Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance

Katherine Cianflone,[1,*] Robert Zakarian,[*] Charles Couillard,[†] Bernadette Delplanque,[§] Jean-Pierre Despres,[†] Allan Sniderman[*]

Mike Rosenbloom Laboratory for Cardiovascular Research,* McGill University Health Centre, Royal Victoria Hospital, 687 Pine Avenue, West Montreal, Quebec H3A 1A1, Canada; Lipid Research Centre,† Centre Hospitalier Universitaire de Québec Research Centre and Department of Food Sciences and Nutrition, Laval University, Sainte-Foy, Canada; and Laboratoire de Physiologie et de la Nutrition,§ Universite Paris Sud, Orsay, France

Abstract Postprandial plasma triglyceride (ppTG) and NEFA clearance were stratified by plasma acylation-stimulating protein (ASP) and gender to determine the contribution of fasting ASP in a normal population (70 men; 71 women). In the highest ASP tertile only, ASP decreased over 8 h (90 ± 9.7 nM to 70 ± 5.9 nM, $P < 0.05$ males; 61.9 ± 4.0 nM to 45.6 ± 6.2 nM, $P < 0.01$ females). Fasting ASP correlated positively with ppTG response. ppTG ($P < 0.0001$, 2-way ANOVA, both genders) and NEFA levels progressively increased from lowest to highest ASP tertile, with the greatest differences in males. By stepwise multiple regression, the best prediction of ppTG was: (fasting ASP + apolipoprotein B + insulin + TG; $r = 0.806$) for men and (fasting ASP + total cholesterol; $r = 0.574$) for women. Leptin, body mass index, and other fasting variables did not improve the prediction. Thus, in men and women, ASP significantly predicted ppTG and NEFA clearance and, based on lower ASP, women may be more ASP sensitive than men. Plasma ASP may be useful as a fasting variable that will provide additional information regarding ppTG and NEFA clearance.— Cianflone K., R. Zakarian, C. Couillard, B. Delplanque, J-P. Despres, and A. Sniderman. **Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance.** ***J. Lipid Res.*** **2004.** 45: **124–131.**

Supplementary key words C3adesArg • fatty acid • insulin • leptin

Zilversmit (1) was the first to propose that postprandial lipemia could be atherogenic. Many investigators have since confirmed the association of delayed triglyceride (TG) clearance with atherosclerosis (2, 3). Delayed TG clearance has also been demonstrated to be a characteristic of patients with visceral obesity (4) and a risk factor for the development of diabetes (5–7). However, much remains to be learned about the physiological determinants of the multiple processes necessary for the rapid and effective removal of TG from plasma.

In adipose tissue, two critical steps must be linked for efficient clearance of chylomicrons. The first is extracellular: the liberation of NEFA from chylomicron TGs by lipoprotein lipase (LPL) (8, 9). The second is intracellular: the uptake of these NEFAs by adipocytes and their resynthesis into TGs (10). The critical interdependence of the two needs to be appreciated. Unless the NEFAs, which are released rapidly and in large quantities by LPL, are taken up and esterified just as rapidly by adipocytes, LPL will be inhibited, and the rate of lipolysis and thus TG clearance will be reduced (11–13). Not only will TG clearance from plasma be delayed but also net uptake by adipose tissue will be reduced. This will result in greater release of NEFAs and partially hydrolyzed chylomicron particles to the systemic circulation, with the result that fatty acid flux to the liver will increase.

The rate at which NEFAs are taken up by adipocytes is determined by the rate at which they can be resynthesized into TGs. In vitro studies have shown that both insulin and acylation-stimulating protein (ASP) markedly stimulate glucose uptake (14–16) and TG synthesis (17) in adipocytes. Importantly, their effects are independent and additive (14–16).

Both insulin and ASP also reduce the rate of fatty acid release from adipose tissue (18). Insulin acts principally via reduction of hormone-sensitive lipase activity and increases fatty acid esterification, but to a lesser extent (18). By contrast, ASP acts principally by increasing reesterification and reduces lipolysis, but to a lesser extent (18). The

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Abbreviations: ASP, acylation-stimulating protein; BMI, body mass index; HOMA, homeostatic model of insulin resistance; iAUC, incremental area under the curve; RM-ANOVA, repeated measures analyses of variance; TG, triglyceride.

[1] To whom correspondence should be addressed.
e-mail: katherine.cianflone@mcgill.ca

effects of both hormones are additive and effectively reduce catecholamine-stimulated fatty acid release from adipocytes by up to 95% (18).

ASP (C3adesArg) is generated through the proximal step of the alternate complement pathway by the initiating action of the specific serine protease enzyme adipsin (complement factor D) on a complex formed by the interaction of factor B with the precursor C3 to produce C3a. ASP is then produced through the rapid cleavage by carboxypeptidase B of the carboxyl terminal arginine (17, 19).

Previous studies on postprandial ASP have demonstrated little significant change in circulating ASP (20–24). On the other hand, arterio-venous studies in human subcutaneous adipose tissue have demonstrated increased release of ASP from human subcutaneous adipose tissue, and the degree to which this occurs correlates with the extent of fatty acid trapping by adipose tissue (19, 25, 26).

Further evidence of a physiologic role for ASP was obtained from studies in rodents, where intraperitoneal injection of ASP enhanced TG clearance in wild-type C57BL/6J mice as well as *ob/ob* and *db/db* mice (27, 28). The delayed TG clearance characteristic of both $C3^{-/-}$ ASP-deficient mice and double knockout *ob/ob* $C3^{-/-}$ ASP-deficient mice was also normalized with ASP administration (29–31). Altogether, these studies support a range of ASP control of dietary fat partitioning.

Insulin and ASP may share another important characteristic: metabolic resistance to their effects. Insulin resistance has been widely studied and is widely accepted as an important risk factor for vascular disease (7). Insulin resistance is closely linked to visceral obesity and is more common in males than in females (32). In vitro studies suggest that ASP resistance may exist as well. In subjects with hyperapolipoprotein B (hyperapoB) and high plasma ASP, there is a decreased TG response to ASP, as assessed in human skin fibroblasts (33, 34), findings that point to ASP resistance. The objectives of the present study, therefore, were to compare plasma TG and NEFA clearance in normals stratified by plasma ASP and gender.

