

Resistance to Diet-Induced Obesity in μ -Opioid Receptor-Deficient Mice

Evidence for a “Thrifty Gene”

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Using pharmacological tools, a role for opioid receptors in the regulation of food intake has been documented. However, the involvement of specific receptor subtypes remains questionable, and little information is available regarding a role for opioid receptors in energy metabolism. Using adult male mice lacking the μ -opioid receptor (MOR) gene ($MOR^{-/-}$), we show that the MOR is not essential for the maintenance of normal levels of ad libitum food intake but does modulate the efficiency of energy storage during high-fat diets through the regulation of energy partitioning. When fed a regular diet, $MOR^{-/-}$ mice displayed only subtle alterations in energy homeostasis, suggesting a relative overuse of fat as a fuel source in the fed state. When fed a high-fat diet, $MOR^{-/-}$ mice were resistant to obesity and impaired glucose tolerance, despite having similar energy intake to wild-type mice. This resistance to obesity was associated with a strong induction of the expression of key mitochondrial enzymes involved in fatty acid oxidation within skeletal muscle. This metabolic role of the MOR, which is consistent with the properties of a “thrifty gene,” suggests that the MOR pathway is a potential target for pharmacological intervention in the treatment of obesity associated with the intake of fatty diets. *Diabetes* 54:3510–3516, 2005

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BAT, brown adipose tissue; COIV, cytochrome oxidase subunit IV; CPT-1, carnitine palmitoyl acetyl transferase 1; FFA, free fatty acid; MOR, μ -opioid receptor; PPAR- α , peroxisome proliferator-activated receptor- α ; UCP, uncoupling protein.

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The endogenous opioid system encompasses cloned receptor populations (μ , δ , and κ) and ligands (β -endorphin, the enkephalins, and the dynorphins). Extensive work indicates that the opioid system plays a role in the regulation of appetite. Opioid receptors and peptides are expressed in sites of the central nervous system that play a role in regulating feeding behavior, and pharmacological experiments using opioid receptor ligands show that agonists promote eating and antagonists decrease food intake, at least in the short term (1–3). Numerous pharmacological studies indicate that opioids also play a role in modulating the rewarding effects of food (3–5). In contrast, little information suggests that opioids have a role in energy metabolism (6–8).

The need to establish the specific role of the opioid receptors in energy homeostasis has led to studies using putative specific opioid receptor ligands or, more recently, antisense probes directed against specific exons of opioid receptor genes (3,9). Pharmacological studies suggest that the μ and κ pathways are part of an interconnected brain network and participate in the orexigenic effect of several peptides that regulate food intake (10–13). However, the interpretation of these data is complicated by our poor knowledge of the in vivo selectivity of ligand-receptor interactions that have been established in vitro (9,14).

Recent studies conducted in mutant mice null for the opioid receptors have complemented pharmacological approaches and clarified the role of each receptor in nociception, anxiety, and drug abuse (14). To further define the role of the μ -opioid receptor (MOR) pathway in energy homeostasis, we characterized mice lacking the MOR gene ($MOR^{-/-}$). Our study results indicated that the MOR is not an essential regulator of energy intake but is involved in the efficiency of fat storage during high-fat diets through the regulation of fatty acid oxidation. This metabolic role of the MOR, consistent with the properties of a “thrifty gene” (15), suggests that the MOR pathway might be a target for pharmacological prevention of high-fat diet-induced obesity.

RESEARCH DESIGN AND METHODS

MOR knockout ($MOR^{-/-}$) mice were obtained as previously described (16). Mice were originally obtained on a hybrid 129 SVJ/C57BL/6 background and were subsequently backcrossed over 12 generations onto a C57BL/6 back-

ground. Only male mice were used in these experiments. All animal protocols complied with National Institutes of Health guidelines and were approved by the Scripps Research Institute. Mature mice were housed singly for at least 4 weeks before the studies. Aged mice were housed in groups of three to four mice per cage. The regular (LM-485; Teklad, Madison, WI) and low- and high-fat (12450B and D12451; Research Diets, New Brunswick, NJ) diets contained 9.5, 10, and 45% kcal as fat with an energy density of 3.75, 3.85, and 4.73 kcal/g, respectively.

Unless otherwise specified, body weight and food intake were measured weekly. Preweighed colored food pellets were placed in cage hoppers; minimal bedding was used in the cages to allow for retrieval of food pieces for weighing. Spillage was taken into account when food intake was determined. For aged, group-housed animals, food intake was measured on a per-cage basis. Body composition was evaluated by dual-energy X-ray absorptiometry (Piximus/Lunar, Lambesc, France). For carcass analysis, mice were killed by decapitation and their carcasses were immediately frozen and shipped to the University of Alabama to be analyzed, as previously described (17). Lean body mass was calculated as the percentage of body fat-free dry mass.

Indirect calorimetry. Mice were housed singly and maintained in a 12-h dark-light cycle in a temperature-controlled colony (21–23°C). Mice were then transferred daily to metabolic chambers similar in size to their home cages and for 5 days were allowed to become habituated to the night/dark onset times used during testing. Metabolic testing was performed for 3 h during the mid-light cycle and at the beginning of the dark cycle, as reported for rats (18). Briefly, O₂ consumption and CO₂ production (milliliters per kilogram per hour) were measured using an AccuScan open-circuit calorimeter (Columbus, OH). The O₂ and CO₂ levels were measured sequentially across chambers for the last 30 s of the 5-min sampling periods. Energy expenditure and respiratory quotient (RQ; calculated as the ratio of VCO₂ to VO₂) were calculated as previously described (18). Data for the 5-min sampling periods were averaged over three periods to generate the 15-min sampling bins reported here.

