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Acylation Stimulating Protein (ASP) Deficiency Alters Postprandial and Adipose Tissue Metabolism in Male Mice*

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Acylation stimulating protein (ASP) is a potent stimulator of triglyceride synthesis in adipocytes. In the present study, we have examined the effect of an ASP functional knockout (ASP(−/−)) on lipid metabolism in male mice. In both young (14 weeks) and older (26 weeks) mice there were marked delays in postprandial triglyceride clearance (80% increase at 14 weeks and 120% increase at 26 weeks *versus* wild type (+/+)). Postprandial nonesterified fatty acids were also increased in ASP(−/−) mice *versus* ASP(+/+) mice by 37% (low fat 10% Kcal) and by 73% (high fat 40% Kcal) diets, although there were no differences in fasting lipid levels. The ASP(−/−) mice had moderately increased energy intake (16% ± 2% $p < 0.0001$) and reduced feed efficiency (33% increase in calories/g of body weight gained on low fat diet) *versus* wild type. The ASP(−/−) mice also had modest changes in insulin/glucose metabolism (30% to 40% decrease in insulin·glucose product), implying increased insulin sensitivity. As well, there were decreases in leptin (29% shift in leptin to body weight ratio) and up to a 26% decrease in specific adipose tissue depots *versus* the wild type mice on both low fat and high fat diets. These results demonstrate that ASP plays an important role in adipose tissue metabolism and fat partitioning.

Acylation stimulating protein (ASP)[1] is a 76-amino acid protein identical to C3adesArg, a cleavage product of complement C3. Extensive *in vitro* data demonstrate an ASP effect on fatty acid esterification in human and murine adipocytes and preadipocytes (1–3) as well as human fibroblasts (4–6). Recently, it has been demonstrated that ASP also inhibits hormone-sensitive lipase through an effect on phosphodiesterase 3 (PDE3) (7). ASP also stimulates glucose transport in human and murine adipocytes and preadipocytes (2, 8) as well as human fibroblasts (4) and differentiated rat muscle cells (9). This effect on glucose transport is consequent to translocation of Glut 1 or Glut 3 or Glut 4 (depending on the particular cell). The effects of ASP are likely mediated through interaction with a specific cell surface receptor that demonstrates high affinity binding and tissue-specific distribution (for a review on ASP see Ref. 3). Adipose tissue mass is determined by the rate of the opposing reactions, triglyceride synthesis, and lipolysis. Since ASP stimulates triglyceride synthesis and inhibits lipolysis and does so independently and additively with insulin (4, 7–9), ASP has the potential to profoundly influence adipose tissue metabolism.

ASP is produced through the interaction of complement C3, factor B, and adipsin (10, 11), and all three factors are expressed and secreted by human and murine adipocytes in a differentiation-dependent manner (1, 12, 13). The production of ASP by cultured adipocytes is stimulated by chylomicrons (14, 15). Although there is substantial *in vitro* data on ASP production, there have been far less *in vivo* studies to date on ASP. In the general circulation there appears to be a slight decrease in ASP over time following a fat load (16). However, generation of ASP does increase postprandially locally across an arterial-venous adipose tissue gradient (17) but not across a muscle gradient.[2] Our hypothesis is that ASP increases the efficiency of dietary energy storage through its cellular effects on lipid and glucose tissue storage.

The development of C3 knockout mice (18) allowed us the opportunity to examine the effects of an obligatory ASP knockout (since the precursor to ASP, C3, is absent in mouse plasma) on postprandial metabolism. In our first study on young (8–10 weeks old) male and female mice, we demonstrated increased postprandial triglyceride (TG) in the ASP(−/−) mice in the absence of any change in fasting TG (19). These effects were more pronounced in the males than in the females. Conversely, administration of ASP to ASP(−/−) mice (19) or to wild type Black6 mice (20) enhanced the clearance of triglyceride and decreased postprandial lipemia.

The aim of the present study was to examine the ASP(−/−) phenotype in more detail in a longitudinal study with different diets (10% low fat and 40% high fat). We postulated that the wild type ASP(+/+) mice on the high fat diet would mimic the knockout ASP(−/−) phenotype and that the high fat diet would amplify the effects of the ASP(−/−) phenotype.

MATERIALS AND METHODS

Ethics—All experimental protocols were approved by the Royal Victoria Hospital Animal Ethics committee and were in accordance with the guidelines set out by the Canadian Committee on Animal Care.

Mice—Dr. H. Colten and Dr. R. H. Wetsel kindly provided the knockout and wild type mice for breeding. Development of the complement C3

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[1] The abbreviations used are: ASP, acylation stimulating protein; ANOVA, analysis of variance; TG, triglyceride; LF, low fat; HF, high fat; NEFA, plasma non-esterified fatty acids; AUC, area under the curve; CHOL, cholesterol; LDL, low density lipoproteins; VLDL, very LDL; GTT, glucose tolerance test; HDL, high density lipoproteins; LPL, lipoprotein lipase.

[2] A. D. Sniderman and K. Cianflone, unpublished observations.

TABLE I
Fasting plasma lipids in ASP (−/−) and ASP (+/+) mice

Fasting samples were obtained from ASP (−/−) and (+/+) mice at the indicated ages (sample size indicated for each group). Values are expressed as the average ± S.E.

Mice	Type	Sample size	Triglyceride	Cholesterol	NEFA
			mM	*mM*	*mM*
10 weeks	(+/+)	7	58.5 ± 5.1	63.0 ± 4.6	0.87 ± 0.18
	(−/−)	12	72.6 ± 11.7	67.0 ± 4.3	1.97 ± 0.52[a]
14 weeks, LF	(+/+)	9	51.1 ± 8.9	91.8 ± 14.4	0.54 ± 0.09
	(−/−)	7	53.2 ± 6.7	74.0 ± 5.1	0.48 ± 0.05
14 weeks, HF	(+/+)	9	70.6 ± 7.3	95.8 ± 7.8	0.47 ± 0.10
	(−/−)	8	67.0 ± 5.6	105.6 ± 10.4	0.61 ± 0.12
26 weeks, LF	(+/+)	9	46.1 ± 6.0	92.1 ± 6.2	0.35 ± 0.05
	(−/−)	7	57.6 ± 4.8	108.2 ± 13.9	0.41 ± 0.07
26 weeks, HF	(+/+)	10	65.6 ± 2.6	127.7 ± 7.4	0.29 ± 0.02
	(−/−)	7	69.9 ± 7.5	148.3 ± 20.4	0.38 ± 0.07

[a] $p < 0.05$ for (−/−) *vs.* (+/+) at the indicated age.

knockout has been described elsewhere in detail (18, 21). The mice were of (129Sv × C57Bl/6) strain, and heterozygous mating produced the littermates (wild type ASP(+/+) and knockout ASP(−/−)) used for the present experiments. Mice were housed in sterile barrier facilities with equal day/night periods. In all cases, littermates were used to randomize genetic variation.

