Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein in C57BL/6 mice

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Murray, Ian, Allan D. Sniderman, and Katherine Cianflone. Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein in C57BL/6 mice. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E474–E480, 1999.—Acylation stimulating protein (ASP), a novel adipocyte-derived autocrine protein, stimulates triglyceride synthesis and glucose transport in vitro in human and murine adipocytes. In vitro, chylomicrons increase ASP and precursor complement C3 production in adipocytes. Furthermore, in vivo, ASP production from human adipose tissue correlates positively with triglyceride clearance postprandially. The aim of the present study was to determine if intraperitoneally injected ASP accelerated triglyceride clearance in vivo after a fat load in C57BL/6 mice. ASP increased the triglyceride clearance with a reduction of the triglyceride area under the curve over 6 h (AUC₁₋₆) from 102.6 ± 30.0 to 61.0 ± 14.5 mg·dl⁻¹·h⁻¹ (P < 0.05), especially in the latter postprandial period (AUC₃₋₆: 56.2 ± 18.0 vs. 24.9 ± 8.9 mg·dl⁻¹·h⁻¹, P < 0.025). ASP also reduced plasma glucose both in the mice with accelerated plasma triglyceride clearance and in those with relatively delayed triglyceride clearance (P < 0.025). Therefore, ASP alters postprandial triglyceride and glucose metabolism.

CLEARANCE AND STORAGE of postprandial dietary triglycerides are an essential part of energy homeostasis. Triglyceride (TG)-rich dietary lipoproteins (chylomicrons) are released into the peripheral blood circulation, and a large proportion of dietary TG within them is cleared by adipose tissue and muscle (5, 12). Chylomicron clearance is effected in a two-step pathway. First, in the interstitial space, lipoprotein lipase (LPL) hydrolyses the chylomicron TG-producing free fatty acids (FFA). Next, these FFA are taken up by muscle (utilization) or by adipocytes, where they are activated to form acyl-CoA and reesterified to be stored as TG in adipose tissue.

Adipose tissue is a metabolically active tissue that regulates its own metabolism via secretion of paracrine and autocrine factors and influences other tissues as well. Adipocytes produce many factors, such as apolipoprotein E (41), leptin (25), tumor necrosis factor-α (22), LPL (2), as well as complement C3, the precursor of acylation stimulating protein (ASP; Ref. 8). Adipocytes secrete C3, factor B, and adipsin (3, 6). C3a is a cleavage product of the amino-terminal α-chain of complement C3 and is formed through the enzymatic interaction of complement C3, factor B, and adipsin (factor D) (18, 23). C3a has a short half-life in plasma and rapid carboxypeptidase cleavage of the carboxy-terminal arginine of C3a produces ASP (C3a des Arg) (15).

ASP is produced by adipocytes and acts back on adipose tissue to stimulate glucose transport and TG synthesis. It therefore appears to act as an autocrine factor (7, 33). ASP has been shown to increase TG synthesis in vitro by increasing the maximum velocity of diacylglycerol acyltransferase (38). ASP also increases glucose transport in adipocytes (21), myotubes (34), and fibroblasts (13). In the latter two cell types, Western blot analysis demonstrated that glucose transport increases consequent to increased translocation of glucose transporters (GLUT-1, GLUT-3, and GLUT-4) to the cell surface (13, 34). Although long-term (24 h) incubation with ASP does not appear to increase total GLUT-1, GLUT-3, or GLUT-4 transporter mass, ASP does appear to augment the insulin effect on increased GLUT-3 and GLUT-4 mass in muscle (L6 myotubes) (34). The effects of ASP are more pronounced in differentiated adipocytes and myotubes than in undifferentiated cells (21, 34).

The importance of these effects is underscored by the conservation of function among species. Thus ASP has been shown to affect both TG synthesis and glucose transport not only in human adipocytes but also in primate and rodent adipocytes and myotubes (24, 34). In an in vivo human study, both adipose tissue ASP production and dietary TG clearance increased within the adipose tissue bed in the latter half of the postprandial period (29). This coordinated change suggests a specific physiological postprandial role of ASP because in vitro, ASP production from adipocytes is increased by specific chylomicron components (20, 31).

