Acylation-stimulating Protein (ASP)/Complement C3adesArg Deficiency Results in Increased Energy Expenditure in Mice*

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Acylation-stimulating protein (ASP) acts as a paracrine signal to increase triglyceride synthesis in adipocytes. In mice, C3 (the precursor to ASP) knock-out (KO) results in ASP deficiency and leads to reduced body fat and leptin levels yet they are hyperphagic. In the present study, we investigated the mechanism for this energy repartitioning. Compared with wild-type (WT) mice, male and female C3(−/−) ASP-deficient mice had elevated oxygen consumption (VO2) in both the active (dark) and resting (light) phases of the diurnal cycle: +8.9% males (p < 0.05) +9.4% females (p < 0.05). Increased physical activity (movement) was observed during the dark phase in female but not in male KO animals. Female WT mice moved 16.9 ± 2.4 m whereas KO mice moved 30.1 ± 5.4 m, over 12 h, +78.4%, p < 0.05. In contrast, there was no difference in physical activity in male mice, but a repartitioning of dietary fat following intragastric fat administration was noted. This was reflected by increased fatty acid oxidation in liver and muscle in KO mice, with increased UCP2 (inguinal fat) and UCP3 (muscle) mRNA expression (p = 0.005 and 0.036, respectively). Fatty acid uptake into brown adipose tissue (BAT) and white adipose tissue (WAT) was reduced as reflected by a decrease in fatty acid incorporation into lipids (BAT −68%, WAT −29%). The decrease of FA incorporation was normalized by intraperitoneal administration of ASP at the time of oral fat administration. These results suggest that ASP deficiency results in energy repartitioning through different mechanisms in male and female mice.

Acylation-stimulating protein (ASP) is an adipocyte-derived protein that has potent anabolic effects on human adipose tissue where it increases glucose uptake and non-esterified fatty acid (NEFA) storage (1, 2) via translocation of glucose transporters (GLUT1, GLUT3, and GLUT4) from intracellular sites to the cell surface (3, 4) and activation of diacylglycerol acyltransferase (DGAT) (2). These effects appear to be mediated through specific cell surface binding (5, 6) resulting in activation of a signal pathway that includes protein kinase C (7). In addition, ASP has been shown to inhibit hormone-sensitive lipase in adipocytes, independently and additively to insulin (8). There is a differentiation-dependent increase in ASP binding and ASP response in human adipocytes (1). The major site of action of ASP is adipocytes, as determined by competitive binding, stimulation of triglyceride synthesis, enhanced glucose transport, and transporter translocation (5).

ASP is identical to C3adesArg, a cleavage product of complement C3. Cleavage of complement C3 is mediated through the alternate complement pathway via the interaction of C3, factor B, and adipsin that generates C3a. Rapid cleavage of the C-terminal arginine of C3a by carboxypeptidase N generates ASP (9). Adipocytes are one of the few cells capable of producing all three factors (factor B, adipsin, and C3) that are required for the production of ASP (10). ASP production increases subsequent to adipocyte differentiation (11) and plasma ASP levels are elevated in obesity (12, 13). In vitro chylomicrons stimulate ASP production by adipocytes (14, 15). In vivo arterial-venous gradients across a subcutaneous adipose tissue bed in humans demonstrate direct postprandial production of ASP (16). The postprandial increase in ASP is adipose tissue specific and is not observed in the general circulation (17). Altogether, these data suggest that ASP and lipid storage are metabolically intertwined.

ASP acts as an adipocyte autocrine factor and we propose that it plays a central role in the metabolism of adipose tissue by increasing the efficiency of triglyceride synthesis in adipocytes, an action that results in more rapid postprandial lipid clearance (18). As ASP is derived through cleavage of complement C3, C3 knock-out mice (C3−/−) are necessarily deficient in ASP. We have previously demonstrated that genetic deficiency of ASP leads to reduced body fat and decreased leptin levels (19, 20). In addition, male mice have delayed triglyceride clearance (16, 20) although this has not been demonstrated in all studies (21). Studies with double knock-out mice (C3−/−, ob/ob) further demonstrated a resistance to development of obesity, delayed triglyceride synthesis and demonstrated that these effects of ASP were independent of leptin.

