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**INCREASED LIPOGENIC POTENTIAL OF RAT ADIPOSE TISSUE
AFTER REPEATED DIETING – THE ROLE OF SREBP-1
TRANSCRIPTION FACTOR**

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Abstract: Repeated dieting is one of the methods used for weight reduction; however, its effectiveness is questionable. We developed an experimental, rat model of repeated dieting, which mimics the dietary approach used in the treatment of obesity in humans. In this experimental model, despite the lower caloric intake, decreased body mass and reduced fat stores, the lipogenic potential of adipose tissue increased. We observed a substantial increase in fatty acid synthase (a key lipogenic enzyme) gene expression in rat adipose tissue accompanied by a 9-fold increase in the serum insulin level. Fatty acid synthase gene expression is controlled at the transcriptional level by SREBP-1. In this study, a remarkable increase (24-fold) in SREBP-1 protein amount, parallel to that in fatty acid synthase mRNA level, protein concentration and enzyme activity was observed after multiple cycles of fasting-refeeding. Although it is possible that the interactions between transcription factors are more complex, we propose that the pivotal role in the increase of the lipogenic potential of adipose tissue after repeated dieting may be played by SREBP-1.

Key Words: Repeated Dieting, SREBP-1, Lipogenic Enzymes, Fatty Acid Synthase, Gene Expression, Adipose Tissue, Rat

INTRODUCTION

Repeated dieting is often used by overweight people as a weight-reduction method; however, its effect on health is controversial. Several investigations have demonstrated that patients lose weight during dieting, then slowly gain it back to a level even greater their previous weight [1, 2]. The underlying

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Abbreviations used: FAS – fatty acid synthase; SRE – sterol regulatory element; SREBP – sterol regulatory element binding protein; WAT – white adipose tissue.

mechanisms contributing to the association between repeated dieting and the “yo-yo” pattern of weight regaining are unknown. We developed an experimental, rat model of repeated dieting, which mimics the dietary approach of weight cycling used in the treatment of obesity in humans. After repeated dieting, rats gained weight faster than control animals [3]. Moreover, we observed that during such dietary manipulation, quantitative and qualitative changes occur in rat white adipose tissue (WAT). In general, in the experimental model investigated, despite the lower caloric intake and a reduction in the fat stores, the lipogenic potential of adipose tissue is growing. We found and reported on an unusual, massive increase in lipogenesis rate, lipogenic enzyme activities and lipogenic enzyme mRNA level in adipose tissue [3, 4]. The observed induction of lipogenic enzyme genes was tissue specific and highly coordinated, suggesting an involvement of a common transcription factor in their regulation. Little is known about the factors that could coordinate lipogenic enzyme gene expression during repeated dieting in adipose tissue.

Recently, it has been proposed that sterol regulatory element binding protein-1 (SREBP-1) could be a major adipogenic transcription factor capable of mediating the effects of insulin on glycolytic or lipogenic enzyme genes [5]. SREBP-1 is involved in the regulation of fatty acid synthase (a key lipogenic enzyme) gene expression [5-7]. Thus, the hypothesis that the increased lipogenic potential of adipose tissue, observed during long-term repeated dieting, may be associated with changes in SREBP-1 protein amount was tested. We investigated changes in the protein concentration of this transcription factor and changes in the mRNA level, protein amount and activity of fatty acid synthase (chosen as a representative of the enzymes involved in the lipogenic pathway) in rat white adipose tissue during repeated dieting.

MATERIALS AND METHODS

Animals and dietary manipulation

Male Wistar rats, weighing approximately 230 g at the start of the experiment, were used in these studies. The animals were given *ad libitum* access to water and were fed a commercial rodent diet. The rats were randomly divided into three groups of eight to ten animals each (1C, 8C and the control group). The one fasting-refeeding cycle group (1C) was fasted for 72 h and subsequently refed for 72 h. The rats from the 8C group (the eight fasting-refeeding cycle group) were subjected to eight fasting and refeeding cycles. In the control group, the rats were given *ad libitum* access to food. All the animals were killed by cervical dislocation between 8 and 10 a.m. (the rats from 1C and 8C had just finished a 72 h feeding period), and their trunk blood was collected. Epididymal adipose tissue was taken from each experimental group. Tissue specimens were split into two portions: one portion was used for enzyme analysis and the second portion was immediately frozen in liquid nitrogen. The tissues were stored at -80°C until required for analysis. The experimental protocol was approved by

the Ethics Committee for Animal Experimentation of the Medical University of Gdańsk.

