Original Article

Melanin-Concentrating Hormone Receptor 1 Deficiency Increases Insulin Sensitivity in Obese Leptin-Deficient Mice Without Affecting Body Weight

Mikael Bjursell,[1,2] Anna-Karin Gerdin,[2] Karolina Ploj,[4] David Svensson,[5] Lennart Svensson,[3] Jan Oscarsson,[1,4] Michael Snaith,[2] Jan Törnell,[1,2] and Mohammad Bohlooly-Y[1,2]

The hypothalamic peptide melanin-concentrating hormone (MCH) plays important roles in energy homeostasis. Animals overexpressing MCH develop hyperphagia, obesity, and insulin resistance. In this study, mice lacking both the MCH receptor-1 (MCHr1 knockout) and leptin (*ob/ob*) double-null mice (MCHr1 knockout *ob/ob*) were generated to investigate whether the obesity and/or the insulin resistance linked to the obese phenotype of *ob/ob* mice was attenuated by ablation of the MCHr1 gene. In MCHr1 knockout *ob/ob* mice an oral glucose load resulted in a lower blood glucose response and markedly lower insulin levels compared with the *ob/ob* mice despite no differences in body weight, food intake, or energy expenditure. In addition, MCHr1 knockout *ob/ob* mice had higher locomotor activity and lean body mass, lower body fat mass, and altered body temperature regulation compared with *ob/ob* mice. In conclusion, MCHr1 is important for insulin sensitivity and/or secretion via a mechanism not dependent on decreased body weight. *Diabetes* 55:725–733, 2006

It is well known that obesity is a major contributing factor to the development of insulin resistance and type 2 diabetes. Diabetic patients can often reverse their insulin resistance by decreasing their body weight. However, the mechanism linking obesity and insulin sensitivity is poorly understood. Mutations in the ob gene results in obesity and insulin resistance, and this may serve as a model for increased food intake leading to obesity and insulin resistance.

Melanin-concentrating hormone (MCH) was originally isolated and sequenced from salmon pituitary and was named MCH due to its role in fish pigmentation (1). In rodents, MCH is predominantly expressed in the lateral hypothalamus and zona incerta, areas involved in the regulation of energy homeostasis and ingestive behavior. MCH fibers project broadly throughout the central nervous system, suggesting that MCH may function as a neurotransmittor and/or a neuromodulator to regulate behavioral functions (2,3). There is much data to support a role for MCH in the regulation of food intake, body weight, and energy balance. This includes the expression profile of MCH in the lateral hypothalamus and the fact that both fasting and leptin deficiency result in elevated MCH levels (4). Overexpression of MCH in transgenic mice given high-fat diet results in weight gain, hyperphagia, obesity, and insulin resistance (5). Chronic infusion of MCH results in hyperphagia and obesity in mice (6). Mice deficient of MCH are hypophagic, lean, and have an increased metabolic rate (7). Also, treatment of rats with an MCH receptor antagonist results in inhibition of food intake and weight loss (8). Furthermore, the central role of MCH and the MCH receptor-1 (MCHr1) for energy balance is supported by the phenotypes of MCHr1 knockout mice (9–11). One important difference between the MCH knockout and MCHr1 knockout mice is that the latter are slightly hyperphagic but still lean due to increased energy expenditure. The rodent receptor for MCH, MCHr1 (or SLC-1/GPR24), is distributed mainly in the central nervous system but also in the pituitary, skeletal muscle, and adipocytes (12–14).

Mice carrying a defective ob gene develop obesity and insulin resistance, making these mice a good model for hyperphagia-induced insulin resistance. The leptin-deficient *ob/ob* mice are obese, hyperphagic, hyperinsulinemic, insulin resistant, and have reduced body temperature and energy expenditure (rev. in 15). Leptin is mainly secreted from adipocytes and functions to suppress appetite, increase energy expenditure, and raise core temperature in mice (15) as well as sympathetic activity in brown adipose tissue (BAT) (16). Furthermore, leptin increases glucose, insulin, and glucagon levels by mechanisms requiring intact sympathetic nerves (17). In contrast, MCH may decrease sympathetic activity since MCHr1 knockout mice have an increased sympathetic tone (11) and MCH infusion reduces body temperature in mice (6). These findings suggest that MCH and leptin have opposing effects on the autonomic nervous system.

The aim of this study was to investigate the role of MCHr1 in the phenotype of the leptin-deficient obese mice. Insulin sensitivity and glucose clearance were studied by performing an oral glucose tolerance test. We also examined body

From the [1]Department of Physiology and Pharmacology, Gothenburg University, Gothenburg, Sweden; [2]AstraZeneca Transgenics & Comparative Genomics, AstraZeneca, Mölndal, Sweden; the [3]Department of Molecular Pharmacology, AstraZeneca, Mölndal, Sweden; the [4]Department of Integrative Pharmacology, AstraZeneca, Mölndal, Sweden; and the [5]Department of Biostatistic, AstraZeneca, Mölndal, Sweden.

Address correspondence and reprint requests to Mikael Bjursell, AstraZeneca, Mölndal S-43183 Mölndal, Sweden. E-mail: mikael.bjursell@astrazeneca.com.

Received for publication 6 October 2005 and accepted in revised form 16 December 2005.

BAT, brown adipose tissue; CRH, corticotrophin-releasing hormone; MCH, melanin-concentrating hormone; MCHr1, MCH receptor-1; MSH, melanocyte-stimulating hormone; RER, respiratory exchange ratio; SCD-1, stearoyl-CoA desaturase-1; UCP-1, uncoupling protein-1.

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

weight, body fat, food intake, and energy expenditure as well as serum chemistry and liver fat accumulation. Moreover, body temperature and expression levels of uncoupling protein-1 (UCP-1) in BAT were measured as an indication of effects on the autonomic nervous system.

RESEARCH DESIGN AND METHODS

MCHr1 knockout mice were generated at AstraZeneca Transgenics and Comparative Genomics as reported previously (11) and backcrossed seven generations to C57BL/6. The ob/+ mice were supplied by Harlan Olac (Blackthorn, Bicester, U.K.) fully backcrossed to C57BL/6. The mice were group housed and fed standard diet containing 4% total lipids, 18.5% protein, 55.7% carbohydrates, and 3.5% fibers (R36; Lactamin, Stockholm, Sweden) in accordance with good animal practice. After 4 weeks of age the mice were single housed. Genotyping was done by PCR. For the MCHr gene, one primer was located upstream in the short arm (5′-GAGTCCCCAGCATTGAGAAC-3′), a second primer located in the exon part (5′-AGCTCCCACTGACATCACCT-3′), and the third located in the PGK-NEO promoter (5′-AGCGCATGCTCCA-GACTGCCTT-3′). The ob mutation was detected using a combined PCR and restriction digest approach utilizing the fact that the ob mutation creates a new restriction enzyme site. PCR fragments were amplified using the primers (5′-GACTTCATTCCTGGGCTTCA-3′) and (5′-ATCCAGGCTCTCTGGCTTCT-3′), and the resultant products were digested with *Dde*I (In Vitro Sweden, Stockholm, Sweden). The study was performed in accordance with the ethical certificate approved by the local ethical committee for animal experimentation.

