0013-7227/08/$15.00/0
Printed in U.S.A.

Endocrinology 149(5):2361–2368

doi: 10.1210/en.2007-0516

Hyperleptinemia without Obesity in Male Mice Lacking Androgen Receptor in Adipose Tissue

I-Chen Yu,* Hung-Yun Lin,* Ning-Chun Liu, Ruey-Shen Wang, Janet D. Sparks, Shuyuan Yeh, and Chawnshang Chang

Departments of Pathology, Urology, and Neuroscience, George Whipple Laboratory for Cancer Research, and the Cancer Center, University of Rochester Medical Center, Rochester, New York 14642

Insulin resistance occurs through an inadequate response to insulin by insulin target organs such as liver, muscle, and adipose tissue with consequent insufficient glucose uptake. In previous studies we demonstrated that whole body androgen receptor (AR) knockout ($AR^{-/y}$) mice develop obesity and exhibit insulin and leptin resistance at advanced age. By examining adipose tissue-specific AR knockout (A-$AR^{-/y}$) mice, we found A-$AR^{-/y}$ mice were hyperleptinemic but showed no leptin resistance, although body weight and adiposity index of A-$AR^{-/y}$ mice were identical with those of male wild-type control mice. Hypotriglyceridemia and hypocholesterolemia found in nonobese A-$AR^{-/y}$ mice suggested a beneficial effect of high leptin levels independent of fat deposition. Further examination showed that androgen-AR signaling in adipose tissue plays a direct regulatory role in leptin expression via enhanced estrogen receptor transactivation activity due to elevated intraadipose estrogens. The present study in A-$AR^{-/y}$ mice suggests a differential tissue-specific role of AR in energy balance control in males. (*Endocrinology* 149: 2361–2368, 2008)

THERE HAS BEEN a paradigm shift from the notion of adipose tissue only as a storage depot for energy to another in which adipose tissue plays an essential role in energy balance as evidenced by the worldwide increase in incidence of obesity and its associated metabolic disorders (1). The predominant type of adipose tissue is commonly known as white adipose tissue (WAT), comprised of mostly adipocytes, surrounded by loose connective tissue that is highly vascularized and innervated, and contains fibroblasts, macrophages, preadipocytes, and various other cell types. WAT provides an unlimited capacity for triglyceride storage crucial for survival. Fatty acids released from adipose tissue during fasting are oxidized by skeletal muscle and liver, generating ketones that serve as alternate fuel sources other than glucose for the brain and peripheral organs.

Adipose tissue responding to different metabolic signals is capable of secreting a variety of proteins known as adipokines, including harmful adipokines such as TNF-α (2), resistin (3), IL-6 (4), and plasminogen activator inhibitor-1 (5) and beneficial adipokines such as leptin (6) and adiponectin (7). These adipokines have been shown to play important roles in regulating a variety of complicated metabolic processes, such as fat metabolism, food intake, energy balance, insulin sensitivity, glucose homeostasis, and vascular tone (8, 9). Leptin, an adipocyte-derived hormone, has been known to play a pivotal role in regulating food intake, energy expenditure, and the neuroendocrine response to altered nutrition (10). The metabolic effects of leptin are not only explained by its effects on food intake alone, but leptin also stimulates fatty acid oxidation (11) and glucose uptake (12, 13) and prevents lipid accumulation in nonadipose tissues causing functional impairments (14). Replacing leptin in leptin-deficient (*ob/ob*) mice and humans results in the depletion of lipid in adipose tissue, liver, and other tissues as well as the improvement of insulin sensitivity (15, 16).

In previous studies, our group has shown that male androgen receptor (AR) knockout ($AR^{-/y}$) mice developed adult-onset visceral obesity and insulin and leptin resistance accompanied with altered lipid metabolic profiles and dyslipidemia (17). Notably, despite a high serum leptin level in proportion to increased body weight and fat mass in obese $AR^{-/y}$ mice, the elevation of serum leptin levels was detected as early as 8 wk of age, even when $AR^{-/y}$ mice showed significantly less body weight and adiposity (17). In addition, in primary cultures of human adipocytes expressing AR, treatment with androgens suppressed expression of leptin mRNA and secretion of leptin (18). These data suggested that androgen-AR signaling might play a direct regulatory role in leptin synthesis and secretion in adipocytes.

Adipose tissue controls the level, bioavailability, and bioactivity of sex steroids (androgens and estrogens) (19), which are important regulators of body fat mass and its distribution. Aromatase (cytochrome P450) within adipose stromal cells and preadipocytes converts androgens to estrogens, such as androstenedione to estrone and testosterone to estradiol. Intraadipose sex steroid metabolism is believed to underline the gender differences in fat distribution, in which young women have larger amounts of sc WAT, compared with a predominance of visceral WAT in aging men and postmenopausal women (20). Visceral adiposity in men has

First Published Online February 14, 2008

* I.-C.Y. and H.-Y.L. contributed equally to this work.

Abbreviations: AR, Androgen receptor; CNS, central nervous system; CPT-1, carnitine palmitoyl transferase 1; Cre, Cre recombinase; ER, estrogen receptor; eWAT, epididymal WAT; FBS, fetal bovine serum; FFA, free fatty acid; MEF, mouse embryonic fibroblast; PGC, peroxisome proliferator-activated receptor-γ coactivator; UCP, uncoupling protein; WAT, white adipose tissue.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

been associated with insulin resistance, type 2 diabetes, and cardiovascular disease (21).

To dissect the tissue-specific role of AR in the development of obesity and insulin resistance and clarify the beneficial high leptin level at early stage and leptin resistance at obese stage of $AR^{-/y}$ mice, we generated adipose-specific AR knockout (A-$AR^{-/y}$) mice by a conditional genetic knockout approach. We found male A-$AR^{-/y}$ mice were hyperleptinemic but showed no leptin resistance, although body weight and adiposity index of A-$AR^{-/y}$ mice were identical with those of wild-type ($AR^{+/y}$) mice. Lower serum triglycerides and cholesterol levels were found in A-$AR^{-/y}$ mice, suggesting a beneficial effect of high leptin levels. Further examination showed that androgen-AR signaling in adipose tissue plays a regulatory role in leptin expression via intraadipose estrogen conversion and increased ER transactivation activity.

