Upregulation of Fatty Acid Synthase Gene Expression in Experimental Chronic Renal Failure

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Hypertriglyceridemia associated with chronic renal failure (CRF) and elevated plasma concentration of very–low-density lipoprotein (VLDL) are thought to be a consequence of the depressed lipoprotein lipase and hepatic lipase activities and impaired clearance of lipoproteins. However, there is some evidence that the lipoproteins overproduction might also contribute to hypertriglyceridemia in CRF. This study was performed to test the hypothesis that the increased rate of lipogenesis consequent to upregulation of fatty acid synthase (FAS), a key lipogenic enzyme, gene expression could contribute to overproduction of triacylglycerols and to hypertriglyceridemia in CRF. FAS activity, FAS protein mass (Western blot analysis), and FAS mRNA level (Northern blot analysis) in liver and epididymal white adipose tissue (WAT) were measured in male Wistar rats 6 weeks after subtotal (5 of 6) nephrectomy or sham operation. Moreover, the rate of lipogenesis in WAT was determined. The CRF group showed significant increase in FAS gene expression (measured as activity, mRNA, and protein abundance) in both liver and WAT. This was associated with the increase in the lipogenesis rate and with the increase in plasma triacylglycerol and VLDL concentrations. Our results suggest that not only decreased removal, but also an increase of triacylglycerol production could contribute, in part, to the CRF-associated hyperlipidemia. Upregulation of FAS gene expression, shown in this report for the first time, reveals another factor involved in disturbed lipid metabolism in CRF. It seems that elevated plasma insulin and cytokine concentration could play an important role in the mechanism responsible for the increased FAS gene expression in CRF.

CHRONIC RENAL failure (CRF) is associated with a number of lipoprotein metabolism abnormalities, which are supposed to contribute to the pathogenesis of the accelerated atherosclerosis observed in uremia.[1] The principal features of the deranged lipid metabolism in CRF include: (1) an increased serum level of both very–low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) fractions leading to hypertriglyceridemia; (2) an unchanged or slightly increased LDL fraction enriched with triacylglycerols; (3) a decreased high-density lipoprotein $(HDL)_2$ subfraction of HDL resulting in decreased concentration of HDL-cholesterol.[1-6] The available data suggest that slow catabolism of the triacylglycerol-rich lipoproteins is responsible for these abnormalities.[3,7] This is probably related to the decreased activity of lipoprotein lipase, which plays a crucial role in lipoprotein metabolism[8] and downregulation of VLDL receptor expression in chronic renal failure.[9,10] The decrease of lipoprotein lipase activity in CRF can be due either to reduced enzyme synthesis and/or increased inhibition of the enzyme activity by circulating inhibitors.[11-13] Marked downregulation of hepatic lipase expression may also, in part, contribute to CRF dyslipidemia.[14] These results support the general concept of impaired removal of plasma triacylglycerol-rich lipoproteins as a primary cause of dyslipidemia in CRF. However, there is some evidence that reduced lipoprotein lipase activity is not a primary pathogenic factor for hypertriglyceridemia in CRF, but it probably accentuates this condition.[11,15] Furthermore, hepatic lipase activity seems to be independent of renal function.[11,15] It has been proposed that both decreased removal and increased production of triacylglycerols contributed to the hyperlipidemia in CRF patients.[16] One may assume, therefore, that increased rate of triacylglycerol-rich lipoprotein production could be a consequence of upregulation of fatty acid synthase (FAS) gene expression in CRF. This is based on the observation that: (1) insulin level, which is known to increase the lipogenic enzyme genes expression[17] is elevated in patients with advanced renal insufficiency[18]; (2) concentration of cytokines, which stimulate lipogenesis[19] and triacylglycerol biosynthesis[20] is often enhanced in chronically uremic patients. [21] However, some animal studies provide evidence against hepatic overproduction of triacylglycerol-rich lipoproteins.[22,23] To solve these issues, we investigated the FAS gene expression, a key lipogenic enzyme in experimental CRF.

