Hepatic Lipase mRNA, Protein, and Plasma Enzyme Activity Is Increased in the Insulin-Resistant, Fructose-Fed Syrian Golden Hamster and Is Partially Normalized by the Insulin Sensitizer Rosiglitazone

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Postheparin plasma hepatic lipase (HL) activity has been shown to correlate with features of the metabolic syndrome and type 2 diabetes in humans. We examined HL postheparin plasma enzyme activity, hepatocyte mRNA, and protein mass in the insulin-resistant, fructose-fed Syrian golden hamster, and the response of the insulin-sensitizing peroxisome proliferator–activated receptor-γ agonist rosiglitazone. Male Syrian golden hamsters were treated for 5 weeks with *1*) normal diet (DIET group), *2*) 60% fructose diet (FRUC group), or *3*) 60% fructose and rosiglitazone (20 mmol · kg^{-1} · day^{-1}) (FRUC+RSG group). Hepatocyte HL mRNA, protein mass, and postheparin plasma HL activity were increased in FRUC compared with DIET hamsters. FRUC+RSG hamsters had partial normalization of HL mRNA, mass, and activity. There was a shift in the size of LDL particles from large to small in FRUC animals and a shift back to large LDL size in FRUC+RSG. This is the first demonstration that HL hepatocyte mRNA, mass, and plasma enzymatic activity increase concomitantly with induction of an insulin-resistant state and can be partially normalized by treatment with an insulin sensitizer. The increase in HL in insulin-resistant states may play an important role in the typical dyslipidemia of these conditions, and reduction of HL could explain some of the beneficial effects of insulin sensitizers on the plasma lipid profile. *Diabetes* 53:2893–2900, 2004

The insulin resistance (or metabolic) syndrome is estimated to affect approximately one-quarter of the North American population (1) and is associated with a high risk for the development of both type 2 diabetes as well as atherosclerotic cardiovascular disease (2,3). The typical dyslipidemia of insulin-resistant states and type 2 diabetes consists of hypertriglyceridemia, low HDL cholesterol, and small, dense LDL particles and is thought to contribute to the accelerated cardiovascular disease in affected individuals (4,5).

Hepatic lipase (HL), a lipolytic enzyme that is a secreted glycoprotein, is synthesized by hepatocytes and bound to heparan sulfate proteoglycans at the surface of liver sinusoidal capillaries (6). HL shares a number of structural and functional homologies with lipoprotein lipase (LPL) but, unlike LPL, it has significant phospholipase as well as triglyceride lipase activity and is more active than LPL in hydrolyzing the triglycerides and phospholipids of LDL and HDL. The activities of HL and LPL are also differentially regulated (7). HL expression is regulated predominantly by cell cholesterol content (through a sterol response element in its promoter region), steroid and thyroid hormones, and possibly glucose and/or insulin, although in adult humans, HL responsiveness to insulin remains controversial (6,8). Some (9,10), but not all (11), in vitro studies have shown a stimulatory effect of insulin on HL secretion from hepatocytes, and regulation of the HL gene by insulin or insulin resistance has not been conclusively demonstrated (6). Clinical studies in humans, in contrast, have consistently shown correlations between postheparin plasma HL activity and parameters of the insulin resistance (metabolic) syndrome, such as direct measures of insulin resistance, obesity (particularly abdominal type/visceral fat obesity), type 2 diabetes, hypertriglyceridemia, and low HDL cholesterol (8,12–18), whereas loss of intra-abdominal fat and reduction of insulin resistance have been found to be significantly correlated with a reduction in postheparin HL (19).

HL plays a key role in the metabolism of lipoproteins, affecting their plasma concentrations as well as their physicochemical properties, although there is no consensus as to whether the overall effect of HL is that of pro- or antiatherogenicity (20,21). HL has a major effect on the remodeling of LDL, converting large, buoyant LDL particles to more atherogenic, smaller, more dense lipoprotein particles (20,22–25). Because the insulin resistance syndrome is also characterized by the presence of small, dense LDL particles (26,27), the elevation of HL activity in insulin resistance may be implicated in promoting the formation of small, dense LDL particles in insulin-resistant

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Apo, apolipoprotein; FFA, free fatty acid; FPLC, fast-protein liquid chromatography; HL, hepatic lipase; LPL, lipoprotein lipase; PPAR, peroxisome proliferator–activated receptor.

