Plasma Acylation Stimulating Protein Concentration and Subcutaneous Adipose Tissue C3 mRNA Expression in Nondiabetic and Type 2 Diabetic Men

Heikki A. Koistinen, Hubert Vidal, Sirkka-Liisa Karonen, Eric Dusserre, Paulette Vallier, Veikko A. Koivisto, Pertti Ebeling

Abstract—We studied the effect of an oral fat load on plasma acylation stimulating protein (ASP) concentrations in 9 lean healthy (age 59 ± 2 years, body mass index [BMI] 23.2 ± 0.4 kg/m²; both mean ± SEM), 9 obese nondiabetic (58 ± 2 years, BMI 29.4 ± 0.5 kg/m²), and 12 type 2 diabetic (60 ± 2 years, BMI 29.6 ± 1.0 kg/m²) men. Because ASP is a cleavage product of complement protein C3 (C3adesArg) and its secretion is regulated by insulin, we also examined the subcutaneous adipose tissue expression of C3 mRNA before and after a 240-minute euglycemic hyperinsulinemic clamp in a subgroup of these men. Plasma ASP concentration and adipose tissue C3 mRNA expression were higher in the obese groups than in the lean men. Plasma ASP concentration did not change significantly after the fat load. Fasting plasma ASP concentration and C3 mRNA expression were correlated negatively with insulin sensitivity and positively with the magnitude of postprandial lipemia in nondiabetic but not in type 2 diabetic men. The expression of C3 mRNA was not regulated by insulin. These data suggest that ASP is associated with whole-body glucose and lipid metabolism in nondiabetic individuals, whereas metabolic disturbances in diabetes may overcome the regulatory role of ASP in lipid and glucose metabolism. (Arterioscler Thromb Vasc Biol. 2001;21:1034-1039.)

Key Words: acylation stimulating protein (ASP) ■ insulin resistance ■ obesity ■ postprandial lipemia ■ RT competitive PCR

Acylation stimulating protein (ASP) is a small (9-kDa), basic human serum protein, and it is identical to a fragment of the third component of the complement system, C3adesArg. ASP has been suggested to be an important determinant of postprandial lipemia, and it is also a potent stimulator of free fatty acid incorporation into triglycerides in human adipocytes in vitro.1-3 ASP is produced by the interaction of the proteins of the alternative complement pathway, C3, factor B, and adipsin, which are all expressed in adipocytes,2-4 suggesting that ASP may be produced locally in adipose tissue and contribute to adipose tissue triglyceride metabolism.

The role of ASP in human postprandial lipid metabolism is still incompletely understood. Previous studies have reported both a rise and no significant change in peripheral plasma ASP concentration after ingestion of an oral fat load.5,6 According to a study that used an arteriovenous cannulation technique, there was an increase in the venoarterial gradient of ASP concentration across the subcutaneous adipose tissue after a mixed meal, suggesting postprandial ASP production in the subcutaneous adipose tissue microenvironment.7 No data exist regarding the plasma ASP response and its relation to postprandial lipemia in obese nondiabetic and type 2 diabetic subjects. Therefore, we examined in the present study whether the plasma ASP concentration is related to postprandial lipemia during an oral fat tolerance test in lean and obese nondiabetic and type 2 diabetic men.

In vitro studies indicate that chylomicrons and insulin increase ASP production.8,9 Moreover, insulin increases the production of ASP precursor protein C3 in adipocytes,8 and the serum C3 concentration is inversely related to insulin sensitivity.10,11 Therefore, to gain insight into the in vivo regulation of adipose tissue C3 mRNA and its relation to insulin sensitivity, we also examined the expression of subcutaneous adipose tissue C3 mRNA before and after a 240-minute euglycemic hyperinsulinemic clamp in a subgroup of the lean and obese nondiabetic and type 2 diabetic men who participated in the oral fat tolerance test.