Materials and Methods

Mice with adipose-specific deletion of AR

Generation of floxAR mice was as described previously (17, 22), and floxAR mice were bred into a C57BL/6 background. We obtained aP2-Cre mice in a C57BL/6 background from Jackson Laboratories (Bar Harbor, ME), in which Cre recombinase (Cre) expression is specifically in adipose tissues as driven by the aP2 promoter. By crossing female floxAR mice with male aP2-Cre mice, we generated mice with deleted floxed AR fragments specifically in adipose tissues. Genotyping of A-$AR^{-/y}$ mice was done with primers as described (22).

Animals were housed in pathogen-free facilities, maintained on a 12-h light, 12-h dark schedule and had free access to standard laboratory chow (no. 5010; PMI Lab Diet, St. Louis, MO) and water. All animal studies were approved by the Department of Laboratory Animal Medicine of the University of Rochester, in accordance with National Institutes of Health guidelines.

WAT histology

Epididymal fat pads harvested from 20-wk-old mice were fixed in 4% paraformaldehyde (grams per volume), embedded in paraffin, and stained with hematoxylin/eosin. Images were acquired using an E800 microscope (Nikon, Melville, NY) and a SPOT camera (Diagnostic Instruments, Sterling Heights, MI) and were analyzed using SigmaScan Pro software (version 5.0; SPSS, Chicago, IL).

Analytical procedures

Blood samples were withdrawn from overnight (16–18 h) fasted mice at the age of 20 wk. Blood glucose concentrations were measured using a glucometer (One Touch Ultra; Lifescan, Milpitas, CA). Triglyceride levels in serum were determined using a GPO-Trinder assay (Sigma Aldrich, St. Louis, MO). Serum free fatty acid levels were measured using a NEFA-Kit-U (Wako Pure Chemical, Richmond, VA). Leptin and insulin levels were determined by mouse leptin and insulin ELISA kit (Crystal Chem, Downers Grove, IL) according to the manufacturer's instructions. For testosterone and estradiol determinations, blood samples were withdrawn from 12-wk-old mice and serum levels of testosterone and estradiol were measured by ELISA kits (Assay Designs, Ann Arbor, MI) according to the manufacturer's instructions.

Tissue triglyceride content

Epididymal fat pads harvested from 20-wk-old A-$AR^{-/y}$ and $AR^{+/y}$ mice were homogenized on ice in the extraction buffer [20 mM Tris-HCl (pH 7.3) containing 1 mM β-mercaptoethanol and 1 mM EDTA] and centrifuged. The glycerol content of the supernatant fluid supernatants was determined using the GPO-Trinder assay (Sigma-Aldrich) according to the manufacturer's instructions.

Establishment of $AR^{+/y}$ and $AR^{-/y}$ mouse embryonic fibroblast (MEF) cell lines

MEF cell lines were self-immortalized following the 3T9 protocol. Briefly, primary $AR^{+/y}$ and $AR^{-/y}$ MEFs were isolated from embryonic d 12.5 littermate embryos and cultured in DMEM/10% fetal bovine serum (FBS). Early-passage (<5) MEFs were then plated at a density of 2.5×10^6 cells per 25-ml flask. Every 3 d, cells were gently trypsinized and replated at the same density. Cells were immortalized after 5 months of continuous culture.

Cell culture of 3T3-L1 preadipocytes and stably transfected 3T3-L1-siAR cells

Mouse 3T3-L1 preadipocytes (CL173; American Type Culture Collection, Manassas, VA), $AR^{+/y}$, and $AR^{-/y}$ MEF cell lines were maintained in DMEM/10% FBS. For differentiation, the medium was changed to DMEM supplemented with 10% (vol/vol) FBS, 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 1 μM dexamethasone at 2 d after reaching confluence. The media were renewed every other day. Nine days thereafter, leptin levels were determined in duplicate 5-μl samples, using a mouse leptin ELISA kit (Crystal Chem). Stable 3T3-L1-siAR and 3T3-L1-scr (scramble) preadipocytes were generated by infecting with retrovirus pSuperior-mAR short hairpin RNA or scramble control constructs to manipulate AR expression. Infected cells were allowed to grow 48 h before antibiotics selection (G418; 600 μg/ml). Stably transfected 3T3-L1-siAR cells were then monitored for expression of AR by Western blotting to confirm AR knockdown.

RNA extraction and real-time PCR analysis

Total RNA was extracted from epididymal fat pads using TRIzol reagent (Invitrogen, Carlsbad, CA). cDNA synthesis was carried out by RT-PCR with Superscript RNase H-reverse transcriptase and cDNA cycle kit (Invitrogen) using 4 μg total RNA according to the manufacturer's instructions. Expression levels of RNA were determined by quantitative real-time PCR performed on an iCycler real-time PCR amplifier (Bio-Rad Laboratories, Hercules, CA) using iQ SYBR Green supermix reagent (Invitrogen). The relative copy number of *Gapdh* RNA was quantified and used for normalization. The cycle threshold 2-$\Delta\Delta C_T$ method was used to calculate relative differences between wild-type and knockout mice. Primer sequences used for lipid metabolism genes were as described (17). The hypothalamus was dissected from 16-wk-old $AR^{+/y}$ and A-$AR^{-/y}$ mice and was subjected to total RNA extraction as described above. Expression of hypothalamic neuropeptides were quantified using real-time PCR using the primer sequences as follows: *Npy* (5′-CTCCGCTCTGCGACACTACA-3′, 5′-AATCAGTGTCTCAGGGCTGGA-3′); and *Agrp* (5′-GCGGAGGTGCTAGATCCACA-3′, 5′-AGGACTCGTGCAGCCTTACAC-3′); *Pomc* (5′-ACCTCACCACGGAGAGCAAC-3′; 5′-GCGAGAGGTCGAGTTTGCA-3′).

Leptin sensitivity test

$AR^{+/y}$ and A-$AR^{-/y}$ mice (16 wk old) were ip treated with saline or leptin (R&D Systems, Minneapolis, MN) once daily (1 μg/g body weight) for 3 d. Food intake and body weight were monitored daily. We monitored food intake and body weight for 7 d before leptin administration for base line. Changes in food intake and body weight were calculated according to baseline to estimate the effects of exogenous leptin administration.

Transfection and reporter gene assay

3T3L1-siAR and 3T3-L1-scr preadipocyte cells were seeded into 24-well dishes for 16–18 h (overnight). Transient transfection into cells was performed using the Superfect reagent (QIAGEN, Valencia, CA) according to the manufacturer's instruction. The pRL-TK vector, which expresses Renilla luciferase (Promega, Madison, WI), was cotransfected as an internal control. Both luciferase activities were measured using dual-luciferase reporter assay system (Promega). Each experiment was repeated at least three times.

