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PAPER

Acylation stimulating protein (ASP) acute effects on postprandial lipemia and food intake in rodents

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BACKGROUND: *In vitro* studies have shown that acylation stimulating protein (ASP) stimulates triglyceride (TG) synthesis and storage in adipocytes. We have previously demonstrated that intraperitoneal (i.p.) injection of ASP in C57BL/6J mice accelerated TG clearance following an orally-administered fat load as well as reducing postprandial glucose levels.
RESULTS: In the present study, we first examined the effect of i.p. and intracerebroventricular (i.c.v.) injection of ASP on food intake in Sprague–Dawley rats. Intraperitoneal injection resulted in a short-term increase in food intake (maximum increase 29.3% within the first hour, $P < 0.025$) decreasing thereafter as compared to vehicle alone. i.c.v. Administration of a comparable dose of ASP resulted in a similar but delayed increase in food intake with a maximum at 2–4 h, suggesting that the actions of ASP are peripherally mediated. However, there was no significant difference in 24 h food intake with either i.p. or i.c.v. injection. We also examined the effects of ASP on TG clearance in two obese mouse strains with different metabolic profiles: *ob/ob* (C57BL/6J-*Lep*^{ob}) and *db/db* (C57BLKS/J-*Lepr*^{db}). In a crossover design, the response to an oral fat load was determined with and without i.p. injection of exogenous ASP. In *ob/ob* mice, there was a 44% greater clearance of postprandial TG (area under the curve (AUC) $= 245 \pm 49$ control *vs* 138 ± 43 mg/dl h with ASP; $P < 0.05$ by RM ANOVA). The *db/db* mice showed a greater response, with a 62% decrease in postprandial TG (AUC $= 4080 \pm 1489$ control *vs* 1540 ± 719 mg/dl h with ASP; $P = 0.004$ by RM ANOVA). In addition there were decreases in postprandial glucose and non-esterified fatty acid (NEFA) levels in response to ASP.
CONCLUSION: These results are the first to report that ASP can increase food intake in rats and also enhance postprandial TG clearance in obese animals. These data therefore support previous *in vitro* evidence pointing to ASP as a regulator of lipid metabolism.
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Keywords: complement C3a; adipose tissue; non-esterified fatty acid; glucose; triglyceride; acylation stimulating protein

Introduction

There are many factors which regulate food intake, including glucocorticoids, growth hormone, catecholamines, numerous gastrointestinal factors and others.[1–3] Relatively few of these have been shown to have a direct effect on nutrient partitioning of ingested food in tissues, in particular triglyceride (TG) storage in adipose tissue.[4] Insulin and leptin are examples of two such metabolic hormones that have received wide attention for their roles in these processes.

Insulin is a potent metabolic hormone, which increases glucose transport, decreases hormone sensitive lipase activity and increases fatty acid incorporation into adipose tissue.[4] Insulin is transported into the central nervous system where it acts as a long-term regulator of energy balance and adiposity via its central actions to inhibit food intake.[5–7] Animals with genetically induced deficits in insulin signalling in the central nervous system exhibit increased food intake and adiposity.[8] Circulating insulin concentrations increase in response to dietary influxes of glucose and protein.[3,9] Circulating insulin concentrations are often increased in obesity as a consequence of insulin resistance.

Leptin is an adipocyte-derived hormone whose circulating levels closely relate to adipose tissue mass and recent energy

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intake.[10] Leptin is most well known for its central actions in the brain where, like insulin, it acts potently to inhibit food intake and increase energy expenditure. However, leptin also has direct peripheral actions on adipose tissue to increase lipolysis,[11,12] oxidation,[13] and inhibit insulin mediated stimulation of glucose transport, lipogenesis, fatty acid synthase expression and insulin binding,[13–17] although not all studies have reported such effects.[18–20]

Acylation stimulating protein (ASP) is a recently described metabolic factor, which like insulin and leptin has metabolic effects on adipocytes. Like leptin, ASP is produced by adipocytes and like insulin, ASP demonstrates anabolic effects on glucose and fat storage.[review21] ASP is produced through the interaction of complement C3, factor B and adipsin, all three of which are produced by adipocytes. C3, the precursor to ASP, binds factor B, generating an active site for the enzymatic action of adipsin, which ultimately produces C3a. Carboxypeptidase removes the carboxyl terminal arginine of C3a to generate ASP (C3adesArg).

