis) were found to be considerably higher in WAT of MFR rats refed for 3 days in comparison to control rats, and remained elevated even after 12 days of refeeding. These observations suggest that the elevation of lipogenic enzyme activities induced by multiple cycles of dietary deprivation followed by refeeding persists for several days, markedly increasing the lipogenic capacity of adipose tissue, which, accompanied by a decrease in circulating leptin levels, may promote weight gain.

Key words: weight cycling, food restriction, refeeding, leptin, lipogenic enzymes, fatty acid synthase, malic enzyme, white adipose tissue, rat

INTRODUCTION

Obesity is a major risk factor for metabolic disorders such as diabetes, hypertension and atherosclerosis (1). Due to an increasing interest in the
prevention and treatment of obesity several weight reduction strategies have been developed, with the so called “yo-yo” dieting being the most prevalent. Despite the apparent widespread use of this approach, its effectiveness and health benefits remain controversial. Numerous studies have demonstrated that patients lose weight during dieting, then slowly gain it back even over their previous weight (2, 3). The underlying mechanisms contributing to weight regaining are far from being clear. One of the possibilities is that repeated loss and gain of weight may promote fat deposition in adipose tissue (3).

Triacylglycerols stored in adipose tissue originate either from the diet or from de novo synthesis. Fatty acid synthase (FAS) plays a central role in de novo lipogenesis in mammals, catalysing the synthesis of fatty acids from acetyl-CoA and malonyl-CoA as substrates and NADPH as the reducing equivalent (4 - 6). NADPH is generated by malic enzyme (ME) and the pentose phosphate shunt (6). As for many other lipogenic genes involved in the maintenance of energy balance, the expression of FAS- and ME-encoding genes in adipose tissue is highly dependent on nutritional conditions (6 - 8). We have previously reported that the long-term dietary manipulation, such as multiple cycles of food deprivation and refeeding, is associated with a dramatic increase in lipogenesis and concomitant up-regulation of genes involved in fatty acid synthesis in rat white adipose tissue (9, 10). These data revealed a potent pro-lipogenic effect of repeated dieting on adipose tissue metabolism.

In addition to its role as a specialised store of body fat, adipose tissue is considered to be the major source of circulating leptin, a cytokine-like hormone involved in appetite control and metabolic responses to nutrients (11, 12). Leptin is synthesized and secreted by adipocytes in response to increased energy storage in adipose tissue (12, 13). It has been established that leptin signals body energy status to the hypothalamus where it inhibits the synthesis and release of orexigenic peptides, thus reducing food intake (13). The effects of leptin on food intake have been documented in rodents and primates (14, 15).

To test the hypothesis that weight cycling may enhance the lipogenic capacity of adipose tissue and stimulate fat deposition, we investigated serum leptin levels and lipogenic enzyme (FAS and ME) activities in adipose tissue during twelve days of ad libitum feeding subsequent to cycles of alternating food deprivation and refeeding.

**MATERIALS AND METHODS**

*Animals and dietary manipulation*

Fifty-five male Wistar rats weighing 232 ± 10 g at the start of the experiment were housed individually in wire-mesh cages at 22°C in an animal room with a 12:12-h light-dark cycle (08:00 to 20:00). All animals were given ad libitum access to water and were fed a commercial rodent diet (9). The rats were randomly assigned to eleven experimental groups of 5 animals each. The
scheme of the experiment is shown in Fig. 1. One group of rats (F) was deprived of food for 3 days. Four groups were subjected to 3-day food deprivation and subsequently refed for three (F+3R), six (F+6R), nine (F+9R) or twelve days (F+12R). Rats from MFR group were subjected to eight food deprivation and refeeding cycles, consisting of 3 days of food deprivation followed by 3 days of refeeding each. Another four groups of rats were subjected to eight food deprivation-refeeding cycles and, after the eighth period of food deprivation, refed for three (MFR+3R), six (MFR+6R), nine (MFR+9R) or twelve days (MFR+12R). In the control group (C), rats were given *ad libitum* access to food. The rats were killed by cervical dislocation between 08:00 and 10:00, and their trunk blood was collected. Epididymal adipose tissue was taken from each experimental group and used for enzyme analysis. The experimental protocol was approved by the Local Animal Ethics Committee.