Methods

Study 1: Oral Fat Tolerance Test

Nine lean healthy men, 9 obese nondiabetic men with normal glucose tolerance as determined with a 75-g oral glucose tolerance test, and 12 male type 2 diabetic patients participated in the study...
TABLE 1. Characteristics of the Men Participating in the Oral Fat Tolerance Test

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lean (n=9)</th>
<th>Obese (n=9)</th>
<th>Type 2 Diabetic (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59±2</td>
<td>58±2</td>
<td>60±2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.2±0.4</td>
<td>29.4±0.5*</td>
<td>29.6±1.0*</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>17.5±1.4</td>
<td>27.8±1.3*</td>
<td>28.8±2.6*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.89±0.01</td>
<td>0.99±0.01*</td>
<td>1.03±0.02*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>5.8±0.1</td>
<td>5.6±0.1</td>
<td>9.2±0.9†</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.3±0.1</td>
<td>5.5±0.1</td>
<td>7.8±0.3†</td>
</tr>
<tr>
<td>Serum free insulin, mU/L</td>
<td>4±1</td>
<td>7±1*</td>
<td>16±3†</td>
</tr>
<tr>
<td>Serum apoB, mg/dL</td>
<td>95±5.1</td>
<td>91.2±5.0</td>
<td>81.2±4.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.6±0.3</td>
<td>5.7±0.2</td>
<td>4.9±0.3</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.66±0.08</td>
<td>1.24±0.09*</td>
<td>1.04±0.08*</td>
</tr>
<tr>
<td>Serum TG, mmol/L</td>
<td>0.85±0.09</td>
<td>1.74±0.3*</td>
<td>1.78±0.37*</td>
</tr>
<tr>
<td>Serum NEFAs, mmol/L</td>
<td>0.61±0.11</td>
<td>0.69±0.04</td>
<td>0.83±0.13</td>
</tr>
<tr>
<td>Plasma ASP, µg/L</td>
<td>237±9</td>
<td>404±65*</td>
<td>298±17*</td>
</tr>
<tr>
<td>AUC TG, mmol/L×min</td>
<td>596±59</td>
<td>1332±168*</td>
<td>1409±219*</td>
</tr>
<tr>
<td>Incr AUC TG, mmol/L×min</td>
<td>84±20</td>
<td>286±59*</td>
<td>340±69*</td>
</tr>
<tr>
<td>AUC NEFAs, mmol/L×min</td>
<td>381±29</td>
<td>578±21*</td>
<td>519±36*</td>
</tr>
<tr>
<td>Incr AUC NEFAs, mmol/L×min</td>
<td>13±49</td>
<td>162±25*</td>
<td>24±54†</td>
</tr>
<tr>
<td>TG max, mmol/L</td>
<td>1.63±0.3</td>
<td>3.21±0.3*</td>
<td>3.41±0.4*</td>
</tr>
</tbody>
</table>

TG indicates triglyceride; NEFA, nonesterified fatty acid; Incr, incremental; and AUC, area under the curve.

Significant differences (P<0.05) are *obese nondiabetic or type 2 diabetic vs lean men and †type 2 diabetic vs obese nondiabetic men.

The diabetic patients had a duration of diabetes of 12±2 years, and they were treated with diet alone (n=1), oral hypoglycemic agents (sulphonylurea, metformin; n=4), a combination of oral hypoglycemic agents and insulin (n=4), or insulin alone (n=3). One obese nondiabetic man and 2 type 2 diabetic patients were using lovastatin for treatment of hypercholesterolemia, which was discontinued for at least 4 weeks before the study. Subjects were asked to avoid strenuous exercise for at least 1 day before the studies and to maintain their normal weight-maintaining diet containing at least 200 to 250 g carbohydrates per day. None of the subjects had any intercurrent infection for at least 2 weeks before the study. All subjects gave their written, informed consent before participating in the study, which was approved by the Ethics Committee of the Helsinki University Central Hospital.

After an overnight fast (12 to 14 hours) and baseline blood sampling, the subjects received a fat meal consisting of 200 mL cream and an egg yolk, which contains 78 g fat and 760 kcal energy.12 Plasma ASP concentration was determined in blood samples at 0, 1, 2, 3, 4, 6, 8, and 10 hours after the fat meal by a radioimmunoassay equipped with a commercially available kit specific for C3adesArg (human complement C3adesArg, Amersham International).13 Insulin sensitivity was determined on a separate occasion by the euglycemic hyperinsulinemic clamp technique13 (insulin infusion rate of 72 mU/m² body surface area per minute for 240 minutes). Data on the insulin sensitivity of the subjects have been previously published.10 One type 2 diabetic man took part in the oral fat tolerance test but not in the clamp study.

