Hyperphagia, not hypometabolism, causes early onset obesity in melanocortin-4 receptor knockout mice


Previous studies on mice with melanocortin-4 receptor gene (*MC4r*) knockout have focused on obese adults. Because humans with functional *MC4r* mutations show early-onset obesity, we determined the onset of excessive fat deposition in 10- to 56-day-old mice, taking into account sex and litter influences. Total body fat content of *MC4r*−/− on day 35 and *MC4r*+/− on day 56 significantly exceeds that of *MC4r*+/+. Plasma leptin levels increase in proportion to fat mass. According to cumulative food intake and energy expenditure measurements from day 21 to 35, onset of excessive fat deposition in *MC4r*−/− is fueled by hyperphagia and counteracted partially by hypermetabolism. In 35- to 56-day-old mice, arcuate nucleus neuropeptide Y (NPY) mRNA decreases and pro-opiomelanocortin (POMC) mRNA increases with fat content and plasma leptin levels independently of genotype. Taking into account fat content by ANCOVA reveals, however, increases in both NPY mRNA and POMC mRNA due to melanocortin-4 receptor (*MC4R*) deficiency. We conclude that hyperphagia, not hypometabolism, is the primary disturbance initiating excessive fat deposition in *MC4R*-deficient mice at weaning and that the overall changes in NPY and POMC expression tend to antagonize the onset of excessive fat deposition.

**neuropeptide Y; pro-opiomelanocortin; genetic obesity; energy expenditure**

In several studies about 2–4% of extremely obese patients were found to have mutations in the melanocortin-4 receptor gene (*MC4r*), presumably leading to haploinsufficiency of the receptor (*MC4R*) (6, 8, 11, 27, 35, 38, 39, 40). Mutation carriers mostly had early-onset obesity (4, 6, 11, 35, 40). A corresponding knockout model, producing mice lacking *MC4r*, has formally been classified as dominant, displaying late-onset obesity (12). Although adult animals were thoroughly analyzed in the original and several follow-up studies (1, 2, 22, 36), physiological changes in early postnatal stages were not investigated. Assessing the incipient phase of obesity onset is, however, important because a multitude of secondary metabolic disorders will obscure cause-and-effect relationships, once obesity is established. Adult, obese mice homozygous (−/−) for the *MC4r* gene defect showed hyperphagia and increased energy expenditure compared with wild-type (+/+). The *MC4r*−/− mice (2), characteristic features of both animal and human obesity (29). On the other hand, results obtained from the comparison of 8-wk-old −/− and +/+ mice where body masses are still similar have suggested generation of obesity by depressed energy expenditure without accompanying hyperphagia (36). According to the detailed analysis of obesity onset in another animal model, the leptin receptor-defective *LEPR*−/− rat, however, excessive fat deposition starts long before differences in body mass become apparent from population data. Moreover, information obtained by only short term measurements of metabolic rate (MR), as well as the use of body mass-normalized MR, may be misleading (13, 14, 21, 31). Thus, for the *MC4R* deficiency, the available data do not provide conclusive answers about the time scale within which obesity starts to develop and what is the underlying primary metabolic disorder. Moreover, the same need for discrimination between primary and secondary alterations applies to the central nervous signaling system. For example, the orexigenic neuropeptide Y (NPY) and the anorexigenic pro-opiomelanocortin (POMC) pathways, which are under the control of the circulating hormone, leptin, produced by adipocytes in proportion to body fat content, should be assessed in this way. Furthermore, except in the original study (12), potential abnormalities in heterozygous carriers (+/−) of the *MC4r* defect have received little attention, although this state represents the most interesting analogy to human *MC4R* haploinsufficiency. The present study was designed to deal with these open questions. 1) The time of onset and the degree of excessive fat deposition in both −/− and +/+ mice were determined by study-
ing animals at ages between 10 and 56 days. 2) We analyzed to what degree the proportionality between plasma leptin levels and body fat content might be modified by genotype. 3) We tried to identify the primary disturbance of energy balance responsible for the earliest stages of obesity development. 4) We analyzed how the hypothalamic expression of NPY and POMC was related to body fat and plasma leptin levels and whether there was evidence for an additional genotype influence.