*Fig. 1. The scheme of the experiment. The dark grey boxes represent 3 days of food deprivation, while the light grey boxes indicate 3 days of *ad libitum* feeding.*
Leptin and insulin radioimmunoassay

Serum leptin and insulin levels were measured with rat-specific radioimmunoassay kits (Linco Research, St. Charles, USA). Assays were performed according to the manufacturer’s instructions.

Enzyme assays

One gram of white adipose tissue was homogenised in 7 ml of ice-cold homogenising buffer (25 mM Tris-HCl pH 7.8, 0.2% Triton X-100). The homogenate was centrifuged at 30,000 g at 4°C for 20 min. The resulting pellet was homogenised in 5 ml of homogenising buffer and centrifuged again. Both supernatants obtained were pooled and were immediately used for enzyme assays.

The activity of FAS (EC 2.3.1.85) was measured spectrophotometrically by monitoring the rate of NADPH oxidation, according to the method of Nepokroeff et al. (16) with modifications. Briefly, 930 μl of the reaction mixture (100 mM potassium phosphate buffer pH 7.0, 175 μM NADPH, 125 μM acetyl-CoA, 3 mM DTT) was pre-incubated at 37°C with 50 μl of supernatant for 5 min. The reaction was initiated by the addition of 20 μl of malonyl-CoA (final concentration 25 μM) and the oxidation of NADPH was followed at 340 nm at 37°C for 5 min using Beckman DU 68 spectrophotometer (Beckman Instruments, Fullerton, USA). A correction was made for the rate of NADPH oxidation in the absence of malonyl-CoA as a background.

ME (EC 1.1.1.40) activity was estimated by the addition of 50 μl of supernatant into 950 μl of assay buffer (50 mM Tris-HCl pH 7.8, 10 mM L-malate, 250 μM NADP+ and 1 mM MnCl₂). The reduction of NADP+ was followed at 340 nm at 37°C for 5 min using Beckman DU 68 spectrophotometer. The background activity was measured in the absence of L-malate according to the method described previously (17).

Determination of serum glucose and triacylglycerol levels

Glucose concentration in serum was determined by a spectrophotometric method using hexokinase and glucose 6-phosphate dehydrogenase (Alpha Diagnostics, Warszawa, Poland). Serum triacylglycerol levels were measured by an enzymatic method (Roche Diagnostics, Mannheim, Germany).

Statistical analysis

The results are presented as means ± standard error of the mean (S.E.M.). The statistical significance of the differences between groups was assessed by one-way analysis of variance (ANOVA). An unpaired Student’s t-test or a Kruskal-Wallis test was used to compare the groups. P < 0.05 was considered as a significant difference.

RESULTS

Changes in body weight and epididymal adipose tissue mass after multiple cycles of food deprivation and refeeding

We examined changes in body weight and epididymal adipose tissue mass in rats deprived of food for 3 days (F) and subsequently refed for 3, 6, 9 and 12 days; as well as in rats subjected to eight cycles of food deprivation and refeeding prior to ad libitum feeding (MFR). As can be seen from the Table 1, F rats lost some
In the course of subsequent refeeding F rats were gaining weight (F+3R weighted 310 ± 22 g, F+6R: 314 ± 10 g, F+9R: 329 ± 11 g) and after 12 days their body weight reached control values (334 ± 7 g). Rats in the MFR group showed a greater reduction in body weight when compared with the control group (232 ± 8 g versus 334 ± 18 g, P < 0.001). Furthermore, although they have been gaining weight during ad libitum feeding (MFR+3R weighted 246 ± 19 g, MFR+6R: 258 ± 21 g, MFR+9R: 279 ± 16 g), the body weight of the MFR+12R rats remained lower than that of the control rats (288 ± 19 g versus 334 ± 18 g, P < 0.01).