TABLE 1
Characteristics of fructose-fed (FRUC), fructose-fed and rosiglitazone-treated (FRUC+RSG), and normal diet–fed control (DIET) hamsters

	DIET	FRUC	FRUC+RSG
Weight (g)	128 ± 1 (33)	134 ± 2 (60)	136 ± 2 (62)
FFAs (mmol/l)	0.618 ± 0.060 (23)	0.762 ± 0.113 (22)	0.610 ± 0.063 (23)
Total triglycerides (mmol/l)	1.07 ± 0.15 (23)	1.67 ± 0.31 (20)	1.08 ± 0.19 (19)
Insulin (pmol/l)	250 ± 65 (28)	373 ± 50 (34)	278 ± 48 (32)
Glucose (mmol/l)	3.7 ± 0.2 (28)	4.3 ± 0.3 (32)	3.9 ± 0.2 (32)

Data are means ± SE (n). These are cumulative data from our laboratory for fasting blood samples taken from animals treated as described in the RESEARCH DESIGN AND METHODS. There were no significant differences between treatment groups for any of the variables presented.

states. To the best of our knowledge, no studies have prospectively examined the molecular regulation of HL in vivo with induction of insulin resistance and subsequent insulin sensitization.

We have previously shown that a 60% fructose diet in the Syrian golden hamster results in insulin resistance and dyslipidemia (28) and that rosiglitazone, a peroxisome proliferator–activated receptor (PPAR)γ agonist insulin sensitizer, ameliorates insulin resistance and dyslipidemia (29). In the present study, we found that HL activity is increased in the fructose-fed hamster and reduced with rosiglitazone treatment. The changes in HL activity were associated with similar changes in HL mRNA and protein mass, suggesting that HL gene transcription is regulated in some manner by one of the components of the insulin-resistant state.

RESEARCH DESIGN AND METHODS

Male Syrian golden hamsters (*Mesocricetus auratus*) were purchased from Charles River (Quebec, Canada). All animals were housed in pairs and were given free access to food and water. Hamsters were fed a normal diet for 7 days to allow acclimatization to the new environment and recovery from the stress of shipping. They were then placed on one of three feeding protocols as previously described (29): *1*) normal diet for 5 weeks (DIET), *2*) high-fructose diet (hamster diet with 60% fructose; Dyets, Bethlehem, PA) for 5 weeks (FRUC), and *3*) high-fructose diet for 5 weeks with rosiglitazone (20 μmol · kg^{-1} · day^{-1}) (GlaxoSmithKline, Philadelphia, PA) diluted in water and given once daily by gavage for the last 3 weeks of the fructose feeding period (FRUC+RSG). The hamsters' weights were monitored every week. At the end of the 5 weeks, 14 h after removal of food, the animals received an intraperitoneal injection of heparin for determination of postheparin plasma HL activity as described below. Additional animals did not receive heparin but were killed for fast-protein liquid chromatography (FPLC) determination of plasma lipoproteins and HL mRNA and protein mass on snap-frozen liver biopsy samples. Plasma HL enzymatic activity was also determined in the absence of heparin administration in four normal diet–fed hamsters to determine whether HL can be detected in preheparin plasma, as has been suggested by others (30). In the present study, comparative data between groups are reported for postheparin samples because the administration of heparin allowed us to measure both LPL and HL activities. In addition, the administration of heparin ensured that we were measuring the total HL activity (i.e., bound plus unbound), not just the unbound HL.

We have found that retro-orbital plexus blood sampling from Syrian golden hamsters induces considerable stress and results in an elevation of plasma glucose and free fatty acid (FFA) concentrations. Because hamsters do not have a tail, it is also not possible to obtain blood by tail vein sampling as it is in other rodents. Consequently, measurements of fasting plasma insulin, triglyceride, glucose, and FFAs that are reported in Table 1 represent cumulative data from our laboratory from DIET, FRUC, and FRUC+RSG hamsters that had placement of femoral and arterial catheters for in vivo studies, as previously described by us (29). All animal protocols were approved by the Animal Ethics Committee of the University Health Network.

Protocol for measurement of plasma LPL and HL activity. Following the 5-week treatment period, 50 units heparin was injected intraperitoneally into the hamster, and after 30 min, blood was collected under anesthesia from the abdominal aorta into chilled EDTA tubes on ice to prevent ongoing lipolysis of the samples in the test tubes (DIET, $n = 20$; FRUC, $n = 19$; and FRUC+RSG, $n = 21$). Samples were spun at 3,000 rpm for 15 min, and plasma was stored at −70°C for later analysis of LPL and HL activity.