Statistical analysis

The data were evaluated by Student's *t* test or ANOVA followed by *post hoc* comparisons using the Student-Newman-Keuls test.

Results

Adipocytes derived from AR$^{-/y}$ mouse embryonic fibroblasts contain higher leptin secretion capacity

In the present study, we first examined leptin secretion of adipocytes derived from general AR$^{-/y}$ and AR$^{+/y}$ MEFs, and found that differentiated adipocytes induced from AR$^{-/y}$ MEFs secreted more leptin, compared with that from AR$^{+/y}$ MEFs (Fig. 1A). Increased leptin secretion of AR$^{-/y}$ derived adipocytes suggested that androgen-AR signaling might regulate leptin production and secretion by adipocytes.

Generation of A-AR$^{-/y}$ mice

Using a Cre-loxP conditional knockout strategy, we crossed female AR$^{flox/+}$ mice with male aP2-Cre transgenic mice to generate male A-AR$^{-/y}$ and AR$^{+/y}$ (aP2-Cre^{+}) littermates. Genomic DNA extracted from tail was analyzed to verify the presence of floxed *ar* and *cre* transgene in A-AR$^{-/y}$ mice (Fig. 1B). In addition, various tissues harvested from A-AR$^{-/y}$ and AR$^{+/y}$ mice were subjected to RT-PCR analysis using primers for AR exon 1 and exon 3 designed according to our AR deletion strategy (22). Only adipose tissue from A-AR$^{-/y}$ mice showed deletion of AR exon 2 with a 180-bp RT-PCR product (Fig. 1C). Quantitative real-time PCR analysis by AR exon 2 primers was performed to confirm reduced AR expression in epididymal WATs (eWATs) of A-AR$^{-/y}$ mice, compared with AR$^{+/y}$ controls (Fig. 1D).

Normal body fat composition with increased leptin production in A-AR$^{-/y}$ mice

The body weight of A-AR$^{-/y}$ mice was indistinguishable from that of AR$^{+/y}$ counterparts at the age of 20 wk (Fig. 1E), although there was a reduction of heart weight in A-AR$^{-/y}$ mice (Fig. 1F). Reflecting their equivalent body weight, A-AR$^{-/y}$ mice had normal adiposity as shown by the adiposity index (Fig. 2A) and unaltered morphology in eWATs (Fig. 2B). Furthermore, there were no significant differences in sizes of adipocytes (Fig. 2C) and triglyceride content of eWATs (Fig. 2D) resulting from loss of AR specifically in adipose tissues.

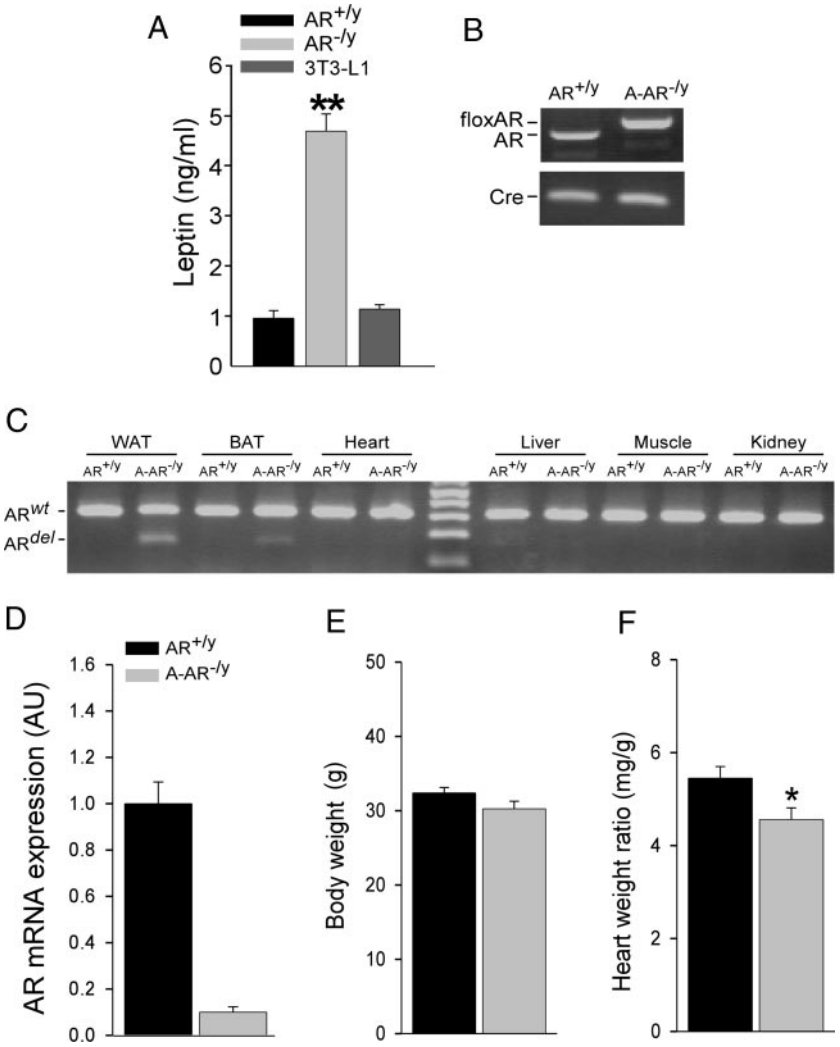

FIG. 1. Generation of A-AR-$^{-/y}$ mice with conditional knockout of AR in adipocytes. A, Leptin concentrations in culture medium in differentiated MEF cells and 3T3-L1 cells. Data are mean ± SEM; n = 3. **, $P <$ 0.01 *vs.* AR$^{+/y}$. B, Identification and confirmation of A-AR$^{-/y}$ mice. Genomic DNA was isolated from tail snips and used as template for PCR with primers select and 2–3. The detailed method and primer sequences have been described previously (22). The expression of floxed AR and aP2-Cre in the tail genomic DNA of A-AR$^{-/y}$ male mouse was confirmed by PCR. C, RT-PCR analysis of different tissues from AR$^{+/y}$ and A-AR$^{-/y}$ mice. Only adipose tissues (WAT; BAT: brown adipose tissue) of A-AR$^{-/y}$ mice show deleted AR mRNA when primers exon 1 and exon 3 were used. Data are representative images from the two experimental groups (n = 4–5). D, Quantitative real-time PCR confirmed loss of AR mRNA expression in WATs of A-AR$^{-/y}$ mice. E, Body weights in 20-wk-old AR$^{+/y}$ mice and A-AR$^{-/y}$ mice. F, Heart weight in 20-wk-old mice. Data are mean ± SEM; n = 5–6. *, $P < 0.05$; A-AR$^{-/y}$ *vs.* AR$^{+/y}$.