MATERIALS AND METHODS

Animals

All animal procedures were conducted in agreement with our institutional guidelines for the care and use of laboratory animals. Male Wistar rats weighing approximately 250 g at the start of the investigation were housed in wire-mesh cages at 22^0C under a light/dark (12/12 hour) cycle with lights on at 7 AM. Renal failure was induced by 2-stage subtotal (5 of 6) nephrectomy using a dorsal incision as described previously.[24] Sham-operated animals served as control. The rats (both control and CRF) were allowed free access to food (commercial diet, composition described in Kochan et al[25]) 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 induction of renal failure. Blood samples were collected from the abdominal aorta

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under thiopental anesthesia. Determination of blood urea, creatinine, triacylglycerols, and insulin was performed in the Department of Clinical Biochemistry, Medical University of Gdansk, using routine methods. Small pieces of epididymal white adipose tissue (WAT) (approximately 1 g) and liver (approximately 0.2 g) were collected and rapidly frozen in liquid nitrogen. The tissues were stored at -80^0C until analysis.

Probes Designing and Labeling

FAS mRNA levels were detected using a 32-mer oligonucleotides (5′-GAT AGA GGT GCT GAG CCA GCG TGC TGA GCG TG-3′)[26] complementary to the rat FAS (Gene Bank accession no. M84761). The 31-mer antisense oligonucleotide (5′-CGC CTG CTG CCT TCC TTG GAT GTG GTA GCC G-3′) was used as a probe for the 18S rRNA.[27] The oligonucleotides were synthesized commercially (GENSET SA, Paris, France) with a single digoxigenin ligand at the 3′ end.

Isolation of RNA and Northern Blot RNA Analysis

Total cellular RNA was extracted from frozen tissue by a guanidinium isothiocyanate-phenol/chloroform method[28] 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 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 ultraviolet (UV) light. Prehybridization and hybridization were performed as described previously. [24,26,29] Signals were scanned and quantified using the Sigma Scan software program, and the levels of FAS mRNA were estimated. The values were normalized for the corresponding amount of 18S rRNA. Results expressed in arbitrary units are presented as means ± standard error (SEM) of the sample from 12 rats.

Western Blot Analysis

Polyclonal rabbit anti-FAS antibody was kindly provided by Dr Marcelle M. Lavau, INSERM Unite 465, Paris, France. Liver and WAT extracts were mixed with sample buffer, boiled, separated by sodium-dodecyl sulfate-polyacrylamide (SDS-PAGE) and electroblotted to Immobilon P membrane. The Immobilon P membrane was blocked by incubation in blocking buffer, incubated with polyclonal anti-FAS antibody, washed, and incubated with alkaline phosphatase-conjungated secondary antibody. Signal was revealed by membrane incubation in buffer containing 0.1 mol/L Tris, 0.1 mol/L NaCl, 50 mmol/L $MgCl_2$, 5-bromo-4-chloro-3-imdolyl phosphate (BCIP), and nitro blue tetrazolium (NBT) (pH, 9.5). Signals were scanned and quantified using the Sigma Scan software program (Jandel Scientific, Chicago, IL), the level of FAS protein was estimated. The values were normalized for β actin.

Enzyme Activity Assay

One-gram pieces of liver and epididymal WAT were placed in 8 mL ice-cold 20 mmol/L Tris-Cl buffer (pH, 7.8) containing 0.2% Triton X-100. The tissue was minced finely with scissors, homogenized manually with a Teflon-pestle homogenizer, and centrifuged at 30,000 x *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 enzyme assay. The fatty acid synthase (EC 2.3.1.85) was assayed as described previously. [30]

Lipogenesis In Vivo

The rate of fatty acid biosythesis was assayed after intraperitoneal injection of ^3H_2O (ICN Biomedicals, Costa Mesa, CA) 10 mCi in 1 mL per animal (both control and CRF rats). After 1 hour, WAT pieces were removed and rapidly frozen in liquid nitrogen. Samples of WAT (approximately 1 g) were saponified, and the fatty acid fraction was extracted as described previously.[31] Radioactivity was measured using Beckman LS 6000 IC Scintillation Counter. The results are expressed as micrograms of ^3H incorporated/h/g of wet tissue from the total radioactivity in the lipid residue and the specific radioactivity of plasma water measured in blood samples taken at the same time as tissue samples.

