Expression of AMPK, SIRT1, and ACC Differs between Winter- and Summer-Acclimatized Djungarian Hamsters

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ABSTRACT

The wintering strategy of the Djungarian hamster (*Phodopus sungorus*) includes a naturally occurring decrease in food intake and body mass. Our aim was to investigate the conceivable role of the metabolic regulators, AMP-activated protein kinase (AMPK) and sirtuin 1 (SIRT1), in the seasonal adaptation of the Djungarian hamster. In addition, a rate-limiting enzyme in fatty acid synthesis and oxidation, acetyl CoA carboxylase (ACC), was studied. Relative protein expressions and phosphorylated forms (pAMPK and pACC) were determined by Western blot from subcutaneous white adipose tissues (sWAT), abdominal white adipose tissues (aWAT), interscapular brown adipose tissues (iBAT), skeletal muscle, and hypothalamus of winter- and summer-acclimatized hamsters. The winter group had higher AMPK expression in sWAT, aWAT, and iBAT, but the relative amount of phosphorylated protein (pAMPK/AMPK ratio) was lower in these tissues. Furthermore, ACC expression was higher in sWAT and iBAT of the winter animals. pACC (inactive form) levels were higher in all adipose tissues, yet a lower pACC/ACC ratio was detected in iBAT of the winter hamsters. Muscle AMPK expression was lower but pAMPK/AMPK ratio higher in the winter group. SIRT1 expression was higher in muscle and all adipose tissues of the winter hamsters. Hypothalamic protein expressions did not differ between the groups. Higher expressions of AMPK, ACC, and SIRT1 in WAT and iBAT of the winter hamsters suggest a role in the regulation of lipid reserves and increased thermogenic capacity characteristic to the winter-adapted Djungarian hamsters.

Keywords: body mass, energy metabolism, seasonal adaptation, white adipose tissue, brown adipose tissue, Siberian hamster.

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Introduction

Seasonal changes in food intake, body weight, and adiposity are common for mammals living at northern latitudes where food resources are limited during winter. The autumnal accumulation of body fat stores and hibernation throughout the winter is a survival strategy especially for many small species. However, some species have quite the opposite physiological response before winter. The Djungarian hamster (*Phodopus sungorus*), also known as the Siberian hamster, is a photoperiodic species that voluntarily reduces energy intake approximately 20%–30% when exposed to a short photoperiod despite ad lib. access to food (Heldmaier and Steinlechner 1981*a*; Wade and Bartness 1984; Knopper and Boily 2000). Wintertime body mass of the animals can be up to 30%–40% lower compared to the long photoperiod, with most of the lost mass being adipose tissue (Heldmaier and Steinlechner 1981*a*; Klingenspor et al. 2000). Thus, the energy required for the maintenance of body mass is reduced and consequently the needed amount of food and foraging activity is decreased. In addition, Djungarian hamsters display daily torpor as an energy-saving strategy (Heldmaier and Steinlechner 1981*a*, 1981*b*). This naturally occurring reduction in body adiposity offers an interesting model to study molecular mechanisms that participate in seasonal metabolic adjustments.

One key enzyme in the control of energy metabolism is AMP-activated protein kinase (AMPK). It is a cellular energy and nutrient sensor that is activated in response to decreased energy levels in the cell (Hardie et al. 2012). Moreover, AMPK has a broad role in the maintenance of whole-body energy balance, including the hypothalamic control of food intake and appetite as well as the regulation of glucose and lipid metabolism in the peripheral tissues (Andersson et al. 2004; Minokoshi et al. 2004; Mulligan et al. 2007). AMPK regulates the expression and activity of numerous metabolic enzymes. For instance, AMPK modulates lipid metabolism through acetyl CoA carboxylase (ACC), a rate-limiting enzyme in fatty acid (FA) synthesis and oxidation. AMPK inhibits ACC by phosphorylation, which consequently increases mitochondrial β-oxidation and decreases FA synthesis (Kahn et al. 2005). Another essential metabolic regulator is sirtuin 1 (SIRT1), a member in the family of mammalian NAD^+-dependent histone/protein deacetylases (SIRT1–7). SIRT1 expression and activity have been shown to increase under conditions of reduced nutrient availability, such as fasting and calorie restriction. SIRT1 has numerous target substrates, including regulators of metabolic pathways related to lipid metabolism and mitochondrial biogenesis (Rodgers et al. 2005; Gerhart-Hines et al. 2007). In general, AMPK and SIRT1 are activated in response to changes in energy balance. As a result, the

energy-producing pathways are enhanced and energy-consuming processes are downregulated (Fulco and Sartorelli 2008). In addition to common activators, such as fasting, calorie restriction, and exercise, AMPK and SIRT1 have overlapping functions in cellular energy metabolism. They have common target molecules in glucose and lipid metabolism, such as peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), a transcriptional coregulator that promotes mitochondrial biogenesis (Haigis and Sinclair 2010; Ruderman et al. 2010).