Evidence suggests that there is a specific saturable receptor for ASP, which is highly expressed in adipocytes.[22] Interaction of ASP with adipocytes generates a metabolic cascade similar to that of insulin: ASP increases glucose transport, increases fatty acid incorporation into adipose tissue and inhibits hormone sensitive lipase.[21,23] However the signal transduction pathway of ASP would appear to be different from insulin, although the effects are additive to those of insulin.[21,23–25] As with insulin and leptin, circulating ASP increases in obesity.[26] Within the adipose tissue bed (as determined by arterial–venous adipose tissue gradient studies) there is an increased production of ASP postprandially with a maximum at 3–5 h; however the levels in the general circulation demonstrate little change.[9,27] This is later than the peak circulating insulin, but occurs at a time when LPL mediated TG hydrolysis is maximal and is consistent with the *in vitro* effect of chylomicrons on ASP production in human adipocytes.[28,29]

Acute *in vivo* intraperitoneal (i.p.) ASP administration in C57BL/6J mice demonstrated that ASP accelerated postprandial TG and non-esterified fatty acid (NEFA) clearance following a fat load.[30] ASP also had an effect on plasma glucose, enhancing the return to basal levels over the 6 h time course. On the other hand, C3 knockout mice (which are ASP deficient) demonstrated delayed TG clearance following a fat load,[31,32] although this was not confirmed elsewhere.[33] The mice also have moderate reductions in adipose tissue mass,[32,34] but normal fat absorption,[34] consistent with the proposed role of ASP in enhancing dietary storage mechanisms.[32]

The aim of the present study was to further examine the acute effects of ASP. Since both leptin and insulin have central effects on feeding behaviour, in addition to their peripheral actions on adipocyte metabolism, we wondered whether ASP might also exert an effect on food intake. It has been demonstrated that adipsin, factor B and C3 are synthesized in brain cells (astrocytes),[35] C3adesArg (ASP) is present in cerebrospinal fluid at concentrations approximately 15 times lower than plasma,[36] and intra-cerebroventricular (i.c.v.) injection of C3a increased the feeding response to norepinephrine in rats.[37] We first examined the effects of i.p. and i.c.v. injections of ASP on food intake in rats. Second, we also examined the acute postprandial response to ASP in two obese mice models, *ob*/*ob* (C57BL/6J-*Lep*^{ob}) and *db*/*db* (C57BLKS/J-*Lepr*^{ob}), which both present with insulin abnormalities and non-functional leptin pathways. A fat load was used, rather than a mixed meal, in order to minimize insulin changes and examine only the effects of ASP.

Methods

Surgical implantation of i.c.v. cannulas in rats

Adult, male Sprague–Dawley rats (220–250 g) were obtained from Simonsen Laboratories (Gilroy, CA). Animal care was according to the National Institutes of Health guidelines and the animal protocols were approved by the University of California, Davis Animal Use and Care Committee. Rats were surgically implanted with cannulas directed towards the lateral ventricle as previously described.[38] In brief, animals were anaesthetized with a ketamine cocktail as described previously[39] and placed in a stereotoxic apparatus. Unilateral stainless steel guide cannulas were inserted using the coordinates of Paxinos and Watson[40] and fastened to the skull with dental acrylic. Penicillin (60 000 units i.m.) was administered at the completion of surgery. Following implantation, rats were fed a moistened 20% casein protein diet for at least 8 days prior to the i.c.v. injections. During this time, they were adapted to handling and to a 1 h period of fasting prior to the onset of the dark cycle. To verify cannula placement, angiotensin II was administered 3–4 days prior to (100 ng/5 μl) and 3–4 days following (100 ng/15 μl) the test (control and ASP) injections.[41] Results from animals not drinking greater than 5 ml during the 30 min angiotensin II test were excluded from further analysis. At the completion of the experiments, the animals were sacrificed, the brain fixed and sectioned. Animals with injection sites outside the lateral ventricle were excluded from the analysis.

i.c.v. and i.p. injections and food intake in rats

To determine baseline food intake, food bowls were weighed (±0.1 g) during two consecutive days prior to the i.c.v. injections. For the i.c.v. injection experiments, each animal received injections of 15 μl of aCSF (artificial cerebrospinal fluid) or ASP (5 or 25 μg in 15 μl of aCSF) in random order. Injections were made by lowering the 33 gauge injector, connected to a microinjection pump (CMA/100, Bioanalytical Systems, West LaFayette, IN) into the lateral ventricle. The infusion rate was 3.75 μl/min over 4 min administered 90 min prior to the start of the dark cycle. The injection needle was left in place for 60 s before being removed. There was a 2–3 day washout period between each injection of ASP. For i.p. administration, animals were given an injection

of 48 μg ASP/kg body weight or saline (1 μl/g body weight, average body weight 313 g). Using a crossover design, rats were injected i.p. with ASP or vehicle 30–90 min prior to the initiation of the dark cycle, food intake was measured over specific time intervals and the cumulative food intake calculated. Differences (with *vs* without ASP) were calculated for each animal for each time interval. The same group of animals was used for i.c.v. and i.p. injections.