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 (Systat, Melbourne, Australia). Differences between groups were considered significant at $P < .05$.

RESULTS

To validate our experimental model, we have determined several parameters characteristic for CRF (Table 1). As expected, serum urea and creatinine concentrations in CRF animals increased approximately 3-fold, and serum insulin concentration approximately 2-fold as compared with control. The absence of nephrotic syndrome was documented by similar plasma albumin concentration in sham-operated and CRF rats. Significant end study body weight differences between CRF rats and control animals were found (Table1). This was due to smaller body weight gain, since at start point, all the animals tested had identical body weights. An average, daily food intake by control and CRF rats was 32±3 and 27±3 g, respectively ($P < .01$).

The effect of CRF on liver and WAT FAS activity and on plasma triacylglycerol and VLDL-triacylglycerol concentration is presented in Fig 1A through D. The significant increase of FAS activity both in liver (Fig 1A) and WAT (Fig 1B) was observed in rats with CRF. Simultaneously, total triacylglycerol (Fig 1C) and VLDL-triacylglycerol (Fig 1D) concentrations were found to be significantly higher in this group of animals as compared with the controls. To determine whether upregulation of liver FAS activity was accompanied by a modulation of liver FAS gene expression, immunoblot analysis was performed (Fig 2, top panel). The membrane was quantified by densitometry, and the level of liver FAS protein in CRF and control rats was compared with the corresponding β-actin

Table 1. Comparison of Some Studied Parameters in Rats With CRF and Controls

	Control (n = 12)	CRF (n = 12)	Statistical Analysis
Urea (mg/dL)	58.6 ± 11.1	182.8 ± 38.6	$P < .001$
Creatinine (mg/dL)	0.45 ± 0.2	1.26 ± 0.3	$P < .001$
Insulin (IU/L)	5.3 ± 1.4	9.9 ± 1.6	$P < .001$
End-study body weight (g)	450.0 ± 51.0	372.9 ± 26.7	$P < .05$

NOTE. Values are means ± SEM.