Genotyping—For genotyping, tail DNA was extracted, and polymerase chain reaction was performed. Polymerase chain reaction was performed using 800 nM each of the following primers: C3 sense, CTT AAC TGT CCC ACT GCC AAG AAA CCG TCC CAG ATC; C3 antisense, CTC TGG TCC CTC CCT GTT CCT GCA CCA GGG ACT GCC CAA AAT TTC GCA AC; neomycin sense, ATC GCA TCG AGC GAG CAC GTA CTC GGA; neomycin antisense, AGC TCT TCA GCA ATA TCA CGG CTA GCC. Polymerase chain reaction conditions were 30 cycles at 94 °C for 1 min, 67 °C for 2 min, and 72 °C for 3 min. Products were separated by electrophoresis on a 7% polyacrylamide gel and visualized with ethidium bromide staining.

Diet, Feeding, and Weighing—ASP(+/+) and ASP(−/−) male mice were weighed once weekly from weaning at 4 weeks of age. At 8 weeks, the mice were housed individually and allowed to acclimatize for 2 weeks. At 10 weeks of age, the mice were placed on a pelleted low fat diet (LF) consisting of 19.3% protein, 67.3% carbohydrate, and 4.3% fat (w/w) or high fat diet (HF) consisting of 22.9% protein, 45.8% carbohydrate, and 20.3% fat w/w modified from Van Heek *et al.* (22) and obtained from Research Diets, Inc. (New Brunswick, NJ) (Diets D12477 and D12478, respectively). The diets contained 10% Kcal (LF) and 40% Kcal (HF) energy from fat, with a 1:1:1 ratio of saturated:monounsaturated: polyunsaturated fats and were stored at 4 °C. Carbohydrate was in the form of cornstarch rather than sucrose (70% LF and 40% HF Kcal). The vitamin and mineral content conformed with the AIN (American Institutes of Nutrition) guidelines. The food was weighed 3 times weekly over a period of 16 weeks, and food intake was determined over the time period of 10 to 26 weeks of age. After 1 month on the diet, a fat load was performed, and 2 weeks later a fasting blood samples was taken. The same treatment was repeated at 4 months on the diet (26 and 28 weeks of age). On a subset of mice, a glucose tolerance test was performed at 30 weeks of age, and mice were sacrificed at 32 weeks or 48 weeks. Mice were anesthetized (0.01 ml/10 g of body weight intramuscularly) with a mixture composed of 5 ml of ketamine (100 mg/ml), 2.5 ml of xylazine (20 mg/ml), 1 ml of acepromazine (10 mg/ml), and 1.5 ml of sterile saline. Blood was drawn from the tail (0.5 ml), and the mice were sacrificed by cervical dislocation. Tissues were dissected, weighed, and frozen in liquid nitrogen. Four adipose tissues depots were collected: inguinal, pectoral and suprascapular together, gonadal fat up to the apex of the ovary, and perirenal adipose tissue with the adrenal gland removed. Additional tissues collected were heart, liver, intrascapular and scapular brown adipose tissue, both kidneys, and quadriceps muscles with all visible fat removed.

Plasma Assays—Blood was collected by tail bleeding into EDTA-containing tubes by tail bleeding as described previously (19, 20) from mice fasted overnight (16 h) with water *ad libitum* at 10, 16, 28, and 32 weeks of age. Blood was separated by centrifugation, and the plasma was stored at −80 °C. Leptin was measured using a mouse leptin radioimmunoassay (Linco Inc. St Charles, MO) as described previously (23). Fasting insulin was measured using a rat insulin radioimmunoassay kit that had 100% cross-reactivity to mouse insulin (as described by the manufacturer, Linco Inc). Glucose was measured using a Trinder glucose kit (Sigma). Plasma free fatty acids (NEFA), cholesterol, and triglycerides were measured using colorimetric enzymatic kits (Roche Molecular Biochemicals).

Fat Load—After an overnight fast (16 h), 400 μl of olive oil (followed by 100 μl of air) was given by gastric gavage using a feeding tube (1-cm curved ball tipped feeding needle 20), according to standard procedures as published previously (19, 20) and similar to previously published methods (24–27). Blood (40 μl) was collected by tail bleeding at 0, 1, 2, 3, 4, and 6 h.

Glucose Load—For glucose tolerance tests, mice were fasted overnight for 16 h with water *ad libitum*. Basal blood was taken (80 μl), and mice were injected intraperitoneally with a sterile D-glucose solution in saline, 2 mg/g of body weight from a stock solution of 200 mg/ml (0.010 ml/g of body weight). Blood was collected at 15, 30, 60, 90, and 120 min. Insulin and glucose were measured 0, 30, 60, and 120 min (80 μl collected), and glucose only was measured at 15 and 90 min (20 μl collected).

Statistical Analyses—Results are presented as the mean ± S.E. The two groups were compared by repeated measures of two-way ANOVA followed by Bonferroni post-test or by the area under the curve (AUC) (for time course data), *t* test, ANOVA (fasting data), or Pearson correlation using computer-assisted analysis (Sigma Stat, Jandel Scientific, San Rafael, CA and Prism, GraphPad San Diego, CA).

RESULTS

Mice were examined at three time points, at 10 weeks of age, 14 weeks of age (after 1 month on a low fat or a high fat diet), and again at 26 weeks of age (after a total of 4 months on a low fat or high fat diet). The diets contained a fixed amount of protein (20%), and as the fat content was increased (10% to 40% of energy), the carbohydrate contribution was proportionally reduced (70 to 40% of energy). Basal fasting lipids for male wild type ASP(+/+) and male knockout ASP(−/−) mice are shown in Table I. Overall there was no change in plasma TG between the wild type ASP(+/+) and ASP(−/−) mice, nor was there any effect of diet or age. Plasma cholesterol (CHOL) on the other hand increased with age and also increased on a high fat diet, as has been reported elsewhere (28–30). Although there appeared to be a trend toward increased plasma CHOL in the (−/−) mice, especially in the older mice, the differences were not significant and were due primarily to increases in HDL CHOL in the ASP(−/−) knockout with no change in VLDL + LDL CHOL (results not shown). Plasma NEFA decreased with age as seen previously (31), although there was no effect of the high fat diet on these parameters. Although young ASP(−/−) mice (10 weeks old) had higher plasma NEFA and slightly increased VLDL + LDL CHOL *versus* wild type, as shown previously (19), this was not apparent as the mice aged.

