Lipoprotein lipase deficiency is associated with elevated acylation stimulating protein plasma levels

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Abstract **Acylation stimulating protein (ASP, C3adesArg) is an adipose tissue derived hormone that stimulates triglyceride (TG) synthesis. ASP stimulates lipoprotein lipase (LPL) activity by relieving feedback inhibition caused by fatty acids (FA). The present study examines plasma ASP and lipids in male and female LPL-deficient subjects primarily with the P207L mutation, common in the population of Quebec, Canada. We evaluated the fasting and postprandial states of LPL heterozygotes and fasting levels in LPL homozygotes. Homozygotes displayed increased ASP (58–175% increase, $P < 0.05$–0.01), reduced HDL-cholesterol (64–75% decrease, $P < 0.0001$), and elevated levels of TG (19–38-fold, $P < 0.0001$) versus control (CTL) subjects. LPL heterozygotes with normal fasting TG (1.3–1.9 mmol/l) displayed increased ASP (101–137% increase, $P < 0.05$–0.01) and delayed TG clearance after a fatload; glucose levels remained similar to controls. Hypertriglyceridemics with no known LPL mutation also had increased ASP levels (63–192% increase, $P < 0.001$). High-TG LPL heterozygotes were administered a fatload before and after fibrate treatment. The treatment reduced fasting and postprandial plasma ASP, TG, and FA levels without changing insulin or glucose levels. ASP enhances adipose tissue fatty-acid trapping following a meal; however in LPL deficiency, high ASP levels are coupled with delayed lipid clearance.**—Paglialunga, S., P. Julien, Y. Tahiri, F. Cadelis, J. Bergeron, D. Gaudet, and K. Cianflone. **Lipoprotein lipase deficiency is associated with elevated acylation stimulating protein plasma levels.** ***J. Lipid Res.*** **2009.** 50: **1109–1119.**

Supplementary key words C3adesArg • chylomicron • postprandial lipemia • fenofibrate

Lipoprotein lipase (LPL) is a key enzyme involved in fat metabolism. In its active form as a dimer, LPL catalyzes the hydrolysis of triglyceride (TG) from both chylomicrons and VLDL, releasing nonesterified fatty acids (NEFA) which can be taken up by adipose tissue, to store as TG, or by the muscle to be used as an energy source (as reviewed in Ref. 1). LPL activity is highly regulated by several factors, including apolipoproteins, hormones, and even NEFA levels (1). ApoCIII and TNF-α decrease LPL function, while apoCII, apoAV and both insulin and acylation stimulating protein (ASP, C3adesArg) enhance LPL activity (as reviewed in Ref. 2).

ASP is an adipose tissue derived hormone generated through the alternative complement pathway and is the cleavage product of complement C3 by adipsin (as reviewed in Ref. 3). ASP stimulates TG synthesis in adipocytes by increasing diacylglycerol acyltransferase (DGAT) activity, the final enzyme in the TG synthesis cascade (4). ASP acts through C5L2, a G protein-coupled receptor, to stimulate TG synthesis and glucose uptake (5, 6). C5L2 is highly expressed in adipose tissue, muscle, and liver (5, 7). While insulin directly increases LPL activity in adipocytes (8), ASP stimulates LPL activity indirectly by enhancing cellular uptake and esterification of NEFA, thereby relieving feedback inhibition on LPL (8). Like insulin (9, 10), ASP exerts an inhibitory effect on LPL activity in the muscle (11). Furthermore, in vivo antibody treatment that blocked ASP function in mice resulted in decreased LPL activity in adipose tissue and increased muscle LPL activity (12).

Circulating ASP levels tend not to change during a fatty meal (13, 14); however, there is a postprandial increase in local adipose tissue ASP production (15, 16). While little is known regarding ASP regulation, chylomicrons have been shown to directly increase production of ASP through

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Abbreviations: Apo, apolipoprotein; ASP, acylation stimulating protein; AUC, area under the curve; BMI, body mass index; CTL, control; DGAT, diacylglycerol acyltransferase; high-TG, hypertriglyceridemic; FA, fatty acid; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; normo-TG, normotriglyceridemic; TG, triglyceride.

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TABLE 1. Identified LPL mutations for heterozygote and homozygote subjects

	Males			Females		
	HE NTG (n = 4)	HE HTG (n = 12)	HO (n = 15)	HE NTG (n = 4)	HE HTG (n = 13)	HO (n = 6)
G188E	—	—	—	—	1	2
P207L	3	11	10	4	11	3
D250N	1	—	—	—	—	—
D9N and P207L	—	—	1	—	1	—
D9N and N291S	—	—	1	—	—	—
G188E and P207L	—	—	2	—	—	1
P207L and N291S	—	1	—	—	—	—
Unknown	—	—	1	—	—	—

HE HTG, heterozygotes with high fasting triglyceride; HE NTG, heterozygotes with normal fasting triglyceride; HO, homozygotes.

increased production of C3 and increased conversion of C3 to ASP in adipocytes (17, 18). Furthermore, previous studies on postprandial TG clearance in normal healthy subjects indicated that fasting ASP positively correlates with TG area under the curve (AUC), where higher ASP levels are associated with inefficient TG clearance (19).

Since ASP plays a pivotal role in dietary fatty acid uptake and esterification through modulation of LPL activity, we evaluated the impact of total and partial LPL deficiency on ASP levels and the association with lipid levels. Homozygote mutations in the LPL gene are rare autosomal recessive disorders with prevalence in the general population of approximately one in a million (20). However two LPL mutations, G188E and P207L, that cause complete loss of postheparin LPL activity in homozygotes and 50% loss in heterozygotes, are commonly seen in Quebec, Canada (1). The present study examines the plasma ASP levels in total or partial LPL-deficient patients in the fasting state and after a fatty meal (heterozygotes only). In addition, plasma ASP was measured in hypertriglyceridemic LPL heterozygotes before and following 3 months of fibrate treatment intended to lower TG levels.

METHODS

Subjects

Non-obese, healthy free living Caucasians from Montreal and Quebec City, Canada, were recruited from the general population. For control (CTL) subjects, those with a history of heart disease, diabetes, hypertension, and lipid lowering drug treatment were excluded. Patients with a history of hypertriglyceridemia without LPL deficiency (fasting triglyceride levels greater than 2.5 mmol/l), with or without cardiovascular disease, were recruited from the McGill University Health Centre (MUHC), Royal Victoria Hospital in Montreal as well as the Lipid Clinic, Lipid Research Centre (CHUQ Research Centre), Quebec City. A total of 21 LPL homozygotes and 33 LPL heterozygotes were also recruited from the CHUQ and the Chicoutimi Lipid Clinic. At the time of sampling, no subject was taking any lipid lowering medications, or other medications known to impact metabolic profiles. The protocol was approved by local ethics committees at the MUHC, CHUQ, and Chicoutimi Lipid Clinic, and written informed consent was obtained from each subject.

