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Leptin decreases lipogenic enzyme gene expression through modification of SREBP-1c gene expression in white adipose tissue of aging rats

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Abstract

Aging is associated with a significant reduction of lipogenic enzyme gene expression and lipogenesis in white adipose tissue (WAT). The age-related increase of *lep* gene expression could be, in part, responsible for these changes. Considering that sterol regulatory element binding protein 1c (SREBP-1c) plays an important role in regulation of lipogenic enzyme gene expression, it is likely that the age-related decrease of WAT lipogenic potential could be a consequence of the inhibition of SREBP-1c gene expression by leptin. We determined whether the increase of *lep* gene expression would account for the age-related decrease in SREBP-1c and its direct target, main lipogenic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), by assaying the messenger RNA (mRNA) levels of SREBP-1c, FAS, ACC , and leptin in WAT of 2-month-old (young) and 20-month-old (old) rats. Leptin mRNA level was much higher in the old animals, whereas in contrast, old rats displayed much lower mRNA levels of SREBP-1c and lipogenic enzymes. Moreover, experimentally increased plasma leptin concentration in young rats to the value observed in old rats resulted in the decrease of SREBP-1c, FAS, and ACC mRNA levels in WAT. Thus, the increase of *lep* gene expression could, in part, account for the reduced SREBP-1c gene expression and, consequently, the diminished lipogenic activity in WAT of old animals.

1. Introduction

It has been shown that aging is associated with significant reduction of some lipogenic enzyme activity in white adipose tissue (WAT) [1]. Moreover, these data suggest that resistance to insulin and T_3 is not directly related to significant reduction in lipogenic enzyme activity in WAT of aged rats [1]. This suggests that other factor(s) must contribute to diminish lipogenic enzyme activity in old rats.

A number of experiments have shown that lipid metabolism is regulated by leptin, a protein hormone primarily produced by adipose tissue [2-4]. Our recently published data suggest that the age-related increase of serum leptin concentration might be a cause of decreasing WAT lipogenic activity with age [5-7], although the exact mechanisms underlying this process remain unknown.

Sterol regulatory element binding proteins (SREBPs) are transcription factors, which are involved in regulation of lipids biosynthesis in animal cells. The proteins have basic-helix-loop-helix-leucine zipper domain and bind to sterol regulatory elements in the promoters of target genes [8-12]. Three SREBPs have been identified so far: SREBP-1a and SREBP-1c are produced from the same gene through the use of alternate promoters, and SREBP-2 is encoded by a separate gene [11,13]. SREBP-1a and SREBP-1c preferentially activate genes involved in lipogenesis, whereas SREBP-2 is primarily responsible for the transcriptional regulation of genes involved in cholesterol homeostasis [13,14]. SREBP-1a is the main isoform in cell cultures and spleen, whereas SREBP-1c and SREBP-2 are predominant in most organs of adult animals including WAT [11]. It was suggested that SREBP-1a is expressed

This work is dedicated to Prof Mariusz M. Zydowo on his 80th birthday.

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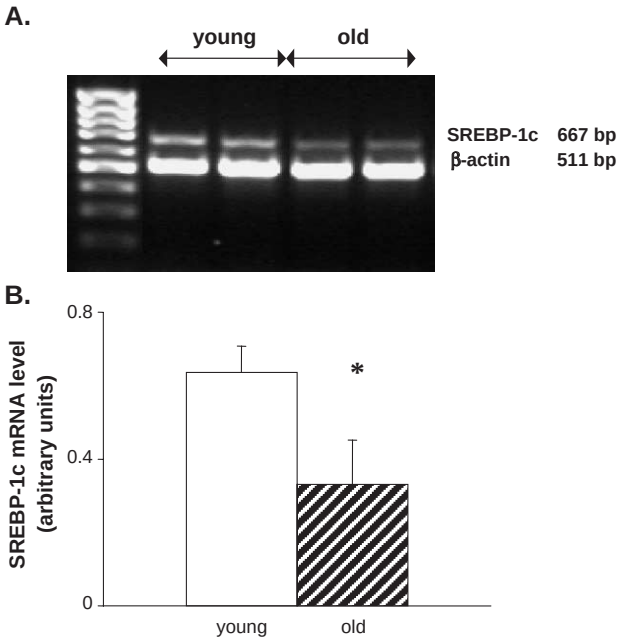