The use of murine models to elucidate in vivo metabolic pathways has increased over the past years. The murine strain C57BL/6, especially the leptin (ob/db) and leptin receptor (db/db) mutants, has commonly been used to determine lipid-related parameters (1, 14, 16, 36) as well as for oral fat loads (36). Because we have demonstrated that ASP increases TG formation in vitro in adipocytes and glucose transport in myotubes, we hypothesized that in vivo administration of ASP would accelerate TG clearance after a fat load in a murine model.

METHODS

Animals. The Royal Victoria Hospital Research Institute Animal Ethics Committee approved all procedures, which were in accord with the Canadian Animal Care Committee...
Guidelines. Twelve female C57Bl/6 NHsd mice were obtained from Harlan Sprague Dawley (Chicago, IL) and were fed a Purina chow 5075 diet. Mice were housed in sterile barrier facilities with controlled humidity, temperature, and light-dark cycles. Experiments were conducted on acclimatized mice at 14–16 wk of age with 2 wk between experiments to allow for restoration of blood volume.

Postprandial fat load on mice. Fat loads were performed on mice; one-half were injected intraperitoneally with human ASP and the other one-half with vehicle. Two weeks later, with a crossover design, the mice that received ASP at the time of the first fat load received vehicle and vice versa. After an overnight fast (16 h), 400 µl of olive oil (followed by 100 µl air) were given by gastric gavage with a feeding tube (1/2-cm curved ball tipped feeding needle no. 20) according to standard procedures published previously (19, 32, 36, 39) and the ASP intraperitoneal injection was performed immediately afterward. The mice were free running, with water ad libitum but no food. The unanesthetized mice were restrained in a plastic cylinder (modified 50-ml Falcon tube) for the duration of the bleed (2 min). The tail was cut with a scalp knife for the first bleed only. For repeated bleeds, the scab was removed and blood was collected by gently stroking the tail (lubricated with Vaseline). Blood samples (40 µl) were collected with heparinized capillary tubes by tail bleeding at 0, 1, 2, 3, 4, and 6 h into EDTA-containing tubes on ice (maximum 10% blood vol of the mouse for the total fat load). The plasma was separated by centrifugation within 30 min of collection at 12,000 g for 5 min and stored at –80°C. TG, glucose, FFA, and human ASP were measured in these samples as described in ASP injections and Assays.

ASP injections. ASP (8932.5 MW) was prepared as described previously (4), and its purity (99%) was ascertained by ion spray mass spectrophotometry (3). Each mouse received intraperitoneally either sterile ASP (500 µg) dissolved in PBS, pH 7.4, containing 1 mg/ml fatty acid free BSA (Sigma, St. Louis, MO) or sterile vehicle (same solution without ASP). Preliminary experiments demonstrated that injection of the vehicle solution (1 mg/ml BSA in PBS, 300 µl) had no effect of postprandial TG clearance in the mice compared with the same mice without vehicle. The 2-wk interval permitted restoration of blood volume. Mice were injected intraperitoneal (300 µl), immediately after receiving the oral fat load.

Assays. TG, FFA (Boehringer Mannheim, Laval, QC, Canada), and glucose (Trinder Kit, Sigma) were measured with commercial enzymatic colorimetric kits. ASP was measured by a sandwich ELISA as described previously (29). In all assays, each sample was measured in duplicate. The anti-human ASP murine monoclonal capture antibody does not cross-react with mouse ASP. Results at each time point during the postprandial period are expressed as means ± SE (mg/dl) of difference in TG levels from fasting (34.8 ± 2.1 mg/dl, n = 24) by subtracting basal value (time zero) from each time point. Comparisons of time points vs. fasting TG, *P < 0.01 and **P < 0.005, and between mice with ASP vs. without ASP were performed, #P < 0.025.