**Study 2: Expression of Adipose Tissue C3 mRNA**

Abdominal subcutaneous adipose tissue biopsies14 were taken under local anesthesia (1% Lidocaine without epinephrine) by needle aspiration at the level of the umbilicus before and at the end of the 240-minute euglycemic hyperinsulinemic clamp in 6 of the lean (age 58±3 years, BMI 23.5±0.4 kg/m²) men, 6 of the obese nondiabetic (56±3 years, BMI 30.4±0.7 kg/m²) men, and 6 of the type 2 diabetic men (59±3 years, BMI 31.8±0.9 kg/m², HbA1c 8.4±0.4%). Biopsies were frozen in LN₂ and stored at −70°C until analyzed. During the last hour of the clamp, plasma glucose (5.5±0.1 vs 5.2±0.1 vs 5.3±0.1 mmol/L, P=0.104) and serum free insulin concentrations (121±5 vs 155±10 vs 140±16 mU/L, P=0.108) were not statistically different in lean nondiabetic, obese nondiabetic, and type 2 diabetic men, respectively. The rate of glucose infusion during the last hour of the clamp, corrected for changes in glucose pool size, was used as a measure of insulin sensitivity.10,13,14 and it was higher in lean nondiabetic (65.9±2.8 µmol·kg⁻¹·min⁻¹) than in obese nondiabetic (38.0±2.7 µmol·kg⁻¹·min⁻¹, P<0.01 vs lean) or in type 2 diabetic (15.8±3.0 µmol·kg⁻¹·min⁻¹, P<0.01 vs lean or obese nondiabetic men).

**RNA Preparation and Quantification of C3 mRNA**

Total RNA from adipose tissue (~100 to 200 mg frozen tissue) was obtained with the use of the RNeasy total RNA kit (Qia-gen).14,16 The yield of RNA from adipose tissue samples was ~1.7 µg/100 mg adipose tissue, and it was not different in biopsies taken before and after the clamp. Human C3 mRNA was quantified by reverse transcription–competitive polymerase chain reaction (RT–competitive PCR), which consists of the coamplification of target cDNA with known amounts of a specific DNA competitor molecule added in the same PCR tube. The construction of competitor, sequences of C3-specific sense and antisense primers, validation of the assay, and the conditions used in the RT–competitive PCR assay have been recently described elsewhere in detail.17 All RNA preparations and C3 mRNA determinations were performed in the INSERM U449 laboratory in Lyon, France.

**Other Determinations**

The estimation of body fat percentage was done by bioelectric impedance analyzer (Holtain Ltd). Circumference of the waist was measured to the nearest 0.5 cm midway between the lower rib margin and the iliac crest, and the circumference of the hip was measured at the level of the trochanters with the use of a soft measuring tape. Serum free insulin (Pharmacia) and serum leptin (Linco Research) concentrations were determined with commercial radioimmunoassays. Plasma glucose concentration was determined with the glucose oxidase method (Beckman glucose analyzer, Beck-
man Instruments). Serum apoB concentrations were determined with an immunoturbidimetric method (Orion Diagnostica) on a Cobas Mira analyzer. Serum free fatty acid concentrations were determined enzymatically (NEFA C test kit, Wako Chemicals GmbH). Serum total and HDL cholesterol and serum triglyceride concentrations were determined enzymatically as previously described.\(^{10,11}\) The areas under the serum triglyceride and free fatty acid concentration curves during the oral fat tolerance test were calculated according to the trapezoidal rule. Incremental areas under the serum triglyceride curve or free fatty acid curves were calculated in the same way after subtracting the fasting serum triglyceride or free fatty acid concentration from all subsequent time points. HbA1\(_c\) (reference range 4.0% to 6.0%) was determined by ion-exchange high-performance liquid chromatography. Serum C3 concentrations were determined by an automated (Hitachi 911) in-house immunoturbidimetric method with commercial rabbit anti-human C3c antibodies (Dako code Q368).

### Statistical Analysis

The results are given as mean±SEM. Wilcoxon’s test and the Kruskal-Wallis 1-way ANOVA, followed by pairwise comparisons with the Mann-Whitney U test when total ANOVA indicated a significant difference, were used in the comparisons between paired and grouped items, respectively. Correlation analysis was done with Spearman’s test. The plasma ASP, serum C3, and serum triglyceride responses over time were evaluated with Friedman’s test. \(P<0.05\) was considered statistically significant.

