

# Anti-obesity effects of $\alpha$ -lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase

Min-Seon Kim<sup>1</sup>, Joong-Yeol Park<sup>1</sup>, Cheryl Namkoong<sup>2</sup>, Pil-Geum Jang<sup>2</sup>, Je-Won Ryu<sup>2</sup>, Hai-Sun Song<sup>2</sup>, Ji-Young Yun<sup>2</sup>, Il-Seong Namgoong<sup>1</sup>, Joo-hun Ha<sup>3</sup>, In-Sun Park<sup>4</sup>, In-Kyu Lee<sup>5</sup>, Benoit Viollet<sup>6</sup>, Jang Hyun Youn<sup>7</sup>, Hong-Kyu Lee<sup>8</sup> & Ki-Up Lee<sup>1</sup>

**AMP-activated protein kinase (AMPK) functions as a fuel sensor in the cell and is activated when cellular energy is depleted. Here we report that  $\alpha$ -lipoic acid ( $\alpha$ -LA), a cofactor of mitochondrial enzymes, decreases hypothalamic AMPK activity and causes profound weight loss in rodents by reducing food intake and enhancing energy expenditure. Activation of hypothalamic AMPK reverses the effects of  $\alpha$ -LA on food intake and energy expenditure. Intracerebroventricular (i.c.v.) administration of glucose decreases hypothalamic AMPK activity, whereas inhibition of intracellular glucose utilization through the administration of 2-deoxyglucose increases hypothalamic AMPK activity and food intake. The 2-deoxyglucose-induced hyperphagia is reversed by inhibiting hypothalamic AMPK. Our findings indicate that hypothalamic AMPK is important in the central regulation of food intake and energy expenditure and that  $\alpha$ -LA exerts anti-obesity effects by suppressing hypothalamic AMPK activity.**

Body weight is maintained at a relatively constant level over days and months despite variability in food intake and physical activity. To achieve energy homeostasis, the hypothalamus receives information related to energy surplus or shortage from the periphery, and controls food intake and energy expenditure. Leptin, an adipocyte-derived hormone, is a principal mediator that signals the brain about the stored energy status. An increase in leptin signaling in the brain prevents excess energy stores by suppressing food intake and increasing energy expenditure<sup>1</sup>. In addition, insulin and nutrients themselves, such as glucose and free fatty acids, also regulate food intake<sup>2–4</sup>.

The heterotrimeric serine/threonine protein kinase AMPK is a principal cellular regulator of lipid and glucose metabolism<sup>5</sup>. AMPK is activated when cellular energy is depleted. Activation of AMPK in skeletal muscle increases glucose uptake<sup>6</sup>. In addition, AMPK activation increases fatty acid oxidation by inhibiting acetyl-coenzyme A (acetyl-CoA) carboxylase (ACC) activity and by decreasing malonyl-CoA concentrations<sup>7</sup>. AMPK is expressed in the central nervous system (CNS)<sup>8</sup>, but little is known about its role there, although AMPK in the hypothalamus has been implicated in the regulation of food intake<sup>9,10</sup>.

The naturally occurring short-chain fatty acid  $\alpha$ -LA contains two sulfur molecules and is an essential cofactor of mitochondrial respiratory enzymes.  $\alpha$ -LA has a powerful antioxidant capacity and is used

clinically for treating diabetic neuropathy<sup>11</sup>.  $\alpha$ -LA is a unique antioxidant because it has beneficial effects on fuel metabolism: it enhances glucose transport into the skeletal muscle of lean and insulin-resistant obese animals<sup>12</sup>. Here we show that  $\alpha$ -LA exerts potent anti-obesity effects by suppressing hypothalamic AMPK activity.

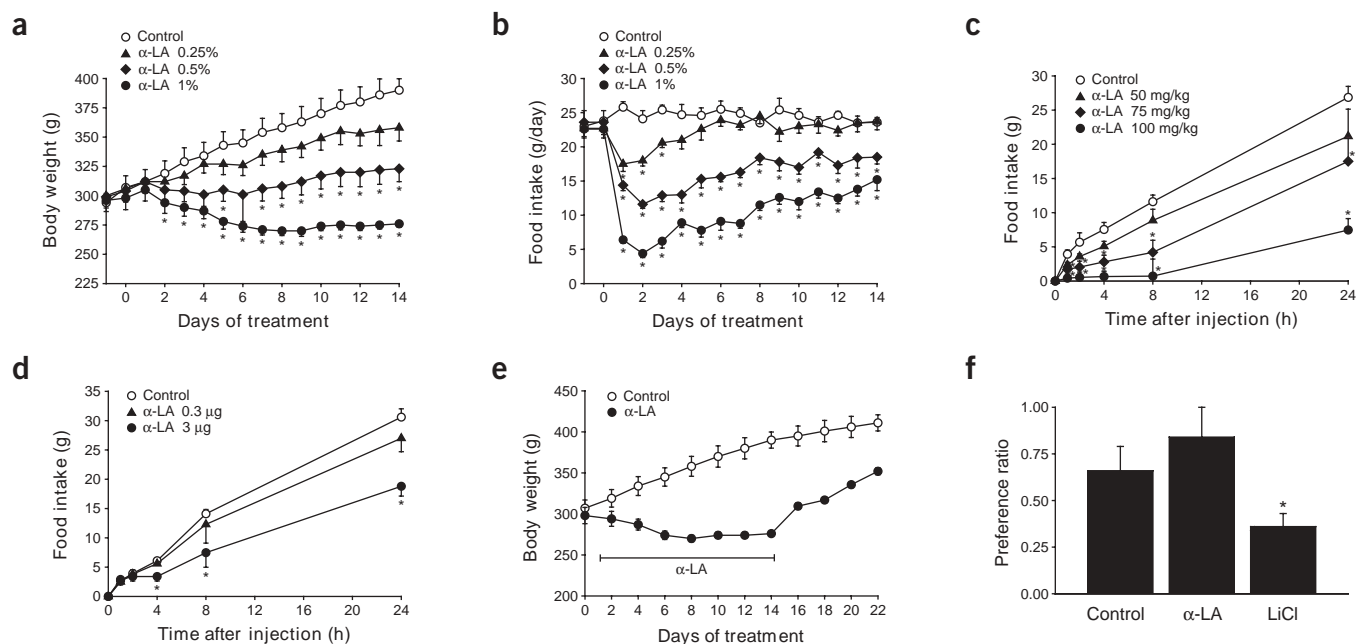
## RESULTS

### $\alpha$ -LA reduces food intake and body weight in rodents

We gave male Sprague-Dawley rats standard rat chow containing  $\alpha$ -LA (0.25, 0.5 and 1%, wt./wt.) for 2 weeks.  $\alpha$ -LA significantly reduced food intake and body weight in a dose-dependent manner (Figs. 1a,b). Acute administration of  $\alpha$ -LA by intraperitoneal (i.p.) injection (50, 75 and 100 mg per kg (bodyweight)) also suppressed food intake (Fig. 1c). In addition, the i.c.v. injection of small doses of  $\alpha$ -LA (0.3 and 3  $\mu$ g) reduced food intake (Fig. 1d), suggesting that the CNS is the primary site of the anorexic effect of  $\alpha$ -LA.