METHODS

Animals. We used animals derived from our colony founded in 1999 with heterozygous breeding pairs from brother-sister matings of the original knockout line kindly provided by D. Huszar (Millennium Pharmaceuticals). Animals were maintained on standard pelleted food (type 1314; Altromin, Lage, Germany) at 25°C in a 12:12-h light-dark cycle. To maximize the number of pups available for genotype-related intra-litter comparisons, we mainly used +/- x +/- matings rather than +/- x +/- matings that produced only two genotypes in each litter. Pups in each litter were individually marked by subcutaneous injections of India ink at about 4 days of age and were weaned at 21 days of age.

Genotype identification. Mouse genomic DNA was isolated from the tail tip using a commercially available kit (QI Amp Tissue Kit; Qiagen, Hilden, Germany). Genotypes were identified by PCR using MC4 F3 primer (5’-GGA AGA TGA ACT CCA CCC ACC-3’), MC4 R1 primer (5’-GAC GAT GGT TTC CGA CCC ATT-3’), and pGK R3 primer (5’-TTC CCA GCC TCT GAG CCC AGA-3’) kindly provided by D. Huszar. Amplifications in a thermocycler (GeneAmp PCR System 2400; Perkin-Elmer, Weiterstadt, Germany) started with initial denaturation at 94°C, and 72°C, and 72°C, each for 45 s, and a final extension of 72°C for 10 min. PCR products were separated on a 2.5% agarose gel by electrophoresis followed by ethidium bromide staining. We obtained a PCR product of 313 bp for the wild-type allele and of 405 bp for the mutated allele.

Determination of body composition and plasma leptin concentrations. Two hours before the end of their daily light phase, mice were exposed to CO2 gas for about 30 s and then decapitated. Brains were quickly removed, and blood was collected in heparinized tubes on ice and centrifuged. Brains were first frozen on dry ice and then stored, as were the plasma aliquots, at -80°C for further measurements. Carcass mass was determined after removing stomach and intestines and emptying the bladder. Body composition [water, fat, and fat-free dry mass (FFDM)] was evaluated by drying the carcasses at 75°C to constant weight followed by total-body chloroform extraction in a Soxhlet apparatus and drying again to constant weight (21).

For leptin measurements we used a mouse RIA kit (Linco, St. Charles, MO). Measurements were independently duplicated, and variability was decreased by correcting the data for interassay variability and buffer dilution using internal correction factors. Samples from littersmates were always measured in the same RIA. For each animal we averaged the results of at least two measurements carried out in different RAs.

In situ hybridization. From the animals used for body composition analysis, in situ hybridization was carried out on coronal hypothalamic slices from a total of 59 mice at the age of 35 and 56 days, selected so that they covered the entire range of body fat contents found in body composition analysis. The measurements were carried out in three different runs, each containing pups of all genotypes in about equal numbers: female mice were used in two of the runs, and males were used in the third run. NPY and POMC expression in the hypothalamic arcuate nucleus (ARC) was quantified in adjacent 20-μm coronal sections, equivalent to Bregma -1.22 mm to -2.54 mm in the mouse brain according to Franklin and Paxinos (7). We performed in situ hybridization in brain slices mounted on poly-L-lysine-coated slides, employing techniques described in detail elsewhere (23, 24, 25).

NPY probes were generated from a 0.5-kb fragment of a rat NPY cDNA generously provided by Dr. Stephen Sabol (10). A 344-bp fragment of POMC cDNA was cloned from Siberian hamster hypothalamic cDNA showing 85% identity to exon three of mouse POMC (26).

Autoradiographic images were quantified using the Image-Pro Plus system (Media Cybernetics, Silver Spring, MD). Data were standardized with 14C-autoradiographic scales (Amersham Biosciences, Little Chalfont, UK). Gene expression was measured as the integrated intensity of the autoradiographic signal, i.e., as the background-corrected optical density integrated over all pixels in the hybridization area. Normally, three sections for each brain were analyzed and data averaged.

Determination of energy expenditure and food intake. Oxygen consumption of an additional set of 26 weanlings from 4 litters was individually recorded continuously from postnatal day 21 to 35 in an open flow system as previously described (21, 42). The intake of chow was recorded daily at the end of the light phase. Body composition at the start of the experiment was estimated from the close correlations of fat and FFDM with body mass found for the population data of 21-day-old animals. Final body composition at 35 days of age was determined as described above. For this part of the study only offspring of +/- and +/- mothers were used.