Strikingly, however, although ASP-deficient mice (C3−/−) have decreased body weight and delayed TG and NEFA clearance, they have a marked increased in food intake. This raised the question: why are ASP-deficient mice leaner if they ingest more caloric energy? We addressed that question by examining

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† The abbreviations used are: ASP, acylation-stimulating protein; BAT, brown adipose tissue; WAT, white adipose tissue; FA, fatty acid; TG, triglyceride; KO, knock-out; ANOVA, analysis of variance; WT, wild type; UCP, uncoupling protein; NEFA, non-esterified fatty acid; DGAT, diacylglycerol acyltransferase; RQ, respiratory quotient; AUC, area under curve; VO2, oxygen consumption; NS, not significant.

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ASP Deficiency and Energy Expenditure in Mice

energy expenditure and postprandial fat partitioning during resting and active phases of the diurnal (light:dark) cycle.

MATERIALS AND METHODS

Generation and Genotyping of Mice—The genetic background of the mice used in this study was 129Sv C3 knock-out (C3−/−) mice (129Sv × C57Bl6 genetic background), as well as wild-type mice, were originally obtained from Dr. Harvey Coulton. Mice were backcrossed 8–10 generations to obtain a homogenous 129Sv genetic background. C3−/− mice were sacrificed for RNA analysis. Tissues were collected and immediately frozen in liquid nitrogen, then stored at −80 °C until analysis. The following tissues were excised: spleen, kidney, heart, skeletal muscle (quadriceps), liver, intrascapular brown adipose tissue (BAT), peritoneal adipose tissue, gonadal adipose tissue, pectoral adipose tissue, inguinal adipose tissue, intestine, and stomach. The samples were weighed and frozen in liquid nitrogen at −80 °C for later analysis. Tissue fragments (10–50 mg) were extracted to measure total lipid and separated from fatty acid oxidation products. Following extraction with 2 ml of chloroform/methanol (2:1), samples were mixed overnight. On the following day, 1 ml of 50 mM CaCl2 was added, and the suspension was centrifuged at 3000 rpm 4 °C for 20 min to achieve a phase separation. The total lipids in the chloroform/methanol layer were separated, evaporated and resuspended in chloroform/methanol (2:1) for separation by thin layer chromatography as previously published to quantify triglyceride, diglyceride, free fatty acid, and polar lipids by scintillation counting (29). The aqueous phase containing oxidation products was counted directly.

UCP mRNA Expression—Separate sets of wild-type and KO mice were sacrificed for RNA analysis. Tissues were collected and immediately frozen in liquid nitrogen, then stored at −80 °C until analysis. The primers used for testing were: UCP-1: sense 5′-AGCAA GAGGAAGG-GACGTC-3′ (38–57 bp), antisense 5′-TTCGGAATTTGTCGGTT TC-3′ (255–237 bp). UCP-2: sense 5′-GTTCCTGCTCCACCGATT T-3′ (61–80 bp), antisense 5′-TGAATTGCTGATCCCTCCA-3′ (290–271 bp) and UCP-3: sense 5′-ACTGTATGCGAGTGGTGCC T-3′ (925–946 bp), antisense 5′-AATGTGACGATCACCAG-3′ (1315–1297 bp). 18 S was used as housekeeping gene (Ambion).

Total RNA was isolated from the four tissue samples from each mouse, using the TRIzol method (Invitrogen, Life Technologies, Inc.). Reverse-transcription was performed using 3 μg RNA and Moloney murine leukemia virus reverse transcriptase in buffer containing dNTPs, RNAse inhibitor and oligo(dT) primer (Invitrogen, Life Technologies, Inc.). The resulting cDNA (RT mixture) was subjected to PCR. Briefly, 2 μl of cDNA and UCP-1, 2, or 3 primers (1 μl final concentration) were mixed with 0.5 units of TaqDNA polymerase/reaction tube in standard buffer (0.2 mM dNTPs, 100 μM TAMAC, and MgCl2 diluted to 2 mM). The cDNA was amplified for 5 min at 95 °C followed by 30 cycles of (1 min 95 °C, 1 min 60 °C and 1 min 72 °C), followed by 7 min 72 °C. The PCR components (Taq polymerase, MgCl2, PCR buffer, primers, 10 mM TAMAC (tetramethylammonium chloride)) were all obtained from Invitrogen, Life Technologies, Inc.