Dietary administration of  $\alpha$ -LA (0.5%, wt./wt.) for 14 weeks also decreased body weight and visceral fat mass in genetically obese Otsuka Long-Evans Tokushima Fatty (OLETF) rats (Supplementary Fig. 1 online); these effects were accompanied by a reduction in plasma glucose, insulin, free fatty acid and leptin (Supplementary Table 1 online).

<sup>1</sup>Department of Internal Medicine and <sup>2</sup>Asan Institute for Life Sciences, University of Ulsan College of Medicine, 138-736 Poongnap-dong, Songpa-ku, Seoul 138-736, Korea. <sup>3</sup>Department of Molecular Biology, Kyunghee University College of Medicine, 1 Hoegi-dong Tongdaemun-gu, Seoul 130-701, Korea. <sup>4</sup>Department of Anatomy, College of Medicine, Inha University, 7-241 Shinheung-dong, Choong-ku, Incheon 400-103, Korea. <sup>5</sup>Department of Internal Medicine, Keimyung University School of Medicine, 194 Dongsan-dong, Taegu 700-310, Korea. <sup>6</sup>Department of Genetics, Development and Molecular Pathology, Institut Cochin, INSERM, CNRS, Rene Descartes University, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France. <sup>7</sup>Department of Physiology and Biophysics, University of Southern California Keck School of Medicine, 1333 San Pablo St. MMR626, Los Angeles, CA 90089, USA. <sup>8</sup>Department of Internal Medicine, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-ku, Seoul 110-744, Korea. Correspondence should be addressed to K.-U.L. (kulee@amc.seoul.kr).



**Figure 1**  $\alpha$ -LA causes weight loss and anorexia. **(a,b)** Body weight **(a)** and daily food intake **(b)** during dietary administration of  $\alpha$ -LA ( $n = 6$ ).  $*P < 0.05$  versus control. **(c)** Effects of i.p. administration of  $\alpha$ -LA on food intake ( $n = 6$ ).  $*P < 0.05$  versus control. **(d)** Effects of i.c.v. administration of  $\alpha$ -LA on food intake ( $n = 7$ ).  $*P < 0.05$  versus control. **(e)** Recovery of weight loss after termination of  $\alpha$ -LA treatment (0.5% dietary administration) ( $n = 5$ ). **(f)** Conditioned taste aversion (CTA) was induced by lithium chloride but not by  $\alpha$ -LA ( $n = 8$ ).  $*P < 0.05$  versus control.

### Anti-obesity effect of $\alpha$ -LA is not due to toxicity

We wanted to exclude the possibility that the anorexic effect of  $\alpha$ -LA was caused by systemic toxicity or an illness induced by  $\alpha$ -LA. We found that Sprague-Dawley rats lost weight during treatment with  $\alpha$ -LA (0.5%) but rapidly gained weight on termination of the treatment (Fig. 1e), indicating that  $\alpha$ -LA does not induce a persistent state of wasting. Histological analysis of the major organs of rats that had been treated with  $\alpha$ -LA showed no adverse pathology, and blood cell counts and blood chemistry were unchanged (data not shown).

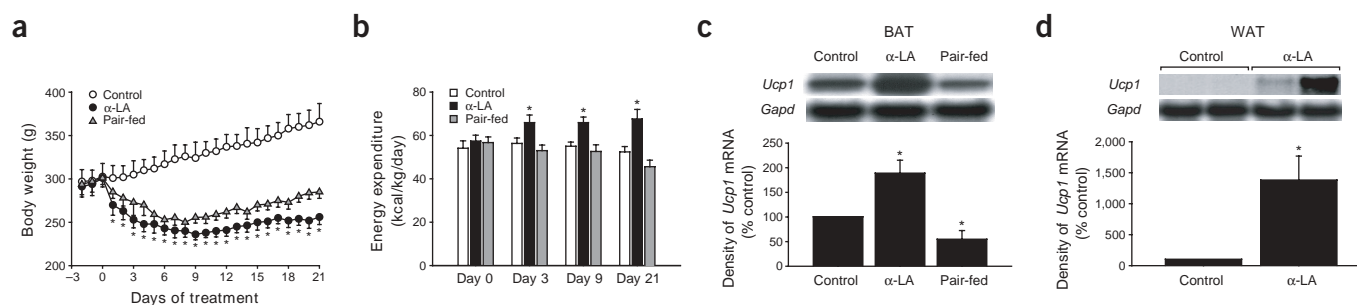
Conditioned taste aversion (CTA) is commonly used to assess whether a particular substance or treatment renders an animal ill<sup>13</sup>. Rats given  $\alpha$ -LA (75 mg/kg) consumed a similar amount of saccharin compared to rats given saline in a two-bottle test (Fig. 1f), indicating that  $\alpha$ -LA does not cause a CTA. By contrast, administration of lithium chloride (22 mg/kg) reduced saccharin consumption by 46%. Plasma corticosterone, an indicator of stress, was also not altered by

7 d of  $\alpha$ -LA treatment (data not shown). Collectively, these results indicate that the anorexic effect of  $\alpha$ -LA is not due to systemic toxicity or to an illness induced by  $\alpha$ -LA.

