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Changes of peripheral α-melanocyte–stimulating hormone in childhood obesity

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Abstract

Relationships of blood circulating melanocortins to childhood obesity are not well established. We evaluated serum α-melanocyte–stimulating hormone (α-MSH) in lean children and different study groups of childhood obesity. We examined serum α-MSH in 52 otherwise healthy children with childhood obesity (Ob; mean age, 11 years; 32 girls/20 boys), 27 normal-weight children of same age, 7 additional obese patients with reduced melanocortin-4 receptor function (MC4Rmut), and 22 patients with craniopharyngioma (CP). Fasting serum α-MSH and leptin were measured by radioimmunoassay. Serum α-MSH was also evaluated 1 hour after 500-kcal liquid meal (CP and Ob) and at the end of 1-year lifestyle intervention in 24 Ob patients. The α-MSH levels were similar in obese vs lean children but significantly lower in CP ($P < .001$) and significantly higher ($P < .05$) in MC4Rmut patients compared with Ob. One hour after liquid meal, α-MSH increased in patients with Ob but not with CP. After 1 year, α-MSH levels increased significantly in the successful weight reduction Ob subgroup despite unchanged cortisol levels. The α-MSH changes correlated to weight status changes ($r = 0.67$, $P = .0003$) but not to changes of cortisol, insulin, or homeostasis model assessment of insulin resistance index. Persistently low α-MSH levels in CP patients are suspected to be due to pituitary or hypothalamic damage. High peripheral levels in MC4Rmut carriers indicate up-regulation of α-MSH. Changes of weight status are associated with changes of peripheral α-MSH.

1. Introduction

Whereas most energy homeostasis studies have focused on central melanocortin action, the relevance of peripheral, blood-circulating melanocortins is not well established. Although considered to be primarily of pituitary origin, small quantities of these proopiomelanocortin (POMC)-derived peptides have been identified in a variety of peripheral tissues such as the thyroid, pancreas, and gastrointestinal tract [1-3]. The α-melanocyte–stimulating hormone (α-MSH) is a posttranslational product of the prohormone POMC [4], and the pituitary pars intermedia lobe melanotrophs are considered to be the major source of circulating α-MSH in most mammals [5]. Five melanocortin receptors are known, 2 of which are believed to be involved in energy balance signaling: melanocortin-4 receptor (MC4R) and MC3R. Mutations in the *MC4R* have solidly been shown to exert major genetic effects in obesity [6]; data on *MC3R* mutations are more limited. In addition, reduced MC4R function leads to severe hyperinsulinemia, increased linear growth, and hyperphagia [6].

Craniopharyngioma (CP) is the most common childhood tumor in the hypothalamic-pituitary region, derived from the Rathke cleft—the embryonal precursor to the adenohypophysis. Although the tumor histology is benign, most

The studies are registered at ClinicalTrials.gov (NCT00435734 and NCT00258453).

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patients require iterative hormonal substitution. In addition, more than 50% of patients diagnosed with CP during childhood will eventually develop severe obesity after tumor treatment (some with or without increased caloric intake) accompanied by exacerbating phenomena of decreased physical activity and increased need for sleep after tumor removal, all of which likely result from destruction of parts of the medial hypothalamus by the tumor itself as well as its surgical excision and irradiation [7-12]. These sequelae are no surprise, as the hypothalamic structures integrate afferent hormonal feedback signals from the body such as insulin, leptin, and gut-brain hormones such as ghrelin and peptide YY, and energy homeostasis–regulating signals released from hypothalamic neurons such as α-MSH, cocaine-amphetamine–regulated transcript, neuropeptide Y, and agouti-related peptide [13].

Besides the central appetite-suppressing (anorexigenic) effect of α-MSH [14], melanocortins increase the metabolic rate mediated by peripheral cells expressing different melanocortin receptors such as muscle cells, expressing MC1R, MC3R, MC4R, and especially MC5R [15,16]. As part of their anorexigenic effect on adipocytes, melanocortins both stimulate lipolysis and inhibit leptin secretion [17,18]. In a recent study of α-MSH effects on skeletal muscle, it was shown that α-MSH plays a role in thermal regulation by increasing free fatty acid oxidation in skeletal muscle [16].

It was our aim in this study to compare peripheral α-MSH levels in otherwise healthy children with obesity (Ob) with levels of (*a*) healthy lean children, (*b*) obese patients with reduced melanocortin-4 receptor function (MC4Rmut), as well as patients with CP, with the hope of learning more about the role of peripheral, human α-MSH in obesity. We hypothesized that α-MSH levels differ between the 4 groups and that CP patients have reduced α-MSH levels. Furthermore, we hypothesized that changes of α-MSH levels correlate with body mass index (BMI) changes after obesity intervention.

2. Patients and methods

The study protocols were approved by the local standing committees for clinical studies and the committees on ethical practice of the Universities Witten/Herdecke and Bonn (Ob and MC4Rmut patients), as well as Würzburg (CP). Written parental and/or patient consent was obtained, and the investigations were conducted according to the principles expressed in the Declaration of Helsinki.