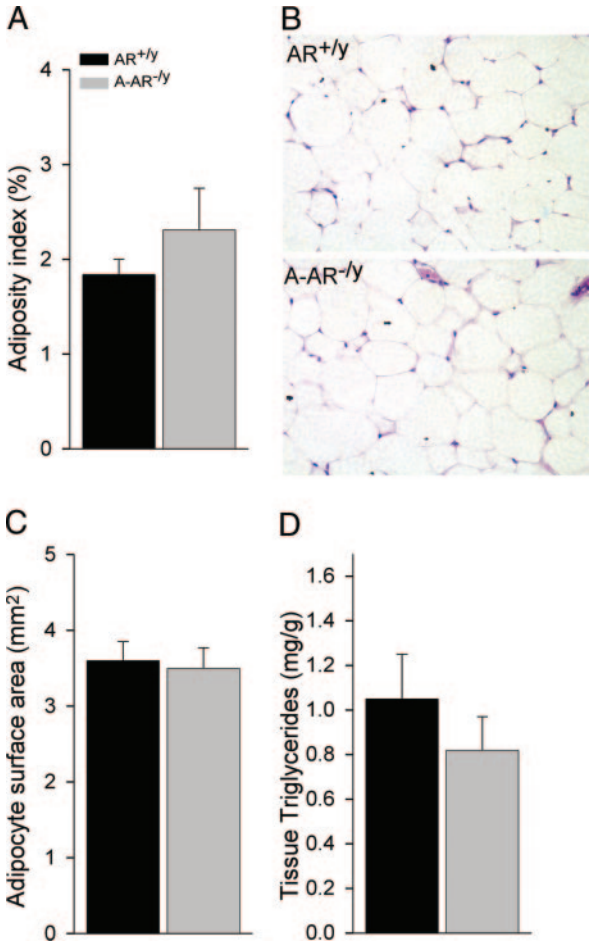

FIG. 2. No alteration of adiposity and morphology of eWATs in A-AR$^{-/y}$ mice. A, Adiposity index in 20-wk-old mice. The adiposity index was the ratio of epididymal and perirenal adipose tissue to body weight for mice. B, There is no genotype difference in the morphology of WAT from epididymal fat pad. Data are representative images from the two experimental groups (n = 3). C, Distribution of cell size of epididymal WAT from 20-wk-old mice. E, Triglyceride contents in WATs from 20-wk-old mice. Data are mean ± SEM; n = 5–6.

A-AR$^{-/y}$ mice exhibited increased serum levels of leptin, compared with AR$^{+/y}$ littermate controls, despite identical adiposity (Fig. 3A). Hyperleptinemia, a hallmark of the leptin-resistant state, is usually associated with obesity as a consequence of increased fat mass. However, even with hyperleptinemia, A-AR$^{-/y}$ mice exhibited an increase in leptin sensitivity responding to exogenous leptin treatment as significantly reduced food intake, compared with controls (Fig. 3B), although there are no significant changes of body weight (data not shown). Furthermore, serum leptin levels in A-AR$^{-/y}$ mice showed an elevated linear relationship with fat percentage, compared with AR$^{+/y}$ controls (Fig. 3C). We therefore examined leptin expression in eWATs and found a significant increase of leptin mRNA in eWATs of A-AR$^{-/y}$ mice, indicating that their increased serum leptin levels reflected enhanced leptin expression in adipocytes (Fig. 3D). On the other hand, loss of AR in eWATs had no direct effect on expression of adiponectin and resistin, the other two metabolic adipokines produced by adipocytes (Fig. 3, E and F).

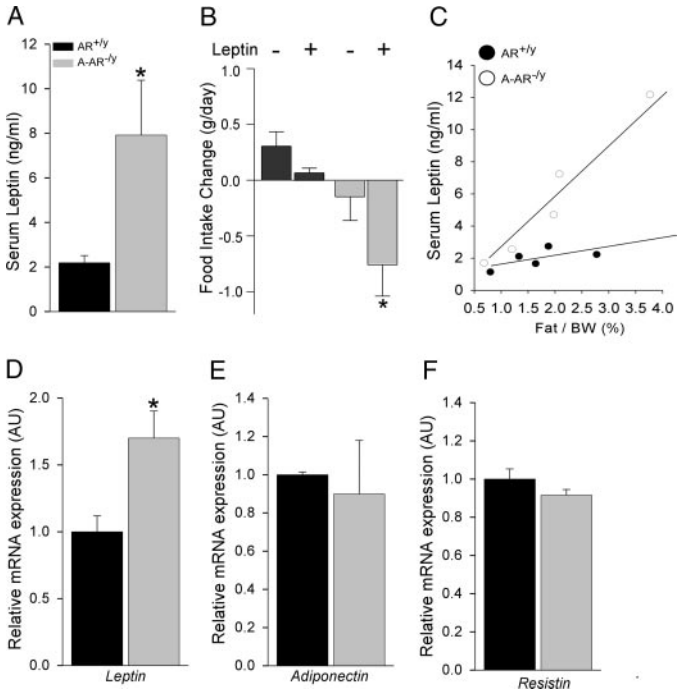

FIG. 3. Hyperleptinemia resulted from increased leptin synthesis in A-AR$^{-/y}$ mice. A, Increased serum leptin levels in A-AR$^{-/y}$ mice. B, Leptin sensitivity is increased in A-AR$^{-/y}$ mice. AR$^{+/y}$ and A-AR$^{-/y}$ mice were injected with leptin (1 μg/g body weight per day) or saline for 3 d. Daily food intake was measured for 7 d before leptin administration (for baseline) and daily after leptin injection. Leptin-treated A-AR$^{-/y}$ mice showed significant reduced food intake, compared with leptin-treated AR$^{+/y}$ mice. Data are mean ± SEM; n = 5. *, $P < 0.05$; A-AR$^{-/y}$ *vs.* AR$^{+/y}$. C, Relationship between fed leptin concentrations and body fat percentage of 20-wk-old mice (n = 5). BW, Body weight. D, Increased leptin mRNA expression in eWATs of A-AR$^{-/y}$ mice. Data are mean ± SEM; n = 5–6. *, $P < 0.05$, A-AR$^{-/y}$ *vs.* AR$^{+/y}$. Expression of adiponectin (E) and resistin (F) mRNA were not changed in A-AR$^{-/y}$ mice.