Three days of food deprivation resulted in a decrease in epididymal white adipose tissue mass (1.69 ± 0.13 g versus 3.57 ± 0.19 g, P < 0.001; compared with control). However, within the subsequent 12 days of ad libitum feeding WAT mass increased progressively (1.93 ± 0.19 g in the F+3R, 2.39 ± 0.14 g in the F+6R and 2.83 ± 0.13 g in the F+9R rats) and returned to control values (3.77 ± 0.30 g in the F+12R rats). Eight cycles of food deprivation and refeeding had significant effect on adipose tissue mass, which decreased to 1.20 ± 0.15 g in the MFR rats. Although in the course of ad libitum feeding a continuous increase in WAT mass was found (1.64 ± 0.06 g in the MFR+3R, 2.33 ± 0.07 g in the MFR+6R and 2.60 ± 0.15 g in the MFR+9R rats), it remained below the control values after 12 days of refeeding (2.85 ± 0.16 g in the MFR+12R rats, P < 0.05).

**Effects of multiple cycles of food deprivation and refeeding on serum insulin and glucose levels**

Serum insulin and glucose concentrations were determined by radioimmunoassay and an enzymatic method, respectively. Compared with control rats, F+3R rats showed significantly elevated serum insulin levels: 5.23 ± 1.77 ng/ml versus 2.38 ± 0.57 ng/ml, P < 0.05 (Table 1). In MFR+3R rats, the increase in serum insulin was even more pronounced (7.82 ± 1.19 ng/ml versus 2.38 ± 0.57 ng/ml in the MFR+3R group and control group, respectively; P < 0.001). In F+3R and MFR+3R group, serum insulin levels returned to control

### Table 1. Body and WAT mass of rats and serum metabolite and hormone levels

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>F+3R</th>
<th>MFR+3R</th>
<th>F+12R</th>
<th>MFR+12R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass [g]</td>
<td>334±18</td>
<td>310±22</td>
<td>246±19***</td>
<td>334±7</td>
<td>288±19**</td>
</tr>
<tr>
<td>WAT mass [g]</td>
<td>3.57±0.19</td>
<td>1.93±0.18***</td>
<td>1.65±0.06***</td>
<td>3.77±0.30</td>
<td>2.85±0.16*</td>
</tr>
<tr>
<td>Serum triacylglycerols [mg/dl]</td>
<td>133.9±9.3</td>
<td>75.1±3.2***</td>
<td>78.1±3.5***</td>
<td>116.6±8.8</td>
<td>91.5±5.6**</td>
</tr>
<tr>
<td>Serum cholesterol [mg/dl]</td>
<td>67.7±2.5</td>
<td>73.9±2.6</td>
<td>72.0±2.1</td>
<td>65.8±3.9</td>
<td>66.0±4.2</td>
</tr>
<tr>
<td>Serum insulin [ng/ml]</td>
<td>2.38±0.57</td>
<td>5.23±1.77*</td>
<td>7.82±1.19***</td>
<td>2.19±0.29</td>
<td>2.20±0.47</td>
</tr>
<tr>
<td>Serum glucose [mmol/l]</td>
<td>6.85±0.23</td>
<td>6.87±0.24</td>
<td>6.63±0.26</td>
<td>6.94±0.19</td>
<td>6.95±0.22</td>
</tr>
</tbody>
</table>

Data are reported as means ± S.E.M., *P < 0.05, ***P < 0.001 compared to control.
values throughout the subsequent 12 days of *ad libitum* feeding. There were no significant differences in circulating glucose levels between the groups (serum glucose: 6.85 ± 0.23 mmol/l in the control group versus 6.87 ± 0.24 mmol/l and 6.63 ± 0.26 mmol/l in F+3R and MFR+3R group, respectively).

