Original Article

Opposing Effects of Adiponectin Receptors 1 and 2 on Energy Metabolism

Mikael Bjursell,[1,2] Andrea Ahnmark,[1] Mohammad Bohlooly-Y,[1,2] Lena William-Olsson,[1] Magdalena Rhedin,[1] Xiao-Rong Peng,[1] Karolina Ploj,[1] Anna-Karin Gerdin,[1] Gunnel Arnerup,[3] Anders Elmgren,[1] Anna-Lena Berg,[3] Jan Oscarsson,[1,2,4] and Daniel Lindén[1,4]

The adipocyte-derived hormone adiponectin regulates glucose and lipid metabolism and influences the risk for developing obesity, type 2 diabetes, and cardiovascular disease. Adiponectin binds to two different seven-transmembrane domain receptors termed AdipoR1 and AdipoR2. To study the physiological importance of these receptors, *AdipoR1* gene knockout mice (*AdipoR1*$^{-/-}$) and *AdipoR2* gene knockout mice (*AdipoR2*$^{-/-}$) were generated. *AdipoR1*$^{-/-}$ mice showed increased adiposity associated with decreased glucose tolerance, spontaneous locomotor activity, and energy expenditure. However, *AdipoR2*$^{-/-}$ mice were lean and resistant to high-fat diet–induced obesity associated with improved glucose tolerance and higher spontaneous locomotor activity and energy expenditure and reduced plasma cholesterol levels. Thus, AdipoR1 and AdipoR2 are clearly involved in energy metabolism but have opposing effects. *Diabetes* 56:583–593, 2007

The prevalence of obesity is increasing rapidly, reaching epidemic proportions in several developed countries (1–4). Obesity is usually associated with type 2 diabetes, hypertension, and dyslipidemia, thereby increasing the risk for coronary artery disease and mortality (5). The adipose tissue is important not only because it constitutes the major reservoir for energy storage, but also due to the secretion of factors regulating energy balance in the body (6). The importance of adipose tissue as an endocrine organ became evident with the identification and characterization of the hormone leptin (7). Leptin suppresses appetite and increases insulin sensitivity and energy expenditure by acting both in the hypothalamus (8) and in peripheral tissues by inhibiting lipid storage (9). Leptin-deficient (*ob/ob*) mice or mice lacking a functional leptin receptor (*db/db*) are obese, hyperphagic, hyperinsulinemic, and insulin resistant and have reduced spontaneous locomotor activity and energy expenditure (6,9).

In 1995, another hormone exclusively secreted by the adipose tissue termed adiponectin (Acrp30, AdipoQ, apM1, or GBP28) was characterized independently by four groups (10–13). Low adiponectin levels are found in obesity and type 2 diabetes (11,14,15), and reduced adiponectin levels have also been correlated to increased risk of developing obesity, type 2 diabetes (16,17), and myocardial infarction (18). Adiponectin treatment has both antidiabetic (19–25) and anti-atherosclerotic (25,26) effects in animals. Mice deficient in adiponectin are insulin resistant and prone to develop atherosclerosis (22,27).

Two adiponectin receptors (AdipoR1 and AdipoR2) have been found. The genes encoding these receptors are located on different chromosomes, and the gene products constitute a novel class of seven-transmembrane domain receptors (28). The immediate downstream signaling events are largely unknown, but stimulation of the receptors has been shown to activate AMP-activated protein kinase (AMPK) and induce peroxisome proliferator–activated receptor α (PPARα) signaling (20,23–25,28).

To study the in vivo importance of these receptors, *AdipoR1* gene knockout mice (*AdipoR1*$^{-/-}$) and *AdipoR2* gene knockout mice (*AdipoR2*$^{-/-}$) were generated. These mice were studied in terms of body weight, body fat, food intake, glucose tolerance, energy expenditure, spontaneous locomotor activity, receptor downstream signaling, and plasma biochemistry.

RESEARCH DESIGN AND METHODS

Creation of *AdipoR1* and *AdipoR2* gene knockout mice. Heterozygous *AdipoR1* and *AdipoR2* gene knockout mice were obtained from Deltagen (San Carlos, CA). Briefly, the mice were generated by homologous recombination in 129/OlaHsd ES cells using targeting vectors containing the following cassette: lacO-splice acceptor–internal ribosomal entry site–lacZ–poly A–phospho glycerate kinase promoter–neomycin resistance–poly A. This cassette drives expression of the *lacZ* gene by the endogenous adiponectin receptor promoter. *AdipoR1*$^{-/-}$ mice were created by deleting 105 bp corresponding to base 107–198 in the AdipoR1 cDNA (Genbank no. BC014875), and *AdipoR2*$^{-/-}$ mice were created by deleting 1,561 bp corresponding to base 616–802 in the AdipoR2 cDNA (Genbank no. BC024094). The insertion of the targeting cassettes did not affect immediate downstream gene expression in either of the knockouts (data not shown), indicating that the phenotypes of the two receptor knockouts are not due to interference with expression of flanking genes. The mouse models were generated by breeding chimeras carrying a disrupted *AdipoR1* or *AdipoR2* gene with C57BL/6 females, resulting in F1

From the [1]AstraZeneca R&D, Mölndal, Sweden; the [2]Department of Physiology/Endocrinology, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden; [3]Safety Assessment Sweden, AstraZeneca R&D, Södertälje, Sweden; and the [4]Wallenberg Laboratory for Cardiovascular Research, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden.

Address correspondence and reprint requests to Daniel Lindén, AstraZeneca R&D, Department of Integrative Pharmacology, SE-431 83 Mölndal, Sweden. E-mail: daniel.linden@astrazeneca.com.

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ABCA1, ATP binding cassette transporter-1; AGRP, agouti-related peptide; AMPK, AMP-activated protein kinase; apoAI, apolipoprotein AI; BAT, brown adipose tissue; CPT-1, carnitine palmitoyl transferase-1; CRH, corticotrophin-releasing hormone; DEXA, dual-energy X-ray absorptiometry; HFD, high-fat diet; NPY, neuropeptide Y; PPARα, peroxisome proliferator–activated receptor α; SNP, single nucleotide polymorphism; WAT, white adipose tissue.

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heterozygous offspring. F1 generation mice were backcrossed to C57BL/6 females for seven (*AdipoR1*) or six (*AdipoR2*) generations before intercrossing. Both intercrosses resulted in normal litter sizes and Mendelian distribution of genotypes (data not shown). All experiments in this study were performed using littermate wild-type mice as controls. Genotyping was done by a PCR strategy. For the *AdipoR1* gene, one primer was located upstream of the deleted region in the short arm (5′AGGCAGGGTAAGCTGATTAGCTATG 3′), a second primer located in exon 2 (5′ TCCACTGTGTCAGCTTCTCTG TTAC 3′), and a third located in the targeting cassette (5′ GGGTGGGATTAG ATAAATGCCTGCTCT 3′). For the *AdipoR2* gene, one primer was located upstream of the deleted region in the short arm (5′GACGGAGTTTGTATGTG GTAGCGTC 3′), a second primer was located in exon 5 (5′ TCTCTGCCTTT CCTTTTCATGGCTC 3′), and a third was located in the targeting cassette (5′ GGGCCAGCTCATTCCTCCCACTCAT 3′).