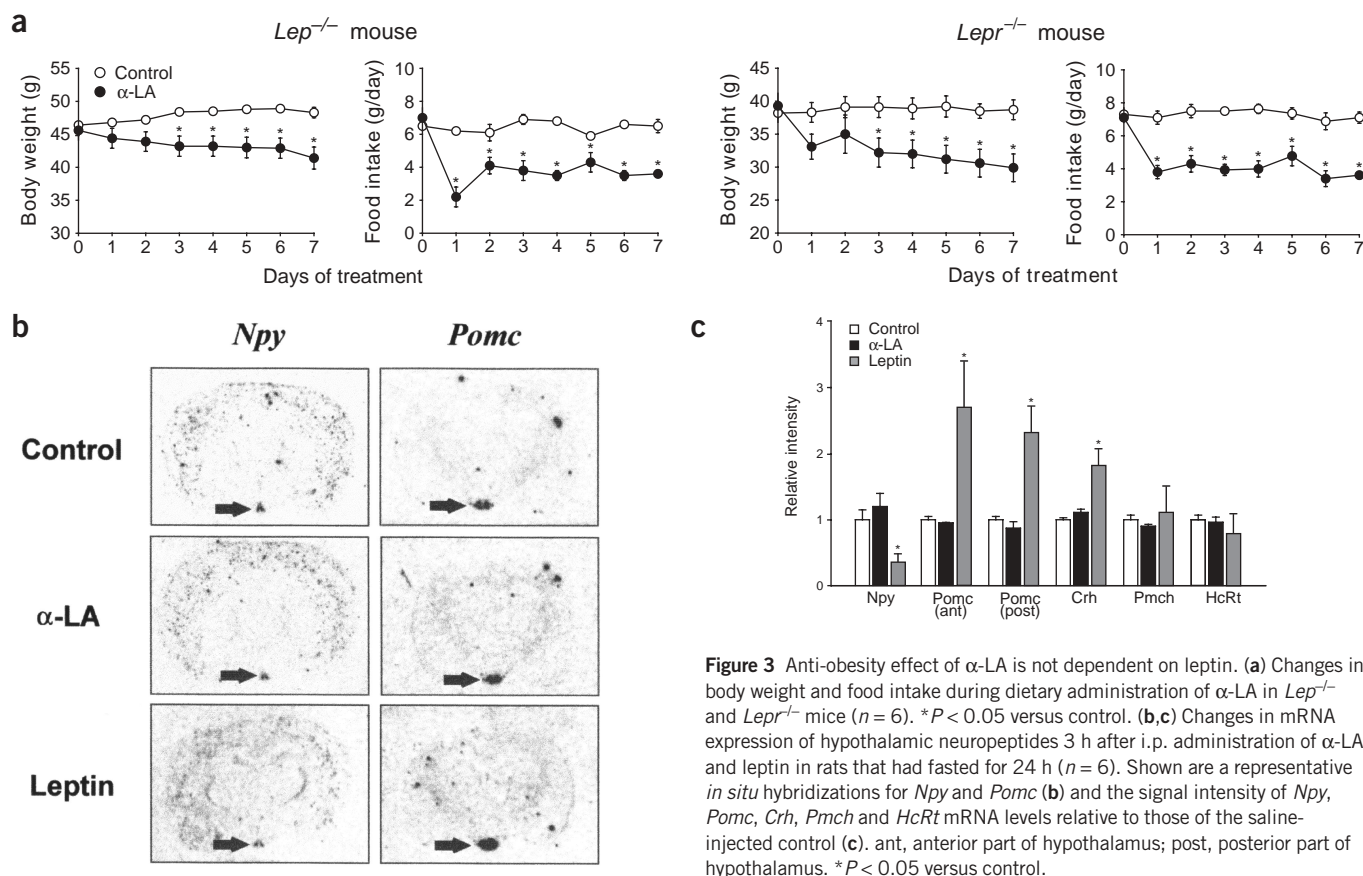
### $\alpha$ -LA stimulates whole-body energy expenditure

Body weight is determined by the balance between food intake and energy expenditure. To test whether  $\alpha$ -LA increases whole-body energy expenditure, we compared body weight changes in Sprague-Dawley rats given dietary  $\alpha$ -LA (0.5%) and in pair-fed rats given the same amount of food as that consumed by the  $\alpha$ -LA group on the previous day. The  $\alpha$ -LA-treated rats weighed significantly less than the pair-fed rats (Fig. 2a), indicating an enhanced use of ingested energy. Energy expenditure measured by indirect calorimetry was indeed higher in rats given  $\alpha$ -LA than in control or pair-fed rats (Fig. 2b).

Uncoupling protein-1 (Ucp1) in brown adipose tissue (BAT) is a chief regulator of energy expenditure in rodents<sup>14</sup>. Ucp1 is located in the



**Figure 2**  $\alpha$ -LA increases energy expenditure and expression of *Ucp1* in BAT. **(a,b)** Changes in body weight **(a)** and energy expenditure **(b)** during dietary administration of  $\alpha$ -LA ( $n = 6$ ).  $*P < 0.05$  versus control and pair-fed group. **(c,d)** Northern blot analysis of *Ucp1* mRNA expression in BAT **(c)** and WAT **(d)** in control,  $\alpha$ -LA-treated and pair-fed rats after 3 weeks of treatment ( $n = 6$ ).  $*P < 0.05$  versus control group.



**Figure 3** Anti-obesity effect of  $\alpha$ -LA is not dependent on leptin. **(a)** Changes in body weight and food intake during dietary administration of  $\alpha$ -LA in *Lep<sup>-/-</sup>* and *Lepr<sup>-/-</sup>* mice ( $n = 6$ ).  $*P < 0.05$  versus control. **(b,c)** Changes in mRNA expression of hypothalamic neuropeptides 3 h after i.p. administration of  $\alpha$ -LA and leptin in rats that had fasted for 24 h ( $n = 6$ ). Shown are a representative *in situ* hybridizations for *Npy* and *Pomc* **(b)** and the signal intensity of *Npy*, *Pomc*, *Crh*, *Pmch* and *HcRt* mRNA levels relative to those of the saline-injected control **(c)**. ant, anterior part of hypothalamus; post, posterior part of hypothalamus.  $*P < 0.05$  versus control.

mitochondrial inner membrane and dissipates the proton electrochemical energy as heat. The pair-fed rats showed reduced expression of *Ucp1* in BAT, whereas rats given  $\alpha$ -LA showed increased *Ucp1* mRNA in BAT (Fig. 2c;  $P < 0.05$ ) and ectopic expression of *Ucp1* in white adipose tissue (WAT) (Fig. 2d). These data indicate that weight loss induced by  $\alpha$ -LA is due, in part, to an enhancement of energy expenditure.

### Anti-obesity effect of $\alpha$ -LA is not dependent on leptin

The effects of  $\alpha$ -LA on food intake and energy metabolism are similar to the reported effects of leptin<sup>1,2</sup>. To determine whether the action of  $\alpha$ -LA is mediated by leptin or leptin receptor signaling, we fed leptin-deficient (*Lep<sup>-/-</sup>*) or leptin receptor-deficient (*Lepr<sup>-/-</sup>*) mice a diet containing  $\alpha$ -LA (0.5%).  $\alpha$ -LA reduced food intake and caused weight loss in both strains of mice (Fig. 3a), indicating that leptin and its receptor are not essential for  $\alpha$ -LA-induced anorexia.

Leptin exerts its anorexic effect through the regulation of hypothalamic neuropeptides<sup>15</sup>. The i.p. administration of leptin (1 mg/kg) to Sprague-Dawley rats reduced the expression of hypothalamic neuropeptide Y (*Npy*) mRNA and increased that of pro-opiomelanocortin (*Pomc*) and corticotropin releasing hormone (*Crh*) mRNA. By contrast, the i.p. administration of  $\alpha$ -LA (75 mg/kg) did not cause any acute changes in the levels of hypothalamic *Npy*, *Pomc*, *Crh*, pro-melanin concentrating hormone (*Pmch*) or hypocretin (*HcRt*; also called orexin) mRNA (Figs. 3b,c).

