Increase of Lipogenic Enzyme mRNA Levels in Rat White Adipose Tissue After Multiple Cycles of Starvation-Refeeding

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Recently, we have found that despite the significant reduction of body weight after multiple starvation-refeeding cycles, white adipose tissue (WAT) exhibits surprisingly high rates of lipogenesis and lipogenic enzyme activities. The purpose of this study was to determine the response of WAT lipogenic enzyme mRNAs of rats subjected to multiple cycles of 3 days fasting and 3 days of refeeding. Despite the body weight reduction, significant increase of lipogenic enzymes (ie, fatty acid synthase [FAS], acetyl-coenzyme A [CoA] carboxylase [ACC], adenosine triphosphate (ATP)-citrate lyase [ACL], NADP-linked malic enzyme [ME], and glucose 6-phosphate dehydrogenase [G6PDH]) mRNAs in WAT was found after multiple cycles of starvation-refeeding of rats on standard laboratory diet. These findings, together with the results published recently, indicate that multiple cycles of starvation-refeeding cause the increased lipogenesis in WAT by upregulation of the lipogenic enzymes gene expression.

RECENTLY, AN UNUSUAL increase of lipogenesis in rat white adipose tissue (WAT) was found after multiple (more than 2) cycles of starvation-refeeding.[1] A parallel increase in enzymatic activities of fatty acid synthase (FAS), acetyl-coenzyme A (CoA) carboxylase (ACC), adenosine triphosphate (ATP)-citrate lyase (ACL), NADP-linked malic enzyme (ME), and hexose monophosphate shunt dehydrogenases suggests that the increased rate of lipogenesis in WAT is a consequence of increased lipogenic enzyme activities.[1] However, the mechanism of the increase of lipogenic enzyme activity after multiple cycles of starvation-refeeding is not well understood. In this report, we extend our previous observation by studying the lipogenic enzyme mRNAs abundance in rat WAT after multiple cycles of starvation-refeeding. A significant increase of lipogenic enzyme mRNAs in WAT was found after multiple cycles of starvation-refeeding of rats on standard laboratory diet. Parallel increase in enzymatic activities related to fatty acid synthesis and the rate of lipogenesis[1] suggests that the increased rate of lipogenesis in WAT is a consequence of increased lipogenic enzyme mRNA levels.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing approximately 230 g at the start of the investigation were maintained in wire mesh cages at 22°C with alternating 12-hour light/12-hour dark (the light cycle running from 8:00 AM to 8:00 PM) and were allowed free access to food and tap water (control group). Starving for 72 hours began at 8:00 AM, and the animals were then fed a finely ground commercial diet (LSM-Centralne Laboratorium Przemyslu Paszowego;, Motycz, Poland) for 72 hours (1 cycle of starvation-refeeding). This procedure was repeated 2 (2 cycles of starvation-refeeding) or 8 times (8 cycles of starvation-refeeding). All of the rats were allowed free access to tap water.

Designing and Labeling of Probes

The fatty acid synthase (FAS; EC. 2.3.1.85), acetyl-CoA carboxylase (ACC; EC. 6.4.1.2), ATP-citrate lyase (ACL; EC. 4.1.3.8), glucose 6-phosphate dehydrogenase (G6PDH; EC. 1.1.1.49), and malic enzyme (ME; EC. 1.1.1.40) coding sequences and 18S rRNA sequence were obtained from the EMBL database. Antisense oligonucleotides (32-mers in size: 5′-GAT CAT GTT CAC GTT CCA GGA TCT GGC GCA CT-3′, 5′-CAT ATA CCT CCA GAG CCG CCA TCC TCA CCA CC-3′, 5′-GCA GAT GTA GTC AGC AGT GGC GTC CAC CTT GG-3′, 5′-GTC TCT CCC GAA GGG CTT CTC CAC TAT GAT GC-3′, 5′-CTC ACT CGC CTG TGC CGC AGC CCA ATA TAC AA-3′, and 5′-CCA TTA TTC CTA GCT GCG GTA TCC AGG CGG CT-3′ for FAS, ACC, ACL, G6PDH, ME, and 18S rRNA, respectively) were designed by using the GeneRunner program (Hastings Software, Hastings, NY). Their uniqueness was checked against the EMBL data library using the Fasta program (*http://www.ebi.ac.uk/fasta3/*). The oligonucleotides were synthesized commercially (GENSET, Paris, France) with a single digoxigenin ligand at the 3′ end.