Diets, body weight, food intake, body composition, fecal energy content, and termination. The mice were given either standard laboratory chow containing (energy percentage) 12% fat, 62% carbohydrates, and 26% protein, with a total energy content of 12.6 kJ/g (R3; Lactamin, Kimstad, Sweden) or a high-fat diet (HFD) containing 39.9% fat (mainly saturated), 42.3% carbohydrates, and 17% protein with a total energy content of 21.4 kJ/g and supplemented with 0.15% cholesterol (R638; Lactamin). Mice were weighed weekly after 5 weeks of age. Food intake was analyzed for 24 h in food-deprived (12 h) mice housed individually as described before (29). Body composition analysis was performed on isoflurane (Forene; Abbot, Scandinavia, Sweden)-anesthetized mice by dual-energy X-ray absorptiometry (DEXA) (PIXImus Lunar; GE Medical Systems, Madison, WI). Feces from the animals were dried at 55°C overnight and stored in airtight containers at −20°C until assayed. The gross energy content of the fecal pellets was determined using a bomb calorimeter (C5000; IKA, Werke, Staufen, Germany). At termination (9–11 A.M.), plasma was isolated from isoflurane-anesthetized mice and organs collected, weighed, snap frozen in N_2, and stored at −80°C. Experimental procedures were approved by the local ethics review committee on animal experiments (Gothenburg region).

Indirect calorimetry and activity. Oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were measured using an open-circuit calorimetry system (Oxymax; Columbus Instruments International, Columbus, OH) as described before (29). Energy expenditure (in kilocalories per hour) was calculated as follows: (3.815 + 1.232 RER) × V_{O_2}, where RER is the respiratory exchange ratio (volume of CO_2 produced per volume of O_2 consumed [both in ml/kg/min]) and V_{O_2} is the volume of O_2 consumed per hour per kilogram of mass of the animal. The value of energy expenditure was correlated with individual body weights. Locomotor and rearing activity and corner time were measured in activity boxes (Kungsbacka mät-och reglerteknik, Kungsbacka, Sweden) over 1 h, 10:00–11:00 A.M., on 2 consecutive days.

Oral glucose tolerance test. Oral glucose tolerance tests were performed 12.30 h after a 4-h fast by oral administration of 2 g glucose/kg body wt. Blood (12 μl) was sampled from the tail vein at 0, 15, 30, 60, 90, and 120 min for determination of glucose (2 μl, Accu-Chek; Roche Diagnostics, Mannheim, Germany) and insulin (2 × 5 μl, Ultra-sensitive rat insulin ELISA kit; Crystal Chem, Downers Grove, IL) levels.

Blood biochemistry and liver triglyceride content. Plasma adiponectin was measured using a radioimmunoassay from Linco Research (St. Charles, MO) and plasma leptin with an ELISA from Chrystal Chem. Total plasma thyroxine (T_4), triiodothyronine (T_3), and testosterone levels were determined using radioimmunoassays (Coat-A-Count; Diagnostic Products, Los Angeles, CA). Plasma cholesterol and triglyceride levels were measured with enzymatic colorimetric assays (Roche Diagnostics). The cholesterol distribution profiles were measured using a size-exclusion high-performance liquid chromatography system, SMART, with column Superose 6 PC 3.2/30 (Amersham Pharmacia Biotech), as described before (30). The lipoproteins in a 10-μl sample were separated within 60 min, and the area under the curves represents the cholesterol content. Liver triglyceride content was measured after homogenization in isopropanol (1 ml/50 mg), incubation at 4°C for 1 h, and centrifugation at 2,500 rpm for 5 min.

Histology. Tissues were fixed in 4% buffered paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin or β-galactosidase. For electron microscopy, fixated sections were treated with osmium, dehydrated in graded ethanol (70, 85, 95, and 100%) and propylene oxide, and embedded in TAAB 812 (TAAB Laboratories, Berkshire, England).

RNA preparation and quantitative real-time PCR. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from DNAse-treated total RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real-time PCR analysis was performed with an ABI Prism 7900 Sequence Detection System using FAM- and TAMRA-labeled fluorogenic probes or the SYBR-Green detection system (Applied Biosystems). Expression data were normalized against mouse acidic ribosomal phosphoprotein P0 (m36B4). The relative expression levels were calculated according to the formula $2^{-\Delta CT}$, where ΔCT is the difference in cycle threshold values between the target and the m36B4 internal control. Sequences for primers and probes are presented in supplemental Table 1 (available in an online appendix at http://dx.doi.org/10.2337/db06-1432).

Immunoblotting. Antibodies against human AdipoR1 and AdipoR2 were generated by immunizing rabbits with either of the following peptides: GAPASNREADTVELAELGP (AdipoR1) and CSRTPEPDIRLRKGHQLDG (AdipoR2). These sequences had 89% (AdipoR1) and 94% (AdipoR2) homology with the corresponding mouse sequences. The antibodies were affinity purified and used at 1 μg/ml in phosphate-buffered saline with Tween, 5% milk powder. Tissues were homogenized in 10 mmol/l Hepes-KOH (pH 7.4), 2 mmol/l EDTA, 40 mmol/l sucrose, and 125 mmol/l mannitol in the presence of protease inhibitors and centrifuged at 800*g* to remove the nuclei. The supernatant was collected and centrifuged at 100,000*g* to collect total membranes. Phosphorylation and protein levels of AMPKα1 were determined as described (31) using AMPKα1 antibodies from Upstate Cell Signaling (Lake Placid, NY) and phospho AMPK (Thr172) antibodies from Cell Signaling Technology (Danvers, MA).

Statistics. Values are presented as means ± SEM. Differences between two groups were examined for statistical significance using Mann-Whitney *U* test. For multiple groups, *P* values were calculated using the MIXED procedure in SPSS. A mixed ANOVA model is assumed with treatment and time as factors and their interaction as fixed effects and subject within treatment group as random effects. Each *P* value corresponds to a test of no difference between treatments at the specified time point, derived from the corresponding contrast in the ANOVA model.

RESULTS

AdipoR1-deficient mice show increased adiposity. *AdipoR1*$^{-/-}$ mice were created by exchanging part of exon 2 with a targeting vector containing *LacZ* and *Neomycin resistance* genes (Fig. 1*A*). Wild-type controls, mice heterozygous for the *AdipoR1* gene, and *AdipoR1*$^{-/-}$ mice were genotyped with PCR (Fig. 1*B*). The absence of AdipoR1 protein in *AdipoR1*$^{-/-}$ mice was confirmed by immunoblot analysis of brain (Fig. 1*C*) and quadriceps skeletal muscle (Supplemental Fig. 1*A* [available at http://dx.doi.org/10.2337/db06-1432]) protein preparations. Male but not female *AdipoR1*$^{-/-}$ mice had increased body weight gain (Fig. 1*D* and *E*) in spite of food intake similar to that of wild-type controls (data not shown). Furthermore, male *AdipoR1*$^{-/-}$ mice had increased total body fat mass at 15 weeks of age, as determined by DEXA (Fig. 1*F*). At 31 weeks of age, *AdipoR1*$^{-/-}$ mice had increased reproductive white adipose tissue (WAT), perirenal WAT, and brown adipose tissue (BAT) weights (Fig. 1*G*). In line with the increased fat mass, *AdipoR1*$^{-/-}$ mice tended to have higher plasma leptin levels than wild-type controls, while plasma adiponectin levels were unchanged (Table 1). Thus, *AdipoR1* deficiency results in increased fat mass.