Enzyme activity assay

One gram of white adipose tissue was homogenized at 4°C in 7 ml of homogenizing 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 minutes. The resulting pellet was homogenized in 5 ml of homogenizing buffer and centrifuged again. Both of the obtained supernatants were pooled and immediately used for enzyme assays. The activity of FAS (EC 2.3.1.85) was measured spectrophotometrically at 37°C by monitoring the rate of NADPH oxidation [3].

Northern blot analysis

Total RNA was extracted from rat adipose tissue and subjected to Northern blot analysis. The RNA was size-fractionated by agarose gel electrophoresis and transferred onto a positively charged nylon membrane by capillary blotting. FAS mRNA was detected by hybridization with a 32-mer oligonucleotide probe labelled with digoxigenin at the 5'-end (5'-GAT CAT GTT CAC GTT CCA GGA TCT GGC GCA CT-3') [4]. Hybridization was carried out overnight at 42°C. After exposure to the Kodak XAR film for 5-30 minutes, the membranes were stripped and re-probed for 18S rRNA using a 32-mer digoxigenin-labelled antisense oligonucleotide (5'-CCA TTA TTC CTA GCT GCG GTA TCC AGG CGG CT-3'), as described previously [4]. The chemiluminescent detection of mRNAs on Northern blots was performed with CDP-Star used as a substrate (Roche Diagnostics GmbH, Mannheim, Germany). The intensity of the chemiluminescent signal was quantified using a GS-710 scanning densitometer and Quantity One software (Bio-Rad, Hercules, USA). The values for FAS mRNA were normalized to those for 18S rRNA.

Western blot analysis

Epididymal WAT was homogenized in lysis buffer containing 50 mM Tris-HCl, pH 7.5, 0.2% Triton X-100 and a protease inhibitor mixture. Then, small aliquots of homogenates containing 20 µg of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10% gels (ICN, Costa Mesa, USA) and transferred to Hybond membrane (Amersham Pharmacia Biotech, Little Chalfont, UK). The membranes were subsequently incubated in buffers containing antibodies against SREBP-1, FAS or β-actin. Rabbit polyclonal anti-FAS antibodies were obtained from Dr. I. Dugail (INSERM, Paris, France). The antibody against SREBP-1 (sc-367), reacting with SREBP-1a and SREBP-1c isoforms, and all the other antibodies were from Santa Cruz (La Jolla, USA). The proteins of interest were detected by enhanced chemiluminescence with CDP-Star as a substrate (Roche Diagnostics GmbH, Mannheim, Germany). Protein levels were corrected for differences in gel loading or blotting using the β-actin band as a reference.

Insulin radioimmunoassay

Serum insulin level was measured with a radioimmunoassay kit (Linco Research, St. Charles, USA).

Statistical analysis

The arbitrary densitometric values were normalized based on the mean mRNA or protein levels of the appropriate controls, which were assigned an arbitrary value of one. Statistical analysis of the data was performed using SYSTAT software (Systat Software, Richmond, USA). The results are presented as means \pm 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) followed by the Student's unpaired t-test or by the Mann-Whitney test. The differences between the groups were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

At the beginning of the study, all rats weighed about 230 g; however, after 48 days, the body mass of the rats subjected to repeated dieting (the 8C group) was significantly lower (240 ± 15 g) than that of the control animals (335 ± 20 g) (Fig. 1a). Epididymal adipose tissue mass was also significantly lower in both 1C and 8C rats. Furthermore, the effect of dieting was more pronounced in the animals subjected to 8 cycles of fasting-refeeding (Fig. 1b).