Fig. 1. SREBP-1c mRNA level in perirenal WAT of young and old rats. A, Representative agarose gel electrophoresis of products corresponding to SREBP-1c and βactin, amplified by Multiplex PCR method. The individual lanes represent individual animals. The measurements were in the linear range of the assay. B, SREBP-1c mRNA level (mean ± SEM) in each group (n = 6 per group) was quantified as described in the Materials and Methods section. The cDNA amplified from β-actin mRNA was used to normalize corresponding SREBP-1c results. Data are expressed in arbitrary units ($P < .05$).

under conditions in which increased lipid synthesis is required, whereas SREBP-1c expression preferentially occurs during physiological conditions in which relatively low but regulated levels of lipid synthesis are important [15]. SREBP-1c was also identified as an adipocyte determination and differentiation factor [16]. Thus, SREBP could be a strong candidate responsible for the age-related differences in lipogenic enzyme gene expression in WAT. The increased expression of SREBP-1c in adipose tissue of young and its down-regulation in old rhesus monkey [17] suggest that SREBP-1c could, in part, contribute in the age-related differences in adipose tissue lipogenic enzyme gene expression also in rats. There is evidence that leptin affects SREBP-1c level [18-20]. Thus, it is very likely that leptin might decrease SREBP-1c gene expression and, consequently, suppress lipogenic enzyme gene expression in WAT of old rats.

To verify this hypothesis, our present study investigated the relationship between key lipogenic enzymes (fatty acid synthase [FAS] and acetyl-CoA carboxylase [ACC]), *lep*, and SREBP-1c gene expression in WAT of 2-month-old (young) and 20-month-old (old) rats. In further studies, using a single intraperitoneal injection of recombinant leptin in 2-month-old rats, we tested the effect of increased serum leptin concentration on SREBP-1c, FAS, and ACC messenger RNA (mRNA) levels.

2. Materials and methods

2.1. Animals

2.1.1. Young and old animals

Male Wistar rats (this strain has been considered as an adequate model for aging studies [21]) aged 2 and 20 months (n = 6 per age) were housed in wire-mesh cages at 22°C under a light-dark (12:12 h) cycle with lights on at 7:00 AM. Food (commercial diet, composition described in Ref [22]) and tap water were provided ad libitum. Average daily food intake was measured by the differences in weight between the amount of food provided and the amount remaining over a 1-day period.

2.1.2. Leptin-treated young animals

Two-month-old animals were fasted 24 hours and divided into 2 groups (n = 6 per group): (*a*) control rats, which were intraperitoneally injected with vehicle (0.4 mL phosphate-buffered saline per rat); and (*b*) leptin-treated rats, which were intraperitoneally injected with 1 mg of recombinant rat leptin (R&D System, Minneapolis, Minn) in 0.4 mL phosphate-buffered saline per rat; 1 hour after leptin injection, rats were refed ad libitum. Animals were killed 24 hours after leptin administration.