AdipoR1-deficient mice have decreased glucose tolerance. Male *AdipoR1*$^{-/-}$ mice had decreased glucose tolerance as determined by oral glucose tolerance testing (Fig. 1*H*). The area under the curve for the glucose response was 34% higher ($P < 0.05$) in *AdipoR1*$^{-/-}$ mice compared with wild-type controls. The insulin response was not significantly altered (Fig. 1*H*). *AdipoR1*$^{-/-}$ females had 22% higher fasting glucose levels (wild-type 7.68 ± 0.42 mmol/l and *AdipoR1*$^{-/-}$ 9.37 ± 0.44 mmol/l, $P < 0.05$, $n = 4$–7) but unchanged fasting insulin levels (wild-type 0.53 ± 0.06 μg/l and *AdipoR1*$^{-/-}$ 0.73 ± 0.11 μg/l, $n = 4$–7). Thus, *AdipoR1* deficiency leads to decreased glucose tolerance. *AdipoR1* deficiency did not affect plasma triglyceride levels, but plasma cholesterol levels were elevated in *AdipoR1*$^{-/-}$ females compared with wild-type controls. In addition, liver triglyceride

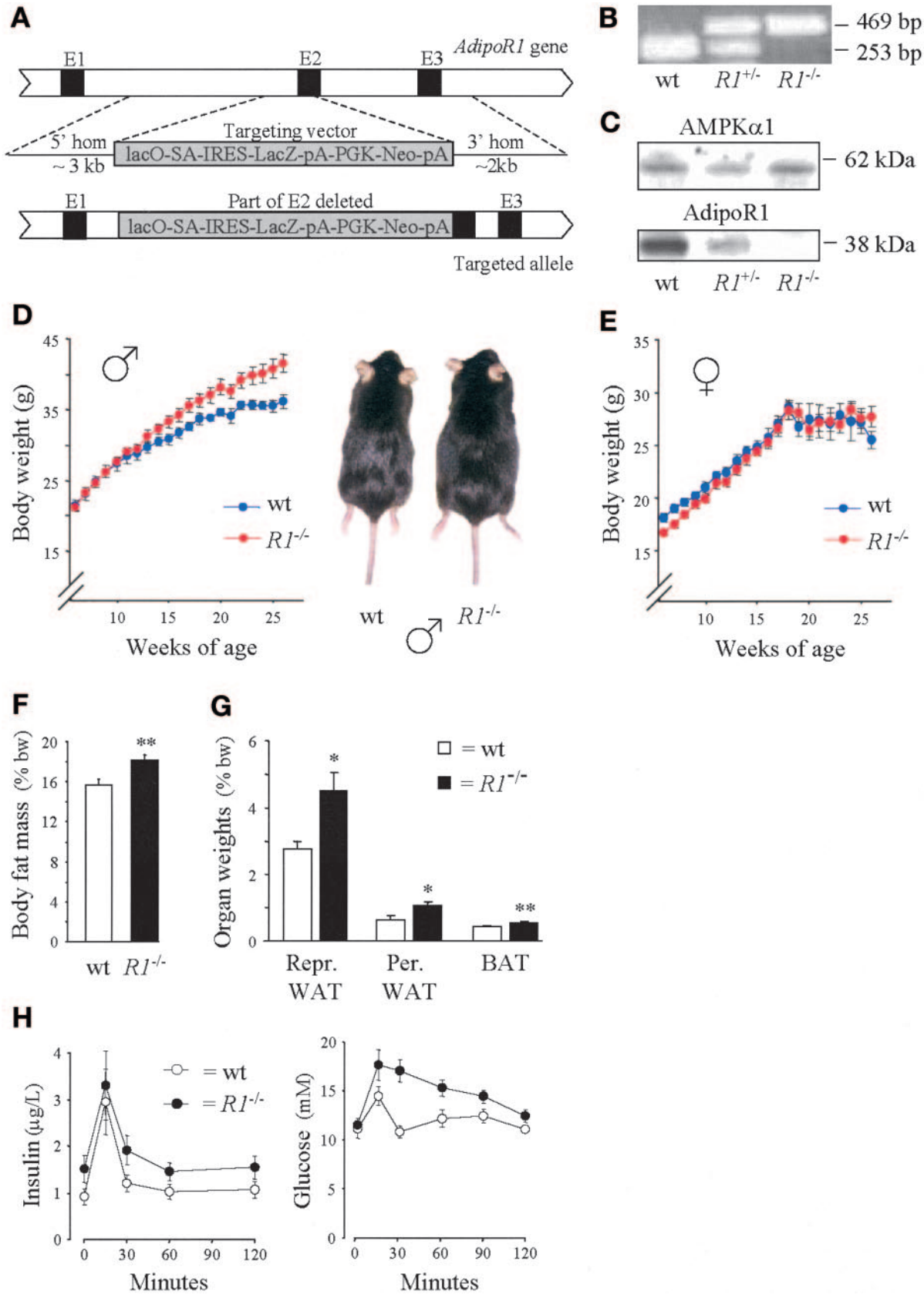

FIG. 1. Creation and phenotype of *AdipoR1* gene knockout mice ($R1^{-/-}$). *A*: Part of exon 2 in the *AdipoR1* gene was homologously replaced with a targeting vector containing *lacZ-Neo* genes. *B*: Wild-type (wt) mice, mice heterozygous for the *AdipoR1* gene ($R1^{+/-}$), and $R1^{-/-}$ were genotyped with PCR. *C*: Lack of AdipoR1 protein in $R1^{-/-}$ was confirmed using immunoblot analysis of brain protein preparations. AMPKα1 subunit served as loading control. *D*: Body weight curves for males fed regular chow (n = 5–8) and representative image of 31-week-old $R1^{-/-}$ and wild-type male mice. *E*: Body weight curves for females (n = 4–7). *F*: Quantitative assessment of body fat mass by DEXA on 28-week-old $R1^{-/-}$ and wild-type males (n = 5–8). *G*: Dissected reproductive (Repr.) WAT, perirenal (Per.) WAT, and BAT from 31-week-old males and females (n = 8–14). *H*: Oral glucose tolerance test on 30-week-old $R1^{-/-}$ and wild-type males (n = 4–7). Values are means ± SEM. *P < 0.05, **P < 0.01, Mann-Whitney U test.

TABLE 1
Plasma biochemistry and liver triglyceride content in *AdipoR1* gene knockout mice ($R1^{-/-}$)

	n	Leptin (ng/ml)	Adiponectin (nmol/l)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	Liver triglycerides (% liver wt)	T_3 (nmol/l)	T_4 (nmol/l)
Males								
Wild-type	4	3.5 ± 0.7	611 ± 60	3.0 ± 0.3	1.0 ± 0.2	3.13 ± 0.19	1.1 ± 0.0	55.6 ± 4.1
$R1^{-/-}$	8	4.8 ± 1.7	574 ± 31	3.4 ± 0.3	0.9 ± 0.1	4.75 ± 1.25	1.2 ± 0.1	52.4 ± 2.0
Females								
Wild-type	4	2.5 ± 0.9	782 ± 148	2.2 ± 0.1	0.7 ± 0.1	3.88 ± 0.61	ND	ND
$R1^{-/-}$	7	6.2 ± 1.1	1,064 ± 140	3.7 ± 0.4*	1.0 ± 0.2	6.30 ± 1.22	ND	ND

Data are means ± SEM. The mice were 31 weeks old. Plasma levels of leptin, adiponectin, cholesterol, triglycerides, liver triglyceride content, T_3, and T_4 were measured as described in RESEARCH DESIGN AND METHODS. ND, not determined. *$P < 0.05$, Mann Whitney *U* test.

content tended to be increased in $AdipoR1^{-/-}$ mice compared with wild-type controls (Table 1).