Generation of the MCHr1 knockout ob/ob mice. In the first step of breedings, MCHr1 knockout (−/−) mice were crossed to ob/+ mice to generate offspring that were heterozygous for both MCHr1 (+/−) and the ob mutation (ob/+). Next, these compound heterozygous mice were intercrossed and MCHr1 knockout (−/−) ob/+ as well as MCHr1 wild-type (+/+) ob/+ were identified. Finally, MCHr1 knockout (−/−) ob/+ were intercrossed to produce male MCHr1 knockout *ob/ob* and MCHr1 knockout ob wild-type (+/+) for the study. Also, MCHr1 wild type ob/+ were intercrossed to give male MCHr1 wild-type *ob/ob* and MCHr1 wild-type ob wild-type (+/+) mice that were studied.

Body growth, food intake, and fecal analyses. Body weights were measured weekly between 4 and 23 weeks of age. To measure food intake, cages (23 × 16 cm) were prepared with normal diet and dried at 80°C for 1 h to correct for any differences in humidity. After 12 h at room temperature the cages were accurately weighed. Mice at 20 weeks of age were fasted for 12 h, over the dark period, before being put in the preweighed cages with free access to food and water. The mice were left in the cage for 48 h and were then returned to their original cages. All excrements were collected for further analysis. The cages were reincubated 1 h at 80°C to dry out water spill and urine and reweighed after 12 h. 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 (C 5000; IKA Werke, Staufen, Germany).

Indirect calorimetry, activity, and cold exposure. Indirect calorimetry was measured as previously described (18) in 24-week-old mice at 22°C. Measures were taken for 5 s every 9 min. Data from the first 2 h was excluded from analysis to allow for acclimatization to the novel environment. Locomotor activity was monitored both in the calorimetric chambers over 48 h and in activity boxes (Kungsbacka mät-och reglerteknik, Kungsbacka, Sweden) over 1 h, 10:00–11.00 A.M. Rectal temperatures were recorded in 26-week-old conscious mice using a rectal probe (components supplied by ELFA, Järfälla, Sweden). Temperature readings were taken 1 min before and at the following time points after the mice had been placed in a 6°C-cold room: 15, 30, 45, and 60 min.

Quantification of body fat and lean body mass. Body fat (%) and lean body mass (g) were determined by densitometry in 25-week-old mice as previously described (19).

Glucose tolerance test. Mice aged 28 weeks were fasted for 12 h (12:00 A.M. to 12:00 P.M.) before oral administration of glucose solution (2 g/kg). Tail blood was collected 1 min before and 5, 15, 30, and 60 min after glucose administration. Glucose levels were measured by using an Accu-chek device and plasma calibrated test strips (Roche Diagnostics, Mannheim, Germany). Insulin levels were determined by using an ultrasensitive insulin enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL). To further investigate insulin sensitivity, a QUICKI calculation was done as follows: $1/[\log(I_0)+\log(G_0)]$, where I_0 is the fasting insulin (ng/μl) and G_0 is the fasting glucose (mmol/l). QUICKI has previously been shown to correlate well to insulin sensitivity in humans (20).

Sample collection. Tissues and serum were collected as previously described (21). Livers and BAT were weighed before freezing. Triglyceride content of liver biopsies were determined using a Triglyceride CP kit (ABX Diagnostics, Montepellier, France).

Serum analysis. Corticosterone was measured using a radioimmunoassay kit (Code RPA 548; Amersham Biosciences, Uppsala, Sweden). For triglycerides and total cholesterol, a CHOD-PAP (TG/GB, no. 12146029216, Cholesterol no. 2016630; Roche Diagnostics, Mannheim, Germany) was used and nonesterified fatty acids levels were analyzed using nonesterified fatty acids C assay kit (Cat. no. 999-75406; Wako Chemicals, Neuss, Germany). The cholesterol distribution profiles were measured as previously described (19).

Determination of mRNA expression. Total RNA extraction, cDNA synthesis and quantification was performed as previously described (21). Sequences for the primers and probes used in the Taqman PCR are presented in Table 1.

Statistics. Values are presented as group means ± SE. Statistical analysis of multiple comparisons was done by using one-way ANOVA followed by Bonferroni post hoc test, and pairwise comparisons were done by using Student's *t* test. In some experiments, more than two groups were investigated over time. In these cases, a type of two-way ANOVA (a standard repeated-measures model) was used with time and group as main effects and with a time and group interaction term included in the model. The within-animal correlation structure was modeled through autoregressive1 covariance structures. Group comparisons were made using simultaneous confidence intervals of Tukey type, and in some cases by post hoc Holm-Bonferroni adjustments. The analysis was conducted using the statistical software SAS PROC MIXED. The level of significance was taken as $P < 0.05$.

TABLE 1
Sequences for Taqman PCR primers and probes

Gene	Sequence 5′-3′
UPC-1 FP	CGATGTCCATGTACACCAAGGA
UPC-1 RP	CCCGAGTCGCAGAAAAGAAG
UPC-1 probe	ACCGACGGCCTTTTTCAAAGGGTTTG
SCD-1 FP	CATCATTCTCATGGTCCTGCT
SCD-1 RP	CCAGTCGTACACGTCATTTT
CRH FP	CGCCCATCTCTCTGGATCTC
CRH RP	TTGCTGTGAGCTTGCTGAGC

UPC-1 was analyzed with FAM-labeled probe, and SCD-1 and CRH were analyzed using SYBR Green dye. FP, forward primer; RP, reverse primer.

RESULTS

To study the importance of a functional MCHr1 for the phenotype of the obese leptin-deficient *ob/ob* mice, MCHr1 knockout *ob/ob* double-null mice were generated and compared with *ob/ob* littermates.

Body weights, food intake, and fecal analyses. Both MCHr1 knockout *ob/ob* and the *ob/ob* mice had significantly higher body weight after 6 weeks of age compared with the wild-type mice (37 and 36% increase, respectively). The body weight of the MCHr1 knockout *ob/ob* and *ob/ob* groups were not distinguishable and did not differ statistically at any time point during the study. At 23 weeks of age, both MCHr1 knockout *ob/ob* and *ob/ob* mice had >40% higher body weight compared with the wild-type mice (Fig. 1*A*). At 30 weeks of age the MCHr1 knockout *ob/ob* and *ob/ob* mice were still similarly overweight (62.8 ± 2.4 g vs. 63.7 ± 2.0 g, respectively). There was no difference in food intake measured over 48 h between the MCHr1 knockout *ob/ob* and *ob/ob* mice. However, both groups consumed more diet than the wild-type mice (Fig. 1*B*). Energy in feces were not different between the MCHr1 knockout *ob/ob*, *ob/ob*, or wild type (data not shown).

