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Reduced Body Weight, Adipose Tissue, and Leptin Levels Despite Increased Energy Intake in Female Mice Lacking Acylation-Stimulating Protein*

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ABSTRACT

Acylation-stimulating protein (ASP) is a potent lipogenic protein produced by adipocytes. *In vitro* studies have shown that ASP increases triglyceride synthesis and glucose transport in both murine and human adipocytes. Our initial study indicated that complement C3-deficient (−/−) mice (and, therefore, ASP deficient) demonstrated altered dietary postprandial triglyceride clearance. In the present study we examined the phenotype of female mice longitudinally on different diets. Female C3(−/−) mice on both low (10% of energy) and high (40% of energy) fat diets displayed an average reduction in total body weight of 10.1 ± 0.5% ($P < 0.0003$, by ANOVA) compared with the C3(+/+) littermates. Reductions in white adipose tissue mass accounted for most of this weight difference (59% reduction; $P < 0.01$ on low fat diet). Plasma leptin levels were significantly reduced in C3(−/−) mice on both high ($P < 0.001$) and low fat diets ($P < 0.01$). This reduction was significant even after adjusting for the reduced body weight and body fat ($P < 0.001$). Leptin reductions in the C3(−/−) were greater on the high fat diet and were associated with increased food intake (18 ± 2% increase; $P < 0.001$). Furthermore, there was a decrease in basal glucose levels and basal insulin levels [12.8% decrease in glucose at 14 weeks (HF; $P < 0.05$) and 41% decrease in insulin at 26 weeks (HF; $P < 0.05$)]. These *in vivo* experiments demonstrate that female mice lacking ASP have marked alterations of body weight, adiposity, plasma leptin, and plasma insulin levels. Decreased adiposity and leptin levels occurred in the ASP-deficient animals despite increased energy intake, suggesting that energy expenditure was elevated in these animals. Thus, ASP appears to have an important role in the regulation of energy balance in mice. (*Endocrinology* **141:** 1041–1049, 2000)

ACYLATION-STIMULATING protein (ASP) stimulates both adipocyte fatty acid esterification into triglyceride and glucose transport (reviewed in Ref. 1). This occurs via an increase in diacylglycerol acyltransferase activity (2) and translocation of glucose transporters (GLUT1, GLUT3, and GLUT4) from intracellular sites to the cell surface (3, 4). These dual effects are mediated through specific cell surface binding (5, 6), resulting in activation of a signaling pathway that includes protein kinase C (7). In addition to stimulation of fatty acid esterification, ASP has recently been shown to also inhibit hormone-sensitive lipase in adipocytes, independently and additively to insulin (8). The major site of action of ASP appears to be on adipocytes, as determined by competitive binding, stimulation of triglyceride synthesis, and enhanced glucose transport and transporter translocation (reviewed in Ref. 1). There is also a differentiation-specific increase in ASP binding and ASP response in adipocytes (1).

ASP is identical to complement C3adesArg. Cleavage of complement C3 through the alternate complement pathway via the interaction of C3, factor B, and adipsin generates C3a (9). Rapid cleavage of the C-terminal arginine by carboxypeptidase N generates ASP (9). Adipocytes are one of the few cells capable of producing all three of the factors (factor B, adipsin, and C3) that are required for the production of ASP (10, 11). ASP production increases consequent to adipocyte differentiation (10, 12) and also after chylomicron stimulation *in vitro* in adipocytes (13, 14). *In vivo* arterial-venous gradients across a sc adipose tissue bed in humans demonstrate postprandial production of ASP (15), and plasma ASP levels are elevated in obesity (16, 17). The postprandial increase in ASP is adipose tissue specific and is not observed in the general circulation (15, 18) or in arterial venous differences across muscle tissue (our unpublished observations).

The correlation between *in vivo* postprandial arterial-venous adipose tissue ASP production associated with dietary triglyceride clearance and *in vitro* functionality data with chylomicron-stimulated ASP production suggests that ASP might play an important role in dietary fat clearance and tissue deposition. Our initial studies in murine models with excess exogenous ASP confirm this. Administration of ASP to C57BL/6 mice resulted in decreased plasma triglyceride levels after an orally administered fat load (19). This increase in triglyceride clearance was dependent on the degree of postprandial lipemia (19). Initial studies were performed in complement C3 knockout mice (20), which lack circulating

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plasma C3 and complement activity (21). Being deficient in C3, these mice are unable to produce ASP. In young male mice (129Sv × C57BL/6 strain), although fasting triglyceride levels were not different from those in wild-type littermates, elevated triglyceride levels were observed postprandially after an oral fat load, suggesting delayed triglyceride clearance in the absence of ASP (22). Furthermore, administration of exogenous ASP to these functional ASP knockout male mice was able to reduce this postprandial lipemia and decrease plasma triglyceride levels (22). The female mice displayed similar, but less striking, differences in postprandial lipid metabolism (22).

Like ASP, leptin is produced by adipocytes (23) and is involved in the regulation of body fat stores. Leptin production is regulated by insulin responses to meals (24), an effect that appears to involve increased adipocyte glucose metabolism (25). Leptin is critically involved in the regulation of body energy balance via its central actions on food intake and energy expenditure (26). However, leptin also appears to have peripheral actions on substrate fluxes in the liver (27) and may act directly on adipocytes (28, 29), where it has been reported to increase lipolysis and impair insulin-mediated lipogenesis. Therefore, leptin and ASP may have opposing effects on adipocyte triglyceride stores. It is possible that ASP and leptin interact in a paracrine fashion to directly regulate adipocyte metabolism. In the present study longitudinal experiments were conducted to examine the effects of age and diet on adipose stores, energy intake, and circulating leptin and insulin levels in female mice that are functionally deficient in ASP due to genetic knockout of the C3 gene.

Materials and Methods

Ethics

All experimental protocols were approved by the Royal Victoria Hospital animal care committee and were in accordance with the guidelines set out by the Canadian committee on animal care.

Mice

Drs. H. Colten and R. H. Wetsel provided the knockout and wild-type mice for breeding. Development of the complement C3 knockout has been described previously in detail (20, 21). The mice were of (129Sv × C57BL/6) strain, and heterozygous mating produced the littermates [wild-type C3(+/+) and knockout C3(−/−)] used for the present experiments. Mice were housed in sterile barrier facilities with equal day/night periods. In all cases paired littermates were used for the study to randomize genetic variation.

Genotyping

For genotyping, tail DNA was extracted, and PCR was performed. PCR 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; and neomycin antisense, AGC TCT TCA GCA ATA TCA CGG CTA GCC. PCR 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

C3(+/+) and C3(−/−) female 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 pelleted low fat diet (LF) consisting of 19.3% protein, 67.3% carbohydrates, and 4.3% fat (wt/wt/wt) or high fat diet (HF) of 22.9% protein, 45.8% carbohydrate, and 20.3% fat (wt/wt/wt) modified from the report by Van Heek *et al.* (30) and obtained from Research Diets, Inc. (New Brunswick, NJ; diets D12477 and D12478, respectively). The diets contained 10% Cal (LF) and 40% Cal (HF) energy from fat, with a 1:1:1 ratio of saturated/monounsaturated/polyunsaturated fat and were stored at 4 C. Carbohydrate was in the form of cornstarch rather than sucrose (70% LF and 40% HF Cal). The vitamin and mineral content conformed with the American Institutes for Nutrition guidelines. The food was weighed twice weekly over a period of 16 weeks, and food intake was determined over the time period of 10–26 weeks of age.

