Differential effects of central fructose and glucose on hypothalamic malonyl–CoA and food intake

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The American diet, especially that of adolescents, contains highly palatable foods of high-energy content and large amounts of high-fructose sweeteners. These factors are believed to contribute to the obesity epidemic and insulin resistance. Previous investigations revealed that the central metabolism of glucose suppresses food intake mediated by the hypothalamic AMP-kinase/malonyl–CoA signaling system. Unlike glucose, centrally administered fructose increases food intake. Evidence presented herein indicates that the more rapid initial steps of central fructose metabolism deplete hypothalamic ATP level, whereas the slower regulated steps of glucose metabolism elevate hypothalamic ATP level. Consistent with effects on the [ATP]/[AMP] ratio, fructose increases phosphorylation/activation of hypothalamic AMP kinase causing phosphorylation/inactivation of acetyl–CoA carboxylase, whereas glucose has the inverse effects. The changes provoked by central fructose administration reduce hypothalamic malonyl–CoA level and thereby increase food intake. These findings explain the paradoxical fructose effect on food intake and lend credence to the malonyl–CoA hypothesis.

acetyl–CoA carboxylase | AMP kinase | high-fructose corn syrup | hypothalamic ATP | obesity

Over the past three decades there has been an alarming increase in the incidence of obesity and type 2 diabetes in the United States (1). Particularly troubling is the rise of these conditions in youth (2). Paralleling this rise has been the extensive use of high-fructose sweeteners in the diet and increasing evidence that fructose may be a contributing factor to the obesity epidemic (3). These correlations are consistent with the finding that high-fructose diets promote insulin resistance, glucose intolerance, and increased rates of hepatic lipogenesis in laboratory animals (4).

Although both glucose and fructose enter metabolism via the glycolytic pathway, the initial steps of hepatic fructose metabolism differ from those of glucose. Likewise, recent evidence suggests that sugar metabolism in regions of the central nervous system (CNS) that control food intake and energy expenditure, fructose metabolism also differs from that of glucose. It is known that the initial steps of hepatic fructose metabolism use a different set of enzymes that allow this sugar to bypass the rate-limiting step [catalyzed by phosphofructokinase (PFK)] in the glycolytic pathway. Similar enzymes of fructose metabolism are found in regions of the CNS that play an important role in monitoring energy balance and satiety control (5–7). These findings are consistent with a recent report (ref. 8 and findings reported herein) that centrally-administered fructose provokes feeding. In contrast, the central administration of glucose causes satiety (8, 9). In this article, we provide a molecular basis for these differences. It should be noted, however, that uncertainty remains regarding the extent to which fructose in systemic circulation can cross the blood–brain barrier to enter these regions of the brain (10–12).

Under conditions of energy surplus cellular ATP level rises and AMP level falls, this inverse relation being determined by the adenylate kinase (AK)-catalyzed equilibrium (ATP + AMP $\leftrightarrows$ ADP + ADP) (13, 14). Because AMP is an activator of AMP kinase (AMPK), a drop in AMP level causes the dephosphorylation/inactivation of AMPK. Because AMPK catalyzes the phosphorylation/activation of acetyl-CoA carboxylase (ACC), it follows that a decrease in AMPK activity leads to dephosphorylation and activation of hypothalamic ACC. Consistent with this scenario we found that the central administration of glucose increases hypothalamic malonyl–CoA, decreases orexigenic neuropeptide expression, increases anorexigenic neuropeptide expression and suppresses food intake (9). Similar effects were elicited by feeding a high-carbohydrate diet after food deprivation.

Glucose, the major circulating sugar in animals, is the primary fuel of the brain in the fed state (15) and an indicator of energy status. It is not surprising, therefore, that the central metabolism of glucose produces satiety. It does so by inactivating AMPK and depressing ACC activity, which in turn increases hypothalamic malonyl–CoA to produce an anorectic effect (9). A recent seemingly counterintuitive finding (8) revealed that fructose has the inverse effect, i.e., when administered centrally fructose increased food intake. The present article provides the metabolic basis for this observation and provides a rationale for the possible deleterious effect of excessive fructose consumption. Here, we show that, unlike glucose, the rapid initial steps of fructose metabolism in the CNS provoke an immediate drop in the ATP/AMP ratio, increased AMPK activity, decreased ACC activity, lowered malonyl–CoA in the hypothalamus, and as a result increased food intake.

