The Central Sirtuin1/p53 Pathway Is Essential for the Orexigenic Action of Ghrelin

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OBJECTIVE—Ghrelin is a stomach-derived peptide that increases food intake through the activation of hypothalamic AMP-activated protein kinase (AMPK). However, the molecular mechanisms initiated by the activation of the ghrelin receptor, which in turn lead to AMPK activation, remain unclear. Sirtuin1 (SIRT1) is a deacetylase activated in response to calorie restriction that acts through the tumor suppressor gene p53. We tested the hypothesis that the central SIRT1/p53 pathway might be mediating the orexigenic action of ghrelin.

RESEARCH DESIGN AND METHODS—SIRT1 inhibitors, such as Ex527 and sirtinol, and AMPK activators, such as AICAR, were administered alongside ghrelin in the brain of rats and mice (wild-type and p53 knockout [KO]). Their hypothalamic effects on lipid metabolism and changes in transcription factors and neuropeptides were assessed by Western blot and in situ hybridization.

RESULTS—The central pretreatment with Ex527, a potent SIRT1 inhibitor, blunted the ghrelin-induced food intake in rats. Mice lacking p53, a target of SIRT1 action, failed to respond to ghrelin in feeding behavior. Ghrelin failed to phosphorylate hypothalamic AMPK when rats were pretreated with Ex527, as it did in p53 KO mice. It is noteworthy that the hypothalamic SIRT1/p53 pathway seems to be specific for mediating the orexigenic action of ghrelin, because central administration of AICAR, a potent AMPK activator, increased food intake in p53 KO mice. Finally, blockade of the central SIRT1 pathway did not modify ghrelin-induced growth hormone secretion.

CONCLUSIONS—Ghrelin specifically triggers a central SIRT1/p53 pathway that is essential for its orexigenic action, but not for the release of growth hormone.

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hrelin is the only known endogenous signal stimulating adiposity and feeding (1–3). At the hypothalamic level, ghrelin activates AMP-activated protein kinase (AMPK), causing relevant changes in hypothalamic mitochondrial respiration and production of reactive oxygen species (4–6), altering the expression of transcription factors Bsx, Forkhead box class O (FoxO1), and cAMP-responsive element–binding protein (pCREB), and leading to the final activation of agouti-related peptide/neuropeptide Y (AgRP/NPY) neurons. However, the molecular mechanisms occurring after GHS-R1a activation and before AMPK phosphorylation are completely unknown. Ghrelin is the only gut peptide with orexigenic properties in rodents and humans; thus, the ghrelin system is uniquely positioned as a drug target for the treatment of cachexia. The current study tested the hypothesis that the central sirtuin1 (SIRT1)/p53 pathway might be mediating the orexigenic action of ghrelin.

SIRT1 is a NAD+-dependent deacetylase that acts on important tumor suppressors like p53. In addition to their biologic actions on cancer, SIRT1 and p53 are also important in several metabolically relevant tissues. SIRT1 controls divergent metabolic pathways in adipose tissue (7), liver (8), pancreatic β cells (9), and skeletal muscle (10), mainly through the regulation of rate-limiting enzymes involved in glucose and lipid metabolism. Recent reports have shown that central SIRT1 also regulates energy and glucose homeostasis (11–17). On the other hand, p53 is activated by the lack of nutrients through the activation of AMPK, and p53 senescence activity contributes to the development of insulin resistance (18).

Because the molecular mechanisms that link the effects of the ghrelin/GHS-R1a system to AMPK are unknown, the current study tested the hypothesis that the hypothalamic SIRT1/p53 pathway might be mediating the orexigenic action of ghrelin.

RESEARCH DESIGN AND METHODS

Animal models. Male Sprague-Dawley rats (8 weeks old, 200–250 g) and C57/Bl6 mice (8 weeks old) were housed in air-conditioned rooms (22–24°C) under a 12/12-h light/dark cycle and fed standard chow. p53-null (8–10 weeks old, mixed background C57BL6/J and 129/Sv) mice were described previously (19). Homozygous wild-type (WT) and knockout (KO) mice were originated from heterozygous mating, so only littermate WT and KO animals were compared in each experiment. Animals were treated and killed when they were 12 to 14 weeks of age before any sign of morbidity resulting from tumor development occurred. Animals were killed by decapitation between 1000 and 1200 h. Animal experiments were conducted in accordance with the standards approved by the Faculty Animal Committee at the University of Santiago de Compostela, and the experiments were performed in accordance with the Rules of Laboratory Animal Care and International Law on Animal Experimentation.

