Secondary hyperparathyroidism downregulates lipoprotein lipase expression in chronic renal failure

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Vaziri, N. D., X. Q. Wang, and K. Liang. Secondary hyperparathyroidism downregulates lipoprotein lipase expression in chronic renal failure. Am. J. Physiol. 273 (Renal Physiol. 42): F925–F930, 1997.—In a recent study, we found marked downregulation of lipoprotein lipase (LPL) gene expression in fat, myocardium, and skeletal muscle of rats with chronic renal failure (CRF). Recently, hepatic lipase expression was shown to be depressed in CRF rats, and parathyroidectomy (PTX) was shown to reverse this abnormality. This study was undertaken to determine whether downregulation of LPL expression in CRF is due to secondary hyperparathyroidism. Accordingly, LPL mRNA (Northern analysis), protein mass (Western analysis using mouse antiovine LPL monoclonal antibody, SD2), and catalytic activity of the fat and soleus muscle were compared in five-sixths-nephrectomized male rats (CRF), parathyroidectomized CRF rats, and sham-operated control animals. The CRF animals exhibited marked hypotriglyceridemia and significant reductions of fat and skeletal muscle LPL mRNA abundance, protein mass, and catalytic activity (P < 0.05 vs. controls, for all parameters). PTX completely normalized the LPL mRNA, protein mass, and enzymatic activity and partially ameliorated the CRF hypotriglyceridemia (P < 0.05 vs. CRF group, for all parameters). Thus secondary hyperparathyroidism is responsible for impaired LPL expression in experimental CRF. This abnormality is completely corrected by PTX.

CHRONIC RENAL FAILURE (CRF) is commonly accompanied by increased plasma triglyceride and very-low-density lipoprotein (VLDL) concentrations (3, 5, 11). This is associated with triglyceride enrichment of VLDL, intermediate-density lipoprotein, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) particles (3, 20, 26). Moreover, clearance of VLDL and chylomicrons and their remnants, as well as LDL and HDL, is impaired in CRF (4, 13, 25, 26, 33). Lipoprotein lipase (LPL) and hepatic lipase play an important role in catabolism of plasma triglyceride-laden lipoproteins. Deficiencies of these enzymes result in accumulation and triglyceride enrichment of these lipoproteins, resembling the plasma lipoprotein profile observed in CRF. In this regard, several previous studies (1, 3, 6, 9, 10, 15, 19) have pointed to the presence of acquired LPL and hepatic lipase deficiencies in CRF, as evidenced by depressed postheparin lipolytic activity, reduced adipose tissue LPL activity, and diminished plasma immunodetectable LPL protein in clinical and experimental CRF. In a recent study (30), we showed a marked downregulation of LPL gene expression in the heart, skeletal muscle, and adipose tissue of rats with experimental CRF. Although it elucidated the molecular basis of CRF-induced LPL deficiency, the latter study did not address the mechanism by which CRF downregulates LPL gene expression. It has been speculated that the CRF-associated reduction in LPL activity may be mediated by hyperparathyroidism and the resulting insulin resistance (1, 3). However, to our knowledge, the role of CRF-induced secondary hyperparathyroidism and its reversal by parathyroidectomy on LPL gene expression have not yet been demonstrated. Interestingly, hepatic lipase expression has been shown to be markedly depressed in rats with CRF (17). This abnormality was completely reversed by parathyroidectomy, indicating that the effect of CRF on hepatic lipase must be mediated by excess parathyroid hormone (PTH), which is a constant feature of CRF (17). On the basis of these observations, we hypothesized that downregulation of LPL expression in CRF may be due to the associated secondary hyperparathyroidism. The present study was designed to test this hypothesis.

METHODS

Animal Models

Male Sprague-Dawley rats weighing 225–250 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). They were housed in a climate-controlled, light-regulated facility with 12-h-day (~500 lx) and 12-h-night (~5 lx) cycles. The animals were fed regular rat chow (Purina Mills, Brentwood, MO) and water ad libitum. They were then randomly assigned to one of the following groups.

CRF group. The animals assigned to the CRF group were subjected to five-sixths nephrectomy, i.e., surgical resection of the upper and lower one-thirds of the decapsulated left kidney followed by right nephrectomy 5 days later. The procedures were carried out under general anesthesia, using a dorsal incision as described previously (30).