Results

Previous studies showed that glucose administered by i.p. injection rapidly enters and is metabolized by the brain, increasing the level of hypothalamic malonyl–CoA (9), which is known to suppress food intake (16–18). Because an earlier study showed that fructose administration had the opposite effect on feeding behavior (8), we set out to determine the effect of fructose on hypothalamic malonyl–CoA. Preliminary experiments were conducted to assess the feasibility of delivering fructose to the CNS indirectly via systemic circulation of food-deprived mice. As illustrated in Fig. 1*A*, fructose rapidly entered systemic circulation after i.p. injection and reached a maximal level within 6 min then returned to baseline within 45–50 min. After a short lag of 10–12 min blood glucose began to increase (Fig. 1*A*), presumably a result of a lag in the hepatic conversion from fructose.

To verify that i.p. fructose is directly metabolized to malonyl–CoA in the brain without first being converted to glucose, mice were given 2-deoxyglucose (2-DG) by i.c.v. injection just before the peripheral (i.p.) administration of fructose. 2-DG undergoes phosphorylation by hexokinase to produce 2-DG-6-phosphate, a

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The authors declare no conflict of interest.

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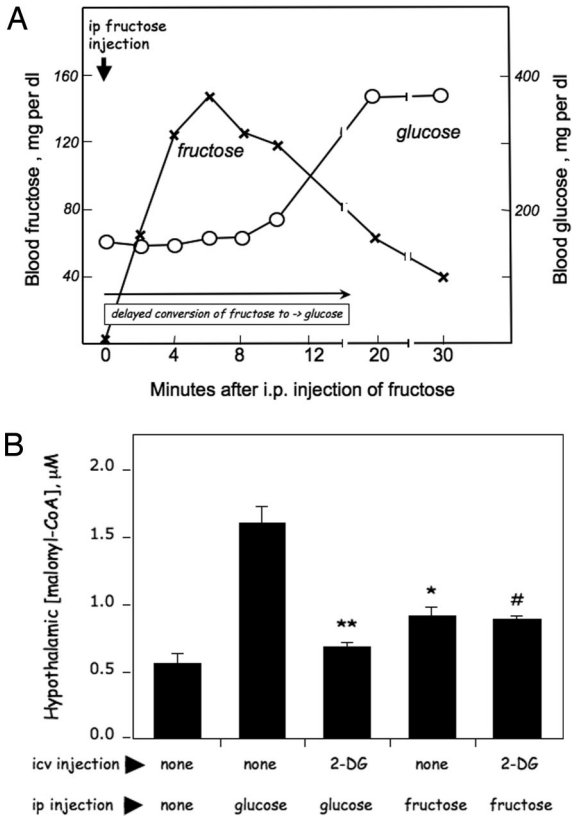

Fig. 1. Effects of i.p. fructose injection on blood sugar and hypothalamic malonyl–CoA levels. (*A*) Food-deprived mice were given i.p. injections of fructose (4 g/kg of body weight) after which blood fructose and glucose were quantified ($n = 4$ per group). (*B*) Mice treated as in *A* were given an i.c.v. injection of 2-DG (2 μl of 5 mM 2-DG) or vehicle and 10 min later hypothalami were extirpated for malonyl–Co A analysis ($n = 4$ per group). **, $P < 0.001$; *, $P < 0.01$ versus i.p. glucose only; #, $P < 0.05$ versus i.c.v. 2DG/i.p. glucose.

potent inhibitor of glucose-6-phosphate metabolism and the glycolytic pathway. Thus, downstream events including those required to produce malonyl–CoA from peripheral administration are disrupted. It should be noted that we previously showed (9) that i.c.v. injection of 2-DG blocks the conversion of glucose to malonyl–CoA in the hypothalamus. As shown in Fig. 1*B*, 2-DG prevented the increase in hypothalamic malonyl–CoA caused by i.p. glucose. However, a blockade of central glucose metabolism by 2-DG had no effect on the fructose-induced increase in hypothalamic malonyl–CoA. Thus, it appears that fructose entry into glycolysis in the hypothalamus bypasses the entry point of glucose in the pathway. The extent to which peripherally administered fructose enters the hypothalamus remains uncertain (see *Discussion*).