Isolation of RNA and Northern Blot RNA Analysis

The animals were killed by decapitation. WAT (epididymal) were rapidly removed and frozen in liquid nitrogen. Total RNA was extracted from frozen tissues by a guanidinium isothiocyanate-phenol/chloroform method[2] and dissolved in dimethyl pyrocarbonate-treated water. The RNA concentration of the extracts was determined from the absorbance at 260 nm. All samples had a 260/280 nm absorbance ratio of about 2.0. RNA samples were applied (10 μg/lane) to a 1% agarose gel containing 0.41 mol/L formaldehyde and fractionated by horizontal gel electrophoresis. RNA was transferred overnight to a positively charged nylon membrane by capillary blotting and fixed with ultraviolet (UV) light. Hybridization was performed as described previously[3-5] in solutions containing oligonucleotide probes specific for FAS, ACC, ACL, G6PDH, ME, and 18S rRNA. After posthybridization washing, the membranes were incubated with a polyclonal antibody against digoxigenin conjugated to alkaline phosphatase and then in a chemiluminescent substrate solution-CDP-*Star* (Boehringer-Mannheim, Mannheim, Germany). Membranes were exposed to Kodak XAR film (Eastman-Kodak, Rochester, NY) for 60 minutes at 37°C.

Data Analysis

Signals were scanned and quantified using the NIH-Image software (National Institutes of Health, Bethesda, MD). The levels of FAS, ACC, ACL, G6PDH, and ME mRNA were estimated by using PeakFit

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software (Jandel Scientific, Erkrath, Germany). The values for mRNA levels were normalized to the corresponding amount of 18S rRNA. Results expressed in arbitrary units are presented as mean ± SEM for samples from 10 rats. The statistical significance of differences between groups was assessed by 1-way analysis of variance (ANOVA) followed by Student's *t* test or by Mann-Whitney test, using Systat software (SPSS, Chicago, IL).

RESULTS

The effect of 1, 2, and 8 cycles of starvation-refeeding on FAS mRNA level is presented in Fig 1, which exhibits 3 (for each treatment) representative Northern blot analyses (top panel). The films were quantified by densitometry, and the levels of WAT FAS mRNA in rats undergoing starvation-refeeding were compared with the corresponding mRNA level in control animals (bottom panel). A quantitative analysis showed that after 1 cycle of starvation-refeeding, the FAS mRNA level increased approximately 14-fold in WAT as compared with control animals (Fig 1). Further increase of FAS mRNA level was observed after 2 and 8 cycles of starvation-refeeding. The level of FAS mRNA was at least 22 times higher after 8 cycles of starvation-refeeding, respectively, as compared with control. Subsequent stripping and reprobing of the membrane with an antisense oligonucleotide for 18S rRNA showed that satisfactory transfer of RNA did occur (Fig 1). The effect of multiple cycles of starvation-refeeding on acetyl-CoA carboxylase mRNA level in WAT (Fig 2) was essentially similar to that found for FAS mRNA. It seems, therefore, that the level of key lipogenic enzyme mRNAs is coordinately regulated in WAT by multiple cycles of starvation-refeeding. Furthermore, mRNAs of ACL (Fig 3) and of the enzymes participating in reduced nicotinamide adenine dinucleotide phosphate (NADPH) production required for fatty acid synthesis, ie, G6PDH mRNA (Fig 4) and ME mRNA (Fig 5) were also affected in WAT by multiple cycles of starvation-refeeding. After 1 cycle of starvation-refeeding, the ME mRNA increased approximately 12-fold in WAT as compared with rats fed ad libitum (Fig 5). A second episode of starvation-refeeding caused approximately an 18-fold increase of ME mRNA in WAT. After 8 cycles of 3 days of fast followed by 3 days of refeeding, the ME mRNA in WAT increased about 25 times as compared with control (Fig 5). The results presented above