We have previously demonstrated that young male ASP(−/−) mice (8–10 weeks old) demonstrate small but significant delays in TG clearance following a fat load with a 52% increase in TG AUC (19). As shown in Fig. 1, at 14 weeks of age (on a low fat diet) there is a substantial difference in postprandial TG in the ASP(−/−) as compared with ASP(+/+) (893 ±

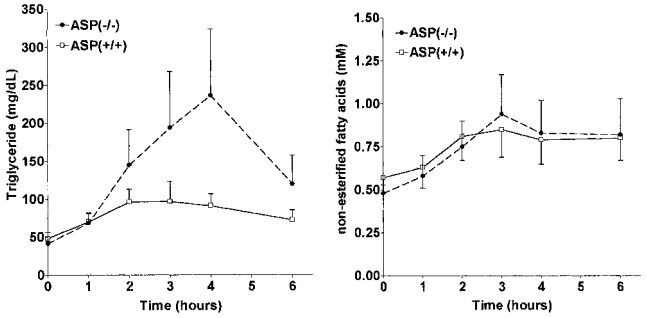

FIG. 1. **Postprandial lipemia in wild type (+/+) and ASP knockout (−/−) mice on low fat diet.** An oral fat load was given to the mice after 1 month on a low fat diet (14 weeks of age) and triglycerides (*left panel*), and nonesterified fatty acids (*right panel*) were measured over the 6-h time course for $n = 7$ ASP(−/−) and $n = 8$ ASP(+/+). Results are shown as average ± S.E. Two-way repeated measures of ANOVA results indicate that there is a significant time ($p < 0.02$) and genotype ($p < 0.01$) effect for triglyceride, but there is no significant change for plasma nonesterified fatty acids.

279 (−/−) *versus* 497 ± 90 (+/+) AUC mg/dl·h, an 80% increase, $p < 0.02$). There is no difference in the NEFA postprandial profile between the two groups (Fig. 1, *right panel*). The postprandial TG curves after 1 month on the HF diet are shown in Fig. 2. The high fat diet results in a delay in postprandial TG clearance in the ASP(+/+) mice such that the TG AUC increases by 65% *versus* the ASP(+/+) control on LF (820 ± 149 mg/dl·h, $p < 0.01$). The postprandial curve of the ASP(−/−) mice slightly increased as compared with the ASP(−/−) on LF diet (1094 ± 242 mg/dl·h AUC). However, in the ASP(−/−) mice on HF there was also a pronounced increase in the postprandial plasma NEFA (2.5 fold) that did not occur in the ASP(+/+) mice on the high fat diet (Fig. 2, *right panel*).

After a total of 4 months on the respective diets, we reexamined postprandial responses in the mice. The results for TG AUC for 10 weeks of age, 1 month on diet (14 weeks of age), and 4 months on diet (26 weeks of age) are shown in Fig. 3. In the ASP(+/+) mice, there are progressive diet-induced increases in TG AUC. In the ASP(−/−) mice there are also marked changes in TG AUC that in most instances are larger than the wild type. The differences in TG AUC between ASP(+/+) and ASP(−/−) are not secondary to differences in basal TG levels, since there is no change in fasting TG levels (Table I). At 1 month (low and high fat diet) and 4 months (low fat diet) the TG AUC in ASP(−/−) is significantly increased compared with ASP(+/+). At 4 months on the high fat diet, the TG AUC for ASP(−/−) is reduced compared with HF ASP(+/+) but is still significantly increased over the LF ASP(+/+) control.

The results for NEFA at 4 months are shown in Fig. 4 for low fat (*left panel*) and high fat (*right panel*). In the wild type mice, the increase in postprandial NEFA AUC is modest (20.6% increase over basal AUC for LF and 30.8% for HF diet). By contrast, the increase in NEFA in the ASP(−/−) mice is greater than the (+/+) mice, 45% on LF and 70% on HF ($p < 0.003$ and $p < 0.0001$, respectively, by two-way repeated measures ANOVA).

We also examined glucose and insulin metabolism in the mice. Fasting glucose and insulin are given in Table II. High fat diets are recognized for their effects on inducing insulin resistance and disordered glucose metabolism (28–30, 32–34). In the ASP(+/+) mice, there is a slight increase in glucose with age, as well as an effect of the high fat diet that increases glucose by 9% on average. There is a similar high fat effect on insulin that increases by 2-fold at both 1 month and 4 months on the diet. In the ASP(−/−) mice, there is a similar increase in insulin with age and diet. However, the ASP(−/−) have significantly lower glucose levels than the wild type at 14 and 26 weeks old

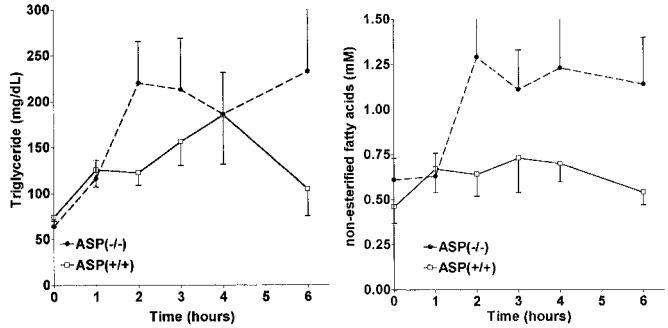

FIG. 2. **Postprandial lipemia in wild type (+/+) and ASP knockout (−/−) mice on high fat diet.** An oral fat load was given to the mice after 1 month on a high fat diet (14 weeks of age) and triglycerides (*left panel*), and nonesterified fatty acids (*right panel*) were measured over the 6-h time course for $n = 8$ ASP(−/−) and $n = 10$ ASP(+/+). Results are given as average ± S.E. Two-way repeated measures of ANOVA results indicate that there is a significant time ($p < 0.01$) and genotype ($p < 0.04$) effect for triglyceride and a significant genotype ($p < 0.0003$) and time ($p < 0.05$) effect on plasma nonesterified fatty acids.

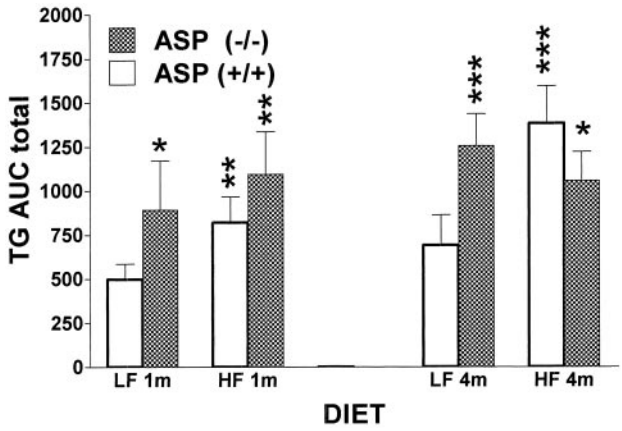

FIG. 3. **Postprandial area under the curve (AUC) for triglyceride and nonesterified fatty acids in wild type (+/+) and knockout (−/−) mice.** An oral fat load was given to the mice at 10 weeks old (basal), 14 weeks old (1 month (*1 m*) on LF or HF diet) and at 26 weeks old (4 months (*4 m*) on LF or HF diet). Results are the average ± S.E. for 7 to 10 mice in each group (as indicated in Table III). Significance was calculated by 2-way repeated measures of ANOVA using the individual time points for all three treatment groups (wild type HF, knockout LF, and knockout HF) *versus* the control group (wild type LF), where * represents $p < 0.03$, ** represents $p < 0.003$, and *** represents $p < 0.0001$.