The time window for distinguishing the downstream effects of fructose metabolism in the CNS from those of glucose is short, i.e., ≈10 min (see Fig. 1*A*), as peripherally administered fructose is rapidly converted to blood glucose in the liver. This view is supported by the finding (results not shown) that 30–45 min after i.p. fructose injection when blood glucose levels had risen, hypothalamic malonyl–CoA rises and i.c.v. 2-DG blocked this effect, responses characteristic of glucose (ref. 9 and Fig. 1*B*) rather than fructose. Therefore, in all subsequent experiments fructose and glucose were administered by i.c.v. injection to ensure that only the short-term central effects of fructose metabolism were assessed.

Effect of Centrally Administered Fructose and Glucose on Hypothalamic ATP and the Phosphorylation State of AMPK. The rapid initial steps of fructose metabolism that bypass the slower, regulated

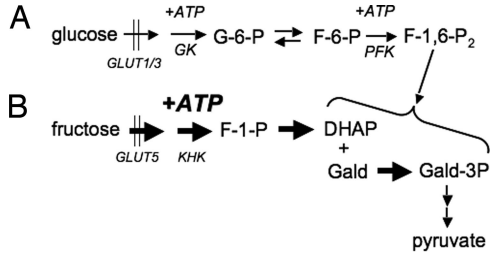

Fig. 2. Metabolic pathways of entry of (*A*) glucose and (*B*) fructose into the glycolytic pathway of the CNS. GK, glucokinase; KHK, ketohexokinase; DHAP, dihypoxyacetone; Gald, glyceraldehyde.

steps of glucose metabolism in the CNS are outlined in Fig. 2. Glucose enters the glycolytic pathway via glucokinase-catalyzed phosphorylation and its further metabolism is subject to control by the PFK reaction, which is considered to be the rate-limiting step of the pathway. In contrast, fructose bypasses this step entering the glycolytic pathway at the level of the triose phosphates and thus is metabolized at a far faster rate than glucose. The possibility was considered that the rapid unregulated fructokinase step might deplete hypothalamic ATP, leading to an increase in AMP level, a response that would activate the hypothalamic AMPK signaling pathway and thereby food intake. Recent evidence has shown that in the CNS energy status is monitored by AMPK through changes in the cellular adenine nucleotide concentration, i.e., the [ATP]/[AMP] ratio (19–22). In view of the fact that adenine nucleotides are in rapid equilibrium (ATP + AMP ⇆ ADP + ADP) catalyzed by AK, when ATP increases, AMP decreases, and vice versa.

As shown in Fig. 3 the central administration of fructose caused a rapid decrease in hypothalamic ATP level, whereas glucose led to a rapid rise. Because of the AK-catalyzed equilibrium, the level of AMP would be the inverse of ATP. Consistent with a decrease in the ATP/AMP ratio, centrally administered fructose produced a rapid rise in the phosphorylation state/activation of AMPK, whereas i.c.v. glucose that increased the level of ATP, thus the ATP/AMP ratio, caused a decrease of phospho-AMPK (P-AMPK) (Fig. 4). Importantly, these events occurred within the same time frame (10–20 min).

Effect of Centrally Administered Fructose and Glucose on the Phosphorylation State of Hypothalamic ACC and the Level of Malonyl–CoA. ACC, the key regulatory enzyme of fatty acid biosynthesis (23–28), is phosphorylated/inactivated by AMPK (13, 14). As

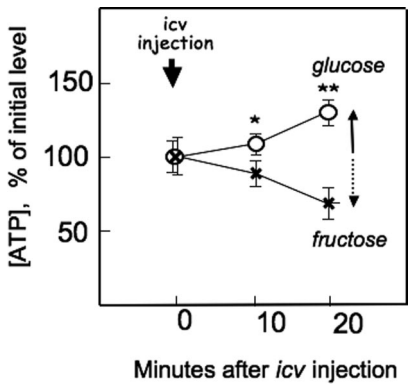

Fig. 3. Effect of i.c.v. injection of fructose on hypothalamic ATP level. Food-deprived mice were given i.c.v. injections (400 μg/2 μl) of fructose or glucose and hypothalamic ATP concentration determined at 0, 10, and 20 min after injection ($n = 4$ per group). **, $P < 0.001$; *, $P < 0.01$ versus glucose.