**Effects of multiple cycles of food deprivation and refeeding on circulating leptin levels**

To determine whether cycles of food deprivation and refeeding had any effect on circulating leptin, we analysed serum leptin levels by radioimmunoassay. Three days of food deprivation reduced circulating leptin levels from 3.33 ± 0.13 ng/ml in control rats to 1.89 ± 0.07 ng/ml in F rats, *P < 0.01* (*Fig. 2*). Although leptin levels steadily increased during refeeding, reaching 2.52 ± 0.15 ng/ml in F+3R rats and 2.71 ± 0.10 ng/ml in F+6R rats, they were lower than those in control rats. Serum leptin levels returned to control values after 9 days of *ad libitum* feeding.

Rats subjected to eight cycles of food deprivation and refeeding showed significantly decreased circulating leptin levels when compared with control rats.

![Fig. 2. Circulating leptin in rats subjected to 8 cycles of food deprivation and refeeding (MFR group) and MFR rats fed 3, 6, 9, and 12 days (■) compared to serum leptin levels in rats subjected to 3 days of food deprivation and then refed for 3, 6, 9, and 12 days (○). Data are reported as means ± S.E.M., *P < 0.05, **P < 0.01 compared to control.](image-url)
nearly 50% decrease in leptin levels, P < 0.01). Throughout 12 days of *ad libitum* feeding, serum leptin levels increased modestly from 2.26 ± 0.08 ng/ml in MFR+3R to 2.55 ± 0.27 ng/ml in MFR+12R but remained significantly (24%, P < 0.05) lower than control levels.

**FAS activity in rat adipose tissue after repeated dieting**

Fatty acid synthase activity was measured in homogenates prepared from epididymal white adipose tissue of rats subjected to 3 days of food deprivation, 3 days of food deprivation followed by 3, 6, 9 and 12 days of refeeding, eight food deprivation-refeeding cycles and eight food deprivation-refeeding cycles followed by 3, 6, 9 and 12 days of refeeding.

Compared with the control group, rats subjected to 3 days of food deprivation showed a dramatic (60%, P < 0.05) decrease in FAS activity in adipose tissue (Fig. 3). Three successive days of refeeding led to a substantial increase in the activity of FAS - in adipose tissue of F+3R rats it was 2-fold higher than that measured in WAT of control rats. Through the course of subsequent refeeding

![Fig. 3. FAS activity in adipose tissue of rats subjected to 8 cycles of food deprivation and refeeding (MFR group) and MFR rats fed 3, 6, 9, and 12 days (■) compared to FAS activity in adipose tissue of rats subjected to 3 days of food deprivation and then refeed for 3, 6, 9, and 12 days (○). Data are reported as means ± S.E.M., *P < 0.05, **P < 0.01 compared to control.](image-url)
FAS activity in adipose tissue declined progressively and, after 12 days of feeding, returned to control values.

Eight cycles of food deprivation and refeeding resulted in a significant increase in FAS activity in rat WAT. FAS activity in adipose tissue of MFR rats, measured after the eighth period of food deprivation, was by 47% higher than that found in WAT of control rats (Fig. 3). Moreover, the activity of FAS in MFR rats rose dramatically during the first 3 days of refeeding, to the level 9-fold higher than that in adipose tissue of control rats. Fatty acid synthase activity decreased slightly during refeeding, however until ninth day of feeding it remained 6-fold higher than control values. Even after 12 days of ad libitum feeding FAS activity in adipose tissue of rats subjected previously to eight cycles of food deprivation-refeeding remained significantly (3-fold, P < 0.01) higher in comparison to that measured in WAT of rats subjected to one cycle of food deprivation-refeeding and that measured in adipose tissue of control rats.