The amplified cDNA was separated by electrophoresis on 7.5% polyacrylamide gel in 1.5 mM Tris-HCl, pH 8.8 buffer at 200 V, with silver staining (BioRAD) and dried. Quantification was done by gel scanning, using the Molecular Analyst software (BioRAD), referenced against DNA concentration standards (BioRAD) that were electrophoresed with every gel. Results were expressed as a ratio of specific UCP/18 S RNA.

Statistical Analysis—All results are presented as average ± standard error (S.E.). Statistical comparisons were by Student’s t tests or ANOVA, as indicated in the text and figure legends. Statistical significance was set at p < 0.05, where NS indicates not significant.

RESULTS

We have previously reported that C3(−/−) ASP-deficient mice (KO) have reduced body weight, adipose tissue mass, and plasma leptin but consume 30% more energy compared with WT mice (19, 20). This was also true of the present study where KO mice had a reduced body weight and increased food intake. Leptin levels were reduced: WT 6.23 ± 1.45 versus KO 4.23 ± 0.70 ng/ml, p < 0.05. Body composition analysis demonstrated decreases in percent fat in KO as compared with WT: WT male 3.64 ± 0.05, g fat/g carcass weight, ANOVA 

In addition, we have previously demonstrated that ASP-deficient C3(−/−) mice have increased insulin sensitivity as reflected by decreased HOMA (homeostatic model assessment) based on fasting insulin and glucose levels (19, 20), even when the mice are backcrossed onto an obese (C57Bl/6) background (30). ASP-deficient mice also demonstrate enhanced clearance of glucose following an oral fat load and an oral glucose tolerance test (19, 20). In the present study, the KO mice also demonstrated decreases in insulin relative to glucose and lower HOMA where the values were: WT 3.48 ± 0.19 versus KO 3.64 ± 0.14 mU/ml of glucose, WT 9.46 ± 1.22 versus KO 7.90 ± 0.56 μU/ml insulin and WT 1.46 ± 0.22 versus KO 1.21 ± 0.11 mU/mU for HOMA.

WT and KO mice were randomly selected except for the experiments with intragastric fat administration on male mice. In this case, we deliberately matched the body weights of the
KO and WT mice to avoid a partitioning bias resulting from a difference in adipose tissue mass and body weight (thus the KO mice were, on average, 2 weeks older).

Oxygen Consumption and Physical Activity (Movement) Monitoring—Mice were monitored for 42 h continuously, from 14:00 on day 1 to 8:00 on day 3. To allow for acclimation, only data from the final 24 h are reported (8:00 day 2 to 8:00 day 3). The mice were allowed to eat, drink, and move about freely. Movement was recorded, and the ambient gas was sampled and analyzed every 3 min. As shown in Fig. 1, in wild-type mice (both male and female), oxygen consumption (VO₂ measured as ml/kg/min) was greater in the active phase (20:00–8:00) and less in inactive phase (8:00–20:00) and the KO mice maintained this pattern. Interestingly, female mice had greater oxygen consumption than the male mice (both WT and KO) and this was confirmed in additional experiments where the mice were tested in a pairwise fashion. Both male and female KO mice had higher VO₂ in the active phase compared with wild-type mice (12 h active phase mean VO₂, 2-way ANOVA, \( p = 0.0039 \) males, \( p = 0.027 \) females). The differences were also significant when calculated as area under the curve (measured as ml O₂/BW·67/min; \( p = 0.032 \) males, \( p = 0.031 \) females). The KO males had higher inactive phase VO₂ than WT, calculated as area under the curve (AUC) of VO₂ (\( p < 0.05 \), Table I). VO₂ during the inactive phase was not different between the KO and WT females.