Effects of AdipoR1-deficiency on AMPK and PPARα signaling and AdipoR2 expression. Adiponectin treatment may increase insulin sensitivity by stimulating fatty acid oxidation via AMPK (23,24,28) and PPARα activation (20,25,28) in liver and skeletal muscle. The expression of PPARα mRNA was unchanged, and its downstream target gene carnitine palmitoyl transferase-1 (CPT-1)α was increased in $AdipoR1^{-/-}$ livers compared with wild-type controls, while PPARα or CPT-1β mRNA levels did not differ between the genotypes in skeletal muscle (Supplemental Fig. 1*B*). In addition, $AdipoR1^{-/-}$ mice did not show changed phosphorylation of AMPK in liver, skeletal muscle, or heart (Supplemental Fig. 1*C*). Thus, $AdipoR1^{-/-}$ mice do not have decreased glucose tolerance as a result of changed PPARα activity or basal AMPK phosphorylation. $AdipoR1^{-/-}$ mice showed increased AdipoR2 mRNA expression in BAT, while it was unchanged in WAT, skeletal muscle, and brain. In addition, both mRNA and protein levels of AdipoR2 tended to be elevated in $AdipoR1^{-/-}$ livers (Supplemental Fig. 1*D* and *E*). Thus, $AdipoR1^{-/-}$ deficiency results in higher expression of AdipoR2 in liver and BAT but not in WAT, skeletal muscle, or brain.

AdipoR1-deficient mice show decreased energy expenditure and locomotor activity. $AdipoR1^{-/-}$ mice had decreased energy expenditure but unchanged respiratory exchange ratio compared with wild-type controls (Fig. 2*A*). The decreased energy expenditure in $AdipoR1^{-/-}$ mice was observed during both the light and dark phase of the 24-h period. $AdipoR1^{-/-}$ mice had decreased spontaneous locomotor activity and increased time spent in the corners of the cages (Fig. 2*B*). The decreased activity in $AdipoR1^{-/-}$ mice was not due to alterations in plasma levels of thyroid hormones (T_3 or T_4) (Table 1). Thus, the increased fat mass in $AdipoR1^{-/-}$ is due to decreased energy expenditure. Decreased spontaneous locomotor activity could contribute to the decreased energy expenditure.

AdipoR2-deficient mice show resistance to HFD-induced obesity. $AdipoR2^{-/-}$ mice were created by exchanging exon 5 with a targeting vector containing *LacZ* and *Neomycin resistance* genes (Fig. 3*A*). Wild-type controls, mice heterozygous for the *AdipoR2* gene, and $AdipoR2^{-/-}$ mice, were genotyped with PCR (Fig. 3*B*). The absence of AdipoR2 protein in $AdipoR2^{-/-}$ mice was confirmed by immunoblot analysis of liver (Fig. 3*C*) and quadriceps skeletal muscle (Supplemental Fig. 2*A*) protein preparations. Surprisingly, both male and female $AdipoR2^{-/-}$ mice were lean on regular chow and resistant to HFD-induced weight gain (Fig. 3*D* and *E*). The resistance was not due to decreased food intake. If anything, a trend toward increased food intake (28%) was observed in female $AdipoR2^{-/-}$ mice compared with wild-type controls given HFD (Supplemental Fig. 2*B*). In accordance with a higher food intake, the hypothalamic mRNA levels of the orexigenic peptides agouti-related peptide (AGRP) and neuropeptide Y (NPY) were increased by >150 and 60%, respectively (Supplemental Fig. 2*C*), while the expression of another orexigenic peptide, melanin-concentrating hormone, was unchanged in female $AdipoR2^{-/-}$ mice (data not shown). Intracerebroventricular administration of adiponectin has been shown to increase the hypothalamic expression of the anorexigenic corticotrophin-releasing hormone (CRH) (32). $AdipoR2^{-/-}$ mice had 48% increased hypothalamic mRNA levels of CRH (Supplemental Fig. 2*C*). $AdipoR2^{-/-}$ mice did not show any signs of malabsorption. The total fecal energy output measured over 48 h was not different between $AdipoR2^{-/-}$ mice and wild-type controls (data not shown). Thus, $AdipoR2^{-/-}$ mice were resistant to HFD-induced weight gain in spite of a trend toward increased food intake and unchanged fecal energy loss.

After 8 weeks of HFD, female $AdipoR2^{-/-}$ mice had markedly lower total body fat mass and moderately lower lean body mass compared with wild-type controls as determined by DEXA (Fig. 3*G*). However, crown-rump length was not different (data not shown). After 11 weeks

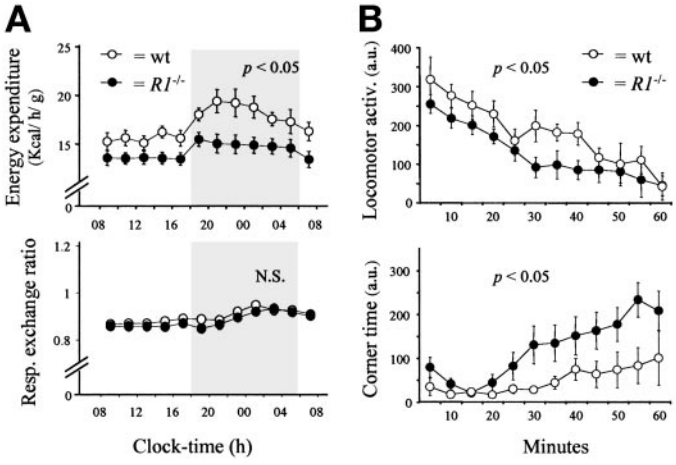

FIG. 2. Energy expenditure and spontaneous locomotor activity of *AdipoR1* knockout mice ($R1^{-/-}$). *A*: Energy expenditure and respiratory exchange ratio measured with an open circuit calorimetry system in 28-week-old $R1^{-/-}$ and wild-type males (dark period shaded, n = 5–7). *B*: Locomotor activity and corner time measured with activity boxes in 29-week-old $R1^{-/-}$ and wild-type males (n = 5–7). a.u., arbitrary units; N.S., nonsignificant. Values are means ± SEM. A mixed ANOVA was used for statistical analyses.