### Results

#### Serum C3 Response During the Oral Fat Tolerance Test

Because complement protein C3 is the precursor of ASP, we examined whether there were changes in its serum levels after the oral fat load. Serum samples for the determination of C3 protein were taken at times 0, 2, 4, 6, 8, and 10 hours. Serum C3 concentration did not change significantly after an oral fat load in lean \((P=0.14)\) or in type 2 diabetic men \((P=0.09,\) Friedman’s test). In obese nondiabetic men, C3 concentration was slightly higher and lower in comparison with fasting values at 2 and 6 hours after the fat load, respectively (Table 2).

#### Plasma ASP Response During the Oral Fat Tolerance Test

The fasting plasma ASP concentrations were not statistically different in obese nondiabetic \((404±65 \mu\text{g/L})\) and in type 2 diabetic \((298±17 \mu\text{g/L}, P=0.26)\) men, but they were slightly higher than in lean healthy men \((237±9 \mu\text{g/L}; P<0.005\) vs both groups; Table 1). In the obese nondiabetic men, 2 subjects had 2-fold higher plasma ASP values \((706 and 762 \mu\text{g/L},\) respectively) than the others. Without these 2 outliers, the plasma ASP concentration was \(310±24 \mu\text{g/L}\) in obese nondiabetic men \((n=7),\) and it was higher than in lean men \((P=0.005)\) but not different from that in type 2 diabetic men \((P=0.74)\). Despite significant changes in serum triglyceride concentrations after the oral fat load in all 3 groups \((P<0.001,\) Friedman’s test; please see http://atvb.ahajournals.org), plasma ASP concentrations did not change significantly in lean healthy men \((n=9, P=0.07),\) in obese nondiabetic \((n=9, P=0.30),\) or in type 2 diabetic men \((n=12, P=0.21,\) Friedman’s test; Figure 1). There was no significant change in plasma ASP levels after the oral fat load, even when data from all of the subjects were analyzed together \((n=30, P=0.51)\).

### Correlations With Fasting Plasma ASP in the Oral Fat Tolerance Test

There was no correlation between fasting plasma ASP concentration and the area under the serum triglyceride concentration curve in lean healthy men \((r=0.43, P=0.22),\) in obese nondiabetic \((r=−0.08, P=0.81),\) or in type 2 diabetic men \((r=−0.36, P=0.24).\) However, when all subjects were analyzed collectively \((n=30),\) there was a significant, positive correlation between fasting plasma ASP concentration and both the total and the incremental \((r=0.39, P<0.05\) for both) areas under the serum triglyceride concentration curve. In addition, fasting plasma ASP concentration was correlated with fasting \((r=0.43, P<0.05)\) and maximal \((r=0.39, P<0.05)\) serum triglyceride concentrations during the oral fat tolerance test, the area under the serum free fatty acid concentration curve \((r=0.37, P<0.05),\) BMI \((r=0.51, P<0.01),\) total fat mass \((r=0.51, P<0.01),\) and waist-to-hip ratio \((r=0.62, P<0.01).\) Moreover, fasting plasma ASP concentration was correlated negatively with the whole-body glucose disposal rate \((r=−0.58, P<0.01)\) and HDL cholesterol concentration \((r=−0.45, P<0.02).\) Fasting plasma ASP

### Table 2. Serum C3 Concentrations (During the Fat Tolerance Test)

<table>
<thead>
<tr>
<th>Time</th>
<th>Lean</th>
<th>Obese</th>
<th>Type 2 diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.20±0.05</td>
<td>1.29±0.05</td>
<td>1.30±0.06</td>
</tr>
<tr>
<td>2 h</td>
<td>1.19±0.05</td>
<td>1.32±0.05*</td>
<td>1.31±0.06</td>
</tr>
<tr>
<td>4 h</td>
<td>1.16±0.05</td>
<td>1.29±0.05</td>
<td>1.31±0.07</td>
</tr>
<tr>
<td>6 h</td>
<td>1.17±0.06</td>
<td>1.25±0.05*</td>
<td>1.30±0.07</td>
</tr>
<tr>
<td>8 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 h</td>
<td>1.16±0.05</td>
<td>1.28±0.05</td>
<td>1.32±0.07</td>
</tr>
</tbody>
</table>

*Significant difference from baseline.

There was no significant change in mean serum C3 concentration in lean men or in type 2 diabetic men. Values are in g/L.