KO female mice showed a substantial (78.4%, \( p < 0.05 \)) increase in active phase movement (physical activity, Fig. 2) compared with WT females, but there were no differences in either active or inactive phase movement between KO and WT male mice. The diurnal pattern of the RQ was similar in the female KO and WT mice, while the KO males had a very slight increase in AUC of RQ during the active phase compared with WT males (Table I). On the other hand, measurement of rectal body temperature did not detect any significant differences between the groups when tested over a range of ages (5–30 weeks) and body weight (20–40 g); WT male 37.8 ± 0.13, KO male 37.7 ± 0.12, WT female 37.7 ± 0.10, and KO female 37.9 ± 0.07 °C, \( n = 20–30 \) mice per group).

Fat Administration in Male Mice—Three groups of body weight matched male mice were tested: 6 wild-type mice, 6 KO mice and 6 KO mice that received intraperitoneal (IP) injections of ASP at the same time as the intragastric fat bolus. Body weight for the groups was chosen so as to not be different (WT 25.9 ± 0.7 versus KO 25.0 ± 0.8 g), thus the KO mice were slightly older. Following an overnight fast, a fat load of olive oil containing \(^{3}H\)Oleate was given to the mice, and serial blood samples was taken over 6 h (0, 1, 2, 3, 4, and 6 h). All the mice were sacrificed at 6 h after the fat load to determine \(^{3}H\)Oleate distribution in various tissues. Even though the KO mice were matched for body weight to WT mice, KO mice had clearly delayed TG clearance (incremental AUC: WT 1.7 ± 0.4 mU/h, KO 6.1 ± 1.2 mU/h, \( p < 0.01 \); KO with IP ASP 3.8 ± 0.7 mU/h, \( p < 0.05 \) versus KO and versus WT) and NEFA clearance (AUC: WT 5.6 ± 0.1 mU/h, KO 7.0 ± 0.5 mU/h, \( p < 0.05 \); KO with IP ASP 6.0 ± 0.5 mU/h, \( p < 0.05 \) versus KO) after the fat load. Injecting ASP at the time of fat administration (time = 0) partially normalized both TG and NEFA clearance (Fig. 3). There was no significant difference in the amount of \(^{3}H\)Oleate remaining in intestine and stomach in the three groups of animals (data not shown). We have previously reported that ASP deficiency did not alter fat absorption (19, 20). \(^{3}H\)Oleate incorporation was measured in spleen, kidney, heart, skeletal muscle, liver, BAT, peritoneal adipose tissue, gonadal adipose tissue, pectoral adipose tissue, and inguinal adipose tissue in all three groups of mice (WT, KO, KO + IP ASP). Data are reported as total \(^{3}H\)Oleate radiolabel incorporated per organ (Fig. 4). For muscle, total body muscle mass was estimated as 25% of body weight according to previously published data (28). For both WT and KO mice, most of the absorbed radiolabel (average 92%) was present in the combined tissues of liver, muscle, and BAT. For the male KO mice, there was a +63% increase (\( p < 0.05 \)) in muscle radiolabel (WT 5.14 ± 0.39, KO 8.37 ± 0.79, KO with IP ASP 5.66 ± 0.65 million dpm) and a +50% increase in liver radiolabel (WT 2.32 ± 0.27, KO 3.50 ± 0.26, KO with IP ASP 2.80 ± 0.06 million dpm) compared with WT; but a −59% decrease (\( p < 0.05 \)) in \(^{3}H\)Oleate radiolabel in BAT (WT 1.75 ± 0.31, KO 0.71 ± 0.25, KO with IP ASP 2.20 ± 0.61 million dpm, \( p < 0.05 \)) (Fig. 4). Administration of ASP in KO mice normalized \(^{3}H\)Oleate tissue uptake in these three tissues (Fig. 4). However, there was no difference in total counts in white adipose tissue (Fig. 4) or in spleen, kidney, or heart for all three groups (data not shown).