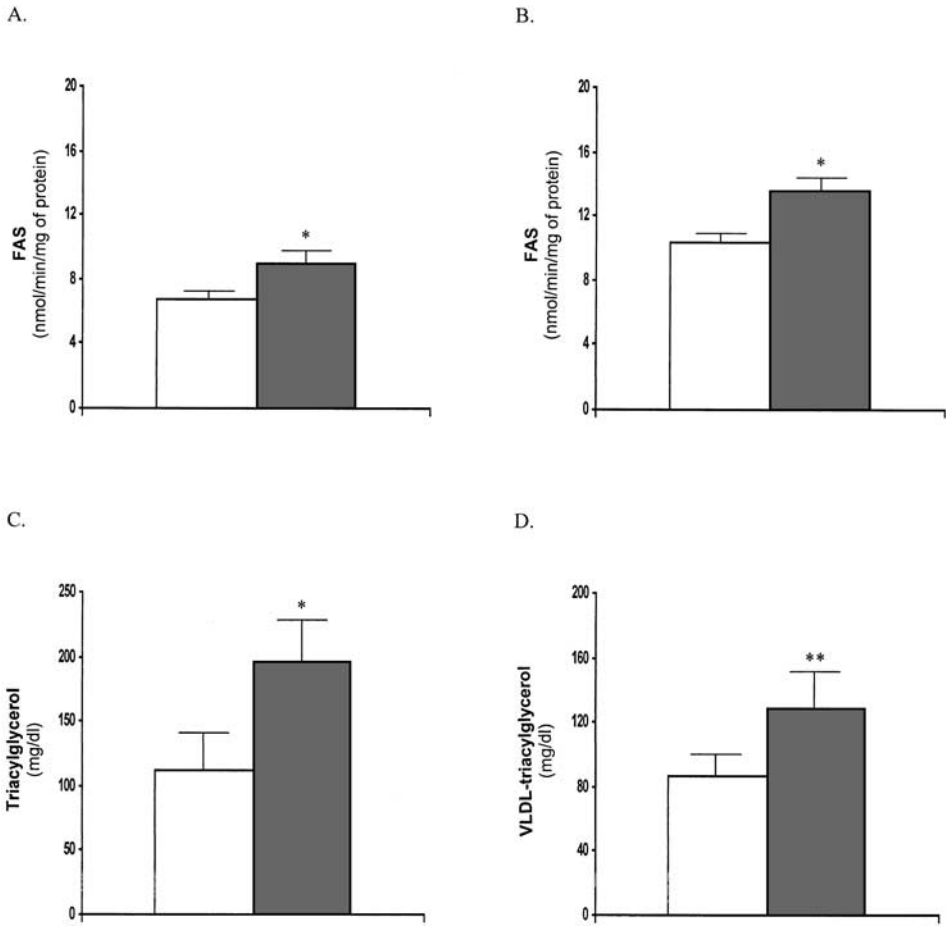

Fig 1. FAS activity in (A) liver and (B) WAT and serum concentration of (C) total triacylglycerol and (D) VLDL- triacylglycerol of chronically uremic (■) and control (□) rats. Values are means ± SEM. (* $P < .01$, ** $P < .005$).

level in these animals (Fig 2, bottom panel). Western blot analysis indicates that the increase of liver FAS activity accounted for the increase in the enzyme protein abundance. Finally, to determine whether the increase of FAS activity is accompanied by an increase of FAS mRNA abundance, Northern blot analysis was performed. The effect of CRF on liver FAS mRNA level is presented in Fig 3, which exhibits a representative Northern blot analysis (top panel). The films were quantified by densitometry, and the level of FAS mRNAs in CRF and control rats was compared with the corresponding 18S rRNA level in these animals (Fig 3, bottom panel). These results indicate that CRF induced a substantial increase in FAS mRNA level in liver. The pattern of changes in the FAS activity and FAS protein level resemble that of changes in abundance of FAS mRNA (compare Figs 1A, 2, and 3). Thus, the observed upregulation of FAS activity in the CRF animals appears to occur at a pretranslational step (presumably at a transcriptional step and/or by an increase in stability of mRNA). The significant increase in WAT FAS activity has also been observed in CRF animals as compared with control rats (Fig 1B). Western blot analysis (Fig 4) indicates that the increase of WAT FAS activity accounted for the increase in the enzyme protein abundance. The effect of CRF on WAT FAS mRNA level is presented in Fig 5, which exhibits a representative Northern blot analysis (top panel). The films were quantified by densitometry, and the level of FAS mRNA in CRF and control rats was compared to the corresponding 18S rRNA level in these animals (Fig 5, bottom panel). These results indicate that CRF induced a substantial increase in FAS mRNA level in epididymal WAT. The pattern of changes in the FAS activity and FAS protein level resemble that of changes in abundance of FAS mRNA (compare Figs 1B, 4, and 5). As a consequence of increase in FAS gene expression, the rate of fatty acid synthesis was also enhanced in CRF animals (Fig 6).

DISSCUSSION

The present study demonstrates that the FAS gene expression in liver and epididymal WAT measured as the enzyme activity, protein, and mRNA abundance increased in response to experimental CRF, while FAS gene expression was constant in the control animals. This suggests (but does not prove) that the increase in FAS gene expression is due to the renal insufficiency as opposed to the possible effect of either the surgical procedure or the ageing process. However, the close analysis let us note that there are significant differences in liver FAS mRNA abundance also among the uremic individuals. The reason for that phenomenon is obscure, particularly because we