### $\alpha$ -LA suppresses hypothalamic AMPK activity

The i.p. administration of  $\alpha$ -LA (75 mg/kg) to Sprague-Dawley rats caused a significant decrease in AMPK and ACC phosphorylation in the medial part of the hypothalamus 30 and 60 min after injection

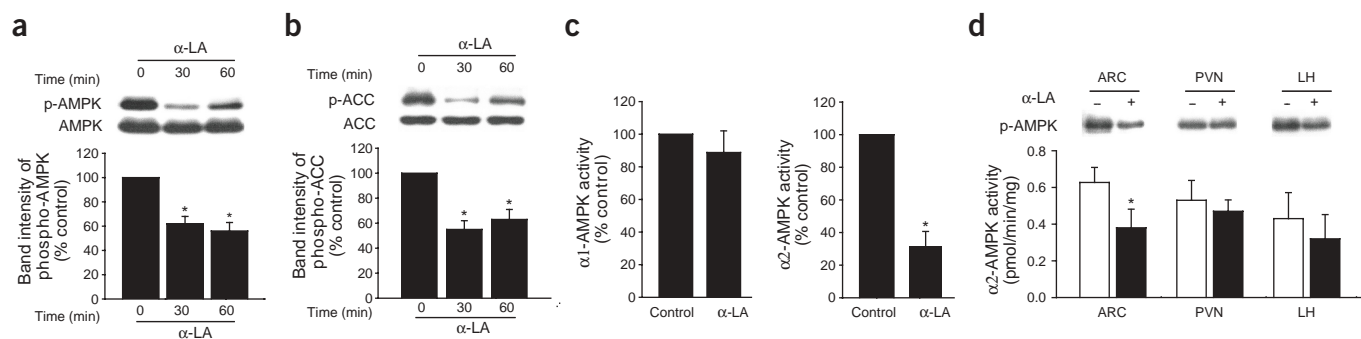
(Figs. 4a,b). The i.c.v. administration of  $\alpha$ -LA (3  $\mu$ g) also suppressed hypothalamic AMPK phosphorylation 1 h after injection (data not shown). Both the  $\alpha$ 1 and  $\alpha$ 2 isoforms of AMPK are present in the CNS<sup>8</sup>. Measurement of isoform-specific AMPK activity showed that  $\alpha$ 2-AMPK activity was reduced 1 h after administration of  $\alpha$ -LA, whereas  $\alpha$ 1-AMPK activity did not change significantly (Fig. 4c).

To dissect further the hypothalamic areas involved in  $\alpha$ -LA-induced anorexia, we prepared individual hypothalamic nuclei by using a 'micropunch' technique<sup>16</sup> and measured AMPK activity in the arcuate nucleus, paraventricular nucleus and lateral hypothalamic area separately after the i.p. injection of  $\alpha$ -LA. We found that  $\alpha$ -LA significantly ( $P < 0.05$ ) reduced AMPK phosphorylation and  $\alpha$ 2-AMPK activity in the arcuate nucleus but not in the paraventricular nucleus or the lateral hypothalamic area (Fig. 4d).

### AMPK activation reverses the effect of $\alpha$ -LA

To determine whether AMPK inhibition mediates the anorexic effect of  $\alpha$ -LA, rats that had fasted for 24 h were given an i.c.v. injection of 5'-aminoimidazole-4-carboxamide ribonucleoside (AICAR; 10 nmol), an AMPK activator<sup>6</sup>, and  $\alpha$ -LA. AICAR alone did not affect food intake but completely blocked the suppression of food intake induced by an i.c.v. injection of  $\alpha$ -LA (3  $\mu$ g; Fig. 5a). To confirm that the effect of AICAR was mediated by activation of AMPK, we examined the effect of AICAR in rats overexpressing dominant-negative AMPK (DN-AMPK) in the bilateral mediobasal hypothalamus (MBH) centered on the arcuate nucleus. In these rats, the blocking effect of AICAR on the  $\alpha$ -LA-induced anorexia was reduced (Fig. 5b).

To verify further that AMPK is involved in the  $\alpha$ -LA-induced suppression of food intake, we overexpressed a constitutively active



**Figure 4**  $\alpha$ -LA suppresses hypothalamic AMPK activity. (a–c) Western blot analysis of AMPK (a) and ACC phosphorylation (b) and measurement of  $\alpha$ 1- and  $\alpha$ 2-AMPK activity (c) in the medial hypothalamus after i.p. administration of  $\alpha$ -LA ( $n = 6$ ). \* $P < 0.05$  versus saline-injected control. (d) AMPK phosphorylation and  $\alpha$ 2-AMPK activity in hypothalamic nuclei after i.p. administration of  $\alpha$ -LA ( $n = 6$ ). ARC, arcuate nucleus; LH, lateral hypothalamic area; PVN, paraventricular nucleus. \* $P < 0.05$  versus saline injected control.

AMPK (CA-AMPK) variant<sup>17</sup> in the bilateral MBH. Four days after *in vivo* gene transfer, rats were given an i.p. injection of vehicle or  $\alpha$ -LA (75 mg/kg). In these rats, AMPK activity and ACC phosphorylation in MBH increased about twofold (Fig. 5c) and the anorexic effect of  $\alpha$ -LA was significantly attenuated as compared with the rats overexpressing a control  $\beta$ -galactosidase ( $\beta$ -gal) gene (Fig. 5d). A single i.c.v. injection of  $\alpha$ -LA (3  $\mu$ g) increased energy expenditure and expression of *Ucp1* mRNA in BAT after 15 h, and the i.c.v. coadministration of AICAR completely reversed these effects (Figs. 5e,f). Collectively, these results indicate that a decrease in hypothalamic AMPK activity is responsible for the  $\alpha$ -LA-induced changes in food intake and energy expenditure.