Core body temperature. Body temperatures of individually housed mice were measured during the mid-portion of the light phase using a digital thermometer and rectal probe for mice (VWR, Bridgeport, NJ).

Locomotor activity. Locomotor activity of individually housed mice was evaluated over 24 h using a cage rack activity system (San Diego Instruments, San Diego, CA). Two sets of photobeams that allowed the recording of horizontal (locomotion) and vertical (rearing) movements were used. Mice were placed in the activity boxes for 7 h to habituate them to the testing environment and then tested during a 12-h dark and a 12-h light phase.

Glucose homeostasis. For the glucose tolerance test, animals were tested in the morning after a 15-h fast. Glucose was measured on blood samples obtained by tail bleeding before and 30, 60, 90, and 120 min after the intraperitoneal injection of glucose (2 g/kg). The insulin tolerance test was performed in fed mice during the mid-portion of the light cycle. Mice were injected with regular insulin (1 unit/kg i.p.; Lilly, Indianapolis, IN). Blood samples for glucose measurement were obtained before and 30, 60, and 90 min after insulin injection.

Hypothalamic-pituitary-adrenal axis evaluation. Blood samples for corticosterone measurement were obtained by tail bleeding in the same mice 1 h before the onset of the dark phase and 1 h after the onset of the light phase. All samples were collected within 1 min of the mice being removed from their cages. After the light-phase sampling, mice were exposed to 15 min of restraint; blood was sampled again at the end of the stress experiment.

Serum measurements. Glucose concentration was immediately analyzed using a Glucometer Elite (Bayer, Elkhart, IN). Plasma was collected in heparinized capillary tubes, immediately centrifuged, and stored at –80°C until assayed. Triglycerides and free fatty acids (FFAs) were measured using an enzymatic kit (Wako NEFA-C, Neuss, Germany). Specific immunoassays were used to measure plasma leptin and insulin (Linco, St. Charles, MO), corticosterone (ICN, Costa Mesa, CA), and total thyroxine (Diagnostic Products, Los Angeles, CA).

Fat stools. Fresh stools were collected after mice were handled and immediately frozen. Total fat was measured by acid hydrolysis at Covance (Madison, WI).

Expression of mitochondrial genes involved in fatty acid oxidation. Total RNA from frozen liver and rectus femoris muscle samples was extracted using the Extract-All solution (Eurobio, Courtaboeuf, France). Complementary DNA was synthesized with the Super Script reverse transcriptase and poly dT primers (Invitrogen, Cergy Pontoise, France). The real-time PCR measurement of individual cDNAs was performed using SYBR green dye to measure duplex DNA formation with the Gene Amp 5700 (Applied Biosystems) and normalized to the amount of cyclophilin RNA.

The following primers were used: cytochrome oxidase subunit IV (COIV) sense 5'-CCGACTGGAGCAGCCTTTC-3', antisense 5'-TCGGCGAAGCTCTCG TTAAG-3'; cyclophilin sense 5'-GATGAGAACCTTCATCCTAAAGCATACA-3', antisense 5'-TCAGTCTTGGCAGTGCAGATAAA-3'; liver carnitine palmitoyl

acetyl transferase 1 (CPT-1) sense 5'-AGCAGAGGCTCAAGCTGTTCA-3', antisense 5'-GCCAGCGCCCGTCAT-3'; muscle CPT-1 sense 5'-CCAAACGTCCAC TGCCTAAGCT-3', antisense 5'-GGCCGCACAGAATCCAAGT-3'; peroxisome proliferator-activated receptor- α (PPAR- α) sense 5'-GACAAGGCCTCAGGG TACCA-3', antisense 5'-GCCGAATAGTTCGCCGAAA-3'; and uncoupling protein type 3 (UCP-3) sense 5'-CCTCTGCACTGTATGCTGAAGATG-3', antisense 5'-CAGAAAGGAGGGCACAAATCC-3'.

Uncoupling protein type 1 measurement. Interscapular brown adipose tissue (BAT) was dissected and immediately frozen until it was analyzed. After mitochondria were prepared, the mitochondrial protein content was assayed using bicinchoninic acid. Mitochondrial protein extracts were loaded onto polyacrylamide gels, separated by electrophoresis, and transferred onto a nitrocellulose membrane. Western blots were hybridized with anti-UCP-1 (19) at a 1:4,000 dilution and goat anti-IgG at a 1:3,000 dilution. Direct recording of the chemiluminescence corresponding to UCP-1 was performed using the CCD camera of the GeneGnome analyzer, and quantification was achieved using the Genetool software (Ozyme, Saint-Quentin en Yvelines, France).