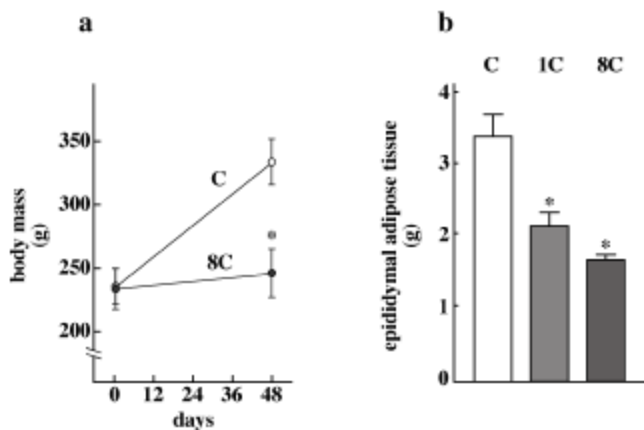


Fig. 1. The effect of repeated cycles of fasting-refeeding on body mass (a) and epididymal adipose tissue mass (b) of control (C), 1C and 8C rats. * $P < 0.01$ as compared to the control.

However, while fed *ad libitum* after dieting, the rats previously subjected to fasting-refeeding cycles gained weight faster than the control animals (5.53 ± 0.33 g/kg/day vs. 2.93 ± 0.54 g/kg/day $P < 0.005$). Moreover, despite the

decreased adipose tissue mass, an increased lipogenic potential of this tissue was observed. The gene for fatty acid synthase – the main lipogenic enzyme – was induced to a remarkable level in this experimental model. The FAS mRNA level increased 15- and 20-fold in the adipose tissue of rats subjected to one and 8 cycles of fasting-refeeding, respectively (Fig. 2a). The long-term regulation of enzyme gene expression involves changes in the concentrations of the regulated enzymes. Indeed, as presented in Fig. 2b, the amount of FAS protein in rat WAT, determined by Western blot analysis with anti-FAS antibody, increased about 17-fold after repeated fasting and refeeding cycles. In addition, repeated dieting resulted in an increase in FAS enzyme activity in the white adipose tissue of rats: 10-fold in the 1C group and 30-fold in the 8C group, compared to the control (not shown).

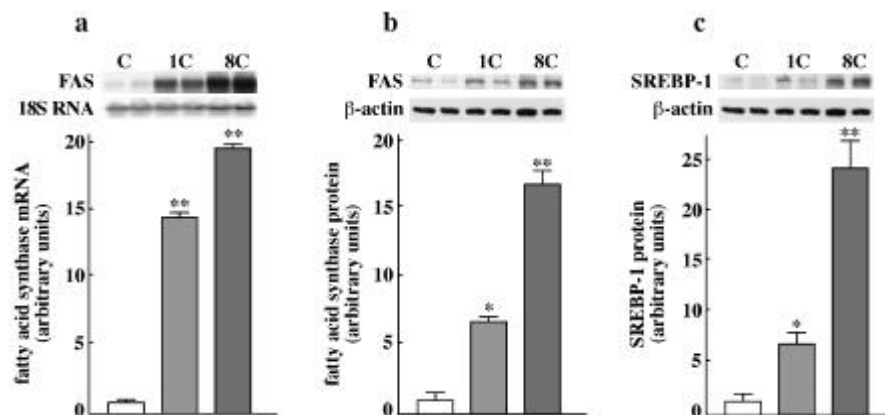


Fig. 2. The effect of repeated cycles of fasting-refeeding on the amounts of FAS mRNA (a), FAS protein (b) and the mature (68 kDa) form of SREBP-1 protein (c) in the white adipose tissue of control (C), 1C and 8C rats. Representative blots are shown. * $P < 0.01$, ** $P < 0.001$ as compared to the control.

Fatty acid synthase gene expression in the liver and adipose tissue has been reported to be controlled at the transcriptional level by SREBP-1c [5, 6]. SREBP-1c belongs to the sterol response element binding protein family of transcription factors, which comprises three members: SREBP-1a and SREBP-1c, alternative transcripts derived from a single gene, and SREBP-2, encoded by a distinct gene [7].