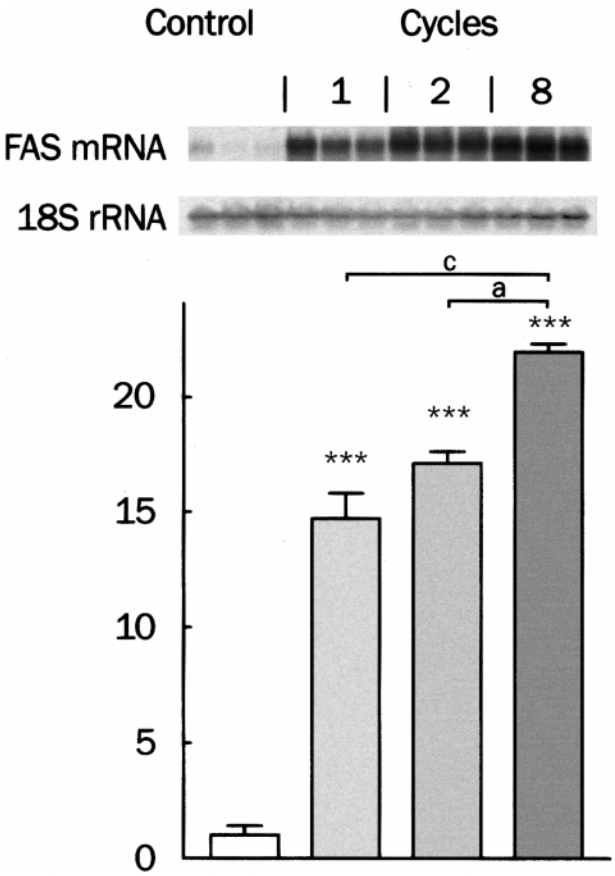

Fig 1. The effect of repeated cycles of starvation-refeeding on the level of FAS mRNA in rat WAT. The mRNA concentration was measured by Northern blot analysis (top panel shows representative Northern blots), calculated as described in Materials and Methods and expressed in arbitrary units (bottom panel). [a]*P* < .05, [c]*P* < .001 for differences between groups; ***P* < .001 compared with control.

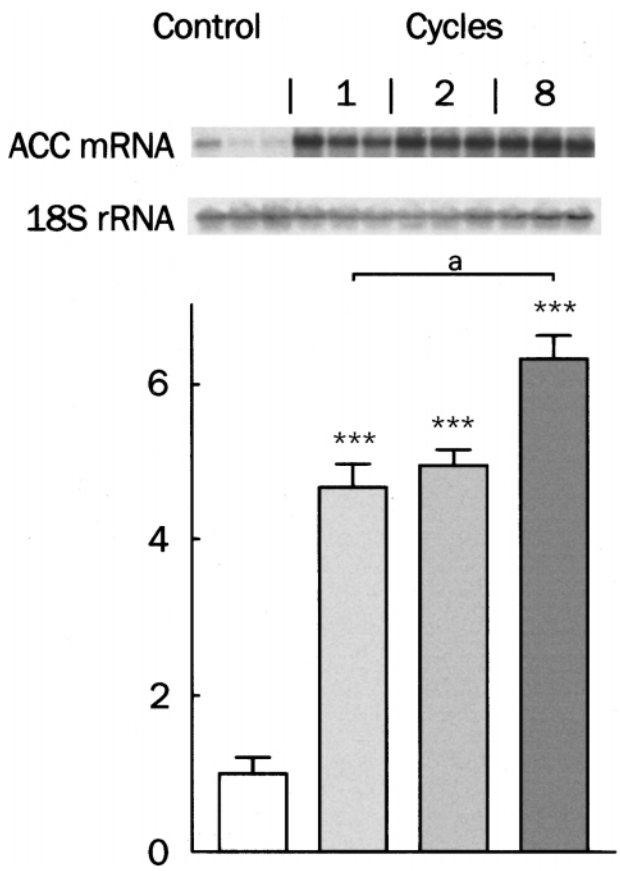

Fig 2. The effect of repeated cycles of starvation-refeeding on the level of ACC mRNA in rat WAT. The mRNA concentration was measured by Northern blot analysis (top panel shows representative Northern blots) calculated as described in Materials and Methods and expressed in arbitrary units (bottom panel). [a]*P* < .05 for differences between groups, ***P* < .001 compared with control.

