Upregulation of Lipogenic Enzymes Genes Expression in White Adipose Tissue of Rats With Chronic Renal Failure Is Associated With Higher Level of Sterol Regulatory Element Binding Protein-1

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Chronic renal failure (CRF) frequently results in hypertriglyceridemia and elevated plasma concentration of very-low-density lipoprotein (VLDL). These abnormalities are thought to be primarily due to depressed lipoprotein lipase and hepatic lipase activities, as well as impaired clearance of plasma lipoproteins. Some results suggest that not only lipoproteins catabolism but also their overproduction might contribute to hypertriglyceridemia in CRF. Because sterol regulatory element binding protein (SREBP) plays an important role in the regulation of lipid homeostasis, increased level of this transcription factor might be involved in modulating lipid metabolism in CRF. The purpose of the present study is to determine whether there is an altered regulation of the SREBP-1 in CRF rats and whether the altered regulation of SREBP-1 is associated with the upregulation of lipogenic enzymes genes expression in CRF rats. In the white adipose tissue (WAT) of CRF rats, marked increases in the microsomal (precursor) and nuclear (mature) forms of SREBP-1 have been found. The increase in SREBP-1 was associated with an increased level of lipogenic enzymes (acetyl–coenzyme A [CoA] carboxylase [ACC], adenosine triphosphate–citrate lyase [ACL], fatty acid synthase [FAS], glucose 6-phosphate dehydrogenase [G6PDH], 6-phosphogluconate dehydrogenase [6PGDH], and malic enzyme [ME]) genes expression. In turn, this was associated with an increased rate of fatty acids synthesis in WAT and a significant increase in plasma triacylglycerol (TAG) and VLDL concentration. Our study indicates that WAT SREBP-1 expression is increased in CRF rats and that SREBP-1 may play an important role in the increased fatty acid synthesis. These results reveal another facet of disturbed lipid metabolism in CRF.

CHRONIC RENAL FAILURE (CRF) is associated with abnormal concentration and composition of plasma lipoproteins. The resulting disturbances, at least, contribute to the pathogenesis of the atherosclerosis found in uremic syndrome.[1] The lipid abnormalities are characterized mainly by: (1) an increase in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) fractions, leading to hypertriglyceridemia; (2) an unchanged or slightly increased low-density lipoprotein (LDL) fraction enriched with triacylglycerols (TAGs); and (3) a decrease in the HDL_2 subfraction, resulting in a decreased concentration of HDL-cholesterol.[1-6] The principal disturbance of the lipoprotein metabolism appears to be the reduced catabolism and clearance of TAG-rich apolipoprotein B–containing lipoproteins.[3,7] The main factors contributing to decreased lipoproteins catabolism include reduced activity of lipolytic enzyme, compositional abnormalities of lipoproteins as substrate for lipolysis, and decreased receptor-mediated uptake of lipoproteins.[8-10] The decrease of lipoprotein lipase activity in CRF can be due either to reduced enzyme synthesis and/or to increased inhibition of the enzyme by the circulating inhibitors.[11-13] Marked downregulation of hepatic lipase expression may also contribute in part to the CRF dyslipidemia.[14] In addition, impaired HDL maturation (due to downregulation of lecithin–cholesterol acyltransferase), which plays a critical role in TAG metabolism, may be responsible for impaired plasma TAG clearance in CRF.[15] Moreover, expression of hepatic acyl–coenzyme A (CoA):cholesterol acyltransferase, the enzyme that plays an important role in packaging and secretion of HDL, is markedly elevated in CRF.[16] Thus, this phenomenon can also contribute to hypertriglyceridemia in CRF.

Recently we have shown that fatty acid synthase (FAS) and adenosine triphosphate–citrate lyase (ACL) genes expression is upregulated in uremic subjects.[17,18]This was associated with an increase in the lipogenesis rate and plasma TAG concentration.[17] These results suggest that not only the decrease of removal, but also an increase of fatty acid production, could contribute in part to the CRF-associated hyperlipidemia.