CRF-parathyroidectomy group. Animals assigned to this group were first subjected to surgical parathyroidectomy (PTX) without removal of the thyroid tissue. The procedure was carried out by electrocautery under a surgical microscope as described by Ni et al. (21). The success of the procedure was ascertained by demonstrating a fall in serum calcium concentration by at least 2 mg/dl below the baseline after PTX. The animals that failed this test were excluded. In an attempt to discern the effect of PTX from that of the associated hypocalcemia, the drinking water supply for these animals was supplemented with calcium gluconate at a concentration of 50 g/l. This intervention was sufficient to restore normocalcemia, despite PTX in these animals, as demonstrated by other investigators (21). The animals were then allowed to recover for 1 wk, after which they were subjected to five-sixths nephrectomy as noted above.

Control group. The animals assigned to the control group were subjected to sham operation and were provided free access to food and water.

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Protocol

The study groups were observed for 5 wk, at which point they were placed in metabolic cages for a 24-h urine collection. They were then killed between 9 and 11 AM by exsanguination, using cardiac puncture. Soleus muscle and suprasarticular fat pad were harvested immediately, snap frozen in liquid nitrogen, and stored at −70°C until processed. All surgical procedures were carried out under general anesthesia (pentobarbital sodium, 50 mg/kg ip) while strict hemostasis and aseptic techniques were observed. Serum calcium concentration was measured by atomic absorption spectrometry (model 305; Perkin-Elmer, Norwalk, CT). Serum cholesterol, triglyceride, creatinine, and albumin concentrations and urinary protein and creatinine contents were determined, using standard laboratory procedures. Arterial blood pressure was determined by a tail sphygmomanometer (Harvard Apparatus, South Natick, MA). Briefly, the conscious animal was placed in a restrainer and permitted to rest for 10–15 min. The cuff was then placed on the tail and was inflated and released several times to condition the animal to the procedure. After stabilization, blood pressure was measured three times, and the average of the three values was used.

RNA Preparation and Northern Blot Analysis

The rats were killed, and the tissues were removed immediately, snap frozen in liquid nitrogen, and stored at −70°C until processed. Total RNA was prepared from 0.5 g of tissue with RNAzol, using the manufacturer’s recommended procedure (Tel-Test, Friendswood, TX). RNA concentration was determined from the absorbance at 260 nm, using a spectrophotometer (Gene-Quat; Bio-Rad, Hercules, CA). Twenty-five microgram aliquots of total RNA were denatured in 2.2 M formaldehyde at 65°C for 15 min and run on 1% agarose-2.2 M formaldehyde gel at 40 V for 5 h. The separated RNA was irradiated (Ultraviolet Crosslinker; Fisher Scientific, Pittsburgh, PA). The membrane was incubated at 65°C in a solution containing 5× SSPE (0.75 M NaCl, 0.05 M NaH2PO4, 0.005 M EDTA, pH 7.4), 5× Denhardt’s (1 g/l each of Ficoll type 400), polyvinylpyrrolidone, and bovine serum albumin (fraction 5%), 1% sodium dodecyl sulfate (SDS), and 100 µg/ml salmon sperm DNA for 2 h. The CDNA probes for mouse LPL [1.5-kilobase (kb) EcoR I fragment of PGmL D (16)] and rat glyceroldehyde-3-phosphate dehydrogenase (GAPDH) (1.3-kb Pst I fragment) were obtained from American Type Culture Collection (Rockville, MD) and labeled with [32P]dCTP (3,000 Ci/mmol; NEN, Boston, MA) by the random primer method (Promega, Madison, WI). Hybridization was carried out at 60°C in a prehybridization solution with 32P-labeled CDNA. The blots were washed twice in 2× SSPE-0.5% SDS solution at room temperature, twice in 1× SSPE-0.5% SDS solution at 25°C, and twice in 0.1× SSPE-0.5% SDS solution at 37°C, for 15 min each. The washed blots were exposed to X-ray film (NEN) at −80°C for 2–6 h for GAPDH and 2–3 days for LPL. The autoradiographs were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA) to determine relative mRNA levels. The values obtained for GAPDH were used as an internal control.