Evaluation of energy balance. In the small metabolic chambers used, energy intake in terms of consumed standard pelleted food was difficult to correct for losses due to the extensive crumbing which occurs in weanlings. For this reason and since energy extraction from ingested food has not been determined for juvenile MC4R-deficient mice, energy balance was calculated from body composition data and from measurements of energy expenditure, which can be determined with high precision (15, 21). The energy equivalents were used as 38 kJ/g for fat mass, 20 kJ/g for FFDM, and 20.4 kJ/liter O2 corresponding to a respiratory quotient of 0.85. When this was done independently for all genotypes and when the values obtained were compared with the recorded gross food intake, net energy extraction values of 10.6, 10.5, and 10.8 kJ/g food were estimated for +/-, +/- and +/- animals, respectively. These values were 20% lower than the energy extraction per gram of food consumed, as determined in adult C57BL/6J mice by analyzing caloric input and output, but they did not differ from each other. Therefore, when studying these young animals, gross pellet consumption could be taken as a reliable indicator for genotype-related differences in energy intake.

Statistical evaluation. Growth of rat pups reared in different litters varies considerably (17, 21, 33, 37). Consequently, taking this factor into account for mice as well appears necessary for the detection of more subtle differences in body composition due to genotype and sex among sucklings and weanlings. Body composition data were evaluated by three-way ANOVA (StatSoft, Tulsa, OK) with the factors genotype, litter, and sex for each age group. Because most litters contained only two of three possible genotypes at MC4r, separate comparisons between +/- and +/-, and between
+/- and -/- mice had to be carried out. To determine the relationship between plasma leptin concentration and body fat content, regression analysis was applied separately to the data from each age group. For the analysis of the energy balance data obtained between 21 and 35 days of age, two-way ANOVA (SigmaStat program; SPSS, Chicago, IL) could be applied, following the approach developed for a preceding study (33), because the investigated litters contained pups of both genotypes. Genotype effects were separated from possible sex and litter effects by considering, as the second factor, a combined litter-sex effect, assigning different litter numbers to male and female littermates. In this way, we could clearly separate the genotype effects from the highly significant litter-sex effect. We abstained from separating the sex and litter effects in these data for two reasons. First, in litters containing 6–8 pups of three genotypes with a more or less uneven sex distribution, this separation would not have been possible with the relatively small number of weanling litters used for the energy expenditure and food intake measurements. Second, separating the influences of the sex and litter factors was not considered important for the conclusions to be drawn from this part of the study.

The in situ hybridizations from three experimental runs were analyzed in different ways. 1) Both NPY and POMC expression data of 35- and 56-day-old mice were analyzed by three-way ANOVA with experimental run, genotype, and age as the factors, using only animals of the same sex in each run. 2) To obtain further information, for each of the three experimental runs, regression analysis was performed separately, using the NPY and POMC expression data as dependent variables and body fat content or plasma leptin concentrations as the independent variables. Linear and parallel relationships were obtained after logarithmic transformation of the variables. 3) On this basis, the NPY and POMC expression data were standardized for common evaluation by z-transformation, using the SigmaStat program. 4) Regression analysis was applied to the relationship between the standardized data for NPY or POMC expression and body fat content or plasma leptin concentration. 5) In addition, the general relationships existing between neuropeptide (NPY, POMC) expression and body fat content of 35- and 56-day-old mice was analyzed for potential influences of MC4R deficiency. For this purpose, analysis of covariance (ANCOVA) was carried out (Statistica) with log body fat content (respectively, log plasma leptin concentration) as covariable and genotype as factor.

RESULTS

Body composition. The genotype effects on body fat content, as the parameter most relevant for characterizing obesity, are documented in Fig. 1 after taking into account the persistently strong litter and weak sex effects. At 35 days of age, body fat content of -/- mice first exceeds that of their +/- littermates. By 56 days of age, not only is body fat content of -/- mice more than twice that of +/+ littermates, but body fat content of the latter is also significantly higher by 20% than that of their wild-type littermates. Even in these young adults, a strong litter effect (P < 0.01) was observed, indicating its importance as a factor to be taken into account also in the analysis of the MC4R defect in postweaning mice. Although significant (P < 0.05) sex differences were observed from day 21 onward, no genotype × sex interactions were found, i.e., the development of obesity did not differ significantly between males and females.