Baseline characterization of mice

ob/*ob* (C57BL/6J-*Lep*^{ob}) and *db*/*db* (C57BLKS/J-*Lepr*^{db}) male and female mice were obtained from Jackson Laboratories, Bar Harbour, Maine. In the studies performed, no difference was seen in male *vs* female mice, and the data are presented as pooled data. The mice were weighed and a fasting blood sample (150 μl) was obtained by tail bleeding. Plasma NEFA and TG were measured using colorimetric enzymatic kits (Boehringer Mannheim, Laval, Quebec, Canada). Fasting insulin was measured using a rat insulin RIA kit with rat insulin standards which crossreact with mouse insulin as indicated by the supplier (Linco Research Inc., St Charles, MO). Fasting plasma glucose was measured using a Trinder glucose kit (Sigma, St Louis, MO). All animal studies were approved by the Animal Care Committee at the Royal Victoria Hospital/McGill University and were in accordance with standard procedures.

Postprandial fat load on mice

An oral fat load was administered by intragastric feeding to *ob*/*ob* and *db*/*db* mice (male and female), 11–14 weeks old, as described previously[30–32] according to published methods.[42–44] Following an overnight fast, 400 μl of olive oil (followed by 100 μl air) were given based on 10 μl/g mouse weight.[42–44] Half the mice received an i.p. injection of human ASP (500 μg) in phosphate buffered saline (PBS, pH 7.4) containing 1 mg/ml bovine serum albumin (BSA) in a maximum volume of 300 μl (the other half were sham injected with placebo—buffer solution containing BSA but no ASP). Two weeks later the procedure was repeated with the other half of the mice receiving human ASP. Human ASP is very similar to mouse ASP[45] and has been shown to have equal bioactivity on human and mouse cells *in vitro*.[46] Blood samples (40 μl) were collected by tail bleeding at 0, 1, 2, 3, 4 and 6 h into EDTA-containing tubes and centrifuged. Plasma isolated was used to measure TG, NEFA, glucose (as above) and plasma human ASP.

ASP purification and quantitation

Human plasma ASP was prepared as previously described in detail.[47] Following the last step of purification on an HPLC Vydac C4 column (1.0×25 cm, Separation Group, Hesperia, CA), the fractions containing ASP were pooled and aliquoted in siliconized microtubes (Diamed, Mississauga, Ontario, Canada) and the solvent was evaporated in a vacuum centrifuge. Stock solutions of fatty acid free BSA (10 mg/ml) and 10× stock of PBS were added to the vials prior to evaporation of the solvent and the ASP/BSA/PBS was reconstituted in sterile water to yield a final concentration of ASP from 0.5–1.0 mg/ml in 1 mg/ml BSA in PBS and stored at −80°C. For i.c.v. injections, ASP was reconstituted in aCSF solution. Repeated freezing and thawing was avoided since this inactivates ASP. As well, it is essential to store ASP in siliconized tubes since it sticks easily to glass and plastic, a common feature of small basic proteins. The concentration of human ASP in mouse plasma was determined by a polyclonal-monoclonal sandwich enzyme linked immunosorbent assay (ELISA) specific for human ASP.[9]

Statistics

Results are expressed as average ± standard error of the mean (s.e.m.). Results of the curves are compared by two-way repeated measures ANOVA (RM ANOVA) with Bonferroni post-test to examine the differences at each time point. As well, the area under the postprandial curve (AUC) was determined using a linear trapezoidal equation (Sigma Stat, Jandel Scientific, San Rafael, CA) and results with *vs* without ASP administration were compared by paired *t*-test, where NS = not significant.