Previous studies in hibernating species suggest involvement of AMPK and SIRT1 in the metabolic adjustments during seasonal adaptation. In marmots (*Marmota flaviventris*) and ground squirrels (*Callospermophilus lateralis*) hypothalamic AMPK participates in the regulation of food intake during hibernation season (Florant et al. 2010; Healy et al. 2011*a*). In peripheral tissues, such as white adipose tissues (WAT) and brown adipose tissues (BAT) and liver, AMPK expression and phosphorylation vary in response to different stages of the torpor bout and also between the summer- and winter-acclimatized euthermic ground squirrels (*C. lateralis* and *Ictidomys tridecemlineatus*; Horman et al. 2005; Healy et al. 2011*b*; Lanaspa et al. 2015). Furthermore, tissue-specific alterations of expression levels and activity of different sirtuins were observed during the torpor-arousal cycle of the ground squirrel (*I. tridecemlineatus*; Rouble and Storey 2015). However, data supporting a role for AMPK and SIRT1 in seasonally occurring metabolic changes mainly come from studies in species exhibiting autumnal fattening, total aphagia, and deep hibernation in winter. The diverse wintering strategy of the Djungarian hamster presents a comparative model that could reveal further information about the function of these proteins in the seasonal regulation of energy metabolism. In this study, we evaluated AMPK expression and phosphorylation (active form) level and SIRT1 expression in different tissues of winter- and summer-acclimatized hamsters. In addition, the expression and phosphorylation of ACC, a downstream target of AMPK, were analyzed. We hypothesized that due to their central roles in the metabolic processes, especially in lipid metabolism, these proteins are involved in the maintenance of energy homeostasis in the lean winter phenotype of the Djungarian hamster and are differentially expressed in winter- and summer-acclimatized animals.

Material and Methods

Djungarian hamsters (age 12–13 mo; 16 males and 8 females) were obtained from the breeding colony at the Department of Biology, University of Oulu. Before the experiment, the hamsters were housed individually in an animal room under a 12L∶12D cycle and at an ambient temperature of 22° ± 2°C. Animals had free access to water and food (Lactamin) and weekly supplement of apple and curd. Animals used in this study received the same diet throughout the experiment.

The experimental protocol was approved by Finland's national Animal Experiment Board (license no. ESAVI-2010-06711/Ym-23). Hamsters were randomly divided into two groups: winter acclimatization ($N = 16$; 11 males and 5 females) and summer acclimatization ($N = 8$; 5 males and 3 females). Winter animals were exposed to a short photoperiod (8L∶16D, lights on at 0800 hours) at 10°C, and summer animals were housed in a long photoperiod (14L∶10D, lights on at 0600 hours) at 22°C for 15 wk (December 30–April 13). Hamsters were weighed weekly throughout the experimental period, and incidence of possible torpor bouts was monitored visually every other day. At the end of the experiment, hamsters were killed during the light phase (between 0900 and 1500 hours) by cervical dislocation in a nonfasted and nontorpid state. Whole hypothalamus, hind limb muscle (*m. rectus femoris*), samples from subcutaneous (inguinal) and abdominal (retroperitoneal) white adipose tissues (sWAT and aWAT), and interscapular brown adipose tissue (iBAT) were collected and frozen immediately in liquid nitrogen and stored at −80°C for later analyses.

Tissue samples and whole hypothalamus were prepared, and Western blot analysis was conducted as previously described (Kinnunen et al. 2016). Briefly, samples ranging from 20 to 150 mg were homogenized in 6 vol ice-cold buffer, and the insoluble material was removed by centrifugation (17,000 *g* for 10 min). Samples containing an equal amount of protein were resolved by SDS-PAGE and transferred to nitrocellulose membrane. The total amount of protein loaded into each well of the gels was 10 μg for hypothalamus, 12 μg for muscle, 16 μg for iBAT, and 20 μg for sWAT and aWAT. Membranes were incubated overnight at +4°C in primary antibodies against pAMPKα (Thr172) 2535, AMPKα 2532, pACC (Ser79) 3661, ACC 3662 (Cell Signaling Technology, Beverly, MA), and SIRT1 12193 (Abcam, Cambridge, UK). The antibodies for pACC and ACC recognize both isoforms of ACC: ACC1 (265 kDa) and ACC2 (280 kDa). Anti-β-actin ab8227 (Abcam) or anti-α-tubulin 2125 (Cell Signaling Technology) antibodies were used as loading controls. Antibodies were validated for the Djungarian hamster using rat and mouse samples as positive controls. All antibodies gave corresponding bands at the expected molecular masses in the hamster samples. AP-conjugated goat anti-rabbit (Bio-Rad Laboratories) was used as secondary antibody. Antibody detection was performed with NBT/BCIP stock solution (Roche Diagnostics). Optical densities of the detected bands were analyzed with the FluorS MultiImager program (Bio-Rad). Results were normalized with the corresponding loading control.