When the genotype effects on body mass, fat mass, and FFDM were analyzed in the same way by three-way ANOVA (not shown), the development of significant genotype differences in body mass and fat mass exactly paralleled that shown for body fat content. For FFDM, however, a significantly (P < 0.01) higher value was found only for -/- vs. +/- mice at 56 days of age.

Plasma leptin concentration. Across all genotypes, plasma leptin concentration is closely correlated with both total fat mass and body fat content at a given age (Fig. 2). Correlation coefficients are 0.6–0.8 for the 10- to 35-day-old animals and 0.9 for the 56-day-old animals, whose fat mass and body fat content varies over
a much greater range than in the younger pups. The slope is steepest for the correlation between plasma leptin and fat mass in the 10-day-old pups that have higher plasma leptin levels than weanlings over approximately the same range of body fat mass. When plasma leptin is related to body fat content, the slope decreases significantly with age until day 35, but is significantly steeper again at 56 days of age (day 10 > day 56 > day 21 > day 35). Visual inspection indicates no genotype-related difference in these correlations, i.e., −/− mice have higher plasma leptin concentrations than +/− and +/+ mice, but only in proportion to their increased body fat. Considering fat content, genotype, sex, and litter in a multiple regression analysis of the logarithm of plasma leptin concentration of 56-day-old mice yields $R^2 = 0.86$, with 85% of the variability of plasma leptin being explained by body fat content ($P < 0.001$) and only 1% ($P < 0.01$) by genotype. In younger pups, body fat content is the only parameter significantly influencing plasma leptin. Thus, under the premise that body fat content normally determines the plasma leptin level, the data provide no indication for a disturbance of the hormonal feedback regulation by the MC4R deficiency.

**Primary factors fueling the onset of excessive fat deposition.** To determine whether hypometabolism is a primary consequence of the MC4R deficiency, we continuously recorded oxygen consumption and food intake in +/+ , +/− , and −/− mice of four litters from

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**Fig. 2.** Plasma leptin concentration as a function of total body fat mass (top) and of percent body fat content (bottom) in +/+ (black symbols), +/− (gray symbols), −/− (open symbols) mice at 10 days old (d10, hexagons), 21 (squares), 35 (triangles), and 56 days old (circles). Symbols with a cross mark indicate data from females. Correlation coefficient ($r$) for 10- to 35-day-old mice (left) $0.6 < r < 0.8$, and for 56-day-old mice (right) $r = 0.9$.

**Fig. 3.** Daily food intake and continuously measured metabolic rates (MR) of seven littermates reared individually from weaning on postnatal day 21 until day 35. Symbols with a cross mark indicate data from females; +/+ , black symbols; +/− ; gray symbols; and −/− , open symbols.
weaning until 35 days of age. As shown in Fig. 3 for one of the litters, daily food intake of \(-/-\) pups clearly exceeds that of their wild-type littermates during the first 2 wk after weaning. Moreover, in paralleling their increased food intake, oxygen consumption of the \(-/-\) mice is also higher rather than lower, with the +/- mice showing intermediate values in both parameters.

The energetics of the onset of excessive fat deposition in this animal model were analyzed more closely by taking into account the tight correlation of total fat mass and of FFDM with body mass observed at 21 days of age (Fig. 4). Proceeding from these relationships, the initial energy contents of the individual weanlings used for the metabolic studies were estimated from their body masses on day 21. Figure 5 demonstrates the changes in body mass (Fig. 5A), FFDM (Fig. 5C), and fat mass (Fig. 5E) developing from day 21 to day 35. As shown by 2-way ANOVA, the group of weanlings used in this part of the study unexpectedly was found to have grown somewhat larger until day 21 than weanlings of the groups used at the same age for the main analysis of body composition, with each parameter being significantly, although only slightly greater in the \(-/-\) animals than in their wild-type littermates. However, by 35 days of age, these differences have become much larger, and significant differences in body mass and fat mass now exist between +/- and +/- mice as well. Within these 2 wk after weaning, cumulative food intake (Fig. 5B), cumulative oxygen

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**Fig. 4.** Correlation of fat-free dry mass (FFDM, \(r = 0.99\)) and fat mass (\(r = 0.88\)) with body mass of 21-day-old +/- (black symbols), +/- (gray symbols), and -/- (open symbols) mice. Symbols with a cross mark indicate data of females; because no differences in the correlations can be detected, common regressions for males and females can be used at this age.