Plasma assays

Blood was collected at 10, 14, 26, 32, and 48 weeks of age into EDTA-containing tubes by tail bleeding as previously described (19, 22) from mice fasted overnight (16 h) with water *ad libitum*. Blood was separated by centrifugation and stored at −80 C. Leptin was measured using a mouse leptin RIA assay (Linco Research, Inc., St. Charles, MO) as described by Ahren *et al.* (31). Fasting insulin was measured using a rat insulin RIA kit, which has 100% cross-reactivity to mouse insulin (as described by the manufacturer, Linco Research, Inc.). Glucose was measured using a Trinder glucose kit (Sigma, St. Louis, MO). Plasma nonesterified fatty acids, cholesterol and triglyceride were measured using colorimetric enzymatic kits (Roche, Laval, Canada).

Fat load

After an overnight fast (16 h), 400 μl olive oil (followed by 100 μl air above the oil) was given by gastric gavage using a feeding tube (12-cm curved ball tipped feeding needle (20) according to standard procedures as previously described (19, 22) and similar to previously published methods (32–35). There was a 2-week interval between the fasting blood sample and the fat load. Blood (40 μl) was collected at 0, 1, 2, 3, 4, and 6 h by tail bleeding.

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 then injected ip with a sterile D-glucose solution in saline and 2 mg/g BW from a stock solution of 200 mg/ml (0.010 ml/g BW). Blood was collected by tail bleeding at 0,15, 30, 60, 90, and 120 min (80 μl at 0, 30, 60, and 120 min for insulin and glucose, 20 μl at 15 and 90 min for glucose only).

Tissues

Mice were anaesthetized (0.01 ml/10 g BW, im) with a cocktail composed of 5 ml ketamine (100 mg/ml), 2.5 ml xylazine (20 mg/ml), 1 ml acepromazine (10 mg/ml), and 1.5 ml sterile saline. Blood was drawn (0.5 ml) by tail bleeding, and the mice were killed by cervical dislocation. Tissues were dissected, weighed, and frozen in liquid nitrogen. Four adipose tissues depots were collected: inguinal, pectoral (together with suprascapular), gonadal (up to the apex of the ovary) and perirenal (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.

Fecal analysis

The fat content of the feces was analyzed as described previously (36). The animals were placed in a chamber with a metabolic screen, and feces were collected over 24 h on normal chow (6% fat) or collected for 24 h after a fat load (6 h) with 6 h fasting, followed by food *ad libitum*. The stool was weighed and dried to constant weight, and the fat was extracted as described by Schwartz (36).

Statistical analyses

Results are presented as the mean ± SEM. The two groups were compared by repeated measures two-way ANOVA followed by Bon-

ferroni post test (where indicated), *t* test, or Pearson's correlation using computer-assisted analysis (SigmaStat, Jandel Scientific, San Rafael, CA; and Prism, GraphPad Software, Inc., San Diego, CA).

Results

In this study of female C3(+/+) and C3(−/−) mice, growth curves were measured from 4–26 weeks of age. Specific diets were fed from the age of 10 weeks on: LF: C3(−/−), n = 12; C3(+/+), n = 8; and HF: C3(−/−), n = 11; C3(+/+), n = 6. On the LF diet (Fig. 1 *left panel*), the C3(−/−) mice weighed 10.9 ± 0.5% less ($P < 0.0001$, by ANOVA), and this occurred primarily after 9 weeks of age (after puberty) and maturation of the fat pad [6 weeks cited by Shimomura (37)]. The results for the HF diet are also shown in Fig. 1 (*right panel*). Average weight gains in the knockout and wild-type mice (4 weeks to 25 weeks) were similar and greater than that in mice on the LF diet [11.5 ± 2.0% more in C3(+/+) and 20.5 ± 3.9% more in C3(−/−)]. Nonetheless, even on the high fat diet, the C3(−/−) mice weighed significantly less (−9.3 ± 0.4%; $P < 0.0003$).

To investigate which body tissues were responsible for this reduced weight, after 4 months on specific diets, tissues were collected from a subset of both genotypes and diets at 32 and 48 weeks of age. As shown in Fig. 2, at 32 weeks in C3(−/−) mice there were marked decreases in white adipose tissue depots on both LF (*left panel*) and HF (*right panel*) consistent with the decrease in body weight [27.9 ± 1.0 C3(−/−) *vs.* 34.2 ± 2.3 C3(+/+) g]. There were no significant changes in liver, kidney, brown adipose tissue, or heart weights. The total adipose tissue weight (sum of all four adipose depots, excluding brown adipose tissue) was decreased by 59% on LF [1.78 ± 0.27 C3(−/−) *vs.* 4.34 ± 1.0 C3(+/+)] and by 40% on HF [6.29 ± 1.28 C3 (−/−) *vs.* 10.41 ± 0.38 (+/+)] g/mouse. The C3(+/+) mice always had significantly greater adiposity (measured as the sum of the four adipose tissue depots/BW) on both diets [12% ± 2% C3(+/+) *vs.* 6% ± 1% C3(−/−) on LF diet and 24% ± 2% C3(+/+) *vs.* 18% ± 1% C3(−/−) on HF diet]. This difference in adipose tissue was also observed at 48 weeks on the low fat diet although to a lesser extent (35.3% decrease in total adipose tissue; $P < 0.04$, data not shown), whereas there was no difference on the HF diet.

Leptin levels are highly correlated with adipose tissue mass and were thus measured in these animals at 10, 14, and 26 weeks on the corresponding diets (Fig. 3). Leptin levels increased with age and even more so with fat content of the diet, as previously reported (30, 31, 38), accurately reflecting increases in adipose tissue mass. The leptin levels were significantly reduced in the knockout animals on both diets (LF, $P < 0.01$, ANOVA; HF, $P < 0.0001$, by ANOVA). Leptin levels were also measured at 32 and 48 weeks of age in a subset of mice at the time of death and dissection. At 32 weeks there were significant differences in leptin in the knockout *vs.* the wild-type mice on the LF diet [8.95 ± 2.0 (n = 4) *vs.* 18.7 ± 5.6 (n = 5) ng/ml; $P < 0.05$] and on the HF diet [17.4 ± 1.8 (n = 3) *vs.* 32.5 ± 3.5 (n = 4) ng/ml; $P < 0.025$], which corresponded to the reduced adipose tissue mass (Fig. 2). By 48 weeks of age, although the differences were maintained on a LF diet, on the HF diet the differences between C3(+/+) and C3(−/−) were no longer evident, again consistent with the adipose tissue weights [LF diet, 20.6 ± 6.1 C3(−/−) (n = 3) *vs.* 27.3 ± 5.7 C3(+/+) (n = 3); HF diet, 60.4 ± 5.5 C3(−/−) (n = 3) *vs.* 62.3 ± 5.2 C3(+/+) (n = 3) ng/ml leptin].