Decreased serum levels of total cholesterol and triglycerides in A-AR$^{-/y}$ mice

In A-AR$^{-/y}$ mice, fasting blood glucose levels are relatively normal, compared with AR$^{+/y}$ controls, suggesting no critical alteration in glucose homeostatic control due to loss of AR in adipose tissue (Fig. 4A). In addition, serum levels of insulin and free fatty acids were similar in A-AR$^{-/y}$ mice, compared with controls (Fig. 4, B and C). However, A-AR$^{-/y}$ mice had significantly lower serum triglyceride and cholesterol levels (Fig. 4, D and E).

Up-regulated transcription factors and enzymes involved in lipid oxidation in eWATs of A-AR$^{-/y}$ mice

Consistent with unchanged morphology and size of adipocytes, mRNA levels of transcription factors involved in lipid synthesis, including CCAAT/enhancer binding protein-α, peroxisome proliferator activated receptor-γ, sterol regulatory element binding protein-1c, and carbohydrate regulatory element binding protein, were not significantly different in eWATs of A-AR$^{-/y}$ mice, compared with AR$^{+/y}$ mice (Fig. 5A). However, mRNA levels of transcription factor, peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, and carnitine palmitoyl transferase 1 (CPT-1), in-

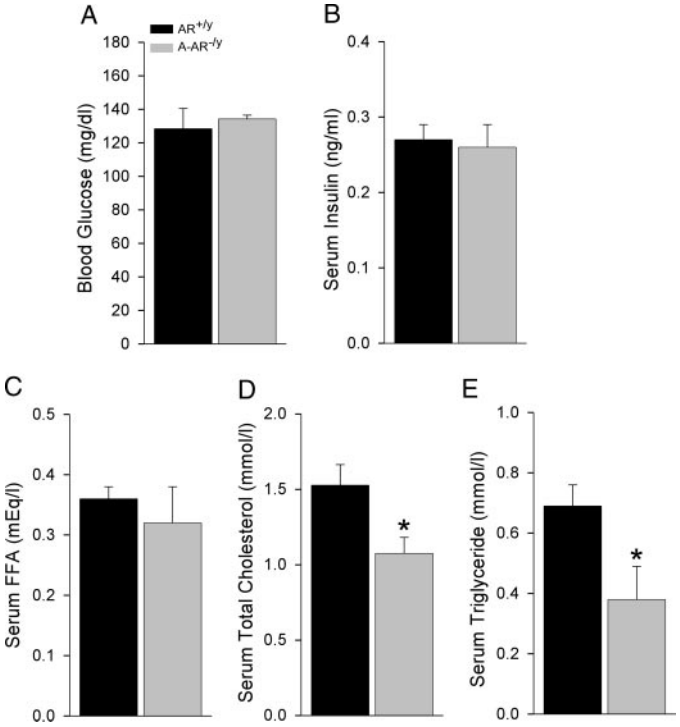

FIG. 4. Serum metabolic parameters in 20-wk-old $AR^{+/y}$ and A-$AR^{-/y}$ mice. A, Fasting blood glucose. B, Serum insulin levels in $AR^{+/y}$ and A-$AR^{-/y}$ mice. C, Serum free fatty acid (FFA) levels in $AR^{+/y}$ and A-$AR^{-/y}$ mice. D, A-$AR^{-/y}$ mice had significantly reduced serum total cholesterol levels. E, A-$AR^{-/y}$ mice had significant reduced serum triglyceride levels. Data are mean ± SEM; n = 5. *, $P < 0.05$, A-$AR^{-/y}$ *vs.* $AR^{+/y}$.

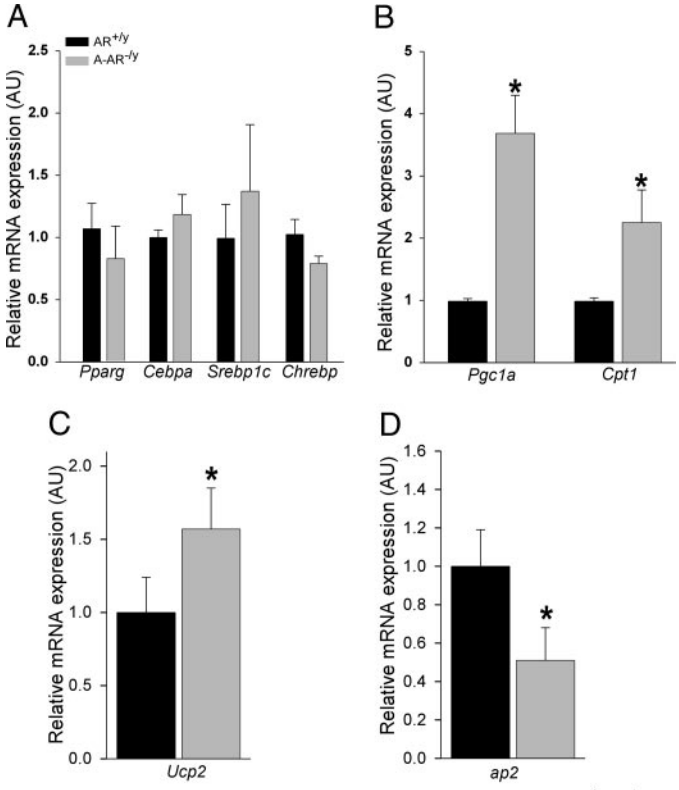

FIG. 5. Increased expression of molecules involved in fatty acid oxidation within eWATs of A-$AR^{-/y}$ mice. A, No significant differences in the expression of transcription factors involved in lipid synthesis between $AR^{+/y}$ and A-$AR^{-/y}$ mice. AU, Arbitrary unit; Pparg, peroxisome proliferator-activated receptor-γ; Cebpa, CCAAT/enhancer binding protein-α; Srebp1c, sterol regulatory element binding protein-1a; Chrebp, carbohydrate regulatory element binding protein. B, Increased *Pgc1a* and *Cpt1* mRNA expression in eWATs of A-$AR^{-/y}$ mice. C, *Ucp2* mRNA expressions in response to high leptin was increased in A-$AR^{-/y}$ mice. D, Decreased *ap2* mRNA expressions in eWATs of A-$AR^{-/y}$ mice. Data are mean ± SEM; n = 5–6. *, $P < 0.05$, A-$AR^{-/y}$ *vs.* $AR^{+/y}$.