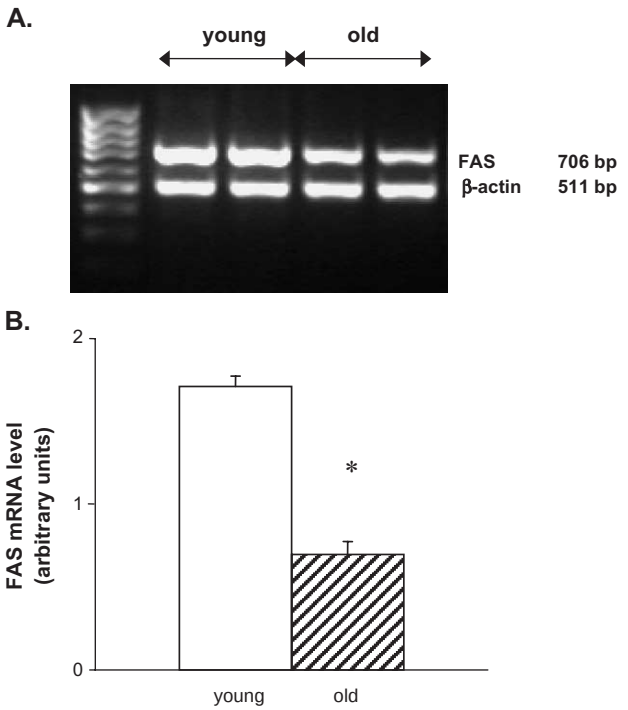

Fig. 2. FAS mRNA level in perirenal WAT of young and old rats. A, Representative agarose gel electrophoresis of products corresponding to FAS and β-actin, amplified by Multiplex PCR method. The individual lanes represent individual animals. The measurements were in the linear range of the assay. B, FAS mRNA level (mean ± SEM) in each group (n = 6 per group) was quantified as described in the Materials and Methods section. The cDNA amplified from β-actin mRNA was used to normalize corresponding FAS results. Data are expressed in arbitrary units ($P < .05$).

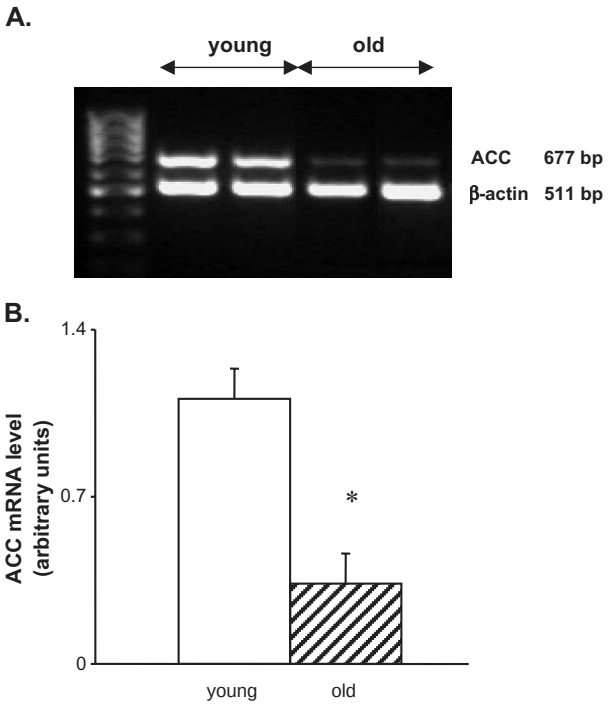

Fig. 3. ACC mRNA level in perirenal WAT of young and old rats. A, Representative agarose gel electrophoresis of products corresponding to ACC and β-actin, amplified by Multiplex PCR method. The individual lanes represent individual animals. The measurements were in the linear range of the assay. B, ACC mRNA level (mean ± SEM) in each group (n = 6 per group) was quantified as described in the Materials and Methods section. The cDNA amplified from β-actin mRNA was used to normalize corresponding ACC results. Data are expressed in arbitrary units ($P < .05$).

All rats were killed from 8:00 to 10:00 AM. Perirenal WAT was collected, rapidly frozen in liquid nitrogen, and then stored at −80°C until analysis.

All studies were approved by the local ethics committee in Gdansk, Poland.

2.2. RNA isolation

Total cellular RNA was extracted from frozen WAT by the guanidinium isothiocyanate–phenol/chloroform method and then performed as previously described [6,23]. The RNA concentration was determined from the absorbance at 260 nm and all samples had the 260:280 nm absorbance ratios about 2.0.