Tissue Preparation

Tissue samples for measurements of LPL protein and activity were prepared as previously described (8). Briefly, 200-mg aliquots of frozen tissues (muscle or fat) were homogenized in 1 ml of 50 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride (pH 8.0) buffer containing 1 M polyethylene glycol, 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 1 µg/ml pepstatin A. The crude extracts were then centrifuged at 14,000 revolutions/min for 20 min at 4°C, using a microcentrifuge to remove tissue debris. The supernatant was saved for determination of total protein concentration and LPL activity and for Western analysis. Protein concentration in the supernatant was measured, using a biochumonic acid protein assay reagent kit (Pierce, Rockford, IL).

LPL Enzymatic Activity Assay

A [13C]triolein-containing substrate (Amersham Life Science, Arlington Heights, IL) was emulsified with lecithin (Sigma) and mixed with human serum as a source of apolipoprotein CII, as described previously (14). Two hundred microliters of the test sample were incubated with the substrate at 37°C for 60 min. The incubation was terminated, and 13C-labeled free fatty acids were extracted with a methanol-chloroform-heptane mixture and assayed for radioactivity, using standard liquid scintillation spectrometry (model LS 9000; Beckman, Fullerton, CA). Activity was expressed as nanoequivalent free fatty acid released per minute per gram of the original tissues (27).

Western Blot Analysis

Aliquots of the supernatants containing 50 µg protein were size fractionated on 4–12% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 2 h. After electrophoresis, proteins were transferred to Hybond enhanced chemiluminescence membrane (Amersham Life Science). The membrane was incubated in milk-free Super Block blocking buffer (Pierce) overnight. The membrane was then incubated for 2 h in the same buffer, to which a mouse anti-bovine LPL monoclonal antibody (SD1) was added at a final concentration of 0.1 µg/ml. This antibody was generously provided by Professor John Brunzell (Univ. of Washington, Seattle, WA). The specificity of this antibody has been documented previously (23). After incubation, the membranes were washed four times (15 min each) in a solution containing 1× Tris-buffered saline and 0.1% Tween 20 before a 1-h incubation in Super Block blocking buffer to which diluted (1:250,000) Immunopure peroxidase-linked goat anti-mouse immunoglobulin G (Pierce) was added. The washes were repeated before the membranes were developed with the chemiluminescent agent (Ultraluminol/Enhance Solution, Pierce) and subjected to autoradiography for 5 s. In all instances, the membranes were stained with Ponceau SX stain, which verified uniformity of protein load across all samples. Thus the difference in LPL protein among the study animals was not due to differences in total protein loading.

Statistical Analyses

Analyses of variance, regression analysis, and Duncan’s multiple range test were used in statistical evaluation of the data, which are expressed as means ± SE. P ≤ 0.05 was considered significant.

RESULTS

General Data

Data are depicted in Table 1. As anticipated, the CRF and CRF-PTX groups showed a marked reduction in creatinine clearance (P < 0.003, analysis of variance). No significant difference was found in creatinine clear-
ance between the CRF and CRF-PTX groups. The CRF group exhibited a marked increase in serum triglyceride concentration when compared with the normal control group ($P < 0.001$). Serum triglyceride concentration in the CRF-PTX group was significantly lower than that found in the CRF group. However, serum triglyceride level was not fully normalized by PTX. Hence, PTX ameliorated the CRF-induced hypertriglyceridemia significantly but not completely. In contrast, PTX had no effect on serum cholesterol concentration, which is moderately elevated in this model of CRF (12, 17, 30). The PTX group showed severe hypocalcemia (5.42 ± 0.4 mg/dl) before calcium gluconate supplementation. However, with addition of calcium gluconate to drinking water, serum calcium in the CRF-PTX group (9.5 ± 0.25 mg/dl) rose to a value that was comparable with those found in the CRF (9.1 ± 0.3 mg/dl) and the control (9.6 ± 0.2 mg/dl) groups. Thus the observed hypocalcemia in the CRF-PTX group confirmed the success of the PTX procedure, and restoration of normocalcemia with calcium gluconate administration demonstrated the effectiveness of the latter therapy in maintaining normal serum calcium concentration, despite PTX as shown earlier (17, 22). Body weight at the conclusion of the study was significantly greater ($P < 0.05$) in the control group (393 ± 12 g) than in either the CRF (326 ± 16 g) or CRF-PTX (320 ± 10 g) group. Thus the animals with CRF showed a significant growth retardation. The CRF group exhibited a moderate rise in arterial blood pressure that was partially mitigated by PTX.