METHODS

Subjects

A healthy, normal population group was recruited consisting of 70 men and 71 women. Of the 71 women, 65 were known to be premenopausal. Information was not available on 6 women; exclusion of these subjects did not change the results presented. All subjects fasted and drank only water for 12 h before the study. None of the subjects were known to be on any medication that could affect lipoprotein metabolism. All studies were approved by the Royal Victoria Hospital, McGill University Health Centre Ethics Committee, and subjects gave their informed consent.

Experimental design

Subjects were given a mixed meal equivalent to ~50% of their daily caloric requirement. The high-fat/high-energy meal was selected to provide an equal acute challenge above the normal fat and energy intake per meal, in which average North American daily fat intake is 35–40% (35). The meal contained 700 kcal/m^2 of body surface area, and contained 63% fat (25% saturated, 26% monounsaturated, 10 polyunsaturated plus 6% other sources), 11% protein, and 26% carbohydrate (by weight). The meal was composed of eggs, cheese, toast, butter, peanut butter, peaches, whipped cream, and milk and was well tolerated by all subjects. Blood samples were taken at 0, 2, 4, 6 and 8 h after eating the meal. The samples were immediately centrifuged, and plasma was stored at −80°C until analysis.

Analyses

Human ASP was assayed as previously described (25, 36). Plasma total cholesterol (TChol) and TGs were measured by commercial enzymatically colorimetric methods (Roche Diagnostics, Laval, Quebec, Canada). Plasma HDL was measured following precipitation of apolipoprotein B (apoB)-containing lipoproteins (37), and LDL cholesterol was calculated based on the Friedewald formula (38). Plasma NEFA was measured by an enzymatic method (Wako Pure Chemicals, Osaka, Japan). Total apoB was measured by commercial nephelometric assay, using a commercial standard and controls. Plasma insulin was measured by a radioimmunoassay kit (Medicorp, Montreal, Quebec, Canada). Glucose was measured using a commercial colorimetric enzymatic kit (Sigma, St. Louis, MO). Leptin was analyzed using a radioimmunoassay kit (Linco Research, Inc., St. Charles, MO).

Statistical analyses

All results are expressed as means ± SEM. Fasting baseline lipid measurements were analyzed by *t*-test or Mann-Whitney rank sum test for abnormal distributions (as indicated in Results). Postprandial data were analyzed by repeated measures analyses of variance (RM-ANOVA), with all pairwise multiple comparisons (including interactions) using Bonferroni *t*-tests, or RM-ANOVA on ranks for abnormal distributions. Statistical analyses were performed using computer-assisted analysis (GraphPad Prism, San Diego, CA) and SigmaStat (Jandel, San Rafael, CA). Correlations between values of selected parameters were conducted by Pearson product moment correlation. Forward stepwise analysis was used for multiple regression. Significance was set at $P < 0.05$, where P = ns indicates not significant.

RESULTS

Seventy healthy men and 71 healthy women with no known disease, including diabetes or cardiovascular disease, were studied. The baseline data are given in **Table 1**. All average lipid levels are within published normal ranges (39). Fasting plasma ASP and apoB were significantly higher in males than in females, whereas plasma insulin and leptin were significantly higher in females than in males (all $P < 0.0001$). For the remaining parameters, no significant differences were noted.

In **Table 2**, males and females are shown divided into tertiles based upon fasting levels of plasma ASP, with an equal number of subjects in each group. Several points are of interest. In males, based on separation from lowest to highest tertile of ASP, there is a significant progressive increase in body mass index (BMI), fasting plasma TGs, NEFA, insulin, homeostatic model of insulin resistance (HOMA) and leptin. In the female subjects, there are similar increases from lowest to highest ASP tertile in BMI, fasting TGs and NEFA (as with men), and also apoB and glucose, but no significant changes in leptin, insulin, or HOMA (although the trends are similar).

TABLE 1. Fasting lipid profiles of all men and women

	Males (n = 70)	Females (n = 71)	*P*
ASP (nM)	49.4 ± 4.9	35.7 ± 2.8	<0.001
Leptin (ng/ml)	6.1 ± 0.63	22.4 ± 3.19	<0.001
Insulin (pM)	70.9 ± 5.51	88.7 ± 7.84	<0.001
Glucose (mM)	5.0 ± 0.07	4.6 ± 0.11	ns
HOMA	1.9 ± 0.19	1.8 ± 0.26	ns
TG (mM)	1.5 ± 0.13	1.0 ± 0.05	ns
NEFA (mM)	0.6 ± 0.04	0.5 ± 0.03	ns
TChol (mM)	4.9 ± 0.11	4.5 ± 0.09	ns
HDLC (mM)	1.0 ± 0.04	1.4 ± 0.05	ns
LDLC (mM)	3.1 ± 0.10	2.6 ± 0.09	ns
apoB (mg/dl)	93.9 ± 2.84	81.5 ± 2.51	<0.01
BMI (kg/m^2)	26.2 ± 0.53	26.1 ± 0.79	ns
Age (years)	41.3 ± 1.73	39.6 ± 1.41	ns

apoB, apolipoprotein B; ASP, acylation-stimulating protein; BMI, body mass index; HDLC, HDL-cholesterol; HOMA, homeostatic model of insulin resistance; LDLC, LDL-cholesterol; ns, not significant; TChol, total cholesterol; TG, triglyceride. Values are given as average ± SEM. Groups were compared by *t*-test or Mann Whitney rank sum test for abnormal distributions (ASP, leptin, insulin, HOMA, and TG).

Following an overnight fast, a fat load meal was consumed, and blood samples were taken over an 8 h time period. Postprandial levels of ASP for each tertile for both males and females are shown in **Fig. 1**. For both genders, there are no significant changes over time for ASP in the lowest and middle tertiles of ASP. In the highest tertile, however, there is a significant decrease in plasma ASP over the course of the fat load (8 h vs. 0 h: 90 ± 9.7 nM to 70 ± 5.9 nM, $P < 0.05$ in males and 61.9 ± 4.0 nM to 45.6 ± 6.2 nM, $P < 0.01$ in females).