**Fig. 5.** Least square means (±SE) of body mass (A), FFDM (C), and fat mass (E) on day 21 and on day 35, and for the period from day 21 to day 35, of cumulative food intake (\(\Sigma\) food, B), cumulative oxygen consumption (\(\Sigma\) VO\(_2\), D), and energy gain, i.e., the increase in body energy content determined from FFDM and fat mass accumulation (F), for +/- (solid black bars), +/- (gray bars), and -/- (open bars) mice. Fat mass and FFDM were estimated for the 21-day-old animals from the regression line for the population data (see Fig. 4) and directly measured at 35 days of age. To permit common evaluation of genotype effects from the data of males and females, genotype and litter-sex were used as factors in 2-way ANOVA (see METHODS). *\(P < 0.05\). **\(P < 0.01\). ***\(P < 0.001\).
consumption (Fig. 5D) and energy gain, i.e., the increase in body energy contents determined from the individual increases in FFDM and fat mass (Fig. 5F), of the −/− animals significantly exceed those of the +/+ animals, and the +/+ animals keep an intermediate position. As the sums of energy gains and energy expenditures, net energy intakes of 399, 424, and 485 kJ could be estimated for the +/+, +/−, and −/− genotypes, respectively. Because their relationships directionally correspond to those found for the gross chow intakes, the latter are incorporated, as the directly measured parameter, into Fig. 5. Comparison of calculated net energy intakes and oxygen consumption for each genotype shows that the higher energy gain of the −/− mice compared with the +/+ mice is fully explained by their 20% (86 kJ) higher net energy intake, which exceeds their only 10% (36 kJ) higher energy expenditure. Similarly, the small but significant excess energy gain of the +/− relative to the wild-type mice results from their 6% (25 kJ) higher net energy intake, which exceeds their 4% (12 kJ) higher energy expenditure. Thus the difference between net energy intake and energy expenditure, although both are individually not significant, would explain the significant excess energy gain determined for the heterozygous genotype relative to the wild type.

**NPY and POMC expression.** Analyzing the expression data of 35- and 56-day-old animals by three-way ANOVA with the factors genotype, age, and experimental run as factors (not shown), revealed, for NPY mRNA, a significant decrease with age and a tendency (P = 0.08) for a lower level in −/− vs. +/+ and +/− animals. For the POMC mRNA data from the same individuals, however, both a significant increase with age and a clear increase with the number of copies of the defective MC4R gene (P < 0.01, +/+ < +/− ≤ −/−) were found. Deducing potential genotype effects in the conventional manner only from mean value differences, however, ignores the potential influence of body fat content. It might act as an intervening variable because of its well-known influence on plasma leptin level, which, in turn, might secondarily influence NPY and POMC expression in the ARC and conceivably primary effects of the MC4R deficiency on neuropeptide expression. Therefore, as a further analytical approach, the mRNA expression data of the 35- and 56-day-old animals from the three separate in situ hybridization runs were standardized (see methods) to permit evaluation of their common relationships with body fat content and plasma leptin. Because visual inspection of the data showed no overt influence of genotype and age, common regressions were calculated, with genotype and age being indicated in the graphical presentations of Fig. 6. The standardized NPY expression data of the 35- and 56-day-old animals are negatively correlated with body fat content (Fig. 6A) and with plasma leptin concentration (Fig. 6C). In contrast, the standardized POMC mRNA data are positively correlated with body fat content (Fig. 6B) and with plasma leptin concentration (Fig. 6D).

Presuming that potential genotype effects might be obscured by the predominant influence of body fat content, we applied ANCOVA to the standardized mRNA expression data of the 35- and 56-day-old animals, with log body fat content as covariable and genotype as factor. Indeed, significant genotype influences (P < 0.001) are found in this analysis for NPY (Fig. 6E) and POMC (Fig. 6F). Not shown are the very similar genotype effects found when log plasma leptin concentration was used as the covariable in ANCOVA (P < 0.01 for NPY and P < 0.001 for POMC). Thus taken together the data suggest that NPY and POMC expression in the ARC is dominantly and oppositely influenced by body fat content and plasma leptin, respectively, whereas a less distinct but significant equidirectional genotype influence exists, exerting a stimulatory action on NPY and POMC expression in −/− compared with +/+ animals.

