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Hypothalamic Inhibition of Acetyl-CoA Carboxylase Stimulates Hepatic Counter-Regulatory Response Independent of AMPK Activation in Rats

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Abstract

Background: Hypothalamic AMPK acts as a cell energy sensor and can modulate food intake, glucose homeostasis, and fatty acid biosynthesis. Intrahypothalamic fatty acid injection is known to suppress liver glucose production, mainly by activation of hypothalamic ATP-sensitive potassium (K(ATP)) channels. Since all models employed seem to involve malonyl-CoA biosynthesis, we hypothesized that acetyl-CoA carboxylase can modulate the counter-regulatory response independent of nutrient availability.

Methodology/Principal Findings: In this study employing immunoblot, real-time PCR, ELISA, and biochemical measurements, we showed that reduction of the hypothalamic expression of acetyl-CoA carboxylase by antisense oligonucleotide after intraventricular injection increased food intake and NPY mRNA, and diminished the expression of CART, CRH, and TRH mRNA. Additionally, as in fasted rats, in antisense oligonucleotide-treated rats, serum glucagon and ketone bodies increased, while the levels of serum insulin and hepatic glycogen diminished. The reduction of hypothalamic acetyl-CoA carboxylase also increased PEPCK expression, AMPK phosphorylation, and glucose production in the liver. Interestingly, these effects were observed without modification of hypothalamic AMPK phosphorylation.

Conclusion/Significance: Hypothalamic ACC inhibition can activate hepatic counter-regulatory response independent of hypothalamic AMPK activation.

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Introduction

The hypothalamus actively participates in energy expenditure, satiety signals and counter-regulatory response [1–3]. Neuropeptides such as NPY, AGRP, POMC and CART are known to be expressed in the hypothalamic nucleus, to participate in the regulatory mechanism of energy expenditure, satiety signals and counter-regulatory response, and be modulated by hormones and nutrients.

AMP-activated protein kinase (AMPK) can integrate signaling circuits between peripheral tissues and the hypothalamus to regulate food intake and whole-body energy expenditure [3,4]. This important cell energy sensor can activate the catabolic pathways that produce ATP when energy availability is low. On the other hand, when energy is sufficient for cellular activity, it shuts down pathways that produce energy [5]. Additionally, hypothalamic AMPK has an important role in the expression of hypothalamic neuropeptides [6–8] and counter-regulatory response [2], modulating energy expenditure and plasma concentrations of corticosterone, glucagon, and catecholamines.

Acetyl-CoA carboxylase (ACC) is responsible for catalyzing the reaction that produces malonyl-CoA, an intermediate in the biosynthesis of fatty acids. ACC is an AMPK target, which phosphorylates on Ser72 and thereby inactivates ACC under conditions of energy surplus [9]. Several studies have shown that pharmacological activation of AMPK, which promotes the inhibition of ACC and a decrease in hypothalamic levels of malonyl-CoA, leads to an increase in food intake [10,11]. Furthermore, recently Kinote and colleagues showed that fructose activates hypothalamic AMPK and stimulates hepatic PEPCK and gluconeogenesis [12]. On the other hand, refeeding and fatty acid synthase inhibitor increase the hypothalamic availability of malonyl-CoA and decrease food intake [13–16]. The hypothalamic level of malonyl-CoA increases (4.0-fold) in response to

transition from fasted to fed state. In fasted rats, the reduction of hypothalamic level of malonyl-CoA occurs even in the presence of acetyl-CoA [17].

Hypothalamic lipid metabolism is important for the control of energy metabolism [18–20]. Obici and colleagues demonstrated that oleic acid [21] and inhibition of carnitine palmitoyltransferase-1 [22] decrease food intake and liver glucose production. More recently, Ross and collaborators demonstrated differential effects of hypothalamic long-chain fatty acid infusion on the glucose production [23]. In this study, they showed that a low dose of oleic acid administered to the medium basal hypothalamus is sufficient to markedly reduce liver glucose production, whereas a polyunsaturated fatty acid (linoleic acid) and a saturated fatty acid (palmitic acid) did not show any effect or only in high dose, respectively. Mammalian cells are not capable of producing polyunsaturated fatty acids, but the biosynthesis of saturated and monounsaturated fatty acids that occurs in the cytoplasm is very important for this pathway.

We hypothesized that inhibition of ACC independent of nutritional status and AMPK activation has an important role in the positive modulation of hepatic counter-regulatory response. To test this hypothesis, intracerebroventricular injection of antisense oligonucleotide (ASO) to acetyl-CoA carboxylase (ACC) was performed in rats with free access to food; the analyses were performed at noon because it is a time when the animal has no counter-regulatory stimulus resulting from long fasting.

Materials and Methods

Ethics Statement

This study was carried out in strict accordance with the recommendations of the COBEA (Brazilian College of Animal Experimentation) guidelines, which was approved by the Ethical Committee for Animal Use (ECAU) (ID protocol: 1970–1) of the Universidade Estadual de Campinas (UNICAMP), Campinas, São Paulo, Brazil.