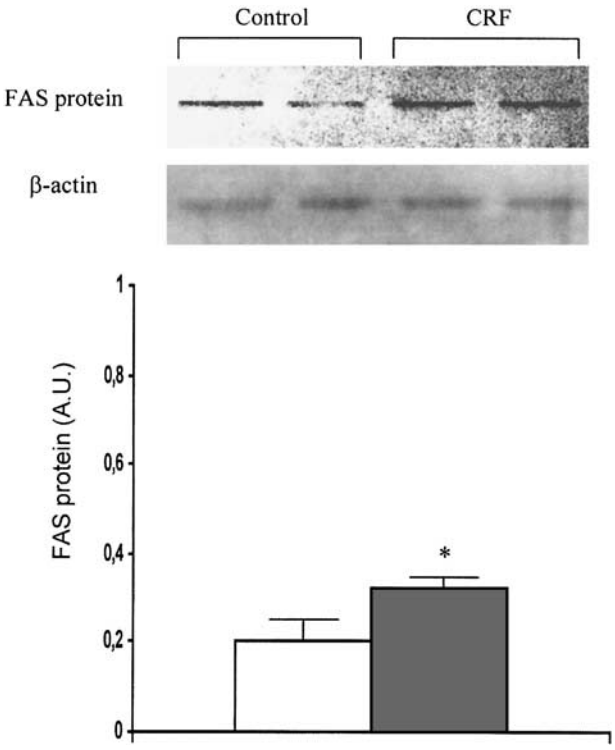

Fig 2. (A) The representative Western blot analyses of the liver FAS protein from chronically uremic (■) and control (□) rats . (B) Signals were scanned and quantified. Values are means ± SEM. (* *P* < .05).

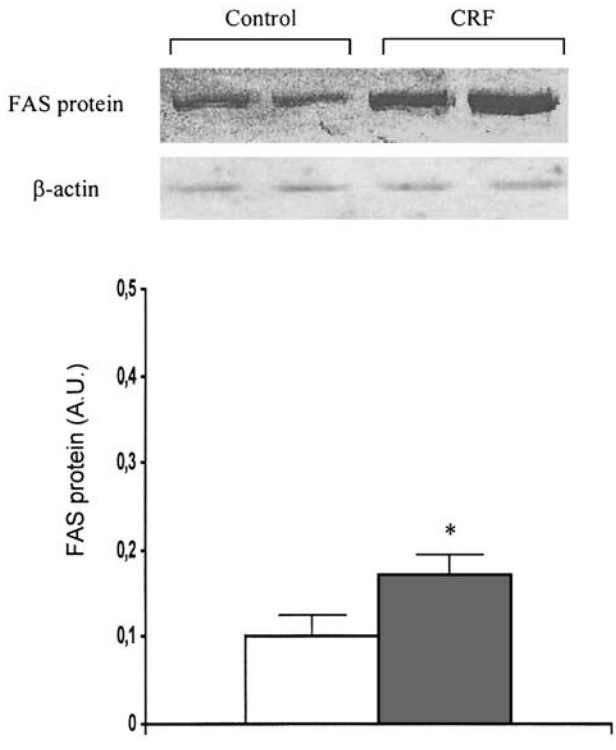

Fig 4. (A) The representative Western blot analyses of the FAS protein from WAT of chronically uremic (■) and control (□) rats . (B) Signals were scanned and quantified. Values are means ± SEM. (P* < .01).**