Statistics. Data are presented as means \pm SE. Statistical analyses were performed using Statview (SAS Institute, Cary, NC) and Statistica (Statsoft, Maison-Alfort, France). Main and interaction effects were analyzed by two-factor ANOVA for repeated or factorial measures or by using *t* and Mann-Whitney tests where appropriate. When justified by the ANOVA analysis, differences between individual group means were analyzed by the Newman-Keuls post hoc test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Adult *MOR*^{-/-} mice fed a regular diet have no overt change in body weight, food intake, or energy expenditure but display subtle alterations in fuel utilization. The 16- to 28-week-old mutant and wild-type mice fed a regular diet had similar body weights (*MOR*^{-/-} 29.2 \pm 0.8 g, $n = 12$; *MOR*^{+/+} 28.8 \pm 1.0 g, $n = 13$) and body composition, as determined by carcass analysis (Fig. 1A). There were no differences in 24-h food intake during 1 week of daily measurements or in 24-h locomotor activity between the genotypes (Fig. 1B and C). Energy expenditure, calculated using indirect calorimetry, was similar during the resting and active periods of the light cycle (Fig. 1D). The RQ was also similar during the dark phase. However, during the mid-light cycle, mutant mice exhibited lower RQ than wild-type mice (mean RQ: 0.72 \pm 0.01 [$n = 7$] vs. 0.76 \pm 0.01 [$n = 6$], respectively; $P < 0.03$) (Fig. 1E). Thus, in the fed/resting state and despite replenished carbohydrate stores, fat was utilized relatively more than carbohydrates as an energy substrate in *MOR*^{-/-} mice. We hypothesized that this constitutive difference in fuel utilization might result in less efficient storage of fat when the mice were challenged with a high-fat diet and, conversely, greater depletion of fat stores during starvation (20,21).

Adult *MOR*^{-/-} mice show reduced vulnerability to the weight-promoting effect of a high-fat diet. A separate set of 17- to 21-week-old mice raised on a regular diet ($n = 10$ per genotype) were switched to a high-fat diet. Mice again had similar body weights and compositions on the regular diet. After being switched to a high-fat diet, the *MOR*^{-/-} mice showed a dramatically reduced increase in weight and adiposity (Fig. 2A and B), despite having a food intake similar to that of *MOR*^{+/+} mice. Daily measurement of food intake demonstrated the same transient burst of hyperphagia in the mice after the switch to high-fat diet (Fig. 2C), and the cumulative caloric intake over 10 weeks was similar between the *MOR*^{-/-} and *MOR*^{+/+} mice (896.5 \pm 24.4 vs. 938.5 \pm 29.5 kcal), indicating a decreased food efficiency in *MOR*^{-/-} mice (Fig. 2D).

Rectal temperature with a regular diet was similar between the *MOR*^{-/-} and *MOR*^{+/+} mice (37.3 \pm 0.2 [$n =$

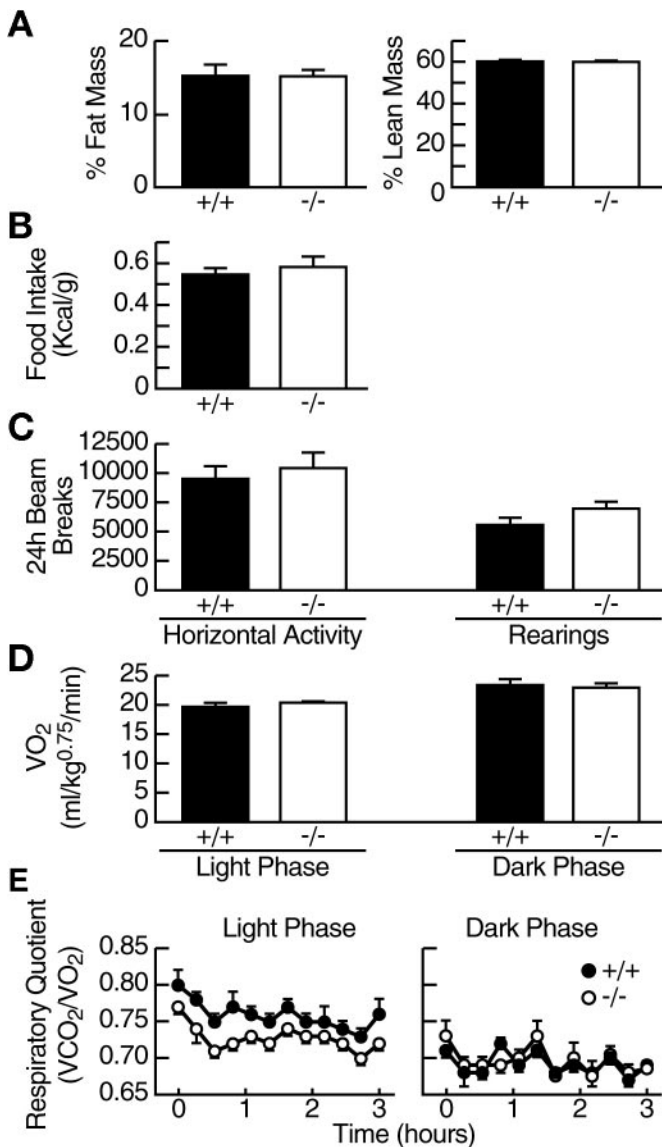


FIG. 1. Energy homeostasis of mature *MOR*^{+/+} and *MOR*^{-/-} mice on a regular diet. Shown here are body composition (A; *n* = 8 per genotype) as well as daily food intake (B) and 24-h locomotor activity in *MOR*^{+/+} (*n* = 12) and *MOR*^{-/-} (*n* = 14) mice. D: Indirect calorimetry: the estimated energy expenditure is similar between the genotypes (*n* = 6 and 7 for *MOR*^{+/+} and *MOR*^{-/-} mice, respectively) during the light and dark periods. E: RQ (15 min averages during 3 h): *MOR*^{-/-} mice exhibit reduced RQ during the light period only. Repeated ANOVA, genotype effect: $F_{1,11} = 6.39$, $P < 0.03$.