### Figure 1

The plasma ASP response during the 10-hour oral fat tolerance test in lean (black circles) and obese nondiabetic (black boxes) and type 2 diabetic (open triangles) men. There was no significant change in plasma ASP levels in lean \((n=8, P=0.07),\) in obese nondiabetic \((n=9, P=0.30)\) or in type 2 diabetic \((n=12, P=0.21)\) men (Friedman’s test).
concentration was correlated with the fasting levels of its precursor protein complement C3 (r=0.58, P<0.01) and with fasting insulin concentration (r=0.52, P<0.01) but not with serum apoB concentration (r=-0.03).

When all nondiabetic subjects (n=18) were analyzed separately, the aforementioned correlations were stronger. Fasting plasma ASP concentration was correlated with the area under the serum triglyceride concentration curve (r=0.73, P<0.01), fasting (r=0.75, P<0.01) and maximal (r=0.64, P<0.01) serum triglyceride concentrations during the oral fat tolerance test, the area under the serum free fatty acid concentration curve (r=0.62, P<0.02), BMI (r=0.58, P<0.02), total fat mass (r=0.65, P<0.01), and the waist-to-hip ratio (r=0.69, P<0.01). In addition, fasting plasma ASP concentration was correlated negatively with the whole-body glucose disposal rate (r=-0.69, P<0.01) and HDL cholesterol concentration (r=-0.59, P<0.01). These correlations were not seen when type 2 diabetic subjects were analyzed separately.

Expression of C3 mRNA in Subcutaneous Adipose Tissue
The expression of subcutaneous adipose tissue C3 mRNA was higher in obese nondiabetic men (148±16 amol/μg total RNA, P<0.05, n=6) and type 2 diabetic men (124±18 amol/μg total RNA, P=0.08, n=6) than in lean nondiabetic men (64±16 amol/μg total RNA, n=6), and it was not different between obese nondiabetic and type 2 diabetic men (P=0.26). During a 240-minute physiological hyperinsulinemia, the expression of subcutaneous adipose tissue C3 mRNA did not change in any of the 3 groups (Figure 2).

Correlations With C3 mRNA Expression in the Clamp Study
When nondiabetic subjects (n=12) were analyzed together, the basal expression of subcutaneous adipose tissue C3 mRNA was correlated positively with BMI (r=0.72, P<0.02; Figure 3, upper panel) and negatively with whole-body glucose disposal rate (r=-0.79, P<0.01; Figure 3, lower panel). Subcutaneous adipose tissue C3 mRNA expression was also correlated with waist-to-hip ratio (r=0.70, P<0.05), fasting leptin (r=0.64, P<0.05), and HDL cholesterol concentrations (r=-0.70, P<0.05). These associations were not seen in type 2 diabetic men. Similarly, when both nondiabetic and type 2 diabetic men were analyzed collectively (n=18), none of the above-mentioned correlations reached statistical significance, with the exception of waist-to-hip ratio (r=0.53, P<0.05) and serum HDL cholesterol concentration (r=-0.60, P<0.02), which were significantly associated with C3 mRNA expression.

Correlations With C3 mRNA Expression and Postprandial Lipemia
ASP has been suggested to play a role in postprandial lipemia, and in the present study, we observed a positive correlation with the fasting plasma ASP concentration and the magnitude of serum triglyceride response after the oral fat load. Therefore, we next analyzed whether the subcutaneous adipose tissue expression of C3 mRNA was related to the magnitude of postprandial lipemia, which was measured on a separate occasion in the same subjects. The time interval between the oral fat tolerance test and the insulin clamp study with adipose tissue biopsies was 3±1 months. The body weight of the subjects was stable during this time, as reflected by its coefficient of variation of 1.2±0.2%.

In both nondiabetic and type 2 diabetic men (n=18), basal C3 mRNA expression in the clamp study was correlated positively with total serum triglyceride (r=0.52, P<0.05) and total serum free fatty acid (r=0.49, P<0.05) areas under the curve during the oral fat tolerance test. When nondiabetic men (n=12) were analyzed separately, these associations were stronger, with basal C3 mRNA expression in the clamp study correlating with both total (r=0.62, P<0.05) and incremental (r=0.73, P<0.02; Figure 4) triglyceride areas under the curve and with both total (r=0.63, P<0.05) and incremental (r=0.65, P<0.05) serum free fatty acid areas under the curve during the oral fat tolerance test. These associations were not significant in type 2 diabetic men.
Discussion