Postprandial TG levels for males and females in each tertile of ASP are depicted in **Fig. 2**. Again, the greatest differences were seen in males. In men, postprandial TG levels were progressively higher and TG clearance progressively delayed from the lowest to the highest tertile of plasma ASP ($P < 0.0001$, 2-way ANOVA). The maximal increase in TG at 4 h was 180%, 235%, and 235% from lowest to highest ASP tertile, respectively. At 8 h, plasma TG was still significantly increased in the two highest tertiles ($P < 0.0001$).

In women, plasma TGs were higher and clearance delayed only in the highest tertile, compared with the middle and lowest tertile ($P < 0.0001$, 2-way ANOVA). In all three subgroups, TG clearance, calculated as TG incremental area under the curve (TG iAUC), was significantly more rapid in women compared with men (Fig. 2, inset).

Fasting and postprandial levels of NEFA are shown in **Fig. 3**. Again the differences among the tertiles are much greater in males than in females. In men, NEFA increased significantly from 0.36 ± 0.06 mM at 0 h to 0.64 ± 0.08 mM at 8 h, $P < 0.001$ in the lowest tertile; from 0.55 ± 0.04 mM to 0.98 ± 0.11 mM, $P < 0.001$ in the middle tertile; and from 0.75 ± 0.09 mM to 1.3 ± 0.15 mM, $P < 0.001$ in the highest tertile of plasma ASP. In all three subgroups in women, plasma NEFA also increased significantly, although the differences were less than in the men. As was the case with TGs, plasma NEFA levels were significantly higher in the highest tertile of plasma ASP. Thus, NEFA concentrations increased from 0.41 ± 0.004 mM to 0.60 ± 0.06 mM, $P < 0.01$ in the lowest tertile; from 0.47 ± 0.06 mM to 0.67 ± 0.05 mM, $P < 0.001$ in the middle tertile; and from 0.70 ± 0.07 mM to 0.79 ± 0.07 mM, $P < 0.05$ in the highest tertile. In addition, in the women only, there was a significant drop in NEFA at 2 h, usually indicative of inhibition of adipose tissue hormone-sensitive lipase.

Pearson's correlation coefficients were calculated to determine whether there were significant associations between the hormones ASP, insulin, and leptin and BMI with TG clearance. In both genders, as shown in **Table 3**, there were significant and strong associations between both fasting and 8 h plasma ASP with fasting plasma TG, TG iAUC, NEFA AUC, and apoB. In men, insulin, leptin, and BMI also displayed strong significant correlations with these variables. Further, ASP indices also correlated with HOMA, BMI, and leptin. In females, insulin, leptin, and BMI correlated significantly only with fasting but not with postprandial plasma TGs or NEFA. To compare the relative effects between men and women, the slopes of the correlations of fasting ASP and TG with postprandial TG

TABLE 2. Fasting lipid profiles for men and women separated based on ASP tertile

	Men				Women			
	Group 1	Group 2	Group 3	ANOVA	Group 1	Group 2	Group 3	ANOVA
Tertile ASP (nM)	19.9 ± 0.89	36.7 ± 1.0	90.0 ± 9.7		13.8 ± 0.76	29.1 ± 1.2	61.9 ± 4.0	
(range)	(13.2–28.3)	(29.5–45.3)	(50.5–253.2)		(8.4–18.6)	(19.1–38.8)	(38.8–123.0)	
(n)	23	23	24		23	23	25	
Age (years)	46.0 ± 4.0	39.1 ± 2.3	40.0 ± 2.5	ns	37.0 ± 2.2	41.2 ± 2.8	40.5 ± 2.3	ns
BMI (kg/m^2)	23.7 ± 0.7	26.7 ± 0.9	28.7 ± 0.8	$P < 0.001$	23.6 ± 0.8	26.3 ± 1.0	28.2 ± 0.2	$P < 0.05$
TG (mM)	1.1 ± 0.1	1.4 ± 0.2	2.1 ± 0.3	$P < 0.01$	0.8 ± 0.1	0.9 ± 0.1	1.3 ± 0.10	$P < 0.001$
NEFA (mM)	0.36 ± 0.1	0.55 ± 0.0	0.75 ± 0.1	$P < 0.001$	0.41 ± 0.0	0.47 ± 0.1	0.70 ± 0.1	$P < 0.01$
TChol (mM)	4.9 ± 0.2	4.7 ± 0.2	4.9 ± 0.2	ns	4.2 ± 0.1	4.5 ± 0.2	4.7 ± 0.2	ns
HDLC (mM)	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	$P < 0.01$	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	ns
LDLC (mM)	3.2 ± 0.2	3.1 ± 0.2	3.0 ± 0.2	ns	2.3 ± 0.1	2.8 ± 0.2	2.8 ± 0.2	ns
apoB (mg/dl)	87.0 ± 3.4	95.8 ± 5.3	98.1 ± 5.4	ns	71.7 ± 3.5	82.0 ± 4.0	90.4 ± 4.5	$P < 0.01$
Insulin (pM)	45.5 ± 4.2	66.6 ± 9.1	121.1 ± 10.2	$P < 0.001$	75.8 ± 10	95.3 ± 11	125.8 ± 17	ns
Glucose (mM)	4.8 ± 0.2	5.0 ± 0.1	5.1 ± 0.1	ns	4.2 ± 0.2	4.6 ± 0.1	5.0 ± 0.2	$P < 0.01$
HOMA	1.3 ± 0.2	1.8 ± 0.3	3.7 ± 0.8	$P < 0.01$	1.3 ± 0.3	1.9 ± 0.3	3.5 ± 1.3	ns
Leptin (ng/ml)	3.5 ± 0.7	5.2 ± 0.7	9.5 ± 1.2	$P < 0.001$	18.6 ± 4.1	19.6 ± 5.2	29.5 ± 7.0	ns

Values are given as average ± SEM. ANOVA on ranks was used for parameters with abnormal distribution (leptin, insulin, HOMA, and TG).