2.1. Patient groups

All lean controls and obese patients were without any regular medication. Anthropometric markers and fasting serum were obtained from 3 discrete groups of patients. Twenty-seven normal-weight children served as lean controls (group lean: 15 girls/12 boys; mean age, 10 years) (Table 1). The second group consisted of 52 obese children (group Ob: 32 girls/20 boys; mean age, 11 years) who were consecutively treated for obesity either at the Department of Pediatrics, University of Bonn, or at the Department of Pediatric Nutrition Medicine, Vestische Kinderklinik, Datteln, Germany (ClinicalTrials.gov NCT00435734). Screening for MC4R mutations was negative; syndromal obesity was excluded in this group. The third group consisted of additional 7 obese patients with identified MC4R mutations entailing a reduced receptor function (group MC4Rmut: 3 girls/4 boys; mean age, 12 years), identified in a cohort of 328 obese children treated in Datteln or in Bonn, who were screened for MC4R mutations (see below for the different mutations) [6,19]. All mutation carriers were unrelated to exclude the influence of potential familial factors. The fourth group consisted of 22 CP patients (group CP: 13 girls/9 boys; mean age, 17 years) who were involved in the German pediatric CP study KRANIOPHARYNGEOM 2000 (ClinicalTrials.gov NCT00258453) and had all undergone cranial tumor surgery. In most patients, hypothalamic involvement,

Table 1
Age, sex, BMI, blood glucose, HOMA insulin resistance index, and serum levels of insulin and α-MSH in lean and obese cohorts

	Lean	Ob	MC4Rmut	CP
n	27	52	7	22
Age (y)	10 (8-13)	11 (8-12)	12.0 (10.4-14.7)	17 (14-22)*,†
Sex (% female)	55%	61%	43%	59%
BMI	17.4 (15.9-20.7)	27.1 (24.8-29.9)*	32.2 (27.2-36.9)*	33.4 (28.2-38.6)*,†
SDS-BMI	0.15 (-0.74-0.73)	2.45 (2.20-2.77)*	2.84 (2.55-2.94)*	2.42 (1.88-3.25)*
BG (mg/dL)	ND	90 (85-97)	79 (75-85)†	108 (103-121)†,‡
Insulin (mU/L)	ND	13.3 (6.8-19.4)	18.3 (14.6-39.6)§	17.0 (11.5-38.5)§
HOMA	ND	2.97 (1.44-4.32)	3.70 (2.70-7.58)	4.70 (3.04-10.16)†
α-MSH (fmol/mL)	22.8 (16.5-29.2)	21.6 (16.2-29.4)	34.6 (26.1-49.6)†,§	6.6 (4.1-8.7)*,†,‡

Median values and interquartile ranges are shown. BG indicates blood glucose; ND, not determined.

* $P < .001$ vs lean.

† $P < .001$ vs Ob.

‡ $P < .001$ vs MC4Rmut.

§ $P < .01$ vs Ob.

assessed by intraoperative microscopic inspection and/or imaging, was present. A complete tumor resection was performed in 6 patients, whereas 16 (73%) received percutaneous cranial irradiation. In approximately half of the patients, the tumor had already relapsed at the time he or she was studied. Of the 15 CP patients at pubertal/postpubertal age, 11 were treated with growth hormone replacement, 14 with levothyroxine, 12 with desmopressin acetate (DDAVP), all with hydrocortisone, and 10 with sex steroids. Panhypopituitarism patients were assessed for hormonal deficiencies and were adequately treated as required.

2.2. Obesity intervention

Of the 52 Ob patients, 24 children participated in the 1-year German obesity intervention program "Obeldicks" [20,21], whereas family schedules of the other children did not permit participation. The Obeldicks intervention program (named for a popular European comic figure) was based on physical exercise, nutrition education, and behavioral therapy, including the individual psychologic care of the child and his or her family [20,21]. The aims were to reduce overweight and to improve the cardiovascular disease risk factor profile by lifestyle modification. An interdisciplinary team of pediatricians, dietitians, psychologists, and exercise physiologists was responsible for the training. The children were divided into groups according to their sex and age. The 1-year training program was divided into 3 phases. In the intensive phase (3 months), the children took part in the nutritional course and in the eating-behavior course in 6 group sessions, each lasting for 1.5 hours. At the same time, the parents were invited to attend 6 parents' evenings. In the establishing phase (6 months), individual psychologic family therapy was provided (30 min/mo). In the follow-up phase of the program (3 months), further individual care was possible if necessary. The exercise therapy took place once a week throughout the year and consisted of ball games, jogging, trampoline jumping, and instructions in physical exercise as part of everyday life, as well as a reduction in the amount of time spent watching television. Fifteen of the 24 Ob participants did not show weight reduction (group Ob no WR), whereas 9 patients lost weight effectively (group Ob WR), showing greater than 0.4 reduction of standard deviation score BMI (SDS-BMI).

2.3. Genotypes

The following 5 MC4R mutations were detected heterozygously in the 7 individuals of the MC4Rmut group: (*a*) Two patients had a haplotype [Y^{35}Stop; 110A>T] comprising a nonsense mutation leading to a loss of MC4R function. (*b*) Two patients had nonsynonymous P48S. In silico analyses by PolyPhen (http://genetics.bwh.harvard.edu/pph/index.html) indicated that this leads to a possibly damaging effect. (*c*) One patient had the nonsynonymous T112M that has been described as similar to the wild-type receptor or as entailing a reduced receptor function [6,22,23]. (*d*) One patient had a combination of the V103I polymorphism and the S127L nonsynonymous mutation, the latter leading to reduced receptor function [24]. (*e*) One patient had the nonsynonymous Y302F that leads to a loss of function [25].

2.4. Meal test

To investigate the secretion dynamics, we quantified α-MSH before and 1 hour after ingestion of a 500-kcal liquid meal (protein, 15%; fat, 30%; carbohydrate, 55%) in patients with CP vs Ob.

2.5. Anthropometric data

Obesity was defined according to the International Task Force of Obesity using population-specific data [26,27]. Height was measured to the nearest centimeter using a rigid stadiometer. Weight was measured in underwear to the nearest 0.1 kg using a calibrated balance scale. The SDS-height, SDS-weight, and SDS-BMI were calculated according to German percentiles [27]. Pubertal developmental stage was assessed using the standards from Marshall and Tanner.