levels are shown as difference from basal (mg/dl) where average fasting plasma TG level was 34.8 ± 2.1 mg/dl (n = 24). TG of control mice (vehicle only) increased rapidly within 1 h, reaching a maximum at 4 h, and then returned toward baseline levels, similar to published studies (19, 32, 39). In separate studies, we verified that the TG profiles of the mice were not altered by the procedure of intraperitoneal injection (data not shown). At all time points postprandially, the plasma TG were significantly elevated compared with fasting TG levels (Fig. 1). With injection of ASP, there was no effect on the initial rise (1st h) in plasma TG, but the clearance from 2 h on was markedly altered. At 1 h, plasma TG was significantly elevated compared with fasting TG (P < 0.01). However, at all time points past 1 h, ASP effected a decrease in plasma TG such that the levels were no longer significantly different from fasting TG. The total TG AUC from 0 to 6 h (AUC0–6) was reduced from 102.6 ± 30.0 to 61.0 ± 14.5 mg·dl⁻¹·h (P < 0.05). Although the first half of the AUC0–3 (from 0 to 3 h postprandially) was not significantly different with ASP (46.4 ± 15.3 control vs. 36.1 ± 7.9 with ASP), there was a twofold reduction in the latter half of the postprandial period (AUC3–6 h 56.2 ± 18.0 control vs. 24.9 ± 8.9 mg·dl⁻¹·h with ASP, P < 0.025). The differences were due to the administration of ASP because basal lipemic response was the same whether the mice received vehicle first or second in the crossover design. Fat loads repeated in the same mouse differed

![Fig. 1. Change in plasma triglyceride (TG) in C57Bl/6 mice after injection with human acylation stimulating protein (ASP) after an oral fat load: an oral fat load was administered by gastric gavage as described in METHODS. Twelve C57Bl/6 mice were studied either with ASP or vehicle (1 mg/ml BSA in PBS). Results at each time point (30) by ion spray mass spectrophotometry (3). Each mouse received intraperitoneal injection immediately after receiving the oral fat load.](http://ajpendo.physiology.org/)

Downloaded from http://ajpendo.physiology.org/ by [10:20:33.2 on June 17, 2017](http://ajpendo.physiology.org/)
by only 20 ± 2.6% for AUC over 6 h (n = 6). As well, when a sham fat load was performed (saline only given), plasma TG, FFA, and glucose have small changes compared with fasting plasma levels over a 3-h time course (−6 ± 4, 19 ± 3, and −19 ± 3% change, respectively; n = 6 mice).

ASP entered the plasma compartment after its intraperitoneal administration (Fig. 2). There was no cross-reaction of the murine monoclonal anti-human ASP capture antibody with mouse ASP. ASP levels peaked rapidly at 1 h to 771 ± 78 nM and declined rapidly to baseline at 3 h with an average total AUC 0–6 of 1,091 ± 86 nM/h. The ASP peak preceded its effects on plasma TG, with TG changes beginning after 1 h.

As dietary plasma TG are hydrolyzed by LPL, FFA are generated. The plasma increase in FFA is shown in Fig. 3, again as difference from basal where the fasting FFA plasma concentration was 0.408 ± 0.036 mM (n = 24). As with TG, FFA increased rapidly by 1 h, corresponding to the rise in postprandial TG. Throughout the postprandial period (up to 6 h), the fasting FFA remained significantly elevated above fasting in the mice both with and without ASP injections. At 6 h, postprandial FFA was still significantly above baseline levels, with a significant difference between mice with ASP vs. without at 6 h (P < 0.05).

ASP has been shown in vitro to increase glucose transport, and in the present fat load studies we also measured plasma glucose. As shown in Fig. 4, postprandial glucose levels increased slightly but significantly from baseline, up to a maximum of 9% by 1 h, with basal glucose levels being 111 ± 4 mg/dl (n = 24). The glucose changes were similar to those of the TG curves.
Table 1. Fasting basal parameters in mice with normal postprandial lipemia and accelerated clearance