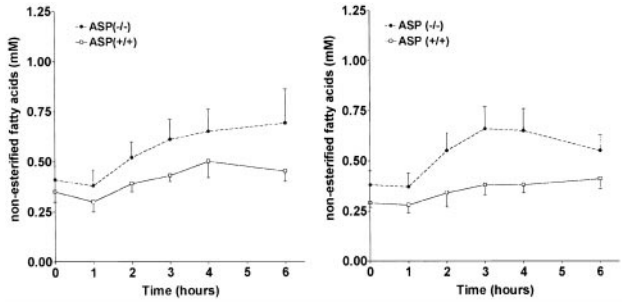

FIG. 4. **Postprandial nonesterified fatty acids in ASP(−/−) and ASP(+/+) mice.** An oral fat load was given to the mice after 4 months on a low fat (*left panel*) or high fat (*right panel*) diet, and nonesterified fatty acids were measured over the 6-h time course (ASP(−/−) $n = 7$ LF and 7 HF, and ASP(+/+) $n = 9$ LF and 9 HF). Results are expressed as average ± S.E. where $p < 0.003$ (LF) and $p < 0.0001$ (HF) for ASP(−/−) *versus* ASP(+/+) by 2-way repeated measures of ANOVA.

at any given insulin level, and this is evidenced by the reduced insulin·glucose product, which is lower in the ASP(−/−) mice. This relationship between insulin and glucose in ASP(+/+) and

TABLE II
Fasting glucose and insulin in ASP (−/−) and ASP (+/+) Mice

Fasting samples were obtained from ASP (−/−) and (+/+) mice at the indicated ages (sample size indicated for each group). Glucose · Insulin represents the product of the two values. Values are expressed as average ± S.E.

Mice	Type	Sample size	Glucose	Insulin	Glucose · Insulin
			mg/dl	*ng/ml*	
10 weeks	(+/+)	5	112 ± 15.8	0.32 ± 0.08	27 ± 5.5
	(−/−)	11	99 ± 14.0	0.31 ± 0.03	28 ± 4.7
14 weeks, LF	(+/+)	8	113 ± 12.7	0.20 ± 0.06	43.4 ± 16.8
	(−/−)	7	91 ± 3.9[a]	0.23 ± 0.02	20.5 ± 1.6
14 weeks, HF	(+/+)	10	123 ± 5.6	0.42 ± 0.10	52.6 ± 12.0
	(−/−)	8	104 ± 4.0[b]	0.51 ± 0.11	46.3 ± 10.0
26 weeks, LF	(+/+)	9	126 ± 9.1	0.42 ± 0.08	56.1 ± 13.9
	(−/−)	10	104 ± 5.8[a]	0.31 ± 0.03	31.2 ± 2.5[a]
26 weeks, HF	(+/+)	10	136 ± 8.9	0.84 ± 0.12	111 ± 13.2
	(−/−)	7	117 ± 10.3	0.91 ± 0.20	78 ± 11.6[a]

[a] $p < 0.05$ for (−/−) *vs.* (+/+).
[b] $p < 0.025$ for (−/−) *vs.* (+/+).

ASP(−/−) was analyzed by linear regression analysis, and for any given value of insulin, the corresponding glucose is significantly lower in the ASP(−/−) as compared with ASP(+/+) ($p < 0.002$).

Postprandial glucose after a fat load at either 1 month or 4 months on LF or HF was no different in ASP(+/+) *versus* (−/−) (Table III). In a subset of mice we also measured plasma glucose after a glucose tolerance test (GTT) at 4.5 months on LF or HF diet, and again there was no difference in ASP(+/+) *versus* (−/−) (Table III). There was a clear effect of the high fat diet in both wild type and knockout mice (Table III) where glucose AUC increased 29 to 47% and was significant in all cases. Finally, GTT insulin response increased with a high fat diet in both ASP(+/+) and (−/−) but to a lesser extent in (−/−), and on the LF diet only the insulin response to GTT was significantly lower in ASP(−/−) *versus* ASP(+/+), $p < 0.02$.

We also looked at other factors related to energy nutrient disposition and fat mass: growth curves of the mice, body composition, leptin and food efficiency. The mice were weighed each week from 4 to 26 weeks old, and the food intake monitored over the dietary period (16 weeks from 10 to 26 weeks of age). Although the knockout mice were slightly heavier at all ages *versus* (+/+) by 9% ± 0.9% (LF) and 4.1% ± 0.3% (HF), the differences were not significant. Total body weight on high fat diets, relative to low fat diet, increased to the same extent in both ASP(+/+) and ASP(−/−) by 22.5% ± 2.1% (+/+) and 18.7% ± 1.7% (−/−) over the 4-month period. Leptin, which correlates very highly to body weight and adipose tissue mass, was measured at several time points from 10 to 32 weeks of age as an index of adiposity in the mice. Leptin increases both with age and with diet in wild type mice, as reported by others (22, 23, 35), and this was also true of the ASP(−/−) mice. However, as shown in Fig. 5, relative to body weight, there was always significantly less leptin in ASP(−/−) *versus* (+/+), $p < 0.006$, suggesting reduced whole body fat. This decrease in leptin was reflected by small but significant decreases in adipose tissue mass (Table IV) particularly in the gonadal and perirenal fat (−26% and −13% (LF), respectively), representing a decrease from 6.3% ASP(+/+) to 4.2% ASP(−/−) fat *versus* body weight. On the other hand, there was a significant increase in intrascapular brown adipose tissue in the ASP(−/−) mice *versus* ASP(+/+) on both the low and high fat diets (89 and 39%, respectively, $p < 0.05$).