Animals and Surgical Procedures

Male Wistar rats (12 wk old, 250–280 g) were taken from the University's central breeding colony and maintained in polypropylene cages in a room at 24±1°C with lights on from 6:00 to 18:00 h and fed diets and water *ad libitum*. The rats were chronically instrumented with an ICV cannula and kept under controlled temperature and light-dark conditions in individual metabolic cages. Surgery was performed under anesthesia, and all efforts were made to minimize animal suffering. Briefly, the animals were anesthetized with 50 mg/kg ketamine and 5 mg/kg diazepam (ip) and positioned onto a Stoelting stereotaxic apparatus after the loss of cornea and foot reflexes. A stainless steel 23 gauge guide cannula with an indwelling 30 gauge obturator was stereotaxically implanted into the lateral cerebral ventricle at pre-established coordinates, anteroposterior, 0.2 mM from bregma, lateral, 1.5 mM; and vertical, 4.2 mM, according to a previously reported technique [24]. The cannulas were considered patent and correctly positioned by dipsogenic response elicited after injection of angiotensin II (2 μL of solution 10^{-6} M) [25]. After test for cannula function and position, rats were randomly assigned to one of the experimental groups.

Food intake and Body Weight Measures

Body weight was evaluated from the first to the fourth days of treatment with ASO. Food intake was evaluated on the third day after the ACC-ASO administration was started. Pre-weighed food was provided in individual cages 10 min before the start dark period. Cumulative food intake was measured after 12 h by weighing the residual food in the cages. The amounts of food left over on the bottom of the cages were recorded. Intake was calculated as the weight (g) of food provided less that recovered.

AICAR Injection

The fed rats received 3 μL of bolus injection of 2 mmol/L 5-amino-1-β-D-ribofuranosyl-imidazole-4-carboxamide (AICAR) into the lateral ventricle.

Sense (SO) and Antisense Oligonucleotide (ASO) Injection

Phosphorothioate-modified sense and antisense oligonucleotides (produced by IDT, Munich, Germany) were diluted to a final concentration of 1 nmol/μL in dilution buffer containing 10 mmol/L Tris–HCl and 1.0 mmol/L EDTA. The oligonucleotides sequences consisted of 5′-GCC AGT CAG TAA GAG CAG-3′ (sense) and 5′-TGA GAT CTG CAA TGC A-3′ (antisense). Wistar rats were injected into the lateral ventricle with two daily doses of 4 nmoles oligonucleotides in dilution buffer containing either sense (ACC-SO) or antisense oligonucleotides (ACC-ASO) for three days. Fragments of liver and hypothalamus were obtained at about 12:00 h on the fourth day of treatment with oligonucleotide. The animals were provided free access to water and rat chow. Control animals received saline solution.

Pyruvate Challenge

Rats with free access to food were injected intraperitoneally with sodium pyruvate (0.5 g/kg). Blood samples were collected from the tail vein immediately before and at various time points (0–120 min) after the pyruvate load to measure blood glucose.

Tissue Extraction and Immunoblotting

The rats were anesthetized after specific treatments and tissue samples were obtained and homogenized in freshly prepared ice cold buffer (1% Triton X 100, 100 mM TRIS, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF, and 0.01 mg aprotinin.mL^{-1}). The insoluble material was removed by centrifugation (10,000 g) for 25 min at 4°C. The protein concentration in the supernatant was determined by the Bradford dye-binding method. The supernatant was resuspended in Laemmli sample buffer and boiled for 5 min before separation in SDS PAGE using a miniature slab gel apparatus (Bio Rad, Richmond, CA). Electrotransfer of proteins from the gel to nitrocellulose was performed for 90 min at 120 V (constant). The nitrocellulose transfers were probed with specific antibodies. The phophoenolpyruvate carboxykinase (PEPCK) and glyceraldeyde-3-phosphate dehydrogenase (GAPDH) antibodies were obtained from Santa Cruz Biotechnology, Inc., Santa Cruz, CA. The total AMP activated protein (AMPK), phospho-AMPK (p-AMPK), total ACC (ACC) and phospho-ACC antibodies were obtained from Cell Signaling Technology, Inc., Danvers, MA. Subsequently, the blots were incubated with HRP-conjugate antibodies (KPL, Gaithersburg, MD- USA). The results were visualized by autoradiography with preflashed Kodak XAR film. Band intensities were quantified by optical densitometry of developed autoradiographs (Scion Image software, ScionCorp) and the intensities of the bands were normalized to those of either total protein or GAPDH to correct for protein loading in the case of cellular lysate extracts.