2.3. Reverse transcriptase–polymerase chain reaction

First-strand complementary DNA (cDNA) was synthesized from 1 μg of total RNA. Before amplification of cDNA, each RNA sample was treated with RNase-free DNase I (Fermentas, Vilnius, Lithuania) at 37°C for 30 minutes. The cDNA was used as a template in Multiplex polymerase chain reaction (PCR) reaction [24,25] with the housekeeping β-actin gene as an internal control. The cDNA samples were amplified for 30 cycles at a final volume of 20 μL containing 1× PCR buffer, 3.5 mmol/L $MgCl_2$, 0.5 mmol/L dNTP Mix, 0.5 mmol/L of sense and antisense primers, and 0.5 U *Taq* DNA Polymerase (Fermentas). The conditions of all reactions were experimentally checked to ensure that the signals were in the linear range of the assay. Specific sense and antisense primers used for the preparations of respective cDNA were the following: SREBP-1c, 5′-TAGCTCATCAACAACCAAGAC-3′ and 5′-TTATGGCACCTGTGTCTGTC-3′; FAS, 5′-GCAACTGTGCGTTAGCCAC-3′ and 5′-TGTTTCAGGGGAGAAGAGACC-3′ [26]; ACC, 5′-CCGAGATGTCATTGTCATC -3′ and 5′-ACACCAGTGTAAGACTGTG-3′; leptin, 5′-CCCATTCTGAGTTTGTCCA-3′ and 5′-GCATTCAGGGCTAAGGTC-3′ [27]; and β-actin, 5′-GAAATCGTGCGTGACATTAAG-3′ and 5′-GCTAGAAGCATTTGCGGTGGA-3′. The PCR products were analyzed by 1.5% agarose gel electrophoresis. Band intensities were compared by imaging ethidium bromide staining and quantified using the Sigma Scan software program (Jandel Scientific, San Rafael, Calif). The values were normalized for the corresponding amount of β-actin mRNA. Results are expressed in arbitrary units and presented as means ± SEM of the 6 samples.

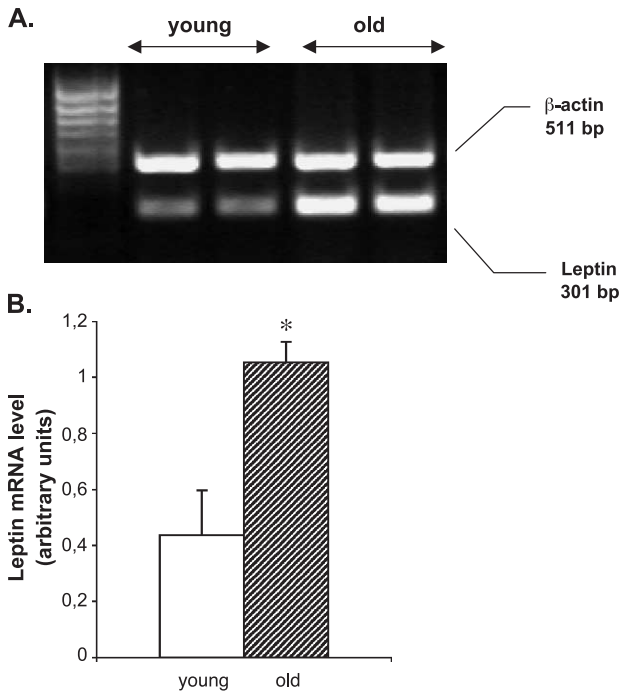

Fig. 4. Leptin mRNA level in perirenal WAT of young and old rats. A, Representative agarose gel electrophoresis of products corresponding to leptin and β-actin, amplified by Multiplex PCR method. The individual lanes represent individual animals. The measurements were in the linear range of the assay. B, Leptin mRNA level (mean ± SEM) in each group (n = 6 per group) was quantified as described in the Materials and Methods section. The cDNA amplified from β-actin mRNA was used to normalize corresponding leptin results. Data are expressed in arbitrary units ($P < .05$).

2.4. Quantification of serum leptin concentration

Serum leptin concentration was measured by radioimmunoassay with Rat Leptin RIA Kit (Linco Research, St Charles, Mo).

2.5. Statistics

The statistical significance of differences between groups was assessed by 1-way analysis of variance followed by Student t test using the Systat software (Point Richmond, Calif). Differences between the groups were considered significant when $P < .05$.