Finally, the ASP(−/−) mice appeared to be mildly hyperphagic, possibly as a result of decreased leptin levels. As shown in Fig. 6, (low fat diet) there was little difference in energy intake in ASP(+/+) *versus* (−/−) in the first 5 weeks after the change to a low fat or high fat diet. However, after this adjustment period the ASP(−/−) mice consistently consumed a greater caloric load (112 ± 1.8 ASP(−/−) *versus* 95.4 ± 0.8 ASP(+/+) cal/week, a 17% increase for ASP(−/−) *versus* wild type, $p < 0.0001$ by two way repeated measures ANOVA). This was also true on the high fat diet (similar profiles), although to a lesser extent, since all mice tended to increase their energy intake on a high fat diet (average 131.4 ± 3.8 ASP(−/−) *versus* 125.7 ± 2.8 ASP(+/+) cal/week, a 6% increase for ASP(−/−) *versus* wild type, $p = 0.07$). When energy intake is calculated relative to an increase in body weight (feed efficiency) over the 16-week period, the ASP(−/−) mice had a lower feed efficiency (*i.e.* required more energy intake relative to body weight gained) as compared with the ASP(+/+) on both the low fat diet (148 ± 19 ASP(−/−) *versus* 111 ± 16 ASP(+/+) cal/g of body weight gained, 33% increase, $p < 0.001$) and high fat diet (109 ± 18 ASP(−/−) *versus* 98.3 ± 8 ASP(+/+) cal/g of body weight gained, 11% increase, $p = 0.07$).

DISCUSSION

In the present study we have examined in detail the ASP knockout phenotype in male mice. These mice are characterized by 1) marked postprandial lipemia (triglycerides) and increased postprandial nonesterified fatty acid levels on both low and high fat diets, 2) increased energy intake/feed efficiency, and 3) modest changes in insulin/glucose metabolism and leptin/adiposity. On the other hand, in the female mice (reported elsewhere), although the changes were directionally the same, the female mice demonstrated greater changes in insulin/glucose metabolism and leptin/adiposity, with smaller changes in free fatty acids and, surprisingly, no postprandial lipemia. In both types of mice, however, there were changes in energy intake/feed efficiency.

Two characteristics of the ASP(−/−) male mice were (i) the presence of marked postprandial lipemia in the ASP(−/−) mice and (ii) increased energy intake/reduced feed efficiency. Increased postprandial lipemia as well as increased energy intake are also characteristic of wild type mice that have been maintained on a high fat diet (24, 36). However, the similarities between the ASP(−/−) phenotype and high fat diets in wild type mice end there. Although the ASP(−/−) mice demonstrate improved insulin/glucose, reduced leptin/adiposity, and increased postprandial free fatty acids, wild type mice on a high fat diet are characterized by increased glucose and insulin, insulin resistance, increased leptin, increased adipose tissue stores, and no dietary effect on free fatty acids. In fact, the ASP(−/−) phenotype is maintained even when the mice are placed on a high fat diet. The metabolic consequences (as discussed below) might be expected to be different between the two phenotypes.

Postprandial triglycerides are cleared from the circulation in

TABLE III
Postprandial and GTT, glucose and insulin AUC

Postprandial glucose over 6 h was measured at 14 weeks (1 month on diet) and 26 weeks (4 months on diet). Glucose and insulin were measured following a 2-h GTT after 4.5 months on diet. In all cases, values are given for average AUC ± S.E. and the % increase for HF *vs.* LF is indicated (with significance).

Assay	Type	Sample size	LF	HF	% Increase
Postprandial glucose (1 month)	(+/+)	(7/10)	649 ± 57	875 ± 62	35%, $p < 0.0001$
	(−/−)	(7/8)	632 ± 67	857 ± 79	32%, $p < 0.0001$
Postprandial glucose (4 months)	(+/+)	(9/9)	672 ± 51	887 ± 42	32%, $p < 0.0001$
	(−/−)	(7/7)	749 ± 28	887 ± 61	32%, $p < 0.0001$
GTT glucose (4.5 months)	(+/+)	(3/4)	287 ± 36	423 ± 34	47%, $p < 0.0001$
	(−/−)	(3/3)	330 ± 10	371 ± 34	29%, $p < 0.001$
GTT insulin	(+/+)	(3/4)	2.06 ± 0.44	3.22 ± 0.43	57%, $p < 0.0001$
	(−/−)	(3/2)	1.00 ± 0.13[a]	2.91 ± 0.49	90%, $p < 0.001$

[a] $p < 0.02$ for ASP (−/−) *vs.* ASP (+/+).

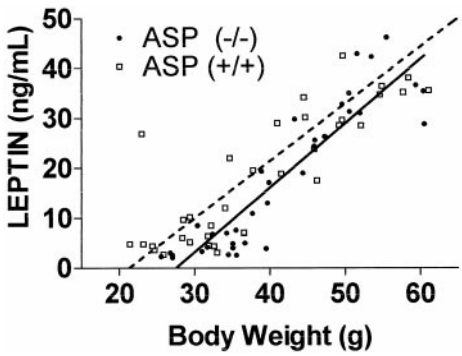

FIG. 5. **Linear regression of leptin *versus* body weight in ASP(−/−) and ASP(+/+) mice.** Plasma leptin was measured in fasting plasma from the age of 10 weeks to 48 weeks old on both low fat and high fat diets and graphed *versus* body weight at the time of sampling. Linear regression was calculated for ASP(+/+) *versus* ASP(−/−); the two lines are significantly different at $p < 0.006$.

a two-step coupled process. First, triglycerides are hydrolyzed by the rate-limiting enzyme lipoprotein lipase (LPL), and second, the free fatty acids generated are cleared from the circulation by uptake into target tissues (white and brown adipose tissue, muscle, and liver). Thus clearance of triglycerides will, in the first instance, be regulated by the mass and activity of LPL, which can be influenced genetically by mutation (37), overexpression (38), or knockout of LPL (26). Environmentally, the major influences on LPL are gender and the influence of sex hormones (39), insulin levels and insulin sensitivity (40), and inhibition by the local level of NEFA (41–43). On a high fat diet, wild type (+/+) mice develop postprandial lipemia, probably due to reduced insulin sensitivity or its reduced effects on LPL. On the other hand, in the male ASP(−/−) mice, the lack of ASP results in increased circulating concentrations of NEFA and delays in tissue free fatty acid uptake, and this may inhibit LPL. As compared with the female mice, male wild type mice have reduced adipose tissue mass (44), decreased LPL (45), and increased insulin levels (46) and are more sensitive to high fat diet-induced insulin resistance (31). These factors may compound the effect of ASP deficiency, resulting in a pronounced delay in postprandial TG clearance. Conversely, in the female ASP(−/−) mice, the postprandial NEFA do not reach the same levels as in the males, and there is no enhanced postprandial lipemia. The most likely explanations are (i) increased adipose tissue mass (relative to the males), which thus providing more LPL and more tissue available for fatty acid uptake and esterification, coupled to (ii) increased insulin sensitivity to stimulate LPL activity and fatty acid esterification. Thus alternate mechanisms in female mice may help to compensate for the lack of ASP.