2.6. Blood parameters

Blood sampling was performed in the fasting status at 8:00 AM, and the samples were put on ice immediately after withdrawal. After centrifugation, serum specimens for α-MSH were frozen at −80°C, whereas all other laboratory determinations were performed directly. The α-MSH immunoreactivity was measured with a rabbit anti–α-MSH (Catalog RAB-043-01; Phoenix Pharmaceuticals, Belmont, CA). ^{125}I-labeled α-MSH was prepared by the iodogen method and purified by high-pressure liquid (University of Mississippi Peptide Radioiodination Service Center, University, MS). The sensitivity was 0.30 fmol/mL. The intraassay variation (coefficient of variation [CV%]) was determined by replicate analysis (n = 10) of 2 samples at α-MSH concentrations of 2 and 10 fmol per tube, the results being 7.8% and 7.5%, respectively. The interassay CV% was 10.7% and 12.1% for the range of value measured. α-Melanocyte–stimulating hormone is a tridecapeptide with an amino acids sequence that is identical to residues 1 to 13 of adrenocorticotropic hormone (ACTH). It differs only in the N-terminal acetyl and C-terminal valine-amide. Because the expected α-MSH levels overlap with ACTH, the cross-reactivity of the anti–α-MSH antibody with ACTH was determined by displacement technique in 24-hour incubation experiments ("Results"). Plasma ACTH levels were measured by a commercially available immunoradiometric assay (IRMA ACTH; Mitsubishi Kagaku Iatron, Tokyo, Japan). According to the manufacturer, the antibody does not cross-react with α-MSH or ACTH 1 to 24; but it can detect human and rat ACTH 1 to 39. The sensitivity was 0.55 pg/mL. The intraassay CV% was 3.1%, and interassay CV% was 4.5%. The ACTH human plasma pool was prepared mixing 10 human plasma samples and adding human ACTH (Sigma, A-0423, ACTH 1-39; Sigma

Chemical, St Louis, MO). Blood was collected in iced plastic tubes with 250 μL EDTA plus 20 μL of protease inhibitor cocktail (Sigma). To test the degradation product of ACTH, we incubated 5 aliquots of 500 μL of ACTH human plasma pool at 25°C and measured the degradation of endogenous α-MSH and ACTH in 24 hours (times 0, 1, 4, 8, and 24). The antibody cross-reacted fully with the acetylated α-MSH and partially (46%) with deacetylated α-MSH, but not with ACTH.

Serum leptin levels were measured at baseline in Ob patients participating in the lifestyle intervention by a commercially available radioimmunoassay (RIA) (Human Leptin RIA; Mediagnost, Reutlingen, Germany); the intra- and interassay CVs were defined at 5% and 8%, respectively. The sensitivity was 0.1 ng/mL. Serum insulin concentrations were measured by microparticle enhanced immunometric assay (Abbott, Wiesbaden, Germany). Specimen for quantification of plasma glucose was collected in tubes with glucolytic inhibitor (sodium fluoride–potassium oxalate; Sarstedt, Nümbrecht, Germany), and plasma glucose levels were determined by colorimetric test using a Vitros analyzer (Ortho Clinical Diagnostics, Neckargmuend, Germany). Intra- and interassay CVs were less than 5% in all methods apart from α-MSH and leptin. Homeostasis model assessment (HOMA) was used to detect the degree of insulin resistance as described previously. Insulin resistance was assessed from the fasting glucose and insulin concentrations using the following formula: resistance (HOMA) = [insulin (in microunits per liter) × glucose (in millimoles per liter)]/22.5 [28]. Serum cortisol concentrations were determined in Ob patients participating in the lifestyle intervention by solid-phase technique chemiluminescence immunoassays (Immulite; DPC, Los Angeles, CA) (intraassay CV, 6.8%; interassay CV, 9.9%).

2.7. Statistical analysis

Statistical analysis was performed using the Winstat (Bad Krozingen, Germany) and GraphPad (San Diego, CA) Prism 4.0 software packages. Correlations were calculated by Pearson correlations. Normal distribution was tested for all continuous variables using the Kolmogorov-Smirnov test. Normally distributed variables in obese and normal-weight children as well as pre- and postmeal values were compared by Student *t* test for unpaired observations. Nongaussian variables were compared by Mann-Whitney *U* test. Longitudinal changes in SDS-BMI over the course of the 1-year Obeldicks program were evaluated. *Effective weight loss* was defined by reduction of SDS-BMI greater than 0.4. Because the distribution of BMI is not comparable in children and adults, not even among the various childhood age groups, we used the LMS method to calculate SDS-BMI as a measurement for the degree of overweight. The LMS method was chosen because it summarizes the data in terms of 3 smooth age-specific curves called *L* (λ), *M* (μ), and *S* (σ) based on German population-specific data [26,27]. The M and S curves correspond to the median and CVs of BMI for German children at each age and sex, whereas the L curve allows for the substantial age-dependent skewness in the distribution of BMI. The assumption underlying the LMS method is that, after Box-Cox power transformation, the data at each age are normally distributed [26]. Data were analyzed by 1-way analysis of variance for multiple groups, followed by Bonferroni multiple comparison test. Changes of α-MSH were correlated to changes of weight status (SDS-BMI), cortisol, insulin, and insulin resistance index HOMA by Pearson correlation. As there were no effects of age and sex upon α-MSH levels, levels from all girls and boys within groups were combined for further analyses. For the interventions, we calculated that 9 subjects studied under different experimental conditions would provide greater than 80% power to detect changes in the mean effect estimates larger than 1.24 times the respective SD at the conventional $\alpha = .05$ level and will be of adequate size in terms of assessing α-MSH changes (2-tailed test). A P value < .05 was considered as significant. Values are expressed as both the median and the interquartile range in the table and as the mean ± SEM in text and figures, if not otherwise stated.