Leptin levels correlated very closely with the size of all adipose tissue depots (r = 0.799, 0.728, 0.909, and 0.844 for inguinal, pectoral, perirenal, and gonadal, respectively) as well as with total adipose tissue (r = 0.911; n = 24) for combined data of female mice at 32 and 48 weeks, LF and HF diets, and C3(−/−) and (+/+). However, the highest correlation was with body weight (r = 0.934, by Pearson correlation). The relationship between body weight and leptin levels (which is an indicator of adiposity) in both the C3(+/+) and C3(−/−) groups for all mice from 10–48 weeks of age was also significantly different. The data show that there is a linear relationship between body weight and

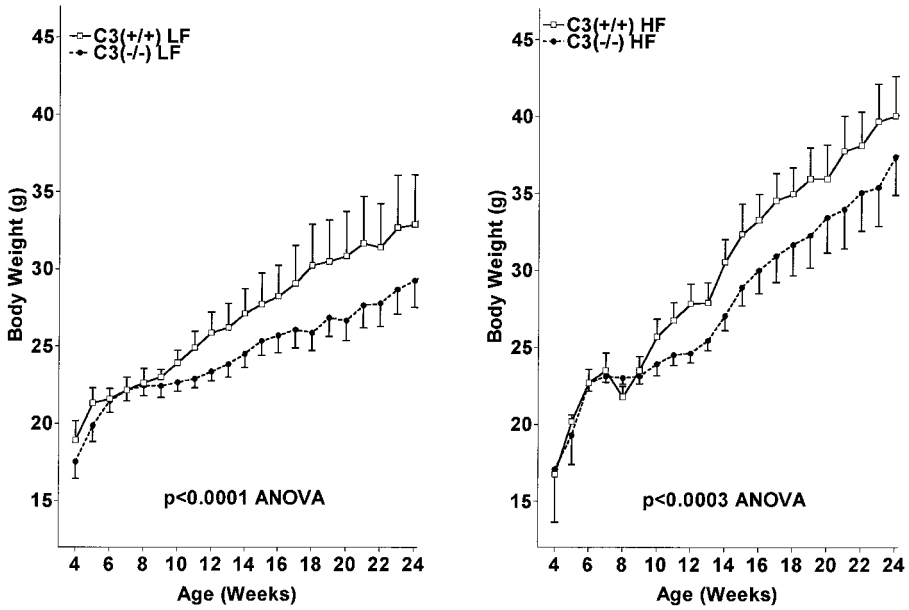

FIG. 1. Growth curves in mice on LF and HF diets. Knockout C3(−/−) mice (*filled circles, dotted line*) and wild-type C3(+/+) mice (*open squares, solid line*) on LF (10% Cal; *left panel*) and HF (40% Cal diet; *right panel*) diets were weighed twice weekly from 4–26 weeks of age. The diets were started at 10 weeks of age. The data are given as the mean ± SEM with C3(−/−) (LF, n = 12; HF, n = 11) and C3(+/+) (LF, n = 8; HF, n = 6). Statistical differences were determined by two-way repeated measures ANOVA.

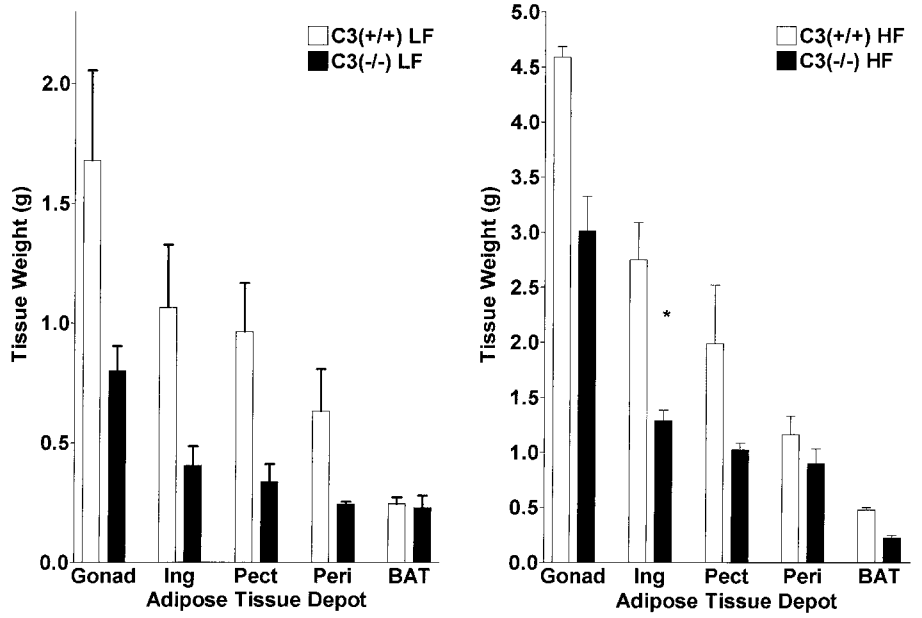

FIG. 2. Adipose tissue weights in mice on LF and HF diets. The adipose tissue weights of female knockout C3(−/−) mice (*filled bars*) and wild-type C3(+/+) mice (*open bars*) on a LF [10% Cal diet; *left panel*; C3(−/−), n = 4; C3(+/+), n = 5] or a HF [40% Cal diet; *right panel*; C3(−/−), n = 3; C3(+/+), n = 4] were measured at 32 weeks of age, and the data are represented as the mean ± SEM. Ing, Inguinal; Pect, pectoral; Peri, perirenal; Gonad, gonadal; BAT, brown adipose tissue. **, $P < 0.001$; *, $P < 0.05$ [C3(−/−) *vs.* C3(+/+)].

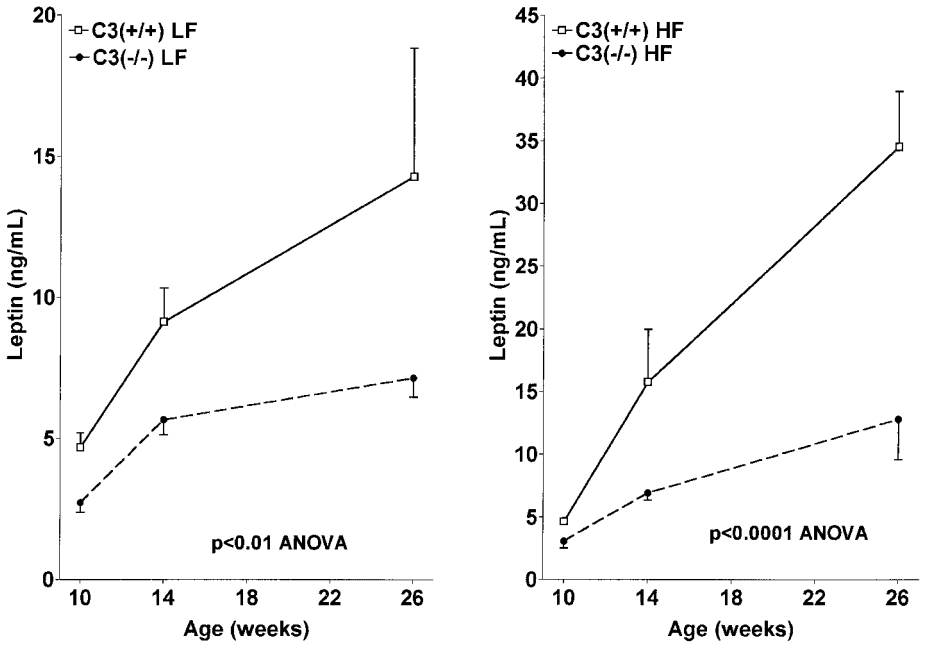

FIG. 3. Plasma leptin levels in mice on LF and HF diets. Plasma leptin levels at 10, 14, and 26 weeks were measured in wild-type C3(+/+) (*open squares*) and knockout C3(−/−) (*filled circles*) on the LF and HF diets (*left* and *right panels,* respectively). The sample sizes are five and three for the C3(−/−) and four and four for the C3(+/+) on the LF and HF diets, respectively. Results are shown as the mean ± SEM, and statistical differences were measured by two-way repeated measures ANOVA.

leptin in both sets of animals [$r^2 = 0.85$, slope = 1.08 ± 0.123, *x*-intercept = 20.1, n = 36 for C3(+/+); $r^2 = 0.70$, slope = 1.36 ± 0.095, *x*-intercept = 18.9, n = 38 for C3(−/−)]. For any given body weight, leptin levels were significantly lower in C3(−/−) mice ($P < 0.0001$).