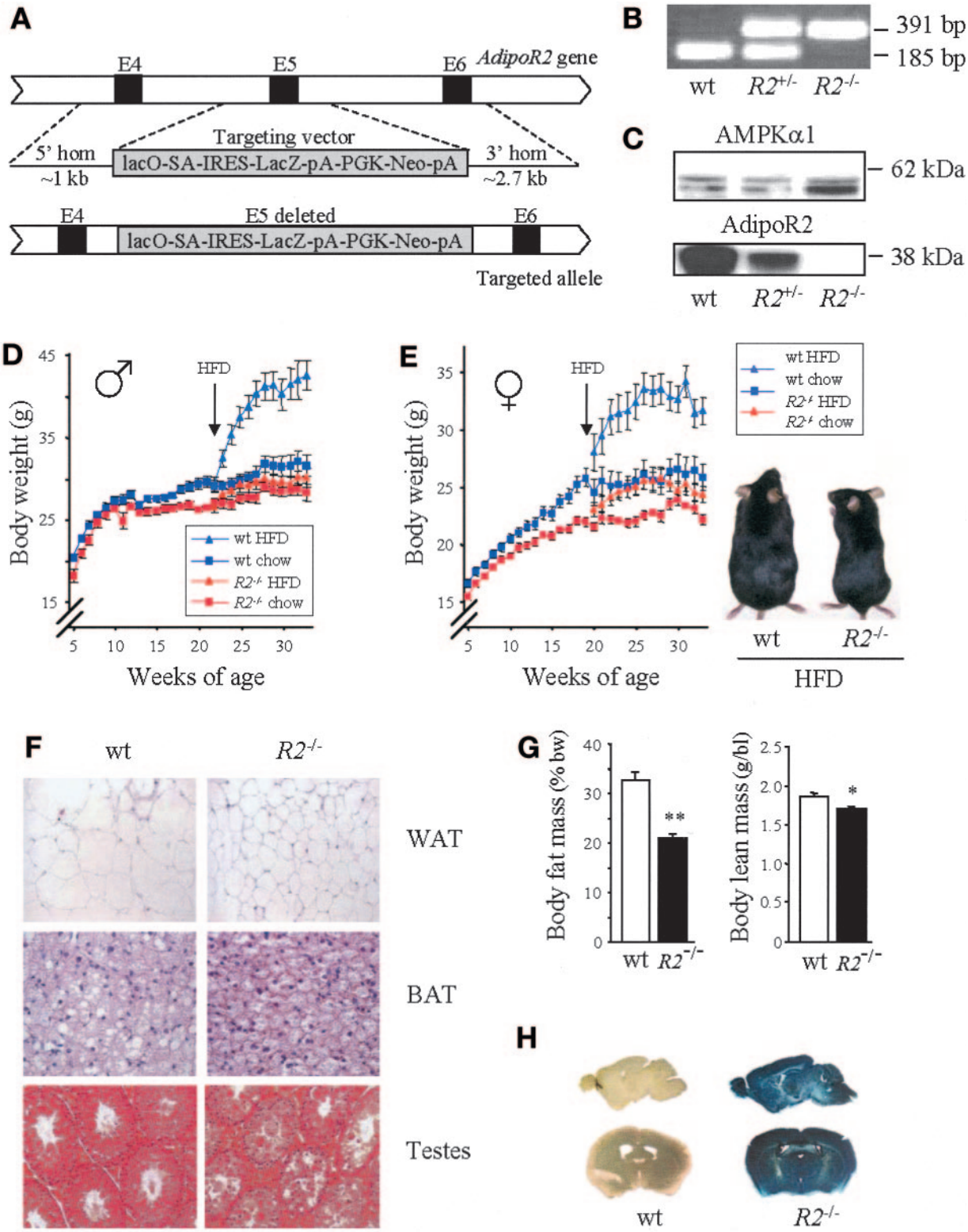

FIG. 3. Creation, body weight-gain, histological analyses, and body fat mass assessment of *AdipoR2* gene knockout mice ($R2^{-/-}$). *A*: Exon 5 in the *AdipoR2* gene was homologously replaced with a targeting vector containing *lacZ-Neo* genes. *B*: Wild-type (wt) mice, mice heterozygous for the *AdipoR2* gene ($R2^{+/-}$), and $R2^{-/-}$ were genotyped with PCR. *C*: Lack of AdipoR2 protein in $R2^{-/-}$ was confirmed using immunoblot analysis of liver protein preparations. AMPKα1 served as loading control (upper band). *D*: Body weight curves for males fed regular chow or HFD ($n = 5$). *E*: Body weight curves for females fed regular chow or HFD ($n = 5$–10) and representative image of $R2^{-/-}$ and wild-type females after 14 weeks of HFD. *F*: Representative histology of testes from 15-week-old males on regular chow, WAT, and BAT from 33-week-old females fed HFD for the last 14 weeks. *G*: Quantitative assessment of body fat and lean mass by DEXA on 32-week-old $R2^{-/-}$ and wild-type females fed HFD for the last 13 weeks ($n = 10$). *H*: Representative histology and β-galactosidase staining of brains. Values are means ± SEM. *$P < 0.05$, **$P < 0.01$, Mann-Whitney *U* test.

TABLE 2
Organ weights of *AdipoR2* gene knockout mice ($R2^{-/-}$)

	n	Reproductive WAT (% body wt)	Perirenal WAT (% body wt)	BAT (% body wt)	Brain (% body wt)	Brain (g)
Males						
Chow fed						
Wild-type	6	3.25 ± 0.43	1.06 ± 0.18	0.44 ± 0.03	1.46 ± 0.06	0.465 ± 0.005
$R2^{-/-}$	7	2.36 ± 0.23	0.69 ± 0.09	0.44 ± 0.05	1.69 ± 0.06	0.502 ± 0.007*
HFD fed						
Wild-type	5	6.25 ± 0.17	1.89 ± 0.09	0.95 ± 0.06	1.08 ± 0.04	0.459 ± 0.005
$R2^{-/-}$	5	2.79 ± 0.25*	0.89 ± 0.11*	0.53 ± 0.06*	1.66 ± 0.08*	0.500 ± 0.009†
Females						
Chow fed						
Wild-type	6	4.28 ± 0.64	0.67 ± 0.11	0.37 ± 0.03	1.89 ± 0.09	0.475 ± 0.005
$R2^{-/-}$	6	2.15 ± 0.16*	0.29 ± 0.03*	0.32 ± 0.02	2.32 ± 0.03*	0.520 ± 0.005*
HFD fed						
Wild-type	6	8.87 ± 0.61	1.40 ± 0.09	0.43 ± 0.01	1.43 ± 0.07	0.457 ± 0.013
$R2^{-/-}$	6	2.90 ± 0.34*	0.44 ± 0.07*	0.32 ± 0.02*	2.31 ± 0.06*	0.508 ± 0.007*

Data are means ± SEM. The mice were 33 weeks old and fed normal chow or HFD for the last 11 weeks. Reproductive WAT, perirenal WAT, BAT, and brain were weighed and presented as grams or percentage of body weight. *$P < 0.01$, †$P < 0.05$, Mann Whitney *U* test.

of HFD, $AdipoR2^{-/-}$ mice had markedly less reproductive WAT (males −55% and females −67%), perirenal WAT (males −53% and females −69%), and BAT (males −44% and females −26%) weights and plasma levels of leptin (−70% in both males and females) compared with corresponding wild-type controls (Table 2 and 3). After 14 weeks of HFD, female $AdipoR2^{-/-}$ adipocytes in WAT were smaller in size and their BAT contained less vacuoles than wild-type controls (Fig. 3*F*). Female $AdipoR2^{-/-}$ mice had lower liver triglyceride content when fed regular chow and a trend toward decreased levels after HFD compared with wild-type controls. In addition, male $AdipoR2^{-/-}$ mice had markedly lower liver triglyceride content (−61%) than wild-type controls when fed HFD (Table 3). Plasma levels of adiponectin tended to be elevated in $AdipoR2^{-/-}$ mice and was significantly elevated in female $AdipoR2^{-/-}$ mice on regular chow (Table 3). In summary, $AdipoR2^{-/-}$ mice were lean and resistant to HFD-induced body weight gain, obesity, and hepatic steatosis.

AdipoR2-deficient mice have reduced testes weight and increased brain weight. From extensive weighing and histological microscopic examination of >40 different tissues, testes weight was found to be reduced in $AdipoR2^{-/-}$ males compared with wild-type controls. This weight reduction was associated with an atrophy of the seminiferous tubules and aspermia (Fig. 3*F*) but no significant change in plasma testosterone levels (data not shown). Because of the testicular phenotype of $AdipoR2^{-/-}$ males, female $AdipoR2^{-/-}$ mice were analyzed in more detail.