3. Results

The standard displacement curve for α-MSH RIA showed that the ACTH standard curve and the α MSH displacement curve were not parallel, demonstrating that the antibody cannot detect ACTH in the concentration studied (Fig. 1A). To test that α-MSH detected by the assay is not a degradation product of ACTH, we added ACTH to a human pool of plasma. The degradation of both peptides, endogenous α-MSH and ACTH, was measured for 24 hours at 25°C. Although ACTH degraded at room temperature, no increase of α-MSH as a degradation product was detected (Fig. 1B), even when ACTH was added to a human pool of plasma (Fig. 1C).

There were no appreciable differences in baseline α-MSH levels between the obese and normal-weight children (Table 1). In analyzing values of lean controls with the Ob group, α-MSH levels were comparable in both sexes (α-MSH in femtomoles per milliliter: lean girls, 25.3 ± 3.6; lean boys, 26.7 ± 3.3; Ob girls, 24.4 ± 1.8; Ob boys, 21.6 ± 2.4) and were not age dependent ($r = 0.052$, $P = .648$). Serum α-MSH values did not correlate with SDS-BMI values in lean controls ($r = 0.086$, $P = .671$), Ob patients ($r = 0.256$, $P = .073$), or lean and Ob combined ($r = 0.058$, $P = .616$). The MC4Rmut patients with identified MC4R mutations and reduced MC4R function had significantly higher α-MSH values compared with lean controls as well as Ob patients of comparable SDS-BMI ($P < .05$). Conversely, patients with CP had significantly reduced α-MSH levels compared with all other groups ($P < .001$) (Table 1). In the liquid meal stimulation test, low pre- and postmeal α-MSH levels were measured in patients with CP 1 hour after 500-

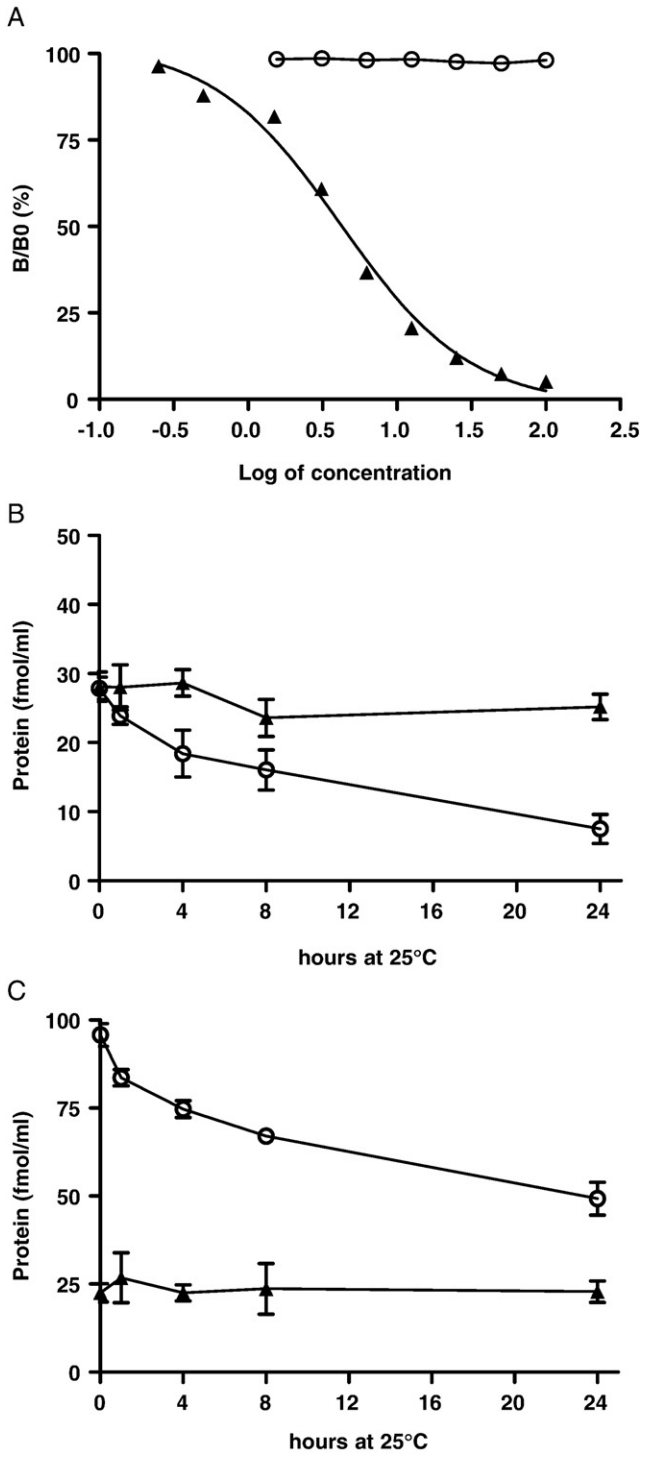

Fig. 1. Standard displacement curve for α-MSH RIA and cross-reactivity in the used RIA for α-MSH detection. The α-MSH standard curve (triangles) showed a not-parallel displacement curve, demonstrating that the antibody cannot detect ACTH added (circles) in the concentration studied (A). Using a human pool of plasma, degradation of ACTH (circles) at room temperature (25°C) did not result in an increase of α-MSH (triangles) detected by the α-MSH assay (B). Even if ACTH (circles) was added to a human pool of plasma, the degradation of ACTH did not result in an increase of α-MSH (triangles) as a degradation product (C).

kcal liquid meal compared with Ob patients (α-MSH in femtomoles per milliliter: CP before meal, 4.8 ± 0.6; after meal, 5.3 ± 0.7; Ob before meal, 17.9 ± 3.3; after meal, 21.6 ± 3.0; $P < .01$ Ob before compared with Ob after meal; Fig. 2).