Lipid homeostasis in animal cells is regulated by a family of transcription factors called sterol regulatory element binding proteins (SREBPs).[19-24] 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.[23,24]

The SREBPs are inserted into the membrane of the endoplasmic reticulum (an inactive precursor form). Under conditions of cholesterol deprivation, the NH_2 terminal domain is released through a coupled 2-step proteolytic process and the active, mature form of SREBP enters nucleus. It binds to the sterol regulatory elements (SRE) and activates the expression of genes involved in the synthesis and uptake of cholesterol, fatty acids, TAGs, and phospholipids.[23-27] SREBP-1 preferentially activates genes involved in lipogenesis, whereas SREBP-2 is primarily responsible for the transcriptional regulation of genes involved in cholesterol homeostasis.[23,24] Because SREBP-1 plays an important role in the regulation of lipogenesis, altered regulation of this transcription factor in

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CRF could be involved in the regulation of lipogenic enzymes genes expression and consequently may be in part responsible for hypertriglyceridemia in CRF. Thus, the main goal of the present study was to investigate the SREBP-1 level together with lipogenic enzymes genes expression and the rate of fatty acid synthesis in white adipose tissue (WAT) of CRF rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing approximately 250 g at the start of the investigation were housed in wire-mesh cages at 22°C under a light: dark (12:12 hour) cycle with lights on at 7 AM. Renal failure was induced by 2-stage subtotal (five-sixths) nephrectomy as described previously.[28] Sham-operated rats (ie, with incision of skin, muscle, and layered suture) served as controls. Ten animals per group (both control and CRF) were used. The rats (both control and CRF) were allowed free access to food (commercial diet composition described previously[29]) and tap water. Average daily food intake was measured by the difference in weight between the amount of food provided and the amount remaining over a 1-day period. The rats were killed (from 8 to 10 AM) 6 weeks after the induction of renal failure. Blood samples were collected from the abdominal aorta under thiopental anesthesia. Determination of blood urea, creatinine, TAGs, and VLDL were performed in the Department of Clinical Biochemistry, Medical University of Gdansk, using routine methods. Small pieces (~1 g) of epididymal WAT were collected and rapidly frozen in liquid nitrogen. The tissues were stored at −80°C until analysis.

Probes Designing and Labeling

Lipogenic enzymes mRNA level has been detected using synthesized commercially (Genset S.A, Paris, France) oligonucleotides with a single digoxigenin ligand at the 3′ end, complementary to the rat lipogenic enzymes coding sequence: acetyl–CoA carboxylase (ACC) 5′CATATAC-CTCCAGAGCCGCCATCCTCACCACC-3′,[30] ACL 5′GCAGATG-TAGTCAGCAGTGGCGTCCACC3′,[30] FAS 5′-GAT AGA GGT GCT GAG CCA GCG TGC TGA GCG TG-3′,[31] glucose 6-phosphate dehydrogenase (G6PDH) 5'GTCTCTCCCGAAGGGCTTCTCCACTATGA-TGC3′,[30] 6-phosphogluconate dehydrogenase (6PGDH), 5′GATCAG-CTGCATGTC(A/C)CCATYACTCTATCCCGTTCTG3′,[32] and malic enzyme (ME) 5′CTCACTCGCCTGTGCCGCAGCCCAATATACAA.[31] The 31-mer antisense oligonucleotide (5′CGC CTG CTG CCT TCC TTG GAT GTG GTA GCC G-3″) was used as probe for the 18S rRNA.[33]

Isolation of RNA and Northern Blot RNA Analysis

Total cellular RNA was extracted from frozen tissue (~1 g of tissue was used) by a guanidinium isothiocyanate–phenol/chloroform method[34] and finally dissolved in dimethyl pyrocarbonate–treated water. The RNA concentration of the extracts was determined from the absorbance at 260 nm and all samples had a 260/280-nm absorbance ratio of about 2.0. RNA samples were applied (10 μg per lane) to a 1% agarose gel containing 0.41 mol/L formaldehyde, and fractionated by horizontal gel electrophoresis. After electrophoretic fractionation, RNA was transferred overnight to a positively charged nylon membrane by capillary blotting and fixed with UV light. Prehybridization and hybridization were performed as described previously.[30-32]

Signals were scanned and quantified using the Sigma Scan software program (Jandel Scientific, San Rafael, CA), the levels of mRNA for lipogenic enzymes were estimated. The values were normalized for the corresponding amount of 18S rRNA.