Immunofluorescence Staining

For histological evaluation, hypothalamic tissue samples were fixed in 4% formaldehyde and processed routinely for embedding in paraffin block. The samples were dehydrated (alcohol at 70, 80, 90, 95%, and absolute), diaphanized by immersion in xylol, and embedded in paraffin. Hydrated (alcohol at absolute, 95, 90, 80, and 70%) 5.0-mm paraffin sections were processed for immunofluorescence staining. The expression of ACC (Cell Signaling Technology, Inc., Danvers, MA) was analyzed employing goat anti-rabbit-FITC was used as secondary. The images were obtained using a Leica FW 4500 B microscope, software LAS V3.8.

Real Time PCR Analysis

Hypothalamic total RNA was extracted using Trizol reagent (Life Technologies, Gaithersburg, MD, USA) according to the manufacturer's recommendations. Total RNA was rendered genomic DNA-free by digestion with Rnase-free Dnase (RQ1, Promega, Madison, WI, USA). Reverse-transcription was performed using total RNA from hypothalamic samples. Intron-skipping primers for NPY, POMC, CART, TRH, and CRH mRNAs were obtained from Applied Biosystems. Real-time PCR analysis of gene expression was performed in an ABI Prism 7700 sequence detection system (Applied Biosystems, California). The optimal cDNA and primer concentrations, as well as the maximum efficiency of amplification, were obtained through five-point, two-fold dilution curve analysis for each gene. Each PCR contained 20 ng of reverse-transcribed RNA and was run according to the manufacturer's recommendation using the TaqMan PCR Master Mix (Applied Biosystems, California). Target mRNA expression was normalized to GAPDH expression and expressed as a relative value using the comparative threshold cycle (Ct) method ($2-\Delta\Delta Ct$) according to the manufacturer's instructions.

Biochemical and Hormonal Measurements

Blood glucose was determined using a glucometer. Serum insulin, glucagon, and corticosterone were analyzed simultaneously and measured in duplicate at all time points using a commercially available rat endocrine Linco-plex kit (Rendo-85 K, Linco Research, St Charles, MO, USA). All blood samples were collected from the tail vein.

For the determination of the level of hepatic glycogen, a tissue fragment was evaluated as described by Burant and colleagues [26].

Data Presentation and Statistical Analysis

All numerical results are expressed as means ± SE of the indicated number of experiments. Blot results are presented as direct band comparisons in autoradiographs and quantified by densitometry using the Scion Image software (ScionCorp). Student's t-tests of unpaired samples and variance analyses (ANOVA) for multiple comparisons were used as appropriate. Post hoc test (Tukey) was employed when required at significance level of $p<0.05$.

Results

AMPK Phosphorylation and ACC Expression

Initially, we evaluated the levels of hypothalamic phosphorylation of AMPK and the expression of ACC. As expected, fasted rats showed higher AMPK phosphorylation than fed rats. The ICV treatment of fed rats with either antisense or sense oligonucleotide (ASO and SO, respectively) to ACC proteins did not affect the hypothalamic phosphorylation of AMPK (Fig. 1A). In addition, we evaluated the effect of intracerebroventricular (ICV) injection of ACC-ASO (antisense oligonucleotide) on the hypothalamic, hippocampal and brainstem expression of ACC. For this purpose, rats were treated with either antisense or sense oligonucleotide (4 nmol/animal) twice a day for three days. As shown in Fig. 1B, the ACC-ASO treatment reduced ACC expression in the hypothalamus by 50%, when compared to both the control group (treated with saline) and the ACC-SO group (sense nucleotide). As expected, the treatment with ACC-SO did not affect the expression of ACC. ACC expression was also evaluated in the brainstem and hippocampus of control and ACC-ASO rats. As shown in Fig. 1C, ACC-ASO rats presented reduced ACC expression when compared to control rats in both investigated areas. Furthermore, to evaluate whether the ACC-ASO treatment affected the neuronal populations in the arcuate nucleus (ARC), paraventricular nucleus (PVH) and lateral hypothalamus (LH) differently, we performed immunoflorescence staining of hypothalamus samples from control and ACC-ASO rats. ACC expression was detected in all studied nuclei. However, as expected, it decreased in the ARC, PVH and LH after administration of ACC-ASO (Fig. 1D).

Food intake, Body Weight and Epididymal Fat Mass

Food intake was measured on the third night after the treatment with ACC-ASO had been started. As can be observed in Fig. 2A, rats treated with ACC-ASO presented higher food intake (20.0±0.7 g/12 h) than control (saline) and ACC-SO (15.9±1.3 g/12 h and 16.0±1.0 g/12 h, respectively). At the end of the experimental period, the epididymal fat mass and body weight gain were measured. Figure 2B shows that the treatment with ACC-ASO diminished epididymal fat mass (1.5±0.4 g), when compared to control (5.0±0.8 g) and ACC-SO animals (4.5±0.7 g). Furthermore, we evaluated body weight gain. Although, body weight gain was lower in ACC-ASO than in control and ACC-SO rats, the difference was not significant (Fig. 2C).