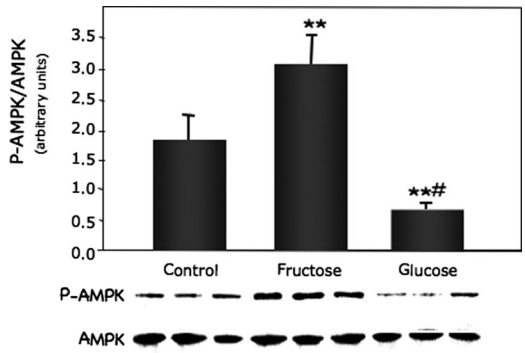

Fig. 4. Effect i.c.v. injection of fructose on the phosphorylation state of AMPK. Food-deprived mice were given i.c.v. injections (400 μg/2 μl) of fructose or glucose, and 10 min later hypothalami were lysed and subjected to SDS/PAGE and immunoblotting with antibodies to P-AMPK and AMPK ($n = 4$ mice per group). **, $P < 0.001$ versus control; #, $P < 0.001$ versus fructose.

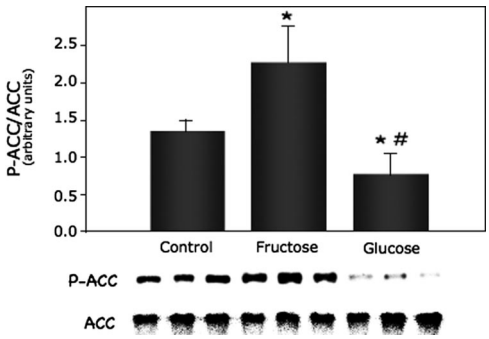

Fig. 5. Effect i.c.v. injection of fructose on the phosphorylation state of ACC. Food-deprived mice were given i.c.v. injections (400 μg/2 μl) of fructose or glucose, and 10 min later hypothalami were lysed and subjected to SDS/PAGE and immunoblotting with antibodies to ACC and P-ACC ($n = 4$ mice per group). *, $P < 0.01$ versus control; #, $P < 0.01$ versus fructose.

shown in the present study, the central administration of glucose, which increased hypothalamic ATP (Fig. 3) and lowered P-AMPK (Fig. 4) and AMPK activity, decreased phospho-ACC (P-ACC) (Fig. 5), the catalytically-inactive state of ACC. In contrast, the central administration of fructose produced the inverse effect on these variables (Figs. 3–5). As predicted by these changes, central/i.c.v. glucose promoted a rapid rise in the level of malonyl–CoA in the hypothalamus, whereas central fructose had the inverse effect (Fig. 6). These findings indicate that the metabolism of fructose in the CNS rapidly depletes hypothalamic ATP (Fig. 2), initiating a cascade of events including increased AMPK activity, decreased ACC activity, and a drop in the level of hypothalamic malonyl–CoA.

A large body of recent physiological, pharmacological, and genetic evidence (9, 16–18, 21, 27, 28) predicted that these effects on hypothalamic malonyl–CoA level would affect feeding behavior.

Effect of Centrally Administered Fructose and Glucose on the Expression of Anorexigenic and Orexigenic Neuropeptides and Food Intake. Previous studies have shown that increasing the level of malonyl–CoA in the hypothalamus triggers changes in the expression of anorexigenic [α-melanocyte-stimulating hormone and cocaine- and amphetamine-regulated transcript (CART)] and orexigenic [neuropeptide Y (NPY) and agouti-related peptide (AgRP)] neuropeptides that suppress appetite (9, 16–22, 26–28). To assess the acute effects of centrally administered fructose and glucose on the expression of these neuropeptides, food-deprived mice were given an i.c.v. injection of glucose, and 10 min later hypothalamic mRNA was isolated. The levels of proopiomelanocortin (POMC), CART, NPY, and AgRP mRNA were then determined by quantitative RT-PCR. As illustrated in Fig. 7*A*, central glucose increased levels of hypothalamic POMC and CART mRNAs and decreased levels of NPY and AgRP mRNAs compared with the responses in control and fructose-injected animals. Centrally-administered fructose lowered POMC mRNA, predicting a rise in food intake, but had little effect on CART, NPY or AgRP mRNAs. The net effect of these changes is consistent with the observed effect on food intake shown below (Fig. 7*B*).

To compare the effects of centrally administered fructose and glucose on feeding behavior, food intake was measured during the short time window (0–30 min) after the i.c.v. injection of glucose or fructose to food-deprived mice (Fig. 7*B*). Like the inverse response patterns of centrally-administered fructose and glucose on hypothalamic ATP, P-AMPK, and P-ACC and malonyl–CoA levels, reciprocal effects on food intake were observed. Thus, central fructose administration rapidly increased food intake, whereas central glucose suppressed food intake (Fig. 7*B*).