Food intake was also measured and relative energy intake calculated as caloric intake per g BW over the 16-week diet period. Despite the decreased body weight in the knockout mice, there was no decrease in food intake noted for the C3(−/−) mice on the low fat diet [96.8 ± 1.1 C3(+/+) *vs.* 97.9 ± 1.4 C3(−/−) cal/week]. On the high fat diet, the food intake of the knockout mice increased markedly compared with that of the wild-type mice after 14 weeks of age, and this difference was maintained for the duration of the study (Fig. 4, left panel). The average energy intake was 107.3 ± 1.8 cal/week C3(+/+) *vs.* an energy intake of 125.9 ± 2.3 cal/week in the C3(−/−), an average increase of 17.5 ± 2.1% ($P < 0.0001$). Energy intake (in calories) per g BW was substantially greater (and therefore less efficient) in the knockout animals than in the wild-type mice on both diets, but especially the HF [Fig. 4, *right panel*; increase, 33.2 ± 2.7% cal/g BW; C3(−/−) *vs.* C3(+/+), $P < 0.0001$), and over the 16-week period, the average calories per week/g increase in BW was substantially greater in the C3(−/−) mice on both LF [172 ± 20 C3(+/+) *vs.* 196 ± 32 C3(−/−); 14% increase] and HF [160 ± 30 C3(+/+) *vs.* 192 ± 36 C3(−/−); 20% increase].

To determine whether this phenotype could be attributed to a lack of intestinal absorption, fecal fat content was analyzed in a subset of mice (16–20 weeks old) on a chow diet equal in fat content to the LF diet. There was no change in

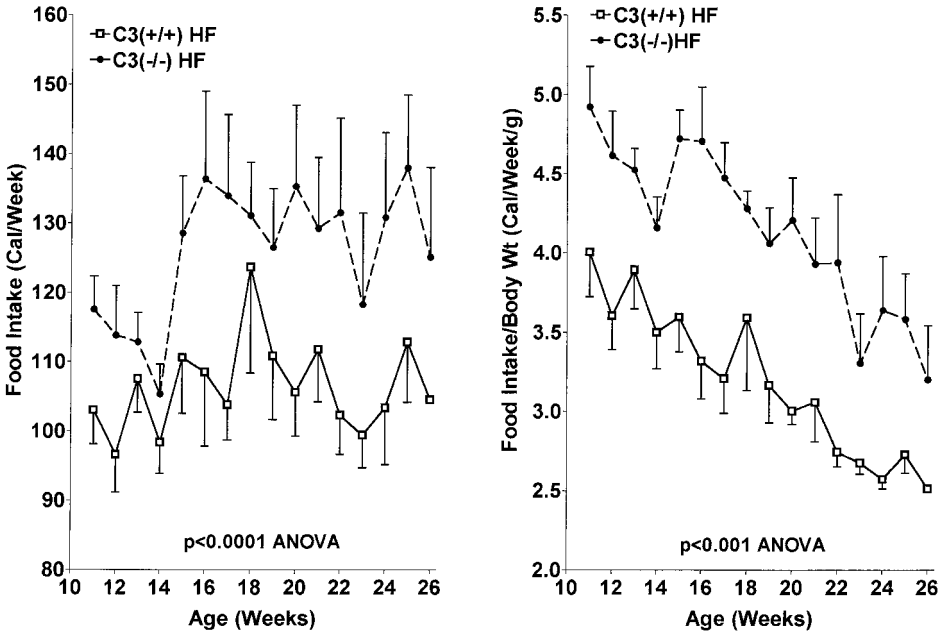

FIG. 4. Food intake and food efficiency on HF diets in wild-type and knockout mice. Food was weighed three times weekly from the age of 10–26 weeks and expressed as calories consumed per week (*left panel*) and food intake per g BW (calories consumed per week per g BW; *right panel*) for the wild-type C3(+/+) (*open squares*; n = 6) and knockout C3(−/−) (*filled circles;* n = 11) as the average ± SEM. Differences between wild-type and knockout were determined by two-way repeated measures ANOVA.

the fecal fat weight [5.7 ± 0.5% *vs.* 6.1 ± 1.0% on chow diet (n = 5 and 3) and 11.3 ± 1.1% *vs.* 10.0 ± 0.2% (wt/wt; n = 3 and 2) after fat load for C3(−/−) *vs.* C3(+/+) mice, respectively] or fecal lipid profile on TLC (data not shown).

Fasting plasma parameters were measured as shown in Table 1. There was no significant difference in fasting plasma triglyceride, cholesterol, or nonesterified fatty acid between C3(+/+) and C3(−/−), although in both there was an age-dependent increase in cholesterol and decrease in nonesterified fatty acids (NEFA) as noted previously (39, 40). Interestingly, glucose and insulin were significantly decreased in the C3(−/−) as well as the glucose × insulin product, and these differences were more marked on the HF diet. The correlation between insulin and glucose (all ages and diets) indicated that for any given concentration of insulin, glucose levels were always lower in the C3(−/−) ($P < 0.002$).

The glucose levels were investigated further with a glucose tolerance test in a subset of mice at 30 weeks of age (after 4.5 months on LF or HF diet). The glucose area under the curve (AUC) increased slightly on the HF fat diet *vs.* the LF diet, but there was no significant difference between the C3(+/+) and C3(−/−) (Table 2). What was striking, however, was that serum insulin in the C3(−/−) mice was markedly lower, both fasting (Table 1) and after the glucose tolerance test, as shown by the insulin AUC even on the HF diet (Table 2).

In the early postprandial period adipose tissue hormone-sensitive lipase is inhibited. Both insulin and ASP have been shown to inhibit hormone-sensitive lipase (8). We therefore examined the mice for changes in plasma NEFA and triglyceride postprandially after a fat load of 400 μl olive oil at 14 and 26 weeks of age (Table 3). At 14 weeks of age, there was no difference in triglyceride AUC between C3(+/+) and C3(−/−) on either LF or HF diet. However, at 26 weeks of age, on both LF and HF, the triglyceride AUC was reduced in C3(−/−) *vs.* C3(+/+) (Table 3). With regard to the NEFA, although there was no significant difference in fasting NEFA, there was an increase in NEFA AUC in the C3(−/−) *vs.* C3(+/+), especially at 26 weeks (Fig. 5, *left panel*). As shown in Fig. 5, *right panel*, there was also a consistent drop in NEFA at 1 h postprandially in the C3(+/+) mice on both LF and HF diets (HF, −24.0 ± 8.5%; HF, −32.3 ± 11.3%) at 4 months on the diet (26 weeks of age). This drop was absent in the C3(−/−) mice (average 1 h change: LF, 1.2 ± 7.2%; HF, 2.4 ± 8.6%).

Thus, in the female mice significant decreases were observed in body weight, adipose tissue, leptin levels, and food efficiency, and moderate changes were found in insulin, glucose, and fatty acid metabolism.