LPL-deficient patients

The majority of subjects displayed a mutation at either site P207L or G188E of the LPL gene (**Table 1**). In the homozygote

TABLE 2. Fasting values for control, hypertriglyceridemic, and LPL-deficient subjects

	CTL	HTG	HE NTG	HE HTG	HO	ANOVA
MALES	n = 13	n = 12	n = 4	n = 12	n = 15	
Age (years)	34.4 ± 2.1	43.1 ± 2.6	38.0 ± 3.1	43.6 ± 2.8	35.6 ± 2.8	P = 0.0451
BMI (kg/m^2)	25.3 ± 0.8	30.3 ± 1.0**	22.4 ± 1.2	28.4 ± 1.5	25.3 ± 1.1	P = 0.0013
Chol (mmol/l)	4.38 ± 0.30	5.47 ± 0.20	5.35 ± 0.61	4.81 ± 0.3	6.42 ± 0.81*	P = 0.0588
HDL-chol (mmol/l)	1.02 ± 0.05	0.75 ± 0.03**	0.79 ± 0.09	1.11 ± 0.08	0.36 ± 0.03***	P < 0.0001
ApoB[a] (g/l)	0.87 ± 0.06	1.20 ± 0.05**	1.10 ± 0.17	0.93 ± 0.06	0.40 ± 0.07**	P<0.0001
% ApoE 3	100	100	100	83	80	NS
% nonApoE 3	—	—	—	17	20	
FEMALES	n = 16	n = 6	n = 4	n = 13	n = 6	
Age (years)	35.9 ± 1.4	53.0 ± 4.5***	31.3 ± 2.7	55.6 ± 2.2***	32.3 ± 5.0	P<0.0001
BMI (kg/m^2)	23.0 ± 0.8	31.1 ± 3.5**	24.1 ± 1.2	30.7 ± 1.3***	18.9 ± 0.6	P<0.0001
Chol (mmol/l)	4.12 ± 0.18	5.76 ± 0.55	4.64 ± 0.28	6.19 ± 0.40*	7.56 ± 2.02**	P = 0.0108
HDL-chol (mmol/l)	1.34 ± 0.09	0.96 ± 0.11	0.81 ± 0.11*	1.29 ± 0.12	0.34 ± 0.04***	P<0.0001
ApoB[b] (g/l)	0.70 ± 0.04	1.23 ± 0.18***	0.99 ± 0.05	1.06 ± 0.08**	0.43 ± 0.05	P<0.0001
% ApoE 3	100	100	100	77	83	NS
% nonApoE 3	—	—	—	23	17	

Apo, apolipoprotein; BMI, body mass index; CTL, controls; HE HTG, heterozygotes with high fasting triglyceride; HE NTG, heterozygotes with normal fasting triglyceride; HO, homozygotes; NS, not significant. Values are presented as mean ± SEM. Significant differences were determined by one-way ANOVA followed by Dunnett's posthoc test vs. control. Percentage apoE3 variants were analyzed by chi-square test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[a] n = 3 homozygote subjects with apoB measurements.

[b] n = 2 homozygote subjects with apoB measurements.

group (n = 21), 13 had a mutation at P207L, 2 at G188E, 3 had both P207L and G188E mutations, 2 subjects had combined mutations, one at D9N and P207L, and the other at D9N and N291S, while 1 had an unknown mutation. These mutations have been previously shown to display more than a 90% reduction in LPL postheparin activity (1). While the D9N mutation is known to cause only a small loss in LPL activity (1), in combination with other mutations, enzyme activity can vary and the subjects were classified according to their measured LPL activity. In the heterozygote group (n = 33), 29 had a mutation at P207L of the LPL gene, 1 at G188E, 1 at D250N and 2 subjects had combined mutations, one at D9N and P207L, and one at P207L and N291S, respectively. All mutations were known to cause a nonfunctioning allele with a 50% loss in postheparin LPL activity (1). The mutations were identified by PCR-ASO analysis as described previously (21). The heterozygotes were divided into two groups: heterozygotes with normal fasting TG (HE NTG) and heterozygotes with high fasting TG (HE HTG). Controls were matched for age, body mass index (BMI), and TG levels to the normal-TG LPL heterozygotes, while hypertriglycemic subjects were matched for age and BMI to high-TG LPL heterozygotes. In addition, high-TG heterozygotes were treated with fenofibrate (Lipidil Micro Fournier, Pharma Inc., Montreal) 200 mg per day (once daily) for 3 months.

Plasma measurements

After a 12-h fast, blood samples were collected in 0.15% EDTA containing tubes. Plasma was isolated by centrifugation (1400 *g* at 4°C for 15 min). Cholesterol levels in whole plasma and in lipoprotein fractions and plasma triglyceride levels were measured by enzymatic methods using the Technicon RA-1000 analyzer (Technicon Instruments Corporation, Tarrytown, NY). Total phospholipids were measured by an enzymatic/colorimetric method (Wako Pure Chemicals, Richmond, VA) using the Technicon RA-1000 analyzer. NEFA was analyzed using an enzymatic kit from Wako Pure Chemicals (Osaka, Japan). Plasma VLDL was isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant with heparin and $MnCl_2$ as described previously (22). Apolipoprotein AI (apoAI) and AII (apoAII) as well as apolipoprotein B (apoB) were measured by nephelometry with the BN Prospect analyzer (Dade Behring, Mississauga, ON, Canada) using reagents provided by Dade Behring (23). Apolipoprotein E (apoE) genotype was determined as described by Brisson et al. (24). Plasma ASP was measured as described elsewhere by ELISA assay (25). Insulin and glucose levels were determined as previously indicated (26).

Oral lipid tolerance test (fatty meal)

Following an overnight 12-h fast, the subjects were given a high-fat meal. The meal consisted of a fruit shake (frozen orange juice, peach yoghurt, sugar, and 30 g of Lipomul, Upjohn Laboratories as a source of fat) and two hardboiled egg yolks. The fruit shake was maintained frozen and allowed to defrost in the refrigerator 24 h prior to the study. On the morning of the study, subjects were given 5 min to complete the meal. The total calories per serving were 483 kcal: 91 kcal from carbohydrates, 32 kcal from protein, and 360 kcal from fat. Blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, and 10 h after the meal.

Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). Fasting TG, HDL-cholesterol and ASP values comparing controls and homozygotes were analyzed by unpaired *t*-test. All other fasting values were analyzed by one-way ANOVA followed by Dunnett's posthoc test versus control. Differences in apoE variants were analyzed by chi-square test. Control and normal-TG heterozygote postprandial curves were analyzed by two-way ANOVA followed by Bonferroni posthoc test. Group (G) and time (T) effects are provided in figures. Area-under-the-curve (AUC) data was compared by unpaired *t*-test. Fasting pre- and post-fenofibrate treatment data were analyzed by paired two-tail *t*-test. Pre- and post-fibrate postprandial data were analyzed by repeated measures two-way ANOVA followed by Bonferroni posthoc test, where treatment (G) and time (T) effects are provided in figures. AUC data for pre- and post-fibrate heterozygotes compared with hypertriglyceridemic subjects were analyzed by one-way ANOVA followed by Dunnett's posthoc test. Correlations of selected parameters were analyzed by Pearson linear regression for all subjects that were administered a fatload (control, hypertriglyceridemic, normal-TG heterozygote and pretreatment high-TG heterozygote subjects). Statistical analyses were performed with GraphPad Prism (San Diego,

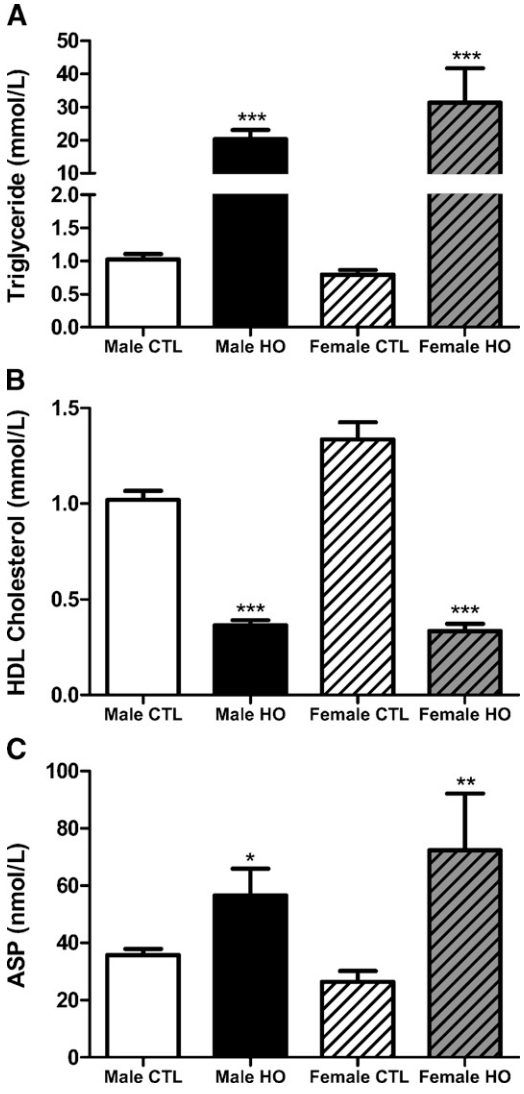

Fig. 1. Fasting triglyceride, HDL-cholesterol, and ASP levels in control and LPL homozygote subjects. Fasting triglyceride (A), HDL-cholesterol (B), and ASP (C) levels in healthy control (CTL) men (*white bars, n = 13*) and women (*white hatched bars, n = 16*) and LPL homozygote (HO) men (*black bars, n = 15*) and women (*gray hatched bars, n = 6*). Values are presented as mean ± SEM and significance was determined by unpaired *t*-test, where * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.0001$ compared with control subjects.

CA) and SigmaStat (San Rafeal, CA) software. Significance was set at $P \leq 0.05$, and NS indicates "not significant."

RESULTS

Three groups of LPL-deficient subjects were evaluated: a cohort of LPL homozygotes (HO); LPL heterozygotes with normal fasting TG (HE NTG); and LPL heterozygotes with high fasting TG (HE HTG). As shown in Table 1, a large proportion of the LPL mutation carriers were identified with either the P207L or G188E mutations (88% to 92%), both which are known to cause a nonfunctioning allele (1). In addition to their LPL mutations, a few homozygotes and heterozygotes also were positive for nonapoE3 variants, while all control subjects were identified with the apoE3 variant (**Table 2**).

Increased TG and low HDL-cholesterol levels are hallmarks of LPL deficiency (1). Male and female LPL homozygotes displayed a substantial 19-fold ($P < 0.0001$) and 38-fold ($P < 0.0001$), increase in fasting TG levels compared with control subjects, respectively (**Fig. 1A**). Meanwhile, HDL-cholesterol was reduced by 64% ($P < 0.0001$) in male and by 75% ($P < 0.0001$) in female LPL homozygote subjects compared with the control subjects (Fig. 1B). In addition, total cholesterol was increased by 46% ($P < 0.05$) in males and 83% ($P < 0.01$) in females (Table 2). Finally, plasma ASP levels were significantly elevated in both male (58%, $P < 0.05$) and female (175%, $P < 0.01$) homozygotes (Fig. 1C).

Control subjects were age, BMI, and TG-matched to heterozygotes with normal fasting TG (HE NTG) (Table 2). Despite having similar TG levels, both male and female HE NTG displayed reduced HDL-cholesterol (23%, NS and 39%, $P < 0.05$, Table 2). Furthermore, when challenged with a fatty meal, exaggerated postprandial TG response was observed for both male HE NTG (**Fig. 2A**, $P < 0.0001$)

