Published in final edited form as:

Physiol Behav. 2009 August 4; 98(1-2): 223–228. doi:10.1016/j.physbeh.2009.05.013.

Up-regulation of apolipoprotein E by leptin in the hypothalamus of mice and rats

Ling Shen^{1,2}, Patrick Tso^{1,2}, David Q.-H. Wang⁴, Stephen C. Woods^{1,3}, W. Sean Davidson¹, Randall Sakai^{1,3}, and Min Liu^{1,2,*}

¹Cincinnati Obesity Research Center, University of Cincinnati College of Medicine, Cincinnati, OH

²Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, OH

³Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, OH

⁴Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Abstract

Apolipoprotein E (apoE) is a satiation factor, playing an important role in the regulation of food intake and body weight. We previously reported that apoE was present in the hypothalamus, but it is unclear which type of the cells in this brain area expressing apoE. In addition, hypothalamic apoE mRNA levels were significantly reduced in both genetically obese *ob/ob* (leptin deficient) mice and high-fat diet-induced obese (leptin resistant) rats, raising the possibility that deficient leptin signaling might be related to the change in apoE gene expression. In the present studies, using double-staining immunohistochemistry, we demonstrated that apoE is mainly present in astrocytes. To characterize the effect of leptin on apoE gene expression, *ob/ob* and *db/db* mice were treated with recombinant mouse leptin (3 µg/g daily, i.p.) or vehicle for 5 days. We found that the increased hypothalamic apoE mRNA levels occurred only in leptin-treated *ob/ob*, but not in pair-fed *ob/ob*, or *db/db*, mice, indicating that leptin up-regulated hypothalamic apoE gene expression depends upon an intact leptin receptor, and this effect is not related to the changes in food intake and body weight. The reduced apoE gene expression caused by fasting, which also results in relatively lower leptin level, is restored by intracerebroventricular administration of leptin. In addition, leptin was significantly less efficacious in apoE KO mice because these animals consumed more food and lost less weight following leptin treatment, compared with wild-type controls. These observations imply that apoE signaling, at least partially, mediates the inhibitory effects of leptin on feeding.

Keywords

apolipoprotein; food intake; obesity

© 2009 Elsevier Inc. All rights reserved.

*Address all correspondence and reprint requests to: Min Liu, Ph.D. Department of Pathology and Laboratory Medicine University of Cincinnati College of Medicine Cincinnati, Ohio 45237-0507, USA Tel: (513) 558-4957 Fax: (513) 558-1312 lium@uc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Numerous peptides and other signals important in the regulation of energy homeostasis by the CNS have been identified in recent years [1;2]. Apolipoprotein E (apoE) is a constituent of several lipoprotein particles and is involved in lipid metabolism [3]. Although some apoE is produced in most organs, its major production sites are the liver and brain [4]. ApoE is mainly considered a lipid transport molecule, but recent data suggest that it has additional roles in the brain regulating both neuronal and astrocyte functions [5-7]. Recently, we reported that apoE in the hypothalamus plays an important role in the control of food intake. Intracerebroventricular (icv) administration of apoE significantly decreased food intake without causing malaise, whereas icv infusion of apoE antibody stimulated feeding, implying that endogenous apoE reduces food intake [8].

The incidence of obesity is increasing dramatically throughout the world with important pathological complications such as type-2 diabetes mellitus and cardiovascular disease [9]. One well-established risk factor for becoming obese is food consumption in excess of energy expenditure. Considerable evidence links a complex circuitry in the mediobasal hypothalamus with the control of food intake and energy expenditure. Based on our previous observations that apoE gene expression is reduced in both genetically obese (*ob/ob*) (leptin deficient) mice and high-fat diet-induced obese (leptin resistant) rats, and that both leptin receptor and apoE are present in the arcuate nucleus (ARC) of the hypothalamus [8], we hypothesized that apoE gene expression is regulated by leptin in the hypothalamus. The purpose of the present series of experiments was to test this hypothesis. It is also possible that apoE may in turn help mediate leptin's action on feeding. ApoE knockout (KO) mice have increased food intake and body weight when chronically fed a diet high in fat and carbohydrate [10], but it is unclear whether the lack of apoE in these mice compromises leptin's anorectic action. We therefore hypothesized that central leptin's action is impaired in animals lacking apoE, and this was tested by administering leptin to apoE KO mice.