Glucose tolerance, serum chemistry, and corticotrophin-releasing hormone expression. Fasting glucose levels were not significantly different between the groups (Fig. 2*A*). Interestingly, fasting insulin was 49% lower in the MCHr1 knockout *ob/ob* mice compared with the *ob/ob* mice (31 ± 8 and 61 ± 9 ng/ml, respectively, Fig. 2*B*). QUICKI calculations revealed that the MCHr1 knockout

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

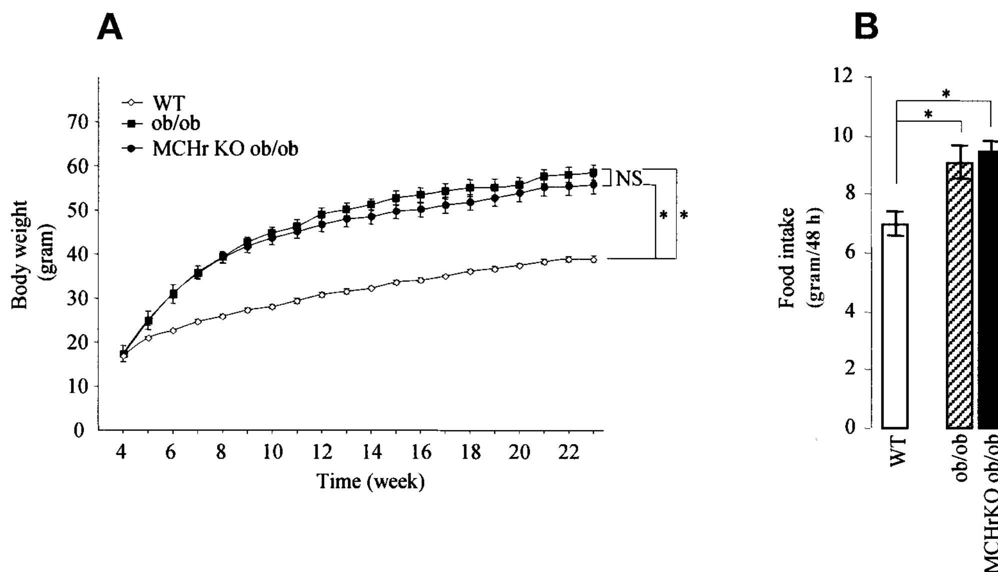

FIG. 1. Body weight and food intake. *A*: Body weights of MCHr1 knockout *ob/ob* (n = 10 ●), *ob/ob* (n = 7 ■), and wild-type (n = 5 ○) mice over the course of 23 weeks. *P < 0.05 differences between groups over time (linear repeated-measure model). *B*: Total food intake of the same MCHr1 knockout *ob/ob*, *ob/ob*, and wild-type mice measured over 48 h. *P < 0.05 (one-way ANOVA followed by Bonferroni post hoc test).

ob/ob mice had significantly higher index compared with the *ob/ob* (0.46 ± 0.04 and 0.38 ± 0.01, respectively, $P < 0.05$). QUICKI in wild-type mice was 0.96 ± 0.05. The MCHr1 knockout *ob/ob* mice had significantly lower glucose levels 15 min after glucose administration when compared with the *ob/ob* mice (23%, $P < 0.05$). At time points 15 and 60 min after glucose administration, the MCHr1 knockout *ob/ob* had 62 and 69% lower insulin levels compared with the *ob/ob* mice and were significantly lower over the entire time course ($P < 0.001$, Fig. 2*B*). At 60 min after the glucose load, both groups had similar glucose levels and neither group differed from wild-type mice (Fig. 2*A*). Thus, the decline in glucose levels 15 min after the glucose load in *ob/ob* mice was significantly faster compared with the MCHr1 knockout *ob/ob* mice ($P < 0.05$). For comparison, MCHr1 knockout mice were also analyzed regarding glucose tolerance. The MCHr1 knockout mice had 43% lower fasting insulin levels compared with wild-type mice (0.7 ± 0.2 and 1.2 ± 0.1 ng/ml, respectively). The glucose administration to the MCHr1 knockout mice resulted in significantly lower serum insulin levels at all time points compared with wild-type mice ($P < 0.01$, Fig. 2*B*). The serum corticosterone levels were >40% lower in the MCHr1 knockout *ob/ob* mice compared with the *ob/ob* mice (Table 2). The expression level of corticotrophin-releasing hormone (CRH) in hypothalamus was not different between the groups (data not shown). Serum levels of triglycerides and cholesterol as well as the cholesterol distribution in the different lipoprotein classes were not different between the MCHr1 knockout *ob/ob* mice and *ob/ob* mice (Fig. 2*C* and Table 2).

Indirect calorimetry, locomotor activity, and body composition. The 48-h indirect calorimetric measurement revealed no difference in respiratory exchange ratio (RER) or energy expenditure between the MCHr1 knockout *ob/ob* and the *ob/ob* mice even though both the groups differed from wild-type mice (Fig. 3*A*). The spontaneous locomotor activity was measured both over 48 h in the indirect calorimetry cages (Fig. 3*C*) as well as over 1 h during daytime in activity boxes (Fig. 3*D*). The MCHr1 knockout *ob/ob* mice had higher locomotor activity compared with the *ob/ob* mice both over the 48 h time course ($P < 0.001$) and over 1 h during light time ($P < 0.05$). The body composition measurements showed that the MCHr1 knockout *ob/ob* mice had lower percentage of fat compared with the *ob/ob* groups (45 ± 1 and 51 ± 1%, respectively; Fig. 4*A*). Also, the MCHr1 knockout *ob/ob* mice had significantly more lean mass normalized for body length compared with the *ob/ob* mice (2.8 ± 0.05 and 2.6 ± 0.04 g/cm, respectively, Fig. 4*B*).

Thermoregulation. At room temperature, there were no statistically significant differences in body temperature between the groups of mice (MCHr1 knockout *ob/ob* 36.8 ± 0.2°C, *ob/ob* 36.6 ± 0.2°C, and wild type 37.2 ± 0.2°C; Fig. 5*A*). After 60 min at 6°C, the body temperatures of the MCHr1 knockout *ob/ob* mice were significantly higher compared with the *ob/ob* mice (36.9 ± 0.3 and 36.2 ± 0.2°C, respectively, $P < 0.05$, Fig. 5*A*). However,

TABLE 2
Serum levels in wild-type, *ob/ob*, and MCHr1 knockout *ob/ob* mice (n = 5–10)

	Wild type	*ob/ob*	MCHr1 knockout *ob/ob*
Corticosterone (ng/ml)	139.7 ± 12.0	620.2 ± 85.7*	355.3 ± 35.3*†
Triglyceride (mmol/l)	1.3 ± 0.1	1.0 ± 0.1	1.5 ± 0.2
Nonesterified fatty acid (mmol/l)	0.9 ± 0.1	0.9 ± 0.2	0.5 ± 0.0*
Cholesterol total (mmol/l)	2.5 ± 0.1	6.9 ± 0.5*	5.8 ± 0.2*

Data are mean ± SE. *$P < 0.05$ compared with wild type. †$P < 0.05$ compared with *ob/ob* (one-way ANOVA followed by Bonferroni post hoc test).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