Nutritional status. Rats (n = 8/group), were assigned to one of the following groups: fed ad libitum, deprived of food for 48 h, and fasted during 48 h and refed during 24 h. All animals had free access to tap water.

Implantation of intracerebroventricular cannulae. Rats were anesthetized by an intraperitoneal injection of ketamine (100 mg/kg body weight [BW])/xylazine (15 mg/kg BW). Mice were anesthetized by an intraperitoneal injection of triethanolamine (480 mg/kg; Sigma-Aldrich, St Louis, MO). Intracerebroventricular cannulae were implanted stereotaxically in rats (20) or mice (21), as described previously.

Intracerebroventricular treatments. Rats received an intracerebroventricular administration of 5 μL of vehicle or ghrelin (5 μg; Bachem, Bubendorf, Switzerland). For the inhibition of SIRT1, we used two potent specific inhibitors of SIRT1: Ex527 (1 to 5-10 μg in a total volume of 5 μL Tocris from the 1Department of Physiology, School of Medicine—Instituto de Investigaciones Sanitarias (IDIS), Capivari de Baixo, Santa Catarina, Brazil; 2CIBER Fisiopatología de la Obesidad y Nutrición (CIBERobn), Capivari de Baixo, Santa Catarina, Brazil; 3Catarinense Institute of Environmental Research and Human Development, IPADIC, Capivari de Baixo, Santa Catarina, Brazil; and the 4Department of Cell Biology and Ecology, University of Santiago de Compostela, Santiago de Compostela, Spain. Corresponding authors: Ruben Nogueiras, ruben.nogueiras@usc.es, and Carlos Dieguez, carlos.dieguez@usc.es. Received 8 June 2010 and accepted 25 January 2011. DOI: 10.2337/db10-0802. This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db10-0802/-/DC1. © 2011 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.
Biosciences, St. Louis, MO) (22) and sertinol (10 to 5-10 μg in a total volume of 5 μL; Tocris Bioscience) (23) before ghrelin administration. For the experiments involving only two groups (vehicle versus ghrelin), the vehicle was saline. For the experiments involving SIRT1 inhibitors, the vehicle was DMSO, because Ex527 and sirtinol both diluted in DMSO. Mice received an intra- cerebroventricular administration of vehicle, ghrelin (5 μg), or AICAR (5 μg; Sigma-Aldrich A9078) in a total volume of 2 μL. For the experiments involving vehicle versus ghrelin and vehicle versus AICAR, the vehicle was saline.

We used the same dose of ghrelin for both rats and mice because this dose has been demonstrated to be effective in both species (2). We used eight rats per group, and the experiments were repeated at least twice. Rats were killed by cervical dislocation. Hypothalami were dissected and stored at −80°C until further processing.

**Western blotting.** Hypothalami were homogenized in ice-cold lysis buffer containing 50 mmol/L Tris-HCl (pH 7.5), 1 mmol/L EGTA, 1 mmol/L EDTA, 1% Triton X-100, 1 mmol/L sodium orthovanadate, 50 mmol/L sodium fluoride, 5 mmol/L sodium pyrophosphate, 0.27 mmol/L sucrose, 0.1% 2-mercaptoethanol, and Complete protease inhibitor cocktail (1 tablet/50 mL; Roche Diagnostics, Mannheim, Germany). Homogenates were centrifuged at 13,000g for 10 min at 4°C, supernatants were removed, and aliquots were snap-frozen in liquid nitrogen. Hypothalamic lysate (40 μg) was subjected to SDS-PAGE on 6% polyacrylamide gels and electrotransferred on a polyvinylidine fluoride membrane.