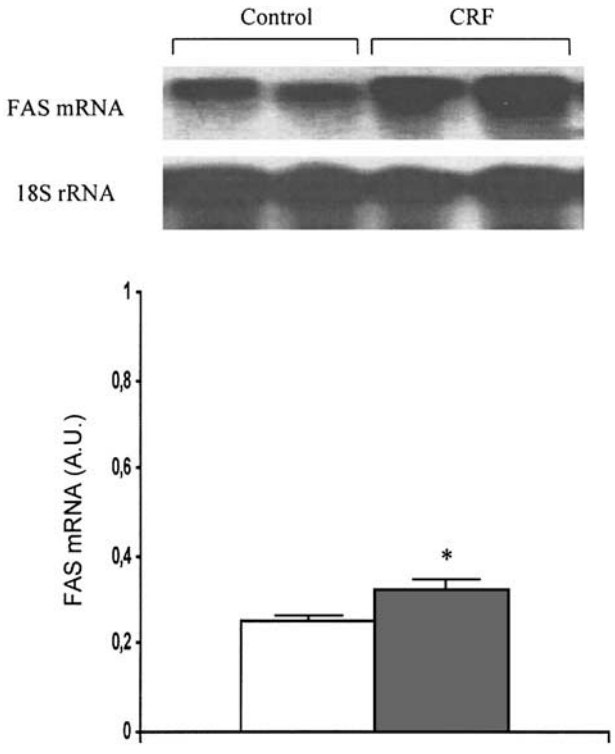

Fig 3. (A) The representative Northern blot analyses of the liver FAS mRNA from chronically uremic (■) and control (□) rats . (B) Signals were scanned and quantified. Values are means ± SEM. (P* < .05).**

know that the animals of the studied group presented a similar level of renal insufficiency and they ate a similar amount of food. Some other, subject-characteristic factors, which may influence liver FAS mRNA level could be involved. This may explain the reasons why the results of studies concerning the role of liver triacylglycerol biosynthesis in the development of renal failure-associated hypertriglyceridemia are diverse.

The marked elevation of plasma triacylglycerols and VLDL concentrations accompanied by an increase of FAS gene expression (Fig 1) and the increase in the rate of fatty acid synthesis (Fig 6) suggest an important role of FAS in pathogenesis of CRF-induced hypertriglyceridemia. The increase of FAS gene expression and consequently the rate of fatty acids synthesis could lead to an increase of triacylglycerol biosynthesis in CRF. It should be noted that serum triacylglycerols concentration increased about 2-fold in uremic rats (Fig 1), while the liver FAS activity, protein, and mRNA abundance increased only approximately 30%. This may corroborate with the generally accepted opinion that an impaired triacylglycerol-rich lipoprotein catabolism is a dominating factor responsible for the hypertriglyceridemia development found in CRF animals and human beings. Overproduction of triacylglycerol-rich lipoproteins could be an additional, not observed in all individuals factor leading to hypertriglyceridemia in CRF. Therefore, our results support the idea that both decreased removal (as a dominating factor) and increased production (as an additional factor) of triacylglycerols contributed to the hyperlipidemia in

CRF.[32] It should be emphasized that the measurements of the FAS gene expression significantly extended the previous observation, which had been mainly concerned with the plasma triacylglycerol turnover.[32] It is likely that the increase of WAT lipogenesis, due to overexpression of FAS, can also contribute to hypertriglyceridemia in CRF animals, since fatty acids released from adipose tissue can be taken up by liver and converted to triacylglycerol-rich lipoproteins (mainly to VLDL).

The mechanism(s) by which CRF increase FAS gene expression in vivo is uncertain and requires further investigation. The CRF animals demonstrated a slight reduction of food intake and slower growth rate as compared with sham-operated rats. However, lower amount of food intake cannot be responsible for the increase of FAS gene expression, since fasting or food restriction causes a decrease of both FAS activity and FAS mRNA level in liver and WAT.[17] Furthermore, a diminished food intake is normally associated with a decrease in plasma triacylglycerol and VLDL levels, [33, 34] as opposed to the significantly elevated levels found in CRF animals (Table 1). Considering that plasma insulin concentration is elevated in CRF animals (Table 1) and it increases FAS gene expression,[17] one may conclude that higher plasma insulin concentration could be responsible for a higher FAS gene expression in CRF rats than in controls. Furthermore, some cytokines are constantly increased in uremic patients[21] and could be an important mediator of the increased rate of lipogenesis[19] and triacylglycerol production. [20] Recently, we have shown that leptin inhibited a FAS gene expression in rat WAT in vivo.[35] Furthermore, we have also found that CRF decreased by about 50% a leptin mRNA level in rat WAT.[24] Therefore, another possible mechanism is that lower leptin concentration leads to the increased FAS gene expression in CRF animals. This conclusion is consistent with a very recent observation of Hasty et al, [36] who showed that leptin deficiency in *ob/ob* mice increased hepatic triacylglycerol production. These data suggest that leptin might have some impact on plasma triacylglycerol concentration. Lower plasma leptin concentration would favor triacylglycerol overproduction in CRF animals. However, other possible mechanisms for the observed upregulation of FAS gene expression in CRF animals must also be considered.