2. Methods

2.1 Subjects

Male adult Long-Evans rats were purchased from Harlan (Indianapolis, IN). Male adult *ob/ob*, apoE KO mice and their wild-type C57BL/6J mice, and *db/db* and their wild-type C57BLKS/J mice, were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were individually housed in a temperature-controlled vivarium on a 12/12 h light/dark (lights on at 0600 h) cycle. Laboratory chow (Purina 5001) and water were provided ad libitum (except where noted) during the experiments. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

2.2. Materials

Recombinant mouse leptin was obtained from Dr. A. F. Parlow, National Hormone and Peptide Program, Torrance, CA. Goat polyclonal anti-apoE antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA) [11;12]. The mouse monoclonal antibodies, including anti-glial fibrillary acidic protein (GFAP), anti-neuronal nuclei (NeuN), anti-rat CD11B, and anti-galactocerebroside were purchased from Chemicon International, Inc. The second antibody for these monoclonal antibodies above was donkey anti-mouse IgG conjugated to fluorescein, Alexa 488, from Molecular Probes, Inc. (Carlsbad, CA).

2.3. Intracerebroventricular cannulation

At least one week after arrival in the laboratory, rats were anesthetized with ketamine (80 mg/kg)/xylazine (1.6 mg/kg) and implanted with 22-gauge stainless steel cannulas (Plastics One)

aimed at the 3rd-cerebral ventricle. Coordinates were 2.2 mm posterior to bregma and 7.4 mm ventral to dura as described previously [13;14]. Placement of cannulas was confirmed by administration of 10 ng of angiotensin II in saline while the animals were water replete. Animals that did not drink at least 5 ml of water within 30 min were considered to have failed cannula placement and were not used in the experiments. All animals were handled for 5 min daily starting at least 5 days before the experiment to equalize their arousal levels.

2.4. Double-labeling Immunohistochemistry

To determine the cell type(s) expressing apoE in the ARC, we dual-labeled tissue, combining apoE immunoreactivity with one of the monoclonal antibodies against different cell-type markers, including anti-neuronal nuclei (NeuN, for neurons), anti-glial fibrillary acidic protein (GFAP, for astrocytes), anti-rat CD11b (for microglia/macrophage cells) and anti-galactocerebroside (for oligodendrocytes). In the sentences below, we use the co-localization of apoE with GFAP as an example of the common procedure. Brain sections (30 μ m) were cut through the hypothalamic ARC from brains of perfusion-fixed rats. After washing, the sections were blocked with 5% donkey normal serum in PBS containing 0.3% Triton X-100 for 2 h. Then, sections were incubated with a goat anti-rat apoE antiserum (1:400 dilution) and mouse monoclonal antibody against GFAP (1:1,500 dilution) overnight at 4° C. After washing, the sections were incubated for 1 h with 2nd antibodies, including 1:400 diluted donkey anti-goat (for apoE) and donkey anti-mouse IgG (for GFAP) conjugated to fluorescein, Alexa 594 or Alexa 488 (Molecular Probes, Inc.), respectively. Confocal imaging was performed on a Zeiss 510 microscope system. Omission of the primary antibodies as well as substituting the primary antibody with apoE pre-absorbed serum or mouse IgG were used to determine the specificity of the antibodies. Quantitative assessments of the percentage of double-labeled cells were made as described previously [15]

2.5. Leptin treatment in ob/ob and db/db mice

ob/ob and *db/db* mice were treated for 5 days with daily injections of either vehicle or recombinant mouse leptin (3 μ g/g body weight, i.p.) at 1700 h (lights off at 1800 h). Food intake, water consumption, and body weight were measured daily. Pair-feeding was performed in a separate group of vehicle-treated mice by assigning each pair-fed *ob/ob* mouse to a matched partner in the leptin-treated group, and the daily food allotment was provided at 1400 h. On the fifth day of the treatments, all mice were fasted 2 h before leptin or vehicle administration, and 2 h later following the injections, the mice were sacrificed. The hypothalamus of each mouse was dissected, and hypothalamic apoE mRNA levels were measured by Quantitative real-time PCR (qPCR).