3. Results

Fig. 1A shows a representative experiment of SREBP-1c mRNA level measurement in perirenal fat depots of young and old rats. SREBP-1c mRNA level was significantly

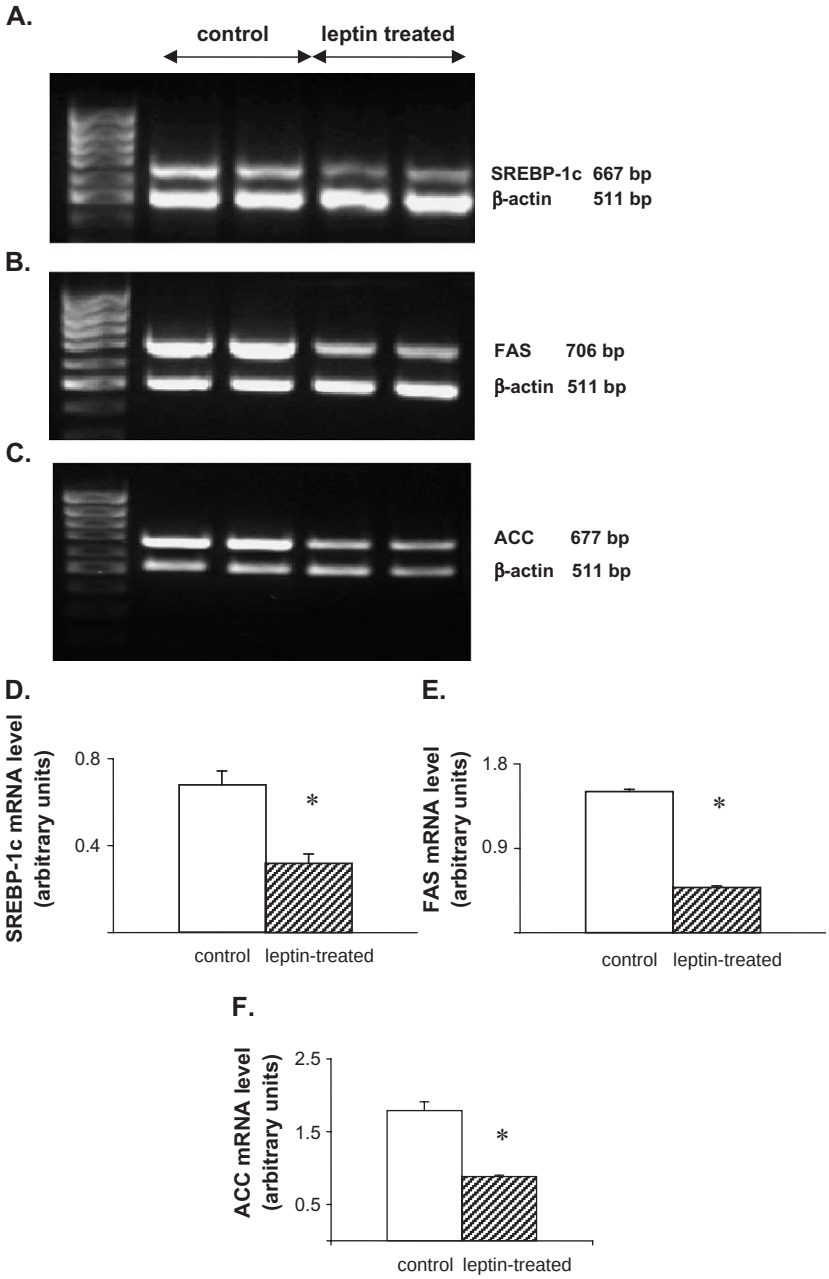

Fig. 5. SREBP-1c (A), FAS (B) and ACC (C) mRNA level in perirenal WAT of control—vehicle-treated young rats; and leptin treated—young rats injected with leptin (1 mg per rat). Representative agarose gel electrophoresis of products corresponding to SREBP-1c, FAS, ACC , and β-actin amplified by Multiplex PCR method. The individual lanes represent individual animals. The measurements were in the linear range of the assay. SREBP-1c (D), FAS (E) , and ACC (F) mRNA level (mean ± SEM) in each group (n = 6 per group) was quantified as described in the Materials and Methods section. The cDNA amplified from β-actin mRNA was used to normalize corresponding SREBP-1c, FAS, and ACC results. Data are expressed in arbitrary units ($P < .05$).