We have previously demonstrated this same profile of postprandial lipemia (although to a lesser extent) in younger male ASP(−/−) mice (19). By contrast, Wetsel *et al.* (47) have not demonstrated a difference in postprandial triglycerides in their study. In their study, they also did not find lower postprandial triglycerides in female *versus* male mice, and their female mice demonstrated higher apoB levels than males. The lack of sexual dimorphism is unusual considering the well recognized differences that have been reported elsewhere (24, 31, 45, 48) and that we have also seen in both the ASP(+/+) and the ASP(−/−) mice (19).

One explanation for the differences may lie in the colony characteristics. Both colonies are a genetic hybrid of C57Bl/6 and 129Sv, and this genetic heterogeneity is controlled for in both studies through the use of ASP(−/−) and (+/+) littermates for the comparisons. However the relative contribution of each background strain in our mice colony may be different from theirs. We have back-crossed the ASP(−/−) onto each of these two different strains to 98% genetic homogeneity (6-generation back-cross). Not only are there strain differences in the fasting lipids (as reported elsewhere (24, 49, 50) [2]) and postprandial profiles in the wild type, more notably there is also differential expression of the ASP(−/−) phenotype, at least as far as postprandial triglycerides are concerned.[3] The different insulin levels measured in these two mice strains may account for these differences. This is consistent with different insulin sensitivity between mouse strains, which may help to compensate for the lack of ASP. In fact, strain-specific phenotypic expression of knockouts or naturally arising mutations are not uncommon.

The association of increased postprandial NEFA, decreased glucose relative to insulin, and decreased leptin/adiposity is interesting with respect to the potential metabolic changes. In the first instance, a decreased glucose-to-insulin ratio would suggest increased insulin sensitivity in ASP(−/−) mice. This increased insulin sensitivity coupled to the reduced leptin and adipose tissue mass would provide a strong metabolic drive toward increased energy intake and partitioning of calories to the adipose tissue, leading to obesity. Thus it is even more striking that, despite the slightly increased calorie-to-body weight intake, even on a high fat diet, the ASP(−/−) mice are not more obese than their (+/+) counterparts. This highlights the important physiological function of ASP, which may be necessary for efficient regulation of adipsoe tissue metabolism.

The increased postprandial NEFA coupled to the lower glucose-to-insulin ratio suggests that there may also be changes in nutrient partitioning. We would hypothesize that the decreased efficiency of NEFA uptake may result in enhanced utilization of glucose, resulting in lowered plasma glucose levels and greater insulin sensitivity. When delivery of NEFA to tissues was disrupted by a targeted knockout of LPL, fasting

[3] I. Murray, A. D. Sniderman, and K. Cianflone, manuscript in preparation.

TABLE IV

Weight of adipose tissue depots in male mice at 8 months of age

Mice were fed the indicated diet from the age of 10 weeks and were sacrificed at 8 months of age. Results are expressed as average ± S.E. % bodyweight, and % decrease for (−/−) *vs.* (+/+). Each group has $n = 3$, and significance level is indicated. BAT = intrascapular brown adipose tissue.

	Low fat diet		High fat diet	
	g	% Bodyweight	g	% Bodyweight
Inguinal				
(+/+)	0.883 ± 0.274	2.7%	2.559 ± 0.047	6.1%
(−/−)	0.834 ± 0.131	2.3%	2.642 ± 0.142	6.2%
% decrease	−6%		−4%	
Pectoral				
(+/+)	0.758 ± 0.136	2.4%	1.828 ± 0.262	4.3%
(−/−)	0.711 ± 0.142	2.0%	2.169 ± 0.181	5.1%
% decrease	−7%		+18%	
Gonadal				
(+/+)	1.421 ± 0.148	4.4%	2.699 ± 0.120	6.4%
(−/−)	1.051 ± 0.143	2.9%	2.378 ± 0.199	5.6%
% decrease	−26%	$p < 0.05$	−12%	
Perirenal				
(+/+)	0.574 ± 0.093	1.8%	1.274 ± 0.098	3.0%
(−/−)	0.501 ± 0.097	1.4%	0.993 ± 0.047	2.3%
% decrease	−13%		−22%	$p < 0.05$
Sum peri-renal + gonadal				
(+/+)	1.995 ± 0.139	6.3%	3.973 ± 0.194	9.4%
(−/−)	1.552 ± 0.240	4.2%	3.372 ± 0.161	7.9%
% decrease	−13%		−16%	$p < 0.05$
BAT				
(+/+)	0.294 ± 0.050	0.9%	0.472 ± 0.074	1.1%
(−/−)	0.556 ± 0.089	1.5%	0.654 ± 0.020	1.5%
% increase	+89%	$p < 0.05$	+39%	$p < 0.05$

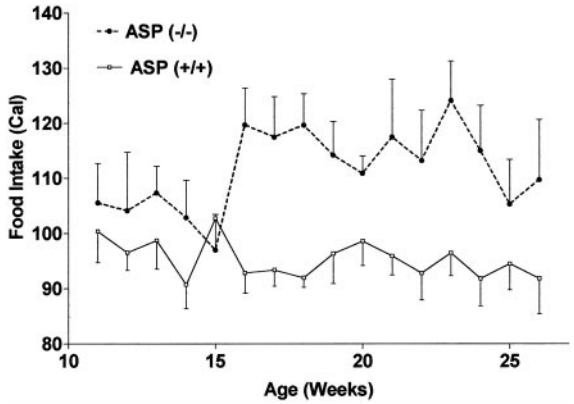

FIG. 6. **Food intake over time on a low fat diet.** Food intake (calories) was measured 2–3 times/week, and body weight was measured time/week on both ASP(+/+) ($n = 9$) and ASP(−/−) ($n = 7$) mice from mice of 10 to 26 weeks old on a low fat diet. On average, the ASP(−/−) mice consumed 16.5 ± 2.1% more calories over the whole time period *versus* the ASP(+/+), $p < 0.0001$ by 2-way repeated measures ANOVA.

plasma glucose was lower and even led to neonatal death (51). Similarily, with GLUT 4 overexpression in mice, it was suggested that the increased glucose uptake and storage into adipose tissue caused decreased NEFA adipose tissue uptake and repartitioning of the available NEFA to muscle and brown adipose tissue, resulting in increased NEFA oxidation (52). This same process may be occurring in the ASP(−/−) mice. The increase in brown adipose tissue mass would point in that direction, and these issues are being explored presently.

In humans, there is considerable evidence suggesting that (i) the association between obesity and glucose/insulin resistance/diabetes as well as (ii) the association between obesity and hyperlipidaemia/hyperapoB are mediated by alterations in plasma NEFA and are collectively known as "syndrome X" (53). Randle *et al.* (54) and more recently Bjorntorp (55) and McGarry (56) as well as others have proposed that the muscle competition for NEFA/glucose utilization as well as NEFA effects on hepatic metabolism, reduced insulin removal, and increased pancreatic insulin secretion are the cause of these complications. These same authors have also documented the link between NEFA and increased VLDL and apoB lipoprotein secretion. In this scenario, any disturbance that increases plasma NEFA would play a causative role, whether it be lack of ASP, lack of response to ASP, or any other factor that disturbs fatty acid metabolism, such as insulin resistance. We have demonstrated increased postprandial NEFA (57) and abnormal response to ASP (in cells) in such hyperapoB human subjects (5, 58).