Interestingly, both male and female $AdipoR2^{-/-}$ mice had higher brain weight compared with wild-type controls (Table 2). In addition, the whole $AdipoR2^{-/-}$ brain was stained with β-galactosidase, demonstrating a general expression of AdipoR2 in the brain (Fig. 3*H*). Gross histological examination did not reveal any major differences in the morphology of the brain. *AdipoR1* deficiency did not

TABLE 3
Liver triglyceride content and plasma biochemistry in *AdipoR2* gene knockout mice ($R2^{-/-}$)

	n	Liver triglycerides (% liver wt)	Leptin (ng/ml)	Adiponectin (nmol/l)	Cholesterol (mmol/l)	Triglycerides (mmol/l)	T_3 (nmol/l)	T_4 (nmol/l)
Males								
Chow fed								
Wild-type	6	1.65 ± 0.22	5.8 ± 1.5	294 ± 21	2.6 ± 0.2	1.3 ± 0.2	ND	ND
$R2^{-/-}$	7	1.51 ± 0.29	3.5 ± 0.5	344 ± 38	2.2 ± 0.1*	1.0 ± 0.1	ND	ND
HFD fed								
Wild-type	5	6.67 ± 1.30	21.1 ± 2.6	308 ± 26	5.6 ± 0.3	0.9 ± 0.2	ND	ND
$R2^{-/-}$	5	2.58 ± 0.29†	6.4 ± 1.2†	321 ± 14	3.0 ± 0.2†	0.9 ± 0.1	ND	ND
Females								
Chow fed								
Wild-type	6	2.88 ± 0.32	3.9 ± 0.9	493 ± 13	1.8 ± 0.1	0.7 ± 0.2	1.0 ± 0.06	71.6 ± 4.8
$R2^{-/-}$	6	1.69 ± 0.16*	2.1 ± 0.3	569 ± 17†	1.5 ± 0.1*	0.7 ± 0.1	1.0 ± 0.04	64.3 ± 3.2
HFD fed								
Wild-type	6	5.29 ± 0.76	11.5 ± 2.3	558 ± 94	3.2 ± 0.3	0.3 ± 0.04	1.4 ± 0.08	88.1 ± 6.8
$R2^{-/-}$	6	4.46 ± 0.63	3.1 ± 0.6†	602 ± 42	1.9 ± 0.1†	0.4 ± 0.05	1.2 ± 0.02	78.5 ± 4.2

Data are means ± SEM. The mice were 33 weeks old and fed normal chow or HFD the last 11 weeks. Liver triglyceride content and plasma levels of leptin, adiponectin, cholesterol, triglycerides, T_3, and T_4 were measured as described in RESEARCH DESIGN AND METHODS. ND, not determined. *$P < 0.05$, †$P < 0.01$, Mann Whitney *U* test.

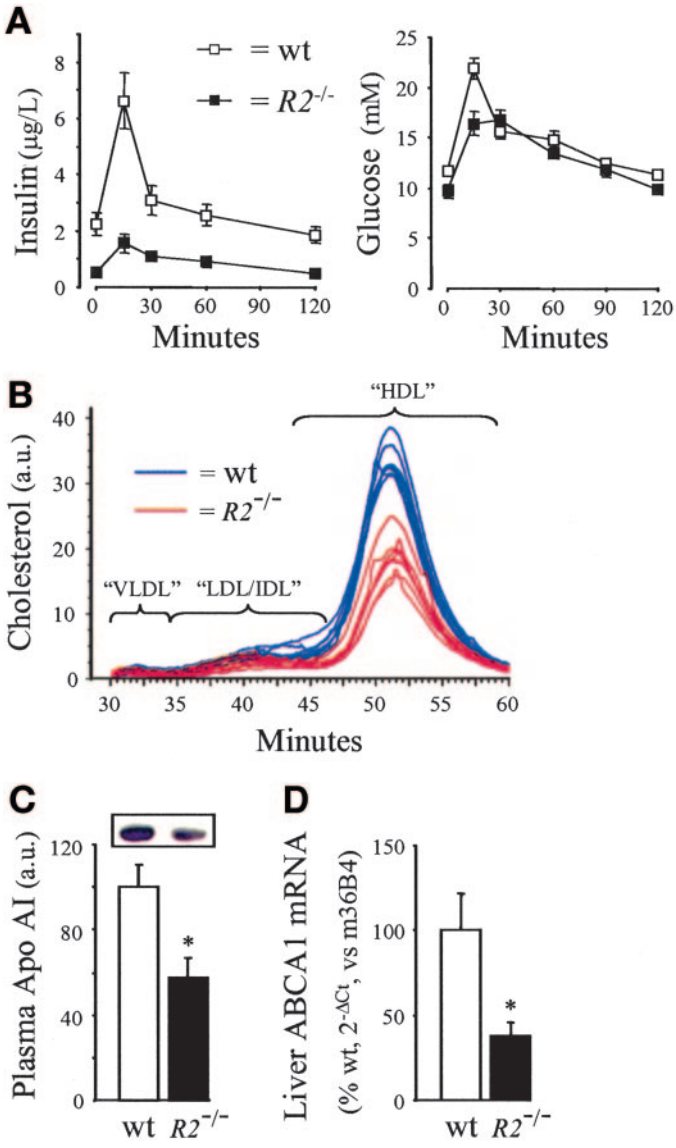

FIG. 4. Oral glucose tolerance, cholesterol distribution profile, plasma Apo AI levels, and liver ABCA1 mRNA expression in *AdipoR2* knockout mice (*R2*$^{-/-}$). *A*: Oral glucose tolerance test on 28-week-old *R2*$^{-/-}$ and wild-type females fed HFD for the last 8 weeks (n = 6). *B*: Cholesterol distribution profiles were measured from individual mice with a size exclusion HPLC system. *C*: Plasma Apo AI was measured with immunoblot analysis. *D*: Liver ABCA1 mRNA expression was measured with quantitative real-time PCR. *B–D*: 33-week-old *R2*$^{-/-}$ and wild-type females fed HFD for the last 14 weeks (n = 5–8). a.u., arbitrary units. Values are means ± SEM. *P < 0.05, Mann-Whitney U test.

affect brain weight (data not shown). Thus, AdipoR2 is of specific importance for brain growth.

AdipoR2-deficient mice have improved glucose tolerance. On HFD, female *AdipoR2*$^{-/-}$ mice had 76% lower fasting insulin levels ($P < 0.01$) and lower insulin and glucose response following an oral glucose tolerance test (Fig. 4*A*). The area under the curve for the insulin response was 70% lower ($P < 0.01$) and the glucose response 9% lower ($P < 0.05$) in *AdipoR2*$^{-/-}$ mice compared with wild-type controls. Thus, *AdipoR2*$^{-/-}$ mice had improved glucose tolerance compared with wild-type controls.

AdipoR2-deficient mice have decreased plasma cholesterol levels. *AdipoR2*$^{-/-}$ mice had reduced total plasma cholesterol levels irrespective of diet, while plasma triglyceride levels were unchanged (Table 3). The decreased plasma cholesterol levels were mainly explained by lower HDL levels (Fig. 4*B*). In addition, plasma apolipoprotein AI (apoAI) levels were reduced in *AdipoR2*$^{-/-}$ mice compared with wild-type controls (Fig. 4*C*), while liver apoAI mRNA expression was unchanged (data not shown). Liver-specific reduction of ATP binding cassette transporter-1 (ABCA1) has been shown to decrease HDL cholesterol and apoAI levels (33). In line with this, *AdipoR2*$^{-/-}$ mice had lower ABCA1 mRNA levels in the liver than wild-type controls (Fig. 4*D*). Thus, *AdipoR2*$^{-/-}$ mice have reduced plasma HDL cholesterol and apoAI levels associated with reduced liver ABCA1 mRNA expression.