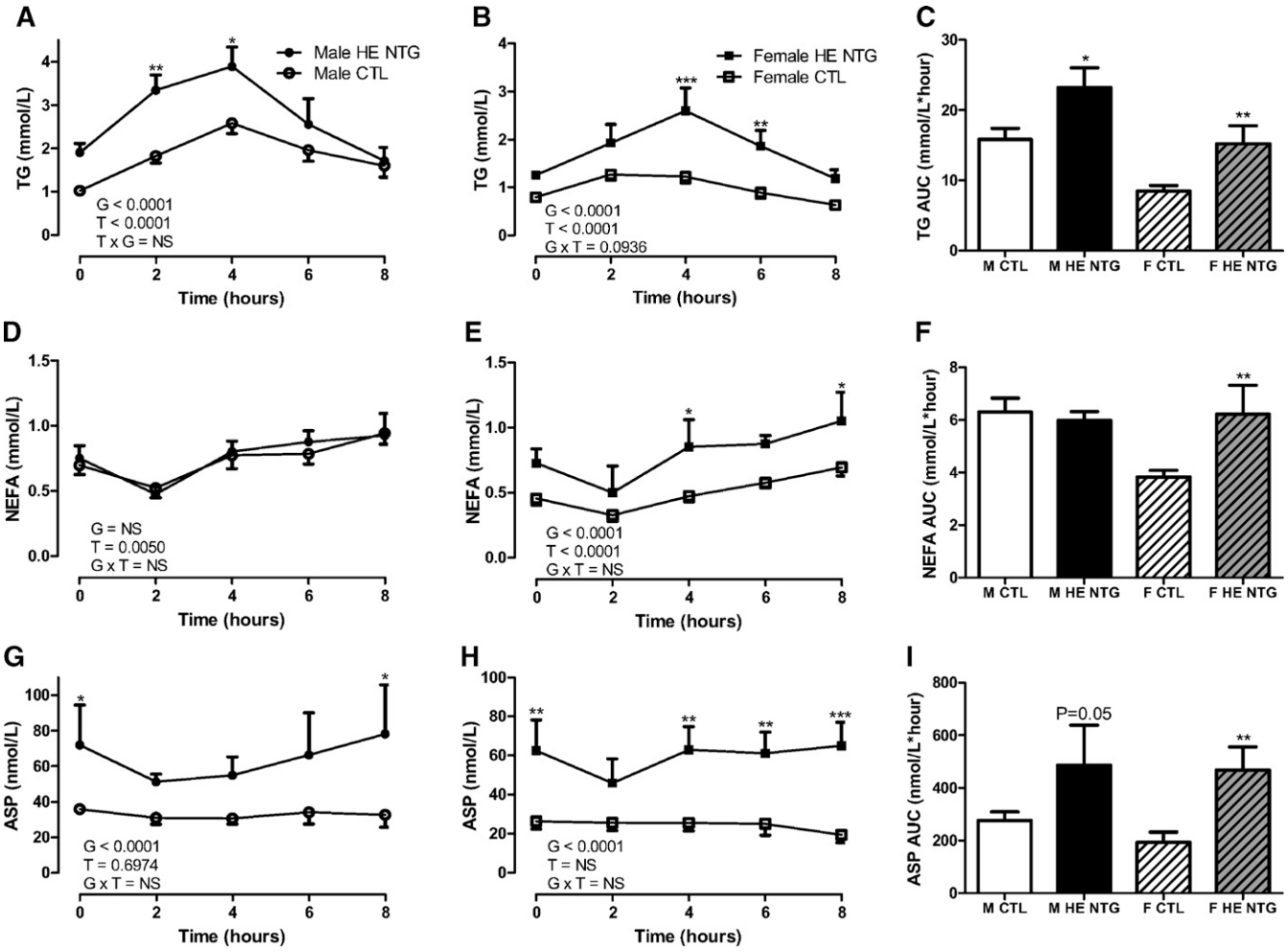

Fig. 2. Normotriglyceridemic LPL heterozygotes postprandial response following a fatty meal. Triglyceride (A and B), NEFA (D and E) and ASP (G and H) postprandial response curves following a fat meal for healthy control men (Male CTL: *white circles, n = 13*), women (Female CTL: *white squares, n = 16*) and normal-TG LPL heterozygote men (Male HE NTG: *black circles, n = 4*) and women (Female HE NTG: *black squares, n = 4*). Statistical significance was determined by two-way ANOVA followed by Bonferroni posthoc test. Group (G) and time (T) P-values are indicated in each graph. Area-under-the-curve (AUC) results are shown for TG AUC (C), NEFA AUC (F) and ASP AUC (I) for male controls (*white bars*), female controls (*white hatched bars*), male heterozygotes (*black bars*) and female heterozygotes (*gray hatched bars*). AUC data was analyzed by unpaired *t*-test. All values are presented as mean ± SEM, where * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and NS is "not significant."

and female HE NTG subjects (Fig. 2B, $P < 0.0001$) compared with control subjects, as reflected by increased AUC (Fig. 2C). Meanwhile, NEFA levels were only significantly delayed in HE NTG females (Figs. 2E, 2F). On the other hand, male HE NTG displayed exaggerated postprandial phospholipids (Male CTL AUC: 17.6 ± 0.2 mmol/l*h and Male HE NTG AUC: 22.4 ± 1.1 mmol/l*h, n = 4/group $P < 0.01$; Female CTL AUC: 20.0 ± 0.6 mmol/l*h and Female HE NTG AUC: 21.7 ± 1.7 mmol/l*h, n = 3/group NS). Similar to previously published results (3), circulating ASP levels did not fluctuate after the fat load; however postprandial ASP levels were significantly higher in the male and female HE NTG subjects versus control subjects (Figs. 2G, H, I). While fasting ASP levels were 101% higher in HE NTG male (Fig. 2G, $P < 0.05$) and 137% higher in HE NTG female (Fig. 2H, $P < 0.01$), fasting insulin levels were significantly lower in male heterozygotes (CTL: 53.9 ± 7.0 pmol/l and HE NTG: 23.8 ± 2.0 pmol/l, $P < 0.05$) and female heterozygotes (CTL: 93.0 ± 10.7 pmol/l and HE NTG: 22.5 ± 7.9 pmol/l, $P < 0.05$). Interestingly, fasting glucose levels were similar between the two groups (Male CTL: 5.0 ± 0.1 mmol/l and Male HE NTG: 4.8 ± 0.2 mmol/l, NS; Female CTL: 4.3 ± 0.1 mmol/l and Female HE NTG: 4.45 ± 0.1 mmol/l, NS).

Hypertriglyceridemic subjects with no known LPL mutation were age and BMI-matched to high-TG LPL heterozygotes (Table 2). While total cholesterol and apoB were similar between the groups, HDL-cholesterol was lower in hypertriglyceridemic subjects (Table 2). However, the heterozygotes displayed slightly elevated fasting TG levels compared with hypertriglyceridemic subjects (Male HTG: 3.2 ± 0.4 mmol/l and Male HE HTG: 4.8 ± 1.1 mmol/l, NS; Female HTG: 2.16 ± 0.4 mmol/l and Female HE HTG: 8.43 ± 2.0 mmol/l, $P = 0.05$), yet no significant difference in ASP levels was detected (Male HTG: 58.3 ± 12.0 mmol/l and Male HE HTG: 32.6 ± 6.4 mmol/l, $P = 0.08$; and Female HTG: 76.9 ± 12.3 mmol/l and Female 55.5 ± 6.7 mmol/l, NS).