Real Time Analysis of Gene Expression

CART, TRH, and CRH mRNA were measured in the hypothalamus by real time PCR. As shown in Figure 3, the treatment with ACC-ASO significantly decreased gene expression of neuropeptides CART (50%), TRH (70%), and CRH (40%) (Figs. 3A, B, and C, respectively). On the other hand, NPY expression increased (2.7-fold) in ACC-ASO rats when compared to control rats. POMC mRNA was not different among the evaluated groups. Interestingly, the analysis of gene expression in fasted rats revealed a behavior similar to that of rats treated with ACC-ASO. ACC-SO treatment did not alter the expression of any of the hypothalamic neuropeptides evaluated.

Serum Hormone Level

Serum glucagon, insulin, and corticosterone levels were quantified on the fourth day. The animals had previous free access to chow and blood samples were collected at 12:00 h. The serum corticosterone level was not affected by the treatments with ACC-ASO, ACC-SO, or saline ICV (data not shown). However, the serum level of glucagon in the ACC-ASO group (14.9±2.7) was higher than in the control and ACC-SO groups (6.2±1.5 and 4.0±2.3 $pmol.L^{-1}$, respectively), but it was similar in the fasted group (11.8±4.0 $pmol.L^{-1}$) (Fig. 4A). On the other hand, the serum level of insulin for ACC-ASO was similar to that of fasted animals (204±38 and 194±102 $pmol.L^{-1}$, respectively), but smaller than the control