Results

Effect of acute ASP on food intake in rodents

The results for i.p. injection of ASP are shown in Figure 1. Following ASP injection, there was an acute increase (average peak increase = 29.3%, $P < 0.025$) in cumulative food intake after 1 h of feeding which decreased gradually thereafter (no

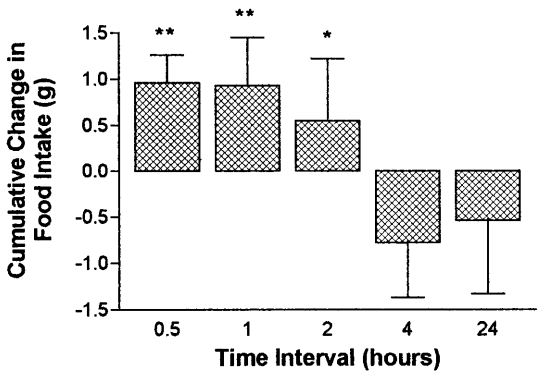

Figure 1 Effect of i.p. administration of ASP on cumulative food intake in rats. ASP (15 μg) or vehicle was injected i.p. prior to initiation of the dark cycle (time 0). Cumulative food intake was then measured at the indicated times. Results are presented as the difference in food intake for ASP *vs* vehicle alone as average ± s.e.m. for $n = 15$ rats, where $^*P < 0.05$ and $^{**}P < 0.025$.

overall difference by 4 h). To determine if this was due primarily to a peripheral *vs* central effect, ASP was also administered i.c.v. Food intake in rats was measured following i.c.v. injection of vehicle and 5 μg ASP given in random order. The results are shown in Figure 2. Although the magnitude of the effect obtained by i.c.v. administration was comparable to the i.p. ASP effect (maximum 36.7%, *P* NS *vs* i.p.), the time that it took to increase food intake and i.c.v., administration was considerably longer (2–4 h post dark cycle initiation for i.c.v. injection *vs* 0.5–1 h i.p. for maximal effect), suggesting that the effect of ASP to increase food intake is mediated primarily through a peripheral mechanism. Increased i.c.v. dose (25 μg) tested on a subset ($n=9$) of rats (given in random order) did not produce any additional effect (maximum 22.4% *P* NS for i.c.v. 25 *vs* i.c.v. 5 by two-way RM ANOVA).

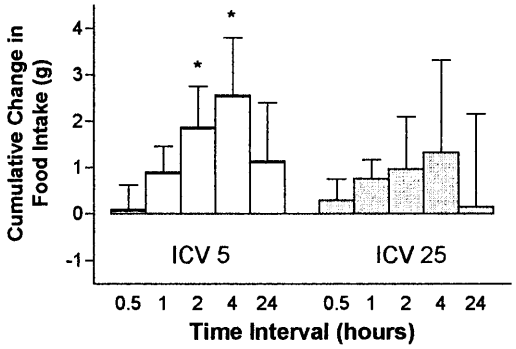

Figure 2 Effect of i.c.v. administration of ASP on cumulative food intake in rats. ASP (5 or 25 μg) or vehicle was injected i.c.v. prior to initiation of the dark cycle (time 0). Cumulative food intake was then measured at the indicated times. Results are presented as the difference in food intake for ASP *vs* vehicle alone as average ± s.e.m. for $n=13$ (5 μg dose) and $n=9$ (25 μg dose) rats, where $^*P<0.025$.

Effect of acute i.p. ASP on postprandial lipemia in obese ob/ob (C57BL/6J-Lep^{ob}) and db/db (C57BLKS/J-Lepr^{db})

Basal plasma lipids (TG, cholesterol and NEFA), glucose and insulin concentrations of both *ob/ob* and *db/db* mice are shown in Table 1. Compared to C57BL/6J (B6 control) mice, the *db/db* mice were hypertriglyceridaemic and hyperglycaemic while plasma total cholesterol and insulin levels were similar to B6 controls. *Ob/ob* mice on the other hand had higher glucose, total cholesterol, HDL-cholesterol and insulin compared to B6 controls.[30,48,49] Figure 3, left panel, shows the plasma postprandial increase in TG from 0 to 6 h following the fat load in the *ob/ob* mice. Postprandial TG levels increased significantly above basal by 1 h postprandially ($P<0.001$ *vs* fasting TG) reaching two-fold over baseline at 2 h (106 ± 17 mg/dl, $P<0.01$ *vs* fasting TG). Plasma TG gradually returned towards fasting levels, although they were still above baseline at the end of the 6 h postprandial period.