Twenty-four of the 52 children in the Ob group participated in the 1-year obesity lifestyle intervention program. The α-MSH levels were compared in 2 subgroups: the Ob no WR group comprising 15 patients without BMI reduction after 1 year was compared with 9 patients in group Ob WR with effective BMI reduction (SDS-BMI before/after: group Ob no WR, 2.41 ± 0.10/2.36 ± 0.13 vs group Ob WR, 2.50 ± 0.15/1.88 ± 0.17). After 1 year, α-MSH levels increased only in group Ob WR (α-MSH in femtomoles per milliliter: before intervention, 16.3 ± 2.0; after intervention, 23.2 ± 3.2; $P = .004$) when compared with their respective baseline levels (Fig. 3A).

In analyzing α-MSH, insulin, and HOMA values of all 52 Ob patients, no correlation between α-MSH and insulin ($r = 0.092$, $P = .489$), between α-MSH and HOMA ($r = 0.074$, $P = .577$), between α-MSH and cortisol ($r = 0{,}116$, $P = .237$), or between α-MSH and leptin ($r = 0.266$, $P = .155$) was found. Patients with CP had significantly higher insulin and HOMA values compared with Ob patients (Table 1). A significant negative correlation between change of α-MSH and change of SDS-BMI (Fig. 3 B), but not changes of insulin, HOMA, or cortisol, was found. Compared with group Ob WR, leptin levels in group Ob no WR were significantly higher (58.8 ± 4.1 vs 22.8 ± 4.1 ng/mL, $P < .01$) at baseline; but blood glucose, insulin, and insulin resistance (HOMA) values were comparable. Serum cortisol levels did not change (serum cortisol before/after: Ob no

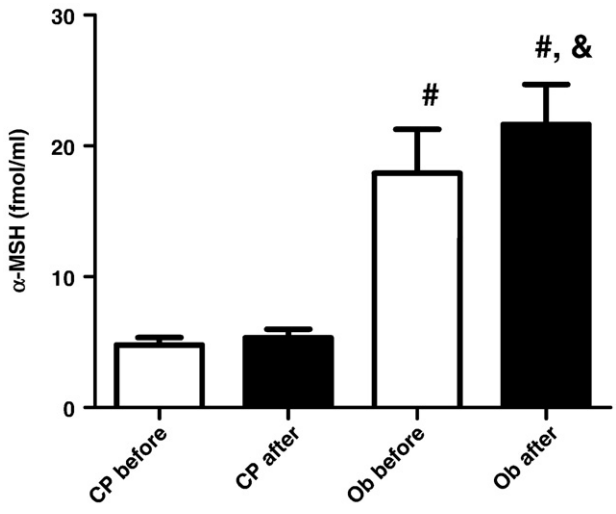

Fig. 2. Mean serum α-MSH levels (±SEM) before and 1 hour after 500-kcal liquid meal. Pre- and postmeal levels in patients with CP were significantly lower than those in Ob patients and did not increase, whereas in Ob patients, postmeal α-MSH levels increased. $^{\#}P < .001$ compared with CP before and CP after meal. $^{\&}P < .01$ compared with Ob before meal.