**DISCUSSION**

The salient findings of this study are as follows. 1) Under standard laboratory conditions, body fat content of mice homozygous (−/−) for the MC4r defect starts to increase shortly after weaning and is three times larger than that of wild-type animals in young (56-day-old) adults. The heterozygous effect, although statistically significant, is still very small at this age. 2) The general relationship between body fat content and plasma leptin concentration is maintained in sucklings and weanlings independent of the genotype differences at the MC4r locus. 3) The onset of excessive fat deposition in +/+ and −/− mice is exclusively fueled by hyperphagia and not by hypometabolism. 4) In young animals up to 56 days of age, hypothalamic NPY mRNA decreases and POMC mRNA increases with body fat content and plasma leptin, irrespective of the genotype. However, if differences in body fat content (respectively, plasma leptin levels) are considered in the statistical analysis, then an enhancing effect of MC4R deficiency on both NPY and POMC expression is revealed.

The MC4R deficiency is only weakly dominant in juvenile animals. The MC4r knockout on the 129/Sv × C57BL/6J background has been formally characterized as dominant in the original study (12), in which the body mass of older +/+ mice ranges roughly in the middle between that of the +/+ and the −/−. The present data, however, show that the −/− mice have already developed a 50% higher body mass and three times higher body fat content than wild-type controls at 56 days of age, whereas in the +/+ mice of the same age body mass is only 7% higher and body fat content less than 30% higher than in their wild-type littermates. The degree of dominance thus seems to change with age. Moreover, a sex difference in the degree of dominance was reported for a second knockout line, in which only the heterozygous males showed a clearly increased body mass (2), different from the absence of sex × genotype interactions after weaning in the present study. As in preceding studies, we investigated non-
backcrossed animals to ensure that genotype effects, if detectable, are not limited to a particular mouse strain and are sufficiently strong in relation to the influences of a heterogeneous genetic background. Such influences and the known roles of litter size and other rearing conditions for postnatal development may be taken as factors adding to the variability of body fat content as a parameter considered in the analysis applied to the data of the present study. As a first estimate, a rather early onset of excessive fat deposition at an age between 21 and 35 days may be concluded from these data. Consideration of the potential influences arising from variations in genetic background, from external impacts such as differences in rearing conditions, and from variations between litters reared by different mothers might, however, explain why in one of the sets of animals investigated in the present study a slight genotype-related influence on body mass was already detectable at the time of weaning (see Fig. 5).

MC4R deficiency leaves regulation of plasma leptin levels virtually unaltered. The close correlation between fat mass and plasma leptin levels found across the genotypes at the MC4r locus in juvenile and adult mice contrasts with effects observed in animals carrying a genetic defect in the leptin receptor (3, 41, 42). The leptin receptor defect results in a more than proportional increase of plasma leptin levels with increasing body fat content. This mismatch is due at least in part to the loss of sympathetically mediated negative feedback control of leptin production by adipocytes that is normally induced by the activation of central leptin receptors (42). The loss of the MC4R function in the brain, in contrast, is most likely not

**Fig. 6.** Correlation of standardized neuropeptide Y (NPY; A and C) and pro-opiomelanocortin (POMC, B and D) expression data (in arbitrary units (a.u.)) in the hypothalamic arcuate nucleus (ARC) with body fat content and with plasma leptin concentration. Data are shown on logarithmic scales with common regression lines for data of 35-day-old (triangles) and 56-day-old (circles) +/+ (black symbols), +/− (gray symbols), and −/− (open symbols) mice. Correlation coefficients: $r = -0.57$ (A), $r = 0.52$ (B), $r = -0.48$ (C), $r = 0.57$ (D), all $P < 0.001$. Logarithmized data from 3 in situ hybridization runs had been standardized (see METHODS) to permit common evaluation. Results of ANCOVA, with log body fat content as covariable and genotype as factor, performed with the standardized NPY and POMC expression data indicate, in addition, genotype effects ($P < 0.001$) for both neuropeptides which are illustrated for NPY (E) and POMC (F) by least square means ± SE.
associated with a disturbance of the feedback regulation of circulating leptin levels.