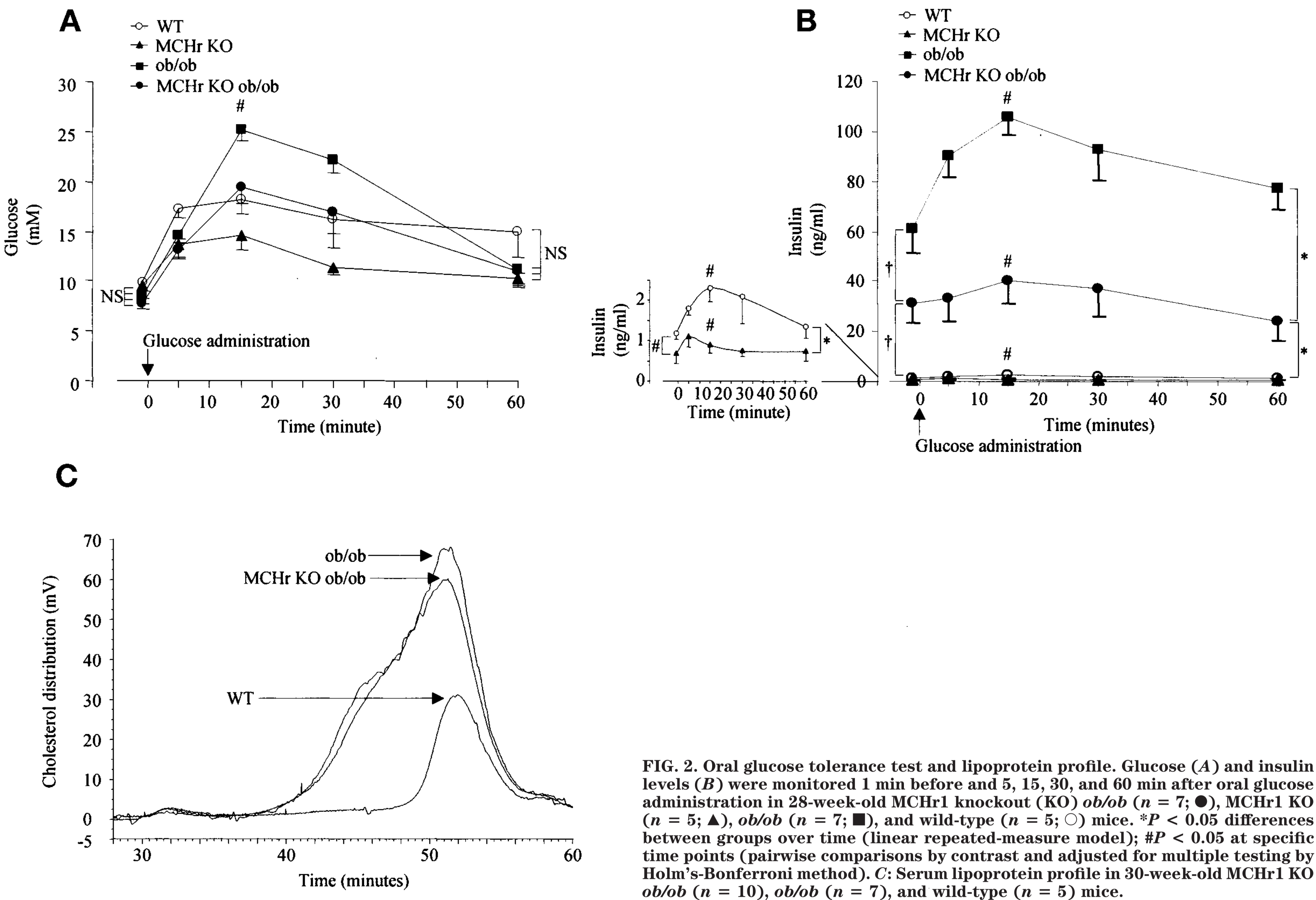

FIG. 2. Oral glucose tolerance test and lipoprotein profile. Glucose (*A*) and insulin levels (*B*) were monitored 1 min before and 5, 15, 30, and 60 min after oral glucose administration in 28-week-old MCHr1 knockout (KO) *ob/ob* ($n = 7$; ●), MCHr1 KO ($n = 5$; ▲), *ob/ob* ($n = 7$; ■), and wild-type ($n = 5$; ○) mice. $^*P < 0.05$ differences between groups over time (linear repeated-measure model); $\#P < 0.05$ at specific time points (pairwise comparisons by contrast and adjusted for multiple testing by Holm's-Bonferroni method). *C*: Serum lipoprotein profile in 30-week-old MCHr1 KO *ob/ob* ($n = 10$), *ob/ob* ($n = 7$), and wild-type ($n = 5$) mice.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