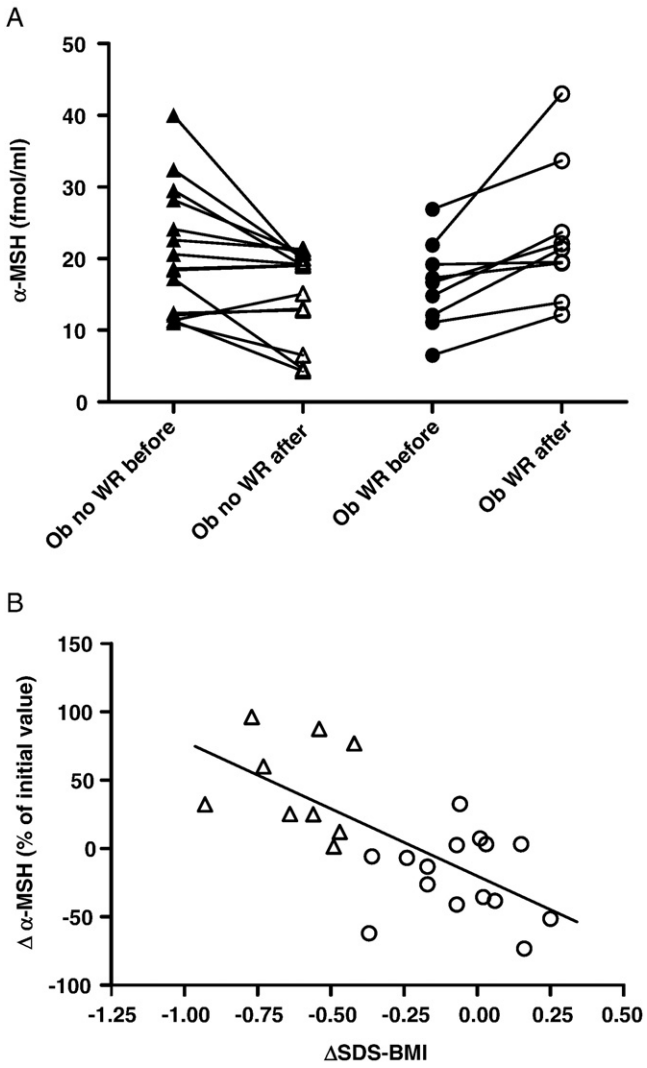

Fig. 3. Changes in α-MSH before and after 1-year obesity intervention in 15 patients without successful weight reduction (Ob no WR, triangles) vs 9 patients with successful weight reduction (Ob WR, circles, ΔBMI >0.4). After 1 year, α-MSH levels were significantly lower in Ob no WR (P = .015), but significantly higher in Ob WR (P = .004) (A). Correlation between ΔBMI (SDS) (SDS-BMI after 1 year − BMI-SDS at baseline) and Δα-MSH (values after 1 year − values at baseline). Triangles represent data from efficient weight losers, and circles represent data from patients with unsuccessful weight reduction. Stronger increase of α-MSH levels were associated with a greater degree of weight reduction (r = 0.67, P = .0003) (B).

WR, 10.5 ± 1.3/10.9 ± 1.3 μg/dL vs Ob WR, 11.1 ± 1.7/10.5 ± 1.0 μg/dL).

4. Discussion

There are only a few reports on serum α-MSH levels in human adults and none in children [29–31]. To gain a more comprehensive insight into the regulation of peripheral blood α-MSH levels and its involvement in energy homeostasis, we studied this hormone under different conditions of childhood obesity and leanness. We for the first time detected both elevated α-MSH serum levels in patients with MC4R mutations and strongly decreased α-MSH levels in obese patients with CP, compared with Ob and lean children. Interestingly, in obese children participating in the Obeldicks obesity intervention program, after the 1-year program was completed, α-MSH levels increased significantly but only in the children with successful weight reduction. No α-MSH levels differences were found in the Ob and lean children groups. We also did not find significant α-MSH differences between girls and boys; and there was also no correlation between α-MSH and age, or correlations between α-MSH levels and any measure of insulin or leptin.

The elevated α-MSH levels determined in the heterozygous MC4R mutation carriers potentially suggest a counterregulation; POMC expression is possibly elevated in the respective individuals. Further studies are warranted to determine whether measurement of α-MSH levels in mutation carriers can help assess the functional implications of MC4R mutations in addition to in vitro studies. It would be worthwhile to investigate α-MSH levels in homozygous or compound heterozygous individuals.

Although no comparable study data of α-MSH levels in children were available in the literature, 2 adults studies demonstrated higher levels of α-MSH in obese compared with lean individuals [29,30]. Hoggard et al [29] found elevated plasma α-MSH in adult obese subjects compared with adult lean individuals; and α-MSH correlated tightly with fat mass and leptin, yet α-MSH levels were not affected by any changes in energy balance in either the lean or the obese volunteers in their short-term intervention study. In a third, slightly more recent study in adults, no differences could be identified [31].

In contrast, in our study, the obese children with significant weight reduction after participating in the 1-year Obeldicks obesity intervention program showed a pronounced increase in α-MSH. We even found a direct correlation between the change of α-MSH levels and the change in BMI-SDS in the obese children who had lost weight compared with those who did not, although α-MSH levels were comparable between nonparticipating Ob patients and lean controls. Although the regulation of α-MSH in these groups is not completely understood by us, these results suggest that α-MSH levels are probably more related to weight change than to static weight status, supporting the probable role of α-MSH in peripheral regulation of energy homeostasis. We also evaluated serum cortisol levels, operating on the assumption that caloric restraint from the obesity intervention could cause stress and therefore might be reflected by changes in ACTH and cortisol levels. The unperturbed cortisol levels in both groups, coupled with the observed differences in the α-MSH concentrations between responders and nonresponders despite unchanged cortisol levels, imply an ACTH-independent effect.

It has been established that intermediate lobe pituitary melanotrophs are the major source of circulating α-MSH [5], although the importance of peripheral α-MSH in energy homeostasis remains unclear. Interestingly, Gavrila et al [31] detected melanin-concentrating hormone as well as α-MSH plasma levels in both lean and overweight adults. Their findings suggest that these centrally expressed hormones secreted from hypothalamic neuron terminals cross the blood-brain barrier to reach the bloodstream. As in our study, the authors did not find a significant correlation between α-MSH and BMI, plasma insulin, or leptin. However, Katsuki et al [30] detected higher α-MSH plasma concentrations in obese vs lean men that correlated with fasting insulin concentrations in obese, but not in nonobese, men. The strongest positive correlation was found between α-MSH and visceral fat mass, which might explain why their results differ from our study, as visceral adiposity, suspected to be a potential source of α-MSH, is usually not as marked in children [32] as it is in obese adult men. Proopiomelanocortin-derived peptides have also been identified in small quantities in a variety of peripheral tissues such as the thyroid, pancreas, and gastrointestinal tract [3]. This helps us explain the high peripheral α-MSH levels we found in MC4R mutation patients, which were most likely reflections of compensatory up-regulations of the substrate due to receptor deficiency.