Fasting plasma ASP concentrations were slightly but significantly higher in obese groups than in lean subjects, and the plasma ASP concentration was correlated with adiposity, in agreement with previous results. Because ASP is produced by the interaction of the complement proteins C3, factor B, and adipin and because insulin increases C3 production in adipocytes, we also investigated subcutaneous adipose tissue expression of C3 mRNA and its regulation by use of a 240-minute insulin infusion. C3 mRNA expression was significantly higher in nondiabetic obese than in lean men and also tended to be higher in type 2 diabetic than in lean men. Thus, obesity is an important determinant of both plasma ASP concentration and subcutaneous adipose tissue C3 mRNA expression. In addition, short-term insulin infusion failed to regulate C3 mRNA expression in all 3 groups. We used whole adipose tissue samples to extract total RNA, and therefore, possible differences in the amount of nonadipose cells and adipocyte size were not corrected for. However, if one assumes that obese subjects had larger adipocytes, then the amount of C3 mRNA per cell would still be increased in obese subjects.

Plasma ASP concentration remained unchanged during the oral fat load, despite marked changes in serum triglyceride concentration. This finding is in agreement with the data of Charlesworth et al. However, sampling plasma from a peripheral vein may not necessarily reflect physiological changes in ASP concentration in the adipose tissue microenvironment, as there is an increase in the venoarterial gradient of ASP concentration across subcutaneous adipose tissue. We observed a significant, positive association between the magnitude of postprandial lipemia and both fasting plasma ASP concentration and subcutaneous adipose tissue C3 mRNA expression. Because ASP may be an important regulator of postprandial lipemia, an increase in basal plasma ASP concentration or an increase in subcutaneous adipose tissue C3 mRNA expression in the face of enhanced postprandial lipemia could be a compensatory phenomenon to a putative ASP resistance, analogous to the hyperinsulinemia in insulin-resistant states.

ASP increases glucose transport in both adipocytes and in the rat L6 muscle cell line, which suggests that ASP may regulate insulin sensitivity. In accordance with a negative correlation between serum C3 concentration and the whole-body glucose disposal rate, we observed that plasma ASP or subcutaneous adipose tissue C3 mRNA expression was correlated negatively with insulin sensitivity. This may partly be a function of obesity, because with increasing BMI there is an increase in plasma ASP or adipose tissue C3 mRNA and a decrease in the glucose disposal rate. In a previous study, no significant correlation between plasma ASP and glucose disposal rate was observed in Pima Indians. Although the putative role of ASP in the pathogenesis of insulin resistance remains to be determined, it has been suggested that reduced fatty acid trapping in the adipocytes due to a defect in the adipin/ASP pathway and the consequentially increased plasma free fatty acid levels could lead to insulin resistance via the Randle cycle.

Fasting plasma ASP concentrations and adipose tissue C3 mRNA expression were similar in type 2 diabetic and matched obese nondiabetic men but were higher than in lean men. In contrast to nondiabetic men, plasma ASP concentration or subcutaneous adipose tissue C3 mRNA expression was not correlated with postprandial triglyceride response or insulin sensitivity in the diabetic men. In the clamp study, this may have partly been due to the small number of diabetic subjects. However, when type 2 diabetic men were combined in statistical analysis, the correlation coefficients with plasma ASP or adipose tissue C3 mRNA and postprandial triglyceride response, as well as other metabolic parameters, were either markedly diminished or no longer significant. These data suggest that the diabetic state and/or the antihyperglycemic therapy of the patients may interfere with these associations.

In summary, our data show that both plasma ASP concentration and subcutaneous adipose tissue C3 mRNA expression are increased in obesity. Furthermore, there is no increase in plasma ASP levels in response to an oral fat load. There is, however, a positive correlation between the magnitude of postprandial lipemia and fasting plasma ASP levels or subcutaneous adipose tissue C3 mRNA expression. Adipose tissue C3 mRNA expression and plasma ASP concentrations are inversely associated with whole-body insulin sensitivity in nondiabetic men. These associations were not seen in type 2 diabetic men. Insulin does not acutely regulate the subcutaneous adipose tissue expression of C3 mRNA in vivo. Taken together, these data suggest that ASP is associated with whole-body glucose and lipid metabolism in nondiabetic men, whereas metabolic disturbances in diabetes may overcome the regulatory role of ASP in lipid and glucose metabolism.

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