Table 1 Plasma parameters in *ob/ob* (C57BL/6J-Lep^{ob}) and *db/db* (C57BLKS/J-Lepr^{db}) obese mice

Plasma parameters	*ob/ob* (n = 8)	*db/db* (n = 9)	*B6 control*[a] (n = 12)
Weight (g)	51.5 ± 1.0	41.2 ± 1.5	23–25
Triglycerides (mg/dl)	44.4 ± 1.7	93 ± 5.9[a]	34.8 ± 2.1
Glucose (mg/dl)	190 ± 27.7[a]	471 ± 34.6[a]	111 ± 4
Total cholesterol (mg/dl)	203.3 ± 6.9	128 ± 5.3	ND
HDL cholesterol (mg/dl)	165.3 ± 5.3	104.3 ± 6.3	ND
LDL cholesterol (mg/dl)	37.97 ± 2.6	23.7 ± 4.2	ND
NEFA (mM)	0.47 ± 0.05	0.66 ± 0.07	0.41 ± 0.04
Insulin (ng/ml)	1.49 ± 0.19	0.46 ± 0.05	ND

Fasting plasma parameters as well as body weight are given for B6 control, *ob/ob* and *db/db* as average ± s.e.m. where ND = not determined. Groups are compared by ANOVA where $^*P<0.05$ *vs* B6 control mice.
[a]As published previously.[30]

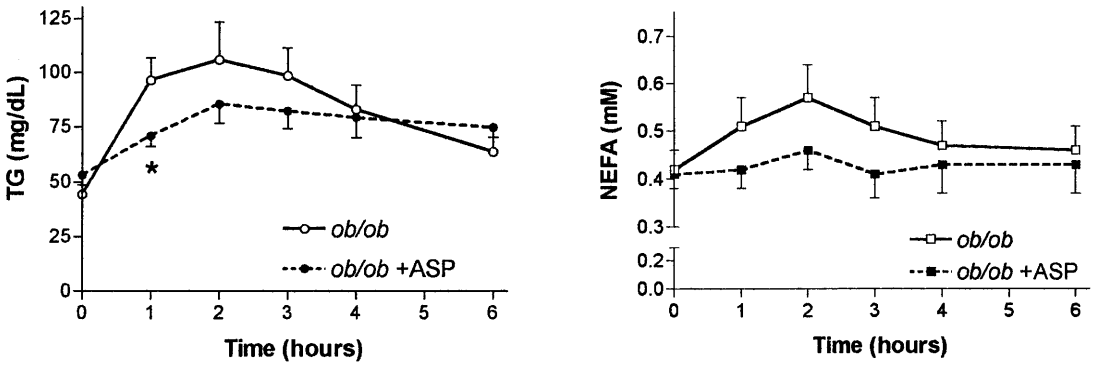

Figure 3 Postprandial TG and NEFA in *ob/ob* (C57BL/6J-Lep^{ob}) mice. A fat load was administered at time zero with or without i.p. injection of ASP (as indicated). Plasma triglycerides (TG) (left panel) and non-esterified fatty acids (NEFA) (right panel) were measured serially. Results are presented as average ± s.e.m. for eight mice. Results were analyzed by RM ANOVA with Bonferroni *post-hoc* test for differences at specific time points with *vs* without ASP where: TG, $P<0.0001$ time, $P<0.05$ group; NEFA, *P* NS time, $P<0.02$ group and $^*P<0.05$ for individual time points with *vs* without ASP.

With ASP injection (given at time 0), a decrease in circulating TG levels was seen, especially at the first hour postprandially ($P<0.05$ *vs* non-ASP control by RM ANOVA). Overall there was a 44% decrease in AUC in the presence of ASP, a decrease which is comparable to the B6 control mice.[30] Figure 3, right panel, shows the changes in NEFA levels in *ob*/*ob* over the same postprandial period in mice. A significant increase in NEFA was seen 2 h postprandially and the overall profile was similar to that seen in B6 control mice.[30] Generally, there was a trend towards lower plasma NEFA following ASP injection compared to *ob*/*ob* control ($P<0.02$ by RM ANOVA).

Following the fat load, TG levels increased significantly in *db*/*db* mice (Figure 4, left panel) with a 10-fold increase over basal by 3 h ($P<0.02$ *vs* fasting TG). Very high TG levels persisted over the whole postprandial period and at 6 h were still 10-fold above basal ($P<0.001$ *vs* fasting TG). i.p. injection of ASP produced a much more profound effect in the *db*/*db* mice compared to the *ob*/*ob* mice. There was a substantial decrease in plasma TG levels over the time course of the postprandial period ($P=0.004$ by RM ANOVA, $P<0.05$ at 2, 3, 4 and 6 h *vs* non-ASP control). Moreover, the AUC was reduced by 62% with ASP. In the mice injected with exogenous ASP, the TG levels were close to baseline levels by 6 h postprandially. Figure 4, right panel, shows the postprandial changes in NEFA in the *db*/*db* mice. There was a significant increase in NEFA by 2 h after the fat load in the control mice ($P<0.01$ *vs* fasting NEFA). This increase was sustained over the course of the postprandial period (as with TG). Injection of ASP resulted in small but not significant change in NEFA.