All statistical analyses were performed using SPSS v20.0 for Windows (IBM). The Shapiro-Wilk test was used to test normality, and homogeneity of variances was tested by the Levene test. Comparisons between the groups were performed using the Student's *t*-test. Correlations were calculated with the Pearson correlation coefficient (r_p). Statistical significance was defined as $P < 0.05$. The results are presented as means ± SEM.

Results

Hamsters lost weight progressively in response to winter acclimatization, and body weight remained stable for the last 2 wk of the experiment, indicating that the animals had reached

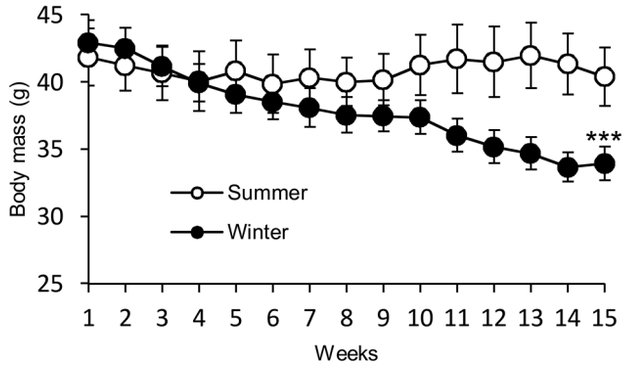

Figure 1. Average body masses of the hamsters during the 15-wk experimental period. Winter-acclimatized Djungarian hamsters ($N = 16$; filled circles) lost 21.4% ± 2.1% (9.7 ± 1.2 g) of their body mass. Asterisks indicate significant difference between body mass at the start and end of the experiment (paired-sample Student's t-test: three asterisks, $P < 0.001$). The body mass of the summer group ($N = 8$; open circles) did not change significantly.

their body fat nadir (fig. 1). After the 15-wk experimental period, the winter group had lost 21.4% ± 2.1% (9.7 ± 1.2 g) of their body mass ($P < 0.001$), but the body mass of the summer group did not change significantly. Occurrence of regular daily torpor was evident in only two individuals. Torpid animals were curled in their dens and did not respond to external stimulation.

In sWAT, expression of AMPK was twofold higher in the winter group compared to the summer animals ($P < 0.05$), but the pAMPK level declined in the winter group ($P < 0.05$) and, consequently, the phosphorylation ratio was threefold lower than in the summer group ($P < 0.05$; fig. 2*A*). Both ACC and pACC levels were higher (1.5-fold, $P < 0.05$, and twofold, $P < 0.001$, respectively) in the winter animals, and also the pACC/ACC ratio was significantly higher in the winter group ($P < 0.05$; fig. 2*B*). There was no correlation between pAMPK and pACC. The SIRT1 level was 1.5-fold higher in the winter animals ($P < 0.05$; fig. 2*C*).

Also in aWAT, total AMPK expression was twofold higher in the winter animals ($P < 0.05$; fig. 3*A*). Although there were no statistically significant differences in the pAMPK level, the pAMPK/AMPK ratio was lower in the winter group, similar to sWAT (fig. 3*A*). ACC expression did not differ between the groups. The pACC level was 2.3-fold higher in the winter animals, yet there was no statistical difference in the pACC/ACC ratio ($P = 0.07$; fig. 3*B*). Positive correlation was observed between pAMPK and pACC ($r_p = 0.519$; $P < 0.05$). SIRT1 protein expression was 2.4-fold higher in the winter group ($P < 0.05$; fig. 3*C*).

In iBAT, AMPK expression was threefold higher in the winter group than in the summer group ($P < 0.05$; fig. 4*A*). No changes were observed in the pAMPK levels, and the pAMPK/AMPK ratio was twofold lower in the winter animals ($P < 0.05$; fig. 4*A*). Also the ACC expression was higher (fivefold) in the winter group ($P < 0.001$), with concomitant twofold elevation in the pACC levels ($P < 0.05$), but the pACC/ACC ratio was twofold lower in the winter animals compared to the summer group ($P < 0.05$; fig. 4*B*). There was no correlation between pAMPK and pACC. SIRT1 expression was 1.7-fold higher in the winter group ($P < 0.05$; fig. 4*C*).