All three isoforms of SREBPs have overlapping functions and regulate lipid synthesis in mammalian tissues. SREBP-2 plays a predominant role in regulating genes involved in the cholesterol biosynthetic pathway, while SREBP-1a and SREBP-1c are more selective for targets involved in fatty acid synthesis [5, 6, 7, 8]. Both isoforms of SREBP-1 have a unique dual DNA-binding specificity and bind to a sterol regulatory element-1 (SRE-1), or to palindromic sequences

called E-boxes, known to be present in the promoter regions of lipogenic enzyme genes [7]. In this study, a remarkable increase in SREBP-1 protein amount in adipose tissue, parallel to that in fatty acid synthase mRNA level, protein concentration and enzyme activity, was observed after 8 cycles of fasting and refeeding (Fig. 2c). The SREBP-1 protein amount increased 24-fold as a result of long-term dietary manipulation. This is the first report presenting increased expression of SREBP-1 in adipose tissue during repeated dieting. The antibody used in this study reacts with both SREBP-1a and SREBP-1c. However, SREBP-1c is the most abundant SREBP isoform in adipose tissue and clearly predominates over SREBP-1a [9]. Moreover, the expression of SREBP-1a is not affected during fasting and refeeding [10]. Thus, we have assumed that the signal obtained in Western blot analysis represented the SREBP-1c protein.

It has been reported previously that in SREBP-1 knockout mice (lacking both SREBP-1a and SREBP-1c), lipogenic enzyme mRNA levels were decreased in the liver but remained normal in adipose tissue [11]. However, the interpretation of this findings may be imprecise, because a compensatory increase in SREBP-2 expression was observed [11]. Lately, the same group reported that SREBP-1c knockout mice had an impaired ability to respond to fasting-refeeding [12]. In recent studies, Horton *et al.* demonstrated that overexpression of SREBP-1a in adipose tissue results in increased cholesterol and fatty acid synthesis, leading to increased lipid accumulation in adipocytes [13]. By contrast, SREBP-1c overexpression in adipose tissue resulted in the disordered differentiation of adipocytes, insulin resistance and diabetes mellitus [14]. These results suggest that SREBP-1a and SREBP-1c have distinct roles in adipocyte lipid metabolism *in vivo*. Recent observations indicate that SREBP-1a contributes to adipogenesis through the activation of lipid synthesis. SREBP-1c, identical to adipocyte determination differentiation dependent factor 1 (ADD1), which was cloned independently by Tontonoz *et al.* [15] from a rat adipocyte cDNA library, is the most abundant SREBP isoform in liver and adipose tissue. It is also a weaker transcription factor than the other SREBPs, with preferential specificity for fatty acid and triacylglycerol synthesis [9]. It seems that the functions of SREBP-1c depend on the state of adipocyte differentiation. Forced overexpression of SREBP-1c in immature adipocytes inhibits adipose tissue differentiation [14], whereas in mature adipocytes this transcription factor regulates lipid metabolism [5, 7]. It has been reported that SREBP-1c is an important downstream target of insulin [16]. The regulation of its proteolysis and enhanced transcription in response to insulin have been documented [16, 17]. In this study, after fasting-refeeding cycles, in spite of the decrease in body and adipose tissue mass, the concentration of serum insulin was elevated: 0.51 ± 0.18 ng/ml in the control group vs. 4.75 ± 0.28 ng/ml in the 8C group. This significantly increased serum insulin level is probably the cause of the increased expression of SREBP-1 during dieting.

It is known that lipogenic enzymes in adipose tissue are dependent on nutritional conditions and plasma insulin level [18, 19]. The precise response to hormones

during repeated dieting may require several regulatory components. Recently published data indicate that overexpression of SREBP-1c does indeed play an important role in the dietary induction of lipogenic enzymes, but is insufficient by itself to account for the full induction observed after high insulin treatment [20]. It has been proposed that the induction of lipogenic enzyme genes after simultaneous addition of insulin and glucose may be a result of the common action of SREBP-1c and carbohydrate responsive element-binding protein (ChREBP) [21]. Although it is possible that the interactions between transcription factors are more complex, we propose that the pivotal role in the increase of the lipogenic potential of adipose tissue after repeated dieting may be played by SREBP-1c. Further studies are necessary to clarify the role of SREBP-1a and SREBP-1c in the induction of lipogenic enzyme mRNAs in adipose tissue.