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<th>Group 1 (NOR)</th>
<th>Group 2 (ACC)</th>
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<tbody>
<tr>
<td>TG, mg/dl</td>
<td>32.8 ± 4.0</td>
<td>36.7 ± 1.2</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>119.8 ± 6.8</td>
<td>101.5 ± 3.3*</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.505 ± 0.055</td>
<td>0.311 ± 0.023†</td>
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Average ± SE basal fasting values (n = 6) of triglyceride (TG), glucose, and free fatty acid (FFA) measured twice for each mouse. NOR, normal postprandial lipemia; ACC, accelerated clearance. *P < 0.025; †P < 0.001.

ence between postprandial glucose and fasting levels. The drop in glucose from 2 to 6 h with ASP was significantly greater compared with mice without ASP (Fig. 4). The overall ASP effect on plasma glucose was a reduction in postprandial AUC0−6 from 67.6 ± 25.9 to −19.5 ± 46.7 mg·dl−1·h (P < 0.025). As with TG clearance, the greatest changes were noted in the second half of the postprandial period AUC3−6 (31.3 ± 18.3 vs. −26.9 ± 16.2 mg·dl−1·h, P < 0.01).

Overall, after the fat load in the control mice, plasma TG AUC0−6 increased by 83% over basal fasting TG. There was considerable interindividual variation of AUC0−6 within the mice, ranging from no change in postprandial TG to a 352% increase in postprandial TG compared with basal TG. We therefore separated the animals into two groups: normal postprandial lipemia (NOR; average 159 ± 52% increase in TG AUC0−6; n = 6; range 44–352%) and those with accelerated dietary TG clearance resulting in less pronounced postprandial TG levels (ACC; average 8 ± 6% change in TG AUC0−6; n = 6; range 26 to −18%). The fasting parameters of the 2 groups are shown in Table 1. Both fasting glucose and FFA levels were significantly lower in the ACC group than the normal NOR group and may be related to the specific breeding colony from which the animals were derived. Postprandial FFA followed a similar trend with higher increases in the NOR group than in the ACC group, although there was no effect of ASP (1.93 ± 0.50 vs. 2.30 ± 0.15 NOR and 0.011 ± 0.16 vs. 0.56 ± 0.16 ACC, without and with ASP, respectively, for AUC0−6. P = nonsignificant). There was, however, no difference in fasting TG between the two groups.

TG clearance of the NOR group vs. the ACC group was examined. As shown in Fig. 5 for the NOR group, plasma TG levels were significantly increased at all time points for these control mice (1 up to 6 h). However, in the presence of ASP, postprandial TG were only increased above baseline at 1 h. At all other time points, the TG were reduced so that there was no difference from fasting TG levels and there was a significant decrease compared with the same time point without ASP (P < 0.005 by 2-way repeated-measures ANOVA). On the other hand, in the ACC group, there was little postprandial change in TG, with a significant increase over fasting only at 1 and 2 h, and therefore ASP had little or no effect.

The ASP effect on glucose in both groups was also examined. The glucose profile in the absence of ASP was similar in both groups of mice (Fig. 6). In the absence of ASP, in NOR and ACC mice, postprandial glucose levels were consistently elevated throughout. Interestingly, in both groups, there was an equivalent effect of ASP on glucose lowering to below fasting levels such that with ASP there was no significant change from fasting glucose at any time point (Fig. 6). In the ACC group, although there was little postprandial change in TG and thus little effect of exogenous ASP, nonetheless there was still an effect of ASP on plasma glucose levels, independent of an effect on TG clearance. In addition, there was a strong negative correlation between total glucose AUC0−6 and ASP AUC0−6 (r = 0.71, P < 0.01).

DISCUSSION

The present study indicates clearly that exogenous administration of human ASP to normal wild-type C57Bl/6 mice altered postprandial metabolism. There were reductions in both postprandial TG and glucose, and these changes were greater in those animals where the postprandial lipemia was greater. The timing of the effects on TG and glucose lowering was consistent with the time lag of appearance of human ASP in plasma, where maximal ASP concentrations occurred at 1 h and...
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The lipid- and glucose-lowering effects occurred after this peak. This time course was consistent with the time delay in achieving ASP effects as shown in the in vitro cellular assays, where it took 30 min (13, 14, 35) to 1 h (21) to detect cellular changes in glucose transport or TG synthesis.