Discussion

In the present report, the ASP phenotype in the female C3(−/−) mice was characterized by marked decreases in adipose tissue, body weight, plasma leptin concentrations, and food efficiency with moderate changes in glucose, insulin, and postprandial NEFA. In male mice, however, the changes in adipose tissue, body weight, leptin, and food efficiency, although significant, were far less pronounced than those in the females, but the NEFA increases were greater (our unpublished observations). On the other hand, postprandial lipemia was greatly increased in the male C3(−/−) mice, a phenomenon that we have seen previously in young mice (22), but which was strikingly absent from female mice in the present study at any age or diet stage. This gender-specific difference may be due to two factors: 1) the greater reservoir of fat tissue in females *vs.* males as noted previously (41), and 2) the greater insulin sensitivity in females *vs.* males in mice (40, 42). Both of these would contribute to enhanced lipoprotein lipase mass and activity and enhanced postprandial clearance, as noted in rodents (43) and humans (44). In general, gender-specific phenotype penetrance is not uncommon in transgenic mice.

We would hypothesize that the increased postprandial

TABLE 1. Fasting parameters measured in plasma

	Basal chow	Low fat	High fat	Low fat	High fat
Age (weeks)	10	14	14	26	26
n	(6/10)	(6/10)	(10/11)	(6/11)	(9/11)
TG					
+/+	54.5 ± 1.8	46.8 ± 5.3	44.2 ± 6.1	44.0 ± 3.6	50.0 ± 6.1
−/−	54.5 ± 4.6	41.4 ± 2.7	50.8 ± 5.6	37.5 ± 2.7	45.0 ± 3.1
Chol					
+/+	56.6 ± 7.0	64.3 ± 7.1	84.8 ± 17.3	79.5 ± 7.3	99.9 ± 11.1
−/−	57.6 ± 3.9	68.8 ± 6.3	65.1 ± 4.3	83.4 ± 6.8	97.3 ± 15.0
NEFA					
+/+	0.91 ± 0.23	0.43 ± 0.06	0.43 ± 0.06	0.37 ± 0.05	0.31 ± 0.06
−/−	1.54 ± 0.41	0.54 ± 0.06	0.37 ± 0.04	0.29 ± 0.03	0.29 ± 0.03
Glu					
+/+	105.6 ± 7.3	100.5 ± 8.1	102.5 ± 6.3	100.9 ± 10.0	105.2 ± 7.9
−/−	90.8 ± 3.2[a]	89.4 ± 6.8	89.4 ± 2.9[a]	96.1 ± 3.5	102.2 ± 5.8
Ins					
+/+	0.19 ± 0.03	0.19 ± 0.02	0.19 ± 0.03	0.30 ± 0.04	0.51 ± 0.05
−/−	0.20 ± 0.01	0.21 ± 0.02	0.23 ± 0.02	0.22 ± 0.02[a]	0.28 ± 0.03[a]
Glu × Ins					
+/+	22.9 ± 2.4	19.2 ± 1.7	21.0 ± 3.3	29.2 ± 3.8	54.1 ± 7.9
−/−	18.2 ± 1.6[a]	18.7 ± 3.2	20.3 ± 1.8	21.7 ± 2.2[a]	30.7 ± 4.4[a]

Fasting plasma parameters were measured in knockout C3(−/−) and wild type C3(+/+) mice at 10, 14, and 26 weeks of age on low fat (10% Cal) and high fat (40% Cal) diets. Data are the mean ± SEM. TG, Triglyceride (milligrams per dl); Chol, cholesterol (milligrams per dl); NEFA, nonesterified fatty acid (millimolar concentrations); Glu, glucose (milligrams per dl); Ins, insulin (nanograms per ml); Glu × Ins, the product of glucose and insulin concentrations. The sample size is displayed at the *top* of the columns for wild-type C3(+/+) and C3(−/−) mice, respectively.

[a] $P < 0.05$, comparing between genotypes within age groups.

TABLE 2. Postprandial glucose area under the curve after a glucose tolerance test

Glucose tolerance test	Low fat		High fat	
	C3(+/+)	C3(−/−)	C3(+/+)	C3(−/−)
n	(5)	(2)	(3)	(3)
Glucose AUC (mg/dl·h)	229 ± 8.3	237 ± 11.4	252 ± 57	327 ± 33
Insulin AUC (mg/dl·h)	1.63 ± 0.19	0.81 ± 0.03[a]	1.63 ± 0.14	0.75 ± 0.01[a]

Results are given as the postprandial glucose area under the curve (AUC) and insulin area under the curve (AUC) after a glucose tolerance test. The data shown are for knockout C3(−/−) and wild-type C3(+/+) mice at 30 weeks of age (4 months on diet) for the glucose tolerance test. The diets are low fat (10% Cal) and high fat (40% Cal). Data are represented as the mean ± SEM. The sample size (n) is displayed at the *top* of the columns for C3(+/+) and C3(−/−) mice, respectively.

[a] $P < 0.005$ for C3(−/−) *vs.* C3(+/+).

TABLE 3. Postprandial triglyceride area under the curve after an oral fat load

Oral fat load	Low fat		High fat	
	C3(+/+)	C3(−/−)	C3(+/+)	C3(−/−)
n	(6)	(10)	(10)	(10)
14 weeks old	441.4 ± 62.5	418.8 ± 91.1	476.8 ± 78.3	564.5 ± 105.3
26 weeks old	563.1 ± 76.8	330.2 ± 22.0[a]	652.2 ± 113.8	553.6 ± 56.1

Results are given as the postprandial triglyceride area under the curve (AUC) after an oral fat load of 400 μl olive oil, as described in *Materials and Methods*. The data shown are for knockout C3(−/−) and wild-type C3(+/+) mice at 14 and 26 weeks of age (4 months on diet). The diets are low fat (10% Cal) and high fat (40% Cal). Data are given as the mean ± SEM. The sample sizes are displayed at the *top* of the columns for wild-type C3(+/+) and C3(−/−) mice, respectively.

[a] $P < 0.003$ significant difference between mice comparing C3(+/+) to C3(−/−).

NEFA would be due to a reduced efficiency of adipose tissue NEFA trapping due to the lack of ASP. This reduced NEFA trapping may lead to disturbances that impact on glucose metabolism. In studies described previously, when delivery of NEFA to peripheral tissues is disrupted (with a targeted lipoprotein lipase knockout), fasting glucose is proportionally lower (45). Similarly, when GLUT4 is overexpressed in adipose tissue, this enhances glucose utilization at the expense of fatty acids (46). Thus, NEFA and glucose metabolism are closely linked, and this may explain the increased postprandial NEFA but reduced glucose and insulin as well as increased insulin sensitivity seen in our C3(−/−) mice. Finally, the decreased glucose and decreased weight gain may explain the protection against high fat diet-induced insulin resistance, as both weight gain and increased plasma glucose contribute directly to insulin resistance (47).