Discussion

Glucose is the primary physiological fuel for brain in fed animals (29) and is an indicator of global energy status (17, 18). The metabolism of glucose in the hypothalamus initiates a signaling pathway that leads to the suppression of food intake (9). Although all steps in this pathway have not yet been defined, it has been established that AMPK, ACC, and malonyl–CoA function in relaying the signal. Thus, increased glucose flux into the hypothalamus/CNS leads to the dephosphorylation/inactivation of AMPK and thereby activation of ACC, which gives rise to an increase in the level of its reaction product, malonyl-CoA (9). Substantial evidence has shown that a rise in hypothalamic malonyl–CoA inactivates expression of the orexigenic neuropeptides while activating the expression of the anorexigenic neuropeptides (9, 16, 27, 28). Together, these events suppress food intake and increase energy expenditure as illustrated:

$$\text{Glucose} \rightarrow \Uparrow[\text{ATP}] \rightarrow \Downarrow[\text{AMP}] \rightarrow \underset{(inactive)}{\text{dephospho-AMPK}} \rightarrow$$

$$\underset{(active)}{\text{dephospho-ACC}} \rightarrow \Uparrow[\text{malonyl-CoA}] \rightarrow \Downarrow\text{food intake.}$$

In contrast to the anorexigenic effect of centrally administered glucose, fructose exerts an orexigenic effect (ref. 8 and Fig. 7).

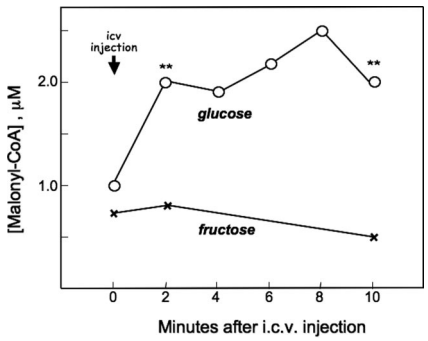

Fig. 6. Effect i.c.v. injection of fructose and glucose on the level of hypothalamic malonyl–CoA. Food-deprived mice were given i.c.v. injections (400 μg/2 μl) of fructose or glucose at the indicated times the malonyl–CoA levels in hypothalami were quantified ($n = 4$ mice per group). **, $P < 0.001$.

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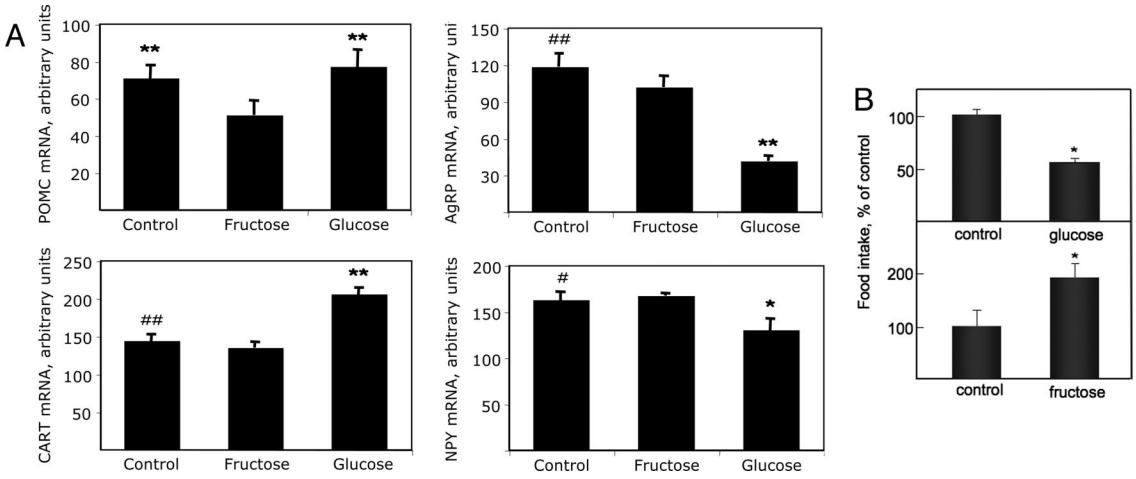

Fig. 7. Effect of i.c.v. injection of fructose and glucose on neuropeptide mRNA expression and food intake. Food-deprived mice were given i.c.v. injections (400 μg/2 μl) of fructose or glucose ($n = 4$ mice per group). (*A*) After 10 min hypothalami were removed, RNA was isolated, and mRNA content was determined as described (9, 16). **, $P < 0.01$; *, $P < 0.05$ versus fructose; ##, $P < 0.01$; #, $P < 0.05$ versus glucose. (*B*) Mice were given access to food and food intake measured over the next 30 min ($n = 4$ mice per group). *, $P < 0.01$ versus control.