High-TG heterozygotes were placed on 200 mg/day fenofibrate treatment for three months. Fibrates are commonly used as pharmaceutical agents to reduce TG levels and may affect other lipid parameters. Indeed, the treatment successfully reduced fasting TG, VLDL apoB, and phospholipid levels, and increased HDL-apoAII levels in both male and female HE HTG (**Table 3**). On average, female HE HTG were significantly older than male HE HTG (55.9 ± 2.0 years versus 43.6 ± 2.8 years, $P < 0.01$) with more severe hypertriglyceridemia. However, only female heterozygotes displayed significantly reduced fasting NEFA and ASP levels following treatment while cholesterol levels were slightly lowered. Male heterozygotes had an increase in LDL-apoB levels post-fibrate regimen. Furthermore, fibrate treatment had no significant effect on BMI, HDL-cholesterol, HDL-apoAI, glucose, or insulin levels in either male or female HE HTG subjects (Table 3).

In addition to lowering fasting TG levels, the fibrate treatment in male HE HTG reduced postprandial TG (**Fig. 3A**), phospholipids (Fig. 3B), NEFA (Fig. 3C), and ASP (Fig. 3D). The fenofibrate treatment had no effect on postprandial insulin (Fig. 3E) or glucose (Fig. 3F) responses. Similar results were observed for female HE HTG subjects, where fibrate treatment significantly decreased postprandial TG (**Fig. 4A**), phospholipids (Fig. 4B), NEFA (Fig. 4C) and ASP (Fig. 4D). Again, no reduction in postprandial insulin (Fig. 4E) or glucose (Fig. 4F) responses was observed following treatment.

AUC analysis of the above-mentioned parameters were lower in HE HTG with treatment compared with HTG subjects with no known LPL mutation. TG AUC for male HE HTG post-treatment subjects was reduced by 53% versus male HTG ($P < 0.05$, **Fig. 5A**). In HE HTG women, after the fibrate regimen, TG AUC was significantly decreased compared with pretreatment values (Fig. 5B). ASP AUC was significantly decreased post-treatment in heterozygotes compared with hypertriglyceridemic subjects (Figs. 5C, 5D). Overall, NEFA AUC was significantly lower for both male HE HTG pre- and post-fibrates compared with male HTG subjects (Fig. 5E). Meanwhile, NEFA AUC for female HE HTG post-fibrates was significantly decreased compared with female HTG subjects (Fig. 5F). Insulin AUC

TABLE 3. Characteristics of high-TG LPL heterozygote deficient subjects pre- and post-fibrate treatment

	Males		Females	
	n = 11		n = 12	
	Pre	Post	Pre	Post
BMI (kg/m^2)	28.9 ± 1.5	28.4 ± 1.4	31.4 ± 1.3	31.3 ± 1.3
TG (mmol/l)	4.77 ± 1.13	2.08 ± 0.36*	8.83 ± 2.13	3.48 ± 0.91**
NEFA (mmol/l)	0.42 ± 0.03	0.39 ± 0.05	0.72 ± 0.05	0.62 ± 0.07*
Chol (mmol/l)	4.88 ± 0.31	4.34 ± 0.40	6.29 ± 0.42	4.82 ± 0.50†
HDL-chol (mmol/l)	1.09 ± 0.09	1.15 ± 0.11	1.25 ± 0.11	1.33 ± 0.08
HDL-apoAI (g/l)	1.25 ± 0.06	1.30 ± 0.08	1.74 ± 0.11	1.73 ± 0.10
HDL-apoAII (g/l)	0.33 ± 0.02	0.40 ± 0.03**	0.38 ± 0.02	0.51 ± 0.03**
VLDL-apoB (g/l)	0.19 ± 0.02	0.11 ± 0.01**	0.31 ± 0.04	0.16 ± 0.03**
LDL-apoB (g/l)	0.50 ± 0.07	0.64 ± 0.09*	0.47 ± 0.06	0.54 ± 0.06
Phospholipid (mmol/l)	2.77 ± 0.15	2.37 ± 0.12*	3.95 ± 0.24	2.96 ± 0.21*
ASP (nmol/l)	32.63 ± 6.46	27.41 ± 2.44	55.53 ± 6.74	40.60 ± 4.19*
Insulin (pmol/l)	106.5 ± 18.2	95.6 ± 15.0	130.6 ± 25.0	117.5 ± 20.0
Glucose (mmol/l)	5.97 ± 0.30	6.05 ± 0.33	6.45 ± 0.43	6.70 ± 0.72

Apo, apolipoprotein; ASP, acylation stimulating protein; BMI, body mass index; NEFA, nonesterified fatty acid; TG, triglyceride. Values are presented as mean ± SEM. Significant differences were determined by paired two-tail *t*-test. * $P < 0.05$, ** $P < 0.01$, † $P = 0.057$.