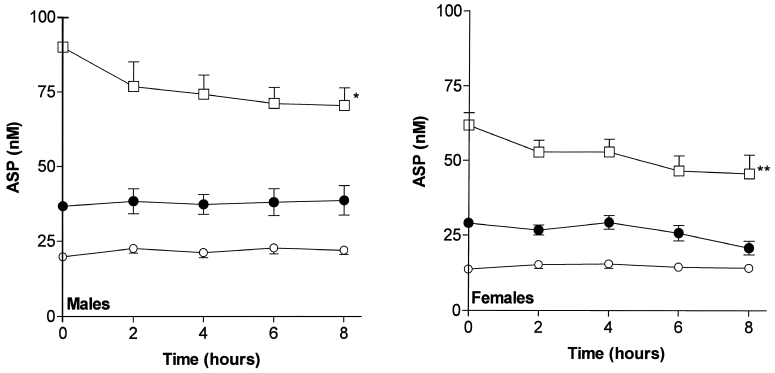

Fig. 1. Plasma acylation-stimulating protein (ASP) levels over time following a fat load for both male and female subjects. Males and females were separated into three tertiles based on fasting plasma ASP. Data are shown for each tertile as average ± SEM for Group 1 (open circles), Group 2 (filled circles), and Group 3 (squares). * $P < 0.05$, ** $P < 0.01$ for ASP at 8 h versus 0 h.

iAUC and NEFA AUC were calculated. Interestingly, although fasting TG correlated closely with postprandial TG iAUC in both men and women, the slopes of the curves were significantly steeper in men (slope = 5.150 men vs. 1.543 women, $P = 0.024$), suggesting that factors other than fasting TG could have a greater effect in men. Fasting TG could account for only a portion ($r = 0.656$ in men and $r = 0.231$ in women) of the variation in postprandial TG. As shown in **Fig. 4**, there is a strong correlation between ASP and TG iAUC as well as NEFA AUC in both men and women. However in this case, although the slopes of the lines are the same for men and women, the y-intercept is significantly greater in men versus women for both TG iAUC and NEFA ($P < 0.01$, see Fig. 4). The ASP values and TG iAUC overall are greater in men (note the axes difference between men and women).

Finally, stepwise multiple regression analysis was used to determine factors that most closely predicted postprandial TG iAUC. ASP, leptin, insulin, and all factors listed in Table 1 were included in the analysis. By forward stepwise regression in men, the model that best predicted postprandial TG iAUC was (fasting ASP [$P = 0.01$] + apo B [$P < 0.001$] + insulin [$P = 0.04$] + TG [$P = 0.03$], $r = 0.806$). No other additional variables improved the prediction. In women, (fasting ASP [$P = 0.006$] + TChol [$P = 0.036$], $r = 0.574$) produced the best model. In both men and women, ASP had a very significant effect on predicting TG iAUC. In neither men nor women did leptin or BMI contribute further.

DISCUSSION

The objective of this study was to examine the relation of plasma ASP in normal men and women to the effectiveness of postprandial fatty acid trapping. Our data demonstrate that TG and NEFA clearance following an oral fat load were inversely related to plasma ASP in both men and women. At the same time, major differences between the genders were documented. Postprandial TG and

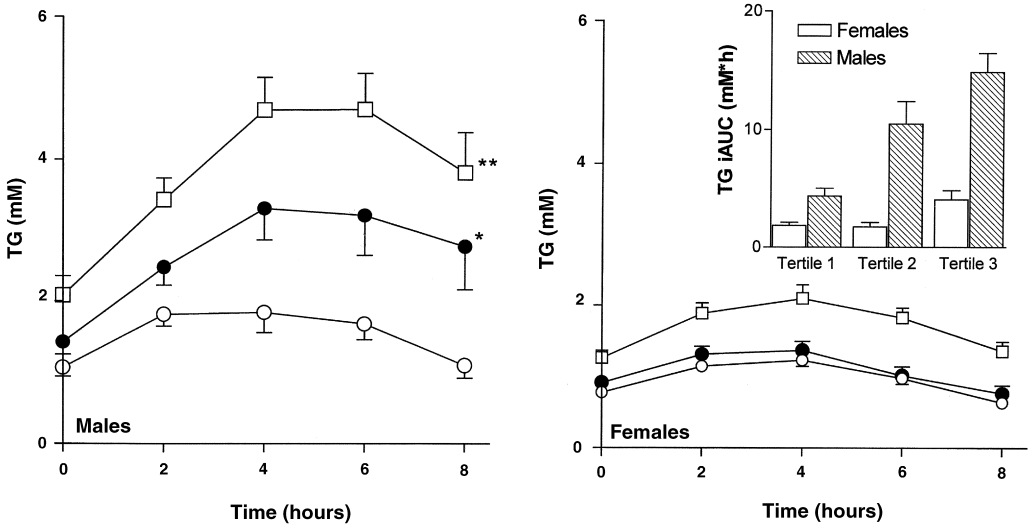

Fig. 2. Plasma triglyceride (TG) levels over time following a fat load for both male and female subjects. Data are shown for each tertile as average ± SEM for Group 1 (open circles), Group 2 (filled circles), and Group 3 (squares). For males, $P < 0.001$ for Group 3 versus Group 1 and $P < 0.05$ Group 2 versus Group 1. Incremental TG area under the curve is shown for both males and females in the inset, where $P < 0.0001$ by ANOVA for the effect of tertile in both men and women. Differences between males versus females: Group 1, no difference; Group 2, $P < 0.01$; Group 3, $P < 0.001$.