volved in lipid oxidation, were significantly increased in eWATs of A-$AR^{-/y}$ mice, suggesting an increase of lipid oxidation within adipocytes (Fig. 5B). Previous studies have shown that leptin administration can up-regulate uncoupling protein (UCP)-2 mRNA expression in eWATs (23). We also found that mRNA levels of UCP2 were significantly increased in eWATs of A-$AR^{-/y}$ mice, compared with $AR^{+/y}$ mice (Fig. 5C). Interestingly, we found that aP2 expression was reduced in eWATs of A-$AR^{-/y}$ mice, compared with $AR^{+/y}$ mice (Fig. 5D).

Increased intraadipose estrogens and estrogen receptor (ER) transactivation due to loss of AR

In adipocytes, leptin (*ob*) gene expression has been reported to be regulated by estradiol and its receptor (24). To investigate the underlying cause of up-regulated *ob* gene expression in eWATs of A-$AR^{-/y}$ mice, we measured circulating sex hormones, testosterone and estradiol, but did not find significant differences between A-$AR^{-/y}$ and $AR^{+/y}$ mice (Fig. 6, A and B). Adipose tissue has been shown to act as a sex hormone-metabolizing organ and active sex steroid levels within adipose tissue may contribute to the regulation of adipocyte metabolism. In adipocytes, estradiol induces various effects including the up-regulation of *ob* gene expression and leptin secretion (25). We hypothesized that the up-regulation of *ob* gene expression in eWATs of A-$AR^{-/y}$ mice is ER dependent. Therefore, we measured estradiol levels within adipose tissue and found elevations in eWATs of A-$AR^{-/y}$ mice, compared with $AR^{+/y}$ controls (Fig. 6C), suggesting enhanced ER activity leads to up-regulated *ob* gene expression in eWATs of A-$AR^{-/y}$ mice. We further confirmed increased ER transactivation activity in AR knocked down 3T3-L1 preadipocytes (Fig. 6D).

Discussion

In the present study, we generated A-$AR^{-/y}$ mice by specifically knocking out AR in adipose tissue to investigate tissue-specific roles of AR in differential body composition and visceral obesity development in males. Our study demonstrated that mice with adipose AR deficiency exhibited hyperleptinemia without an obese phenotype, with decreased serum triglycerides and cholesterol.

The male sex hormone, testosterone, is an important regulator of body composition in men (26). Testosterone replacement therapy in aged hypogonadal men decreases their intraabdominal fat mass (27), indicating a crucial role of androgen-AR in controlling body fat mass. Androgen treatment also suppressed leptin mRNA and secretion of leptin (18), suggesting androgen-AR signaling may be involved in

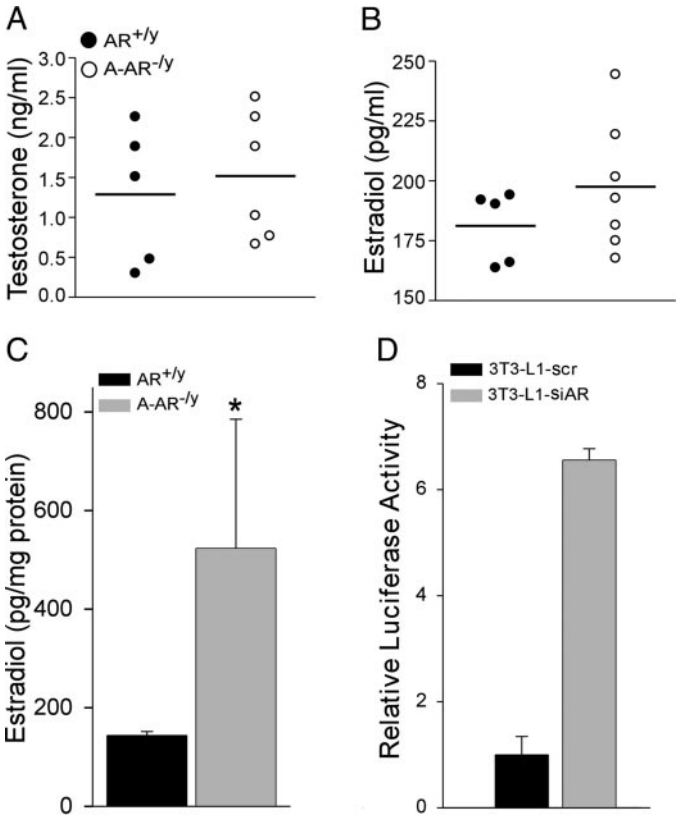

FIG. 6. Enhanced ER transactivation activity due to loss of AR. A, Serum testosterone levels in $AR^{+/y}$ and A-$AR^{-/y}$ mice (n = 5–6). B, Serum estradiol levels $AR^{+/y}$ and A-$AR^{-/y}$ mice (n = 5–7). C, Increased intraadipose estradiol levels in A-$AR^{-/y}$ mice. Data are mean ± SEM; n = 5–6. *, $P < 0.05$; A-$AR^{-/y}$ *vs.* $AR^{+/y}$. D, Increased ER transactivation activity in 3T3-L1-siAR preadipocytes. Stable 3T3-L1-scr and 3T3-L1-siAR preadipocytes were transiently transfected with 0.4 μg estrogen response element-luciferase reporter. Luciferase activity was measured at 48 h after transfection. Luciferase activity in 3T3-L1-scr is set as 1 and relative activities are presented. Data represent means ± SEM; n = 4 independent experiments.

leptin expression. Consistent with previous observations, our results showed that differentiated adipocytes derived from $AR^{-/y}$ mice had higher leptin secretion than those from $AR^{+/y}$ mice. Moreover, A-$AR^{-/y}$ mice are hyperleptinemic, with increased leptin gene expression in eWATs. Our results support that AR plays a direct regulatory role in leptin synthesis in adipocytes.