The MC4R deficiency primarily causes hyperphagia without hypometabolism. Whether total MR or MR per unit of body mass (or a fraction of this) is the best parameter to analyze when comparing the metabolism of animals differing grossly in body mass (and body composition) is a matter of dispute. Contradictory results may be generated from these analyses, for example, in MC4R-deficient mice (1, 2, 36). Moreover, in view of the numerous secondary consequences of obesity for metabolic turnover, it is not possible to deduce its primary disturbance leading to obesity from the metabolic alterations found, once obesity is established in an animal (or human). Therefore, this study aimed at the disclosure of the earliest genotype-related differences in the relationship between energy intake and expenditure. The results of continuous determinations of energy expenditure from weaning to 35 days of age clearly demonstrate for +/− and −/− mice that none of the extra calories accumulated during this time period are attributable to hypometabolism. In this respect, the onset of obesity in MC4R-deficient mice differs markedly from that observed in LEP+/-ada rats. In the latter obesity model, the homozygous genotype starts to deposit excess fat during suckling age and does so by undergoing phases of hypometabolism in which normothermia is temporarily abandoned (21, 31). In contrast, the onset of excessive fat deposition in MC4R-deficient mice is due to enhanced energy intake which overcompensates a slight accompanying hypermetabolism and develops closely parallel to independent solid food ingestion. This gradually starts in mice at the end of the second postnatal week and becomes the only energy source from day 21 onward.

Changes in NPY and POMC expression appear to be chiefly counterregulatory. The predominant, genotype-independent response of the NPY system of the ARC in 35- and 56-day-old mice consists of the reduction of its mRNA signal with increasing body fat content (and plasma leptin levels, respectively). This response suggests downregulation of NPY as a central orexigenic signal, with the effect that excessive fat deposition is mitigated. An analogous interpretation may be put forward for the increase of POMC expression in the ARC with increasing body fat content (and plasma leptin levels, respectively) suggesting upregulation of POMC as the precursor of the anorexigenic peptide α-MSH. These counterregulatory responses were found to be predominant in the juvenile mice of the present study, irrespective of the gene dosage at MC4r.

Taking into account the counterregulatory responses of NPY and POMC expression to increasing body fat content in the statistical analysis has disclosed the much less obvious modifications of the prevailing counterregulatory response, which consist of increases in both NPY and POMC expression in MC4R-deficient mice. It may be hypothesized that they are part of the primary central disturbance leading eventually to gene dose-dependent obesity. Whereas an increased agonist synthesis as a consequence of the MC4R deficiency can be easily explained by a feedback mechanism, we presently cannot explain how NPY synthesis might be increased by the MC4R deficiency.

The observations made in this study on NPY and POMC expression in the ARC have elucidated the juvenile development during the postweaning growth phase toward sexual maturity. Interestingly, no consistent changes in POMC expression were observed in older MC4R-deficient animals in the original study (12). Moreover, in these older mice the genotype at the MC4r locus was reported not to affect NPY expression in the ARC, but to dramatically augment its expression in the dorsal medial hypothalamic nucleus (16). Our data, on the other hand, rather showed an overall decrease in NPY expression in the ARC paralleling the increased body fat content of our young MC4R-deficient mice. Also, no striking increase in NPY expression at any other hypothalamic site was observed in the young −/− mice. These differences between young and adult mice might be explained by the counterregulation observed in the present study becoming eventually disturbed during longer lasting states of obesity (9, 20), even to the degree that obesity would appear as the “normal” state (19).

Perspectives. In view of the human cases with MC4R haploinsufficiency in which childhood-onset obesity has been observed (4, 6, 11, 35, 40), the late development and sometimes only very moderate expression of obesity in the heterozygous animals seems puzzling. Contributions of species-specific differences to this discrepancy cannot be excluded, but it should be noted that the mice of this study were reared under standard laboratory conditions. This means, first, “healthy” food (low fat, high carbohydrate, and high fiber) and, second, usual “room temperature.” A temperature of 22–25°C, however, constitutes a considerable cold load for animals of the size of mice, requiring metabolic rates exceeding those at thermoneutrality by nearly 100%. The human studies are carried out in countries characterized by nearly unlimited access to highly palatable, high-fat food and in a mostly thermoneutral ambiance. The effect of thermoneutrality and attractive food on the onset of excessive fat deposition might well be expected to reveal driving factors for obesity development in MC4R-deficient mice that are different from those under standard rodent rearing conditions, but more relevant for modeling the human case.

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