In the skeletal muscle, AMPK concentration was lower in the winter group than in the summer animals ($P < 0.01$; fig. 5*A*). The difference in the pAMPK level was not statistically significant, but the pAMPK/AMPK ratio was twofold higher ($P < 0.05$) in the winter group (fig. 5*A*). No significant differences were detected in the total ACC, pACC, or pACC/ACC ratio between the groups (fig. 5*B*). No correlation between AMPK and ACC phosphorylation was observed. SIRT1 expression was 1.5-fold

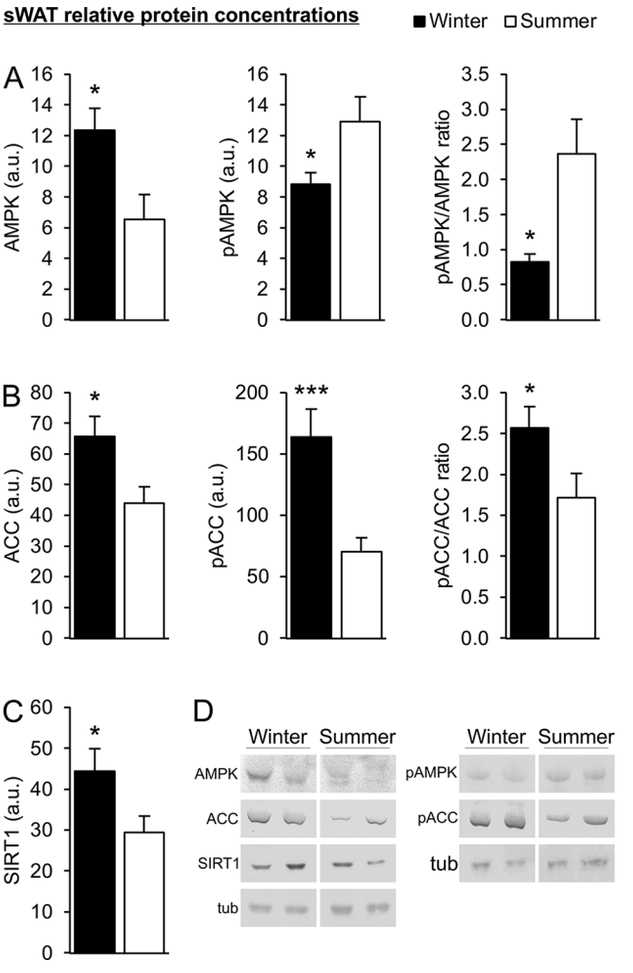

Figure 2. Expression of AMPK, ACC, and SIRT1 in the sWAT of winter- and summer-acclimatized Djungarian hamsters. Relative protein concentrations of total AMPK, phosphorylated AMPK (pAMPK), and pAMPK/AMPK ratio (*A*); total ACC, phosphorylated ACC, and pACC/ACC ratio (*B*); and total SIRT1 (*C*). *D*, Representative Western blots for AMPK, pAMPK, ACC, pACC, SIRT1, and loading control α-tubulin (tub). The results are expressed as mean arbitrary units (a.u.) + SEM of protein band intensities normalized to α-tubulin. Asterisks indicate significant differences between the groups (two-tailed Student's t-test: one asterisk, $P < 0.05$; three asterisks, $P < 0.001$). $N = 16$ for the winter group (filled bars) and $N = 8$ for the summer group (open bars).