Membranes were blocked for 1 h in TBS-Tween (TBST: 50 mmol/L Tris-HCl [pH 7.5], 0.15 mmol/L NaCl, and 0.1% Tween) containing 5% skimmed milk or 3% BSA (for pAMPK Thr172 and pACC Ser79) and probed for 16 h at 4°C in TBST, 5% skimmed milk, or 3% BSA (for pAMPK Thr172, pACC Ser79, SIRT1, and acetyl-p53-Lys99) with the appropriate dilution of the indicated antibodies (acetyl-CoA carboxylase [ACC]: 1:1,500; pACC: 1:2,000; AMPKα1: 1:1,000; AMPKα2: 1:1,000; pAMPK: 1:2,000; β-actin (loading control): 1:2,000). ACC was detected using horseradish peroxidase (HRP)-conjugated coupled streptavidin (Amersham Biosciences, Little Chalfont, UK).

Detection of proteins was performed using HRP-conjugated secondary antibodies and an enhanced chemiluminescence reagent (Amersham Biosciences) at room temperature for 30 min. They were dehydrated using ethanol, and the membranes were air-dried and exposed to Hyperfilm β-Max (Amersham Biosciences) at room temperature for 4 to 6 days for AgRP and NPY and for 21 days for Bsx. The slides were developed in Kodak D-19 developer (Eastman Kodak Co., Rochester, NY), fixed (Kodak fixer), and counterstained with methylene blue.

To confirm the colocalization of ghrelin with orexigenic and anorexigenic markers, the slides were scanned. The specific hybridization signal was quantified by densitometry using the Molecular Analyst digital imaging system (Bio-Rad Laboratories Inc., Richmond, CA). The optical density of the hybridization signal was determined and subsequently corrected by the optical density of its adjacent background value. For this reason, a rectangle with the same background dimensions in each case was drawn enclosing the hybridization signal over each nucleus and over adjacent brain areas of each section (background). We used 16 to 20 sections for each animal (4–5 slides, 4 sections/slide). The mean of these 16 to 20 values was used as the densitometry value for each animal.

**RESULTS**

**Regulation of SIRT1 by nutritional status.** Rats fasted during 48 h exhibited a loss of BW, whereas the refeeding during 24 h partially led to a substantial recovery (Fig. 1A). Acetyl-p53 levels, a marker of SIRT1 activity in vivo (28), were decreased in the hypothalamus of fasted rats (F2,18 = 3.651, P < 0.05), whereas those levels were similar to baseline in rats after refeeding (Fig. 1B). Because ghrelin is a key player for increasing feeding behavior, we tested the hypothesis that central ghrelin administration might stimulate hypothalamic SIRT1 activity. As previously reported, ghrelin increased food intake after 2 h (F1,13 = 24.161, P < 0.001; Fig. 1C) and 6 h (F1,13 = 36.14, P < 0.001; Fig. 1E) (29) and decreased hypothalamic acetyl-p53 levels after 2 h (F1,13 = 7.915, P < 0.01; Fig. 1D) and 6 h (F1,13 = 7.645, P < 0.05; Fig. 1F).

**Blockade of the SIRT1/p53 pathway blunts the orexigenic action of ghrelin.** We next studied the functional relevance of these findings by assessing whether the pharmacologic blockade of SIRT1 activity might regulate the orexigenic action of ghrelin. First, we observed that the central injection of Ex527, a potent inhibitor of SIRT1

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**TABLE 1**

| mRNA   | GenBank accession number | Sequence |
|--------|--------------------------|----------|
| AgRP   | AF2060017                 | 5′-CGAGCCGGAGAGACAGATCGCGGTTTCTGTGATCTGACCCTCTTGCC3′ |
| Bsx    | XM_001064837              | 5′-CTTCACACGGCTTGCGCTGTGAGAACAGATGCC3′ |
| NPY    | M20373                    | 5′-AGATGAGATGTCGCGGGAACACATGGAGAAATCGAGGAGCGAGATTCC3′ |
FIG. 1. Effects of nutritional status on hypothalamic SIRT1. A: Fasting for 48 h caused a significant ($P < 0.001$) decrease in BW, whereas refeeding during 24 h partially recovered the weight loss. B: Hypothalamic acetylated p53 levels decreased in fasted rats and recovered in refed rats. Effects of intracerebroventricular ghrelin injection (5 μg/rat) after 2 h on food intake (C), and hypothalamic acetylated p53 levels (D). Effects of intracerebroventricular ghrelin injection (5 μg/rat) after 6 h on food intake (E), and hypothalamic acetylated p53 levels (F). Values were normalized to those of the internal control β-actin, and the results are expressed as arbitrary units. Mean values were obtained from six animals per group. Values are the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. 