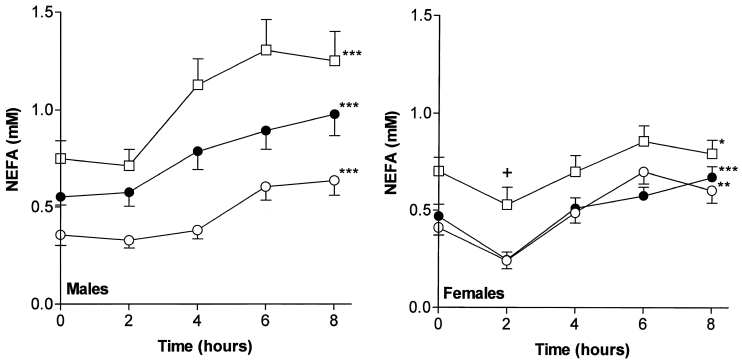

Fig. 3. Plasma NEFA over time following a fat load for both male and female subjects. Data are shown for each tertile as average ± SEM for Group 1 (open circles), Group 2 (filled circles), and Group 3 (squares). For males: Group 1 versus Group 3, $P < 0.001$. For changes over time, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ by ANOVA. +, $P < 0.05$ for 2 h versus 0 h for all three groups of females.

NEFA clearance was substantially more rapid in women than in men. Fasting ASP was significantly higher in men than in women, while fasting insulin and leptin were significantly higher in women than in men. The present data are consistent with previous results pointing to more effective TG clearance and fatty acid trapping in women compared with men (40–42).

Much is already known of the determinants of plasma TG clearance. Fasting plasma TG levels have repeatedly been shown to account for a major portion of the variance in postprandial TG levels (43), and this has been attributed to competition between VLDL and chylomicrons for LPL (44). Once fasting TGs were taken into account, other variables, such as BMI, visceral adipose tissue, apoE, postheparin LPL activity, diabetic status, and insulin resistance (according to the study) contributed little or no additional information (40, 43, 45). Nevertheless, fasting TG only accounts for a portion of the variation in postprandial TG (40, 43, 45) and, therefore, other factors must play a role.

Gender is clearly one such factor. In the present study, not only was NEFA and TG clearance delayed in men versus women, but also the association between fasting TG and postprandial TG iAUC was significantly steeper in men than in women. Although BMI did not differ between males and females, even when stratified into three groups, given the known differences in body composition (46), adipose tissue mass would be expected to be greater in the women. This likely explains their higher plasma leptin. Leptin correlated with postprandial TG iAUC (men and women) as well as postprandial NEFA (men only), and leptin has been shown previously to increase postprandially (47, 48). However, the available evidence is mixed and the mechanisms disputed as to whether leptin itself directly influences postprandial fatty acid metabolism (49). In any event, there was no evidence in this study that leptin was a significant independent determinant of the results.

In men, insulin correlated strongly with fasting TG, TG iAUC, as well as NEFA AUC. Indeed, it has been previously shown that when men are stratified based on fasting TG, there is a correlation with postprandial TG AUC and insulin similar to that we have shown here for ASP (50). By contrast, in females, although fasting insulin correlated with fasting TG, there was no correlation of insulin with postprandial TG iAUC or postprandial NEFA AUC. This

TABLE 3. Pearson correlation coefficients for men and women

	TG	TG (iAUC)[a]	FFA (AUC)	apoB	HOMA	BMI	Leptin
Men							
ASP	0.317[c]	0.409[d]	0.371[c]	ns	ns	0.345[c]	0.308[b]
ASP 8 h	0.335[c]	0.524[d]	0.323[c]	0.235[b]	0.300[b]	0.560[d]	0.421[c]
ASP (8–0 h)	ns	ns	ns	ns	ns	ns	ns
Insulin	0.594[d]	0.673[d]	0.325[c]	0.460[d]	—	0.527[d]	0.729[d]
Leptin	0.380[c]	0.473[d]	0.326[c]	0.343[b]	0.772[d]	0.772[d]	—
BMI	0.451[c]	0.634[d]	0.351[c]	0.477[d]	0.559[d]	—	0.772[d]
Women							
ASP (0 h)	0.513[d]	0.389[d]	0.427[d]	0.419[d]	ns	ns	ns
ASP (8 h)	0.415[c]	0.330[c]	0.607[d]	ns	ns	ns	ns
ASP (8–0 h)	−0.322[c]	−0.292[b]	0.607[d]	−0.297[b]	ns	−0.279[b]	ns
Insulin	0.341[c]	ns	ns	ns	—	0.589[d]	ns
Leptin	0.370[c]	ns	ns	0.372[b]	ns	0.845[d]	—
BMI	0.573[d]	ns	ns	0.387[c]	0.631[d]	—	0.845[d]

iAUC, incremental area under the curve; ASP (8–0 h), drop in ASP from fasting to 8 h. Unless indicated, all variables are fasting.

[a] iAUC increase over fasting levels.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] $P < 0.001$.

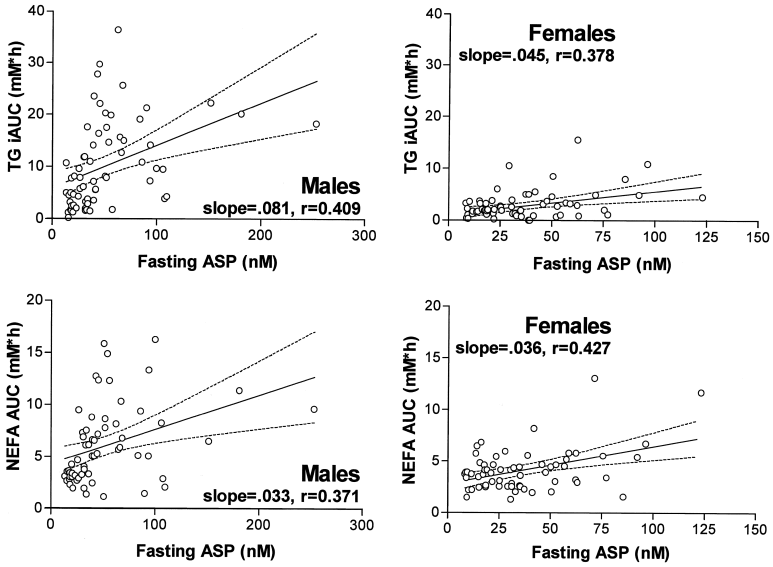

Fig. 4. Correlation between fasting ASP and postprandial TG incremental AUC and NEFA AUC in males and females. Individual data as well as regression lines are shown for males and females. The slopes of the regression curves are not significantly different for males versus females.

was despite the fact that insulin was significantly higher in women than in men, suggesting that fatty acid trapping and TG clearance, while greater in female adipose tissue (51), may be less influenced by insulin than in men (52).