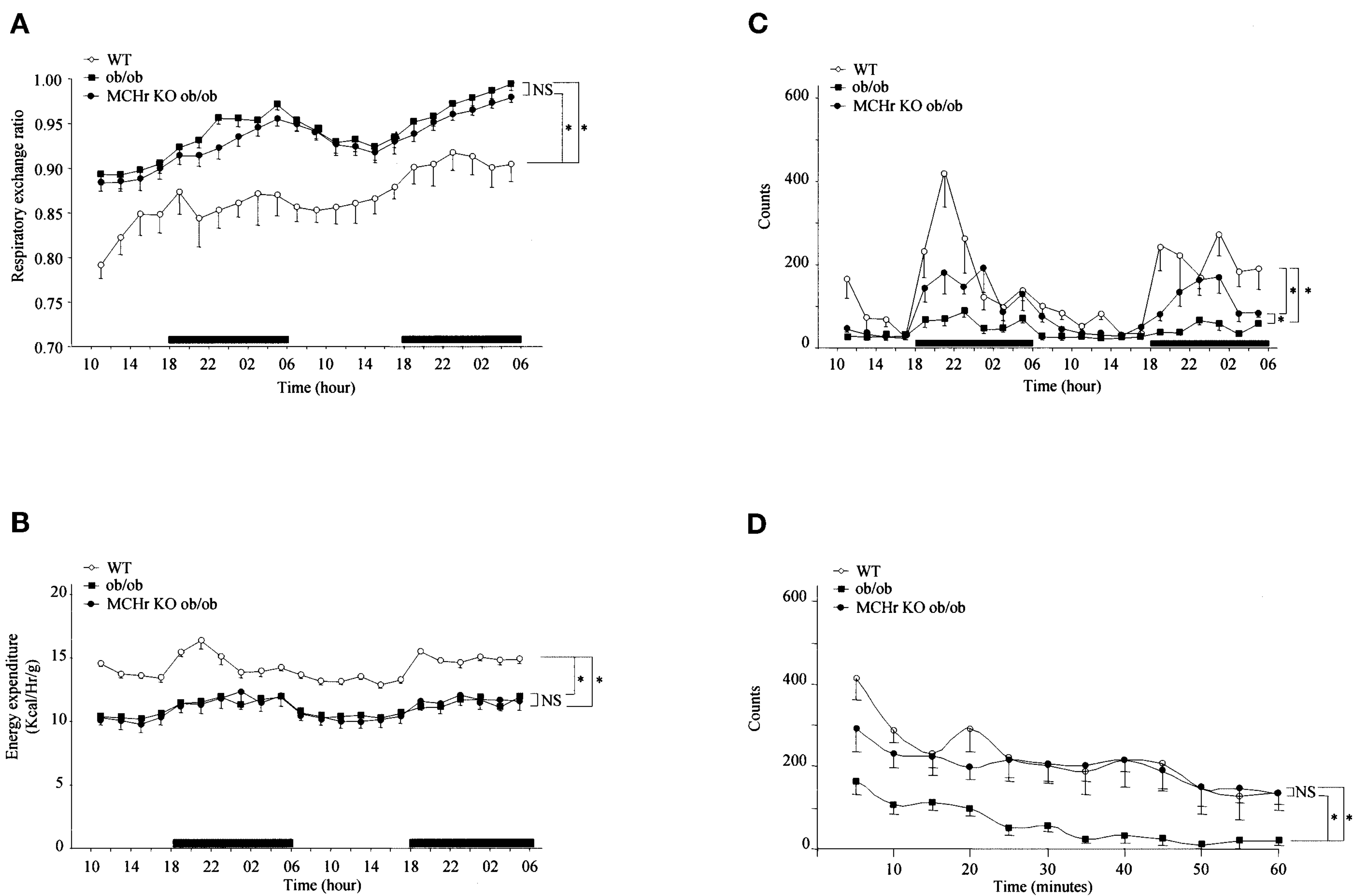

FIG. 3. Indirect calorimetry and locomotor activity of 24-week-old mice. RER (*A*) and energy expenditure (*B*) related to body weights over 48 h. Spontaneous locomotor activity over 48 h (*C*) and locomotor activity in the activity cages during 1 h at daytime (*D*). MCHr1 knockout *ob/ob* (n = 10; ●), *ob/ob* (n = 6; ■), and wild-type (n = 5; ○) mice were studied. *$P < 0.05$ differences between groups over time (linear repeated-measure model).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

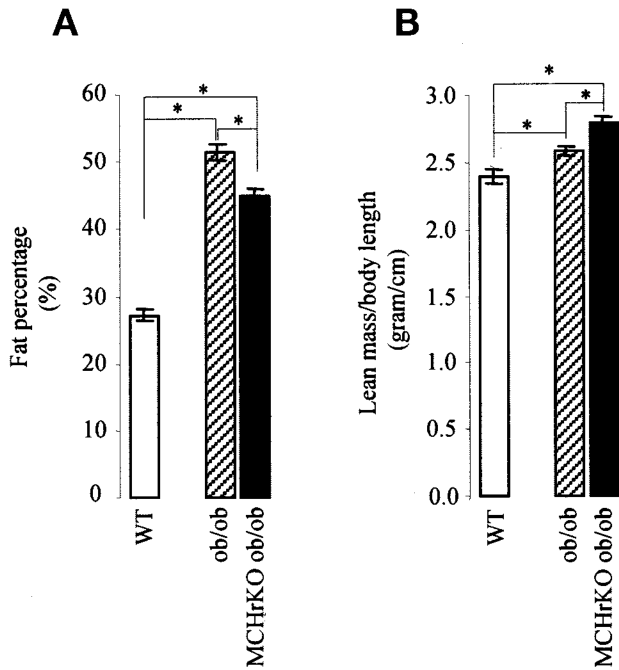

FIG. 4. Body composition of 25-week-old mice. Fat percentage (*A*) and lean mass (*B*) normalized for body length was assessed in MCHr1 knockout *ob/ob* ($n = 10$), *ob/ob* ($n = 7$), and wild-type ($n = 5$) mice by dual-energy X-ray absorptiometry analysis. **$P < 0.05$ **(one-way ANOVA followed by Bonferroni post hoc test).

neither BAT weights normalized for individual lean masses nor mRNA expression of UCP-1 in BAT were significantly different between the MCHr1 knockout *ob/ob* and the *ob/ob* groups (Fig. 5*B*).

Liver triglyceride content and stearoyl-CoA desaturase-1 (SCD-1) mRNA expression. Liver weights normalized to lean mass and liver triglycerides content were not different between the MCHr1 knockout *ob/ob* and *ob/ob* mice (Fig. 6*A*). A high SCD-1 enzyme expression has been shown to be important for the phenotype of the *ob/ob* mice (22). Both the MCHr1 knockout *ob/ob* and the *ob/ob* mice had similar levels of SCD-1 expression, showing that the MCHr1 is not involved in the regulation of SCD-1 (Fig. 6*B*).

DISCUSSION

In this study, leptin-deficient (*ob/ob*) mice also lacking the MCHr1, were examined to investigate the importance of a functional MCHr1 for the obese phenotype of the *ob/ob* mice. We found that MCHr1 knockout *ob/ob* mice had a body weight gain, food intake, RER, energy expenditure, and lipoprotein profile comparable to the *ob/ob* mice. At 30 weeks of age, the MCHr1 knockout *ob/ob* and the *ob/ob* mice were both severely overweight with average body weights of >60 g. Interestingly, an oral glucose tolerance test revealed that the MCHr1 knockout *ob/ob* had lower glucose levels 15 min after glucose administration, lower insulin response, and lower fasting insulin levels when compared with the *ob/ob* mice. QUICKI, which has been previously validated as an index that correlates well with insulin sensitivity (20), was higher in the MCHr1 knockout *ob/ob* mice compared with the *ob/ob* mice. These findings clearly indicate enhanced insulin sensitivity and suggest that the MCHr1 contributes to the poor insulin sensitivity of the *ob/ob* mice. In addition, the MCHr1 knockout *ob/ob* mice had higher lean mass, lower body fat, higher locomotor activity, and a better cold tolerance than the *ob/ob* mice.

It is well known that obesity is the primary risk factor for insulin resistance and type 2 diabetes (rev. in 3). The MCHr1 knockout *ob/ob* mice had a significantly lower glucose and insulin response to an oral glucose load compared with *ob/ob* mice. Lower insulin levels indicate improved insulin sensitivity, suggesting a role for the MCHr1 in the regulation of insulin sensitivity. This is supported by a recent publication showing that central MCH injection to rats induced insulin resistance without affecting body weight (24). The higher lean body mass and the lower body fat may partly explain the improved insulin sensitivity. However, the marked decrease in insulin levels is probably also attributed to other physiological alterations than the small changes in body composition. It is interesting to note that, in humans, exercise improves glucose tolerance. Thus, the increased locomotor activity in MCHr1 knockout *ob/ob* mice may also play a part in the improved insulin sensitivity.

MCH and the MCHr1 also affect glucose metabolism in nonobese animals. MCHr1 knockout mice had lower fasting insulin and insulin response to an oral glucose challenge compared with wild-type mice in line with previous findings of low insulin levels in MCHr1 knockout mice (9,10). An additional mechanism by which MCH may affect glucose metabolism is insulin secretion. MCHr1 mRNA is present in insulin-producing B-cells, and MCH can stimulate insulin secretion (25). It has been suggested that MCH, as a neurotransmitter, plays an important role in the autonomic aspects of metabolism, including control of the pancreas (26). MCH is also present in rat plasma (14). Thus, since the MCHr1 knockout *ob/ob* mice have somewhat slower glucose clearance, we cannot rule out that MCHr1 deficiency in B-cells results in decreased insulin secretion in the MCHr1 knockout *ob/ob* mice.