For measurement of LPL and HL activity, the total plasma triglyceride lipase activity was determined as previously described (31) in pre- and postheparin plasma. In brief, plasma was diluted 1:10 with Krebs-Ringer phosphate buffer, pH 7.4, incubated with a triolein/phosphatidylcholine/albumin emulsion, and trace labeled with glycerol tri[1-^{14}C]oleate (Amersham, Arlington Heights, IL) in 0.178 mol/l Tris-HCl, 0.11 mol/l NaCl, buffer, pH 8.5, containing 55 mg/ml albumin and 0.01 mg/ml heparin, resulting in final incubation pH of 8.2. The incubation was carried out for 60 min at 37°C, and the resultant FFAs were extracted and [^{14}C] content determined. The inclusion of a monoclonal antibody specific for LPL allowed for the determination of LPL activity and HL activity (32). The LPL-specific mAb 5D2 antibody used to block LPL activity in this study recognizes multiple species of LPL. Although this mAb was originally produced against bovine LPL, it cross-reacts strongly with human LPL and with LPL from numerous animals tested (33,34). The antibody is effective against hamster LPL because there was substantial inhibition of total postheparin lipase activity, in some cases inhibiting 70% of the activity. A bovine skim milk LPL standard was included in each assay and used to correct for interassay variation. Human postheparin control plasma was assayed to monitor interassay variation. The intra-assay coefficient of variation was 7% for human LPL and 6% for human HL. The interassay coefficient of variation was 8% for LPL and 10% for HL.

HL mass and mRNA. Blood was drawn from five hamsters in each of the four treatment groups for FPLC determination of plasma lipoproteins. The animals were then killed, and the liver tissue was frozen in liquid nitrogen at −70°C for later analysis of HL mass and mRNA. HL mass was determined from 150 μg of liver tissue lysate from each animal using chemiluminescent Western blots, as previously described (35), with an affinity-purified anti-rat HL antibody provided by Dr. A. Bensadoon (Cornell University, Ithaca, NY). Purified human HL was used as the positive control.

For measurement of HL mRNA, total RNA was extracted from frozen samples using the RNeasy Mini kit (Qiagen, Valencia, CA). Five micrograms of total RNA was converted to single-stranded cDNA using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). Ten percent of this first-strand reaction was used as the template for subsequent PCR. Twenty-four cycles of PCR (95°C for 30 s, 55°C for 60 s, and 72°C for 90 s) were performed using primers specific for HL or β-actin. The primer pairs were as follows: HL: 5′-atg cca att ttg tgg atg cca t-3′, and 5′-ctg ttt tcc cac ttg aac ttg a-3′; and actin: 5′-cct ttt cca gcc ttc ctt c-3′, and 5′-tac tcc tgc ttg ctg atc c-3′.

Lipoprotein composition analysis. Fasting plasma was separated by FPLC. Briefly, 100 μl of fasting plasma was applied to a Superose 6HR column, and the samples were eluted in PBS at a rate of 0.4 ml/min in 60 fractions of 500 μl. Fractions corresponding to VLDL, LDL, and HDL were pooled and lipid composition quantified by enzymatic assays using commercially available kits for triglycerides and total cholesterol (Roche Diagnostics, Laval, Quebec, Canada). Apolipoprotein (apo)B was determined using an in-house electroimmunoassay.

Statistical analysis. All the values are reported as means ± SE. For comparison of HL activity, HL mRNA and protein mass, and VLDL, LDL, and HDL lipid composition between DIET, FRUC, and FRUC+RSG hamsters, one-way ANOVA was used followed by post hoc analysis with Tukey's test. $P < 0.05$ was considered to be significant. The 95% CIs were calculated and illustrated for difference plots of FPLC profiles.

RESULTS

Effect of rosiglitazone treatment on body weight, plasma insulin, FFAs, triglycerides, and glucose (Table 1). There were no significant differences among

TABLE 2
Triglyceride, cholesterol, and apoB composition of lipoprotein fractions

		VLDL			LDL			HDL	
Group	n	Triglycerides	Cholesterol	ApoB	Triglycerides	Cholesterol	ApoB	Triglycerides	Cholesterol
DIET	5	238.7 ± 62	55.8 ± 12	68.7 ± 3	255.8 ± 51	37.2 ± 3	70.2 ± 4	255.8 ± 11	108.0 ± 6
FRUC	5	492.8 ± 33*	49.4 ± 11	78.4 ± 5	290.8 ± 14	82.1 ± 5†	84.2 ± 4	325.9 ± 24‡	171.4 ± 10§
FRUC+RSG	5	248.9 ± 60	26.6 ± 2	66.6 ± 7	267.3 ± 22	73.1 ± 4‖	70.6 ± 8	312.1 ± 5¶	140.1 ± 23

Data are means ± SE (in micrograms per milliliter of the fraction). Significant differences are by ANOVA. *$P < 0.05$ for VLDL-triglycerides in FRUC vs. DIET and FRUC+RSG; †$P < 0.01$ for LDL cholesterol in FRUC vs. DIET; ‡$P < 0.01$ for HDL triglycerides in FRUC vs. DIET; §$P < 0.05$ for HDL cholesterol in FRUC vs. DIET; ‖$P < 0.05$ for LDL cholesterol in FRUC+RSG vs DIET; ¶$P < 0.05$ for HDL triglycerides in FRUC+RSG vs. DIET.

the groups for any of the measured baseline parameters, although there was a trend for FRUC hamsters to have higher FFA, insulin, and triglyceride levels.