2.6. Fasting and leptin treatment in rats

Three groups of male rats (250 – 275 g) with icv cannulas were used. The rats in Group 1 were ad lib fed and treated with vehicle; the rats in Groups 2 and 3 were fasted for 36 h. After 32 h of fasting, rats in Group 2 received leptin (3.5 μ g/rat, icv) and rats in Group 3 received vehicle at 1800 h, and the rats were sacrificed 4 h later (at 2200 h). Hypothalamic apoE mRNA levels were measured by qPCR and compared among groups.

2.7. Leptin administration in apoE KO and WT mice

Adult male apoE KO and wild-type mice (15-16 mice/genotype) were fed standard laboratory chow. Half of each genotype received recombinant murine leptin (3 μ g/g, ip) each day for 5 days, and the other half received vehicle. Daily food consumption and body weight were recorded.

2.8. qPCR for apoE mRNA measurement

Total hypothalamic RNA was isolated with Tri Reagent (Molecular Research Center, Inc.) and 100 ng was reverse-transcribed to first-strand complementary DNA (Amersham Pharmacia Biotech). qPCR was performed in a 25 μ l final reaction volume with an iCycler iQ Detection System using iQTM SYBR Green Supermix (Bio-Rad, Laboratories Inc). The condition of qPCR for apoE, as well as the sequences of the primers for rat, mouse apoE and cyclophilin, were described previously [8]. No other products were amplified because melting curves revealed only one peak in each sample. Threshold cycle (Ct) readings for each of the unknown samples were then used, and the results were transferred and analyzed in Excel using the delta delta CT method (Perkin-Elmer Applied Biosystems) [16]. Cyclophilin mRNA levels from each sample were used as internal controls to normalize the mRNA levels.

2.9. Statistics

Data were analyzed using parametric statistics (Sigma Stat version 3.5). Two-way repeated measures ANOVA and two-way ANOVA followed by Student-Newman-Keuls's test were utilized for analysis of food intake and body weight. For comparing apoE mRNA levels, a one-way ANOVA followed by Tukey's test was used. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. ApoE is mainly present in astrocytes in the hypothalamus

In the present experiment, the co-localization of apoE with specific cell-type markers was used to determine apo E-producing cell type. We observed that 87% of the apoE-positive cells are astrocytes, 6% are neurons, 3% are microglial cells, 2% are oligodendrocytes, and 2% are other brain cells. Examples of co-localization of apoE with GFAP and NeuN are depicted in Fig. 1A and 1B, respectively.

3.2. Reduced hypothalamic apoE mRNA level is restored by leptin in ob/ob, but not in pair-fed ob/ob or db/db mice

The *ob/ob* mice receiving leptin reduced their food intake and body weight progressively over days (Fig. 2). At the end of the experiment, leptin-treated *ob/ob* mice had significantly decreased food intake (3.2 ± 0.2 vs. 6.7 ± 0.3 g/day) and body weight (6.4% reduction, $P < 0.05$), as well as significantly increased hypothalamic apoE mRNA levels, compared with vehicle-treated *ob/ob* mice (91.4 ± 4.6 vs. 68.1 ± 3.8 percentage of apoE mRNA levels in WT mice, $F_{(3, 28)} = 5.89$, $P < 0.05$). The pair-fed *ob/ob* mice had a comparable degree of weight loss (5.9% reduction), but this intervention did not significantly affect hypothalamic apoE mRNA levels (71.0 ± 4.9 percentage of WT control levels; Fig. 3A), implying that the up-regulated apoE mRNA levels is not related to the reduced food intake and body weight.