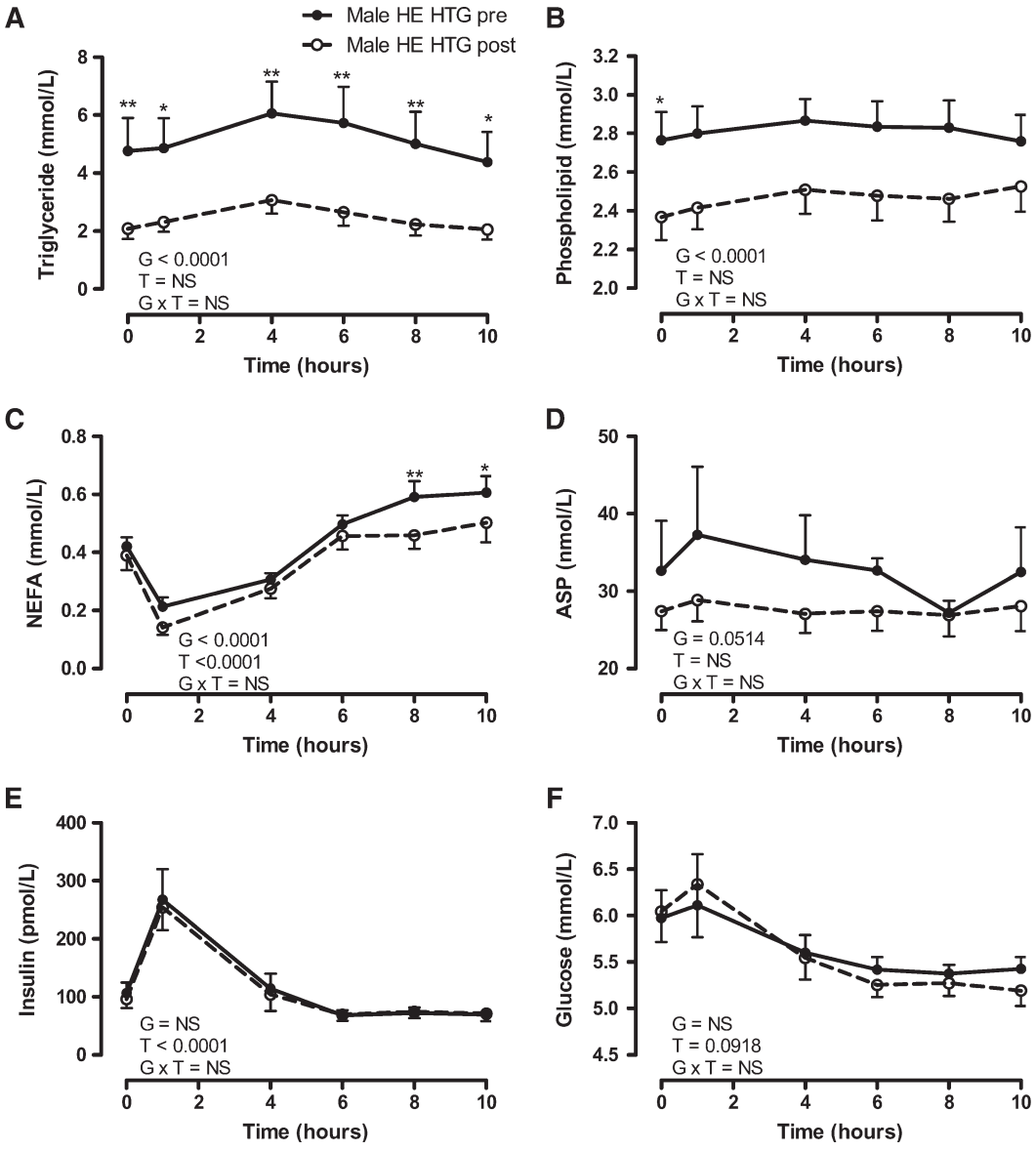

Fig. 3. Male LPL heterozygotes postprandial response before and after a fibrate treatment. Triglyceride (A), phospholipid (B), NEFA (C), ASP (D), insulin (E) and glucose (F) levels following a fat meal. Postprandial response before (pre: *black circles*) and after (post: *white circles*) fibrate treatment in high-TG LPL heterozygote men (n = 11). Significance was determined by two-way repeated measures ANOVA where treatment (G) and time (T) P-values are indicated in each graph. All values are presented as mean ± SEM, where * $P < 0.05$, ** $P < 0.01$, and NS is "not significant".

was lower in both pre- and post-treated males compared with HTG men (Fig. 5G) while glucose AUC was not significantly different among the three groups (Male HTG: 35.1 ± 1.3 mmol/l*h, Male HE HTG Pre: 34.6 ± 1.7 mmol/l*h and Male HE HTG Post: 34.8 ± 1.9 mmol/l*h, NS). In addition, no significant difference was observed in women for insulin AUC (Fig. 5H) or glucose AUC (Female HTG: 34.3 ± 3.2 mmol/l*h, Female HE HTG Pre: 42.7 ± 4.4 mmol/l*h and Female HE HTG Post: 43.6 ± 5.4 mmol/l*h, NS).

Finally, when postprandial data from control subjects, hypertriglyceridemics and LPL heterozygotes (HE NTG and HE HTG pre-treated only) were combined, fasting ASP levels significantly correlated with NEFA AUC in both men (**Fig. 6A**) and women (Fig. 6B). Fasting ASP also correlated with phospholipids AUC ($R = 0.605$, $P = 0.006$, n = 19) in men and glucose AUC ($R = 0.504$, $P = 0.005$, n = 30) in women.

DISCUSSION

Increased fasting TG and low HDL-cholesterol are often referred to as the hallmarks of LPL deficiency (1); this is true of total deficiency yet not necessarily for partial deficiency. In the present study we examined two cohorts of LPL heterozygotes, one with normal and one with high fasting TG. Interestingly, the high-TG heterozygotes had comparable HDL-cholesterol levels compared with control