Glucocorticoids are involved in glucoregulation by reduction of hepatic and peripheral insulin sensitivity (27). It is well known that leptin-deficient mice have increased glucocorticoid levels (28,29). In contrast, both MCH and neuropeptide EI can stimulate the hypothalamic-pituitary-adrenal axis and increase corticosterone levels (30). In our study, the MCHr1 knockout *ob/ob* mice had 40% lower corticosterone level compared with the *ob/ob* mice. However, the expression level of CRH was not statistically different between the groups, suggesting that the effect of MCHr1 was downstream of CRH. Lower corticosterone levels in the MCHr1 knockout *ob/ob* mice might contribute to the lower insulin levels and the improved glucose metabolism. The inhibitory effects of leptin on the hypothalamic-pituitary-adrenal axis may thus involve the MCHr1 and the MCH pathway, as also supported by findings in the MCH knockout *ob/ob* mice (31).

There is a large body of evidence stating that MCH and the MCHr1 affect body weight, food intake, and energy balance as well as leptin and insulin action (4–11,14,25). The orexigenic peptides neuropeptide Y and MCH are upregulated in the *ob/ob* mice, which may be an explanation for the observed hyperphagia (32,33). The MCHr1 knockout *ob/ob* mice used in this study were as hyperphagic as the *ob/ob* mice. Therefore, the contribution of these signals to the hyperphagia of *ob/ob* mice would appear not to require functional MCHr1. Moreover, the MCHr1 is not required as a mediator for another orexigenic peptide: ghrelin (21).

The MCHr1 knockout *ob/ob* mice had increased locomo-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

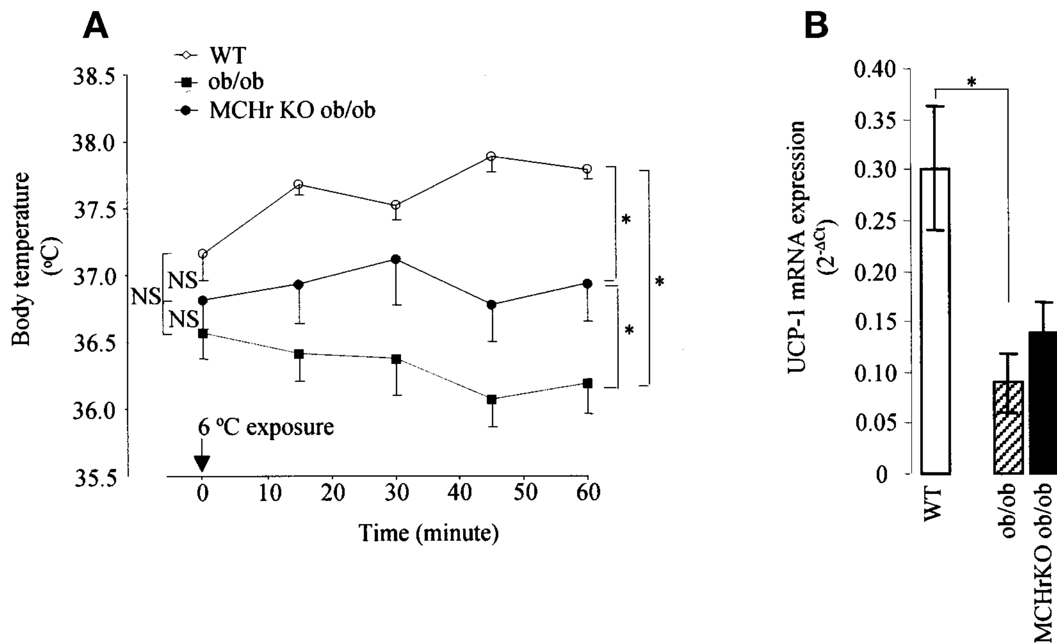

FIG. 5. Body temperature in 26-week-old mice and UCP-1 mRNA expression in BAT in 30-week-old mice. *A*: Rectal body temperature at 1 min before and 15, 30, 45, and 60 min after exposure to a 6°C environment measured in MCHr1 knockout (KO) *ob/ob* (n = 7; ●), *ob/ob* (n = 7; ■), and wild-type (n = 5; ○) mice. *P < 0.05 differences between groups over time (linear repeated-measure model). *B*: mRNA expression of UCP-1 in BAT in MCHr1 knockout *ob/ob* (n = 10), *ob/ob* (n = 7), and wild-type (n = 5) mice. *P < 0.05 (one-way ANOVA followed by Bonferroni post hoc test).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

tor activity compared with their *ob/ob* littermates, which could contribute to the higher lean mass and the lower body fat. It is well established that dopamine and dopamine receptors alter locomotor activity (rev. in 34). Recently, it was shown that MCHr1 knockout mice have upregulated mesolimbic dopamine receptors and norepinephrine transporters, indicating that the MCHr1 might modulate mesolimbic monoamine functions (35). These findings might partially explain the increased locomotor activity.

The high RER indicates that the MCHr1 knockout *ob/ob* and the *ob/ob* mice mainly use carbohydrates as a source of energy while fat is stored. Hepatic fat content was high in both the obese groups. SCD-1 is involved in the biosynthesis of monounsaturated fats and is highly upregulated in *ob/ob* mice (22). The level of SCD-1 mRNA was not different between the MCHr1 knockout *ob/ob* and the *ob/ob* mice. This observation differs from findings in the MCH knockout *ob/ob* mice that had reduced SCD-1 expression compared with *ob/ob* mice, despite high hepatic triglycerides content (31). Thus, MCH and the MCHr1 may differ in the regulation of the SCD-1 gene.

The leptin-deficient MCH knockout and MCHr1 knockout mice also differ with respect to body growth, lean mass, insulin levels, and energy expenditure. Basal core body temperature was not different between MCHr1 knockout *ob/ob* and *ob/ob*, in contrast to findings in the MCH knockout *ob/ob* mice (31). As shown for MCH knockout *ob/ob* mice, the MCHr1 knockout *ob/ob* had improved thermogenesis in response to cold exposure, but the decrease in body temperature was less pronounced in the MCHr1 knockout *ob/ob* mice (this study and 31). The differences between the models indicate that MCH has other ways of signaling than via MCHr1. The MCH gene also encodes other neuropeptides, neuropeptide EI, neuropeptide GE, and MCH gene overprinted polypeptide (2,36). Another possible explanation is that the other neuropeptides derived after proteolytic processing from the MCH preprohormone influence these parameters via separate signaling pathways. Whether neuropeptide GE exists as a functional active peptide is not clear but neuropeptide EI has been shown to be coexpressed with MCH and alter locomotor activity (37,38), whereas MCH gene overprinted polypeptide influences somatostatin secretion (36).