We then subfractionated the radiolabel into lipid and non-lipid (oxidation products) components. As shown in Fig. 5, in both BAT and WAT there were significant decreases in \(^{3}H\)Oleate incorporation into the lipid component (\( p < 0.05 \)), which were normalized to the WT level by ASP administration (BAT: WT 1.67 ± 0.20, KO 0.53 ± 0.20, KO with IP ASP 1.33 ± 0.15 million dpm; WAT: WT 0.65 ± 0.05, KO 0.46 ± 0.04, KO with IP ASP: 0.68 ± 0.04 million dpm). This decrease in \(^{3}H\)Oleate incorporation was reflected primarily in a decrease in the triglyceride/diglyceride ratio (BAT: WT 186.1 ± 57.1, KO 84.8 ± 25.0, \( p < 0.05 \), KO with IP ASP 185.8 ± 31.7 versus KO \( p < 0.05 \); WAT: WT 27.0 ± 5.0, KO 9.8 ± 0.8, \( p < 0.05 \), KO with IP ASP: 8.3 ± 0.5), which indicated that there was relatively less diglyceride converted into triglyceride in KO in these tis-
TABLE I
Oxygen Consumption (VO$_2$) and Respiratory Quotient (RQ) in ASP-deficient C3 ($^{-}$1$^{-}$) and wild-type male and female mice

|                  | Male          | Female         |
|------------------|---------------|----------------|
|                  | Active phase  | Inactive phase | Active phase  | Inactive phase |
| VO$_2$-AUC (L/Kg)|               |                |               |                |
| WT               | 106.5 ± 2.0   | 73.9 ± 1.3     | 133.7 ± 1.5   | 75.4 ± 1.8     |
| KO               | 116.0 ± 2.9   | 81.7 ± 2.9     | 144.0 ± 2.6   | 78.3 ± 1.1     |
| Increase         | 8.9%          | 10.6%          | 9.4%          | 2.8%           |
| p                | <0.05         | <0.05          | <0.05         | NS             |
| RQ-AUC (min)     |               |                |               |                |
| WT               | 1180.0 ± 6.4  | 645.7 ± 13.0   | 1237.9 ± 15.9 | 647.9 ± 16.5   |
| KO               | 1210.0 ± 7.2  | 644.5 ± 13.0   | 1247.9 ± 15.7 | 655.8 ± 8.3    |
| Increase         | 2.5%          | -0.1%          | 0.8%          | 1.2%           |
| p                | <0.05         | NS             | NS            | NS             |

* The active phase was from 20:00-8:00.
* The inactive phase was from 8:00-20:00.
* NS, not significant.

The changes in $[^{3}H]$oleate radiolabel uptake, especially in fatty oxidation products, point to changes in oxidation/thermogenesis, which may be associated with changes in plasma adiponectin or UCPs. No difference in plasma adiponectin was detected in male KO mice versus WT mice ($p < 0.05$), KO with IP ASP 5.48 ± 0.52 million dpm; liver: WT 0.65 ± 0.04, KO 0.99 ± 0.06, KO with IP ASP 0.62 ± 0.02 million dpm; $p < 0.05$ both in muscle and liver resulting in a +52% increase in the liver and a +60% increase in muscle, both $p < 0.05$. On the other hand, there was no difference in the total lipid fraction, nor was there a difference in the proportion in TG in either liver or muscle: in muscle, WT 63.1% ± 7.4, KO 73.3% ± 5.5, and KO + ASP IP 64.9% ± 4.0, $p = NS$ and in liver, WT 88.1% ± 1.5, KO 87.4% ± 1.9, and KO + ASP IP 87.6% ± 1.0, $p = NS$). ASP injection at the same time as the intragastric fat administration in male KO mice resulted in a -37% decrease in liver ($p < 0.05$) and a -31% decrease in muscle ($p < 0.05$) oxidation products with no difference found in total lipid phase. These data suggest that in male KO mice the absorbed fat was partitioned differently compared with WT mice, with greater uptakes into muscle and liver. Specifically, oxidation in liver and muscle appear to be increased significantly.

The results presented here indicate that C3($^{-}$) ASP-deficient KO mice, both male and female, have increased oxygen consumption in the active phase and that male mice may also have increased oxygen consumption in the inactive phase. Increased activity, an important source of the energy expenditure change, was observed in the female KO mice, but not in the male KO mice. Both WT and KO male mice had decreased energy expenditure compared with the female mice. Intragastric fat administration resulted in increased fat oxidation in the liver and muscle of male KO mice, consistent with the observed UCP3 overexpression in muscle.