LPL mRNA

The CRF group exhibited a twofold reduction of the adipose tissue LPL mRNA abundance compared with the normal control group. In addition, skeletal muscle LPL mRNA abundance was reduced by threefold in the CRF group. The reductions in LPL mRNA abundance were completely reversed by PTX. Data are illustrated in Figs. 1 and 2.

LPL Protein Mass

As with the LPL mRNA, the LPL protein mass relative to the total cellular protein mass was markedly reduced in both adipose tissue and skeletal muscles of the CRF animals. The CRF-associated reduction of LPL protein mass in the fat and skeletal muscle tissues was restored to normal by PTX. Data are shown in Figs. 3 and 4.

### Table 1. Serum concentration of triglycerides, cholesterol, and creatinine; urinary protein excretion; and systolic blood pressure in normal control, CRF, and CRF-PTX groups

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>CRF</th>
<th>CRF-PTX</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride, mg/dl</td>
<td>54.7 ± 4.5</td>
<td>157 ± 25.2</td>
<td>103.1 ± 12.1 *</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>57.1 ± 2.7</td>
<td>135.1 ± 20.4</td>
<td>158 ± 15.6</td>
<td>≤ 0.003</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.35 ± 0.02</td>
<td>0.96 ± 0.13</td>
<td>0.93 ± 0.04</td>
<td>≤ 0.003</td>
</tr>
<tr>
<td>Ccr, ml/min</td>
<td>1.9 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>≤ 0.003</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>11.3 ± 1.1</td>
<td>46.5 ± 2.4</td>
<td>44.2 ± 5.1</td>
<td>≤ 0.0034</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td>125 ± 1.6</td>
<td>153 ± 1.8</td>
<td>133 ± 1.7 *</td>
<td>≤ 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ccr, creatinine clearance; CRF, chronic renal failure rats with intact parathyroid glands; CRF-PTX, chronic renal failure rats with parathyroid ablation; ANOVA, analysis of variance. *$P < 0.05$ vs. CRF group.

Fig. 1. Representative Northern blots of adipose tissue and skeletal muscle lipoprotein lipase (LPL) mRNA and corresponding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in 2 normal control animals (NL), 2 chronic renal failure animals with intact parathyroid glands (CRF), and 2 parathyroidectomized chronic renal failure animals (CRF-PTX).

Fig. 2. Group data representing LPL-to-GAPDH mRNA ratios of fat and skeletal muscle of NL, CRF, and CRF-PTX groups. Data are given as means ± SE; $n = 6$ animals/group. *$P < 0.05$ vs. other groups.
LPL Catalytic Activity

The reductions in LPL mRNA and protein mass of the fat and skeletal muscle were accompanied by parallel reductions in LPL catalytic activities of the tested tissues obtained from the CRF animals. The CRF-associated decreases in adipose tissue and skeletal muscle LPL activity were fully reversed by PTX. Data are shown in Fig. 5.

Correlations

LPL catalytic activity was directly related to LPL protein mass in the tested tissues of the study groups (r = 0.86, P < 0.01 for fat tissue; r = 0.81, P < 0.01 for muscle tissue). There was a significant inverse correlation between serum triglyceride concentration and LPL activity (r = -0.79, P < 0.05 for fat; r = -0.76, P < 0.05 for muscle).