Leptin was the first adipocytokine discovered involved in energy balance control (28, 29). Mice with spontaneous null mutation of the leptin gene (*ob/ob*) exhibit hyperphagia and severe obesity due to loss of food intake repression and energy expenditure promotion. Action of leptin depends on binding to its cell surface receptor, leptin receptor, which is highly expressed in the hypothalamus, suggesting many effects of leptin are attributed to controls from the central nervous system (CNS). In hypothalamic neurons, leptin signaling induces the expression of anorexigenic proopiomelanocortin (POMC) to repress appetite and promote energy expenditure. On the other hand, leptin also inhibits the expression of orexigenic neuropeptide Y and agouti-related peptide, which counteract the action of POMC (30, 31). Elevated circulating leptin in A-$AR^{-/y}$ mice may activate leptin signaling. Increased expression of POMC in the hypothalamus derived from A-$AR^{-/y}$ mice (supplementary Fig. 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://endo.endojournals.org) further supports this notion, although expression of neuropeptide Y and agouti-related peptide were not significantly repressed. In contrast to hyperleptinemia resulting from increased fat mass, enhanced leptin production and secretion in AR-deficient adipose tissue did not cause leptin resistance, and better sensitivity in response to exogenous leptin challenge was found in these mice.

In adipose tissue, effects of leptin on lipid metabolism have been shown by adenovirus-induced hyperleptinemia with increased expression of key enzymes involved in fatty acid oxidation, acyl-CoA oxidase and CPT-1, suggesting that leptin favors fatty acid oxidation (32). Moreover, leptin treatment in isolated rat adipocytes up-regulates expression of acyl-CoA oxidase, CPT-1, and UCP2 indicating enhanced fatty acid oxidation (33). According to our gene expression data, leptin leads to up-regulation of PGC-1α, CPT-1, and UCP2 through direct effects on adipocytes. However, the possibility that increased PGC-1α, CPT-1, and UCP2 occur through leptin activated neuronal circuits still remains, as acute (23, 34) and chronic central leptin administration (35) showed up-regulation of these genes as well. The increase of leptin-mediated POMC expression in A-$AR^{-/y}$ mice indicates contribution of CNS in enhanced PGC-1α and CPT-1 expression. Increased expression of PGC-1α may coactivate nuclear respiratory factor-1 and -2, which governs nuclear genes encoding respiratory chain subunits involved in electron transport and oxidative phosphorylation. Enhanced expression of CPT-1 may facilitate fatty acid transportation into mitochondria and β-oxidation. Taken together, our results suggest increased fatty acid oxidation in adipose tissues of A-$AR^{-/y}$ mice.

Down-regulated aP2 expression in eWATs suggested that leptin action, although regulating lipid oxidation centrally or peripherally, may coordinate energy use among different tissues through fatty acid transportation because leptin also promotes fatty acid oxidation in skeletal muscle (36). In addition, leptin enhances fatty acid oxidation and fatty acid uptake in liver when administrated centrally (34). High circulating leptin levels in A-$AR^{-/y}$ mice may also enhance lipid oxidation in liver and skeletal muscle through reducing fatty acid transport into adipose tissues, although no significance reduction of adipocyte size was observed. Increased energy use was also reflected in hypotriglyceridemia and hypocholesterolemia phenotypes observed in A-$AR^{-/y}$ mice. On the other hand, it is also possible there is a compensatory mechanism to prevent lipid accumulation in adipose tissue through enhanced energy expenditure by the action of leptin on the CNS.

In contrast to A-$AR^{-/y}$ mice, mice with whole-body AR deficiency develop insulin and leptin resistance and obesity with hyperlipidemia at an advanced age, showing elevated leptin secretion as early as puberty (17). This suggests that AR deficiency in other tissues, such as brain, liver, and muscle, may impair leptin signaling diminishing the beneficial effects of enhanced leptin production through loss of AR in adipose tissue. Our results in A-$AR^{-/y}$ mice suggested a

differential role of AR in adipose tissue contributing to energy balance control.

Sex hormones participate in sex differences of body fat composition, evidenced by the predisposition to central (abdominal) obesity in men and peripheral obesity in women. This difference has important consequences because visceral obesity, but not sc obesity, is considered as a risk factor for development of metabolic syndrome (37–40). Most studies of sex hormone effects in obesity and on body fat distribution have focused on circulating levels of testosterone and estradiol (41). However, steroid metabolism is much more complex than what can be observed from simple measures of circulating androgens and estrogens. Adipose tissue has been shown to express several steroidogenic enzymes controlling tissue steroid concentrations and ligand bioavailability for intracellular receptors. As suggested, sexual dimorphism of leptinemia is mainly due to ER-dependent stimulation of leptin expression in adipose tissue by estradiol or its precursor (25). Increased estradiol levels in eWATs of A-AR$^{-/y}$ mice leads to enhanced ER transactivation activity that likely contributes to up-regulated leptin gene expression. Because these hydroxysteroid dehydrogenases are also involved in the synthesis of testosterone in testis, their activity may be directly or indirectly influenced by AR (42). It seems that the increases of intraadipose estradiol levels in A-AR$^{-/y}$ mice may be due to altered hydroxysteroid dehydrogenase activity resulting from loss of AR-dependent mechanisms.

In summary, AR plays an inhibitory role in leptin production in adipocytes, and A-AR$^{-/y}$ mice exhibit hyperleptinemia with identical body weight, compared with AR$^{+/y}$ mice. Increased leptin levels lead to increased lipid oxidation centrally and peripherally, resulting in hypotriglyceridemia and hypocholesterolemia phenotypes, and might modulate lipid mobilization and use between different tissues. The present study in A-AR$^{-/y}$ mice provides evidence for a differential tissue-specific role of AR in energy balance control.

Acknowledgments

The authors thank Karen Wolf for manuscript preparation.

Received April 20, 2007. Accepted February 5, 2008.

Address all correspondence and requests for reprints to: Chawnshang Chang, Ph.D., 601 Elmwood Avenue, Box 626, Rochester, New York 14642. E-mail: chang@urmc.rochester.edu.

This work was supported by National Institutes of Health Grant DK073414 and The George Whipple Professor Endowment.

Disclosure Statement: The authors have nothing to disclose.