Lipid composition (from pooled FPLC fractions) in lipoprotein fractions from DIET, FRUC, and FRUC+RSG hamsters (Table 2). VLDL-triglyceride in the FRUC group was significantly higher than in the other two groups ($P < 0.05$). LDL cholesterol was higher in FRUC ($P < 0.01$) and FRUC+RSG ($P < 0.05$) hamsters than in DIET. HDL triglyceride was higher in FRUC ($P < 0.01$) and FRUC+RSG animals compared with DIET ($P < 0.05$), and HDL cholesterol was also higher in the FRUC group than in the DIET group ($P < 0.05$).

Postheparin HL and LPL activity in fasting hamsters (Fig. 1*A* and *B*). HL activity was increased ~40% in fructose-fed hamsters compared with control hamsters (112 ± 7 nmol FFA · ml^{-1} · min^{-1} in FRUC vs. 66 ± 4 in DIET, $P < 0.001$) (Fig. 1*A*). Treatment of fructose-fed hamsters with rosiglitazone resulted in partial normalization of the elevated HL activity (84 ± 7 nmol FFA · ml^{-1} · min^{-1} in FRUC+RSG, $P < 0.001$, versus FRUC, $P = 0.2$, for FRUC+RSG vs. DIET). There were no significant differences in plasma postheparin LPL activity between the various treatment groups (Fig. 1*B*).

Preheparin plasma lipase activities (not shown). Preheparin HL activity from the four normal diet–fed hamsters was 71.2 ± 9.1 nmol FFA · ml^{-1} · min^{-1} (range 60–80), similar to that detected in postheparin plasma from normal diet–fed hamsters. LPL, on the other hand, was lower in preheparin plasma (16 ± 6.3 nmol FFA · ml^{-1} · min^{-1}) than in postheparin plasma.

HL protein mass. Changes in HL protein mass in liver paralleled the changes in HL activity in plasma. HL mass was increased in fructose-fed hamsters ($P < 0.001$ for FRUC vs. DIET) and was partially normalized with rosiglitazone treatment ($P < 0.05$ for FRUC+RSG vs. FRUC). There were no significant differences between the FRUC+RSG and DIET groups (Fig. 2).

HL mRNA levels. Changes in HL mRNA paralleled the changes in postheparin HL activity and HL mass. HL mRNA was increased in fructose-fed hamsters ($P < 0.05$ for FRUC vs. DIET) and tended to be lower with rosiglitazone treatment, although the latter was not significantly different from that of FRUC hamsters. There were no significant differences between the FRUC+RSG and DIET groups (Fig. 3).

Small and large LDL cholesterol in DIET, FRUC, and FRUC+RSG hamsters. Fructose feeding resulted in a significant increase in large (FPLC fractions 22–24) and small (FPLC fractions 26–29) LDL (Fig. 4*A* shows the differences between mean LDL profiles for FRUC and DIET, and 95% CIs that do not cross zero indicate significant differences between FRUC and DIET animals, $P < 0.05$). The addition of rosiglitazone to a high-fructose diet