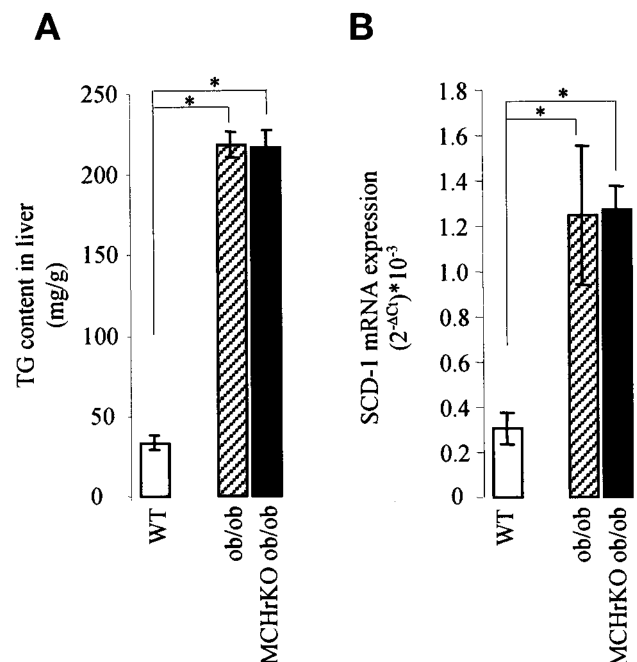

FIG. 6. Triglycerides content and SCD-1 mRNA expression in liver. Triglycerides content (*A*) and SCD-1 mRNA expression (*B*) in MCHr1 knockout (KO) *ob/ob* (n = 10), *ob/ob* (n = 7), and wild-type (n = 5) mice. *P < 0.05 (one-way ANOVA followed by Bonferroni post hoc test).

Sympathetically mediated thermogenesis in BAT depends on activation of UCP-1, which generates heat by uncoupling of the proton gradient across the inner membrane in the mitochondria. Leptin administration increases sympathetic nerve activity in BAT (16), and *ob/ob* mice have lower body temperature (31). MCH on the other hand decreases synaptic activity in neurons from lateral hypothalamus, body temperature, and BAT UCP-1 levels whereas MCHr1 knockout mice have increased heart rate and body temperature (6,11,39). Interestingly, the MCHr1 knockout *ob/ob* mice had significantly higher body temperature than the *ob/ob* mice when exposed to a low-temperature environment. Thus, ablation of MCHr1 in the *ob/ob* mice ameliorates the effect of leptin deficiency on body temperature. This finding suggests that MCH and leptin have opposing effects on the sympathetic nervous system regulated thermogenesis. Both chronic intravenous and intracerebroventricular leptin infusion and overexpression of leptin result in increased arterial pressure (40–42), whereas *ob/ob* mice have lower arterial pressure than control mice (43). It is well known that leptin downregulates orexigenic peptides like neuropeptide Y and MCH and upregulates the anorexigenic peptide α-melanocyte-stimulating hormone (MSH). Intracerebroventricular infusion of α-MSH acutely increases mean arterial pressure and heart rate (44). Thus, since MCHr1 knockout mice have increased heart rate, leptin may affect mean arterial pressure and heart rate via MCH and α-MSH.

This study characterized the MCHr1 knockout on a leptin-deficient background, and our findings clearly show that the absence of MCHr1 affects insulin levels, locomotor activity, body temperature, body fat, and lean body mass. No statistically significant differences on food intake, body weight, energy expenditure, liver triglycerides, or serum lipids were found when compared with *ob/ob* mice. Our data suggests that the MCHr1 knockout *ob/ob* mice have improved insulin sensitivity despite being severely obese. The MCHr1 might also be important for the autonomic control of body temperature acting via sympathetic nervous system. When the MCHr1 knockout *ob/ob* was compared with the previously reported MCH knockout *ob/ob*, there were several differences including body weight, lean body mass, and energy expenditure. The reason for these differences might be attributed to other known peptides from the MCH gene or that MCH exerts its actions via multiple signaling systems in addition to the MCHr1.

ACKNOWLEDGMENTS

We thank Harriet Andersén for her invaluable contribution in generating the MCHr1 knockout mice; Lena Amrot Fors, Anna Tuneld, Charlotte Lindgren, Anne-Cristine Carlsson, Martina Johansson, Gisela Häggblad, and Marie-Louise Berglund Zackrisson who kindly helped with the serum assays; and Johan Johansson who took care of the animals. Also, we thank Brian Middleton for invaluable help with statistical analysis.

REFERENCES

1. Kawauchi H, Kawazoe I, Tsubokawa M, Kishida M, Baker BI: Characterization of melanin-concentrating hormone in chum salmon pituitaries. *Nature* 305:321–323, 1983
2. Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, Vale W, Sawchenko PE: The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *J Comp Neurol* 319:218–245, 1992
3. Skofitsch G, Jacobowitz DM, Zamir N: Immunohistochemical localization of a melanin concentrating hormone-like peptide in the rat brain. *Brain Res Bull* 15:635–649, 1985
4. Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E: A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature* 380:243–247, 1996
5. Ludwig DS, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, Lowell B, Flier JS, Maratos-Flier E: Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest* 107:379–386, 2001
6. Ito M, Gomori A, Ishihara A, Oda Z, Mashiko S, Matsushita H, Yumoto M, Sano H, Tokita S, Moriya M, Iwaasa H, Kanatani A: Characterization of MCH-mediated obesity in mice. *Am J Physiol Endocrinol Metab* 284:E940–E945, 2003
7. Shimada M, Tritos N, Lowell B, Flier J, Maratos-Flier E: Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 396:670–674, 1998
8. Borowsky B, Durkin MM, Ogozalek K, Marzabadi MR, DeLeon J, Lagu B, Heurich R, Lichtblau H, Shaposhnik Z, Daniewska I, Blackburn TP, Branchek TA, Gerald C, Vaysse PJ, Forray C: Antidepressant, anxiolytic and anorectic effects of a melanin-concentrating hormone-1 receptor antagonist. *Nat Med* 8:825–830, 2002
9. Chen Y, Hu C, Hsu C, Zhang Q, Bi C, Asnicar M, Hsiung H, Fox N, Slieker LJ, Yang DD, Heiman ML, Shi Y: Targeted disruption of the melanin-concentrating hormone receptor-1 results in hyperphagia and resistance to diet-induced obesity. *Endocrinology* 143:2469–2477, 2002
10. Marsh DJ, Weingarth DT, Novi DE, Chen HY, Trumbauer ME, Chen AS, Guan XM, Jiang MM, Feng Y, Camacho RE, Shen Z, Frazier EG, Yu H, Metzger JM, Kuca SJ, Shearman LP, Gopal-Truter S, MacNeil DJ, Strack AM, MacIntyre DE, Van der Ploeg LHT, Qian S: Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proc Natl Acad Sci U S A* 99:3240–3245, 2002
11. Astrand A, Bohlooly MY, Larsdotter S, Mahlapuu M, Andersen H, Törnell J, Ohlsson C, Snaith M, Morgan D: Mice lacking the melanin concentrating hormone receptor 1 demonstrate increased heart rate associated with altered autonomic activity. *Am J Physiol Regul Integr Comp Physiol* 287:R749–R58, 2004
12. Hervieu GJ, Cluderay JE, Harrison D, Meakin J, Maycox P, Nasir S, Leslie RA: The distribution of the mRNA and protein products of the melanin-concentrating hormone (MCH) receptor gene, slc-1, in the central nervous system of the rat. *Eur J Neurosci*. 12:1194–216, 2000
13. Saito Y, Nothacker HP, Wang Z, Lin SH, Leslie F, Civelli O: Molecular characterization of the melanin-concentrating-hormone receptor. *Nature* 400:265–269, 1999
14. Bradley RL, Kokkotou EG, Maratos-Flier E, Cheatham B: Melanin-concentrating hormone regulates leptin synthesis and secretion in rat adipocytes. *Diabetes* 49:1073–1077, 2000
15. Muoio DM, Lynis Dohm G: Peripheral metabolic actions of leptin. *Best Pract Res Clin Endocrinol Metab* 16:653–666, 2002
16. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI: Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 100:270–278, 1997
17. Ahren B, Havel PJ: Leptin increases circulating glucose, insulin and glucagon via sympathetic neural activation in fasted mice. *Int J Obes Relat Metab Disord* 23:660–665, 1999
18. Bohlooly-Y M, Olsson B, Bruder CE, Linden D, Sjogren K, Bjursell M, Egecioglu E, Svensson L, Brodin P, Waterton JC, Isaksson OG, Sundler F, Ahren B, Ohlsson C, Oscarsson J, Tornell J: Growth hormone overexpression in the central nervous system results in hyperphagia-induced obesity associated with insulin resistance and dyslipidemia. *Diabetes* 54:51–62, 2005
19. Egecioglu E, Bjursell M, Ljungberg A, Dickson SL, Kopchick JJ, Bergstrom G, Svensson L, Oscarsson J, Tornell J, Bohlooly-Y M: Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity and hypolipidemia in mice. *Am J Physiol Endocrinol Metab*, 2005 [Epub ahead of print]
20. Chen H, Sullivan G, Quon MJ: Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. *Diabetes* 54:1914–1925, 2005
21. Bjursell M, Egecioglu E, Gerdin AK, Svensson L, Oscarsson J, Morgan D, Snaith M, Tornell J, Bohlooly-Y M: Importance of melanin-concentrating hormone receptor for the acute effects of ghrelin. *Biochem Biophys Res Commun* 326:759–765, 2005
22. Cohen P, Friedman JM: Leptin and the control of metabolism: role for stearoyl-CoA desaturase-1 (SCD-1). *J Nutr* 134:S2455–S2463, 2004