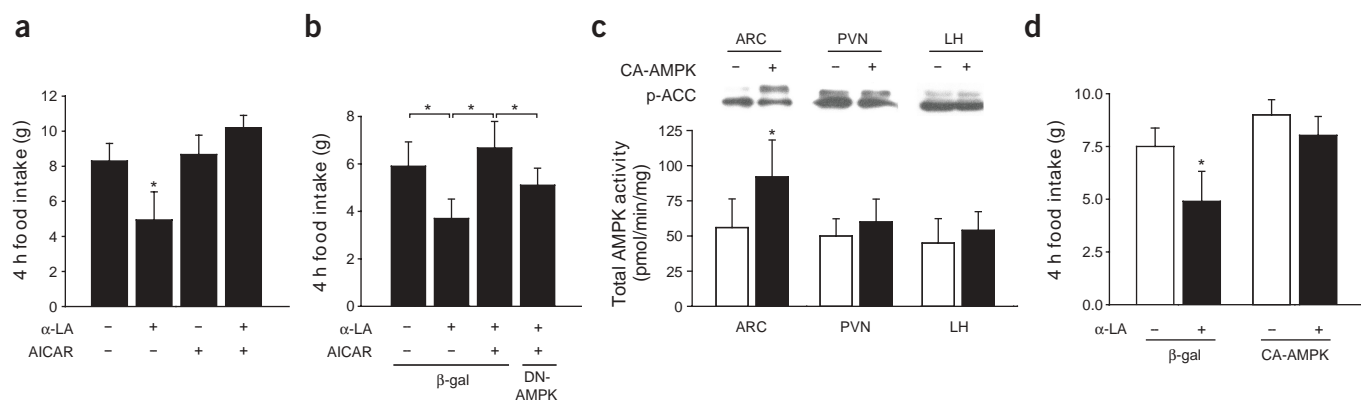
#### Hypothalamic AMPK activity is dependent on energy status

$\alpha$ -LA stimulates glucose transport and ATP synthesis in peripheral tissues<sup>18–20</sup>. Similarly, it may increase glucose uptake in the hypothalamus.

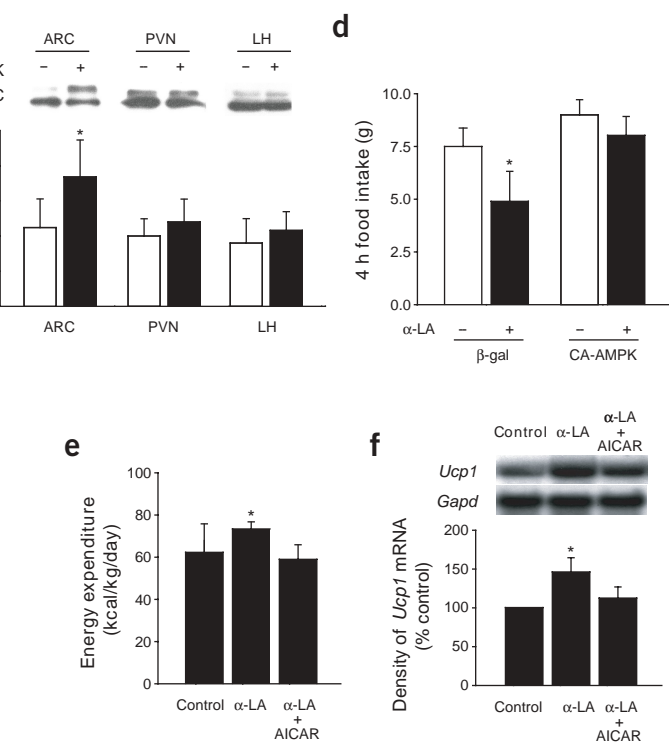
To test the hypothesis that hypothalamic AMPK activity is dependent on local or whole-body energy status, we examined the effects of glucose surplus and glucose depletion on hypothalamic AMPK activity. The i.c.v. administration of glucose (5 mg) to rats that had fasted for 24 h resulted in a decrease in AMPK phosphorylation and  $\alpha$ 2-AMPK activity in the hypothalamus 1 h later (Figs. 6a,b).

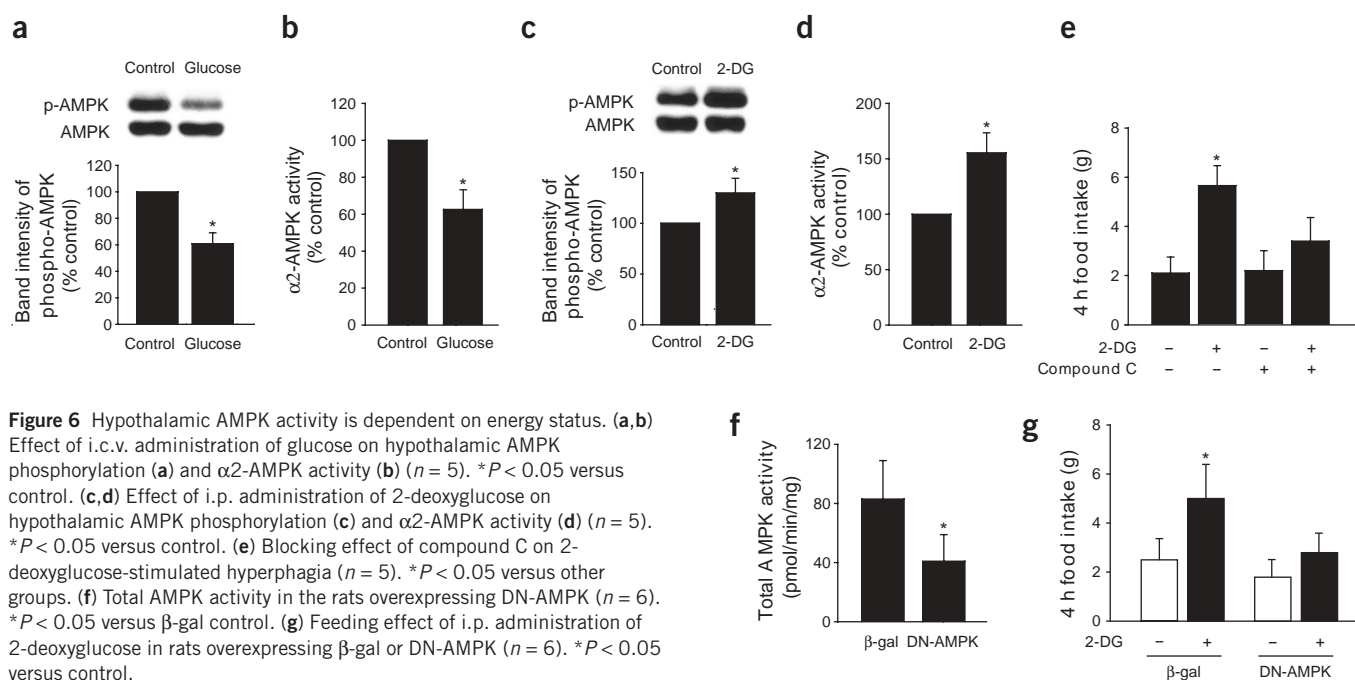
We next examined the effect of 2-deoxyglucose, a known inhibitor of intracellular glucose utilization<sup>21</sup>, in satiated rats. The i.p. administration of 2-deoxyglucose (500 mg/kg) increased food intake as well as hypothalamic AMPK phosphorylation and  $\alpha$ 2-AMPK activity after 1 h (Figs. 6c,d). The i.c.v. administration of the AMPK inhibitor<sup>22</sup> compound C (100 nmol) 1 h before the administration of 2-deoxyglucose prevented this 2-deoxyglucose-induced increase in food intake (Fig. 6e).