7] vs. $36.9 \pm 0.2^\circ\text{C}$ [*n* = 6]) and remained unchanged after 10 weeks of a high-fat diet, suggesting that the *MOR* deletion did not result in large changes in the metabolic rate that could account for the decreased vulnerability to diet-induced obesity in *MOR*^{-/-} mice. Because the *MOR* is expressed in the gut, the possibility of genotype differences in fat absorption was also assessed. However, total fat excreted in stools after the high-fat diet did not differ between the *MOR*^{-/-} and *MOR*^{+/+} mice (5.0 ± 1.2 [*n* = 8] vs. $5.7 \pm 0.9\%$ [*n* = 5]).

Adult *MOR*^{-/-} mice show increased weight loss during food deprivation. When adult age-matched mice were starved for 27 h, the percentage of body weight loss was greater in *MOR*^{-/-} than in *MOR*^{+/+} mice (Fig. 3A). Plasma levels of FFAs were similar between the genotypes in the fed state but were dramatically reduced in *MOR*^{-/-}

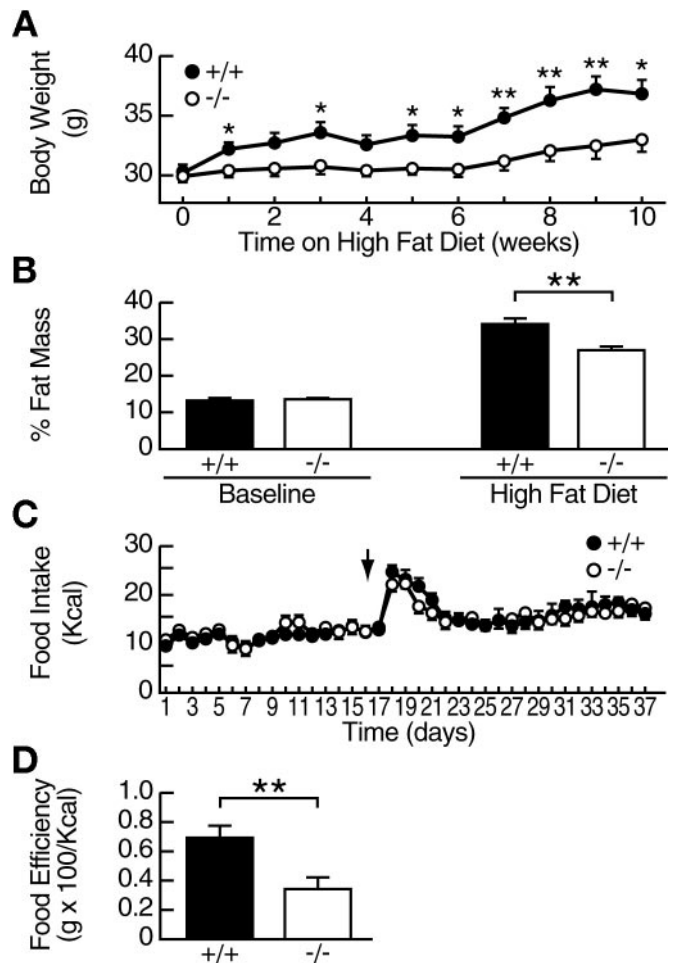


FIG. 2. Energy homeostasis of mature *MOR*^{+/+} and *MOR*^{-/-} mice fed a high-fat diet. A: Body weight after switching from a regular to a high-fat diet. B: Fat mass calculated by dual-energy X-ray absorptiometry before and after 10 weeks of a high-fat diet. C: Daily food intake before and after the switch to a high-fat diet (arrow). D: Food efficiency (100 × gram of body weight gained per kilocalorie consumed) with a high-fat diet. * $P < 0.05$, ** $P < 0.01$.

mice during food deprivation (Fig. 3B). A similar but nonsignificant trend was noted for plasma triglycerides (data not shown). Together these findings suggest that the absence of the *MOR* results in an increased depletion of fat stores during starvation associated with increased fatty acid utilization.