D.A. VELÁSQUEZ AND ASSOCIATES
Increased hypothalamic acetyl-p53 levels at different doses (1, 5, and 10 μg; Supplementary Fig. 1A), indicating that this compound decreased hypothalamic SIRT1 activity in vivo. We then centrally administered ghrelin, Ex527, and Ex527 + ghrelin to the rats. We found that ghrelin increased food intake at 2 h (data not shown) and 6 h (F(1,27) = 12.282, P < 0.001; Fig. 2A), but when the SIRT1 inhibitor was administered 20 min before ghrelin, the orexigenic action of ghrelin was markedly blunted after 6 h (Fig. 2A). Because ghrelin increases food intake through its effects on hypothalamic fatty acid metabolism (5,6), we next assessed the levels of several key enzymes for the synthesis of lipids. We found that 6 h after an intracerebroventricular ghrelin injection, pAMPK levels were increased (F(1,28) = 2.455, P < 0.05), but ACC levels were decreased (F(1,27) = 6.045, P < 0.01). Those effects were abolished when the SIRT1 inhibitor was coadministered (Fig. 2B and C).

It is important to note that even though pAMPK levels were increased, pACC did not reach statistical significance, probably because of the different kinetics of phosphorylation of both enzymes (6). Furthermore, the higher expression of the transcription factors FoxO1 (F(3,26) = 2.509, P < 0.05), pCREB (F(3,26) = 3.686, P < 0.05; Fig. 2D), and Bsx (F(3,26) = 3.526, P < 0.05; Fig. 2E and F) and the neuropeptides NPY (F(3,26) = 4.362, P < 0.05) and AgRP (F(3,26) = 3.33, P < 0.05; Fig. 2E and F) in the hypothalamic arcuate nucleus induced by ghrelin was also abolished when the SIRT1 inhibitor was coadministered. When we used sirtinol, another inhibitor of SIRT1 activity, results were similar to those obtained with Ex527 (Supplementary Fig. 1).

Tumor suppressor protein p53 is a substrate of SIRT1, and it is found hyperacetylated in SIRT1 KO mice (30). Because a recent report showed that p53 is involved in energy metabolism and homeostasis (31), we next inquired...
whether p53 could be a mediator of SIRT1-dependent effects of ghrelin. For this purpose, we treated WT and p53 KO mice with intracerebroventricular ghrelin, following the same protocol as that described above. The p53 KO mice did not show alterations in BW, food intake, fat mass, or nonfat mass compared with WT littermates (Supplementary Fig. 2A–D). As expected, central ghrelin administration increased food intake in WT animals, whereas identical intracerebroventricular ghrelin treatment in p53 KO animals had no effect on food intake after 2 (Fig. 3A) or 6 h (Fig. 3B).

We next assessed the levels of several key enzymes for the synthesis of lipids. No principal differences were found between WT and p53 KO mice regarding the expression of AMPKα1, AMPKα2, or FAS (Fig. 3C and D). It is noteworthy that we found that 6 h after its central injection, ghrelin increased pAMPK levels in WT mice ($F_{1,12} = 4.466$, $P < 0.05$) but failed to do so in p53 KO mice (Fig. 3C and D), suggesting that p53 is an essential mediator of ghrelin actions on AMPK. The levels of pAMPK and pACC are correlated in normal conditions; however, we found that the hypothalamic levels of pACC are downregulated in p53 KO mice ($F_{1,12} = 8.576$, $P < 0.01$) but not in WT mice (Fig. 3C and D).

Although we do not have a clear explanation for these results, it seems that ghrelin is able to activate ACC when

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**FIG. 3.** Mice lacking p53 do not respond to ghrelin injection. Effects of intracerebroventricular ghrelin injection (5 μg/mouse) on food intake after 2 h (A) and 6 h (B) in wild type (WT) and p53 KO mice. Hypothalamic protein levels of pAMPKα, AMPKα1, AMPKα2, pACC, ACCα, and FAS after 6 h of ghrelin injection (C and D). Values were normalized with to those of the internal control β-actin, and the results are expressed as arbitrary units. Mean values were obtained from six animals per group. Values are the mean ± SEM. *$P < 0.05$, **$P < 0.01$.**
p53 is not present, suggesting that p53 might also regulate the actions of ghrelin on different key enzymes modulating fatty acid metabolism. Further studies analyzing not only protein levels but also enzymatic activity and lipolysis/lipogenesis will be necessary to address this issue. Furthermore, we detected that central ghrelin injection decreased ACCα levels in both WT ($F_{1,12} = 2.844, P < 0.05$) and p53 KO mice ($F_{1,12} = 6.699, P < 0.01$; Fig. 3C and D), indicating that p53 is not essential for ghrelin-mediated ACC regulation.