To confirm the involvement of AMPK in 2-deoxyglucose-induced hyperphagia, we investigated the feeding effect of 2-deoxyglucose in the rats overexpressing DN-AMPK in the bilateral MBH. In these rats,



**Figure 5** AMPK activation reverses the effect of  $\alpha$ -LA on food intake and energy expenditure. (a) Blocking effects of AICAR on  $\alpha$ -LA-induced anorexia ( $n = 6$ ). \* $P < 0.05$  versus other groups. (b) Blocking effects of AICAR on  $\alpha$ -LA-induced anorexia in rats overexpressing DN-AMPK in the hypothalamus ( $n = 6$ ). \* $P < 0.05$  between the indicated groups. (c) Total AMPK activity and ACC phosphorylation in rats overexpressing CA-AMPK ( $n = 6$ ). \* $P < 0.05$  versus  $\beta$ -gal control. (d) Effects of  $\alpha$ -LA on food intake in rats overexpressing  $\beta$ -gal or CA-AMPK ( $n = 6$ ). \* $P < 0.05$  versus control. (e,f) Energy expenditure (e) and expression of *Ucp1* (f) in BAT in rats given an i.c.v. injection of  $\alpha$ -LA with or without AICAR ( $n = 6$ ). \* $P < 0.05$  versus other groups.





**Figure 6** Hypothalamic AMPK activity is dependent on energy status. (a,b) Effect of i.c.v. administration of glucose on hypothalamic AMPK phosphorylation (a) and  $\alpha 2$ -AMPK activity (b) ( $n = 5$ ). \* $P < 0.05$  versus control. (c,d) Effect of i.p. administration of 2-deoxyglucose on hypothalamic AMPK phosphorylation (c) and  $\alpha 2$ -AMPK activity (d) ( $n = 5$ ). \* $P < 0.05$  versus control. (e) Blocking effect of compound C on 2-deoxyglucose-stimulated hyperphagia ( $n = 5$ ). \* $P < 0.05$  versus other groups. (f) Total AMPK activity in the rats overexpressing DN-AMPK ( $n = 6$ ). \* $P < 0.05$  versus  $\beta$ -gal control. (g) Feeding effect of i.p. administration of 2-deoxyglucose in rats overexpressing  $\beta$ -gal or DN-AMPK ( $n = 6$ ). \* $P < 0.05$  versus control.

AMPK activity in MBH was reduced by about 40% (Fig. 6f) and the 2-deoxyglucose-induced increase in food intake was attenuated as compared with rats overexpressing  $\beta$ -gal (Fig. 6g).

## DISCUSSION

Studies with compounds that alter fuel metabolism, such as 2-deoxyglucose<sup>21</sup>, fatty acid synthase inhibitor<sup>23</sup> and CPT-1 inhibitor<sup>16</sup> have shown that some populations of hypothalamic neurons can detect changes in local or whole-body energy status and can initiate appropriate behavioral and physiological responses. AMPK is regarded as a principal regulator of cellular metabolism in peripheral tissues that responds to changes in energy status<sup>5–7</sup>. We have shown here that hypothalamic AMPK is involved in sensing energy status and controlling energy balance and that  $\alpha$ -LA exerts potent anti-obesity effects by suppressing hypothalamic AMPK activity.

The i.p. or i.c.v. administration of  $\alpha$ -LA decreased hypothalamic AMPK activity and food intake. Although we did not study the mechanism by which  $\alpha$ -LA decreases hypothalamic AMPK activity or phosphorylation,  $\alpha$ -LA is known to stimulate glucose transport and ATP synthesis in peripheral tissues<sup>18–20</sup> and it may decrease hypothalamic AMPK activity by increasing glucose uptake, glucose metabolism or both in the hypothalamus. In support of this notion, the i.c.v. administration of glucose also decreased hypothalamic AMPK activity. Conversely, the i.p. administration of 2-deoxyglucose, which inhibits glucose metabolism, increased hypothalamic AMPK activity and food intake. The  $\alpha$ -LA-induced anorexia and 2-deoxyglucose-induced hyperphagia were reversed by genetic or chemical activation and inactivation of AMPK, respectively. These data indicate that hypothalamic AMPK is directly involved in the control of food intake.

AMPK phosphorylates and deactivates ACC, the enzyme that produces malonyl-CoA from acetyl-CoA in fatty acid biosynthesis<sup>5,7</sup>. We have shown here that  $\alpha$ -LA activates ACC by decreasing ACC phosphorylation in the hypothalamus, consistent with its effect to decrease AMPK activity. Because activation of ACC would increase intracellular malonyl-CoA<sup>5,7</sup> in hypothalamic neurons, malonyl-CoA might be a

key downstream mediator of AMPK activity responsible for the decrease in food intake that occurs with  $\alpha$ -LA administration, consistent with a previous proposal<sup>23</sup>.

Malonyl-CoA is the main regulator of carnitine palmitoyl transferase-1 (CPT-1), the enzyme responsible for LCAC transport from cytosol to mitochondria<sup>24</sup>. An increase in malonyl-CoA would decrease LCAC transport into mitochondria, consistent with the decrease in fatty acid oxidation that occurs with  $\alpha$ -LA (Supplementary Fig. 2 online), and would increase cytosolic LCAC. In this regard, inhibition of hypothalamic CPT-1 has been reported to decrease food intake, leading to the suggestion that intracellular LCAC may be a key component of hypothalamic fuel sensing and the regulation of food intake<sup>16</sup>. Thus, hypothalamic AMPK activity may regulate food intake by altering malonyl-CoA and/or LCAC in the hypothalamus.

We have also shown that  $\alpha$ -LA treatment increases energy expenditure and *Ucp1* expression in BAT. Expression of *Ucp1* in BAT is regulated by the sympathetic nervous system, the activation of which is governed by the CNS<sup>25</sup>. The i.c.v. administration of very small amounts of  $\alpha$ -LA was sufficient to produce these effects, suggesting that  $\alpha$ -LA-induced enhancement of energy expenditure is mediated by the CNS. In addition, the i.c.v. administration of an AMPK activator abolished the effect of  $\alpha$ -LA on energy expenditure, indicating that hypothalamic AMPK has a key role in this process. Collectively, hypothalamic AMPK seems to function as a major regulator of both food intake and energy expenditure.