higher in perirenal WAT in young than in old rats. β-Actin mRNA (used as an internal control) was expressed at relatively high, essentially similar levels in adipose tissue of both young and old rats (Fig. 1A). Fig. 1B provides a semiquantitative analysis of the data determined from densitometric scans (obtained from all animals used in experiments). Although the results displayed in Fig. 1B represent relative estimates, they clearly indicate that there is the age-related difference in SREBP-1c mRNA abundance. In perirenal adipose tissue of the same rats, the SREBP-1c target genes including FAS and ACC were also studied. Fatty acid synthase (Fig. 2) and ACC (Fig. 3) mRNA levels were significantly higher in young than in old rats. β-Actin mRNA level in perirenal WAT did not show any changes with age (Figs. 2 and 3). Thus, the pattern of the age-related changes in SREBP-1c mRNA level qualitatively resembled that of key lipogenic enzymes mRNA level in perirenal WAT (Figs. 1-3). This close relationship between SREBP-1c and its target genes' mRNAs suggests that the age-related decrease in the FAS and ACC mRNA levels could be a consequence of the lower SREBP-1c mRNA level in old rats.

Considering a suppressive action of leptin on SREBP-1c in mice WAT [19], the effect of age on rat WAT leptin mRNA was performed. The results (Fig. 4) indicate that leptin mRNA level (in contrast to SREBP-1c and lipogenic enzyme mRNA levels; Figs. 1-3) was lower in perirenal WAT of 2-month-old animals as compared with 20-month-old animals. The previously mentioned results indicate a close relationship between the age-dependent increase of *lep* gene expression in perirenal WAT and the age-related decrease of lipogenic enzyme and SREBP-1c gene expression in this tissue. It thus suggests that the age-related suppression of SREBP-1c gene expression by leptin might be a cause of the age-related decrease of lipogenic activity in WAT. To confirm this assumption, we examined the effects of recombinant leptin administration to the 2 month-old-rats (1 mg per rat, approximately equal to 5 mg/kg body weight) on WAT SREBP-1c, FAS, and ACC mRNA levels. Leptin administration significantly decreased SREBP-1c, FAS, and ACC mRNA abundance (Fig. 5). Essentially similar effects of leptin administration on other lipogenic enzymes in WAT were found [7]. It should be noted that leptin administration increases serum leptin concentration approximately 6-fold (from 0.8 ± 0.2 to 5.4 ± 2.9 ng/mL) to the value observed in old animals [7]. Thus, as far as serum leptin concentration is considered, leptin-treated young rats mimic the old animals. It is also noteworthy that the average daily food intake was not significantly different between control (27 ± 3 g) and leptin-treated (25 ± 3 g) rats. An average daily food intake by old rats was 39 ± 3 g. It seems that food intake cannot be responsible for the observed differences in FAS and ACC gene expression because old rats eat more than young ones and still have lower expression of the genes in WAT.

4. Discussion

The age-related increase of rat adipose tissue leptin mRNA level (as well as serum leptin concentration) has been reported [5,6,28]. A suppressive action of leptin on the transcription of SREBP-1c in mice WAT has been shown [19]. Thus, the down-regulation of SREBP-1c gene expression, the main transcription factor that controls the lipogenic enzyme gene expression, may constitute a link between *lep* and lipogenic enzyme gene expression in rat WAT. Our results suggest that the age-associated decrease of SREBP-1c gene expression may, at least in part, account for the decrease in WAT lipogenic potential. This view is based on the following grounds: the data presented in this article indicate a close relationship between the age-dependent decrease of SREBP-1c gene expression and the age-related suppression of the gene expression of key lipogenic enzymes (FAS and ACC) in WAT of the same rats. This close relationship and known stimulatory effect of SREBP-1c on lipogenic enzyme mRNA levels [13,29,30] suggest that the age-related down-regulation of lipogenic enzyme gene expression might occur in response to down-regulation of SREBP-1c gene expression.

Moreover, the results presented in this article indicate a close relationship between the age-dependent increase of *lep* gene expression in perirenal WAT and the age-related suppression of SREBP-1c and lipogenic enzyme gene expression. Considering that (*a*) higher leptin level causes decrease of SREBP-1c in liver and pancreatic islets [18,20] and (*b*) leptin deficiency results in an increase of SREBP-1c gene expression in liver of *ob/ob* mouse [19,31], one may conclude that the high leptin level contributes to suppression of SREBP-1c mRNA level in WAT of old rats. Thus, in old rats, which display higher expression of leptin gene, lower expression of SREBP-1c should be expected. The data previously presented indicate that this may be the case. In addition, the intraperitoneal administration of leptin to young rats suppresses SREBP-1c and lipogenic enzyme gene expression. It is likely that observed down-regulation of lipogenesis in old rats is promoted because of the following events: the age-related induction of the leptin gene expression occurs, and consequently, leptin concentration in adipocytes increases. This higher leptin concentration in adipose tissue may decrease SREBP-1c gene expression, and as a consequence, the coordinated down-regulation of lipogenic enzyme gene expression results.