Unlike what occurred in *ob/ob* mice, no significant changes in food intake or body weight (data not shown), nor in hypothalamic apoE mRNA levels, were observed in *db/db* mice following leptin treatment (Fig. 3B), implying that the up-regulation of apoE mRNA depends upon an intact leptin receptor.

3.3. Fasting-induced reduction of hypothalamic apoE mRNA level is reversed by central leptin administration

The 36 h of fasting resulted in loss of 12% of body weight and significantly reduced hypothalamic apoE mRNA, compared with ad libitum-fed controls (Fig. 4). However, the reduced apoE mRNA level in fasted rats was restored to near-normal levels following icv leptin administration ($F_{(2,18)} = 5.628$, $P < 0.05$, Fig. 4).

3.4. ApoE KO mice have diminished sensitivity to leptin

Daily treatment with leptin resulted in significant reductions of food intake ($F_{(3, 26)} = 4.544$, $P < 0.05$, Fig. 5A) and body weight gain ($F_{(3, 26)} = 4.328$, $P < 0.05$, Fig. 5B) in WT mice compared with vehicle treatment. Leptin also significantly reduced body weight gain in apoE KO mice, but the reductions in both food intake and body weight gain were greatly attenuated and apparent until the 5th day of leptin treatment (Fig. 5A and 5B).

4. Discussion

The brain is a major site of apoE expression in humans and rodents with only the liver making more of this apolipoprotein [3;4]. In previous studies, we reported that apoE is highly expressed in the ARC and PVN of the hypothalamus, key areas in the regulation of energy homeostasis [8]. In the present studies, we further demonstrated that apoE is mainly present in astrocytes within the ARC as revealed by double-staining immunohistochemistry. This observation is consistent with previous reports of glial cells expressing apoE mRNA [4].

There are two primary cell types in the brain: neurons, which are generally considered to carry out most of the integrative and communicatory work of the brain, and glia, which are generally considered to have a number of supporting roles for neurons. Astrocytes are one type of glial cell and it is important to note that astrocytes comprise ~90% of overall brain mass, with numerous studies now highlighting important unique roles for astrocytes in regulating brain function independent of their supportive effects on neurons [17]. Astrocytes have a multitude of functions including buffering the extracellular space against excess K^+ ions [18], sequestering various neurotransmitters [19], forming the blood—brain barrier (BBB) [20], and supplying neurons with various nutrients [21]. Recent evidence also implicates astrocytes as important intrinsic immune regulators of the central nervous system (CNS) [22]. In addition, astrocytes have been reported to release apoE, and this has been demonstrated to regulate neurotransmission [5], growth factor release [6], immune responses [23;24] and energy homeostasis.

In our previous studies, we demonstrated that apoE is a satiation factor and plays an important role in the regulation of food intake and body weight [8]. In those studies, apoE dose-dependently suppressed food intake in both ad lib-fed and fasted rats when administered centrally. Blocking the action of brain apoE with its specific antibody increased food intake, implying that apoE plays a role in the inhibition of feeding. ApoE did not induce a conditioned taste aversion at doses that elicited significant reductions of meal size, indicating that malaise was not a factor in the suppressive activity of apoE on food intake [8].

Obesity is a complex metabolic disorder. Accumulated evidence suggests that many obese animals have blunted satiation, raising the possibility that defective satiation signaling in the brain may contribute to the etiology of obesity [25]. Our previous studies suggested that apoE is a physiological regulator of food intake [8], implying that an impairment in the production of apoE could lead to chronic increases in meal size and the development of obesity. Consistent with this, we observed that there are significantly lower levels of hypothalamic apoE mRNA in both genetically obese mice (*ob/ob*) and HF-induced obese rats, compared with their lean controls [8]. These observations suggested that the reduced apoE levels contribute to the hyperphagia of these obese animals.