Aged *MOR*^{-/-} mice are protected from obesity, glucose intolerance, and insulin-resistance when fed a high-fat diet. The incidence of insulin resistance and type 2 diabetes associated with obesity increases with advancing age (22). Therefore, glucose homeostasis was studied in older mice (age 36–40 weeks) that were given a low- or high-fat diet. Body weights and fasting plasma leptin concentrations were not different between the genotypes at baseline and remained similar on the low-fat diet. In contrast, weight gain in *MOR*^{-/-} mice on the high-fat diet was reduced by 52% compared with that in *MOR*^{+/+} mice. Again, the cumulative caloric intake did not differ between genotypes (Fig. 4A, B, and C)

On the low-fat diet, both genotypes exhibited similar fasting plasma glucose and insulin concentrations and similar glucose clearance during a glucose tolerance test (Fig. 4D and E). After 3 weeks of a high-fat diet, glucose tolerance was reduced in *MOR*^{+/+} mice whereas it re-

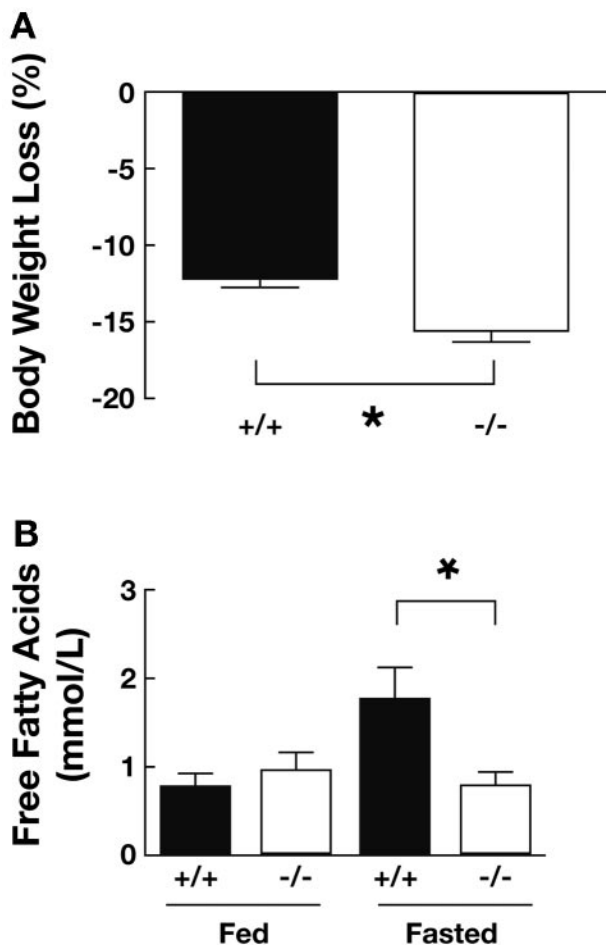


FIG. 3. Response of mature $MOR^{+/+}$ and $MOR^{-/-}$ mice to food deprivation. **A:** $MOR^{-/-}$ mice lost more weight than $MOR^{+/+}$ mice after 27 h of food deprivation ($P < 0.04$; $n = 10$ and 9 , respectively). **B:** Plasma levels of FFAs in fed and fasted conditions. Repeated ANOVA, genotype \times experiment effect: $F_{1,12} = 7.48$, $P < 0.02$.

mained similar in $MOR^{-/-}$ mice to that of the animals fed a low-fat diet (Fig. 4D). After 8 weeks of a high-fat diet, fasting plasma glucose levels were also increased in $MOR^{+/+}$ compared with in $MOR^{-/-}$ mice (152.8 ± 6.6 [$n = 5$] vs. 112.3 ± 4.4 mg/dl [$n = 9$]; $P < 0.01$). Plasma insulin in $MOR^{+/+}$ mice increased together with glucose levels, suggesting that glucose intolerance was due to insulin resistance. In contrast, the plasma insulin-to-glucose ratio in $MOR^{-/-}$ mice fed a high-fat diet remained similar to that of mice on the low-fat diet (Fig. 4E). The preserved insulin sensitivity of $MOR^{-/-}$ mice fed a high-fat diet was confirmed during an insulin-tolerance test (Fig. 4F).

After 14 weeks of a high-fat diet, body weight and plasma leptin in $MOR^{-/-}$ mice were greater than in mice fed a low-fat diet (Fig. 4A and B) and fasting glucose levels became similar between $MOR^{+/+}$ and $MOR^{-/-}$ mice fed a high-fat diet (141.2 ± 4.6 [$n = 5$] vs. 146.5 ± 13.6 mg/dl [$n = 9$]). Thus, the MOR deletion delayed, but did not ultimately prevent, the development of high-fat-diet induced obesity, insulin resistance, and glucose intolerance. **Adult $MOR^{-/-}$ mice show increased gene expression of mitochondrial enzymes involved in fatty acid oxidation.** To elucidate the mechanisms involved in the resistance to diet-induced obesity in $MOR^{-/-}$ mice, we quantified gene expression of several mitochondrial enzymes in liver and skeletal muscle. $MOR^{-/-}$ mice fed a