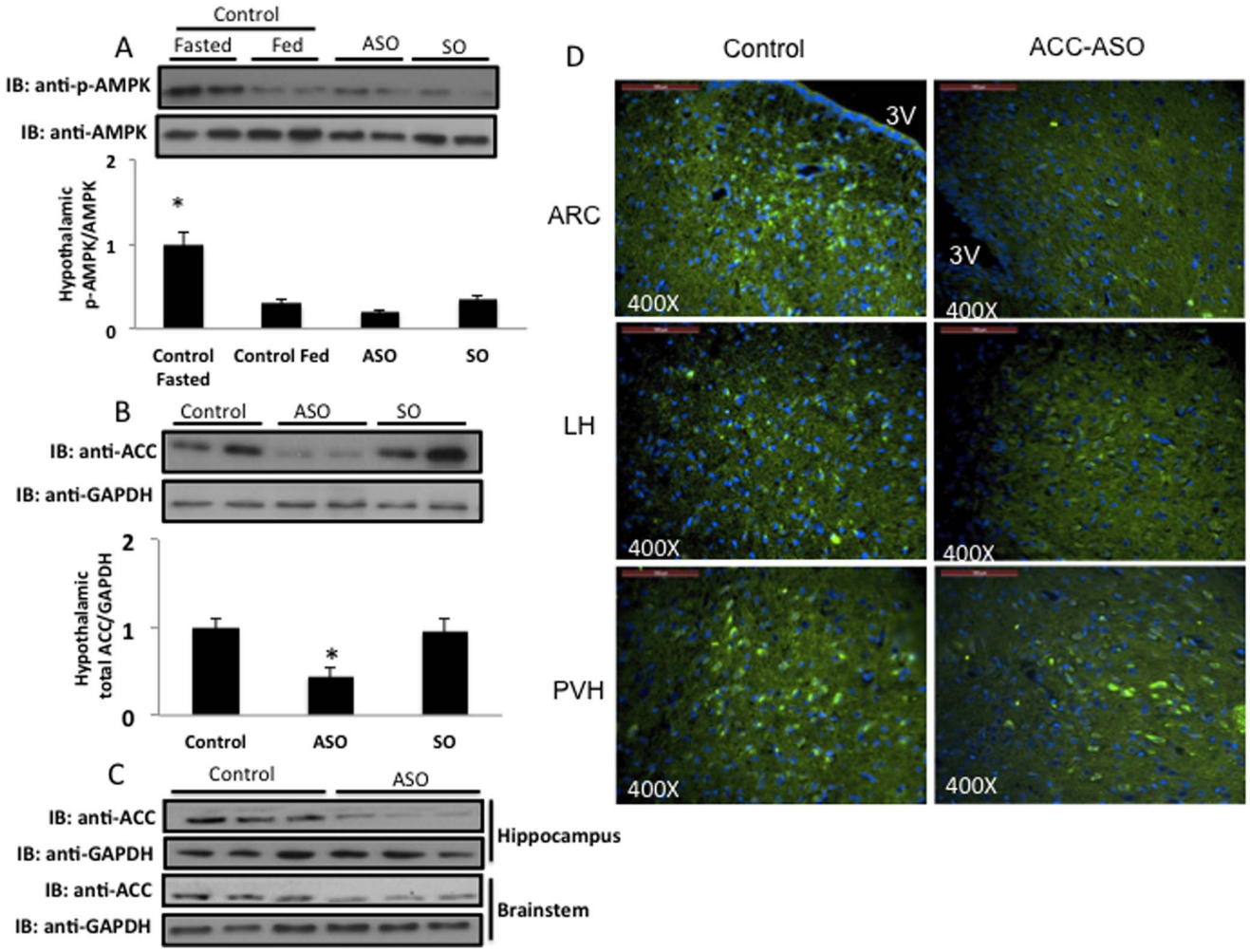

Figure 1. Modulation of AMPK/ACC pathway in central nervous system of ACC-ASO and ACC-SO mice. Representative western blot of hypothalamic p-AMPK (A) and ACC (B) in fasted and fed rats, ACC-ASO (ASO) and ACC-SO (SO). Representative western blot of hippocampus and brainstem area of ACC (C). Representative ACC immunoflorescence staining (green) of samples from hypothalamus (ARC, LH and PVH) of Wistar rats. Cell nuclei were counterstained with DAPI (blue) (D). Bars show quantification of total p-AMPK and ACC proteins normalized by either total GAPDH or AMPK. Data are means ± SEM of five rats. (A)*p≤0.05 vs. control fed, ASO and SO. (B)*p≤0.05 vs. control and SO.
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and ACC-SO treatment values (459±215 and 648±114 pmol.L^{-1}, respectively) (Fig. 4B). Interestingly, the blood level of ketone bodies increased in the ACC-ASO group relative to the control and ACC-SO groups (0.47±0.11, 0.29±0.07 and 0.32±0.09 mmol.L^{-1}, respectively) (Fig. 4C). The hepatic glycogen store was reduced in ACC-ASO (20.1±5.0 mg.g^{-1} of tissue) when compared to control and ACC-SO rats (35±7 and 34±3 mg.g^{-1} of tissue, respectively) (Fig. 4D).

Hepatic Gluconeogenic Profile

To assess whether the liver presented counter-regulatory activity, we evaluated the expression of PEPCK and the capacity of the liver to produce glucose after administration of pyruvate, a gluconeogenic substrate. As can be observed in Figure 5A, the expression of hepatic PEPCK increased significantly (by seven-fold) after treatment with ACC-ASO when compared to control animals, and so did the phosphorylation of AMPK and ACC (Fig. 5B). The treatment with ACC-SO, as expected, did not exert any effect on PEPCK expression and AMPK and ACC phosphorylation relative to control and ACC-SO.

To evaluate liver glucose production, rats received intraperitoneal injection of sodium pyruvate and blood glucose was measured (Fig. 6A and B). As can be observed in Figures 6A and 6B, the glycemic curve and the area under the curve (AUC) were greater in the group treated with ACC-ASO than with ACC-SO and control (3960±700, 2800±400, 2340±450, respectively). Additionally, we evaluated the glycemic curve and AUC in rats previously ICV treated with AICAR, a pharmacological activator of AMPK. As expected, the increase in blood glucose was higher if compared to ACC-SO and control rats.

Discussion

The results presented in this study demonstrate the role of hypothalamic acetyl-CoA carboxylase (ACC) in the control of hepatic glucose production. The studies performed so far have demonstrated the participation of AMPK in the modulation of food intake and glucose homeostasis by different mechanisms in the hypothalamus [1–3,16,18,21–23,27]. A common feature to