The size of the adipose tissue is regulated by the efficiency of triglyceride synthesis and storage as well as inhibition of lipolysis via hormone-sensitive lipase. ASP appears to affect both of these processes *in vitro*. In the present studies, the lack of ASP in female mice results in decreased body weight and adipose tissue mass, implicating ASP not only in the effi-

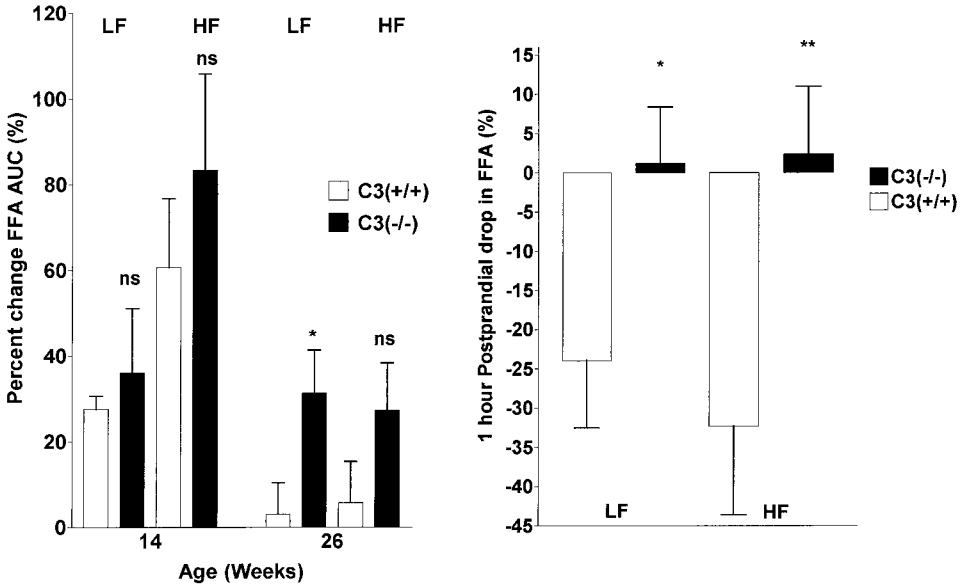

FIG. 5. Increases in NEFA AUC and 1 h postprandial drop after an oral fat load. The plasma fatty acid concentration was measured at 0, 1, 2, 3, 4, and 6 h after an oral fat load. The AUC for NEFA was determined over the whole time course, and the percent change relative to basal NEFA was calculated (*left panel*). The percent drop in NEFA at 1 h *vs.* the basal (time zero) value was also calculated (*right panel*). Basal NEFA concentrations are given in Table 1. Data are shown for C3(−/−) (*filled bars*) and C3(+/+), (*open bars*) for mice on LF (10%) and HF (40%) diets. *, $P < 0.05$; **, $P < 0.01$.

ciency of NEFA trapping (48, 49) but also in the regulation of adipose tissue size. As mice age, body growth diminishes, and adipose tissue stores normally accumulate. With the added input of a HF diet, this overcomes the inefficiency of the adipose tissue seen in the C3(−/−) mice, so that gradually the differences in adipose tissue mass between C3(−/−) and C3 (+/+) mice are reduced. This decrease in adiposity could be due to a selective reduction in gastrointestinal fat absorption, but malabsorption was ruled out because there were no changes in fecal lipid content or lipid composition. As the delivery of fat into the system is normal, and postprandial lipemia is similar in C3(+/+) and C3(−/−) mice, we postulate that the reduction in adipose tissue size is a consequence of reduced storage of dietary triglyceride (through effects on esterification enzymes) and/or enhanced lipolysis of adipose tissue triglyceride. The lack of ASP is consistent with changes in both, as ASP is able to affect both processes.

The lack of ASP not only disrupts adipose tissue balance and NEFA/glucose metabolism (as described above), but also appears to alter overall energy metabolism. This is exemplified by the changes in plasma leptin concentration and feeding behavior/body weight balance. The simplest explanation for the reduced plasma leptin is that it is a consequence of reduced adipose tissue mass, as leptin is highly correlated to adipose tissue mass and body weight (38). However, the correlation analysis suggested that the leptin levels were decreased more than could be explained by the decreased body weight, and this occurred despite the increased food intake, which is an important determinant of leptin production (24, 26). An altered set-point for leptin to body weight has also been demonstrated in β_3-adrenergic knockout mice (50). It may be that ASP causes an indirect effect on leptin via the changes in plasma glucose and insulin, as both of these affect leptin levels (25, 51, 52), or ASP may have an as yet undefined direct effect on leptin secretion in adipose tissue.

The consequences of the decreased leptin are increased eating behavior. However, at the same time that the caloric intake/body weight increases, there must be simultaneous nutrient repartitioning into tissues other than adipose tissue or the body weight in C3(−/−) would be increased relative to that in wild-type mice. The most likely key target tissues would be muscle and brown adipose tissue, and these issues are now being explored.

There are other instances of leanness in murine models exhibiting moderate (53–55) or dramatic (37, 56, 57) losses in adipose tissue. Mice with major adipose tissue reductions demonstrated increased levels of insulin, glucose, and decreased leptin (56, 57). Plasma NEFA levels were unchanged (56) or increased (37). By contrast, with moderate losses of adipose tissue (similar to our mice), there was little change from wild-type mice in fasting plasma cholesterol, triglyceride, NEFA, insulin, and glucose and no evidence of insulin resistance (53). Interestingly, in the aP2/diptheria toxin white adipose tissue ablation mouse (56), adipose tissue reduction only developed postmaturation of the fat pads (after 8 weeks of age) similar to our C3(−/−) murine model, suggesting that it is at the point of puberty and adipose maturation that these differences become apparent. In the protein kinase A subunit knockout (53), females were predominantly affected over males, again similar to the C3(−/−) mice presented here. Our mice have reduced leptin levels, which may suggest increased leptin sensitivity, resulting in increased thermogenesis. Consequently, food intake (hyperphagia) may increase to compensate. Hyperphagia and reduced adipose tissue mass were also observed in both the protein kinase A subunit knockouts (53) and white adipose tissue ablation mice (56).

Adipose tissue loss in humans can also produce metabolic consequences similar to those seen in the C3(−/−) mice. In postobese women there appears to be a drive to regain body weight and reestablish the set-point of adiposity. Increased lipoprotein lipase activity is observed (58), and in some studies greater insulin sensitivity was reported with weight loss (59). Lower insulin levels (60), enhanced triglyceride clearance (Faraj, M., K. Cianflone, and A. D. Sniderman, personal communication), reduced glucose levels (61), reduced adi-

pose tissue mass and reduced serum leptin (62, 63) have been observed in postobese humans. In many ways, the description is similar to our C3(−/−) mice.

Human genetic C3 deficiency is extremely rare, with only 20 cases reported to date in the literature (64). Wetsel *et al.* indicated that these "C3-deficient individuals do not appear to display increased predisposition to hyperlipidemia or coronary artery disease" (65); however, in the review cited (64), no lipid or lipoprotein information is provided. Complement C3 deficiencies are commonly associated with membranoproliferative glomerulonephritus, partial lipodystrophy, and occasionally systemic lupus erythematosus symptoms (reviewed (64, 66–69). Unfortunately, the majority of these patients were examined from an immunological standpoint, and we have been unable to find any detailed lipid and/or lipoprotein analyses of patients with C3 deficiency.

The reduced adiposity in the female C3 knockout mice resembles the phenotype of acquired partial lipodystrophy seen in humans. Partial lipodystrophy may be classified as congenital or acquired. The congenital form often manifests in childhood, affects primarily females, and is associated with hyperlipidemia, hyperinsulinemia, and abnormal glucose tolerance (70–72). Acquired PLD occurs primarily in adults, and there is little documentation of lipid and glucose profiles. The acquired form affects both males and females. Of note, it is associated specifically with hypocomplementemia of C3 (66) due to enhanced catabolism as well as reduced biosynthesis of C3 (73). This enhanced catabolism is the consequence of the presence of a C3 nephritic factor, which acts as an autoantibody, stabilizing the C3 convertase against spontaneous decay and enhancing C3 breakdown and clearance (74), thus specifically affecting the proximal portion of the alternate complement pathway. In a review of 21 cases with acquired partial lipodystrophy, 80.9% demonstrated markedly reduced complement C3 concentrations (66). Our murine model demonstrates similarities to humans with acquired partial lipodystrophy, as evidenced by depleted C3 levels and reduced adipose tissue.

In summary, ASP has been shown previously to play a role postprandially in both humans and mice. The present study demonstrates that a lack of ASP has clear effects on adiposity, energy metabolism, and adipose metabolism in female mice. In the future, the ASP system may represent a potential pharmacological target for reducing obesity.