Plasma levels of exogenous ASP

Following i.p. administration of ASP, a marked surge of exogenous human ASP was seen in the plasma of both *ob*/*ob* and *db*/*db* mice during the first hour (Figure 5). The levels remained elevated and then gradually decreased over the time course of the fat load and were cleared from the plasma by 6 h postprandially. Thus bioactive ASP was present at comparable levels in both *ob*/*ob* and *db*/*db* mice, although the effects on TG clearance appeared to be more pronounced in the *db*/*db* (Figures 3 and 4).

Effect of ASP on postprandial glucose levels in both ob/ob and db/db mouse models

By one hour postprandially, glucose levels increased maximally in both *ob*/*ob* and *db*/*db* mouse models (329 ± 32 and 1108 ± 69 mg/dl, respectively, Figure 6). *Ob*/*ob* mice had a 65% increase in glucose by 1 h and the levels returned to baseline by 3 h (Figure 6, top panel). ASP consistently reduced the glucose level (-41% AUC, $P=0.009$ by RM ANOVA with *vs* without ASP). Fasting glucose levels were substantially higher in *db*/*db* mice (Table 1). The increase at 1 h was much greater ($P<0.0001$ *vs* fasting glucose) and had not returned to baseline levels by the end of the postprandial period (Figure 6). Exogenous ASP induced a decrease compared to control levels only at 4 h ($P<0.05$ *vs* non-ASP control) and 6 h ($P<0.01$ *vs* non-ASP control) in the *db*/*db* mice (Figure 6, bottom panel). Thus the return to fasting glucose levels was delayed in the *db*/*db* mice as compared to *ob*/*ob* mice and B6 control mice,[30] where the drop occurred earlier (by 1 h) and was sustained over the whole postprandial period.

Discussion

In the present study, we have demonstrated that i.p. administration of ASP has effects on energy intake by increasing short-term acute feeding in rats and enhancing dietary TG

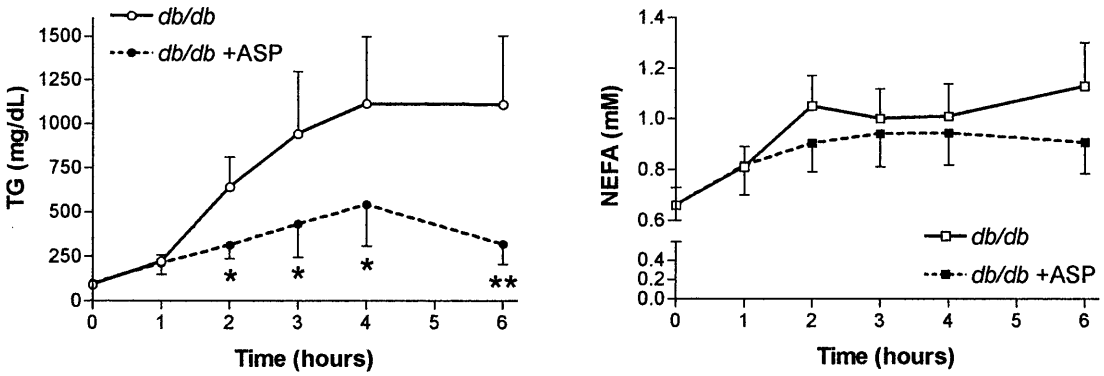

Figure 4 Postprandial TG and NEFA in *db*/*db* (C57BLKS/J-*Lepr*^{db}) mice. A fat load was administered at time zero with or without i.p. injection of ASP (as indicated). Plasma triglycerides (TG) (left panel) and non-esterified fatty acids (NEFA) (right panel) were measured serially. Results are presented as average $\pm$ s.e.m. for nine mice. Results were analysed by RM ANOVA with Bonferroni *post-hoc* test for differences at specific time points with *vs* without ASP where: TG, $P<0.005$ time, $P=0.004$ group; NEFA, $P<0.05$ time, P NS group and $^*P<0.05$, $^{**}P<0.025$ for individual time points with *vs* without ASP.