The hormone leptin is primarily synthesized and secreted from adipose tissue and acts in the hypothalamus to decrease food intake and increase energy expenditure [26]. These actions of leptin are mediated through the long form of its receptor [27;28], which is highly expressed in the rat hypothalamus, especially in the ARC [29]. The data from *ob/ob* (leptin-deficient) mice and HF-induced obese (leptin-resistant) rats [8] suggested to us that the deficiency of leptin

signaling may be related in part to altered apoE gene expression in the hypothalamus. To determine whether leptin influences apoE gene expression, leptin was administered ip to *ob/ob* mice for 5 days, resulting in significantly decreased food intake and body weight (Fig. 2) as well as increased apoE mRNA expression in the hypothalamus (Fig. 3).

To sort out whether leptin up-regulates apoE gene expression directly or whether the altered apoE mRNA levels are secondary to changes of body weight, a pair-fed group of *ob/ob* mice was included. These pair-fed mice had a comparable decrease in body weight as mice receiving leptin, but hypothalamic apoE mRNA levels were not significantly changed. This observation implies that leptin-induced weight loss is not responsible for its ability to increase apoE gene expression in the hypothalamus of *ob/ob* mice. In addition, unlike what occurred in *ob/ob* mice, there was no significant change in food intake, body weight, or hypothalamic apoE mRNA levels in *db/db* mice after leptin treatment (Fig. 3). These data collectively indicate that a mutated leptin-receptor, and/or the chronically impaired downstream signaling from the leptin receptor during development, attributed to the attenuated effect of leptin on apoE gene expression in *db/db* mice.

In our previous studies, we also demonstrated that hypothalamic apoE is significantly reduced after 36 h of fasting [8]. Since fasting also decreases leptin levels in the circulation (10, 11), we asked whether the reduced apoE gene expression induced by fasting could be restored by increasing leptin. Leptin was therefore administered icv at a dose of 3.5 μ g/rat to rats fasted for 32 h. The reduction of brain apoE gene expression induced by fasting was almost completely reversed 4 h after leptin administration.

Unlike the down-regulatory effect of fasting on brain apoE gene expression, it has been reported that fasting increases apo E in white adipose tissue [30]. Huang et al. reported that fasting in C57BL/6J mice for 24 h significantly increased apoE protein and messenger RNA levels in adipose tissue, and this effect did not require leptin because adipose tissue apoE was also significantly increased in *ob/ob* mice after a 48-h fast or after 7 days of caloric restriction [30]. While it is possible that the opposite regulation by fasting in different tissues is species-specific (rats in our experiment and mice in [30], this seems unlikely. A more likely possibility is that apoE has different functions in different tissues. In the brain, apoE reduces food intake, such that it seems teleological that this action should be blunted when animals are fasted and would benefit from consuming larger meals; and this is what occurs. Consistent with this, the down-regulation of apoE gene expression in fasted animals was reversed by leptin administration, consistent with a signal that energy stores have been restored. The action of apoE in adipose tissue is complex and involves changes of lipid flux and lipoprotein trafficking [30], such that the up-regulation of apoE in adipose tissue by fasting presumably supports these activities as adipose stores become depleted.

In the present studies, we focused on apoE gene expression and its modulation by leptin due to the limited size of the tissues isolated from rodent hypothalamus. While our previous reports indicate that apoE protein synthesis changes in parallel with gene expression in the hypothalamus [31], further measurement of apoE protein levels under different treatments will be needed in the future.

The generation of the apoE KO mouse was reported in 1994 [32]. These mice have rampant atherosclerosis and increased lipid peroxidation of plasma [32]. Schreyer *et al.* [10] found that apoE KO mice had consistently higher TG levels, greater food intake, and increased body weight relative to WT mice when fed a diet containing 35.5% fat and 36.6% carbohydrate for 8 weeks. Raber *et al.* [33] reported that apoE KO mice had age-dependent increases in food intake and body weight compared to WT controls. These data suggest that modification in

lipoprotein profiles associated with loss of apoE function have consequences in terms of susceptibility to diet-induced or age-related obesity.