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REFERENCES

1. Brownell, K.D. and Rodin, J. Medical, metabolic, and psychological effects of weight cycling. *Arch. Intern. Med.* **154** (1994) 1325-1330.
2. Rodin, J., Radke-Sharpe, N., Rebuffe-Scrive, M. and Greenwood, M.R. Weight cycling and fat distribution. *Int. J. Obes.* **14** (1990) 303-310.
3. Kochan, Z., Karbowska, J. and Swierczynski, J. Unusual increase of lipogenesis in rat white adipose tissue after multiple cycles of starvation-refeeding. *Metabolism* **46** (1997) 10-17.
4. Karbowska, J., Kochan, Z. and Swierczynski, J. Increase of lipogenic enzyme mRNA levels in rat white adipose tissue after multiple cycles of starvation-refeeding. *Metabolism* **50** (2001) 734-738.
5. Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S. and Yamada, N. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J. Biol. Chem.* **274** (1999) 35832-35839.
6. Boizard, M., Le Liepvre, X., Lemarchand, P., Foufelle, F., Ferre, P. and Dugail, I. Obesity-related overexpression of fatty-acid synthase gene in adipose tissue involves sterol regulatory element-binding protein transcription factors. *J. Biol. Chem.* **273** (1998) 29164-29171.
7. Osborne, T.F. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J. Biol. Chem.* **275** (2000) 32379-32382.

8. Pai, J.T., Guryev, O., Brown, M.S., Goldstein, J.L. Differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in cells expressing individual nuclear sterol regulatory element-binding proteins. **J. Biol. Chem.** 273 (1998) 26138-26148.
9. Shimomura, I., Shimano, H., Horton, J.D., Goldstein, J.L., Brown, M.S. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. **J. Clin. Invest.** 99 (1997) 838-845.
10. Horton, J.D., Bashmakov, Y., Shimomura, I., Shimano, H. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. **Proc. Natl. Acad. Sci. USA** 95 (1998) 5987-5992.
11. Shimano, H., Shimomura, I., Hammer, R.E., Herz, J., Goldstein, J.L., Brown, M.S., Horton, J.D. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. **J. Clin. Invest.** 100 (1997) 2115-2124.
12. Liang G., Yang J., Horton J.D., Hammer R.E., Goldstein J.L., Brown M.S. Diminished Hepatic Response to Fasting/Refeeding and Liver X Receptor Agonists in Mice with Selective Deficiency of Sterol Regulatory Element-binding Protein-1c. **J. Biol. Chem.** 277 (2002) 9520-9528.
13. Horton, J.D., Shimomura, I., Ikemoto, S., Bashmakov, Y., Hammer, R.E. Overexpression of SREBP-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. **J. Biol. Chem.** 278 (2003) 36652-36660.
14. Shimomura, I., Hammer, R.E., Richardson, J.A., Ikemoto, S., Bashmakov, Y., Goldstein, J.L., Brown, M.S. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. **Genes Dev.** (1998) 12 3182-3194.
15. Tontonoz, P., Kim, J.B., Graves, R.A., Spiegelman, B.M. ADD1: A novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. **Mol. Cell. Biol.** 13 (1993) 4753-4759.
16. Le Lay, S., Lefrere, I., Trautwein, C., Dugail, I. and Krief, S. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1c) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein as an SREBP-1c target. **J. Biol. Chem.** 277 (2002) 35625-35634.
17. Azzout-Marniche, D., Becard, D., Guichard, C., Foretz, M., Ferre, P., Foufelle, F. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. **Biochem. J.** 350 (2000) 389-393.
18. Iritani, N., Fukuda, H. and Tada, K. Nutritional regulation of lipogenic enzyme gene expression in rat epididymal adipose tissue. **J. Biochem.** 120 (1996) 242-248.

19. Girard, J., Ferre, P. and Foufelle, F. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. **Annu. Rev. Nutr.** 17 (1997) 325-352.
20. Stoeckman, A.K. and Towle, H.C. The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. **J. Biol. Chem.** 277 (2002) 27029-27035.
21. Uyeda, K., Yamashita, H. and Kawaguchi, T. Carbohydrate responsive element-binding protein (ChREBP): a key regulator of glucose metabolism and fat storage. **Biochem. Pharmacol.** 63 (2002) 2075-2080.