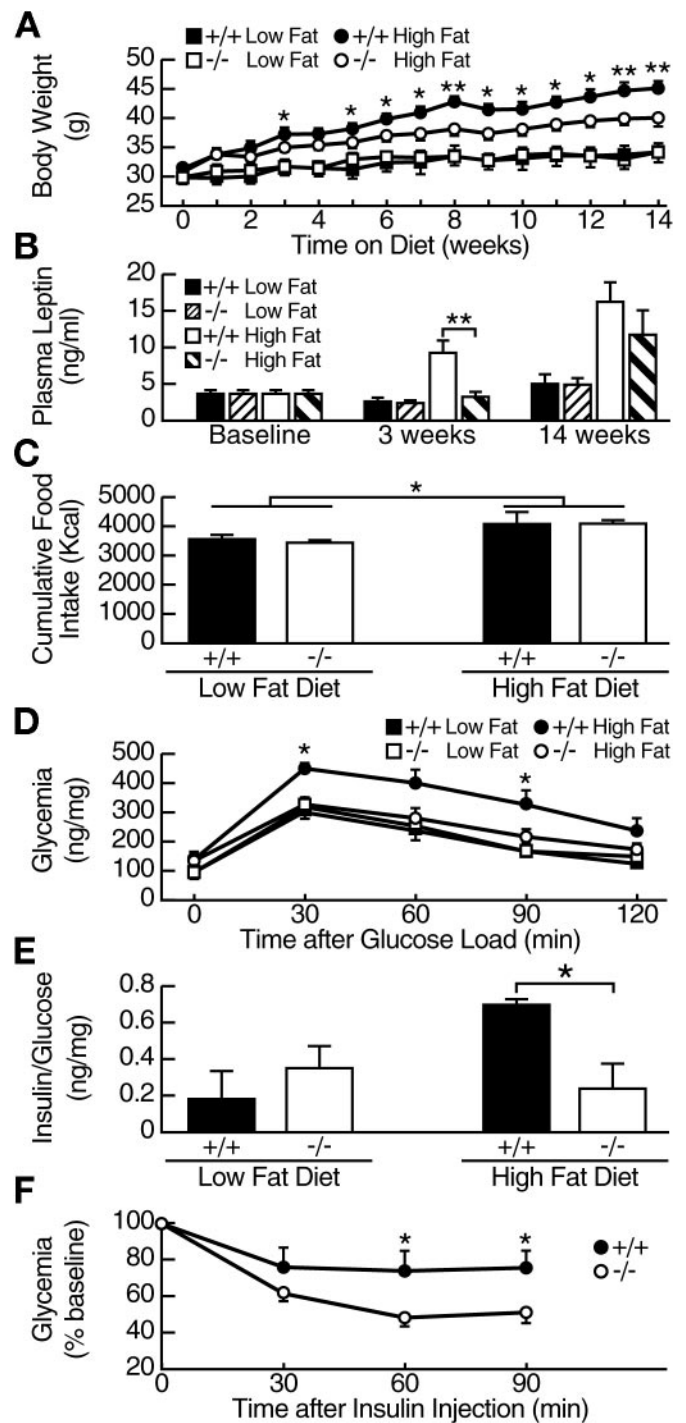


FIG. 4. Aged $MOR^{-/-}$ mice are resistant to high-fat diet-induced impaired glucose homeostasis. **A:** Body weights during low- and high-fat diets ($n = 5$ – 9 per group). Repeated ANOVA, genotype \times experiment effect: $F_{1,25} = 4.4$, $P < 0.05$. **B:** Fasting leptin concentrations before and during diet challenges. Repeated ANOVA, diet effect: $F_{1,23} = 20.8$, $P < 0.001$; genotype \times diet effect: $F_{1,23} = 4.9$, $P < 0.05$. **C:** Cumulative food intake. Diet effect only: $P < 0.03$. **D:** Preserved glucose tolerance after glucose administration in $MOR^{-/-}$ mice after 3 weeks of a high-fat diet. **E:** Fasting plasma insulin-to-glucose ratio (log transformed) after 8 weeks of diet challenge. **F:** Enhanced glucose clearance after insulin administration in $MOR^{-/-}$ mice after 9 weeks of a high-fat diet. * $P < 0.05$, ** $P < 0.01$ for $MOR^{+/+}$ vs. $MOR^{-/-}$ mice on a high-fat diet.

regular diet exhibited an increased hepatic expression of COIV compared with $MOR^{+/+}$ mice (1.34 ± 0.05 vs. 0.96 ± 0.08 ; $P < 0.01$; $n = 5$ in each group). No significant differences were found in liver PPAR- α (1.00 ± 0.16 vs.