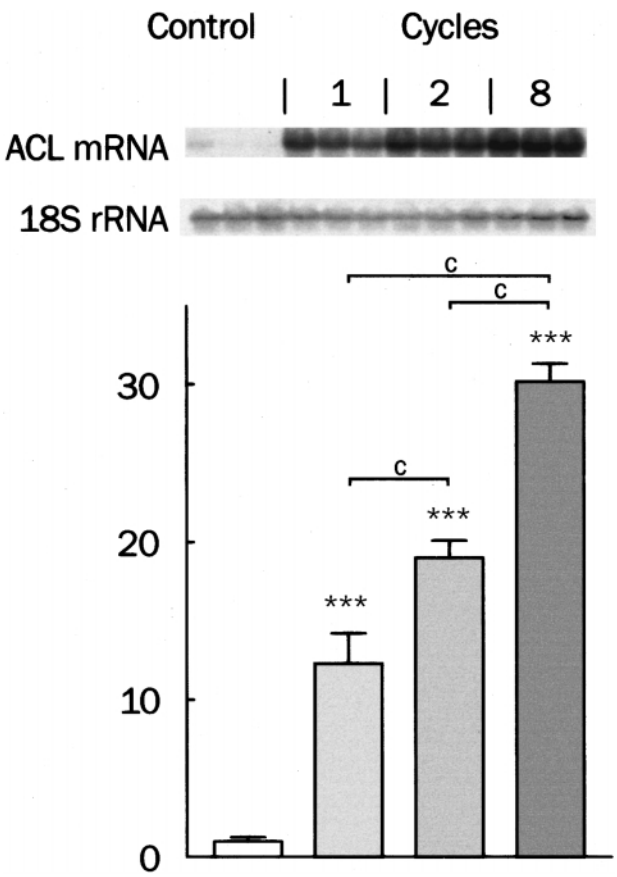

Fig 3. The effect of repeated cycles of starvation-refeeding on the level of ACL mRNA in rat WAT. The mRNA concentration was measured by Northern blot analysis (top panel shows representative Northern blots) calculated as described in Materials and Methods and expressed in arbitrary units (bottom panel). °*P* < .001 for differences between groups, ******P* **< .001 compared with control.**

indicate that the lipogenic enzyme mRNA levels increase gradually after 1, 2, and 8 cycles of starvation-refeeding.

DISCUSSION

It has been established that starvation-refeeding, causes an increase in rat liver[6-9] lipogenic enzymes activities, as compared with the activities of these enzymes in animals fed the same diet ad libitum. The magnitude of the increase is dependent on the diet composition, species, age, and sex of the animal.[10-13] It has been reported also that after the second cycle of starvation-refeeding, the increase in liver of some enzyme activities is even greater than after 1 starve-refeed cycle.[14,15] These changes in liver enzyme activities are exerted at the level of enzyme synthesis, secondary to changes in cellular concentration of specific mRNAs.[16-22] Another major site of lipogenesis is the adipose tissue. However, there are only few data concerning the dietary regulation of lipogenic enzymes expression in this tissue.[23-26] Furthermore, it is not known whether multiple cycles of starvation-refeeding affect lipogenic enzyme mRNA levels in WAT.

The data presented in this report indicate that a significant increase of lipogenic enzyme mRNAs takes place in rat WAT after multiple cycles of starvation-refeeding. These findings together with the results published recently[1] indicate that multiple cycles of starvation-refeeding cause an increased lipogenesis in WAT by upregulation of the lipogenic enzyme genes (ie, the genes for FAS, ACC, and ACL, G6PDH, and NADP-linked ME) expression. Furthermore, our results indicate that the level of key lipogenic enzyme mRNAs and mRNAs of enzymes participating in NADPH production required for fatty acid synthesis are coordinately regulated in WAT by multiple cycles of starvation-refeeding. Therefore, the results presented in this report extend significantly the work published recently.[1] In this report, we have focussed on WAT because this tissue was found to be the most sensitive to multiple cycles of starvation refeeding.[1] However, we have found that multiple cycles of starvation-refeeding caused also the increase of ME mRNA in the liver, but not in other tissues tested (not shown). No response of ME mRNA to multiple cycles of starvation-refeeding observed in kidney cortex, brain, heart, and skeletal muscle indicates that the ME in these tissues is not coordinately regulated along with the enzyme in the lipogenic tissues.

The starvation-refeeding protocol used in our studies may represent abnormal nutritional conditions, which limits extrapolation to human beings. However, such conditions may exist