Western Blot Analysis of SREBP-1

Isolation of nuclei and microsomes. Approximately 2 g of fresh epididymal WAT was placed in ice-cold buffer containing 10 mmol/LTris/HCl, pH 7.8, 0.25 mol/L sucrose, and 0.1 mmol/L EDTA and protease inhibitors cocktail (Sigma Chemical Co, St Louis, MO). Tissue was homogenized manually with Teflon-pestle homogenizer (Fisher Scientific, Schwerte, Germany) and centrifuged at 500 × *g* for 10 minutes at 4°C. The nuclear pellet obtained was suspended in buffer containing: 20 mmol/L HEPES, pH 7.9, 20% glycerol, 0.1 mol/L KCl, 1 mmol/L EDTA, and 0.5 mmol/L phenylmethylsulfunyl fluoride (PMSF). The resulting supernatant was recentrifuged at 15,000 × *g* for 15 minutes at 4°C to remove mitochondria. Supernatant obtained after mitochondria removal was centrifuged at 100,000 × *g* for 60 minutes at 4°C and the pellet containing microsomes was suspended in the same buffer as nuclei.

Aliquots of nuclear extract (100 μg protein) and microsomes (30 μg protein) were subjected to 10% sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS-PAGE) and transferred to Immobilon P (Sigma) membrane. SREBP-1 protein was detected by polyclonal antibodies against the N-terminus of SREBP-1 protein (Santa Cruz Biotechnology, Santa Cruz, CA). Signal was revealed by membrane incubation in buffer containing 0.1 mol/L Tris, pH 9.5, 0.1 mol/L NaCl, and CDP-Star (disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro) tricyclo[3.3.1.1.^{3,7}] decan}-4-yl)-1-phenyl phosphate) (Roche Diagnostics, Mannheim, Germany).

Enzyme Activity Assay

One gram of epididymal WAT was placed in 8 mL ice-cold 20 mmol/L Tris-Cl buffer (pH 7.8) containing 0.2% TritonX-100. The tissue was minced finely with scissors, homogenized manually with a Teflon-pestle homogenizer, and centrifuged at 30,000 × *g* for 20 minutes.

The resulting supernatant was decanted, and the pellet was resuspended in 5 mL isolation medium, rehomogenized, and centrifuged as above. The resulting supernatant was combined with this after the first centrifugation step and used for enzymes assay. ACC (EC 6.4.1.2), ACL (EC 4.1.3.8), FAS (EC 2.3.1.85), G6PDH (EC 1.1.1.49), 6PGDH (EC 1.1.1.44), and ME (EC 1.1.1.40) were assayed as described previously.[29]

Measurement of Lipogenesis In Vivo

The rate of fatty acid synthesis was assayed after intraperitoneal injection of 10 mCi in 1 mL per animal ^3H_2O (ICN Biomedicals, Costa Mesa, CA), as described recently.[17]

The statistical significance of differences between groups was assessed by 1-way analysis of variance (ANOVA) followed by Student's *t* test using the Systat software (Point Richmond, CA). Differences between groups were considered as significant at $P < .05$.

RESULTS

To study the potential association between renal failure, SREBP-1 level, and abnormalities in lipid metabolism, we have determined some serum parameters characteristic for CRF (creatinine, urea), as well as SREBP-1 level, lipogenic enzymes genes expression, and the rate of fatty acid biosynthesis in WAT. Serum urea and creatinine concentrations in CRF animals increased approximately 3-fold. The absence of nephrotic syndrome was documented by similar trace urinary protein excretion and plasma protein concentration in sham-operated and CRF rats. At the end of the experiment, body weights were lower in all CRF rats as compared to the sham-operated controls. This was due to a smaller body weight gain, since at the start point, all tested animals had identical body weights. The average daily food intakes of control and CRF rats were 32 ± 3 and 27 ± 3 g, respectively ($P < .001$).