References

1. **Kershaw EE, Flier JS** 2004 Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 89:2548–2556
2. **Hotamisligil GS, Shargill NS, Spiegelman BM** 1993 Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 259:87–91
3. **Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA** 2001 The hormone resistin links obesity to diabetes. Nature 409:307–312
4. **Fernandez-Real JM, Ricart W** 2003 Insulin resistance and chronic cardiovascular inflammatory syndrome. Endocr Rev 24:278–301
5. **Juhan-Vague I, Alessi MC, Mavri A, Morange PE** 2003 Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost 1:1575–1579
6. **Friedman JM, Halaas JL** 1998 Leptin and the regulation of body weight in mammals. Nature 395:763–770
7. **Chandran M, Phillips SA, Ciaraldi T, Henry RR** 2003 Adiponectin: more than just another fat cell hormone? Diabetes Care 26:2442–2450
8. **Ahima RS** 2006 Adipose tissue as an endocrine organ. Obesity (Silver Spring) 14(Suppl 5):242S–249S
9. **Tilg H, Moschen AR** 2006 Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6:772–783
10. **Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F** 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540–543
11. **Muoio DM, Dohm GL, Fiedorek Jr FT, Tapscott EB, Coleman RA** 1997 Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 46:1360–1363
12. **Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ** 1997 Acute stimulation of glucose metabolism in mice by leptin treatment. Nature 389: 374–377
13. **Minokoshi Y, Haque MS, Shimazu T** 1999 Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes 48:287–291
14. **Unger RH, Zhou YT, Orci L** 1999 Regulation of fatty acid homeostasis in cells: novel role of leptin. Proc Natl Acad Sci USA 96:2327–2332
15. **Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P** 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549
16. **Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM** 2002 Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science 297:240–243
17. **Lin HY, Xu Q, Yeh S, Wang RS, Sparks JD, Chang C** 2005 Insulin and leptin resistance with hyperleptinemia in mice lacking androgen receptor. Diabetes 54:1717–1725
18. **Machinal F, Dieudonne MN, Leneveu MC, Pecquery R, Giudicelli Y** 1999 *In vivo* and *in vitro* ob gene expression and leptin secretion in rat adipocytes: evidence for a regional specific regulation by sex steroid hormones. Endocrinology 140:1567–1574
19. **Meseguer A, Puche C, Cabero A** 2002 Sex steroid biosynthesis in white adipose tissue. Horm Metab Res 34:731–736
20. **Blaak E** 2001 Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care 4:499–502
21. **Wagenknecht LE, Langefeld CD, Scherzinger AL, Norris JM, Haffner SM, Saad MF, Bergman RN** 2003 Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. Diabetes 52:2490–2496
22. **Yeh S, Tsai MY, Xu Q, Mu XM, Lardy H, Huang KE, Lin H, Yeh SD, Altuwaijri S, Zhou X, Xing L, Boyce BF, Hung MC, Zhang S, Gan L, Chang C** 2002 Generation and characterization of androgen receptor knockout (ARKO) mice: an *in vivo* model for the study of androgen functions in selective tissues. Proc Natl Acad Sci USA 99:13498–13503
23. **Tajima D, Masaki T, Hidaka S, Kakuma T, Sakata T, Yoshimatsu H** 2005 Acute central infusion of leptin modulates fatty acid mobilization by affecting lipolysis and mRNA expression for uncoupling proteins. Exp Biol Med (Maywood) 230:200–206
24. **O'Neil JS, Burow ME, Green AE, McLachlan JA, Henson MC** 2001 Effects of estrogen on leptin gene promoter activation in MCF-7 breast cancer and JEG-3 choriocarcinoma cells: selective regulation via estrogen receptors α and β. Mol Cell Endocrinol 176:67–75
25. **Machinal-Quelin F, Dieudonne MN, Pecquery R, Leneveu MC, Giudicelli Y** 2002 Direct *in vitro* effects of androgens and estrogens on ob gene expression and leptin secretion in human adipose tissue. Endocrine 18:179–184
26. **Bhasin S, Woodhouse L, Storer TW** 2001 Proof of the effect of testosterone on skeletal muscle. J Endocrinol 170:27–38
27. **Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, Matsumoto AM, Weber T, Berman N** 2000 Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. J Clin Endocrinol Metab 85:2839–2853
28. **Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM** 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543–546
29. **Friedman JM** 2002 The function of leptin in nutrition, weight, and physiology. Nutr Rev 60:S1–S14; discussion S68–S84; 85–S87
30. **Elmquist JK, Maratos-Flier E, Saper CB, Flier JS** 1998 Unraveling the central nervous system pathways underlying responses to leptin. Nat Neurosci 1:445–450
31. **Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG** 2000 Central nervous system control of food intake. Nature 404:661–671
32. **Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH** 1997 Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci USA 94:4637–4641
33. **William Jr WN, Ceddia RB, Curi R** 2002 Leptin controls the fate of fatty acids in isolated rat white adipocytes. J Endocrinol 175:735–744
34. **Gallardo N, Bonzon-Kulichenko E, Fernandez-Agullo T, Molto E, Gomez-Alonso S, Blanco P, Carrascosa JM, Ros M, Andres A** 2007 Tissue-specific

effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. Endocrinology 148:5604–5610
35. **Cusin I, Zakrzewska KE, Boss O, Muzzin P, Giacobino JP, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F** 1998 Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. Diabetes 47:1014–1019
36. **Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB** 2002 Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339–343
37. **Bjorntorp P** 1997 Hormonal control of regional fat distribution. Hum Reprod 12(Suppl 1):21–25
38. **Bjorntorp P** 1997 Obesity. Lancet 350:423–426
39. **Bjorntorp P** 1997 Body fat distribution, insulin resistance, and metabolic diseases. Nutrition 13:795–803
40. **Arsenault BJ, Lachance D, Lemieux I, Almeras N, Tremblay A, Bouchard C, Perusse L, Despres JP** 2007 Visceral adipose tissue accumulation, cardiorespiratory fitness, and features of the metabolic syndrome. Arch Intern Med 167:1518–1525
41. **Tchernof A, Despres JP** 2000 Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women. Horm Metab Res 32:526–536
42. **Xu Q, Lin H-Y, Yeh S-D, Yu I-C, Wang R-S, Chen Y-T, Zhang C, Altuwaijri S, Chen L-M, Chuang K-H, Brown TR, Chiang H-S, Yeh S, Chang C** 2007 Infertility with defective spermatogenesis and steroidogenesis in male mice lacking androgen receptor in Leydig cells. Endocrine 32:96–106

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**