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024

23. Sharma AM, Chetty VT: Obesity, hypertension and insulin resistance. *Acta Diabetol* 42 (Suppl. 1):3–8, 2005
24. Pereira-da-Silva M, De Souza CT, Gasparetti AL, Saad MJA, Velloso LA: Melanin-concentrating hormone induces insulin resistance through a mechanism independent of body weight gain. *J Endocrinol* 186:193–201, 2005
25. Tadayyon M, Welters HJ, Haynes AC, Cluderay JE, Hervieu G: Expression of melanin-concentrating hormone receptors in insulin-producing cells: MCH stimulates insulin release in RINm5F and CRI-G1 cell-lines. *Biochem Biophys Res Commun* 275:709–712, 2000
26. Buijs RM, Chun SJ, Niijima A, Romijn HJ, Nagai K: Parasympathetic and sympathetic control of the pancreas: a role for the suprachiasmatic nucleus and other hypothalamic centers that are involved in the regulation of food intake. *J Comp Neurol* 431:405–423, 2001
27. Munck A: Glucocorticoid inhibition of glucose uptake by peripheral tissues: old and new evidence, molecular mechanisms, and physiological significance. *Perspect Biol Med* 14:265–269, 1971
28. Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS: Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 138:3859–3863, 1997
29. Vrang N, Kristensen P, Tang-Christensen M, Larsen PJ: Effects of leptin on arcuate pro-opiomelanocortin and cocaine-amphetamine-regulated transcript expression are independent of circulating levels of corticosterone. *J Neuroendocrinol* 14:880–886, 2002
30. Kennedy AR, Todd JF, Dhillo WS, Seal LJ, Ghatei MA, O'Toole CP, Jones M, Witty D, Winborne K, Riley G, Hervieu G, Wilson S, Bloom SR: Effect of direct injection of melanin-concentrating hormone into the paraventricular nucleus: further evidence for a stimulatory role in the adrenal axis via SLC-1. *J Neuroendocrinol* 15:268–272, 2003
31. Segal-Lieberman G, Bradley RL, Kokkotou E, Carlson M, Trombly DJ, Wang X, Bates S, Myers MG Jr, Flier JS, Maratos-Flier E: Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proc Natiol Acad Sci U S A* 100:10085–90, 2003
32. Wilding JP, Gilbey SG, Bailey CJ, Batt RA, Williams G, Ghatei MA, Bloom SR: Increased neuropeptide-Y messenger ribonucleic acid (mRNA) and decreased neurotensin mRNA in the hypothalamus of the obese (ob/ob) mouse. *Endocrinology* 132:1939–1944, 1993
33. Mondal MS, Nakazato M, Matsukura S: Characterization of orexins (hypocretins) and melanin-concentrating hormone in genetically obese mice. *Regul Pept* 104:21–25, 2002
34. Viggiano D, Ruocco LA, Sadile AG: Dopamine phenotype and behaviour in animal models: in relation to attention deficit hyperactivity disorder. *Neurosci Biobehav Rev* 27:623–637, 2003
35. Smith DG, Tzavara ET, Shaw J, Luecke S, Wade M, Davis R, Salhoff C, Nomikos GG, Gehlert DR: Mesolimbic dopamine super-sensitivity in melanin-concentrating hormone-1 receptor-deficient mice. *J Neurosci* 25: 914–922, 2005
36. Allaeys I, Bouyer K, Loudes C, Faivre-Bauman A, Petit F, Ortola C, Cardinaud B, Epelbaum J, Nahon JL: Characterization of MCH-gene-overprinted-polypeptide-immunoreactive material in hypothalamus reveals an inhibitory role of pro-somatostatin1–64 on somatostatin secretion. *Eur J Neurosci* 19:925–936, 2004
37. Bittencourt JC, Elias CF: Melanin-concentrating hormone and neuropeptide EI projections from the lateral hypothalamic area and zona incerta to the medial septal nucleus and spinal cord: a study using multiple neuronal tracers. *Brain Res* 805:1–19, 1998
38. Sanchez M, Baker BI, Celis M: Melanin-concentrating hormone (MCH) antagonizes the effects of alpha-MSH and neuropeptide E-I on grooming and locomotor activities in the rat. *Peptides* 18:393–396, 1997
39. Gao XB, van den Pol AN: Melanin concentrating hormone depresses synaptic activity of glutamate and GABA neurons from rat lateral hypothalamus. *J Physiol* 533:237–252, 2001
40. Shek EW, Brands MW, Hall JE: Chronic leptin infusion increases arterial pressure. *Hypertension* 31:409–414, 1998
41. Correia ML, Morgan DA, Sivitz WI, Mark AL, Haynes WG: Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension* 37:936–942, 2001
42. Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K: Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* 105:1243–1252, 2000
43. Mark AL, Shaffer RA, Correia MLG, Morgan DA, Sigmund CD, Haynes WG: Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens* 17:1949–1953, 1999
44. Hill C, Dunbar JC: The effects of acute and chronic alpha melanocyte stimulating hormone (alphaMSH) on cardiovascular dynamics in conscious rats. *Peptides* 23:1625–1630, 2002

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/55/3/725/651402/zdb00306000725.pdf by guest on 05 October 2024