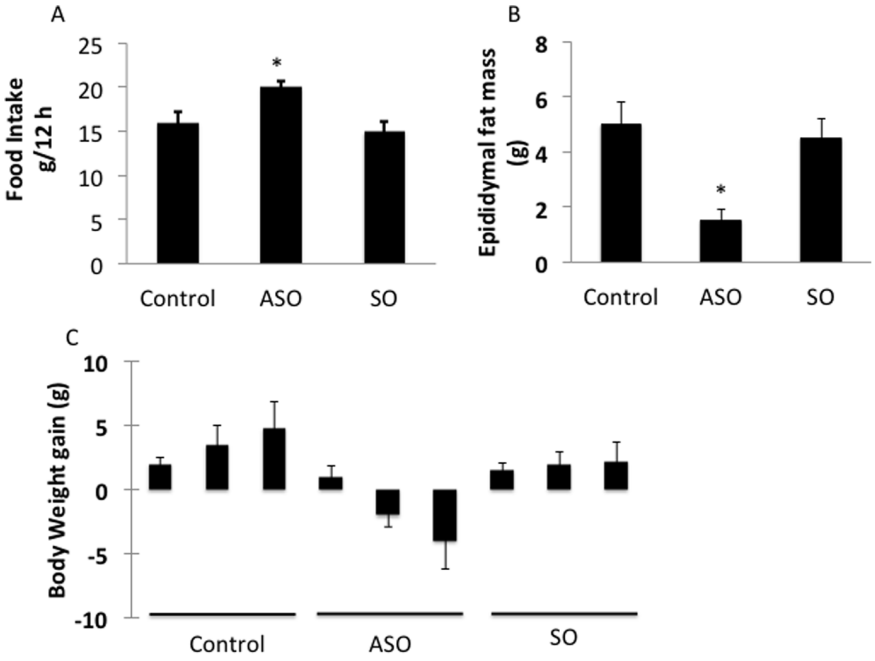

Figure 2. Anthropometric evaluation and feeding behavior in ASO-ACC and ACC-SO mice. Food intake (A), epididymal fat mass (B) and body weight (C) in control, ACC-ASO (ASO) and ACC-SO (SO) rats. Data are means ± SEM of 8–10 rats. (A)*p≤0.05 vs. control and SO rats.
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mechanisms proposed is the modulation of the hypothalamic level of malonyl-CoA. The biosynthesis of malonyl-CoA is controlled by ACC, a key enzyme in the control of biosynthesis of fatty acid and widely expressed in different tissues. Activated AMPK phosphorylates (at ser79) and inhibits ACC, leading to a reduction in the level of malonyl-CoA. The hypothalamic activity of AMPK increases during fasting and decreases during refeeding [4]. To investigate the hypothesis that the hypothalamic level of malonyl-CoA can modulate liver glucose production, we initially evaluated the hypothalamic phosphorylation of AMPK in fasted and freely fed rats (light cycle). As expected, fasted rats showed greater AMPK phosphorylation than fed rats. Reduced AMPK phosphorylation is linked to an increase in ACC activity and biosynthesis of malonyl-CoA. Tokutake and colleagues showed that the hypothalamic level of malonyl-CoA is modulated by the fasting/feeding transition in rats [17]. Interestingly, although the

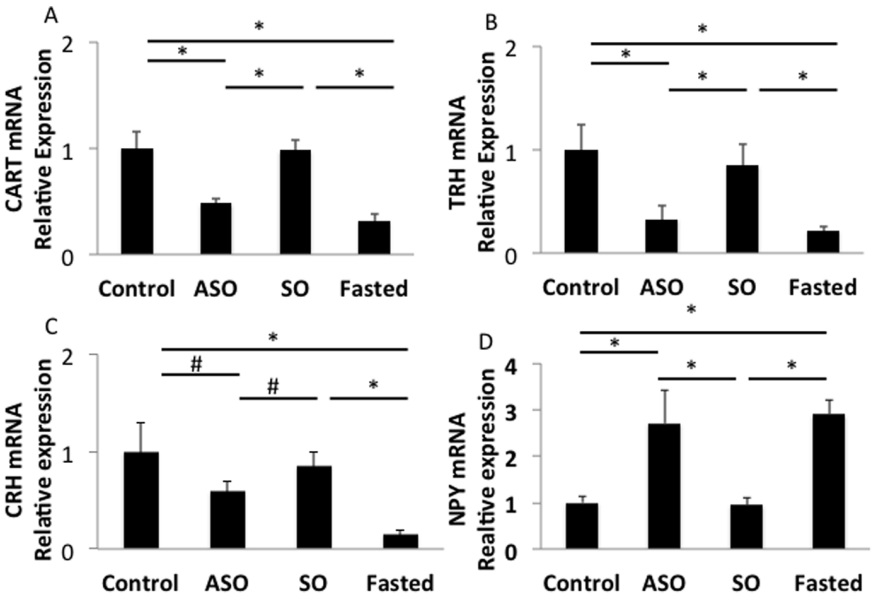

Figure 3. mRNA level of neuropeptides in hypothalamus from ASO-ACC and ACC-SO mice. Analyses of CART, TRH, CRH and NPY mRNA expression in the hypothalamus of control, ACC-ASO (ASO) and ACC-SO (SO) rats by RT-PCR. Data are means ± SEM of 8–10 rats. #p≤0.05 and *p≤0.01.
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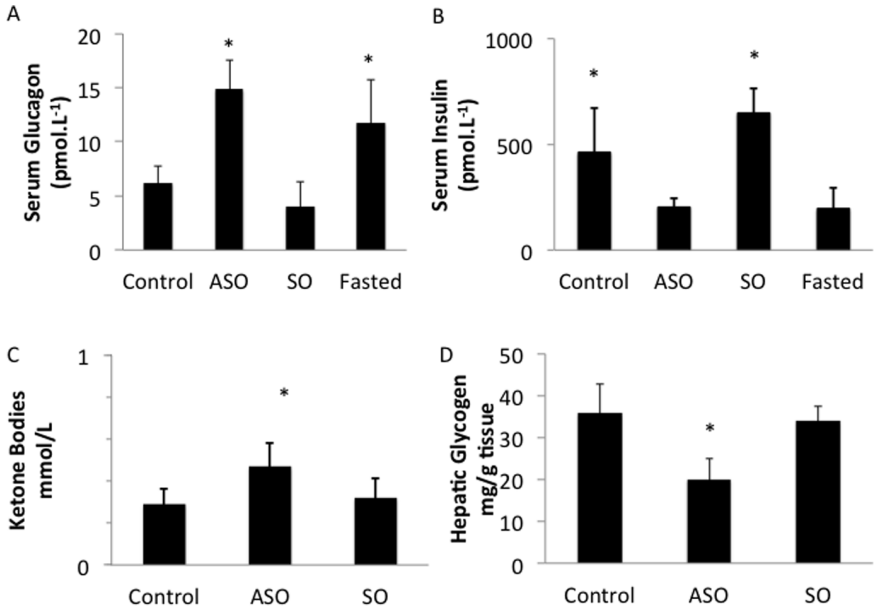