In both men and women, there was a close relation between fasting plasma ASP and plasma TG clearance as well as NEFA, i.e., the higher the fasting plasma ASP, the less effective the postprandial TG and fatty acid clearance. In fact, by stepwise multiple regression analysis, a large proportion of the variance in postprandial TG could be explained by either (ASP + Tchol) for women, or (ASP + apoB + insulin + TG) for men. Addition of either leptin or BMI to the model did not further improve it, although both correlated with fasting TG. The association between ASP and postprandial TG iAUC was similar between men and women, such that for any given ASP, postprandial TG clearance was similar (i.e., there was no difference in the slopes of the regression lines), although both ASP and postprandial TG clearance overall were significantly greater in men.

Although there are a number of studies that have demonstrated an association of fasting ASP with lipoproteins, including TG and NEFA [as reviewed in ref. (19)], to date, only five studies with limited numbers of subjects have examined postprandial ASP. In those studies, postprandial ASP either remained constant (20, 23, 24) or decreased over the fat load (21, 22). In previous studies, the drop in ASP generally occurred in those subjects who had the highest fasting plasma ASP (such as obese and diabetic subjects) (20, 21), but only one report showed fasting ASP correlating with postprandial TG and NEFA (20). In the present study, we have examined this in detail. Only those subjects with increased ASP demonstrated significant drops in postprandial ASP, such that fasting ASP correlated positively with the 8 h drop in ASP. In cultured adipocytes, postprandial chylomicrons were a potent stimulus to ASP production (53, 54), and in the microenvironment of the adipose tissue, ASP production increased postprandially, although this did not result in a general increase in circulating levels (19, 25, 26). Thus, the increase in fasting ASP may be a consequence of chronic increases in postprandial TG, which stimulates ASP production, translating into later increases in ASP in the general circulation. Why ASP then decreases later on in the postprandial phase in these subjects particularly is unknown, although Weyer and Pratley (21) suggested that the decrease in plasma ASP may reflect a postprandial shift from the intravascular to the interstitial compartment, where it exerts its biologic effects.

Delayed postprandial TG and NEFA clearance point to reduced effectiveness of fatty acid trapping by adipose in men versus women. In both genders, plasma ASP was inversely related to these rates. Moreover, plasma ASP was lower in women than in men, notwithstanding their greater adipose tissue mass (for the same BMI). All these associations are consistent with a decreased responsiveness of adipose tissue to ASP in normal males compared with normal females. This suggests the hypothesis that a higher plasma ASP represents an adipose tissue ASP-resistant state, whereas a lower plasma ASP represents an adipose tissue ASP-sensitive state. This hypothesis is supported by in vitro data. Both subcutaneous and visceral adipose tissue plasma membrane preparations from men had a reduced capacity to specifically bind ASP than did comparable adipose tissue preparations from women (55).

We have previously demonstrated that cells from subjects with both increased plasma ASP and hyperapoB demonstrate reduced specific binding and response to ASP, whereas cells from hyperapoB subjects with normal plasma ASP manifest normal binding and a normal stimulatory response to ASP (33, 34). On the other hand, the cellular response to insulin stimulation of TG synthesis was comparable in both groups. The association of increased ASP and/or its precursor, C3, with risk of myocardial infarction, coronary artery disease, insulin resistance, and diabetes has been examined in a number of recent

studies [as reviewed in ref. (19)]. Although only two studies have examined ASP in coronary heart disease and hyperlipidemia, these studies demonstrate correlations of ASP with TG and NEFA (as mentioned above) as well as with apoB (23). Thus, increased plasma ASP appears to be a marker for cellular ASP resistance, just as increased insulin points to insulin resistance. With the recent identification of an ASP receptor in adipose tissue (56), the specific molecular defects in ASP-resistant hyperapoB can now be pursued.

In summary, our data demonstrate gender differences in postprandial fatty acid trapping that are related to plasma ASP. The effectiveness of fatty acid trapping is a major determinant of fatty acid flux to the liver and therefore of the rate at which apoB lipoproteins are secreted by the liver (57). Relative resistance of adipose tissue to ASP and therefore reduced effectiveness of fatty acid trapping could explain why plasma cholesterol, TG, and apoB levels are higher in men compared with women. Measurement of plasma ASP provides information supplementary to that provided by fasting TG alone and may be helpful in identifying individuals likely to have postprandial hyperlipidemia.