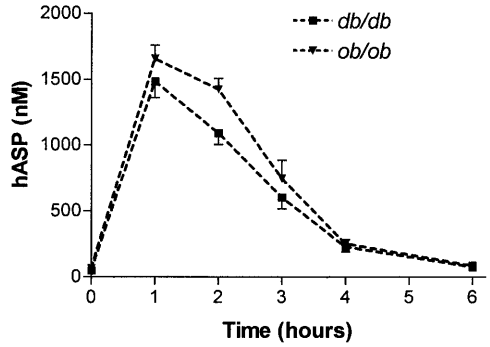

Figure 5 Postprandial human ASP in *ob*/*ob* and *db*/*db* mice. *ob*/*ob* and *db*/*db* mice (as described in Figures 1 and 2) were injected i.p. with ASP at time 0 in eight *ob*/*ob* (C57BL/6J-*Lep*^{ob}) and nine *db*/*db* (C57BLKS/J-*Lepr*^{db}) mice. Human ASP levels in the mouse plasma were then measured serially postprandially as shown (average ± s.e.m.).

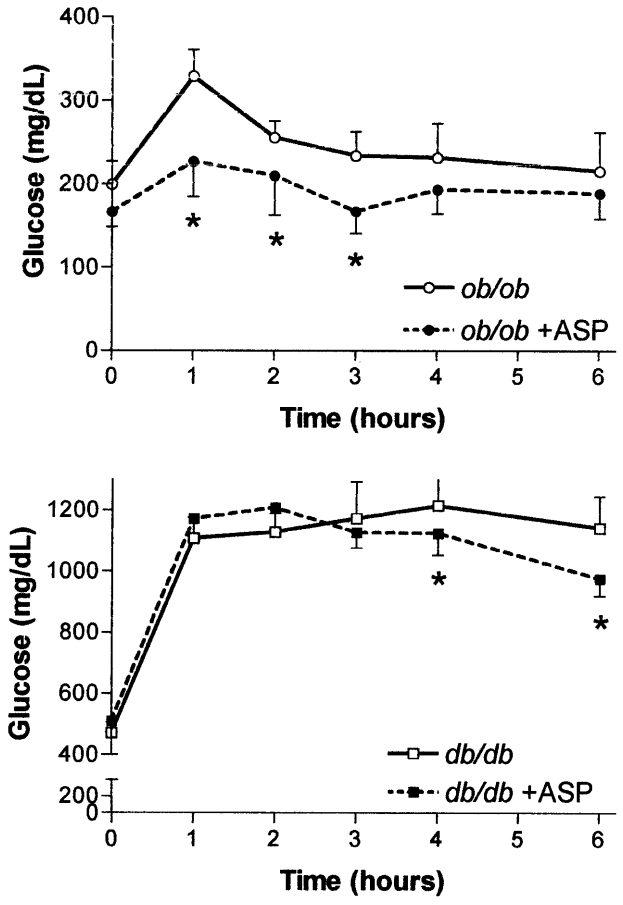

Figure 6 Postprandial glucose in *ob*/*ob* and *db*/*db* mice. A fat load was administered at time zero with or without i.p. injection of ASP (as indicated). Plasma glucose was then measured serially. Results are presented as average ± s.e.m. for eight *ob*/*ob* and nine *db*/*db* mice. Results were analyzed by RM ANOVA with Bonferroni *post-hoc* test for differences at specific time points with *vs* without ASP where: *ob*/*ob*, $P < 0.05$ time, $P = 0.009$ group; *db*/*db* $P < 0.0001$ time, P NS group and $^*P < 0.05$ for individual time points with *vs* without ASP.

and glucose disappearance after an oral fat load in two obese mouse models: *ob*/*ob* which are obese but normotriglyceridemic and *db*/*db* which are both obese and diabetic. This study both extends and complements our previous studies which demonstrated an effect of ASP on postprandial TG lowering in both C57BL/6J mice and ASP deficient mice and delayed TG clearance in ASP deficient (C3 knockout).[30–32]

There is already a long list of factors which have been demonstrated to modulate food intake when administered peripherally. They include members of the gastrointestinal hormone family (CCK, bombesin, gastrin releasing peptide, glucagon-like peptide-1 as well as others), pancreatic polypeptides (insulin, glucagon, peptide YY) as well as other circulating factors (leptin, opioids).[3] Despite evidence that administration of these peptides influences feeding behaviour, it is still unclear whether many of these peptides act via a central or peripheral mechanism. The data presented here suggest that ASP increases food intake acutely, but that food intake equilibrates overall within a 24 h period. This is a similar pattern to CCK where acute administration inhibits food intake in the short-term, however repeated injections result in smaller but more frequent meals without a change in total daily caloric input and body weight.[1,2] The time course of the effect also suggests that ASP is acting peripherally but not centrally, since a more rapid response is observed when ASP is administered i.p. By contrast, leptin action is much more potent when administered centrally than peripherally.[10]