Interestingly, the increased physical activity was only detected in female KO mice, suggesting that the sex hormones (or X-linked genes) may have a function in regulating energy expenditure. Estradiol has been shown to enhance energy expenditure by stimulating voluntary exercise as pointed out by Wade (see review, Ref. 32). Similarly, ovariectomy caused a sharp decrease in running wheel activity in rats and treatment...
with estradiol reinstated high levels of voluntary exercise (32, 33). Intracerebral implants of estradiol in or near these estrogen receptor-containing sites also increased activity in ovarectomized rats (32, 34). Lesions that include the medial preoptic area prevented the increases in activity induced by systemic estradiol treatment, but not those induced by other treatments, such as food deprivation or amphetamines (32, 35). Thus, the estradiol stimulation of physical activity may be one way in which the female KO mice compensate and dispose of the excess energy ingested but not stored in adipose tissue. Whether these effects of estradiol are in any way directly related to the absence of ASP is unknown, but there is evidence that estradiol enhances C3 expression (32, 36). As a hypothesis, the effect of estrogen on energy expenditure may be counterbalanced by stimulation of C3 and thus ASP, increasing storage of fatty acid into adipose tissue. The absence of C3 in the KO mice may unbalance the system leading to estrogen increases in voluntary exercise in female KO mice.

With regard to the mechanism underlying the increased energy expenditure in male KO animals, this could not be explained on the basis of increased physical activity, as this was not increased in males, but only in female mice. We observed a clear increase in fatty acid oxidation products in both muscle and liver and specific increases in UCP mRNA expression in certain tissues. It should be noted that there was an increase in oxidation products, but no increase in lipid (especially triglyceride) products, therefore the increased uptake in these tissues would not be expected to lead to lipotoxicity. Absence of ASP results in reduced TG synthesis in adipocytes and leads to delayed NEFA and TG postprandial clearance. This delay in TG and NEFA clearance may be a function of reduced uptake and esterification into TG (as reflected by the decreased [3H]TG in adipose tissues) as well as reduced inhibition of hormone sensitive lipase (both mediated by ASP) (8). Both of these effects will contribute to the increased circulating fatty acid flux, which in turn can lead to local inhibition of lipoprotein lipase (product inhibition). These substrates may then be redirected to muscle and liver for energy production. The accumulation of radiolabeled lipid in ASP-deficient mice into liver and muscle, caused by impaired lipid transportation into adipocytes, appears to simulate what is observed in the environment of high fat diet consumption. The increased energy expenditure would appear to be a systemic response to this challenge. In mice, thermogenesis in brown adipose tissue appears to be an important regulator of high fat diet induced obesity, mediated by UCP1 and UCP2 (37). In the case of KO mice, the increased energy expenditure may be due to increased brown adipose tissue activity.

Fig. 4. Radiolabeled [3H]oleate incorporation into individual tissues in KO, WT, and ASP injected mice at 6 hours. For the male KO mice, 6 h after fat load, [3H] radiolabel was tested in different tissues. There was a significant increase in muscle and liver of KO mice compared with WT mice but a significant decrease in [3H] radiolabel in BAT. Administration of ASP in KO mice restored the changes. Values are shown as $n = 6$ per group (6 WT, 6 KO, 6 KO with IP ASP) where *, KO versus WT, $p < 0.05$; **, KO with IP ASP versus KO, $p < 0.05$.