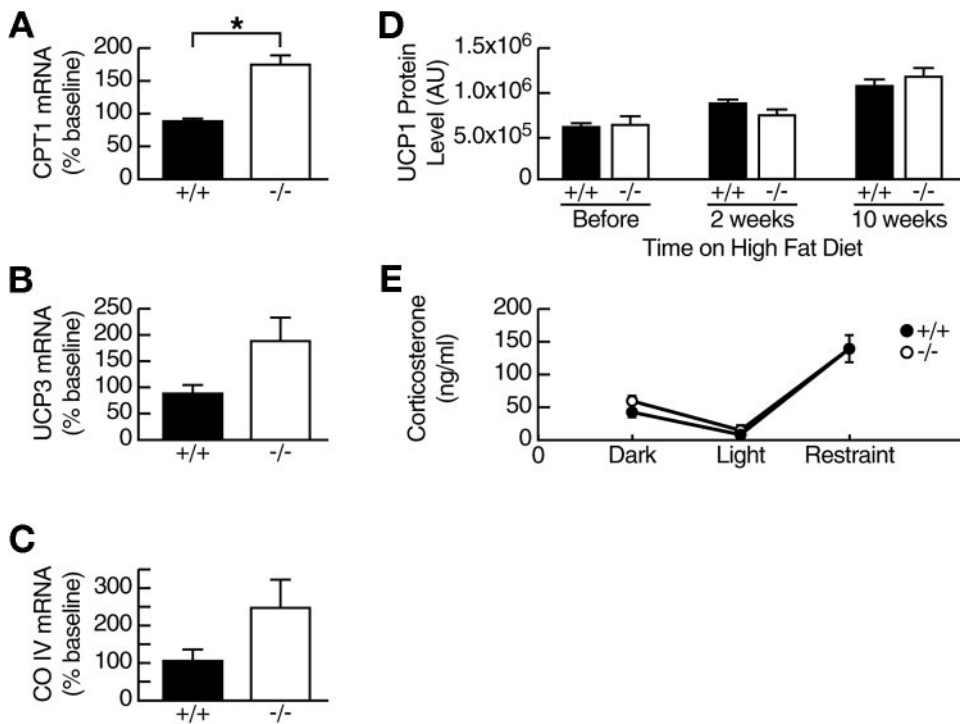


FIG. 5. Molecular correlates of the *MOR*^{-/-} phenotype. **A:** Dramatic increase in muscle CPT-1 expression after 2 weeks of high-fat diet in *MOR*^{-/-} mice but not in *MOR*^{+/+} mice ($P < 0.03$; $n = 4-5$ per genotype). A similar trend is observed for muscle UCP-3 (**B**; $P = 0.06$) and COIV expression (**C**; $P = 0.08$, $n = 4-5$ per genotype). **D:** Similar levels of UCP-1 in BAT of mice ($n = 6-10$ per genotype). ANOVA, diet effect: $F_{2,1} = 22.1$, $P < 0.001$. **E:** Corticosterone levels at the onset of the dark and light periods and after 15 min of restraint are similar between genotypes ($n = 9$ per group).

1.05 ± 0.13) or liver CPT-1 (1.04 ± 0.13 vs. 1.02 ± 0.26) mRNA expression between *MOR*^{+/+} and *MOR*^{-/-} mice ($n = 5$ in each group). After two weeks on a high-fat diet, COIV, PPAR-α, and liver CPT-1 mRNA increased dramatically and reached similar levels in both genotypes (diet effect, $P < 0.01$; data not shown).

In contrast, *MOR*^{+/+} and *MOR*^{-/-} mice fed a regular diet showed equivalent expression of COIV, muscle CPT-1, and UCP-3 mRNA in skeletal muscle. After 2 weeks on high-fat diet, gene expression remained unchanged in *MOR*^{+/+} mice, whereas it was dramatically increased in muscle CPT-1 of *MOR*^{-/-} mice ($P < 0.03$), consistent with increased fatty acid oxidation (Fig. 5A). Similar trends approaching statistical significance were noted for the upregulation of UCP-3 and COIV expression in mutant mice ($P = 0.06$ and 0.08 , respectively) (Fig. 5B and C).

UCP-1 expression in interscapular BAT is unaffected by MOR deletion. BAT UCP-1 protein levels did not differ between *MOR*^{+/+} and *MOR*^{-/-} mice fed a regular diet and increased similarly after 2 and 10 weeks of a high-fat diet (Fig. 5D).

Adult male MOR^{-/-} mice have no associated endocrine alteration that affects energy balance. Corticosterone has a permissive effect on diet-induced obesity, and endogenous opioids are involved in the control of hypothalamic-pituitary-adrenal axis activity (23). However, baseline and stress-stimulated corticosterone concentrations did not differ between the two genotypes in this study (Fig. 5E). Similarly, plasma thyroxine levels were unaffected by *MOR* deletion (*MOR*^{+/+} 2.2 ± 0.1 μg/dl; *MOR*^{-/-} 2.4 ± 0.2 μg/dl; $n = 10$ in each group).

DISCUSSION

Mice lacking the *MOR* have a reduced fat deposition and impairment of glucose metabolism when fed a high-fat diet. These alterations were revealed only by an adipogenic dietary challenge because deletion of the *MOR* had no effect on body weight, fat mass, or glucose metabolism

in adult mice fed a low-fat diet. These results agree with the modest effects of long-acting opioid receptor antagonists on the body weight of rats fed a regular diet and the ability of these agents to prevent weight gain in rats fed an adipogenic diet (6,24). However, in contrast to the findings observed with pharmacological agents, the decreased vulnerability to diet-induced obesity observed in mice selectively deficient for the *MOR* is attributable to changes in energy metabolism and not in caloric intake.

Pharmacological studies have consistently established that selective *MOR* agonists acutely promote eating, whereas selective antagonists decrease food intake, especially when elicited by food deprivation or palatable diets (rev. in 3). Contrary to these results and to the intriguing findings in hyperphagic β-*END*^{-/-} phenotype (25), adult *MOR*^{-/-} mice fed a regular or a high-fat diet were not hypophagic. Similar observations of quantitatively normal food intake with a regular diet have recently been described in *MOR*^{-/-} mice generated by another group of researchers (26). The discrepancies between pharmacological and genetic studies may be related to the short-lived and modest intensity of opioid pharmacological stimulation of feeding (3). In addition, our knowledge of the true in vivo selectivity of ligand-receptor interactions that have been established in vitro remains poor (9,14), and the mode of action of opioid-receptor ligands also relies on the dose, the experimental paradigm (acute versus chronic treatment), and the site of administration (5,14,27). Alternatively, the feeding phenotype of *MOR*^{-/-} mice might reflect the redundancy of brain orexigenic signaling (28) or compensatory brain changes in animals with *MOR* deleted through their lifetime. It should be noted that *MOR*^{-/-} mice show no modification in the number or distribution of δ- and κ-opioid receptors or in the expression of opioid peptide precursor genes (16). However, with regard to the complexity of in vivo opioid receptor signaling, disruption of functional interactions

between opioid receptors in knockout mice cannot be ruled out (29,30).