ASP appears to attenuate the increase in glucose seen after a meal in *db*/*db* and *ob*/*ob* mice (as presented here) and also in B6 control mice.[30] Campfield *et al* have demonstrated that small transient decreases in glucose precede spontaneous meal intake and blocking these decreases can prevent meal initiation.[1,2] Thus the effect of ASP administration is consistent with a short-term increase in food intake induced by small (10%) decreases in plasma glucose levels and follows a similar temporal profile to the changes in plasma glucose (B6 control mice[30] and *ob*/*ob* mice). In the *db*/*db* mice, glucose levels are markedly increased due to increased hepatic gluconeogenesis which is further stimulated by the fatty acid intake[50–52] and ASP has less of an effect.

This acute effect of ASP (on food intake) would appear to contrast with ASP deficient (C3 knockout) mice which have slightly increased food intake.[32] They also manifest reduced adipose tissue mass but normal fat absorption.[34] On the surface, these two effects might appear to be contradictory. However, both effects on food intake are probably indirect, and can be explained more clearly by examining the direct ASP target: dietary substrate utilization. Thus in the first instance, with increased ASP, an ASP effect on tissue fatty acid and glucose uptake reduces circulating glucose and increases food intake. In the ASP deficient mice, a lack of ASP reduces dietary fat storage, adipose tissue mass decreases, plasma leptin decreases, plasma insulin is lower and the mice consequently eat more to re-establish metabolic balance and adipose tissue mass. Thus the different effects on food intake

are indirect and mediated through different metabolic effects.

In *db*/*db* mice, plasma TG and cholesterol increase early in development and are not simply secondary to the obesity which develops.[53] Studies by Li *et al* have demonstrated that the increase in plasma TG is not due to increased hepatic lipogenesis or apolipoprotein B secretion, but decreased plasma TG clearance.[54] This might be explained by a decrease in LPL activity, but there is no published data on adipose tissue or muscle LPL or postprandial TG lipemia in *db*/*db* mice. Nonetheless, as there are many published reports of disordered glucose metabolism and insulin resistance in *db*/*db* mice, presumably this would apply to the insulin-mediated stimulation of LPL mass and activity as well.

One of the features of the ASP induced drop in postprandial lipemia in this study is that the magnitude of the ASP effect is proportional to the postprandial lipemia. Thus, those animals which demonstrate the greatest postprandial lipemia; *db*/*db* mice and ASP-deficient (C3 knockout) mice[31,32] also demonstrated the greatest response to i.p. ASP. On the other hand, in those mice with moderate postprandial lipemia such as *ob*/*ob* and B6 control mice,[30] the overall effect of ASP was moderate. Although we have not measured murine ASP directly in these mice, there is evidence that adipsin, an adipocyte specific enzyme, is markedly reduced in *db*/*db* mice.[55] As adipsin is obligatory to generating ASP via the alternate complement pathway interaction of C3, factor B and adipsin, ASP levels might be expected to be low as well. Thus, in the *db*/*db* mice, and ASP-deficient (C3 knockout) mice the potent effect of administered ASP may be a result of low or no endogenous murine ASP.

What is the potential mechanism of action of ASP, especially in the *db*/*db* mice which have such a proportionally greater response to ASP? First, ASP increases intracellular esterification (and thus uptake) of NEFA, as shown in cultured adipocytes.[4,21] Secondly, ASP may enhance TG clearance by preventing NEFA inhibition of LPL through its tissue NEFA sequestration action. Both *in vitro* and *in vivo* studies have demonstrated an inhibitory and destabilizing effect of NEFA on LPL.[56–60] Finally, ASP also reduces plasma NEFA through inhibition of adipocyte hormone sensitive lipase.[23] All of these mechanisms may contribute to the ASP postprandial effect. In this study we chose to use a fat load rather than a mixed meal in order to avoid insulin changes postprandially so that the changes would reflect primarily an ASP effect. As ASP and insulin effects are additive *in vitro*[21,23,24,28] the interplay between the two will need to be examined *in vivo* in the future.

Endogenously leptin, insulin, glucocorticoids and ASP appear to provide a coordinated balance/counterbalance on energy intake centrally and energy storage peripherally. Thus insulin reduces energy intake, but increases energy storage, while glucocorticoids have opposite effects to those of insulin. On the other hand, leptin inhibits both food intake and the storage of lipid in adipose tissue, whereas ASP stimulates both processes. Thus the ASP system may provide a pharmacologic target to alter adipocyte and lipid metabolism.

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