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REFERENCES

1. Zilversmit, D. B. 1979. Atherogenesis: a postprandial phenomenon. *Circulation.* **60:** 473–485.
2. Simons, L. A., T. Dwyer, J. Simons, L. Bernstein, P. Mock, N. S. Poonia, S. Balasubramaniam, D. Baron, J. Branson, J. Morgan, and P. Roy. 1987. Chylomicrons and chylomicron remnants in coronary artery disease: a case-control study. *Atherosclerosis.* **65:** 181–189.
3. Patsch, J. R., G. Miesenbock, T. Hopferwieser, V. Muhlberger, E. Knapp, J. K. Dunn, A. M. Gotto, Jr., and W. Patsch. 1992. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler. Thromb.* **12:** 1336–1345.
4. Couillard, C., N. Bergeron, D. Prud'homme, J. Bergeron, A. Tremblay, C. Bouchard, P. Mauriege, and J. P. Despres. 1998. Postprandial triglyceride response in visceral obesity in men. *Diabetes.* **47:** 953–960.
5. Despres, J. P. 2001. Health consequences of visceral obesity. *Ann. Med.* **33:** 534–541.
6. Wong, W. W. 1990. Structural and functional correlation of the human complement receptor type 1. *J. Invest. Dermatol.* **94 (Suppl.):** 64–67.
7. Despres, J. P. 1998. The insulin resistance-dyslipidemic syndrome of visceral obesity: effect on patients' risk. *Obes. Res.* **6 (Suppl.):** 8–17.
8. Mead, J. R., S. A. Irvine, and D. P. Ramji. 2002. Lipoprotein lipase: structure, function, regulation, and role in disease. *J. Mol. Med.* **80:** 753–769.
9. Preiss-Landl, K., R. Zimmermann, G. Hammerle, and R. Zechner. 2002. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and energy metabolism. *Curr. Opin. Lipidol.* **13:** 471–481.
10. Coleman, R. A., T. M. Lewin, and D. M. Muoio. 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Annu Rev Nutr.* **20:** 77–103.
11. Xu, X., J. Storkson, S. Kim, K. Sugimoto, Y. Park, and M. W. Pariza. 2003. Short-term intake of conjugated linoleic acid inhibits lipoprotein lipase and glucose metabolism but does not enhance lipolysis in mouse adipose tissue. *J. Nutr.* **133:** 663–667.
12. Saxena, U., L. D. Witte, and I. J. Goldberg. 1989. Release of endothelial lipoprotein lipase by plasma lipoproteins and free fatty acids. *J. Biol. Chem.* **264:** 4349–4355.
13. Posner, I., and J. DeSanctis. 1987. The effects of bovine serum albumin and oleic acid on rat pancreatic lipase and bovine milk lipoprotein lipase. *Comp. Biochem. Physiol. B.* **87:** 137–141.
14. Germinario, R., A. D. Sniderman, S. Manuel, S. Pratt, A. Baldo, and K. Cianflone. 1993. Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. *Metabolism.* **42:** 574–580.
15. Tao, Y., K. Cianflone, A. D. Sniderman, S. P. Colby-Germinario, and R. J. Germinario. 1997. Acylation-stimulating protein (ASP) regulates glucose transport in the rat L6 muscle cell line. *Biochim. Biophys. Acta.* **1344:** 221–229.
16. Maslowska, M., A. D. Sniderman, R. Germinario, and K. Cianflone. 1997. ASP stimulates glucose transport in cultured human adipocytes. *Int. J. Obes. Relat. Metab. Disord.* **21:** 261–266.
17. Cianflone, K., M. Maslowska, and A. D. Sniderman. 1999. Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. *Semin. Cell Dev. Biol.* **10:** 31–41.
18. Van Harmelen, V., S. Reynisdottir, K. Cianflone, E. Degerman, J. Hoffstedt, K. Nilsell, A. Sniderman, and P. Arner. 1999. Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. *J. Biol. Chem.* **274:** 18243–18251.
19. Cianflone, K., Z. Xia, and L. Y. Chen. 2003. Critical review of acylation stimulating protein physiology in humans and rodents. *Biochim. Biophys. Acta.* **1609:** 127–143.
20. Koistinen, H. A., H. Vidal, S. L. Karonen, E. Dusserre, P. Vallier, and V. A. Koivisto. 2001. Plasma acylation stimulating protein concentration and subcutaneous adipose tissue C3 mRNA expression in nondiabetic and type 2 diabetic men. *Arterioscler. Throm. Vasc. Biol.* **21:** 1034–1039.
21. Weyer, C., and R. E. Pratley. 1999. Fasting and postprandial plasma concentrations of acylation-stimulation protein (ASP) in lean and obese Pima Indians compared to Caucasians. *Obes. Res.* **7:** 444–452.
22. Faraj, M., P. Jones, A. D. Sniderman, and K. Cianflone. 2001. Enhanced dietary fat clearance in post-obese women. *J. Lipid Res.* **42:** 571–580.
23. Ylitalo, K., P. Pajukanta, S. Meri, R. M. Cantor, N. Mero-Matikainen, J. Vakkilainen, I. Nuotio, and M. Taskinen. 2001. Serum C3 but not plasma acylation-stimulating protein is elevated in Finnish patients with familial combined hyperlipidemia. *Asterioscler. Thromb. Vasc. Biol.* **21:** 838–843.
24. Charlesworth, J. A., P. W. Peake, L. V. Campbell, B. A. Pussell, S. O'Grady, and T. Tzilopoulos. 1998. The influence of oral lipid loads on acylation stimulating protein (ASP) in healthy volunteers. *Int. J. Obesity Relat. Metab. Disord.* **22:** 1096–1102.
25. Saleh, J., L. K. M. Summers, K. Cianflone, B. A. Fielding, A. D. Sniderman, and K. N. Frayn. 1998. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. *J. Lipid Res.* **39:** 884–891.
26. Kalant, D., S. Phelis, B. A. Fielding, K. N. Frayn, K. Cianflone, and A. D. Sniderman. 2000. Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J. Lipid Res.* **41:** 1963–1968.
27. Murray, I., A. D. Sniderman, and K. Cianflone. 1999. Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein (ASP) in C57Bl/6 mice. *Am. J. Physiol. Endocrinol. Metab.* **277:** E474–E480.
28. Saleh, J., J. E. Blevins, P. J. Havel, J. A. Barrett, D. W. Gietzen, and K. Cianflone. 2001. Acylation stimulating protein (ASP) acute effects on postprandial lipemia and food intake in rodents. *Int. J. Obes. Relat. Metab. Disord.* **25:** 705–713.
29. Murray, I., A. D. Sniderman, and K. Cianflone. 1999. Mice lacking acylation stimulating protein (ASP) have delayed postprandial triglyceride clearance. *J. Lipid Res.* **40:** 1671–1676.
30. Murray, I., A. D. Sniderman, P. J. Havel, and K. Cianflone. 1999. Acylation stimulating protein (ASP) deficiency alters postprandial and adipose tissue metabolism in male mice. *J. Biol. Chem.* **274:** 36219–36225.
31. Xia, Z., A. D. Sniderman, and K. Cianflone. 2002. Acylation-stimulating protein (ASP) deficiency induces obesity resistance and in-