$\alpha$ -LA exerts beneficial metabolic effects on skeletal muscle through an insulin-independent mechanism<sup>26</sup>. We found that  $\alpha$ -LA increases glucose uptake and fatty acid oxidation by activating AMPK in skeletal muscle (K.H.S., J.Y.P., J.M.K., H.S.K., H.S.P. and K.U.L., unpublished data). Thus,  $\alpha$ -LA exerts opposite effects on AMPK activity in different tissues, namely hypothalamus versus skeletal muscle. Similarly, leptin increases  $\alpha 2$ -AMPK activity in skeletal muscle<sup>27</sup> but decreases it in the hypothalamus<sup>9,10</sup>. Although these data suggest that leptin and  $\alpha$ -LA may share common signaling pathways for regulating AMPK and/or

energy balance, it should be noted that  $\alpha$ -LA reduced food intake and body weight in both *Lep<sup>-/-</sup>* and *Lepr<sup>-/-</sup>* mice. We also showed that  $\alpha$ -LA effectively reduced adiposity and visceral fat mass in genetically obese OLETF rats, which are leptin resistant<sup>28</sup>. Because most obese people are resistant to leptin<sup>29</sup>, this agent is ineffective in treating human obesity. Thus,  $\alpha$ -LA may be a promising anti-obesity drug for treatment of leptin-resistant human obesity.

In summary, our results indicate that hypothalamic AMPK may represent a sensor that links energy status in hypothalamic neurons to the regulation of food intake and energy expenditure.  $\alpha$ -LA has previously unknown anti-obesity effects mediated by the suppression of hypothalamic AMPK activity.

## METHODS

**Animals.** OLETF rats were obtained from the Otsuka Research Institute and *Lep<sup>-/-</sup>* and *Lepr<sup>-/-</sup>* mice from Jackson Laboratories. All animal procedures were done in accordance with the guidelines of the Institutional Animal Care and Use Committee of the Asan Institute for Life Sciences.

**ICV cannulation.** Stereotaxic implantation of an intraventricular cannula aimed at the third intracerebral ventricle (1.8-mm caudal to the bregma and 6.5-mm ventral to the sagittal sinus) in Sprague-Dawley rats (250–300 g), aged 8 weeks, was done as described<sup>30</sup>. Placement of the cannula was confirmed by dipsogenic response after the i.c.v. injection of angiotensin II (150 ng). All compounds were dissolved in 0.9% saline or dimethyl sulfoxide (DMSO) and 5  $\mu$ l of each was administered.

**Energy expenditure.** Energy expenditure was measured using an indirect calorimeter (Columbus Instruments). We calculated energy expenditure according to the following formula provided by the manufacturer: energy expenditure (kcal) = (3.815 + 1.232VO<sub>2</sub>/VCO<sub>2</sub>)  $\times$  VO<sub>2</sub>.

**Determination of CTA.** We tested CTA as described<sup>13</sup>. Data are presented as a preference ratio of saccharine intake over total fluid intake.

**Northern blot analysis of *Ucp1* mRNA.** Northern blot analysis of *Ucp1* was done as described<sup>31</sup>. Band intensities were quantified by a densitometer. The results were normalized to *Gapd* to correct for variations in sample loading and are expressed as a percentage of control signals (% control) in each blot to correct for variations between blots.

**In situ hybridization.** Rats that had fasted for 24 h were given an i.p. injection of  $\alpha$ -LA (75 mg/kg) or leptin (1 mg/kg). Three hours after injection, the rats were decapitated and whole brains were immediately frozen in prechilled isopentane and liquid nitrogen and stored at  $-70^{\circ}\text{C}$ . *In situ* hybridization of *Npy*, *Pomc*, *Crh*, *Pmch* and *HcRt* mRNA with antisense oligomers was done as described<sup>32</sup>.

**Microdissection of hypothalamic nuclei.** We dissected the medial part of the hypothalamus in the anterior border of the optic chiasm, posterior border of the mammillary body, upper border of the anterior commissure and lateral border halfway from the lateral sulcus in the ventral side of brain. To dissect further the hypothalamic areas involved in  $\alpha$ -LA-induced anorexia, we prepared individual hypothalamic nuclei by using a 'micropunch' technique as described<sup>16</sup>.

**Western blot analysis.** Phosphorylation of AMPK and ACC in the hypothalamus was assayed by western blotting with antibodies to phosphopeptides based on the amino acid sequence surrounding Thr172 of the  $\alpha$ -subunit of human AMPK (Cell Signaling) and Ser79 of rat ACC (Upstate Biotech), respectively. Band intensities were quantified by a densitometer. Results are expressed as percentage of control signals (% control) in each blot to correct for variations between blots.

**Measurement of AMPK activity.** We lysed the hypothalamus with digitonin buffer and measured total AMPK activity by using a synthetic 'SAMS' peptide and [ $\gamma$ -<sup>32</sup>P]ATP as described<sup>6</sup>. To measure isoform-specific AMPK activity, we immunoprecipitated the medial hypothalamus lysates (40  $\mu$ g of protein) with

specific antibodies (Upstate Biotech) to the  $\alpha$ 1- or  $\alpha$ 2-catalytic subunits of AMPK bound to protein G-Sepharose beads.

**Adenovirus-mediated gene transfer.** Site-directed mutagenesis was used to alter Thr172 to aspartic acid in  $\alpha$ 1-AMPK, and Lys45 to arginine in  $\alpha$ 2-AMPK, as described<sup>17,33</sup>. These mutants act as CA-AMPK and DN-AMPK, respectively. We injected adenovirus expressing either CA-AMPK ( $2.3 \times 10^{12}$  plaque-forming units (p.f.u.) per ml) or DN-AMPK ( $2.0 \times 10^{12}$  p.f.u./ml) mixed 1/1 (v./v.) with  $\beta$ -gal ( $1.8 \times 10^{12}$  p.f.u./ml) into the bilateral MBH using a syringe injector pump (Harvard Apparatus) at a rate of 200 nl/min for 5 min (1  $\mu$ l per injection site) as described<sup>34</sup>. We confirmed injection sites by  $\beta$ -gal staining as described<sup>35</sup>. The rats that did not show  $\beta$ -gal-stained cells in the MBH were excluded from the data analysis.

**Measurement of fatty acid oxidation.** Hypothalamus was collected 1 h after the i.p. injection of  $\alpha$ -LA (75 mg/kg). We assessed palmitate oxidation in the hypothalamus by measuring <sup>3</sup>H-labeled water generated from 9-10-<sup>3</sup>H]palmitate as described<sup>36</sup>.

**Statistical analysis.** Data are reported as the mean  $\pm$  s.e.m. The significance of difference was determined by analysis of variance.

*Note: Supplementary information is available on the Nature Medicine website.*

## ACKNOWLEDGMENTS

We thank M. Schwartz for critically reading the manuscript and E. Souil for technical support. This study was supported by a National Research Laboratory grant from the Ministry of Science and Technology (M1-0104-00-0103) and grants from the Ministry of Health & Welfare (03-PJ1-PG1-CH05-0005) of the Republic of Korea, the Korea Research Council of Fundamental Science and Technology, and the Asan Institute for Life Sciences (2004-006).

## COMPETING INTERESTS STATEMENT

The authors declare competing financial interests; see the Nature Medicine website for details.