Figure 4. Biochemical parameters in ASO-ACC and ACC-SO mice. Serum glucagon (A) and insulin level (B), blood ketone bodies (C), and hepatic glycogen (D) in control (fed), ACC-ASO (ASO), ACC-SO (SO) and fasted rats. Data are means ± SEM of 8–10 rats. (A) *p≤0.01 to ASO and fasted vs. control and SO. (B) *p≤0.05 vs. ASO and fasted. (C and D) *p≤0.05 vs. control and SO.
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hypothalamic level of malonyl-CoA was modified by the fasting/feeding state, the acetyl-CoA level was not altered. To test the hypothesis that the reduction of hypothalamic ACC activity in fed rats would be enough to activate a counter-regulatory response, we injected ICV ACC-ASO in the light cycle in freely fed rats. Although, ACC-ASO injection did reduce hypothalamic ACC protein, it did not affect the hypothalamic phosphorylation of AMPK (Figs. 1A and B). Interestingly, the level of phospho-ACC (inactive form) was not different between groups (data not shown). This result reinforces the idea that treatment with ACC-ASO diminished the availability of active ACC and, consequently, of malonyl-CoA to the cells.

In an elegant study in Kahn's Lab, Minokoshi and colleagues demonstrated that constitutively active-AMPK mice ate more and had increased expression of NPY and AGRP mRNA in ARC [4]. The increase in the AMPK activity was linked to diminished ACC activity, mimicking a fasting condition. In our study, ACC-ASO rats also presented reduced hypothalamic ACC expression (green), if compared to control rats (free access to food) and ACC-SO (Fig. 1B and 1D) in all nuclei studied (ARC, PVH and LH). Furthermore, ACC-ASO also decreased ACC expression in the brainstem and hippocampus (Fig. 1C). Interestingly, lower hypothalamic CART, CRH, and TRH mRNA levels and increased NPY mRNA, an orexigenic neuropeptide, accompanied this effect. CART, CRH, and TRH mRNA present reduced hypothalamic expression in response to fasting and leptin [28,29]. Furthermore, it is important to point out that the analyses were performed in the light cycle, a period of reduced food intake [30].

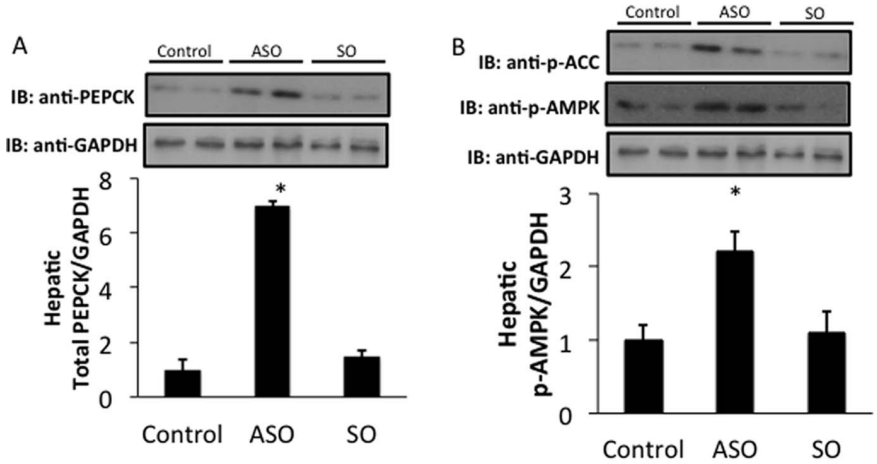

Figure 5. Liver PEPCK expression and AMPK/ACC phosphorylation in ASO-ACC and ACC-SO mice. Representative western blot of PEPCK (A). Bars show quantification of total PEPCK protein normalized by total GAPDH. Representative western blot of p-AMPK and p-ACC protein normalized by total GAPDH (B). Data are means ± SEM of 4–6 rats. *p≤0.05 vs. control and ACC-SO rats.
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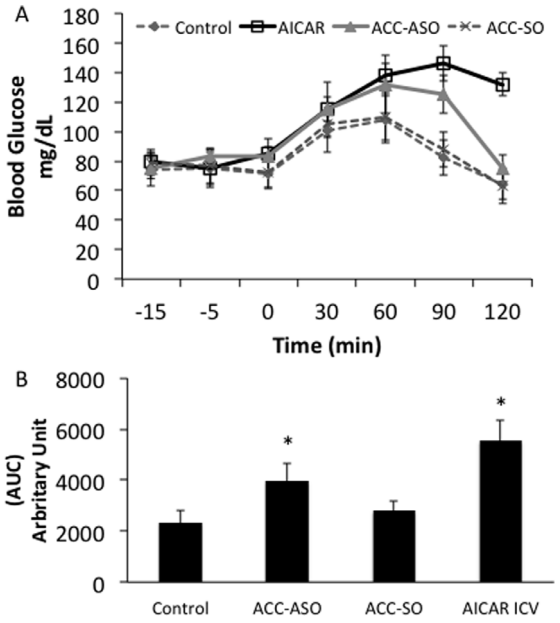