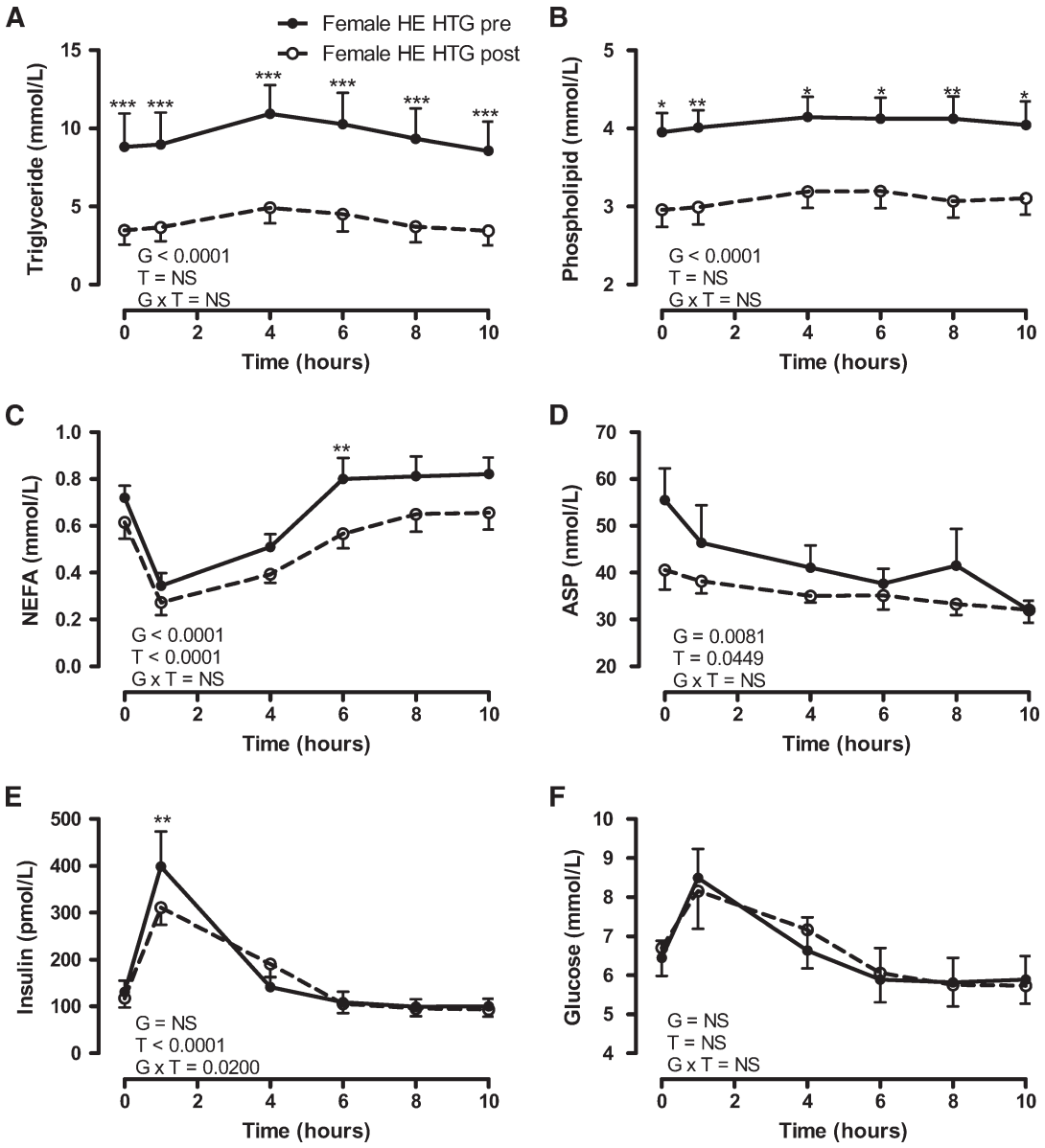

Fig. 4. Female LPL heterozygotes postprandial response before and after a fibrate treatment. Triglyceride (A), phospholipid (B), NEFA (C), ASP (D), insulin (E) and glucose (F) levels following a fat meal. Postprandial response before (pre: *black circles*) and after (post: *white circles*) a fibrate treatment in high-TG LPL heterozygote women (n = 12). Significance was determined by two-way repeated measures ANOVA where treatment (G) and time (T) P-values are indicated in each graph. All values are presented as mean ± SEM, where * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and NS is "not significant."

subjects, while the normal-fasting TG heterozygotes displayed a 23%–39% reduction in HDL-cholesterol compared with control subjects. While these findings are noteworthy, the sample size is small, and the underlying mechanism for these differences in LPL heterozygosity is still unknown. Moreover, regardless of fasting TG levels, we demonstrated that both LPL heterozygotes displayed delayed postprandial clearance coupled with high ASP levels. The G188E and the N291S mutations both have been previously shown to cause delayed postprandial TG clearance (27–29), and the S447X mutation is characterized by enhanced postprandial clearance (30, 31). To our knowledge, only one other group has evaluated postprandial lipids in the P207L mutation (32), a common mutation found in Quebec (1) and the major mutation identified in the LPL-deficient subjects investigated here. Furthermore, in the present study we showed that both LPL homozygotes and heterozygotes display increased fasting ASP levels.

Plasma ASP levels are often elevated in patients with metabolic disorders such as cardiovascular disease, obesity, diabetes, hypoactive thyroid, and polycystic ovary syndrome (PCOS) (25, 33–36). Many of these disorders are also associated with hyperlipidemia, specifically hypertriglyceridemia. In most studies, increased plasma ASP is related to obesity and increased BMI, although ASP can be increased in the absence of obesity as observed in lean type 2 diabetics (34), lean PCOS women (36) and as illustrated in this article in LPL deficiency. Furthermore,

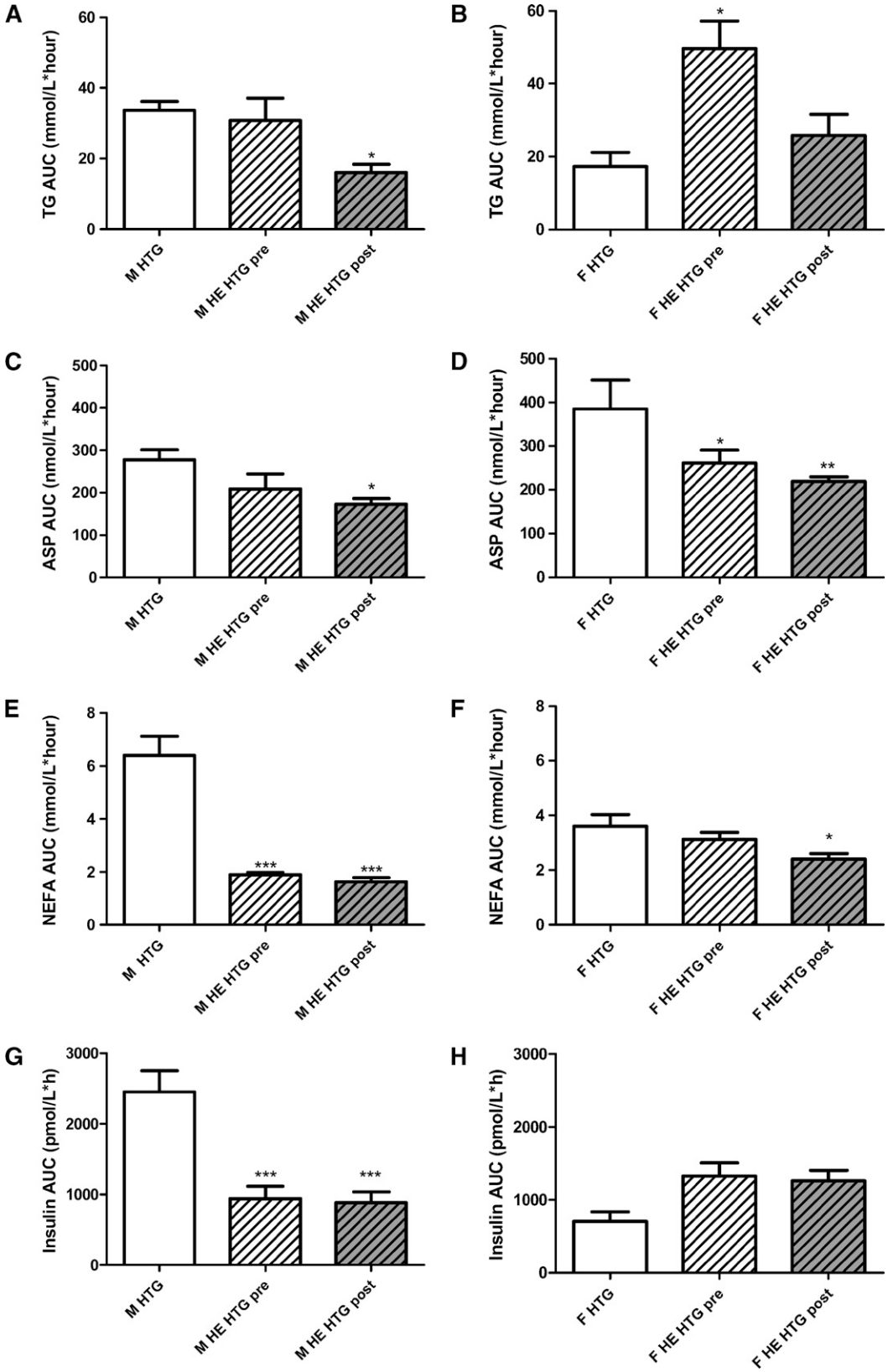

Fig. 5. Postprandial AUC values for hypertriglyceridemic and LPL heterozygote subjects. TG AUC (A and B), ASP AUC (C and D), NEFA AUC (E and F) and insulin AUC (G and H) for hypertriglyceridemic (HTG) men (n = 12) and women (n = 6) (*white bars*), LPL heterozygote (HE HTG) men (n = 11) and women (n = 12) before (pre: *white hatched bars*) and after (post: *gray hatched bars*) 3 month fenofibrate treatment. Significance was determined by one-way ANOVA followed by Dunnett's posthoc test vs. HTG. All values are presented as mean ± SEM, where * indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$.