**ME activity in rat adipose tissue after repeated dieting**

ME activity was measured in homogenates prepared from epididymal white adipose tissue of rats subjected to 3 days of food deprivation, 3 days of food deprivation followed by 3, 6, 9 and 12 days of refeeding, eight food deprivation-refeeding cycles and eight food deprivation-refeeding cycles followed by 3, 6, 9 and 12 days of refeeding.

Throughout the course of the study, the activity of malic enzyme in adipose tissue followed a similar pattern to that of fatty acid synthase: it decreased as a result of food deprivation and increased considerably after refeeding (Fig. 4). In adipose tissue of rats subjected to 3 days of food deprivation ME activity decreased by 33% (P < 0.05, compared with the control group). During the subsequent 3 days of refeeding the activity of ME increased markedly (to 3.36-fold above control); however, it returned to control values by the 9 to 12 days of ad libitum feeding.

In adipose tissue of rats exposed to multiple cycles of alternating food deprivation and refeeding followed by 3 days of feeding ad libitum (MFR+3R group) ME activity increased 10-fold in comparison to that measured in WAT of control rats (Fig. 4). Although the activity of ME in adipose tissue of MFR rats decreased progressively during the subsequent 9 days of ad libitum feeding, it remained elevated by 280% (P < 0.01) at the end of the experiment.

**DISCUSSION**

Repeated dieting is one of the widely used weight reduction strategies. However, the potential benefits of this method to obese patients are questionable. The adverse effects of diet-induced weight cycling include regaining significant amounts of weight (2, 3). The molecular mechanisms
underlying weight gain following cycles of dietary deprivation and refeeding are still poorly understood. It is likely that weight cycling may increase fat deposition (lipogenesis) in people who lose and regain weight. In humans as well as in rodents, lipogenesis occurs both in liver and in adipose tissue (18, 19). Moreover, the activity of lipogenic pathway in adipose tissue is highly dependent on nutritional conditions (6 -8). Previously, we have demonstrated a remarkable increase in lipogenesis rate and lipogenic enzyme activities in rat WAT as a result of multiple cycles of starvation-refeeding (9). In the present study, the activities of FAS and ME in rat adipose tissue throughout ad libitum feeding following multiple cycles of food deprivation and refeeding have been determined. FAS plays a key role in lipogenesis while ME contributes to the production of NADPH in the cell, and therefore they have been chosen as representatives of enzymes directly involved in fatty acid synthesis, which are expected to respond to different nutritional conditions. We found that the activities of both enzymes were elevated in adipose tissue of rats from F+3R (deprived of food for 3 days and then refed for 3 days) and MFR+3R group.

Fig. 4. ME activity in adipose tissue of rats subjected to 8 cycles of food deprivation and refeeding (MFR group) and MFR rats fed 3, 6, 9, and 12 days (■) compared to ME activity in adipose tissue of rats subjected to 3 days of food deprivation and then refed for 3, 6, 9, and 12 days (○). Data are reported as means ± S.E.M., *P < 0.05, **P < 0.01 compared to control.
(exposed to multiple cycles of food deprivation and refeeding followed by 3 days of feeding \textit{ad libitum}), significantly exceeding those measured in WAT of control rats (Figs 3 and 4). In F+3R group of rats the activities of both lipogenic enzymes in adipose tissue decreased continuously in the course of the subsequent \textit{ad libitum} feeding and returned to control values by the end of the experiment. In contrast, FAS and ME activities were considerably higher in WAT of rats previously exposed to multiple cycles of alternating food deprivation and refeeding (MFR+3R group) than in F+3R rats and remained elevated for the duration of the experiment. Thus, the elevation of lipogenic enzyme activities induced by multiple food deprivation-refeeding cycles persists for several days, markedly increasing the lipogenic capacity of adipose tissue. Lipogenesis is stimulated by a carbohydrate diet, whereas it is inhibited by fasting. These effects are partly mediated by hormones, which stimulate (insulin) or inhibit (leptin) lipogenesis. Regulation of the critical enzymes in fatty acid synthesis by nutritional and hormonal factors is carried out mainly at the transcriptional level (7, 8, 10). In our study serum insulin levels were significantly increased after cycles of food deprivation and refeeding (Table 1). Since it is well known that insulin stimulates the transcription of the genes encoding FAS and ME, the increase in FAS and ME activities in adipose tissue of MFR rats is probably the result of elevated circulating insulin levels.