Effects of AdipoR2 deficiency on AMPK and PPARα signaling and AdipoR1 expression. *AdipoR2*$^{-/-}$ mice had decreased AdipoR1 mRNA levels in liver and BAT, while the expression was unchanged in WAT, skeletal muscle, and brain compared with wild-type controls (Supplemental Fig. 2*D*). *AdipoR2*$^{-/-}$ mice had increased AdipoR1 protein levels in BAT and skeletal muscle and a trend toward lower levels in liver (Supplemental Fig. 2*E*). Thus, AdipoR1 protein and mRNA levels were regulated differently in BAT and skeletal muscle. The mRNA expression of PPARα and CPT-1α were downregulated in *AdipoR2*$^{-/-}$ livers, while *AdipoR2*$^{-/-}$ mice and wild-type controls had similar levels of PPARα and CPT-1β in skeletal muscle (Supplemental Fig. 2*F*). *AdipoR2*$^{-/-}$ mice did not show increased phosphorylation of AMPK in liver, skeletal muscle, or heart (Supplemental Fig. 2*G*). Thus, enhanced PPARα or AMPK phosphorylation cannot explain the fact that *AdipoR2*$^{-/-}$ mice are lean and have improved glucose tolerance. However, the results supports a specific role of AdipoR2 in the regulation of hepatic PPARα signaling, since PPARα signaling is increased in *AdipoR1*$^{-/-}$ livers, where AdipoR2 is elevated (Supplemental Fig. 1*B*), and decreased in *AdipoR2*-deficient livers (Supplemental Fig. 2*F*).

AdipoR2-deficient mice show increased energy expenditure and locomotor activity. On HFD, female *AdipoR2*$^{-/-}$ mice had increased energy expenditure but unchanged respiratory exchange ratio compared with wild-type controls (Fig. 5*A*). The increased energy expenditure in *AdipoR2*$^{-/-}$ mice was observed both during the light and dark phase of the measured period. The *AdipoR2*$^{-/-}$ mice had markedly increased locomotor and rearing activity at the expense of decreased corner time in activity boxes in both female (Fig. 5*B*) and male *AdipoR2*$^{-/-}$ mice (Supplemental Fig. *3*). The increased activity in *AdipoR2*$^{-/-}$ mice was not explained by alterations in plasma levels of T_3 or T_4 (Table 3). *AdipoR2*$^{-/-}$ BAT contained more and larger mitochondria, indicating a more active tissue compared with wild-type controls (Fig. 5*C*). In contrast, *AdipoR1*$^{-/-}$ mice had no histological changes in BAT (data not shown). Thus, *AdipoR2*$^{-/-}$ mice are protected from HFD-induced obesity and have increased energy expenditure and spontaneous locomotor activity.

DISCUSSION

Adiponectin is a hormone produced exclusively in adipocytes that has both antidiabetes (19–25) and anti-atherosclerotic (25,26) properties. Thus, it was anticipated that depletion of either of its two receptors (28) would result in a phenotype similar to that of adiponectin deficiency

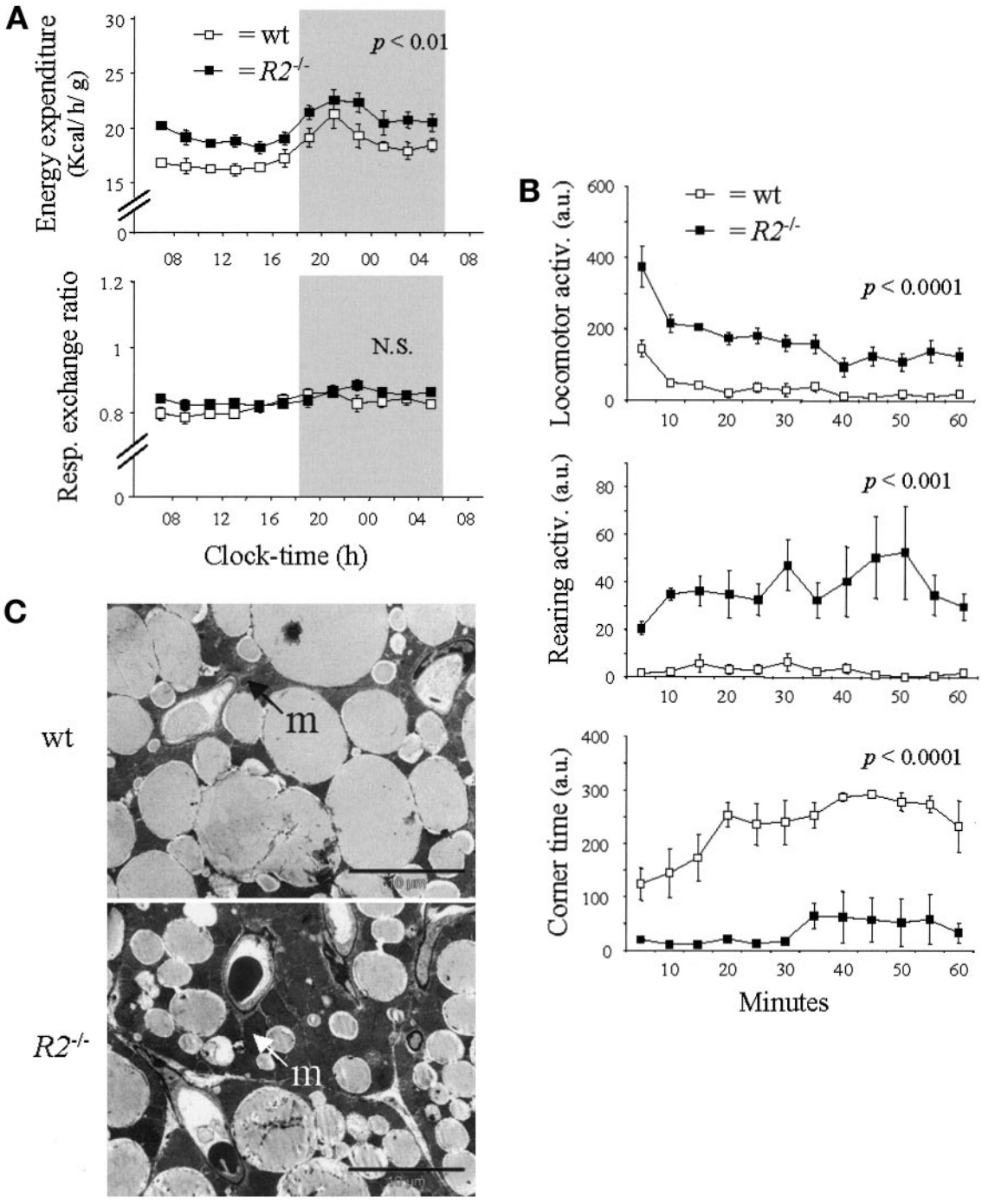

FIG. 5. Energy expenditure, spontaneous locomotor activity, and BAT morphology in *AdipoR2* knockout mice ($R2^{-/-}$). *A*: Energy expenditure and respiratory exchange ratio measured with an open circuit calorimetry system in 32-week-old $R2^{-/-}$ and wild-type females fed HFD for the last 13 weeks (dark period shaded, $n = 8$). *B*: Locomotor and rearing activity and corner time measured with activity boxes in 26-week-old $R2^{-/-}$ and wild-type females fed HFD for the last 6 weeks ($n = 6$). a.u., arbitrary units. *C*: Electron microscopy of BAT from representative 33-week-old $R2^{-/-}$ and wild-type females fed HFD for the last 14 weeks. m, mitochondria. Values are means ± SEM. A mixed ANOVA was used for statistical analyses. N.S., nonsignificant.

(22,27). When the *AdipoR1* gene was deleted, the mice became obese and glucose intolerant. However, *AdipoR2* deficiency resulted in a lean phenotype and resistance to diet-induced obesity associated with improved glucose tolerance and decreased plasma cholesterol levels. The remarkable opposite phenotypes of the two adiponectin receptor knockout mice were associated with opposite effects on energy expenditure and physical activity. *AdipoR1* deficiency resulted in decreased energy expenditure and spontaneous locomotor activity, while *AdipoR2* deficiency had the opposite effect. Thus, AdipoR1 and AdipoR2 are truly yin yang receptors in energy metabolism.