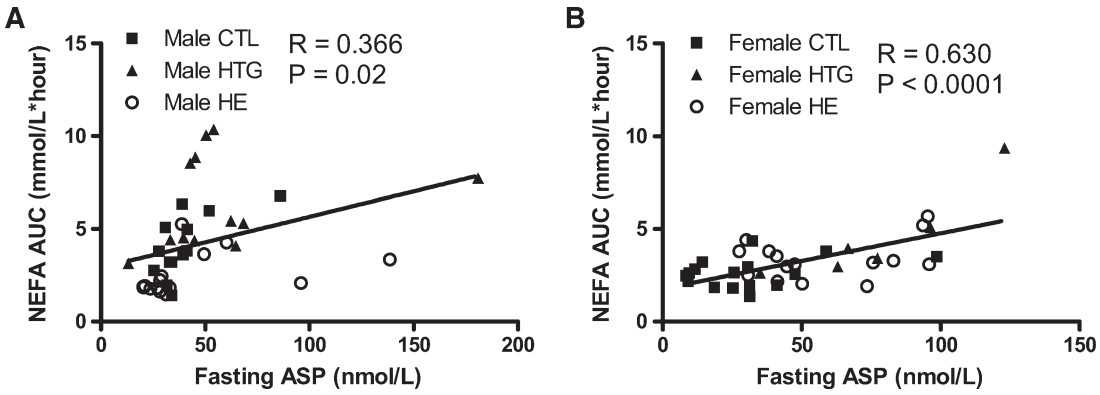

Fig. 6. Fasting ASP positively correlates with NEFA AUC levels. Fasting ASP significantly correlates with NEFA AUC men, n = 40 (A) and women, n = 37 (B). Results for all subjects that underwent the fatload: healthy controls (CTL: *black squares*), hypertriglyceridemics (HTG: *black triangles*) and LPL heterozygotes, both normal-TG and high-TG heterozygotes, (HE: *white circles*). Results were analyzed by Pearson linear regression.

LPL-deficient subjects maintain normal adiposity and TG storage (37, 38). Interestingly, the increased ASP seen in LPL deficiency is not associated with elevated insulin levels. In fact, the LPL heterozygotes appear to be more insulin sensitive than control and hypertriglyceridemic subjects. In addition, others have reported reduced plasma glucose and increased insulin secretion in LPL-deficient subjects during an oral glucose tolerance compared with controls (39).

The accumulation of TG-rich particles associated with LPL deficiency (LPL homozygotes and high-TG LPL heterozygotes) may result in the observed elevated levels of ASP. Previous studies have shown that chylomicrons stimulate the secretion and production of both ASP and its precursor protein C3 (17, 18, 40). Although fasting TG and NEFA were not increased in normo-TG LPL heterozygotes, postprandial TG and NEFA clearance were delayed, and ASP levels remained consistently elevated. Furthermore, fasting ASP positively correlated with NEFA AUC, indicating high ASP is associated with delayed NEFA clearance. While high plasma ASP is often associated with metabolic disorders (as discussed above), the physiological consequences of chronically elevated ASP levels are still unknown. In vitro studies in adipocytes have shown that ASP stimulates in situ LPL activity by increasing FA uptake and relieving product inhibition (8, 11). On the other hand, ASP plays an inhibitory role in skeletal muscle tissue; LPL activity was decreased by 35% in skeletal muscle upon ASP stimulation (11). Taking this into account, exactly how high ASP levels contribute to delayed postprandial clearance, or whether the increased ASP is simply a consequence of the chylomicron effect on ASP production, remains to be elucidated.

Fibrates, the standard pharmacological treatment for hypertriglyceridemia caused by a genetic disorder (41), are peroxisome proliferator-activated receptor α (PPAR-α) agonists. PPAR-α is highly expressed in the liver, muscle, heart, and kidney and regulates the transcription of genes encoding proteins involved in lipid metabolism, including the up-regulation of LPL expression. In addition, PPAR-α activation reduces apoCIII expression which acts as a negative regulator of LPL activity (42). The exact mechanism by which PPAR-α decreases apoCIII expression is still unknown; however, REV-ERB-α (NR1D1), an apoCIII transcriptional repressor, has been shown to be up-regulated by PPAR-α (42). Therefore, fibrates can increase the activity of the unaffected allele in LPL heterozygotes, thus augmenting LPL activity and enhancing chylomicron clearance from circulation. As demonstrated here, female heterozygotes greatly benefited from fibrate treatment, reducing fasting TG by almost 60%, compared with male heterozygotes. However, this may be confounded by a recruitment bias because the women were older, had a higher BMI, higher ASP, and more severe hypertriglyceridemia than the men. Nonetheless, in both males and females the fibrate treatment significantly lowered TG, VLDL-apoB and total phospholipid levels in LPL heterozygotes, indicating a reduction in TG-rich particles. Phospholipids, which are indicative of the total lipoprotein load, tend to be elevated in LPL-deficient subjects (43). In addition, ASP levels were reduced during the fatload. Since the treatment resulted in enhanced TG and NEFA clearance, fibrate treatment may result in decreased ASP production; however, more work is required to confirm this hypothesis.

In addition to the effects PPAR-α has on the liver, PPAR-α is also expressed in adipose tissue, although to a lesser extent (44). Fibrate treatment has direct effects on adipokine expression and secretion; TNF-α and leptin were down-regulated with treatment (45, 46), while adiponectin and visfatin, both insulin sensitizing hormones, were increased (47, 48). Because ASP is an adipose tissue derived hormone, fibrate treatment may regulate its production and the expression of its receptor (C5L2); however this remains to be determined.

In summary, ASP is an important hormone involved in stimulating NEFA esterification and TG synthesis, glucose uptake, and DGAT activity in adipose tissue (3), while having opposing effects on muscle LPL activity. In LPL-deficient subjects, ASP levels are significantly elevated, postprandial lipids are delayed, and ASP levels positively correlate with NEFA AUC. In addition, fibrate treatment designed to

reduce both fasting and postprandial TG levels resulted in decreased ASP levels.

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