The reduced food efficiency that we observed in *MOR*^{-/-} mice fed a high-fat diet and who ate normally definitively indicates a role for this gene in regulating energy metabolism. In contrast to the extensive work on the involvement of opioids in feeding behavior, little information is available about a role of the opioid system in energy metabolism. It has been suggested that opioids favor the conservation of energy because the weight reduction induced by opioid antagonists in rats fed an adipogenic diet exceeded the degree to which caloric intake was reduced (6,24). However, controversial results on energy expenditure have been obtained with pharmacological tools (6,7). Two studies have reported that nonselective opioid antagonists induce a decrease in RQ, thereby indicating an opioid modulation of lipid oxidation, in rats fed regular and high-fat diets (7,8). However, RQ decreases with reductions in caloric intake (8), and it has not been possible thus far to separate the feeding and metabolic effects of opioids by pharmacological manipulation.

Our observations provide evidence that in the absence of any effect on food intake and energy expenditure, the *MOR* plays a specific role in modulating the balance between fat and carbohydrate oxidation. Indirect calorimetric data obtained with a regular diet suggest that *MOR*^{-/-} mice rely more on the oxidation of fatty acids and less on glycolysis during periods when they are not actively eating. This metabolic trait might be related to a constitutive increase in liver COIV expression, a key mitochondrial enzyme involved in cellular respiration (31). This fuel preference may account, at least partially, for the decreased fat deposition seen with a high-fat diet (32). In humans fed a standard diet, irrespective of their energy expenditure, a low "baseline" inherited RQ correlates with subsequent resistance to weight gain (20,21). Early metabolic adaptation to the amount of fat intake has also been shown to be a major determinant of diet-induced weight gain (33,34). We have demonstrated that after 2 weeks of a high-fat diet, the lack of *MOR* strongly induces the expression of key enzymes involved in fatty acid oxidation within skeletal muscle such as CPT-1, UCP-3, and COIV (35,36). These results strongly suggest that the lean phenotype of *MOR*^{-/-} mice could be a consequence of an increased rate of fatty acid oxidation in skeletal muscle in response to a high-fat diet. Consistent with these findings suggesting that the MOR pathway promotes fat deposition in lieu of fat oxidation, body weight changes in *MOR*^{-/-} mice during food deprivation mirrored those seen with an adipogenic diet, including an increased weight loss associated with reduced FFA levels. Other studies using nonselective opioid receptor antagonists suggest that opioids may impair BAT and UCP-1 activity in a way that favors fat deposition (37,38). However, we found no evidence that the MOR pathway is involved in the regulation of UCP-1 expression during high-fat feeding. The primary site of metabolic opioid actions remains to be determined. A brain site of opioid action is suspected (24,39,40). As an alternative, a direct peripheral action might be considered, although the presence of the MOR in liver and skeletal muscle remains poorly documented (41–44).

Obesity results from interactions between environmental and genetic factors that regulate energy intake, energy expenditure, and fuel partitioning. The "thrifty gene" hypothesis suggests that the evolution of the human genome favored the selection of genes promoting efficient storage

of ingested energy as fat (15). This adaptation would confer metabolic advantages in historical periods of food scarcity but predisposes individuals to obesity in modern environments. Because the *MOR* promotes efficient storage of ingested fat, it is metabolically "thrifty." From an evolutionary perspective, the *MOR* may represent a beneficial biological link between the metabolic and hedonic aspects of the regulation of energy balance. The well-established rewarding properties of opioids favor the consumption of energy-dense food (3–5), whereas the metabolic actions of MOR agonists promote the storage of ingested calories as fat. However, the present study has provided evidence that the *MOR* plays a crucial role in the development of obesity and glucose intolerance in a Western diet environment. Previous human trials conducted in obese patients with opioid receptor antagonists have given inconsistent results (8,45). However, in those trials, these agents were nonselective and were given to already obese people. It should be emphasized that more consistent weight-reducing effects in rats were obtained with administration of opioid-receptor antagonists before or during the dynamic phase of obesity development than in animals with preexisting dietary obesity (6,8,24). Also, individual differences in the etiology of obesity (28) may play a role in the efficacy of an opioid receptor antagonist. Again, more consistent weight-reducing effects were obtained in subjects with a putative high endogenous opioid tone, such as rats fed a cafeteria diet (6), binge-eaters (45), or obese women with polycystic ovary syndrome (46). Supporting this hypothesis, high-fat feeding increases hypothalamic MOR expression in rats (47). Therefore, the present findings obtained in mice selectively deficient for the *MOR* combined with data from the literature suggest that inhibition of MOR signaling may be useful in preventing the development of obesity and impaired glucose tolerance associated with chronic intake of fatty diets.

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