Apparently, our conclusion is in contrast with reported data on leptin resistance in old animals [32,33]. But it was also reported that induced hyperleptinemia in old rats causes up-regulation of some genes involved in fatty acid β-oxidation [32]. Thus, down-regulation of lipogenic enzyme gene expression in WAT of old rats might also occur.

Although supported by indirect evidence, the assumption that higher leptin concentration (due to greater leptin production) inhibits lipogenic enzyme gene expression by

reducing the SREBP-1c gene expression in WAT of old rats remains to be proven definitively. One can argue that hyperleptinemia induced by adenovirus-mediated transfer of leptin cDNA to young rats, which have low *lep* gene expression (and, consequently, low serum leptin concentration) and higher SREBP-1c mRNA than in old animals, would be an additional way to prove the effect of leptin on SREBP-1c and lipogenic enzyme gene expression. Such results have been already published by Kakuma et al [18] who found that hyperleptinemia induced in young rats by adenovirus gene transfer (recombinant adenovirus containing rat leptin cDNA) lowered both hepatic SREBP-1c and lipogenic enzyme mRNAs. These data strongly support our proposition. The importance of leptin as a suppressor of SREBP-1c and lipogenic enzyme gene expression in vivo is also disclosed by *ob/ob* mice, which have a mutation in the leptin gene and, consequently, do not synthesize leptin [2]. Accordingly, these animals are characterized by higher level of SREBP-1c and a higher rate of lipogenesis and lipogenic enzyme activities [19,31].

Because food intake has significant impact on SREBP-1c gene expression, one may conclude that this process may also play an important role in the age-related decrease of lipogenic enzyme expression. However, the old rats eat more than young ones. Because starvation decreases SREBP-1c gene expression, it is unlikely that increase of food intake in old animals is responsible for decrease of SREBP-1c and lipogenic enzyme gene expression.

In conclusion, our results indicate that an inverse relationship exists between *lep* and SREBP-1c (as well as key lipogenic enzymes) gene expression in WAT of both young and old rats. Thus, it appears that leptin suppresses lipogenic enzyme gene expression (and, consequently, FAS) at least in part, reducing SREBP-1c gene expression in WAT of old rats. Thus, the suppression of SREBP-1c gene expression and, consequently, lipogenic enzyme gene expression by leptin might constitute a negative feedback loop against excess fat accumulation in adipocytes of old animals.