Received 23 March; accepted 20 May 2004

Published online at <http://www.nature.com/naturemedicine/>

- Halaas, J.L. *et al.* Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* **269**, 543–546 (1995).
- Woods, S.C., Lotter, E.C., McKay, L.D. & Porte, D. Jr. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* **282**, 503–505 (1979).
- Obici, S. *et al.* Central administration of oleic acid inhibits glucose production and food intake. *Diabetes* **51**, 271–275 (2002).
- Davis, J.D., Wirtshafter, D., Asin, K.E. & Brief, D. Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science* **212**, 81–83 (1981).
- Hardie, D.G. & Carling, D. The AMP-activated protein kinase—fuel gauge of the mammalian cell? *Eur. J. Biochem.* **246**, 259–273 (1997).
- Hayashi, T. *et al.* Metabolic stress and altered glucose transport: activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes* **49**, 527–531 (2000).
- Ruderman, N.B., Saha, A.K., Vavvas, D. & Witters, L.A. Malonyl-CoA, fuel sensing, and insulin resistance. *Am. J. Physiol.* **276**, E1–E18 (1999).
- Turnley, A.M. *et al.* Cellular distribution and developmental expression of AMP-activated protein kinase isoforms in mouse central nervous system. *J. Neurochem.* **72**, 1707–1716 (1999).
- Andersson, U. *et al.* AMP-activated protein kinase plays a role in the control of food intake. *J. Biol. Chem.* **279**, 12005–12008 (2004).
- Minokoshi, Y. *et al.* AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428**, 569–574 (2004).
- Ziegler, D. *et al.* Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant  $\alpha$ -lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). *Diabetologia* **38**, 1425–1433 (1995).
- Jacob, S. *et al.* The antioxidant  $\alpha$ -lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. *Diabetes* **45**, 1024–1029 (1996).
- Benoit, S.C. *et al.* Assessment of the aversive consequences of acute and chronic administration of the melanocortin agonist, MTII. *Int. J. Obes. Relat. Metab. Disord.* **27**, 550–556 (2003).
- Dalgaard, L.T. & Pedersen, O. Uncoupling proteins: functional characteristics and role in the pathogenesis of obesity and Type II diabetes. *Diabetologia* **44**, 946–965 (2001).
- Schwartz, M.W., Woods, S.C., Porte, D. Jr., Seeley, R.J. & Baskin, D.G.. Central nervous system control of food intake. *Nature* **404**, 661–671 (2000).

16. Obici, S., Feng, Z., Arduini, A., Conti, R. & Rossetti, L. Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. *Nat. Med.* **9**, 756–761 (2003).
17. Woods, A. *et al.* Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol. Cell. Biol.* **20**, 6704–6711 (2000).
18. Hagen, T.M. *et al.* (R)- $\alpha$ -lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *FASEB J.* **13**, 411–418 (1999).
19. Zimmer, G., Mainka, L. & Kruger, E. Dihydrolipoic acid activates oligomycin-sensitive thiol groups and increases ATP synthesis in mitochondria. *Arch. Biochem. Biophys.* **288**, 609–613 (1991).
20. Yaworsky, K., Somwar, R., Ramlal, T., Tritschler, H.J. & Klip, A. Engagement of the insulin-sensitive pathway in the stimulation of glucose transport by  $\alpha$ -lipoic acid in 3T3-L1 adipocytes. *Diabetologia* **43**, 294–303 (2000).
21. Smith, G.P. & Epstein, A.N. Increased feeding in response to decreased glucose utilization in the rat and monkey. *Am. J. Physiol.* **217**, 1083–1087 (1969).
22. Zhou, G. *et al.* Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* **108**, 1167–1174 (2001).
23. Loftus, T.M. *et al.* Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* **288**, 2379–2381 (2000).
24. Brown, N.F., Esser, V., Foster, D.W. & McGarry, J.D. Expression of a cDNA for rat liver carnitine palmitoyltransferase I in yeast establishes that catalytic activity and malonyl-CoA sensitivity reside in a single polypeptide. *J. Biol. Chem.* **269**, 26438–26442 (1994).
25. Commins, S.P., Watson, P.M., Levin, N., Beiler, R.J. & Gettys, T.W. Central leptin regulates the *UCP1* and *ob* genes in brown and white adipose tissue via different  $\beta$ -adrenoceptor subtypes. *J. Biol. Chem.* **275**, 33059–33067 (2000).
26. Henriksen, E.J. *et al.* Stimulation by  $\alpha$ -lipoic acid of glucose transport activity in skeletal muscle of lean and obese Zucker rats. *Life Sci.* **61**, 805–812 (1997).
27. Minokoshi, Y. *et al.* Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* **415**, 339–343 (2002).
28. Niimi, M., Sato, M., Yokote, R., Tada, S. & Takahara, J. Effects of central and peripheral injection of leptin on food intake and on brain Fos expression in the Otsuka Long-Evans Tokushima Fatty rat with hyperleptinaemia. *J. Neuroendocrinol.* **11**, 605–611 (1999).
29. Maffei, M. *et al.* Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat. Med.* **1**, 1155–1161 (1995).
30. Kim, M.S. *et al.* The central melanocortin system affects the hypothalamo-pituitary thyroid axis and may mediate the effect of leptin. *J. Clin. Invest.* **105**, 1005–1011 (2000).
31. Ryu, J.W. *et al.* DHEA administration increases brown fat uncoupling protein 1 levels in obese OLETF rats. *Biochem. Biophys. Res. Commun.* **303**, 726–731 (2003).
32. Tritos, N.A., Elmquist, J.K., Mastaitis, J.W., Flier, J.S. & Maratos-Flier, E. Characterization of expression of hypothalamic appetite-regulating peptides in obese hyperleptinemic brown adipose tissue-deficient (uncoupling protein-promoter-driven diphtheria toxin A) mice. *Endocrinology* **139**, 4634–4641 (1998).
33. Dyck, J.R. *et al.* Regulation of 5'-AMP-activated protein kinase activity by the noncatalytic  $\beta$  and  $\gamma$  subunits. *J. Biol. Chem.* **271**, 17798–17803 (1996).
34. Morton, G.J. *et al.* Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (*fa<sup>h</sup>/ffa<sup>h</sup>*) rats. *Endocrinology* **144**, 2016–2024 (2003).
35. Mercer, E.H., Hoyle, G.W., Kapur, R.P., Brinster, R.L. & Palmiter, R.D. The dopamine  $\beta$ -hydroxylase gene promoter directs expression of *E. coli lacZ* to sympathetic and other neurons in adult transgenic mice. *Neuron* **7**, 703–716 (1991).
36. Lee, Y. *et al.* Increased lipogenic capacity of the islets of obese rats. *Diabetes* **46**, 408–413 (1997).