To determine whether the loss of apoE gene compromises leptin's anorectic action, the apoE KO mice were treated with leptin for 5 days. Consistent with our hypothesis, apoE KO mice consumed significantly more food and lost significantly less weight than the WT controls over the course of leptin treatment (Fig. 5), indicating that leptin was significantly less efficacious in apoE KO mice. These results imply that apoE, at least partially, mediates the inhibitory effects of leptin on feeding. Further experiments will be needed to investigate the mechanism(s) as to how the apoE KO mice have a reduced response to the leptin. One important approach will be to determine changes in leptin receptor gene expression, as well as downstream signaling pathways [34] in these apoE KO mice.

While our results do not definitively establish hypothalamic apoE cells as downstream mediators of leptin action in the control of food intake and energy balance, they nonetheless strengthen our hypothesis. As indicated in the model depicted in Fig. 6, leptin secreted from adipose tissue is hypothesized to stimulate apoE gene expression and secretion of apoE from hypothalamic astrocytes. This possibility is consistent with a recent report that astrocytes in the ARC express leptin receptors and take up leptin [35]. Since apoE exerts its physiological function through its own receptors and most of them are found on neurons [36], the secreted apoE is consequently hypothesized to bind to its receptors located on pro-opiomelanocortin (POMC) neurons. The activation of apoE receptors in turn would presumably stimulate POMC gene expression [8], from which α -melanocyte stimulating hormone(α -MSH) is cleaved and released. The liberated α -MSH binds to melanocortin-3/4 (MC3/4) receptors in other brain areas, resulting in the reduction of food intake and body weight [8]. This is our working model and further researches are needed to determine its validity as well as the molecular mechanisms as to how leptin stimulates apoE gene expression and how apoE increases POMC gene expression.

ACKNOWLEDGEMENTS

This work was supported in part by research grants DK70992 and DK63907 to ML, DK54012, DK73917 to DQW. DK17844 to SCW.

References

1. Seeley RJ, Woods SC. Monitoring of stored and available fuel by the CNS: implications for obesity. *Nat. Rev. Neurosci* 2003;4:901–909. [PubMed: 14595401]
2. Konturek SJ, Konturek JW, Pawlik T, Brzozowski T. Brain-gut axis and its role in the control of food intake. *J. Physiol Pharmacol* 2004;55:137–154. [PubMed: 15082874]
3. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622–630. [PubMed: 3283935]
4. Boyles JK, Pitas RE, Wilson E, Mahley RW, Taylor JM. Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest* 1985;76:1501–1513. [PubMed: 3932467]
5. Lee Y, Aono M, Laskowitz D, Warner DS, Pearlstein RD. Apolipoprotein E protects against oxidative stress in mixed neuronal-glial cell cultures by reducing glutamate toxicity. *Neurochem. Int* 2004;44:107–118. [PubMed: 12971913]
6. Gutman CR, Strittmatter WJ, Weisgraber KH, Matthew WD. Apolipoprotein E binds to and potentiates the biological activity of ciliary neurotrophic factor. *J. Neurosci* 1997;17:6114–6121. [PubMed: 9236223]
7. Cedazo-Minguez A, Cowburn RF. Apolipoprotein E: a major piece in the Alzheimer's disease puzzle. *J. Cell Mol. Med* 2001;5:254–266. [PubMed: 12067484]