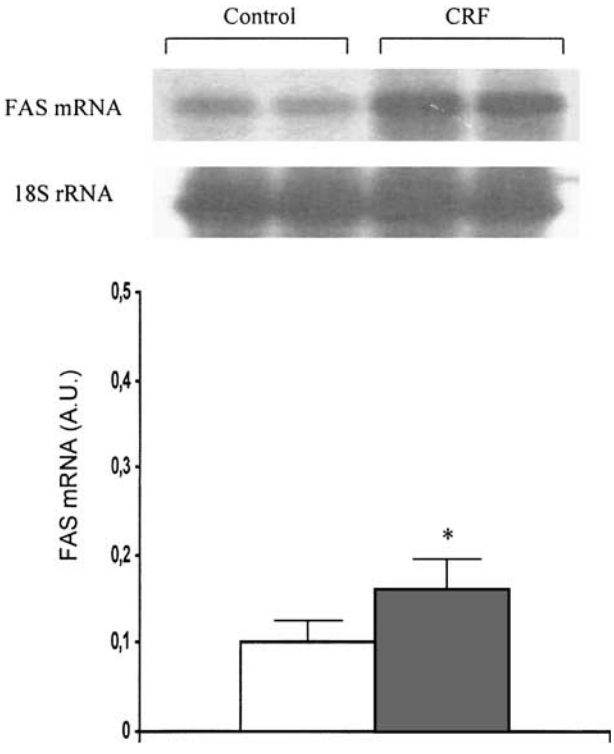

Fig 5. (A) The representative Northern blot analyses of the FAS mRNA isolated from WAT of chronically uremic (■) and control (□) rats . (B) Signals were scanned and quantified. Values are means ± SEM. (*$P < .01$).

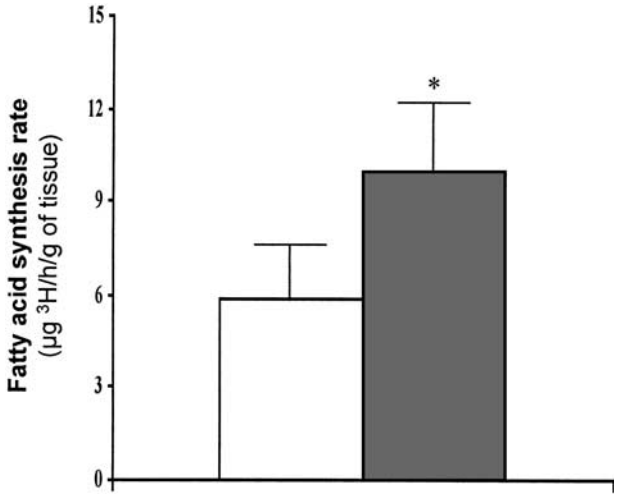

Fig 6. The rate of WAT fatty acid synthesis in chronically uremic (■) and control (□) rats. Values are means ± SEM. (*$P < .05$).

In conclusion, the data reported here suggest that FAS gene expression is increased in both liver and WAT of CRF rats. Furthermore, our results indicate that upregulation of FAS gene expression is accompanied by the marked elevation of plasma total triacylglycerol and VLDL- triacylglycerol concentration. Therefore, upregulation of FAS gene expression, shown here for the first time, reveals another important factor of disturbed lipid metabolism in CRF. The mechanism responsible for the increased FAS gene expression in CRF is unclear. It seems that elevated plasma insulin and cytokine concentrations may play an important role in this phenomenon. It is also of interest that most gene expressions of several proteins involved in lipid metabolism, such as lipoprotein lipase, hepatic lipase, VLDL receptor, LDL-receptor, and leptin have been shown to be depressed in experimental CRF. [9,11,12,24] One may conclude, therefore, a diffuse downregulation of mRNA production in renal failure. The data presented here indicate that this is not the case. The FAS gene belongs to the upregulated genes in uremia.

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