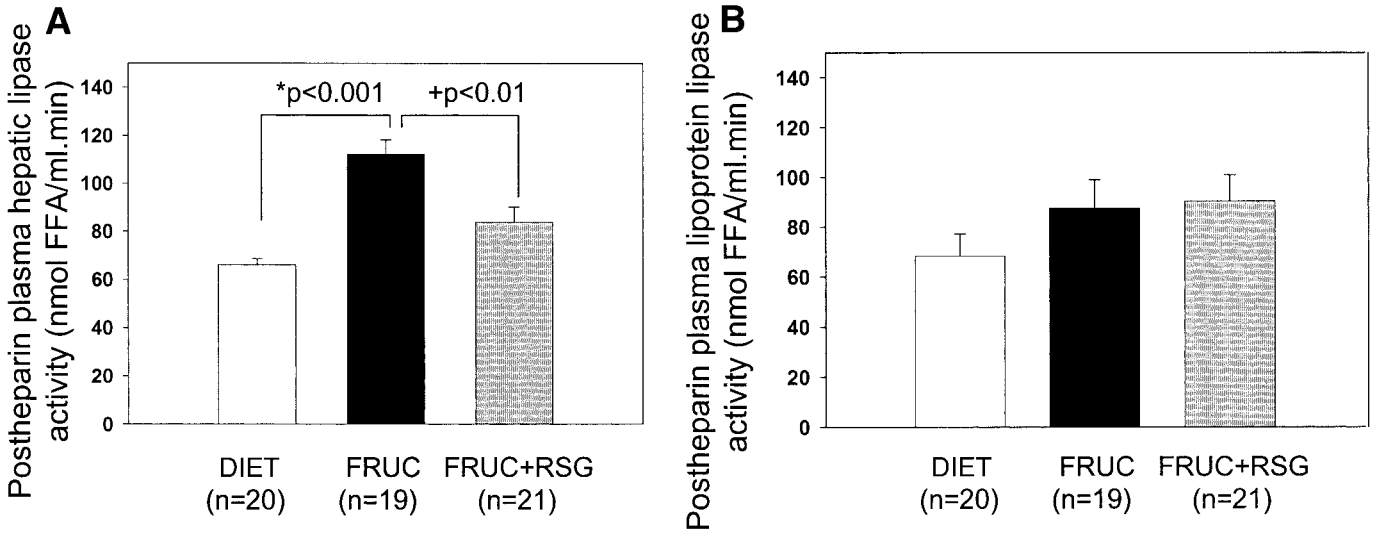

FIG. 1. Postheparin HL and LPL activity in DIET, FRUC, and FRUC+RSG hamsters. Mean postheparin HL (*A*) and LPL (*B*) enzymatic activities are shown for DIET (□, $n = 20$), FRUC (■, $n = 19$), and FRUC+RSG (▨, $n = 21$) hamsters. HL activity was increased in FRUC (*$P < 0.001$, FRUC vs. DIET) and decreased with rosiglitazone (+$P < 0.001$, FRUC+RSG vs. FRUC). There were no significant differences between the DIET and FRUC+RSG groups. There were no significant differences in postheparin LPL activity between the treatment groups.

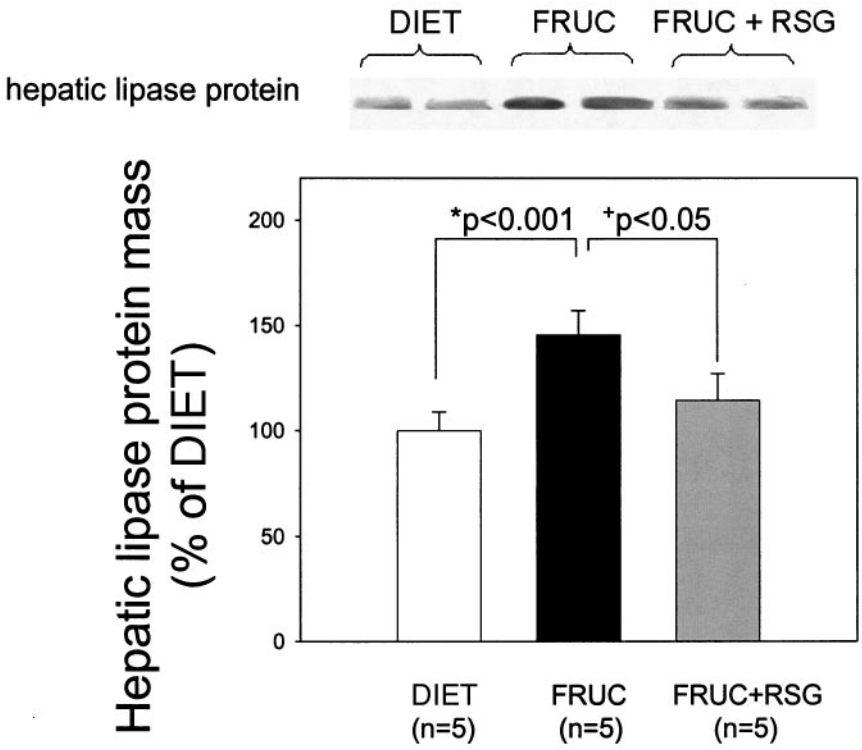

FIG. 2. HL protein mass. Mean HL mass by Western blot and a representative gel are shown for DIET, FRUC, and FRUC+RSG groups (n = 5 in each group). There were no significant differences between the DIET and FRUC+RSG groups. *P < 0.001 for comparison of FRUC vs. DIET; +P < 0.05 for FRUC+RSG vs. FRUC.

decreased small LDL (Fig. 4*B*) ($P < 0.005$ for FRUC+RSG vs. FRUC) and shifted the peak to larger LDL size.