DISCUSSION

In a recent study (30), we demonstrated a marked downregulation of LPL gene expression along with significant reductions of LPL catalytic activity of fat and of skeletal and cardiac muscles in rats with CRF. However, the available data did not allow any conclusion as to the mechanism(s) responsible for the impaired LPL expression and activity in the CRF animals. Klin et al. (17) recently demonstrated that expression of hepatic lipase is depressed in experimental CRF. They further showed that impaired hepatic lipase expression in CRF animals could be reversed by PTX (17). Because LPL and hepatic lipase belong to the large lipase gene family and share considerable structural and functional characteristics, we hypothesized that excess PTH may also account for downregulation of LPL expression in CRF. To this end, LPL mRNA, protein mass, and activity were studied in CRF animals with intact parathyroid gland, CRF animals subjected to PTX but maintained eucalcemic with calcium supplementation, and sham-operated normal control animals. The results confirmed marked downregulation of LPL gene expression and LPL catalytic activity in the CRF animals, as recently demonstrated by our group (30). In addition, the present study revealed parallel reductions in LPL protein mass in the target tissues of animals with CRF. The latter finding had not been documented previously and points to the quantitative as opposed to the functional nature of LPL deficiency in CRF. The study further showed that the CRF-induced downregulation of LPL gene expression, protein mass, and activity can be reversed by PTX, which obviates the CRF-associated hyperparathyroidism. Thus the study results support the original hypothesis that excess PTH is involved in downregulation of LPL. The effect of PTX on LPL expression was not due to the change in serum calcium concentration, since normocalcemia was successfully maintained by calcium supplementation in

Fig. 3. Representative Western blots of adipose tissue and skeletal muscle LPL in 2 NL animals, 2 CRF animals, and 2 CRF-PTX animals.

Fig. 4. Relative optical densities of LPL protein bands of fat and skeletal muscle in NL, CRF, and CRF-PTX groups. Data are given as means ± SE; n = 6 animals/group. *P < 0.05 vs. other groups.

Fig. 5. LPL activity of fat and skeletal muscle of sham-operated NL animals, CRF animals, and CRF-PTX animals. Data are given as means ± SE; n = 6 animals/group. *P < 0.05 vs. other groups.
the CRF-PTX animals. However, excess PTH has been shown to raise cytosolic calcium concentration, and PTX has been shown to restore normal cytosolic calcium concentration in rats with CRF (17, 22). Thus the effect of PTX on LPL gene expression in the CRF animals is most likely mediated by the resulting change in cytosolic calcium concentration.

The restoration of normal tissue LPL expression shown here and that of hepatic lipase shown by Klin et al. (17) were accompanied by a partial, as opposed to complete, correction of CRF-associated hypertriglyceridemia. This observation suggests that together LPL and hepatic lipase deficiencies play a partial role in the pathogenesis of CRF-induced hypertriglyceridemia. The data further indicate that another PTH-independent factor(s) must also contribute to the CRF hypertriglyceridemia. In this regard, we have recently demonstrated a marked downregulation of VLDL receptor gene expression and protein mass in rats with CRF (31). VLDL receptor, which is primarily expressed in the adipose tissue, skeletal muscle, and myocardium, plays an important role in the clearance/catabolism of triglyceride-rich VLDL particles (7, 22, 24, 28, 32). Accordingly, its observed downregulation is expected to contribute to elevation of plasma VLDL and triglycerides in CRF. Interestingly, we have found that in contrast to its effect on LPL and hepatic lipase, PTX does not improve the associated impairment of VLDL receptor expression in CRF animals (unpublished data). The favorable action of PTX in reversing the CRF-mediated downregulations of LPL and hepatic lipase but not VLDL receptor can, in part, account for the partial as opposed to full reversal of CRF-associated hypertriglyceridemia in this model.

We have recently demonstrated a marked downregulation of LPL protein expression in rats with experimental nephrotic syndrome (18). The observed downregulation of LPL expression in the CRF animals employed here is not due to proteinuria. This is because the CRF animals exhibited minimal proteinuria that was approximately sevenfold less than that seen in the nephrotic animals (29).

In a recent study, Arnadottir and Nilsson-Ehle (2) provided evidence that PTH is not an inhibitor of LPL activity. This conclusion was based on 1) lack of a significant correlation between plasma PTH concentration and postheparin lipolytic activity in a group of dialysis patients and 2) failure of PTH to inhibit the enzymatic activity of purified bovine milk LPL in vitro. These observations do not contradict the results of the present study, which showed that excess PTH depresses LPL production (as evidenced by parallel rises in both LPL protein and LPL activity with PTX) as opposed to inhibiting LPL enzymatic activity per se (in which case PTX should have affected LPL activity but not protein mass). In fact, in their concluding paragraph, Arnadottir and Nilsson-Ehle (2) acknowledge that excess PTH may depress LPL production without being an enzymatic inhibitor of LPL.

In conclusion, the CRF-induced downregulation of LPL expression in rat is mediated by the associated secondary hyperparathyroidism and can be reversed by PTX.

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