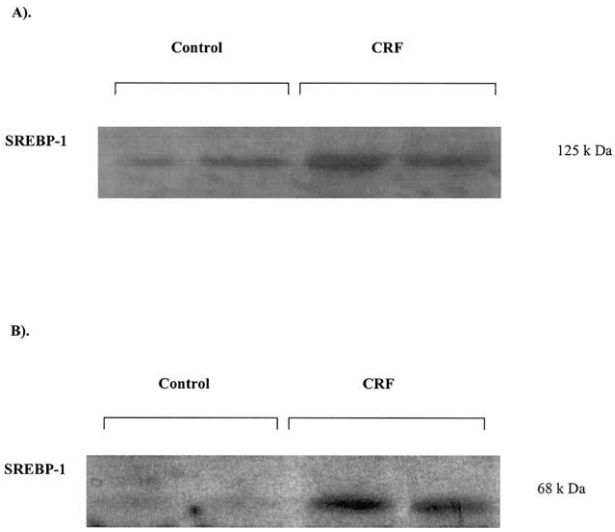
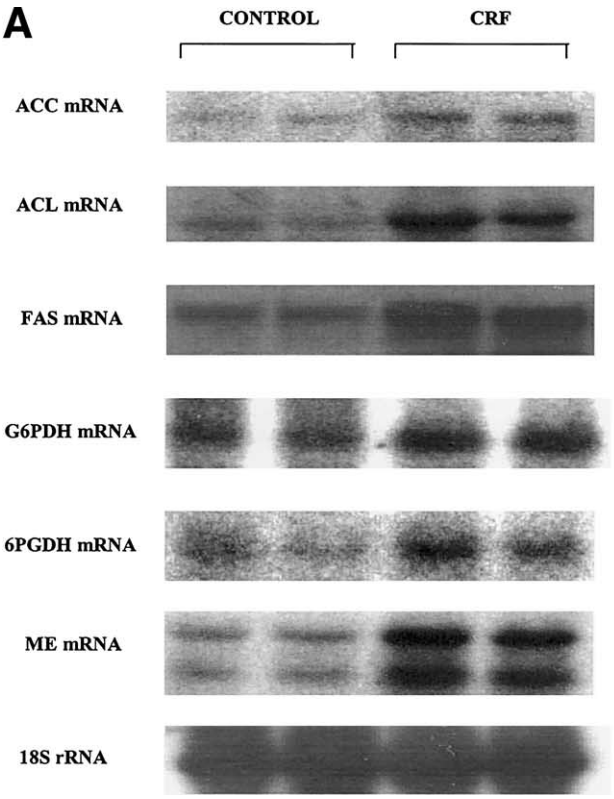


Fig 1. Representative Western blot analysis of SREBP-1 in WAT (A) microsomal and (B) nuclear extracts of control and CRF rats. Series of experiments were performed with 10 controls and 10 CRF rats and the data from 2 representative experiments are shown.

Immunoblotting of WAT microsomal (Fig 1A) and nuclear (Fig 1B) extracts revealed significant increase in both precursor (microsomal) and mature (nuclear) forms of SREBP-1 in CRF rats as compared to controls. The SREBP-1 antibody used for the immunoblots in Fig 1 does not distinguish between SREBP-1a and SREBP-1c. Consequently, the relative abundance of the 2 proteins cannot be quantified. Therefore we name these isoforms generally SREBP-1. The results presented in Fig 1 indicate that CRF caused the increase of both precursor and mature form of SREBP-1. In general, the nuclear content of SREBP-1 reflected the amount of microsomal SREBP-1 (Fig. 1). The mRNA abundance of lipogenic enzymes (ACC, ACL, FAS, G6PDH, 6PGDH, and ME) as determined by Northern blot analysis was upregulated in CRF rats as compared to controls (Fig 2A). As a control for loading equal amounts of RNA, we hybridized the same membrane with a probe against 18S rRNA. The films were quantified by densitometry, and the level of lipogenic enzymes mRNA was compared with the corresponding 18S rRNA (Fig 2B). These results indicate that CRF induced a substantial increase in both SREBP-1 protein abundance (Fig 1) and lipogenic enzymes mRNA level (Fig 2) in WAT. Among the fatty acid synthesizing enzymes, the increase in ACC mRNA level requires a special attention since ACC plays a key role in lipogenesis and this is the first report indicating upregulation of WAT ACC in CRF rats.

As a consequence of upregulation of SREBP-1 and its target genes, we observed a higher activity of lipogenic enzymes in WAT of CRF rats as compared to control (Table 1). To confirm that the elevated mRNAs level for lipogenic enzymes and their activities were leading to enhanced lipid synthesis in CRF rats, we performed in vivo biosynthesis experiments with ^3H_2O as a tracer. The control and CRF rats were given the same dose of ^3H_2O intraperitoneally. After 60 minutes, the WAT was excised, and the content of ^{3}H-labeled total fatty acids was