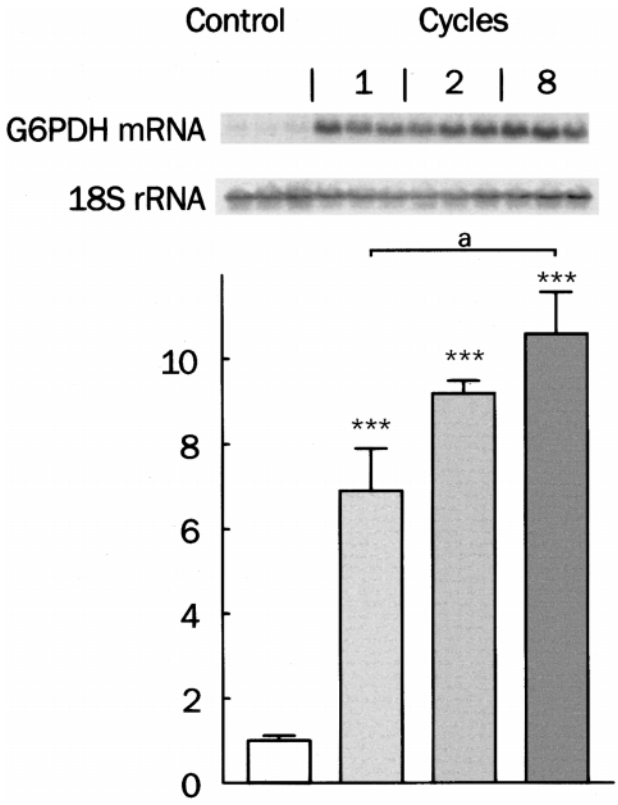

Fig 4. The effect of repeated cycles of starvation-refeeding on the level of G6PDH mRNA in rat WAT. The mRNA concentration was measured by Northern blot analysis (top panel shows representative Northern blots) calculated as described in Materials and Methods and expressed in arbitrary units (bottom panel). [a]*P* < .05 for differences between groups, ******P* **< .001 compared with control.**

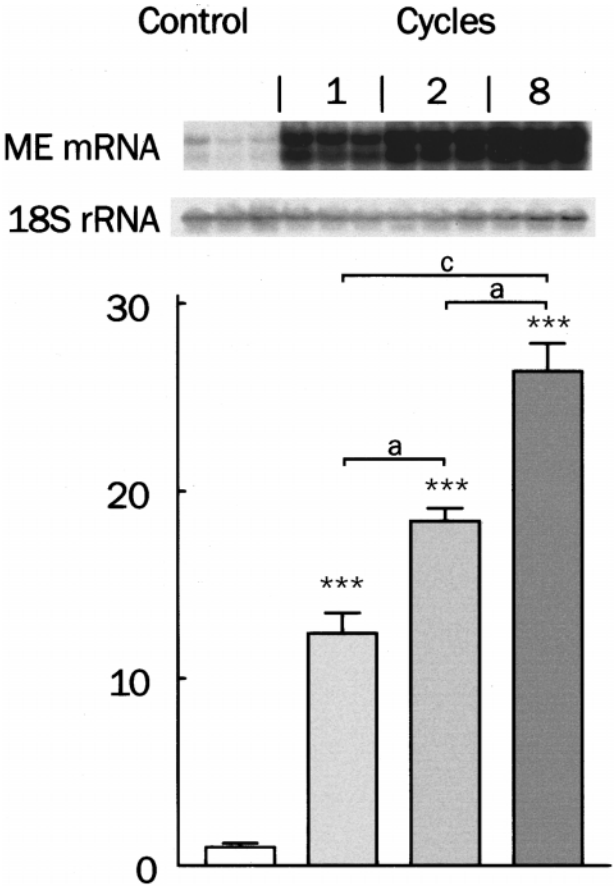

Fig 5. The effect of repeated cycles of starvation-refeeding on the level of ME mRNA in rat WAT. The mRNA concentration was measured by Northern blot analysis (top panel shows representative Northern blots) calculated as described in Materials and Methods and expressed in arbitrary units (bottom panel). $^{a}P < .05$, $^{c}P < .001$ for differences between groups; $^{***}P < .001$ compared with control.

in some patients with various eating disorders or in human beings exposed to multiple cycles of prolonged periods of starvation or food restriction due to treatment of obesity with restriction of food intake followed by uncontrolled refeeding.[27-30] Considering that lipogenic enzymes are present in human adipose tissue and that human adipose tissue is an important site of fatty acid synthesis,[31] it is not excluded that multiple cycles of starvation-refeeding cause also an upregulation of the lipogenic enzyme gene expression in human beings. A sustained elevation of fatty acid biosynthesis due to upregulation of lipogenic enzyme gene expression may be important for the development of obesity.[32,33] This would also explain why repeated dieting induce a resistance to weight loss and even an increase in body weight gain during refeeding periods.[34,35] It is well documented that most patients subjected to weight reduction programs begin to regain weight shortly after the end of active therapy,[36] and in some patients, the regain occurs despite continued therapy.[37] However, it is obvious that further investigation will be necessary to establish whether multiple cycles of starvation-refeeding cause upregulation of lipogenic enzyme gene expression in human adipose tissue.

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