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References

[1] Mooradian AD, Albert SG. The age-related changes in lipogenic enzymes: the role of dietary factors and thyroid hormone responsiveness. Mech Ageing Dev 1999;108:139-49.
[2] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425-32.
[3] Bai Y, Zhang S, Kim K-S, Lee J, Kim K-H. Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipogenic hormones. J Biol Chem 1996;271:13939-42.
[4] Iritani N, Sugimoto T, Fukuda H. Gene expression of leptin, insulin receptors and lipogenic enzymes are coordinately regulated by insulin and dietary fat in rats. J Nutr 2000;130:1183-8.
[5] Nogalska A, Pankiewicz A, Goyke E, Swierczynski J. The age-related inverse relationship between ob and lipogenic enzymes genes expression in rat white adipose tissue. Exp Gerontol 2003; 38:415-22.
[6] Nogalska A, Swierczynski J. The age-related differences in obese and fatty acid synthase gene expression in white adipose tissue of rat. Biochim Biophys Acta 2001;1533:73-80.
[7] Nogalska A, Swierczynski J. Potential role of high serum leptin concentration in age-related decrease of fatty acid synthase gene expression in rat white adipose tissue. Exp Gerontol 2004;39: 147-50.
[8] Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, et al. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 1993;75:187-97.
[9] Wang X, Sato R, Brown MS, Hua X, Goldstein JL. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell 1994;75:53-62.
[10] Bennet MK, Lopez JM, Sanchez HB, Osborne TF. Sterol regulation of fatty acid synthase promoter. Coordinate feedback regulation of two major lipid pathways. J Biol Chem 1995;270:25578-83.
[11] Shimomura I, Shimano H, Horton JD, Golstein JL, Brown MS. 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 1997;99:838-45.
[12] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125-31.
[13] Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res 2001;40:439-52.
[14] Edwards PA, Tabor D, Kast HR, Venkateswaran A. Regulation of gene expression by SREBP and SCAP. Biochim Biophys Acta 2000;1529:103-13.
[15] Toth JI, Datta S, Athanikar JN, Freedman LP, Osborne TF. Selective coactivator interactions in gene activation by SREBP-1a and -1c. Mol Cell Biol 2004;24:8288-300.
[16] Tontonoz P, Kim JB, Graves RA, Spiegelman BM. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol Cell Biol 1993;13:4753-9.
[17] Hotta K, Bodkin NL, Gustafson TA, Yoshioka S, Ortmeyer HK, Hansen BC. Age related adipose tissue mRNA expression of ADD1/SREBP1, PPAR, lipoprotein lipase, and glut4 glucose transporter in rhesus monkeys. J Gerontol Biol Med Sci 1999;54A: B183-8.
[18] Kakuma T, Lee Y, Higa M, Wang Z, Pan W, Shimomura I, et al. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. Proc Natl Acad Sci U S A 2000;97:8536-41.
[19] Soukas A, Cohen P, Socci N, Friedman J. Leptin specific patterns of gene expression in white adipose tissue. Genes Dev 2000;14:963-80.
[20] Unger RH. The physiology of cellular liporegulation. Annu Rev Physiol 2003;65:333-47.
[21] Leighton B, Dimitriadis GD, Parry-Billings M, Lozeman FJ, Newsholme EA. Effect of ageing on the responsiveness of glucose metabolism to insulin in the incubated soleus muscle isolated from Sprague-Dawley and Wistar rats. Biochem J 1989;261: 383-7.
[22] Kochan Z, Karbowska J, Swierczynski J. Unusual increase of lipogenesis in rat white adipose tissue after multiple cycles of starvation-refeeding. Metabolism 1997;46:10-7.
[23] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156-9.

[24] Freeman WM, Walker SJ, Vrana KE. Quantitative RT-PCR: pitfalls and potential. Biotechniques 1999;26:112-25.
[25] Marone M, Mozzetti S, De Ritis D, Pierelli L, Scambia G. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. Biol Proced Online 2001; 3:19-25.
[26] Stoeckman AK, Towle HC. The role of SREBP-1c in nutritional regulation of lipogenic enzyme gene expression. J Biol Chem 2002; 277:27029-35.
[27] Dhillon H, Kalra SP, Prima V, Zolotukhin S, Scarpace PJ, Moldawer LL, et al. Central leptin gene therapy suppresses body weight gain, adiposity and serum insulin without affecting food consumption in normal rats: a long-term study. Regul Pept 2001;99: 69-77.
[28] Li H, Matheny RL, Nicolson M, Tumer N, Scarpace PJ. Leptin gene expression increases with age independent of increasing adiposity in rats. Diabetes 1997;46:2035-9.
[29] Osborne TF. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J Biol Chem 2000;275:32379-82.
[30] Shimano H, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, et al. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 1999;274:35832-9.
[31] Shimomura I, Bashmakov Y, Horton JD. Increased levels of nuclear SREBP-1c associated with fatty livers in two models of diabetes. J Biol Chem 1999;274:30028-32.
[32] Ma XH, Muzumdar R, Yang XM, Gabriely I, Berger R, Barzilai N. Aging is associated with resistance to effects of leptin on fat distribution and insulin action. J Gerontol A Biol Sci Med Sci 2002; 57A:B225-31.
[33] Wang Z, Pan W, Lee Y, Kakuma T, Zhou Y, Unger RH. The role of leptin resistance in the lipid abnormalities of aging. FASEB J 2001; 15:108-14.