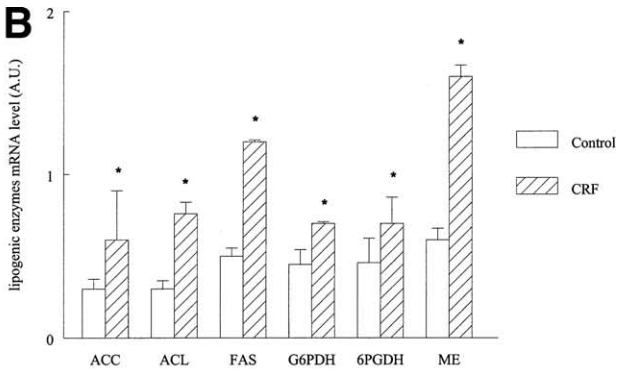


Fig 2. (A) Representative Northern blot analysis of lipogenic enzymes (ACC, ACL, FAS, G6PDH, 6PGDH, ME) and 18S rRNA in WAT of control (sham-operated) and CRF rats. (B) Quantification of the effect of CRF on lipogenic enzymes mRNA level in rat WAT. The blots being sequentially probed for ACC, ACL, FAS, G6PDH, 6PGDH, and ME mRNAs and 18S rRNA. Blots were scanned and quantified by the Sigma Scan software program. The changes in each mRNA of CRF rats (n = 10) as compared to the control rats (n = 10) were calculated after correction for loading differences with 18S rRNA and expressed in arbitrary units (A.U.), $P < .05$.

Table 1. WAT Lipogenic Enzyme Activities and Fatty Acid Synthesis Rate as well as Serum TAG and VLDL Concentrations, in CRF and Sham-Operated (control) Rats

	Control (n = 10)	CRF (n = 10)	Significance *v* Control (*P*)
ACC (nmol/min/mg protein)	2.77 ± 0.37	6.36 ± 1.46	<.05
ACL (nmol/min/mg protein)	4.3 ± 0.5	7.8 ± 0.8	<.05
FAS (nmol/min/mg protein)	7.5 ± 0.5	14.8 ± 0.5	<.01
G6PDH (nmol/min/mg protein)	9.5 ± 1.4	20.4 ± 6.7	<.05
6GPDH (nmol/min/mg protein)	8.2 ± 3.0	15.6 ± 4.9	<.02
ME (nmol/min/mg protein)	13.3 ± 2.3	26.2 ± 4.1	<.005
Fatty acid synthesis rate (μg ^{3}H/h/g tissue)	5.5 ± 1.0	10.9 ± 1.3	<.05
TAG (mg/dL)	110.0 ± 30.0	190.0 ± 50.0	<.01
VLDL-TAG (mg/dL)	85.0 ± 15.0	130.0 ± 20.0	<.005

measured. The CRF rats exhibited a significantly higher rate of fatty acid synthesis than control animals (Table 1). Plasma total TAG and VLDL-TAG concentrations were found to be significantly higher in CRF rats as compared to the control group (Table 1). The data concerning fatty acid synthesis are the same results as presented recently.[17] The data on TAG and VLDL-TAG are new results.

Essentially similar measurements were performed using the liver from the same CRF rats. In some CRF rats we observed significant increase in liver ACC gene expression and SREBP-1 abundance as compared to the control. In other CRF animals, in which a significant increase in WAT ACC mRNA level and SREBP-1 abundance did occur, no effects on ACC gene expression and SREBP-1 levels in the liver were found (not shown). The reason for this phenomenon is not clear. We know that it does not depend on either severity of the renal insufficiency nor food consumption. This suggests that some other factors, which may influence liver lipogenesis in CRF rat, could be involved.