This disparity appears to result from differences in the initial steps by which these sugars are metabolized in the glycolytic pathway and alter the steady-state [ATP]/[AMP] ratio. The brain, like the liver, possesses a unique set of enzymes that enables fructose to bypass the rate-limiting step in glycolysis and thereby rapidly deplete ATP in the hypothalamus (Fig. 3). Thus, the sequential actions of glucose transporter 3/5 (GLUT3/5), 2-ketohexokinase, fructose-1-phosphate aldolase, and triose kinase in the CNS (4–6) allows fructose to enter glycolysis beyond the tightly-regulated PFK-catalyzed step (30, 31) (Fig. 2). Consistent with this proposal, the central administration of fructose rapidly lowers ATP (Fig. 3), increases P-AMPK (Fig. 4) and P-ACC (Fig. 5), and decreases malonyl-CoA (Fig. 6) in the hypothalamus, whereas centrally administered glucose has the inverse effects. Taken together, this evidence supports the following sequence of events after the central administration of fructose:

$$\text{Fructose} \rightarrow \Downarrow[\text{ATP}] \rightarrow \Uparrow[\text{AMP}] \rightarrow \underset{(active)}{\text{phospho-AMPK}} \rightarrow$$

$$\underset{(inactive)}{\text{phospho-ACC}} \rightarrow \Downarrow[\text{malonyl-CoA}] \rightarrow \Uparrow\text{food intake.}$$

In future investigations it will be important to verify that the relevant enzymes of the fructose-1-phosphate metabolic pathway (Fig. 2*B*) are present in the NPY/AgRP- and/or POMC- and CART-expressing hypothalamic neurons that regulate feeding behavior.

Our findings may have broader implications relevant to the extensive use of high-fructose corn syrup as a sweetener in American diets, particularly those of youth, the major consumers of high-fructose-containing soft drinks. It has been shown in animal and human studies that high-fructose diets promote glucose intolerance, insulin resistance, and obesity (32). Evidence presented here suggests that an excessively high fructose intake might suppress the hypothalamic malonyl–CoA signaling pathway and thereby exert an orexigenic effect. This effect would depend, of course, on the ability of fructose to cross the blood–brain barrier or enter the brain through circumventricular structures with weak blood–brain barriers, notably in the arcuate nucleus.

Materials and Methods

Animals. Male C57BL/6NHsd mice (6–7 weeks of age) from Harlan Laboratories were maintained on a 12-h/12-h light/dark cycle and fed rodent chow ad libitum. After food deprivation for 20 h mice ($\geq$3 per group) were given either an i.p. (4 g/kg body weight) or i.c.v. (400 μg/2 μl) injection of glucose, fructose or saline at the start of the dark cycle. In some cases 2-DG (2 μl of 5 mM 2-DG) by given by i.c.v. injection. After euthanization by cervical dislocation, hypothalami were rapidly (within 30 s) extirpated and blood was collected. Each experiment was repeated at least twice with similar results. Animal experiments followed the guidelines of The Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee.

Metabolite Analysis. Glucose was measured by the glucose oxidase method (QuantiChrom; Bioassay Systems), and fructose was measured with a kit using the Hexokinase/PGI method (Sigma). Malonyl–CoA was quantified by using a recycling assay as described (16). For analysis of ATP hypothalami were extracted with cold 25% perchloric acid, neutralized with KOH, and centrifuged. ATP was quantified with a bioluminescence detection kit (Promega).

Immunoblotting and RT-PCR Analysis. Hypothalamic lysates in RIPA buffer (0.15 mM NaCl/0.05 mM Tris·HCl, pH 7.2/1% Triton X-100/1% sodium deoxycholate/0.1% SDS) were subjected to SDS/PAGE separation. Immunoblotting was performed with AMPK, P-AMPK (T172), ACC, and P-ACC antibodies from Cell Signaling Technology). Horseradish peroxidase-conjugated secondary antibody was used and visualized by using SuperSignal chemiluminescent substrate (Pierce). Measurement of hypothalamic neuropeptide mRNA was as described (9, 16).

Statistical Analysis. Results were analyzed by Student's *t* test and are presented as means $\pm$ SEM of multiple determinations.

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