8. Shen L, Tso P, Woods SC, Clegg DJ, Barber KL, Carey K, Liu M. Brain apolipoprotein E: an important regulator of food intake in rats. *Diabetes* 2008;57:2092–2098. [PubMed: 18559657]
9. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell* 2004;116:337–350. [PubMed: 14744442]
10. Schreyer SA, Vick C, Lystig TC, Mystkowski P, LeBoeuf RC. LDL receptor but not apolipoprotein E deficiency increases diet-induced obesity and diabetes in mice. *Am. J. Physiol Endocrinol. Metab* 2002;282:E207–E214. [PubMed: 11739102]
11. Sadowski MJ, Pankiewicz J, Scholtzova H, Mehta PD, Prelli F, Quartermain D, Wisniewski T. Blocking the apolipoprotein E/amyloid-beta interaction as a potential therapeutic approach for Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A* 2006;103:18787–18792. [PubMed: 17116874]
12. Moore ZW, Hui DY. Apolipoprotein E inhibition of vascular hyperplasia and neointima formation requires inducible nitric oxide synthase. *J. Lipid Res* 2005;46:2083–2090. [PubMed: 16061951]
13. Liu M, Maiorano N, Shen L, Pearson K, Tajima D, Zhang DM, Woods SC, Seeley RJ, Davidson WS, Tso P. Expression of biologically active rat apolipoprotein AIV in *Escherichia coli*. *Physiol Behav* 2003;78:149–155. [PubMed: 12536022]
14. Chavez M, Seeley RJ, Woods SC. A comparison between effects of intraventricular insulin and intraperitoneal lithium chloride on three measures sensitive to emetic agents. *Behav. Neurosci* 1995;109:547–550. [PubMed: 7662164]
15. Shen L, Pearson KJ, Xiong Y, Lo CM, Tso P, Woods SC, Davidson WS, Liu M. Characterization of apolipoprotein A-IV in brain areas involved in energy homeostasis. *Physiol Behav* 2008;95:161–167. [PubMed: 18577393]
16. Liu M, Shen L, Liu Y, Tajima D, Sakai R, Woods SC, Tso P. Diurnal Rhythm of Apolipoprotein A-IV in Rat Hypothalamus and Its Relation to Food Intake and Corticosterone. *Endocrinology* 2004;145:3232–3238. [PubMed: 15059955]
17. Kimelberg HK, Norenberg MD. Astrocytes. *Sci. Am* 1989;260:66–72. 74, 76. [PubMed: 2564697]
18. Ransom CB, Sontheimer H. Biophysical and pharmacological characterization of inwardly rectifying K⁺ currents in rat spinal cord astrocytes. *J Neurophysiol* 1995;73:333–346. [PubMed: 7714576]
19. van den Pol AN, Finkbeiner SM, Cornell-Bell AH. Calcium excitability and oscillations in suprachiasmatic nucleus neurons and glia in vitro. *J Neurosci* 1992;12:2648–2664. [PubMed: 1351936]
20. Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permeability. *J Anat* 2002;200:629–638. [PubMed: 12162730]
21. Brown AM, Ransom BR. Astrocyte glycogen and brain energy metabolism. *Glia* 2007;55:1263–1271. [PubMed: 17659525]
22. Teige I, Liu Y, Issazadeh-Navikas S. IFN-beta inhibits T cell activation capacity of central nervous system APCs. *J Immunol* 2006;177:3542–3553. [PubMed: 16951313]
23. Laskowitz DT, Goel S, Bennett ER, Matthew WD. Apolipoprotein E suppresses glial cell secretion of TNF alpha. *J Neuroimmunol* 1997;76:70–74. [PubMed: 9184634]
24. Lynch JR, Morgan D, Mance J, Matthew WD, Laskowitz DT. Apolipoprotein E modulates glial activation and the endogenous central nervous system inflammatory response. *J Neuroimmunol* 2001;114:107–113. [PubMed: 11240021]
25. Woods SC. Gastrointestinal satiety signals I. An overview of gastrointestinal signals that influence food intake. *Am. J. Physiol Gastrointest. Liver Physiol* 2004;286:G7–13. [PubMed: 14665437]
26. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763–770. [PubMed: 9796811]
27. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 1996;379:632–635. [PubMed: 8628397]
28. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996;84:491–495. [PubMed: 8608603]
29. Elmquist JK, Bjorbaek C, Ahima RS, Flier JS, Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. *J. Comp Neurol* 1998;395:535–547. [PubMed: 9619505]