DISCUSSION

The present study for the first time demonstrates higher levels of SREBP-1 (both precursor and mature form) in epididymal WAT of rats with experimental CRF than in controls. This suggests (but does not prove) that the increase in SREBP-1 level is due to the renal insufficiency and not to the possible effect of either the surgical procedure or other factors. Since the increase of precursor form is associated with the increase of mature SREBP-1 protein, one can conclude that the SREBP-1 increase in CRF depends mainly upon the synthesis of SREBP-1 precursor. Recently, we have shown that FAS and ACL mRNA abundance was increasing significantly in CRF.[17,18] Here, we found an increase of ACC, ME, G6PDH, and 6PGDH gene expression. Thus, our results significantly extend our previous observation.[17,18] The most important finding is that the increase of SREBP-1 level is associated with the upregulation of lipogenic enzymes gene expression in CRF rats. The coordinate upregulation of lipogenic enzymes, including ACC, ACL, FAS, G6PDH, 6PGDH, and ME, in CRF rats suggests that a "master switch" type mechanism may be involved in this process. In WAT, nuclear SREBP-1 may serve this function. Thus, as a consequence of upregulation of SREBP-1 and its target genes, we observed a higher rate of fatty acids biosynthesis in WAT of CRF animals (Table 1). The marked rise in plasma TAG and VLDL concentration accompanied by an increase in SREBP-1 level and lipogenic enzymes gene expression suggests a possible role of SREBP-1 in the pathogenesis of CRF-induced hypertriglyceridemia. The increase of SREBP-1 level, which causes an increase in lipogenic enzymes genes expression via transcriptional regulation, would accelerate the rate of fatty acids synthesis in WAT (Table 1). Consequently, this process could lead to the increase of TAG biosynthesis in CRF rats. A marked reduction of insulin sensitivity in WAT (where the hormone-sensitive lipase is expressed) of CRF rats may result in the increased lipolysis in this tissue. It has been proposed that the increased free fatty acid availability from WAT could be of major importance in determining hypertriglyceridemia in CRF.[35] If this is true, the serum concentration of TAG and VLDL in CRF animals could be also affected by the increase of WAT lipogenesis. In fact, this is the case as shown in Table 1. Animals (and humans) with CRF usually exhibit a reduction in adipose tissue mass. This phenomenon may argue against increased lipogenic activity, unless one assumes that adipose tissue is indirectly (via the increase in TAG synthesis and subsequent free fatty acids release) responsible for maintaining plasma (not tissue) lipid levels.

The mechanism(s) by which CRF causes the increase of SREBP-1 level is uncertain and requires further investigation. The CRF animals displayed a slight reduction of food intake and a slower growth rate as compared to the sham-operated rats. However, a lower amount of food intake cannot be responsible for the increase of SREBP level, since fasting or food restriction causes a decrease of SREBP level.[36] It has been reported that insulin increases the amount of liver nuclear SREBP-1c.[37] Considering that plasma insulin concentration is elevated in CRF animals,[17] one may conclude that the higher plasma insulin concentration could be responsible for the higher adipocyte SREBP-1 level in CRF rats than in control animals. However, recently published data suggest that insulin does not stimulate FAS gene expression through increasing SREBP-1 transcription in adipocytes.[38]

It has been shown recently that lipogenic enzymes gene expression (including ACC, FAS, ACL, G6PDH, and ME) and the rate of fatty acid synthesis increase significantly in adipose tissue of lipoprotein lipase–deficient rats.[39] Moreover, the induction of lipogenic enzymes gene expression in WAT of lipoprotein lipase–deficient rats was associated with the increased expression and processing of SREBP.[39] It is known that secondary hyperparathyroidism downregulates lipoprotein lipase expression in CRF rats,[40] which partly mimics conditions

of lipoprotein lipase–deficient rats. Thus, one can conclude that hyperparathyroidism may be a reasonable candidate responsible for the upregulation of SREBP-1 and its consequences in CRF rats. Low activity of lipoprotein lipase causes decreased fatty acids uptake (including essential polyunsaturated fatty acids) by adipose tissue.[41] Dietary polyunsaturated fatty acids (PUFA) are well established as negative regulators of lipogenesis.[42] The suppressive effect of PUFA on lipogenic enzymes is mediated by their reduction of SREBP-1 level.[42] Thus, one can conclude that in CRF rats upregulation of SREBP-1 is taking place, due to the following sequence of events: decrease of lipoprotein lipase activity, decrease of fatty acids uptake, and decrease of WAT PUFA level. This may also partly explain why the results concerning WAT SREBP-1 level (as well as upregulation of lipogenic enzymes) are more consistent than in liver. The experiments presented here were performed on the rat model of CRF. High plasma urea and creatinine, as well as TAG concentrations, suggest that the experimental model of CRF properly mimics human disease. However, one has to keep in mind that there are important species differences in lipid metabolism between rats and humans.

In conclusion, the data reported here indicate that in WAT of CRF rats upregulation of SREBP-1 is taking place, which probably causes the increase in lipogenic enzymes gene expression. Furthermore, our results indicate that upregulation of lipogenic enzymes gene expression in vivo could lead to the increase of fatty acid synthesis and consequently to overproduction of TAG. Therefore, upregulation of SREBP-1 and lipogenic enzymes gene expression, shown here for the first time, reveals another facet of disturbed lipid metabolism in CRF. These observations extend the mechanism of dyslipidemia in CRF beyond the impaired clearance of lipoproteins.

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