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References

1. **Cianflone K, Maslowska M, Sniderman AD** 1999 Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. Semin Cell Dev Biol 10:31–41
2. **Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA** 1991 Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. Lipids 26:495–499
3. **Tao YZ, Cianflone K, Sniderman AD, Colby-Germinario SP, Germinario RJ** 1997 Acylation stimulating protein (ASP) regulates glucose transport in the rat L6 muscle cell line. Biochim Biophys Acta 1344:221–229
4. **Germinario R, Sniderman AD, Manuel S, Pratt S, Baldo A, Cianflone K** 1993 Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation stimulating protein. Metabolism 42:574–580
5. **Kalant D, Zhang ZJ, Cianflone K, Sniderman AD** 1995 Specific binding of ASP to human fibroblasts and human adipocytes. Clin Invest Med [B] 18:B10
6. **Murray I, Parker RA, Kirchgessner TG, Tran J, Zhang ZJ, Westerlund J, Cianflone K** 1997 Functional bioactive recombinant acylation stimulating protein is distinct from C3a anaphylatoxin. J Lipid Res 38:2492–2501
7. **Baldo A, Sniderman AD, Yazruel Z, Cianflone K** 1995 Signal transduction pathway of acylation stimulating protein: involvement of protein kinase C. J Lipid Res 36:1415–1426
8. **Van Harmelen V, Reynisdottir S, Cianflone K, Degerman E, Hoffstedt J, Nilsell K, Sniderman AD, Arner P** 1999 The effect of acylation stimulating protein (ASP) and insulin on the release of fatty acids from isolated human fat cells. J Biol Chem 274:18243–18251
9. **Hugli TE** 1990 Structure and function of C3a anaphylatoxin. Curr Top Microbiol Immunol 153:181–208
10. **Cianflone K, Roncari DAK, Maslowska M, Baldo A, Forden J, Sniderman AD** 1994 The adipsin-acylation stimulating protein system in human adipocytes: regulation of triacylglycerol synthesis. Biochemistry 33:9489–9495
11. **Choy LN, Spiegelman BM** 1996 Regulation of alternative pathway activation and C3a production by adipose cells. Obes Res 4:521–532
12. **Cianflone K, Maslowska M** 1995 Differentiation induced production of ASP in human adipocytes. Eur J Clin Invest 25:817–825
13. **Maslowska M, Scantlebury T, Germinario R, Cianflone K** 1997 Acute *in vitro* production of ASP in differentiated adipocytes. J Lipid Res 38:21–31
14. **Scantlebury T, Maslowska M, Cianflone K** 1998 Chylomicron specific enhancement of acylation stimulating protein (ASP) and precursor protein C3 production in differentiated human adipocytes. J Biol Chem 273:20903–20909
15. **Saleh J, Summers LKM, Cianflone K, Fielding BA, Sniderman AD, Frayn KN** 1998 Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue *in vivo* in the postprandial period. J Lipid Res 39:884–891
16. **Cianflone K, Sniderman AD, Kalant D, Marliss EB, Gougeon R** 1995 Response of plasma ASP to a prolonged fast. Int J Obes 19:604–609
17. **Maslowska M, Vu H, Phelis S, Sniderman AD, Rhodes B, Blank D, Cianflone K** 1999 Plasma ASP, adipsin, and lipids in non-obese and obese populations. Eur J Clin Invest 29:679–686
18. **Charlesworth JA, Peake PW, Campbell LV, Pussell BA, O'Grady S, Tzilopoulos T** 1998 The influence of oral lipid loads on acylation stimulating protein (ASP) in healthy volunteers. Int J Obes Rel Metab Dis 22:1096–1102
19. **Murray I, Sniderman AD, Cianflone K** 1999 Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein (ASP) in C57BL/6 mice. Am J Physiol 40:E474–E480
20. **Circolo A, Garnier G, Fukuda K, Wang X, Tunde H, Szalai AJ, Briles DE, Volanakis JE, Wetsel RA, Colten HR** 1999 Genetic disruption of the murine complement C3 promoter region generates deficient mice with extra-hepatic expression of C3 mRNA. Immunopharmacology 42:135–149
21. **Lubinski JM, Wang L, Soulika AM, Burger R, Wetsel RA, Colten H, Cohen GH, Eisenberg RJ, Lambris JD, Friedman HM** 1998 Herpes simplex virus type 1 glycoprotein gC mediates immune evasion *in vivo*. J Virol 72:8257–8263
22. **Murray I, Sniderman AD, Cianflone K** 1999 Mice lacking acylation stimulating protein (ASP) have delayed postprandial triglyceride clearance. J Lipid Res 40:1671–1676
23. **Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432
24. **Havel PH, Twonsend R, Chaump L, Teff K** 1999 High-fat meals reduce 24-h circulating leptin concentrations in women. Diabetes 48:334–341
25. **Mueller WM, Gregoire FM, Stanhope KC, Mobbs CV, Mizuno TM, Warden CH, Stern JS, Havel PJ** 1998 Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 139:551–558
26. **Havel PJ** 1999 Mechanisms regulating leptin production: implications for control of energy balance. Am J Clin Nutr 70:305–306
27. **Brazilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L** 1997 Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105–3110
28. **Muller G, Ertl J, Gerl M, Preibisch G** 1997 Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J Biol Chem 272:10585–10593
29. **Fuhbeck G, Aguado M, Martinez JA** 1997 *In vitro* lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. Biochem Biophys Res Commun 240:590–594
30. **Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, Davis HRJ** 1997 Diet-induced obese mice develop peripheral, but not central, resistance to leptin. J Clin Invest 99:385–390
31. **Ahren B, Mansson S, Gingerich L, Havel PJ** 1997 Regulation of plasma leptin in mice: incluence of age, high-fat diet, and fasting. Am J Physiol 273:R113–R20
32. **Maeda ND, Li H, Lee D, Oliver P, Quarfordt SH, Osada J** 1994 Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. J Biol Chem 269:23610–23616
33. **Shimada M, Shimano H, Gotoda T, Yamamoto K, Kawamura M, Inaba T, Yazaki Y** 1993 Overexpression of human lipoprotein lipase in transgenic mice. Resistance to diet-induced hypertriglyceridemia and hypercholesterolemia. J Biol Chem 268:17924–17929