Multiple cycles of alternating food deprivation and refeeding had opposite effects on serum insulin and leptin levels. Leptin levels in serum of MFR rats, even in the course of refeeding, were lower than in control rats (Fig. 2) and reflected the size of fat tissue (Table 1). Circulating leptin is mostly derived from adipocytes, where it is synthesized and released in response to increased energy storage in adipose tissue (12, 13). It is well known that serum leptin levels are regulated by adiposity, and they are reduced by the decrease in adipose tissue mass and weight reduction (11, 20, 21). In our study the decrease in serum leptin levels is probably the consequence of the concomitant reduction in adipose tissue mass and a decrease in leptin synthesis and/or secretion from WAT. The decrease in serum leptin levels in MFR rats was associated with the elevation in FAS activity in their adipose tissue (Fig. 5). Recently, Shirai et al. proposed that malonyl-CoA levels may act as a signal of fuel availability to trigger leptin synthesis and/or secretion from adipose cells (22). The inhibition of FAS activity in epididymal adipocytes increased leptin synthesis and release from these cells. In view of Shirai’s results, it seems possible that weight cycling led to a reduction in malonyl-CoA levels in adipose tissue due to elevated activity of FAS, thus decreasing leptin synthesis and/or secretion from adipocytes. Moreover, as shown in Fig. 5, a significant negative correlation was found between FAS activity in adipose tissue and leptin levels in serum ($r = -0.5771$, $P < 0.001$). The negative correlation between FAS activity in adipose tissue and circulating leptin is interesting because FAS-encoding gene has been shown to be a negative target of leptin in rat adipocytes (23, 24). Furthermore, Ceddia et
al. have found a significant decrease in malic enzyme activity in leptin treated adipocytes, suggesting a mechanism by which adipocytes regulate their fat content by an autocrine pathway (25). The inhibitory effect of leptin on lipogenesis may be achieved by down-regulating the expression of genes involved in fatty acid and triglyceride synthesis, as was demonstrated recently by oligonucleotide micro-array analysis (26). Several cycles of food deprivation and refeeding resulted in a long-term decline in serum leptin levels, which may have reduced the suppressive effect of this hormone on lipogenic enzyme gene expression, leading to the increase in FAS and ME activities in adipose tissue of MFR rats. Leptin plays an important role in the regulation of body weight. By stimulating the hypothalamus to release anorexigenic (appetite suppressing) peptides and inhibiting the release of orexigenic peptides leptin decreases food intake and increases energy expenditure. Since most human obesity is diet induced, a decline in leptin serum levels observed after cycles of dietary deprivation followed by refeeding may lead to the increase in food intake, thus promoting weight gain.

From the experiments described above, it appears that the activities of lipogenic enzymes in adipose tissue remain elevated for several days after multiple cycles of alternating food deprivation and refeeding. Thus, cycles of dietary deprivation followed by refeeding enhance the lipogenic capacity of adipose tissue, which, accompanied by a decrease in circulating leptin levels, may contribute to weight gain after weight cycling.

Acknowledgements: This work was supported by the State Committee for Scientific Research Grant 3 P05A 098 23 and ST-41.
REFERENCES


Received: September 15, 2006
Accepted: October 2, 2006

Author’s address Dr Zdzislaw Kochan, Department of Biochemistry, Medical University of Gdansk, ul. Debinki 1, 80-211 Gdansk, Poland. Phone/fax: (+4858) 3491465.
E-mail: kochanz@amg.gda.pl