As mentioned earlier, peripheral effects of melanocortins are not well established. In a recent study, peripheral administration of α-MSH analogue in mice produced an increase in resting energy expenditure measured by indirect calorimetry [33]. This effect was reproduced in leptin-deficient *ob*/*ob* mice, suggesting that α-MSH acts independently from leptin. This could explain why we did not find any correlation between α-MSH and leptin levels. Importantly, there is evidence showing that increased adenosine monophosphate–activated protein kinase (AMPK) activity in skeletal muscle leads to increased free fatty acid oxidation by activation of the protein kinase A (PKA)-AMPK pathway, increasing the activity of carnitine palmitoyltransferase and causing increased rates of glucose transport [16,34]. α-Melanocyte–stimulating hormone had also been shown to play a role in thermal regulation by mobilizing fat stores and increasing free fatty acids [16-18]. These mechanisms lead us to the speculation—which would need to be confirmed by further studies—that in obese individuals increased α-MSH levels after weight loss might support higher energy expenditure and maintenance of a lower body weight compared with individuals with stable or decreasing α-MSH levels. Decreased leptin signaling due to decreased fat mass would on the other hand be expected to result in decreased energy expenditure.

As previously stated, all patients with CP had undergone varying degrees of prestudy tumor excision surgery; and most had cranial irradiation as well. The CP tumor itself and these treatments compromise functions of the hypothalamus pituitary unit, offering likely explanations for their dramatically low α-MSH levels. It was important for us to develop a specific α-MSH RIA without cross-reaction to ACTH, as low ACTH levels in CP patients could have confounded the MSH levels. They were included in this study because most CP patients have hypothalamic obesity, the low α-MSH levels in all likelihood contributing to their disturbed energy homeostasis. As α-MSH inhibits the leptin secretion from adipocytes, elevated leptin in patients with hypothalamic CP as found in a previous study [35] could also be explained, at least in part, by their low α-MSH levels. The low α-MSH levels could furthermore lead to decreased thermogenesis by reduced muscle fatty acid oxidation and reduced lipolysis in adipocytes [16].

Our study has a few potential limitations. The first limitation is that BMI was used to classify study groups as overweight. Although BMI is a good measure for overweight, one needs to be aware of its limitations as an indirect measure of fat mass, which some suspect could be a source in and of itself of energy homeostasis agents. Second, in the meal stimulation test, we used only 1 postmeal time point because otherwise the test would not have been possible in this ambulatory setting. We have chosen the 60-minute postmeal time point because in our previous studies strong changes of postmeal responses of other hormones such as peptide YY, ghrelin, and insulin could be demonstrated at this time point ([36] and unpublished results). Third, α-MSH RIA is a challenging assay mainly because of its very low concentrations in humans. In our assay, both the acetylated and deacetylated circulating α-MSH levels are detected. The 2 forms might have different effects on energy balance. But most importantly, our incubation experiments at room temperature confirmed that α-MSH—and not ACTH or degradation products of ACTH—is detected by the α-MSH assay used in this study.

The intent of this study was to investigate the hypothesis that α-MSH levels differ between the different obesity cohorts and that CP patients have reduced α-MSH levels. In addition, we hypothesized that weight changes in obese children are associated with changes of peripheral α-MSH levels. In summary, the most important results are low α-MSH levels in CP patients and high α-MSH levels in MC4Rmut carriers. Successful weight reduction was associated with an increase of peripheral α-MSH levels. We conclude that, although our measured differences could be explained by underlying hormonal and metabolic changes, they might serve as an indicator for the severity of the metabolic disorder and for success in weight-reducing programs, or even a combination of the two, where hormonal and metabolic changes are not completely ruled out. Although the role of α-MSH in the regulation of muscle glucose intake and thermogenesis needs continued research to be completely understood, the low α-MSH levels we found in CP patients and the increase of peripheral α-MSH levels in children with efficient weight reduction seem to be important to understanding different physiology of energy homeostasis, which should be studied further to help identify potential treatments of childhood obesity.