DISCUSSION

In the present study we have shown that fructose feeding was associated with increases in postheparin plasma HL enzymatic activity, hepatocyte HL protein mass, and HL mRNA. Rosiglitazone, a PPARγ agonist member of the thiazolidinedione class of insulin-sensitizing therapeutic agents, partially normalized the elevated HL mRNA, protein, and plasma enzyme activity in fructose-fed hamsters. Although we cannot prove from these studies that it is the insulin resistance per se that induces these changes in HL, these findings do suggest that the HL gene is up- and downregulated by some factor that is associated with whole-body insulin resistance and insulin sensitization, respectively. The precise molecular mechanism of HL gene regulation in insulin resistance remains to be determined.

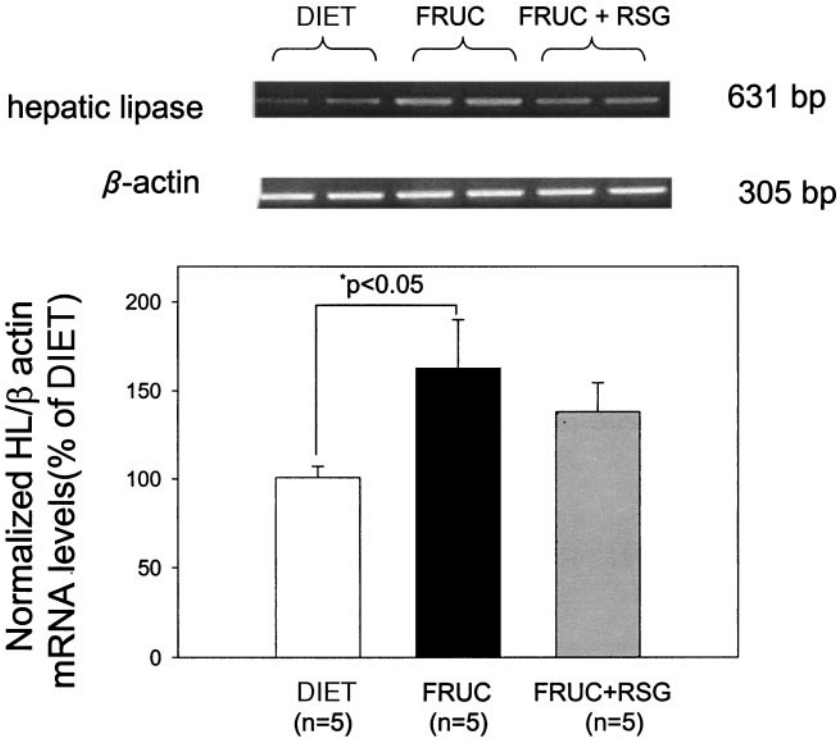

FIG. 3. HL mRNA levels. Mean HL mRNA levels and a representative gel are shown for DIET, FRUC, and FRUC+RSG hamsters (n = 5 in each group). There were no significant differences between FRUC and FRUC+RSG and between DIET and FRUC+RSG hamsters. *P < 0.05 for comparison of FRUC vs. DIET.