Adiponectin treatment has been reported to cause weight loss in mice (19,32). However, adiponectin deficiency is not associated with changes in body weight or obesity (22). *AdipoR1* deficiency resulted in increased body weight and obesity, which is in line with AdipoR1 mRNA levels in adipose tissue being negatively correlated with BMI in humans (34). The obese phenotype of $AdipoR1^{-/-}$ mice was associated with decreased whole-

body energy expenditure and locomotor activity. Conversely, the resistance to diet-induced obesity in *AdipoR2*$^{-/-}$ mice was associated with increased energy expenditure and locomotor activity. Intracerebroventricular administration of adiponectin in mice has been shown to decrease body weight by elevating energy expenditure (32). Furthermore, *AdipoR2*$^{-/-}$ mice showed increased mitochondrial content in BAT, indicating that the fatty acid oxidation is upregulated in BAT. Incubation of human skeletal muscle cells with adiponectin was recently shown to stimulate mitochondrial biogenesis (35). Thus, the phenotype of the *AdipoR2*$^{-/-}$ mice is similar to that observed following adiponectin treatment. Moreover, from the present results it could be speculated that the lack of effect of adiponectin deficiency on adiposity could be due to a similar decrease in AdipoR1 and AdipoR2 signaling.

Interestingly, AdipoR2 was generally expressed in the entire brain in accordance with previous studies measuring AdipoR2 mRNA levels (28,36). It is debated whether adiponectin crosses the blood-brain barrier (32,37). Thus, the effect of *AdipoR2* deficiency on brain size could be due to altered adiponectin signaling via direct or indirect mechanisms (32,37) or alternatively via another yet unidentified factor interacting with AdipoR2 in the brain. Future studies need to explore the physiological importance of AdipoR2 in the brain. It may be speculated that the corticolimbic dopamine and reward mechanisms (38) are important for the observed effects on energy expenditure and spontaneous locomotor activity. Interestingly, in animal models of obesity such as *ob/ob* mice, obese Zucker rats, and obese-prone Sprague-Dawley rats, dopamine activity is reduced and obesity reversed upon treatment with dopamine agonists (39). In addition, hyperactive and lean *melanin-concentrating hormone-1 receptor*–deficient mice (*MCHR1*$^{-/-}$) have upregulated dopamine receptors (40). Despite a decreased body weight and obesity, *AdipoR2*$^{-/-}$ mice had a trend toward increased food intake in combination with increased expression levels of the orexigenic hypothalamic peptides NPY and AGRP. This resembles the phenotype of the *MCHR1*$^{-/-}$ mice, which are hyperphagic but lean due to increased energy expenditure (41,42). Thus, the increased energy expenditure during the entire 24-h period measured in *AdipoR2*$^{-/-}$ mice overrides an effect of increased food intake on body weight in these mice. It is interesting that *AdipoR2* deficiency has the same stimulatory effect on CRH level in hypothalamus as intracerebroventricular injection of adiponectin (32). CRH is known to be anorexigenic and to increase energy expenditure (8). Thus, in spite of increased expression of orexigenic NPY and AGRP and a trend toward increased food intake, *AdipoR2*$^{-/-}$ mice are lean due to increased energy expenditure associated with increased anorexigenic CRH expression in the hypothalamus.

Similar to adiponectin deficiency (22,27), *AdipoR1* deficiency lead to decreased glucose tolerance, indicating decreased insulin sensitivity. In line with this finding, single nucleotide polymorphisms (SNPs) in *ADIPOR1* have been correlated with decreased glucose tolerance also in humans (43). Similar to adiponectin treatment (20,21), *AdipoR2*$^{-/-}$ mice showed improved glucose tolerance and lower insulin response, indicating improved insulin sensitivity. The opposite effect on glucose tolerance in *AdipoR1*$^{-/-}$ and *AdipoR2*$^{-/-}$ mice could be either direct or indirect effects via differences in obesity. Interestingly, β_3-adrenoceptor agonist treatment of *db/db* mice improved glucose tolerance in association with decreased hepatic AdipoR2 levels (44), indicating that lower expression of AdipoR2 is beneficial for glucose homeostasis. In addition, *AdipoR2*$^{-/-}$ mice had markedly lower plasma levels of leptin following HFD compared to wt controls. This finding indicates improved leptin sensitivity in *AdipoR2*$^{-/-}$ mice that may help to explain the resistance to HFD-induced obesity and improved glucose tolerance and increased energy expenditure (9). Moreover, it was recently shown that SNPs in *ADIPOR2* were linked to type 2 diabetes (45), indicating that AdipoR2 affects insulin sensitivity also in humans.

Female *AdipoR1*$^{-/-}$ mice showed increased plasma cholesterol levels. However, *AdipoR2* deficiency resulted in decreased plasma cholesterol levels mainly in the HDL fraction. Recently, an SNP cluster in *ADIPOR2* was shown to be associated with decreased VLDL cholesterol levels (46), indicating that decreased AdipoR2 levels may be associated with decreased VLDL and not HDL cholesterol levels in humans. Whether *AdipoR2* deficiency will be protective against atherosclerosis similar to adiponectin treatment (25) remains to be investigated.

It is difficult to understand the similar phenotype of adiponectin treatment and *AdipoR2* deficiency, since AdipoR2 has been shown to be activated by adiponectin (28). One explanation for the opposing effects of *AdipoR1* and *AdipoR2* deficiency could be receptor signaling. AdipoR1 has been shown to bind globular adiponectin with higher affinity than full-length adiponectin, while AdipoR2 seems to be an intermediate-affinity receptor. In addition, AdipoR1 and AdipoR2 may form both homo- and heteromultimers (28). It has been shown that the relative abundance of hybrids of the insulin and insulin-like growth factor-I receptors is higher in diabetic patients and associated with reduced insulin binding affinity (47). Therefore, it could be speculated that lack of adiponectin receptor heteromultimers in the *AdipoR2*$^{-/-}$ mice may lead to an improved AdipoR1 signaling. Moreover, plasma adiponectin levels were increased or tended to be increased in *AdipoR2*$^{-/-}$ mice, and recently an SNP cluster in *ADIPOR2* was shown to be associated with higher adiponectin levels in humans (46). Thus, decreased AdipoR2 expression may lead to increased adiponectin secretion, which together with increased AdipoR1 signaling may be protective against HFD-induced metabolic alterations. However, improved basal PPARα or AMPK signaling was not detected in *AdipoR2*$^{-/-}$ mice, indicating that other signaling pathways are involved or that the phenotype of *AdipoR2*$^{-/-}$ mice is explained by another mechanism.

In summary, this study shows for the first time that the adiponectin receptors (28) are involved in the control of glucose, fat, and energy metabolism in vivo. *AdipoR1* deficiency resulted in increased adiposity associated with decreased glucose tolerance, physical activity, and energy expenditure, effects that in part overlap the described phenotype of adiponectin-deficient mice (22,27). On the other hand, *AdipoR2* deficiency resulted in resistance to HFD-induced obesity and glucose intolerance associated with increased physical activity and energy expenditure and decreased plasma cholesterol levels. Another remarkable effect of *AdipoR2* deficiency was increased brain size, showing the importance of this receptor for brain development. Thus, activating AdipoR1 or antagonizing AdipoR2 may be attractive therapeutic approaches for treatment of obesity, type 2 diabetes, and cardiovascular disease.

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