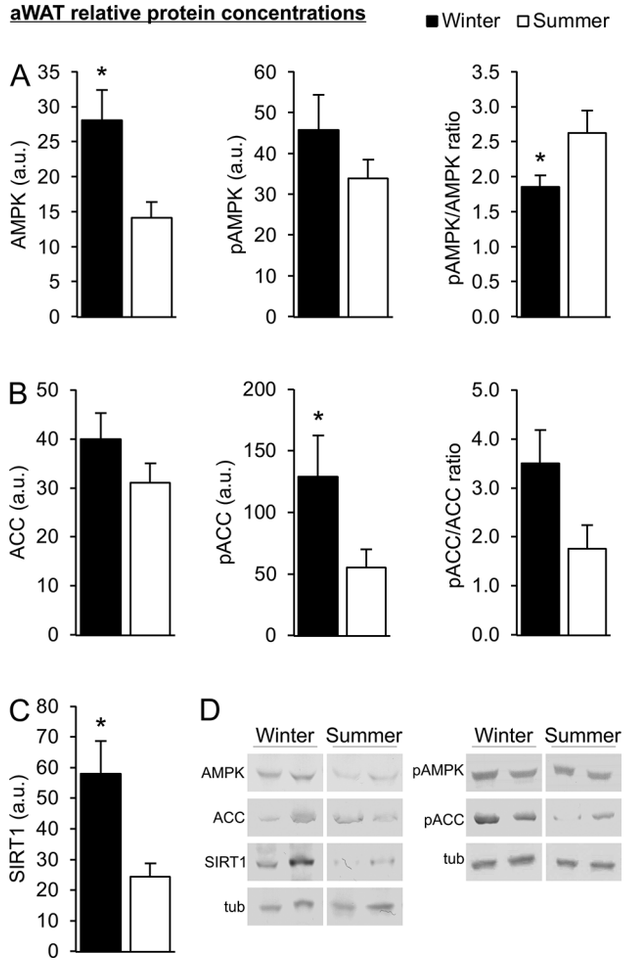

Figure 3. Expression of AMPK, ACC, and SIRT1 in the aWAT of winter- and summer-acclimatized Djungarian hamsters. Relative protein concentrations of total AMPK, phosphorylated AMPK (pAMPK), and pAMPK/AMPK ratio (*A*); total ACC, phosphorylated ACC, and pACC/ACC ratio (*B*); and total SIRT1 (*C*). *D*, Representative Western blots for AMPK, pAMPK, ACC, pACC, SIRT1, and loading control α-tubulin (tub). The results are expressed as mean arbitrary units (a.u.) + SEM of protein band intensities normalized to α-tubulin. Asterisks indicate significant differences between the groups (two-tailed Student's *t*-test: one asterisk, $P < 0.05$). $N = 16$ for the winter group (filled bars) and $N = 8$ for the summer group (open bars).

higher in the muscle of the winter group ($P < 0.05$; fig. 5*C*). There were no differences in hypothalamic AMPK, ACC, or SIRT1 expression or phosphorylation between the groups (fig. 6*A*–6*C*).

Discussion

In this study, we examined the relative protein expression levels of the metabolic regulators AMPK, ACC, and SIRT1 in sWAT, aWAT, iBAT, hypothalamus, and skeletal muscle of winter- and summer-acclimatized Djungarian hamsters. The effect of winter acclimatization was observed in sWAT, aWAT, and BAT, where AMPK, SIRT1, and ACC protein expression was up to fivefold higher. These results indicate that AMPK, ACC, and SIRT1 have a role in the seasonal metabolic adaptation of the Djungarian hamster.

Short day length has been shown to induce adipocyte lipolysis in the WAT depots of the Djungarian hamster (Demas and Bartness 2001; Demas et al. 2002; Bowers et al. 2005). We observed higher SIRT1 and AMPK expression in sWAT and aWAT of the winter hamsters compared to the summer animals. Conversely, AMPK phosphorylation ratios, that is, proportions of active protein, were lower in both tissues of the winter group. However, although phosphorylation is a good indicator of AMPK activity (Suter et al. 2006), it has to be noted that we did not measure enzyme activity per se. Both AMPK and

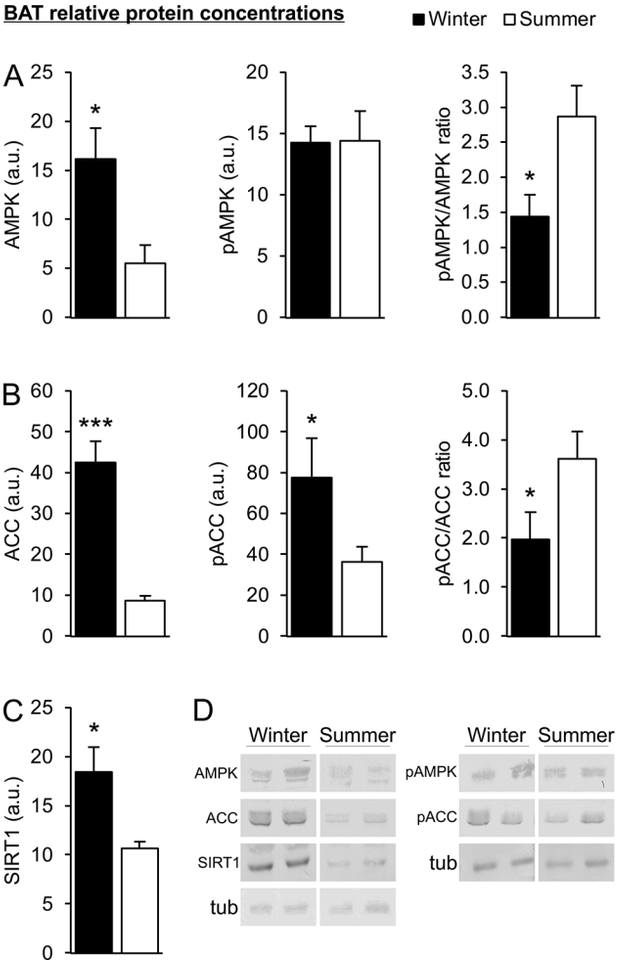

Figure 4. Expression of AMPK, ACC, and SIRT1 in the iBAT of winter- and summer-acclimatized Djungarian hamsters. Relative protein concentrations of total AMPK, phosphorylated AMPK (pAMPK), and pAMPK/AMPK ratio (*A*); total ACC, phosphorylated ACC, and pACC/ACC ratio (*B*); and total SIRT1 (*C*). *D*, Representative Western blots for AMPK, pAMPK, ACC, pACC, SIRT1, and loading control α-tubulin (tub). The results are expressed as mean arbitrary units (a.u.) + SEM of protein band intensities normalized to α-tubulin. Asterisks indicate significant differences between the groups (two-tailed Student's *t*-test: one asterisk, $P < 0.05$; three asterisks, $P < 0.001$). $N = 16$ for the winter group (filled bars) and $N = 8$ for the summer group (open bars).