creased energy expenditure in *ob/ob* mice. *J. Biol. Chem.* **277:** 45874–45879.
32. Couillard, C., N. Bergeron, D. Prud'homme, J. Bergeron, A. Tremblay, C. Bouchard, P. Mauriege, and J. P. Despres. 1999. Gender difference in postprandial lipemia: importance of visceral adipose tissue accumulation. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2448–2455.
33. Cianflone, K., M. Maslowska, and A. D. Sniderman. 1990. Impaired response to fibroblasts in patients with hyperapobetalipoproteinemia to acylation stimulating protein. *J. Clin. Invest.* **85:** 722–730.
34. Zhang, X. J., . Cianflone, J. Genest, and A. D. Sniderman. 1998. Plasma acylation stimulating protein (ASP) as a predictor of impaired cellular biological response to ASP in patients with hyperapoB. *Eur. J. Clin. Invest.* **28:** 730–739.
35. Golay, A., and E. Bobbioni. 1997. The role of dietary fat in obesity. *Int. J. Obesity Relat. Metab. Disord.* **21 (Suppl.):** 2–11.
36. Maslowska, M., H. Vu, S. Phelis, A. D. Sniderman, B. M. Rhode, D. Blank, and K. Cianflone. 1999. Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. *Euro. J. Clin. Inv.* **29:** 679–686.
37. Astrup, A. 1993. Dietary composition, substrate balances and body fat in subjects with a predisposition to obesity. *Int. J. Obesity Relat. Metab. Disord.* **17: (Suppl.)** 32–36.
38. Schectman, G., M. Patsches, and E. A. Sasse. 1996. Variability in cholesterol measurements: comparison of calculated and direct LDL cholesterol determinations. *Clin. Chem.* **42:** 732–737.
39. Contois, J. H., C. J. Lammi-Keefe, S. Vogel, J. R. McNamara, P. W. Wilson, T. Massov, and E. J. Schaefer. 1996. Plasma lipoprotein(a) distribution in the Framingham Offspring Study as determined with a commercially available immunoturbidimetric assay. *Clin. Chim. Acta.* **253:** 21–35.
40. Sharrett, A. R., G. Heiss, L. E. Chambless, E. Boerwinkle, S. A. Coady, A. R. Folsom, and W. Patsch. 2003. Metabolic and lifestyle determinants of postprandial lipemia differ from those of fasting triglycerides: the Atherosclerosis Risk In Communities (ARIC) study. *Arterioscler. Thromb. Vasc. Biol.* **21:** 275–281.
41. Krasinski, S. D., J. S. Cohn, E. J. Schaefer, and R. M. Russell. 1990. Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. Evidence for delayed plasma clearance of intestinal lipoproteins. *J. Clin. Invest.* **85:** 883–892.
42. Ryu, J. E., G. Oward, T. E. Raven, M. G. Ond, A. P. Agaman, and J. R. Crouse. 1992. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. *Stroke.* **23:** 823–828.
43. Syvanne, M., P. J. Talmud, S. E. Humphries, R. M. Fisher, M. Rosseneu, H. Hilden, and M. R. Taskinen. 1997. Determinants of postprandial lipemia in men with coronary artery disease and low levels of HDL cholesterol. *J. Lipid Res.* **38:** 1463–1472.
44. Xiang, S-Q., K. Cianflone, D. Kalant, and A. D. Sniderman. 1999. Differential binding of triglyceride-rich lipoproteins to lipopotein lipase. *J. Lipid Res.* **40:** 1655–1663.
45. Imbeault, P., C. Couillard, A. Tremblay, J. P. Despres, and P. Mauriege. 2000. Reduced alpha(2)-adrenergic sensitivity of subcutaneous abdominal adipocytes as a modulator of fasting and postprandial triglyceride levels in men. *J. Lipid Res.* **41:** 1367–1375.
46. Rosenbaum, M., A. Pietrobelli, J. R. Vasselli, S. B. Heymsfield, and R. L. Leibel. 2001. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. *Int. J. Obes. Relat. Metab. Disord.* **125:** 1365–1371.
47. Evans, K., M. L. Clark, and K. N. Frayn. 2001. Carbohydrate and fat have different effects on plasma leptin concentrations and adipose tissue leptin production. *Clin. Sci. (Lond.).* **100:** 493–498.
48. Imbeault, P., E. Doucet, P. Mauriege, S. St-Pierre, C. Couillard, N. Almeras, J. P. Despres, and A. Tremblay. 2001. Difference in leptin response to a high-fat meal between lean and obese men. *Clin. Sci. (Lond.).* **101:** 359–365.
49. Kalant, D., M. Maslowska, T. Scantlebury, H. Wang, and K. Cianflone. 2003. Control of lipogenesis in adipose tissue and the role of acylation stimulating protein. *Can. J. Diabetes.* **27:** 154–171.
50. Castro Cabezas, M., T. W. A. DeBruin, H. W. DeValk, C. C. Shoulders, H. Jansen, and D. W. Erkelens. 1993. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. *J. Clin. Invest.* **92:** 160–168.
51. Romanski, S. A., R. M. Nelson, and M. D. Jensen. 2000. Meal fatty acid uptake in adipose tissue: gender effects in nonobese humans. *Am. J. Physiol. Endocrinol. Metab.* **279:** E455–E462.
52. Fried, S. K., C. D. Russell, N. L. Grauso, and R. E. Brolin. 1993. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. *J. Clin. Invest.* **92:** 2191–2198.
53. Scantlebury, T., M. Maslowska, and K. Cianflone. 1998. Chylomicron specific enhancement of acylation stimulating protein (ASP) and precursor protein C3 production in differentiated human adipocytes. *J. Biol. Chem.* **273:** 20903–20909.
54. Scantlebury, T., A. D. Sniderman, and K. Cianflone. 2001. Retinoic acid regulation of acylation stimulating protein (ASP) and complement C3 in human adipocytes. *Biochem. J.* **356:** 445–452.
55. Saleh, J., N. Christou, and K. Cianflone. 1999. Regional specificity of ASP binding in human adipose tissue. *Am. J. Physiol.* **276:** E815–E821.
56. Kalant, D., S. A. Cain, M. Maslowska, A. D. Sniderman, K. Cianflone, and P. N. Monk. 2003. The chemoattractant receptor-like protein C5L2 binds the C3a des-Arg77/acylation-stimulating protein. *J. Biol. Chem.* **278:** 11123–11129.
57. Sniderman, A. D., X. Zhang, and K. Cianflone. 1999. Governance of the concentration of plasma LDL: a reevaluation of the LDL receptor paradigm. *Atherosclerosis.* **148:** 215–229.