**p53 does not mediate the orexigenic action of AICAR.** Central injection of AICAR, a potent activator of AMPK activity, stimulates food intake in rodents (32). To determine whether p53 is a crucial player for the orexigenic action of AICAR, we centrally treated p53 KO mice with AICAR (3 μg) and found a stimulation in food intake after 6 h ($F_{1,12} = 3.542, P < 0.05$; Fig. 4A). Hypothalamic pAMPK levels were also increased in p53 KO mice treated with AICAR ($F_{1,12} = 4.479$; Fig. 4B and C). Therefore, our data indicate that p53 is not required for the orexigenic action of direct AMPK activators.

**The central SIRT1 pathway does not modulate ghrelin-induced GH secretion.** Finally, we assessed whether the SIRT1 pathway is mediating other neuroendocrine actions of ghrelin, namely GH secretion. Administration of ghrelin (27) led to the expected increase in plasma GH levels at 5, 10, and 15 min (Fig. 5A), whereas the central blockade of SIRT1 did not alter that response (Fig. 5A). Ghrelin exhibited a similar stimulatory effect in both area under the curve and mean peak GH levels (Fig. 5B and C).

**DISCUSSION**

Our current data demonstrate that the hypothalamic SIRT1/p53 pathway is crucial for the orexigenic effect of ghrelin. Pharmacologic inhibition of SIRT1 or genetic depletion of p53 abolish the effects of ghrelin on AMPK and thereby blunt ghrelin-induced effects on transcription factors, including pCREB, FoxO1, and Bsx, and neuropeptides, such as NPY and AgRP, leading to a suppression of ghrelin-induced food intake.

SIRT1 is a deacetylase that regulates metabolism in multiple peripheral tissues. It has been reported recently that SIRT1 mRNA is located in metabolically relevant areas of the mouse neuroaxis, such as pro-opiomelanocortin neurons, which are critical for energy and glucose homeostasis (11). It seems that SIRT1 regulates the central melanocortin system (13) and that the specific lack of SIRT1 in the brain abolishes the higher physical activity induced by calorie restriction (12). More specifically, the lack of SIRT1...
in pro-opiomelanocortin neurons causes hypersensitivity to diet-induced obesity because of reduced energy expenditure (17). Concurring with those data, we observed that acetylation of p53 in the hypothalamus is decreased after food deprivation, indicating that SIRT1 as a deacetylase increased its hypothalamic activity during starvation. Therefore, the regulation of hypothalamic SIRT1 activity by nutritional status is similar to its regulation in several peripheral tissues (33).

Ghrelin is a stomach-derived hormone that rapidly increases food intake and BW (1,29). Its regulation by nutritional status was controversial because the assays detected both acyl-ghrelin and des-acyl ghrelin and thus were not specific. Studies using new technologies for separately detecting both isoforms indicate that circulating des-acyl ghrelin increases significantly with fasting, whereas blood acyl-ghrelin levels are not changed over the course of fasting (34,35). Most of the effects of ghrelin are exerted through the GH secretagogue receptor 1a (GHS-R1a) (36), which is expressed in AgRP/NPY neurons in the hypothalamic arcuate nucleus (37). The orexigenic effect of ghrelin is mediated by AMPK, a key upstream master regulator of lipid metabolism (5,6). However, the molecular events regulating AMPK phosphorylation after the activation of the GHS-R1a are unknown.

In the present work, we demonstrate that central ghrelin administration increases hypothalamic SIRT1 activity, stimulating the deacetylation of p53. More interestingly and consistent with previous findings (14), the blockade of central SIRT1 activity abolished the potent orexigenic effect of ghrelin. At molecular level, the ghrelin-induced activation of AMPK is prevented when SIRT1 activity is blocked. The interaction between SIRT1 and AMPK has been previously shown in vitro, indicating that resveratrol activates AMPK in neurons (38).