Fig. 5. Radiolabeled [3H]oleate incorporation into total lipids and oxidation products in specific tissues in KO, WT, and ASP injected mice at 6 hours. At 6 h after the fat load, different tissues were extracted and the extractions were separated into total [3H]-radiolabeled lipid (top panel) and [3H]-radiolabeled fatty acid oxidation products (bottom panel). Total radiolabeled lipids were significantly decreased in BAT and WAT, while oxidation products were significantly increased in muscle and livers of KO mice. Administration of ASP in KO mice could restore the changes. Values are shown as $n = 6$ per group (6 WT, 6 KO, 6 KO with IP ASP) where *, KO versus WT, $p < 0.05$; **, KO with IP ASP versus KO, $p < 0.05$.
mice lacking ASP, brown adipose tissue does not appear to function as efficiently as in WT mice since UCP1 is decreased. Unlike UCP1, UCP2 is widely expressed (38), whereas UCP3 is predominantly expressed in skeletal muscle (22, 23). In the ASP-deficient mice, other tissues (muscle and fat) may partially compensate for reduced BAT function through increases in UCP2 and UCP3 expression. It has been clearly shown, both in rodents and humans, that administration of fatty acids increases muscle UCP3 expression (39, 40). However, UCP3 is also regulated by several factors, including thyroid hormone, \( \beta_3 \)-adrenergic agonists and leptin as well as fat feeding in rodents (24–26). One or more of these factors may be responsible for the up-regulation of UCP3 in the ASP-deficient mice.

There is a striking difference in the metabolic adaptations in mice with decreased adipose tissue triglyceride resulting from partial reduction in stored energy (i.e., lean mice), as in the present study, versus those mice that completely lack adipocytes to store lipid (lipodystrophic mice). Mice that lack adipose tissue completely are uniformly insulin resistant and hyperinsulinemic with markedly increased plasma triglycerides (39, 40). However, UCP3 is also regulated by several factors, including thyroid hormone, \( \beta_3 \)-adrenergic agonists and leptin as well as fat feeding in rodents (24–26). One or more of these factors may be responsible for the up-regulation of UCP3 in the ASP-deficient mice.

In conclusion, ASP is an important factor in regulating metabolic balance. The absence of ASP production results in increased energy expenditure in both male and female mice. Male and female KO mice employ different mechanisms to deal with the substrate not stored in adipose tissue resulting from impaired TG synthesis. However, animals of both genders exhibit a lean, insulin sensitive phenotype despite significant hyperphagia.

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![Image](image-url)

**Fig. 6. Expression of uncoupling proteins UCP1, UCP2, and UCP3 in WT and KO mice.** Representative RT-PCR blots are shown for each protein: UCP1, UCP2, and UCP3 where, WT, WT inguinal adipose tissue; KO, KO inguinal adipose tissue; 1, WT gonadal adipose tissue; 3, WT gonadal adipose tissue; 4, KO gonadal adipose tissue; 5, WT brown adipose tissue; 6, KO brown adipose tissue; 7, WT muscle; 8, KO muscle.

| UCP | WT | KO |
|-----|----|----|
| Muscle | 0.41 ± 0.11 | 0.18 ± 0.05 |
| BAT | 1.00 ± 0.18 | 0.61 ± 0.07 |
| Inguinal | 0.51 ± 0.15 | 0.13 ± 0.04 |
| Gondadal | 0.32 ± 0.08 | 0.13 ± 0.04 |

**Table II**

Uncoupling proteins (UCP) 1, 2, and 3 expression in muscle and brown adipose tissue (BAT) in male ASP-deficient C3(−/−) knock-out and wild-type mice.

| UCP 1 | WT | KO |
|-------|----|----|
| Muscle | 0.41 ± 0.11 | 0.18 ± 0.05 |
| BAT | 1.00 ± 0.18 | 0.61 ± 0.07 |
| Inguinal | 0.51 ± 0.15 | 0.13 ± 0.04 |
| Gondadal | 0.32 ± 0.08 | 0.13 ± 0.04 |

| UCP 2 | WT | KO |
|-------|----|----|
| Muscle | 0.41 ± 0.11 | 0.18 ± 0.05 |
| BAT | 1.00 ± 0.18 | 0.61 ± 0.07 |
| Inguinal | 0.51 ± 0.15 | 0.13 ± 0.04 |
| Gondadal | 0.32 ± 0.08 | 0.13 ± 0.04 |

| UCP 3 | WT | KO |
|-------|----|----|
| Muscle | 0.41 ± 0.11 | 0.18 ± 0.05 |
| BAT | 1.00 ± 0.18 | 0.61 ± 0.07 |
| Inguinal | 0.51 ± 0.15 | 0.13 ± 0.04 |
| Gondadal | 0.32 ± 0.08 | 0.13 ± 0.04 |

" NS, not significant.