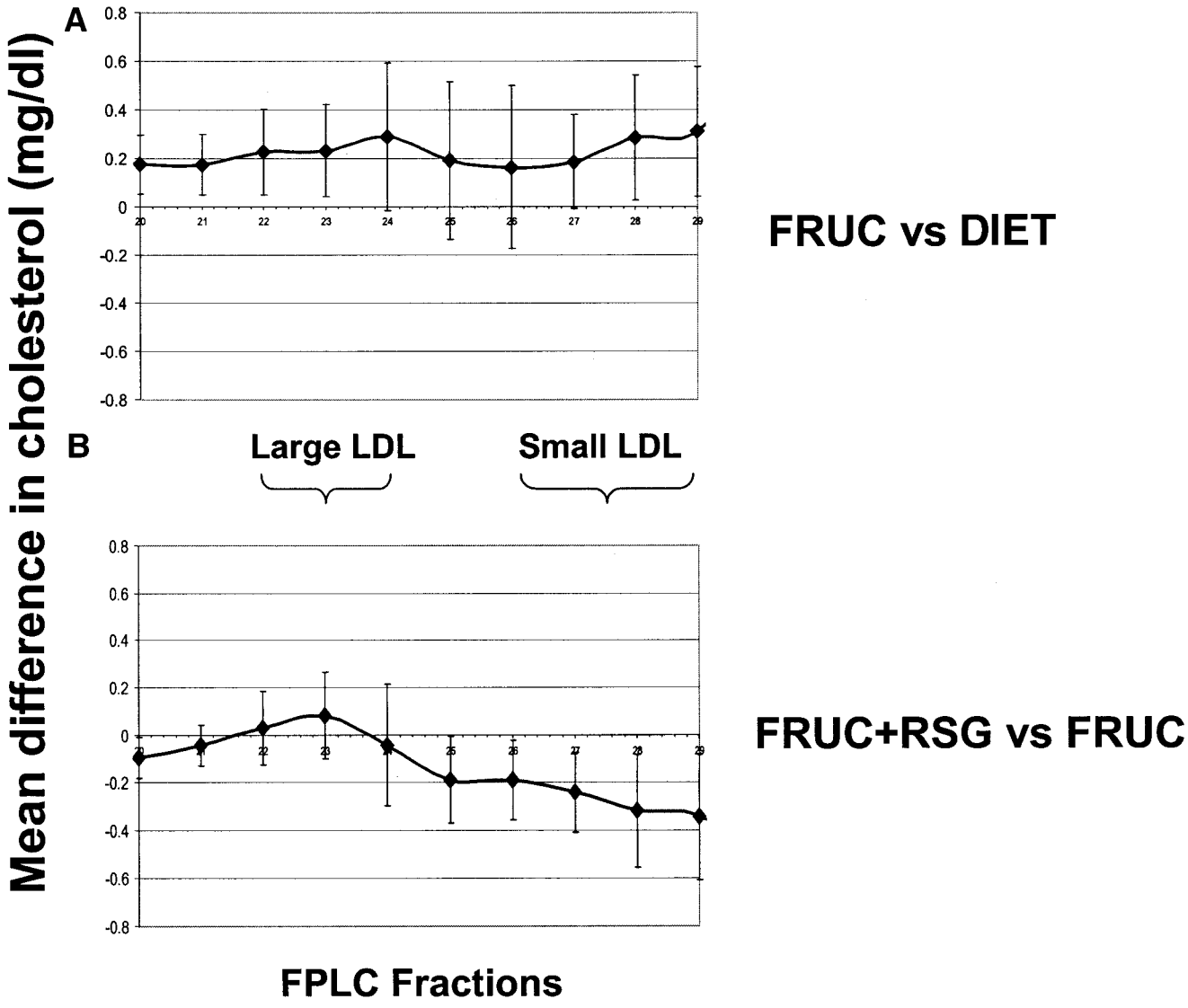

FIG. 4. Small and large LDL cholesterol fraction. Line graphs indicate the differences in LDL cholesterol mean size fractions by FPLC between the different treatment groups (n = 5 in each group). Bars indicate 95% CIs. Fractions 22–24 correspond to large LDL, and fractions 26–29 correspond to small LDL. CIs that do not cross zero indicate significant differences between groups. *A*: Differences between mean LDL profiles for FRUC and DIET are shown, with FRUC feeding resulting in a significant increase in large and small LDL fraction cholesterol ($P < 0.05$). *B*: The addition of rosiglitazone to 60% fructose feeding decreased small LDL ($P < 0.005$ for FRUC+RSG vs. FRUC) and shifted the peak to larger LDL size.

We have previously shown (28,29) that high-fructose feeding for a 3- to 5-week period induced significant hypertriglyceridemia, with increased hepatic VLDL production and the development of whole-body insulin resistance in the Syrian golden hamster. We have also recently shown (29) that these abnormalities were ameliorated with rosiglitazone treatment. As in the present study, we could not determine whether the effects of rosiglitazone on HL activity were mediated by the potent insulin-sensitizing effect of rosiglitazone or by some other effect of PPARγ activation. As one may predict with induction of a hypertriglyceridemic insulin-resistant state, LDL particles shifted from large to small with fructose feeding and back again to large with rosiglitazone treatment. HL is known to play an important role in the metabolic conversion of large, buoyant LDL to potentially more atherogenic, small, dense LDL particles, although triglyceride enrichment of LDL particles per se is also thought to play an important role (24–26). We cannot be certain, therefore, whether the changes in LDL particle size occurred as a direct consequence of the changes in plasma HL activity or are attributable to some other factor. One could speculate though that the insulin resistance–induced increase in HL activity could play an important role in the formation of small, dense LDL particles that typically characterize the insulin-resistant state (8,26).

The plasma LDL cholesterol concentration increased significantly with fructose feeding (FRUC group) compared with a normal diet (DIET group) (Table 2). Rosiglitazone treatment of fructose-fed hamsters (FRUC+RSG) was associated with a plasma LDL cholesterol concentration that was significantly elevated above that of DIET hamsters. An elevation of LDL cholesterol with rosiglitazone treatment has been noted previously in diabetic humans (36). Despite the elevation of plasma LDL cholesterol, there was a net shift in LDL size from small to large

with rosiglitazone in the present study, as discussed above, similar to the LDL size changes that occur in rosiglitazone-treated diabetic humans (36).