34. **Weinstock PH, Bisgaier CL, Aalto-Setala, Radner H, Ramakrishnan R, Levakfrank S, Essenburg AD, Zechner R, Breslow J** 1995 Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. Mild hypertriglyceridemia with impaired very low density lipoprotein clearance in heterozygotes. J Clin Invest 96:2555–2568
35. **Yasushi I, Azrolan N, O'Connell A, Walsh A, Breslow JL** 1990 Hypertriglyceridemia as a result of human Apo CIII gene expression in transgenic mice. Science 249:790–793
36. **Schwarz M, Lund EG, Setchell KDR, Kayden HJ, Zerwekh JE, Bjorkhem I, Herz J, Russell DW** 1996 Disruption of cholesterol 7 alpha-hydroxylase gene in mice. J Biol Chem 271:18024–18031
37. **Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS** 1998 Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital lipodystrophy. Genes Dev 12:3182–194
38. **Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS** 1995 Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1:1311–134
39. **Weibust RS** 1978 Inheritance of plasma cholesterol levels in mice. Genetics 303:303–312
40. **Ranheim T, Dumke C, Schueler KL, Cartree GD, Attie AD** 1997 Interaction between BTBR and C57BL/6J genomes produces an insulin resistance syndrome in (BTBR×C57BL/6J) F_1 mice. Arterio Thromb Vasc Biol 17:3286–3293
41. **Lemonnier D** 1972 Effect of age, sex, and sites on the cellularity of the adipose tissue in mice and rats rendered obese by a high-fat diet. J Clin Invest 51:2907–2915
42. **Moller DE, Chang PY, Yaspelkis BB3, Flier JS, Wallberg-Hendriksson H, Ivy JL** 1996 Transgenic mice with muscle-specific insulin resistance develop increased adiposity, impaired glucose tolerance, and dyslipidemia. Endocrinology 137:2397–2405
43. **Jesmok GJ, Woods EF, Ditzler WS, Walsh G** 1981 Fat emulsion catabolism *in vitro* and *in vivo*-sex related differences. J Parenter Enteral Nutr 5:200–203
44. **Tollin C, Ericsson M, Johnson O, Backman C** 1985 Clearance of triglycerides from the circulation and its relationship to serum lipoproteins: influence of age and sex. Scand J Clin Lab Invest 45:679–684
45. **Merkel M, Weinstock PH, Chajek-Shaul T, Radner H, Yin B, Breslow JL, Goldberg IJ** 1998 Lipoprotein lipase expression exclusively in the liver. A mouse model for metabolism in the neonatal period and during cachexia. J Clin Invest 102:893–901
46. **Gnudi L, Shepherd PR, Kahn BB** 1996 Overexpression of GLUT4 selectively in adipose tissue in transgenic mice: implications for nutrient partitioning. Proc Nutr Soc 55:191–199
47. **Triscari J, Nauss-Karol C, Levin BE, Sullivan AC** 1985 Changes in lipid metabolism in diet-induced obesity. Metabolism 34:580–587
48. **Sniderman AD, Cianflone K, Arner P, Summers L, Frayn K** 1998 The adipocyte, fatty acid trapping and atherogenesis. Arterio Thromb Vasc Biol 18:147–151
49. **Fielding BA, Frayn KN** 1998 Lipoprotein lipase and the disposition of dietary fatty acids. Br J Nutr 80:495–502
50. **Revelli JP, Preitner F, Samec S, Muniesa P, Kuehne F, Boss O, Vassalli JD, Dulloo A, Seydoux J, Giacobino JP, Huarte J, Ody C** 1997 Targeted gene disruption reveals a leptin-independent role for the mouse β_3-adrenoceptor in the regulation of body composition. J Clin Invest 100:1098–1106
51. **Bradley RL, Cheatham B** 1999 Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes 48:272–278
52. **Russell CD, Petersen RN, Rao SP, Ricci MR, Prasad A, Zhang Y, Brolin RE, Fried SK** 1998 Leptin expression from adipose tissue in obese humans: depot-specific regulation by insulin and dexamethasone. Am J Physiol 275:E507—E515
53. **Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, McKnight SG** 1996 Genetically lean mice result from targeted disruption of the RII-β subunit protein kinase A. Nature 382:622–626
54. **Soloveva V, Graves RA, Rasenick MM, Spiegelman B, Ross SR** 1997 Transgenic mice overexpressing the β_1-adrenergic receptor in adipose tissue are resistant to obesity. Mol Endocrinol 11:27–38
55. **Luetteke NC, Lee DC, Palmiter RD, Brinster RL, Sandgren EP** 1993 Regulation of fat and muscle development by transforming growth factor α in transgenic mice and cultured cells. Cell Growth Differ 4:203–213
56. **Burant CF, Sreenan S, Hirano K, Tai TC, Lohmeiller J, Lukens J, Davidson NO, Graves RA** 1997 Troglitazone action is independent of adipose tissue. J Clin Invest 100:2900–2908
57. **Moitra J, Mason M, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C** 1998 Life without white fat: a transgenic mouse. Genes Dev 12:3168–181
58. **Astrup A** 1993 Dietary composition, substrate balances and body fat in subjects with a predisposition to obesity. Int J Obes Relat Metab Disord [Suppl 3] 17:S32—S36
59. **Enzi G, Busetto SS, Del Prato SS, De Kreutzenberg S, Valente P, Lise M, Favretti F** 1994 Modification of glucose tolerance and insulin-mediated glucose and non-esterified fatty acids utilization in morbid obese patients after a surgically induced massive weight loss. In: Angel A, Anderson H, Bouchard C, Lau D, Leiter L, Mendelson R (eds) Progress in Obesity Research, ed 7. Libbey: London, pp 533–539
60. **Fletcher JM, McNurlan MA, McHardy KC** 1989 Residual abnormalities of insulin secretion and sensitivity after weight loss by obese women. Eur J Clin Nutr 43:539–545
61. **Cowan GS, Buffington CK** 1998 Significant changes in blood pressure, glucose and lipids with gastric bypass surgery. World J Surg 22:987–992
62. **Keim NL, Stern JS, Havel PJ** 1998 Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. Am J Clin Nutr 68:794–801
63. **Weigle DS, Duell PB, Conner WE, Steiner RA, Soules MR, Kuijper JL** 1997 Effects of fasting, refeeding, and dietary fat restriction on plasma leptin levels. J Endocrinol Metab 82:561–565
64. **Singer L, Colten HR, Wetsel RA** 1994 Complement C3 deficiency: Human, animal and experimental models. Pathobiology 62:14–28
65. **Wetsel RA, Kildsgaard J, Zsigmond E, Liao W, Chan L** 1999 Genetic deficiency of acylation stimulating protein (ASP/C3asesArg) does not cause hyperapobetalipoproteinemia in mice. J Biol Chem 274:19429–19433
66. **Sissons PJG, West RJ, Fallows J, Williams DG, Boucher BJ, Amos N, Peters DK** 1976 The complement abnormalities of lipodystrophy. N Engl J Med 294:461–465
67. **Skattum L, Martensson U, Sjoholm AG** 1997 Hypocomplementaemia caused by C3 nephritic factors (C3 NeF): clinical findings and the coincidence of C3 NeF type II with anti-C1q autoantibodies. J Intern Med 242:455–464
68. **Walport MJ, Davies KA, Botto M, Naughton MA, Isenberg DA, Biasi D, Powell RJ, Cheung NT, Struthers GR** 1994 C3 nephritic factor and SLE: report of four cases and review of the literature. Q J Med 87:609–615
69. **McLean RH, Hoefnagel D** 1998 Partial lipodystrophy and familial C3 deficiency. Hum Hered 30:149–154
70. **Davidson MB, Young RT** 1975 Metabolic studies in familial partial lipodystrophy of the lower trunk and extremities. Diabetologia 11:561–568
71. **Boucher BJ, Cohen RD, Frankel RJ, Mason SA, Broadley G** 1973 Partial and total lipodystrophy: changes in circulating sugar, non-esterified fatty acids, insulin and growth hormone following administration of glucose and insulin. Clin Endocrinol (Oxf) 2:111–126
72. **Burn J, Baraitser M** 1986 Partial lipodystrophy with insulin resistant diabetes and hyperlipidemia (Dunnigan syndrome). J Med Genet 23:128–130
73. **Charlesworth JA, Williams GD, Sherington WE, Lachman PJ, Peters DK** 1974 Metabolic studies of the third component of complement and the glycine-rich β glycoprotein in patients with hypocomplementemia. J Clin Invest 53:1578–1587
74. **Tanuma T, Ohi H, hatano M** 1990 Two types of C3 nephritic factor: properdin-dependent C3NeF and properdin-independent C3NeF. Clin Immunol Immunopathol 56:226–238