Although pharmacologic experiments based on the administration of SIRT1 inhibitors to animal models are providing important insight into principal effects, targets, and mechanisms of the SIRT1 system, understanding the function of its endogenous role requires more sophisticated approaches, such as genetic disruption of SIRT1 or its substrates. We focused on p53 because this tumor-suppressor protein is a well-known target of SIRT1 action, and there is growing evidence of its role on metabolism and energy balance in peripheral tissues (31). Our results indicate that p53 is required for the ghrelin-induced food intake, because ghrelin failed to increase food intake in p53 KO mice. In accordance with pharmacologic findings, ghrelin stimulated hypothalamic pAMPK levels in WT mice but failed to do so in p53 KO mice. On the other hand, it has been shown that SIRT1 is a target gene of p53 in some but not all of the tissues (31,39), so it might be possible that p53 mediates the changes in SIRT1 upon ghrelin treatment or calorie restriction. However, the observed correlation between acetylated p53 and SIRT1 activity in the hypothalamus on different experimental conditions indicate that p53 is an essential mediator of SIRT1-dependent effects of ghrelin on AMPK. Mice lacking p53 in specific hypothalamic areas will be essential to demonstrating which particular neuronal circuits are responsible for those actions. However, the central SIRT1/p53 pathway is not required by direct AMPK activators, because AICAR stimulated food intake in p53-deficient mice. Therefore, these findings corroborate that the central SIRT1/p53 pathway is not associated with AICAR-induced activation of AMPK.

Finally, our results indicate that ghrelin stimulated GH release as expected, but the blockade of central SIRT1 did not modify GH levels. Therefore, it seems that the central SIRT1/p53 pathway is specifically mediating the ghrelin orexigenic action and suggests that different neuronal pathways modulate ghrelin-induced food intake and GH secretion.
In summary, we provide a combination of pharmacologic and genetic evidence to demonstrate that the central nervous system SIRT1/p53 pathway is essential for the orexigenic response to ghrelin (Fig. 6). The molecular pathway mediating those effects involves alterations in AMPK activation, which leads to changes in hypothalamic fatty acid metabolism, and finally, modifies feeding behavior.

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REFERENCES

1. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature 2000;407:908-913
2. Theander-Carrillo C, Wiedmer P, Cettour-Rose P, et al. Ghrelin action in the brain controls adipocyte metabolism. J Clin Invest 2006;116:1983-1993
3. Egecioglu E, Jerlhag E, Salomé N, et al. Ghrelin increases intake of rewarding food in rodents. Addict Biol 2010;15:304-311
4. Kola B, Forkas I, Christ-Crain M, et al. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. PLoS One 2008;3:e1797
5. Andrews ZB, Liu ZW, Wallingford N, et al. UCP2 mediates ghrelin’s action on NPY/AgRP neurons by lowering free radicals. Nature 2008;454:846-851
6. López M, Lage R, Saha AK, et al. Hypothalamic fatty acid metabolism mediates the orexigenic action of ghrelin. Cell Metab 2008;7:389-399
7. Picard F, Kurtev M, Chung N, et al. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 2004;429:771-776
8. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. Proc Natl Acad Sci USA 2007;104:12861-12866