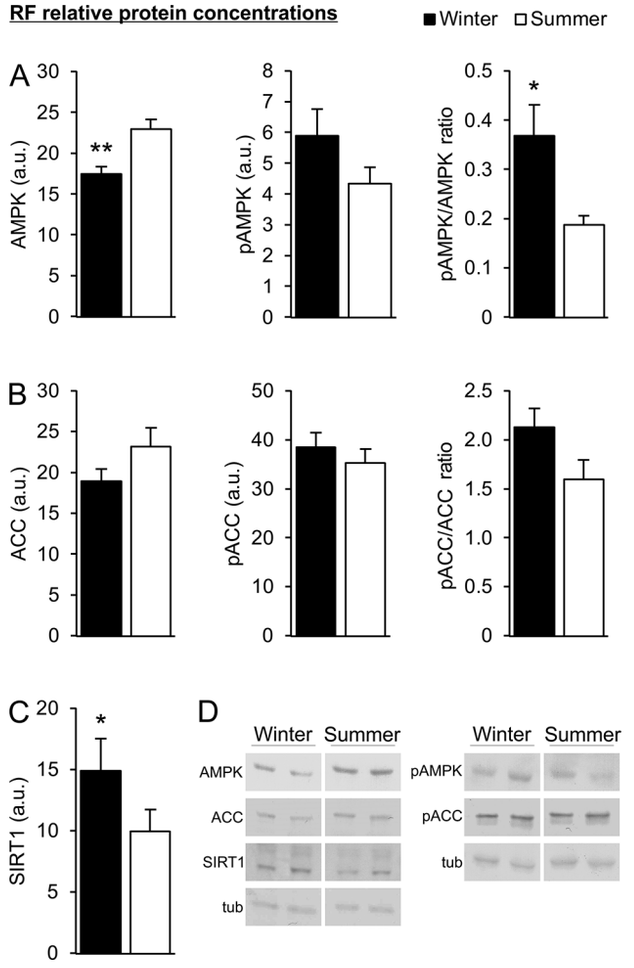

Figure 5. Expression of AMPK, ACC, and SIRT1 in the skeletal muscle (*rectus femoris* [RF]) of winter- and summer-acclimatized Djungarian hamsters. Relative protein concentrations of total AMPK, phosphorylated AMPK (pAMPK), and pAMPK/AMPK ratio (*A*); total ACC, phosphorylated ACC, and pACC/ACC ratio (*B*); and total SIRT1 (*C*). *D*, Representative Western blots for AMPK, pAMPK, ACC, pACC, SIRT1, and loading control α-tubulin (tub). The results are expressed as mean arbitrary units (a.u.) + SEM of protein band intensities normalized to α-tubulin. Asterisks indicate significant differences between the groups (two-tailed Student's *t*-test: one asterisk, $P < 0.05$; two asterisks, $P < 0.01$). $N = 16$ for the winter group (filled bars) and $N = 8$ for the summer group (open bars).

SIRT1 are known to suppress adipocyte differentiation and lipogenesis in rats and mice (Sullivan et al. 1994; Habinowski and Witters 2001; Gaidhu et al. 2009). Furthermore, SIRT1 has been shown to increase lipolysis and fat mobilization in mouse WAT (Picard et al. 2004), whereas AMPK appears to have an antilipolytic effect that depends on the rate of lipolysis (Kim et al. 2016). AMPK is activated in adipocytes in response to increased lipolysis, for example, under fasting condition, and this activation subsequently inhibits the lipolytic pathway (Daval et al. 2005; Gauthier et al. 2008). The antilipolytic effect of AMPK in WAT has been proposed to function as a mechanism that limits energy depletion caused by excessive FA recycling associated with high-rate lipolysis (Gauthier et al. 2008). The more abundant amount of total AMPK and SIRT1 in sWAT and aWAT of the winter hamsters might contribute to efficient lipid mobilization and preservation of adipose tissue at the proper level prescribed by photoperiod. The low AMPK phosphorylation ratio observed in the winter group could indicate that AMPK function is downregulated during the euthermic phase, when hamsters forage and energy availability is not dependent on endogenous lipid reserves. Unfortunately, although all of our hamsters lost body mass during winter acclimatization, only a few animals entered daily torpor regularly. Hence, we were not able to measure protein levels from a sufficient amount of torpid animals to clarify whether the phosphorylation levels differ between the torpid and euthermic stages.