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References

[1] Cone RD. Studies on the physiological functions of the melanocortin system. Endocr Rev 2006;27:736-49.
[2] Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol 2003;149:79-90.
[3] Smith AI, Funder JW. Proopiomelanocortin processing in the pituitary, central nervous system, and peripheral tissues. Endocr Rev 1988;9: 159-79.
[4] Ying J, Gu X, Cai M, Dedek M, Vagner J, Trivedi DB, et al. Design, synthesis, and biological evaluation of new cyclic melanotropin peptide analogues selective for the human melanocortin-4 receptor. J Med Chem 2006;49:6888-96.
[5] Coates PJ, Doniach I, Hale AC, Rees LH. The distribution of immunoreactive alpha-melanocyte–stimulating hormone cells in the adult human pituitary gland. J Endocrinol 1986;111:335-42.
[6] Hinney A, Bettecken T, Tarnow P, Brumm H, Reichwald K, Lichtner P, et al. Prevalence, spectrum, and functional characterization of melanocortin-4 receptor gene mutations in a representative population-based sample and obese adults from Germany. J Clin Endocrinol Metab 2006;91:1761-9.
[7] Müller HL, Bueb K, Bartels U, Roth C, Harz K, Graf N, et al. Obesity after childhood craniopharyngioma—German multicenter study on pre-operative risk factors and quality of life. Klin Pädiatr 2001;213: 244-9.
[8] Müller HL. Childhood craniopharyngioma. Recent advances in diagnosis, treatment and follow-up. Horm Res 2008;69:193-202.
[9] Lustig RH, Post S, Srivannaboon K, Rose S, Danish R, Burghen G, et al. Risk factors for the development of obesity in children surviving brain tumors. J Clin Endocrinol Metab 2003;88:611-6.
[10] Poretti A, Grotzer M, Ribi K, Schönle E, Boltshauser E. Outcome of craniopharyngioma in children: long-term complications and quality of life. Dev Med Child Neurol 2004;46:220-9.
[11] Karavitaki N, Cudlip S, Adams C, Wass J. Craniopharyngiomas. Endocr Rev 2006;27:371-97.
[12] Kendall-Taylor P, Jönsson P, Abs R, Erfurth EM, Koltowska-Häggström M, Price D, et al. The clinical, metabolic and endocrine features and the quality of life in adults with childhood-onset craniopharyngioma compared with adult-onset craniopharyngioma. Eur J Endocrinol 2005;152:557-67.
[13] Schwartz MW, Woods SC, Porte Jr D, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature 2000;404: 661-71.
[14] Rossi M, Kim MS, Morgan DGA, Small CJ, Edwards CMB, Sunter D, et al. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 1998;139:4428-31.
[15] Boston BA, Cone RD. Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. Endocrinology 1996;137:2043-50.
[16] An JJ, Rhee Y, Kim SH, Kim DM, Han DH, Hwang JH, et al. Peripheral effect of alpha-melanocyte–stimulating hormone on fatty acid oxidation in skeletal muscle. J Biol Chem 2007;282:2862-70.
[17] Forbes S, Bui S, Robinson BR, Hochgeschwender U, Brennan MB. Integrated control of appetite and fat metabolism by the leptin-proopiomelanocortin pathway. Proc Natl Acad Sci U S A 2001;98: 4233-7.
[18] Hoggard N, Hunter L, Duncan JS, Rayner DV. Regulation of adipose tissue leptin secretion by alpha-melanocyte–stimulating hormone and agouti-related protein: further evidence of an interaction between leptin and the melanocortin signalling system. J Mol Endocrinol 2004;32: 145-53.
[19] Reinehr T, Hebebrand J, Friedel S, Toschke AM, Brumm H, Biebermann H, et al. Lifestyle intervention in obese children with variations in the melanocortin 4 receptor gene. Obesity (Silver Spring) 2009;17:382-9.
[20] Reinehr T, de Sousa G, Toschke AM, Andler W. Long-term follow-up of cardiovascular disease risk factors in children after an obesity intervention. Am J Clin Nutr 2006;84:490-6.
[21] Reinehr T, Kersting M, Alexy U, Andler W. Long-term follow-up of overweight children: after training, after a single consultation session and without treatment. J Pediatr Gastroenterol Nutr 2003; 37:72-4.
[22] Farooqi IS, Keogh JM, Yeo GSH, Lank EJ, Cheetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Eng J Med 2003;348:1085-95.
[23] Nijenhuis WA, Garner KM, van Rozen RJ, Adan RA. Poor cell surface expression of human melanocortin-4 receptor mutations associated with obesity. J Biol Chem 2003;278:22939-42295.
[24] Valli-Jaakola K, Lipsanen-Nyman M, Oksanen L, Hollenberg AN, Kontula K, Bjørbaek C, et al. Identification and characterization of melanocortin-4 receptor gene mutations in morbidly obese finish children and adults. J Clin Endocrinol Metab 2004;89:940-5.
[25] Roth CL, Ludwig M, Woelfle J, Fan ZC, Brumm H, Biebermann H, et al. A novel melanocortin-4 receptor gene mutation in a female patient with severe childhood obesity. Endocrine 2009.
[26] Cole TJ. The LMS method for constructing normalized growth standards. Eur J Clin Nutr 1990;44:45-60.
[27] Kromeyer-Hauschild K, Wabitsch M, Geller F, Ziegler A, Geiss HC, Hesse V, et al. Percentiles of body mass index in children and adolescents evaluated from different regional German studies. Monatsschr Kinderheilkd 2001;149:807-18.
[28] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[29] Hoggard N, Johnstone AM, Faber P, Gibney ER, Elia M, Lobley G, et al. Plasma concentrations of alpha-MSH, AgRP and leptin in lean and obese men and their relationship to differing states of energy balance perturbation. Clin Endocrinol (Oxf) 2004;61:31-9.
[30] Katsuki A, Sumida Y, Murashima S, Furuta M, Araki-Sasaki R, Tsuchihashi K, et al. Elevated plasma levels of alpha-melanocyte stimulating hormone (alpha-MSH) are correlated with insulin resistance in obese men. Int J Obes Relat Metab Disord 2000;24: 1260-4.
[31] Gavrila A, Chan JL, Miller LC, Heist K, Yiannakouris N, Mantzoros CS. Circulating melanin-concentrating hormone, agouti-related protein, and alpha-melanocyte–stimulating hormone levels in relation to body composition: alterations in response to food deprivation and recombinant human leptin administration. J Clin Endocrinol Metab 2005;90:1047-54.
[32] Reinehr T, Kersting M, Wollenhaupt A, Alexy U, Kling B, Ströbele K, et al. Evaluation of the intervention program "OBELDICKS" for obese children and adolescents. Klin Päd 2005;217:1-8.

[33] Hoggard N, Rayner DV, Johnston SL, Speakman JR. Peripherally administered [Nle4,D-Phe7]-alpha-melanocyte stimulating hormone increases resting metabolic rate, while peripheral agouti-related protein has no effect, in wild type C57BL/6 and ob/ob mice. J Mol Endocrinol 2004;33:693-703.

[34] Fujii N, Jessen N, Goodyear LJ. AMP-activated protein kinase and the regulation of glucose transport. Am J Physiol Endocrinol Metab 2006; 291:E867-877.

[35] Roth C, Wilken B, Hanefeld F, Schröter W, Leonhardt U. Hyperphagia in children with craniopharyngioma is associated with hyperleptinaemia and a failure in the downregulation of appetite. Eur J Endocrinol 1998;138:89.

[36] Roth CL, Enriori PJ, Hinney A, Hebebrand J, Reinehr T, Cowley MA. Peripheral α-MSH in childhood obesity and craniopharyngioma. The Endocrine Society's 89th Abstract, Annual Meeting, San Francisco; 2008.