FIG. 6. Schematic overview summarizing our proposed model for the molecular mechanisms initiated by the activation of the ghrelin receptor leading to AMPK activation and finally to an increased feeding behavior. ROS, reactive oxygen species. (A high-quality color representation of this figure is available in the online issue.)
9. Bordone L, Motta MC, Picard F, et al. Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. PLoS Biol 2006;4:e31
10. Canto C, Gerhart-Hines Z, Feige JN, et al. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 2009; 458:1056–1060
11. Ramadori G, Lee CE, Bookout AL, et al. Brain SIRT1: anatomical distribution and regulation by energy availability. J Neurosci 2008;28:9989–9996
12. Cohen DE, Supinski AM, Bonkowski MS, Donmez G, Guarente LP. Neuronal SIRT1 regulates endocrine and behavioral responses to calorie restriction. Genes Dev 2009;23:2512–2517
13. Cakir I, Perello M, Lansari O, Messier NJ, Vaslet CA, Nillni EA. Hypothalamic Sirt1 regulates food intake in a rodent model system. PLoS One 2009; 4:e8322
14. Dietrich MO, Antunes C, Geliang G, et al. Agrp neurons mediate Sirt1’s action on the melanocortin system and energy balance: roles for Sirt1 in neuronal firing and synaptic plasticity. J Neurosci 2010;30:11815–11825
15. Satoh A, Brace CS, Ben-Josef G, et al. Sirt1 promotes the central adaptive response to diet restriction through activation of the dorsomedial and lateral nuclei of the hypothalamus. J Neurosci 2010;30:10220–10232
16. Sasaki T, Kim HJ, Kobayashi M, et al. Induction of hypothalamic Sirt1 leads to cessation of feeding via agouti-related peptide. Endocrinology 2010;151: 2556–2566
17. Ramadori G, Fujiwara T, Fukuda M, et al. Sirt1 deacetylation in POMC neurons is required for homeostatic defenses against diet-induced obesity. Cell Metab 2010;12:78–87
18. Vousten KH, Ryan KM. p53 and metabolism. Nat Rev Cancer 2009;9:611–700
19. Jacks T, Remington L, Williams BO, et al. Tumor spectrum analysis in p53– mutant mice. Proc Natl Acad Sci USA 2007;104:7217–7222
20. Nogueiras R, Wiedmer P, Perez-Tilve D, et al. The central melanocortin system directly controls peripheral lipid metabolism. J Clin Invest 2007; 117:3475–3488
21. Nogueiras R, Perez-Tilve D, Veyrat-Durebex C, et al. Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. J Neurosci 2009;29:5916–5925
22. Solomon JM, Pasupuleti R, Xu L, et al. Inhibition of Sirt1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. Mol Cell Biol 2006;26:28–38
23. Ota H, Tokunaga E, Chang K, et al. Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells. Oncogene 2006;25:176–185
24. Nogueiras R, Lopez M, Lage R, et al. Bex, a novel hypothalamic factor linking feeding with locomotor activity, is regulated by energy availability. Endocrinology 2008;149:3000–3015
25. Seoane LM, Lopez M, Tovar S, Casanueva FF, Searle R, Diéguez C. Agouti-related peptide, neuropeptide Y, and somatostatin-producing neurons are targets for ghrelin actions in the rat hypothalamus. Endocrinology 2003;144:544–551
26. Paxinos GW. The Rat Brain in Stereotaxic Coordinates. Sydney, Academic Press, 1986
27. Seoane LM, Tovar S, Balldelli R, et al. Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. Eur J Endocrinol 2000;143: R7–R9
28. Kim EJ, Kho JH, Kang MR, Um SJ. Active regulator of Sirt1 cooperates with Sirt1 and facilitates suppression of p53 activity. Mol Cell 2007;28: 277–290
29. Nakazato M, Murakami N, Date Y, et al. A role for ghrelin in the central regulation of feeding. Nature 2001;409:194–198
30. Han MK, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE. SIRT1 regulates apoptosis and Nanog expression in mouse embryonic stem cells by controlling p53 subcellular localization. Cell Stem Cell 2008;2:241–251
31. Hallenborg P, Feddersen S, Madsen L, Kristiansen K. The tumor suppressors pRB and p53 as regulators of adipocyte differentiation and function. Expert Opin Ther Targets 2009;13:235–246
32. Andersson U, Filipsson K, Abbott CR, et al. AMP-activated protein kinase plays a role in the control of food intake. J Biol Chem 2004;279:12005– 12008
33. Cohen HY, Miller C, Bitterman KJ, et al. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science 2004; 305:390–392
34. Kirchner H, Gutierrez JA, Solenberg PJ, et al. GOAT links dietary lipids with the endocrine control of energy balance. Nat Med 2009;15:741–745
35. Prudom C, Liu J, Patric J, et al. Comparison of competitive radioimmunoassays and two-site sandwich assays for the measurement and interpretation of plasma ghrelin levels. J Clin Endocrinol Metab 2010;95: 2351–2358
36. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999;402:656–660
37. Zagnan JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol 2006;494:528–548
38. Dasgupta B, Milbrandt J. Resveratrol stimulates AMP kinase activity in neurons. Proc Natl Acad Sci USA 2007;104:7217–7222
39. Nemoto S, Fergusson MM, Finkel T. Nutrient availability regulates SIRT1 through a forkhead-dependent pathway. Science 2004;306:2105–2108