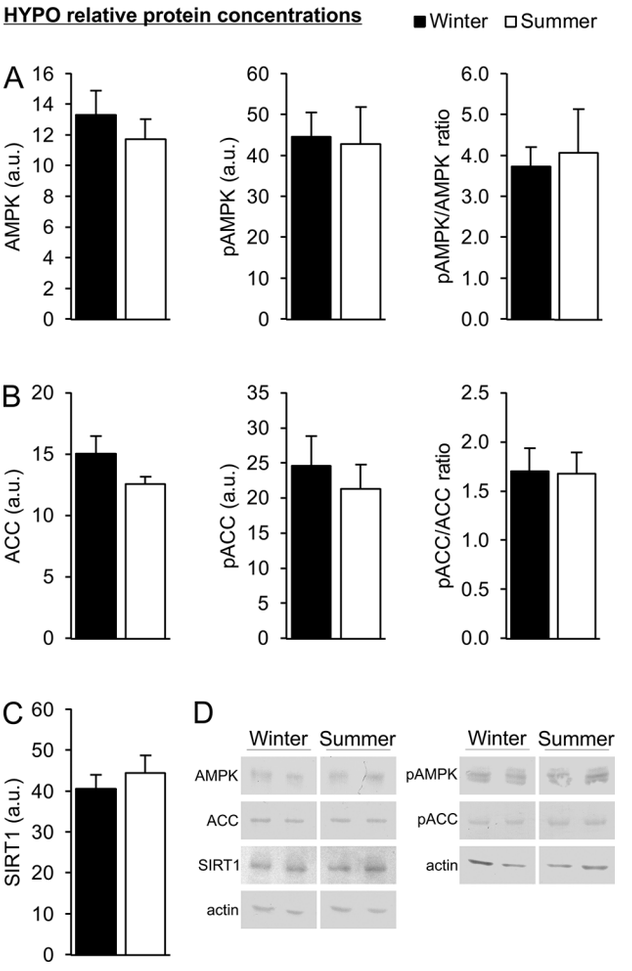

Figure 6. Expression of AMPK, ACC, and SIRT1 in the hypothalamus of winter- and summer-acclimatized Djungarian hamsters. Relative protein concentrations of total AMPK, phosphorylated AMPK (pAMPK), and pAMPK/AMPK ratio (*A*); total ACC, phosphorylated ACC, and pACC/ACC ratio (*B*); and total SIRT1 (*C*). *D*, Representative Western blots for AMPK, pAMPK, ACC, pACC, SIRT1, and loading control β-actin. The results are expressed as mean arbitrary units (a.u.) + SEM of protein band intensities normalized to β-actin. $N = 16$ for the winter group (filled bars) and $N = 8$ for the summer group (open bars).

Interestingly, ACC expression was also higher in sWAT of the winter group, suggesting the potential for enhanced lipogenesis. However, a larger proportion of the total ACC was phosphorylated (inactive form) compared to that in the summer animals. ACC protein expression did not differ in aWAT between the groups, but the pACC level was more than twofold higher in the winter animals. Previous studies have shown that lipid mobilization is not evenly distributed in the short-day-exposed Djungarian hamsters. Large proportions of the lost fat mass are from the internally located fat pads, and the more external subcutaneous fat is utilized to a lesser extent (Bartness et al. 2002; Bowers et al. 2005). Presumably, sWAT has a more dynamic metabolic role as an insulating factor and lipid reserve during winter adaptation. Thus, the higher ACC protein level in sWAT of the winter hamsters could be relevant for the maintenance of the appropriate amount of subcutaneous lipid depots. In turn, aWAT is depleted to maintain the lean winter phenotype, and upregulation of the lipogenic pathway is not required. In addition, lower pACC levels in the summer animals indicate that WAT metabolism is directed toward lipogenesis as excepted since the Djungarian hamster increases body fat stores during summer (Heldmaier and Steinlechner 1981*a*; Klingenspor et al. 2000). The correlation between pAMPK and pACC was inconsistent in all tissues except aWAT, where a slight positive correlation was observed. Similar inconsistence has also been observed by previous studies in winter-acclimatized ground squirrels (Belke et al. 1998; Horman et al. 2005; Healy et al. 2011*b*; Lanaspa et al. 2015). It appears that ACC may be phosphorylated by AMPK-independent pathways during winter adaptation.