In mice, however, this cycle of obesity → NEFA → insulin resistance/hyperlipidaemia does not appear to be present regardless of whether the obesity is genetically or diet induced. In our study, postprandial NEFA increases on either low or high fat diet in ASP(−/−) mice were not associated with increased glucose/insulin or fasting hyperlipidaemia. On the other hand, the high fat diet, which was clearly associated with increased plasma glucose, insulin, and to a lesser extent cholesterol was not associated with any increase in fasting or postprandial NEFA as compared with the low fat diet. This is true for both ASP(+/+) as well as ASP(−/−). There are many other studies that also demonstrate no change in plasma NEFA consequent to high fat diet-induced obesity in wild type (+/+) and genetically obese mutant mice (30, 31, 33, 59–61), and this issue has been discussed in a recent review (62). Although plasma cholesterol may increase in mice on a high fat diet (28–31), there is little change in plasma apoliporpotein B (31). In fact, in a study with 10 different strains of mice (63) it was shown that even in the presence of high fat and high cholesterol supplementation, the effect on apoB was not significant (average 15% ± 7% increase), whereas the overall effect on plasma cholesterol, VLDL CHOL, LDL CHOL, and HDL CHOL was highly significant (37 to 103% increase, $p < 0.001$, calculated by the author) (63). Thus the finding by Wetsel *et al.* (47) that ASP(−/−) does not cause hyperapoB in mice is not unexpected. We could not find any reports of NEFA-linked dietary/drug/

mutations that resulted in increased apoB hepatic production in mice. Clearly, the normal metabolic responses to increased NEFA in mice are very different from those in humans, and this may lie in their capacity to increase thermogenesis and oxidation, issues we are now pursuing in the ASP(−/−) mice.

In summary, these results demonstrate that ASP plays an important role in adipose tissue metabolism, and lack of ASP appears to alter both nutrient partitioning and the balance of energy intake with body weight gain and adiposity. An ASP antagonist could provide a pharmacologic target to alter adipose tissue metabolism.