ASP may achieve these in vivo effects on lipid and glucose metabolism through multiple pathways. In cellular studies, we have shown that ASP increases fatty acid esterification in adipocytes to form storage TG through a direct effect on the lipid synthetic enzymes in this pathway. Although ASP also increased glucose transport, the TG esterification effect was independent of the glucose transport effect (13). Thus we propose that by increasing the amount of fatty acid that is taken up by cells and esterified, the local microenvironmental concentration of fatty acids is decreased, allowing more efficient hydrolysis of dietary TG by LPL, because LPL exhibits end-product inhibition by high FFA (26, 30). This hypothesis is consistent with the data obtained in cellular function assays. In fact, the increased plasma FFA concentrations at 6 h in those mice that received ASP would suggest that LPL exceeds the capacity of tissue fatty acid uptake, resulting in a buildup in circulating fatty acids. The lack of exogenously administered ASP at this time point may account for a reduced fatty acid uptake. However, we cannot rule out a direct effect of ASP on LPL activity or production by the adipocytes as well during the fast load. As the body switches from a fasted to a fed state, LPL activity increases in adipose tissue and decreases in muscle (11, 40), and FFA uptake changes (12). ASP may enhance this FFA partitioning to tissues.

The results also showed an effect of ASP on glucose levels. Although the meal itself was a high-fat meal with no carbohydrate, increases in plasma glucose may be due to fatty acid stimulation of hepatic gluconeogenesis (17). The glucose lowering occurred over the same time frame as the TG effects, and this could be a direct or an indirect effect of ASP. ASP has been shown to stimulate glucose transport directly in adipocytes in vitro through translocation of GLUT-1 and GLUT-4 from intracellular stores to plasma membrane (13, 21, 34), and this could enhance glucose clearance from the plasma. ASP could also indirectly mediate glucose uptake through its effects on fatty acid uptake and storage in adipocytes, which then results in reduced fatty acid inhibition of glucose metabolism in muscle (27, 28). However, even in the absence of an ASP effect on TG clearance (as shown by the low lipemia in 1 group of mice), there was still an effect on glucose clearance, albeit not as marked. Thus both of these explanations probably contribute to the ASP effects on plasma glucose.

We have focused on adipose tissue as the key target tissue for ASP. Certainly in in vivo cellular studies, the major effect of ASP on glucose transport and TG synthesis was found in adipocytes (7). However, administration of ASP may exert actions that increase TG clearance independent of its known effects on adipose tissue determined in vitro thus far. However, muscle and liver also play major roles in lipid and glucose metabolism in vivo. Whereas the liver is capable of taking up and esterifying fatty acids efficiently, ASP appears to have little effect on increasing TG synthesis in these cells. Only a 25% increase was obtained at high doses of ASP in human HepG2 cells (10) in contrast to the 2- to 10-fold increase in TG synthesis obtained in mature fat cells or cultured differentiated adipocytes (9, 35; for review see Ref. 7). We have also found a consistent effect of ASP on stimulation of glucose transport in muscle (cultured differentiated myotubes), which was effected through translocation of GLUT-1, GLUT-3, and GLUT-4 to the plasma membrane (34). Preliminary studies in humans suggest that there is no ASP production in muscle during fasting or postprandial states in arteriovenous studies (K. Cianflone and A. D. Sniderman, unpublished observations), in contrast to the adipose tissue ASP production found postprandially (29). As well, it is possible that the systemic administration of ASP in the present studies might induce responses not only in adipose tissue but also in muscle or other tissues.

In summary, these studies indicate that systemic administration of ASP reduced postprandial lipemia and postprandial glucose and that these effects are consistent with the in vitro cellular function data. Clearly, the exact mechanism and tissue-specific im-
pact need to be examined in much greater detail in future studies. However, the present data are the first in vivo data to demonstrate the potential physiological role that ASP may play in changing clearance of dietary fat.

We appreciate the excellent technical assistance of Steve Phelis in providing purified human ASP and the measurement of the injected human ASP in mouse plasma.

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