One distinct physiological response to short-day conditions in the Djungarian hamster is the increase in the thermogenic capacity of BAT (Heldmaier et al. 1981). It has to be noted that our experiment cannot distinguish between short photoperiod and cold acclimatization. Thus, the changes in protein expression levels are discussed as a result of winter adaptation in general. Chronic cold exposure has been shown to upregulate AMPK expression and activity in BAT of mice, suggesting that AMPK participates in long-term thermoregulation (Mulligan et al. 2007). In ground squirrels, expression of AMPK increases in BAT of hibernating animals compared to that in summertime controls (Horman et al. 2005). Our results show that also in the Djungarian hamster, AMPK expression is elevated in iBAT in response to winter acclimatization. High levels of ACC expression in the winter group (fivefold higher compared to the summer animals) indicate maintenance of efficient FA synthesis and renewal of the tissue in winter conditions. On the other hand, in summer, when the need for nonshivering thermogenesis is reduced, ACC expression and phosphorylation level are downregulated. Similar to sWAT and aWAT, SIRT1 expression was higher in iBAT of the winter hamsters. Studies in the Djungarian hamster have shown that short-day exposure increases PGC-1α mRNA expression in BAT (Demas et al. 2002), and the number of mitochondria is increased 10-fold to enable the increase in nonshivering thermogenesis (Heldmaier et al. 1981). Both AMPK and SIRT1 positively regulate PGC1-α, and increased expression of AMPK and SIRT1 in the winter-acclimatized hamsters might enhance the mitochondrial biogenesis and FA oxidation (Fulco and Sartorelli 2008; Ruderman et al. 2010). Overall, enhanced BAT activity is associated with increased expression and activity of AMPK and SIRT1 (Boutant et al. 2015; van Dam et al. 2015). Thus, higher expressions of AMPK, ACC, and SIRT1 are presumably associated with the increased thermogenic capacity of iBAT in response to winter acclimatization.

Winter acclimatization had less clear effects on skeletal muscle than on adipose tissues. In contrast to WAT and iBAT, AMPK expression declined in muscle of the winter group, and the pAMPK/AMPK ratio was higher compared to that in summer animals. Although not statistically significant, a similar pattern was observed in ACC expression and phosphorylation ($P = 0.07$ for pACC/ACC ratio). However, similar to adipose tissues, SIRT1 expression was higher in muscle of winter hamsters. Both AMPK and SIRT1 increase FA oxidation and mitochondrial biogenesis in mouse and rat skeletal muscle (Zong et al. 2002; Lee et al. 2006; Gerhart-Hines et al. 2007). Higher AMPK phosphorylation ratio, along with higher SIRT1 expression, indicates elevated oxidative capacity in skeletal muscle of the winter-acclimatized hamsters, which could be beneficial during food shortages experienced in winter (e.g., during daily torpor), when muscles are forced to switch from carbohydrates to FA oxidation. Furthermore, transgenic mice overexpressing SIRT1 in skeletal muscles have reduced muscle mass and smaller muscle fiber (Chalkiadaki et al. 2014). Higher SIRT1 expression in muscle of the winter hamsters could contribute to the lower body mass.

Increased expression and activation of hypothalamic AMPK and SIRT1 increases food intake and body weight in mice (Minokoshi et al. 2004; Satoh et al. 2010). Reciprocally, inhibition of AMPK and SIRT1 has an opposite effect (Andersson et al. 2004; Çakir et al. 2009). Studies in marmots (*Marmota flaviventris*) and ground squirrels (*Callospermophilus lateralis*) suggest that suppression of hypothalamic AMPK activity is needed for the successful torpor and maintenance of hypophagia during hibernation season (Florant et al. 2010; Healy et al. 2011*a*). However, differing from deep hibernators, the Djungarian hamster does not become aphagic during winter. Instead, foraging and food intake are decreased to accommodate the lower body weight that remains in the limits of a certain set point determined by photoperiod (Heldmaier and Steinlechner 1981*a*; Steinlechner et al. 1983). Furthermore, energetic balance is maintained despite the lower calorie intake (Scherbarth and Steinlechner 2010). Unchanged levels of hypothalamic AMPK, ACC, and SIRT1 indicate that the winter-acclimatized Djungarian hamsters were in an energetically balanced state. However, as stated previously, we did not compare the winter torpid and euthermic animals, and it is possible that hypothalamic AMPK, ACC, and SIRT1 expression and phosphorylation are altered between these stages.

To conclude, our results show that AMPK, ACC, and SIRT1 are associated with seasonal molecular adaptations in the energy metabolism of the Djungarian hamster. As hypothesized, expression levels differed between winter- and summer-acclimatized

hamsters, observed distinctly as higher AMPK, ACC, and SIRT1 expression in WAT and iBAT of the winter group. This indicates that these proteins are involved in the adjustment of lipid reserves and increased thermogenic capacity of the winter-adapted Djungarian hamster. Although increased protein expression does not necessarily equal increased enzyme activity, higher AMPK and SIRT1 protein levels in different tissues of the Djungarian hamster may facilitate the metabolic adaptation for environmental and nutritional stresses the animals encounter in winter.

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