Figure 6. Liver glucose production after challenge with pyruvate in ASO-ACC and ACC-SO mice. Blood glucose during pyruvate test (A) and area under curve (AUC) (B). Data are means ± SEM of 8–10 rats. *p≤0.05 vs. control and SO rats.
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Although ACC-ASO-treated rats had free access to food, they presented gene expression similar to those of fasted rats (higher NPY level). Additionally, they did not show difference in body weight (Fig. 2C) but epididymal fat mass was reduced (Fig. 2B), due to reduced expression of CART, TRH, and CRH, three neuropeptides linked to pro-thermogenic metabolism and anorexigenic behavior [31,32]. Therefore, these results reinforce the role of malonyl-CoA as a hypothalamic indicator of energy homeostasis. Although, reduced epididymal fat mass seems to be contradictory, since ACC-ASO rats presented higher food intake than control rats (Fig. 2A), the increased lipolysis observed, as indicated by reduced epididymal fat pad, may be due to increased serum levels of glucagon observed in ACC-ASO rats. Furthermore, although the body weight gain was similar, we believe that three days were insufficient to affect body weight gain.

In fasting state, the hepatic metabolism shifts toward fat oxidation and synthesis of glucose as part of a counter-regulatory hormonal response. This shift in metabolism is important for the energy homeostasis, in which the hypothalamus has a fundamental role. Han and colleagues showed that pharmacological inhibition of hypothalamic AMPK or ARC/VMH DN-AMPK overexpression attenuated hypoglycemia-induced increases in plasma concentrations of corticosterone, glucagon, and catecholamine [2]. They concluded that systemic hypoglycemia causes hypothalamic activation of AMPK, which is important for counter-regulatory hormonal responses. In addition, recently, Kawashima and colleagues showed that hypothalamic AMPK activation by glucopenia occurs via a CaMKK-independent pathway [33] and that another AMPK upstream kinase might be involved in 2DG activation of AMPK, such as LKB1 [34]. Considering that ACC-ASO-treated rats mimic fasting condition, we compared their hormonal profile to that of fasted rats. ACC-ASO-treated rats presented reduced serum insulin and hepatic glycogen accompanied by increased serum glucagon, ketone bodies, and AUC in the pyruvate test (Figs. 4 and 6), typical hormonal and biochemical responses to fasting condition. Therefore, these results suggest that modulation of the hypothalamic activity of ACC may be able to trigger a counter-regulatory response, independent of the availability of nutrients, and hypothalamic phosphorylation of AMPK.

In recent years, many studies have shown that the hypothalamus participates in the modulation of hepatic glucose production by activation of hypothalamic ATP-sensitive potassium (K(ATP)) channels and parasympathetic signals delivered by the vagus nerve [35–38], which is associated with reduced hepatic expression of gluconeogenic genes, glucose-6-phosphatase (G6Pase), and phosphoenolpyruvate carboxykinase (PEPCK). The liver is known to play an important role in glucose homeostasis through gluconeogenesis [39]. In ACC-ASO-treated rats, liver expression of PEPCK increased (Fig. 5A) corroborating the results of pyruvate tolerance test. These effects were accompanied by increase in liver AMPK and ACC phosphorylation in ACC-ASO-treated rat (Fig. 5B). Liver activation of AMPK is linked to diminished expression of gluconeogenic enzymes [40] and phosphorylation of glycogen synthase 1 [41], which reduces the glucose output from the liver. Furthermore, the increase in the blood level of ketone bodies (Fig. 4C), as well as ACC inactivation in the liver (Fig. 5B), suggests that fatty acid oxidation is increased under ACC-ASO, when compared to control and ACC-SO groups.

Thus, we believe that the hypothalamic levels of ACC protein and malonyl-CoA are important signals to control liver glucose production by an AMPK-independent mechanism. Although AMPK surely is an important nutrient and energy sensor that maintains energy homeostasis, many proteins can be modulated by kinase activity of AMPK. Thus, in the cell, it can modulate the pathway related to protein synthesis, mitochondrial biogenesis, fatty acid and glucose metabolism, and autophagia [42]. Therefore, ACC may be a better target to control the hepatic metabolism than AMPK.

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Author Contributions

Conceived and designed the experiments: LAV MAT. Performed the experiments: GAS VDP EAFRR DCV LI RFM DSR. Analyzed the data: GAS AST LAV MAT. Contributed reagents/materials/analysis tools: LAV MAT. Wrote the paper: AST MAT.

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