REFERENCES

1. Cianflone, K., Roncari, D. A. K., Maslowska, M., Baldo, A., Forden, J., and Sniderman, A. D. (1994) *Biochemistry* **33,** 9489–9495
2. Murray, I., Parker, R. A., Kirchgessner, T. G., Tran, J., Zhang, Z. J., Westerlund, J., and Cianflone, K. (1997) *J. Lipid Res.* **38,** 2492–2501
3. Cianflone, K., Maslowska, M., and Sniderman, A. D. (1999) *Semin. Cell. Dev. Biol.* **10,** 31–41
4. Germinario, R., Sniderman, A. D., Manuel, S., Pratt, S., Baldo, A., and Cianflone, K. (1993) *Metabolism* **40,** 574–580
5. Zhang, X. J., Cianflone, K., Genest, J., and Sniderman, A. D. (1997) *Eur. J. Clin. Invest.* **28,** 730–739
6. Baldo, A., Sniderman, A. D., St-Luce, S., Avramoglu, R. K., Maslowska, M., Hoang, B., Monge, J. C., Bell, A., Mulay, S., and Cianflone, K. (1993) *J. Clin. Invest.* **92,** 1543–1547
7. Van Harmelen, V., Reynisdottir, S., Cianflone, K., Degerman, E., Hoffstedt, J., Nilsell, K., Sniderman, A., and Arner, P. (1999) *J. Biol. Chem.* **274,** 18243–18251
8. Maslowska, M., Sniderman, A. D., Germinario, R., and Cianflone, K. (1997) *Int. J. Obes.* **21,** 261–266
9. Tao, Y. Z., Cianflone, K., Sniderman, A. D., Colby-Germinario, S. P., and Germinario, R. J. (1997) *Biochim. Biophys. Acta* **1344,** 221–229
10. Lesavre, P. H., Hugli, T. E., Esser, A. F., and Muller-Eberhard, H. J. (1979) *J. Immunol.* **123,** 529–534
11. Muller-Eberhard, H. J., and Schreiber, R. D. (1980) *Adv. Immunol.* **29,** 1–53
12. Cianflone, K., and Maslowska, M. (1995) *Eur. J. Clin. Invest.* **25,** 817–825
13. Choy, L. N., and Spiegelman, B. M. (1996) *Obes. Res.* **4,** 521–532
14. Maslowska, M., Scantlebury, T., Germinario, R., and Cianflone, K. (1997) *J. Lipid Res.* **38,** 21–31
15. Scantlebury, T., Maslowska, M., and Cianflone, K. (1998) *J. Biol. Chem.* **273,** 20903–20909
16. Charlesworth, J. A., Peake, P. W., Campbell, L. V., Pussell, B. A., O'Grady, S., and Tzilopoulos, T. (1998) *Int. J. Obes. Rel. Metab. Dis.* **22,** 1096–1102
17. Saleh, J., Summers, L. K. M., Cianflone, K., Fielding, B. A., Sniderman, A. D., and Frayn, K. N. (1998) *J. Lipid Res.* **39,** 884–891
18. Circolo, A., Garnier, G., Fukuda, K., Wang, X., Tunde, H., Szalai, A. J., Briles, D. E., Volanakis, J. E., Wetsel, R. A., and Colten, H. R. (1999) *Immunopharmacology* **42,** 135–149
19. Murray, I., Sniderman, A. D., and Cianflone, K. (1999) *J. Lipid Res.*, **40,** 1671–1676
20. Murray, I., Sniderman, A. D., and Cianflone, K. (1999) *Am. J. Physiol.,* **277,** E474–E480
21. Lubinski, J. M., Wang, L., Soulika, A. M., Burger, R., Wetsel, R. A., Colten, H., Cohen, G. H., Eisenberg, R. J., Lambris, J. D., and Friedman, H. M. (1998) *J. Virol.* **72,** 8257–8263
22. Van Heek, M., Compton, D. S., France, C. F., Tedesco, R. P., Fawzi, A. B., Graziano, M. P., Sybertz, E. J., Strader, C. D., and Davis, H. R. Jr. (1997) *J. Clin. Invest.* **99,** 385–390
23. Ahren, B., Mansson, S., Gingerich, R. L., and Havel, P. J. (1997) *Am. J. Physiol.* **273,** R113–R120
24. Maeda, N. D., Li, H., Lee, D., Oliver, P., Quarfordt, S. H., and Osada, J. (1994) *J. Biol. Chem.* **269,** 23610–23616
25. Shimada, M., Shimano, H., Gotoda, T., Yamamoto, K., Kawamura, M., Inaba, T., and Yazaki, Y. (1993) *J. Biol. Chem.* **268,** 17924–17929
26. Weinstock, P. H., Bisgaier, C. L., Aalto-Setala, Radner, H., Ramakrishnan, R., Levakfrank, S., Essenburg, A. D., Zechner, R., and Breslow, J. (1995) *J. Clin. Invest.* **96,** 2555–2568
27. Yasushi, I., Azrolan, N., O'Connell, A., Walsh, A., and Breslow, J. L. (1990) *Science* **249,** 790–793
28. Kopecky, J., Hodney, Z., Rossmeisl, M., Syrovy, I., and Kozak, L. (1996) *Am. J. Physiol.* **270,** E768–E775
29. Hamann, A., Flier, J. S., and Lowell, B. B. (1996) *Endocrinology* **137,** 21–29
30. Hotamisligil, G. S., Johnson, R. S., Distel, R. J., Ellis, R., Papaioannou, V. E., and Spiegelman, B. M. (1996) *Science* **274,** 1377–1379
31. Ranheim, T., Dumke, C., Schueler, K. L., Cartree, G. D., and Attie, A. D. (1997) *Arterioscler. Thromb. Vasc. Biol.* **17,** 3286–3293
32. Gnudi, L., Tozzo, E., Shepherd, P. R., Bliss, J. L., and Kahn, B. B. (1995) *Endocrinology* **136,** 995–1002
33. Jensen, D. R., Schlaepfer, I. R., Morin, C. L., Pennington, D. S., Marcell, T., Ammon, S. M., Gutierrez-Hartmann, A., and Eckel, R. H. (1997) *Am. J. Physiol.* **273,** R683–R689
34. Morin, C. L., Eckel, R. H., Marcel, T., and Pagliassotti, M. J. (1997) *Endocrinology* **138,** 4665–4671
35. Frederich, R. C., Hamann, A., Anderson, S., Lollmann, B., Lowell, B. B., and Flier, J. S. (1995) *Nat. Med.* **1,** 1311–1314
36. Surwit, R. S., Feinglos, M. N., Rodin, J., Sutherland, A., Petro, A. E., Opara, E. C., Kuhn, C. M., and Rebuffe-Scrive, M. (1995) *Metabolism* **44,** 645–651
37. Merkel, M., Kako, Y., Radner, H., Cho, I. S., Ramasamy, R., Brunzell, J. D., Goldberg, I. J., and Breslow, J. L. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 13841–13846
38. Shimada, M., Ishibashi, S., Yamamoto, K., Kawamura, M., Watanabe, Y., Gotoda, T., Harada, K., Inaba, T., Ohsuga, J., Yazaki, Y., and Yamada, N. (1995) *Biochem. Biophys. Res. Commun.* **211,** 761–766
39. Bjorntorp, P. (1997) *Hum. Reprod.* **12,** 21–25
40. Appel, B., and Fried, S. K. (1992) *Am. J. Physiol.* **262,** E695–E699
41. Saxena, U., Witte, L. D., and Goldberg, I. J. (1989) *J. Biol. Chem.* **264,** 4349–4355
42. Bengtsson, G., and Olivecrona, T. (1979) *FEBS Lett.* **106,** 345–348
43. Peterson, J., Bihain, B. E., Bengtsson-Olivecrona, G., Deckelbaum, R. J., Carpentier, Y. A., and Olivecrona, T. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 909–913
44. Lemonnier, D. (1972) *J. Clin. Invest.* **51,** 2907–2915
45. Jesmok, G. J., Woods, E. F., Ditzler, W. S., and Walsh, G. (1981) *J. Parenter. Enteral Nutr.* **5,** 200–203
46. Moller, D. E., Chang, P. Y., Yaspelkis, B. B., Flier, J. S., Wallberg-Hendriksson, H., and Ivy, J. L. (1996) *Endocrinology* **137,** 2397–2405
47. Wetsel, R. A., Kildsgaard, J., Zsigmond, E., Liao, W., and Chan, L. (1999) *J. Biol. Chem.* **274,** 19429–19433
48. Liu, M. S., Jirik, F. R., LeBoeuf, R. C., Henderson, H., Castellani, L. W., Lusis, A. J., Ma, Y., Forsythe, I. J., Zhang, H., Kirk, E., Brunzell, J. D., and Hayden, M. R. (1994) *J. Biol. Chem.* **269,** 11417–11424
49. Reue, K., Purcell-Huynh, D. A., Leete, T. H., Doolittle, M. H., Durstenfeld, A., and Lusis, A. J. (1993) *J. Lipid Res.* **34,** 893–903
50. Jiao, S., Cole, T. G., Kitchens, R. T., Pfleger, B. A., and Schonfeld, G. (1990) *Metabolism* **39,** 155–160
51. Merkel, M., Weinstock, P. H., Chajek-Shaul, T., Radner, H., Yin, B., Breslow, J. L., and Goldberg, I. J. (1998) *J. Clin. Invest.* **102,** 893–901
52. Shepherd, P. R., Gnudi, L., Tozzo, E., Yang, H., Leach, F., and Kahn, B. B. (1993) *J. Biol. Chem.* **268,** 22243–22246
53. Despres, J. P. (1991) *Curr. Opin. Lipidol.* **2,** 5–15
54. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963) *Lancet* **1,** 785–789
55. Bjorntorp, P. (1990) *Arteriosclerosis* **10,** 493–496
56. McGarry, J. D. (1998) *Am. J. Clin. Nutr.* **67,** 500–504
57. Sniderman, A. D., and Kwiterovich, P. O., Jr. (1987) *NIH Publication 87-2646*, National Institutes of Health, Bethesda, MD
58. Cianflone, K., Maslowska, M., and Sniderman, A. D. (1990) *J. Clin. Invest.* **85,** 722–730
59. Purcell-Huynh, D. A., Farese, R. V., Jr., Johnson, D. F., Flynn, L. M., Pierotti, V., Newland, D. L., Linton, M. F., Sanan, D. A., and Young, S. G. (1995) *J. Clin. Invest.* **95,** 2246–2257
60. Lombardo, Y. B., Hron, W. T., Sobocinski, K. A., and Menahan, L. A. (1983) *Horm. Metabol. Res.* **16,** 37–42
61. Menahan, L. A. (1983) *Metabolism* **32,** 172–178
62. McKnight, S. L. (1998) *Genes Dev.* **12,** 3145–3148
63. Srivastava, R. A., Jiao, S., Tang, J. J., Pfleger, B. A., Kitchens, R. T., and Schonfeld, G. (1991) *Biochim. Biophys. Acta* **1086,** 29–43