30. Huang ZH, Luque RM, Kineman RD, Mazzone T. Nutritional regulation of adipose tissue apolipoprotein E expression. *Am. J Physiol Endocrinol. Metab* 2007;293:E203–E209. [PubMed: 17389709]
31. Shen L, Carey K, Wang DQ, Woods SC, Liu M. Food-entrained rhythmic expression of apolipoprotein E expression in the hypothalamus of rats. *Brain Res.* 2009
32. Hayek T, Oiknine J, Brook JG, Aviram M. Increased plasma and lipoprotein lipid peroxidation in apo E-deficient mice. *Biochem. Biophys. Res. Commun* 1994;201:1567–1574. [PubMed: 8024602]
33. Raber J, Akana SF, Bhatnagar S, Dallman MF, Wong D, Mucke L. Hypothalamic-pituitary-adrenal dysfunction in Apoe(-/-) mice: possible role in behavioral and metabolic alterations. *J. Neurosci* 2000;20:2064–2071. [PubMed: 10684907]
34. Hoe HS, Harris DC, Rebeck GW. Multiple pathways of apolipoprotein E signaling in primary neurons. *J Neurochem* 2005;93:145–155. [PubMed: 15773914]
35. Cheunsuang O, Morris R. Astrocytes in the arcuate nucleus and median eminence that take up a fluorescent dye from the circulation express leptin receptors and neuropeptide Y Y1 receptors. *Glia* 2005;52:228–233. [PubMed: 15968634]
36. Beffert U, Stolt PC, Herz J. Functions of lipoprotein receptors in neurons. *J. Lipid Res* 2004;45:403–409. [PubMed: 14657206]

Fig. 1. Confocal image of double-immunofluorescent staining for apoE (*red*) and GFAP or NeuN (*green*) in the ARC of the hypothalamus. The yellow color of apoE and GFAP (A), and the surrounding red color (apoE) around green color (NeuN, B), as indicated by *arrows*, depicts the co-localization of apoE with GFAP or NeuN immunofluorescence, respectively. Solid boxes inset in each image are high-magnified images of the area dotted boxes. 3V: Third ventricle.

Fig. 2.

Progressive changes in food intake and body weight of *ob/ob* mice receiving daily ip injections of leptin (3 μ g/g body weight/day) or saline for 5 days. Saline-treated mice were either fed *ad lib* or pair-fed to the food intake of leptin-treated mice. Data are expressed as Mean $\pm$ SEM, $n = 8$ per group. * $P < 0.05$, ** $P < 0.01$, compared with saline-*ad lib* fed mice.

Fig. 3.

Comparison of hypothalamic apoE mRNA levels determined by qPCR in *ob/ob* vs. C57BL/6J and *db/db* vs. C57BLKS/J mice. The *ob/ob* and *db/db* mice were administered daily ip injections of mouse recombinant leptin (3 μ g/g/day) or vehicle for 5 days. Values are expressed as mean $\pm$ SEM, n = 8 per group. * $P < 0.05$ and # $P < 0.05$, between the compared groups.

Fig. 4. Comparison of hypothalamic apoE mRNA levels among ad lib fed, fasted and fasted plus leptin-treated rats. Fasting significantly reduces hypothalamic apoE mRNA levels, which was reversed by leptin administration. Values are expressed as mean $\pm$ SEM, $n = 7$ per group, $^*P < 0.05$, between the compared groups.

Fig. 5.

Comparison of food intake and body weight between apoE KO and WT mice on Day 5 after leptin or vehicle treatment. Mouse recombinant leptin (3 μ g/g body weight/day, i.p.) or vehicle was administered for 5 days. Data are the mean $\pm$ SEM, $n = 8$ per group. * $P < 0.05$, ** $P < 0.01$, between the compared groups. Veh. = vehicle, lep. = leptin.

Fig. 6.

Model for the interaction of apoE with leptin and POMC in the control of food intake and body weight. We demonstrated that apoE gene expression in the hypothalamus is stimulated by leptin. The secreted apoE is hypothesized to bind its receptors located on POMC neurons and stimulate POMC gene expression, resulting in more α -MSH cleaved and released. The secreted α -MSH presumably binds to MC3/4 receptors and consequently reduces the food intake and body weight.