With respect to plasma HDL cholesterol concentrations, there was a significant increase in HDL cholesterol with fructose feeding. Rosiglitazone treatment of fructose-fed hamsters tended to reduce HDL cholesterol back toward the levels seen in the DIET group, but the reduction was not significant. The increase in HDL cholesterol that occurs with fructose feeding of hamsters is not typical of the lowering of plasma HDL cholesterol that is commonly seen in humans with insulin resistance syndromes. We can only speculate on the mechanisms whereby fructose feeding raises HDL in Syrian golden hamsters, since this was not the focus of the present study. First, we point out that others have shown similar elevations of HDL cholesterol with fructose feeding of rats (37) and Syrian golden hamsters (38,39). In the latter study, the elevation of VLDL, intermediate density lipoprotein/LDL, and HDL cholesterol accounted for the increased total cholesterol concentrations, whereas the increase in plasma triglycerides occurred entirely in the VLDL density range. To the best of our knowledge, the mechanism of the fructose-induced elevation of HDL cholesterol has not previously been studied. Although hamsters do have cholesteryl ester transfer protein (38), one major difference between rodents and humans is that rodents carry a greater proportion of their total plasma cholesterol in the HDL rather than in the LDL fraction (40). Human studies (41,42) have shown that nutritional interventions such as high-fat feeding induce insulin resistance with an elevation of HDL cholesterol and apoA-1 production, whereas a reduction of apoA-1 production occurs when the diet is switched from high to low fat. The anticipated lowering of HDL cholesterol in insulin-resistant states may not occur when insulin resistance is induced by short-term high-fructose or high-fat feeding. The exact mechanism of the elevation of HDL cholesterol in the fructose-fed hamster is currently not known and will require further investigation.

Our results are in accordance with numerous studies (8,12–19), which have shown that an elevation in postheparin plasma HL activity is often associated with insulin-resistant states such as central obesity and type 2 diabetes. These findings have led to the hypothesis that the relation between HL and adiposity reflects the modulating effect of secondary factors associated with increased adiposity and insulin resistance, although the precise factors that regulate HL activity are not known. HL activity is not upregulated in a clear-cut fashion by insulin. Although studies (12–14,16,19) in patients with insulin resistance and type 2 diabetes, conditions that are commonly associated with chronic hyperinsulinemia, have shown higher HL activity than in appropriate control subjects, euglycemic-hyperinsulinemic clamp studies in humans have shown (43) that acute hyperinsulinemia actually reduces plasma HL activity. It is likely that instead of hyperinsulinemia per se it is some other aspect of insulin resistance that induces the increase in HL activity.

Although the polymorphism C-514T in the promoter region of the HL gene, which explains up to 38% of the variability in HL activity (44), has recently been (45) associated with fasting hyperinsulinemia and insulin resistance, the functional significance of this site is not known. HL is a secreted glycoprotein, and although we detected a concomitant increase in HL mRNA with induction of insulin resistance, it is also possible that the increase in plasma HL enzymatic activity could have occurred due in part to posttranslational modification of HL. HL is synthesized in the endoplasmic reticulum, first as a high mannose form (52–55 kDa in the rat) and subsequently acquires sialic acid–containing complex oligosaccharides during transit through the Golgi cisternae (46). The mature HL (57–59 kDa, in the rat) is then rapidly secreted and has a residence half-time of ~60 min in the hepatocyte. Trimming of the glucose residues off the high-mannose form appears to be a determining step for rat HL secretion (47,48). In humans, besides remodeling of the N-linked oligosaccharides, association of HL with calnexin, a chaperone protein, may increase the efficiency of HL export from the endoplasmic reticulum (49). Further studies are required to determine the precise molecular regulation of HL that occurs in the setting of insulin resistance.

We demonstrated that HL but not LPL enzymatic activity is easily measurable in preheparin plasma in the Syrian golden hamster, as has been previously reported (30), indicating that a significant proportion of the secreted enzyme is not bound to the endothelium by heparan sulfate proteoglycans but circulated freely in the circulation. Activity levels reported in this work, however, are postheparin values because the administration of heparin allowed us to simultaneously measure changes in LPL in response to the various experimental interventions, in addition to the combination of unbound and hepatic-bound HL activity.

In conclusion, we have shown that plasma HL enzymatic activity and hepatocyte HL mass and mRNA are increased in the fructose-fed, nondiabetic, insulin-resistant Syrian golden hamsters and that the increase is partially normalized by the PPARγ agonist insulin sensitizer rosiglitazone. To the best of our knowledge, this is the first study to examine the molecular regulation of HL in vivo with induction of insulin resistance and subsequent insulin sensitization. Elevated HL in insulin resistance may play an important pathophysiological role in the atherogenic dyslipidemia of insulin-resistant states, particularly in the formation of potentially atherogenic, small, dense LDL particles. Further studies are required to confirm these findings in insulin-resistant humans and to determine the molecular regulation of HL in insulin-resistant states.

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