SEVERE PEDIATRIC OBESITY

Diagnostic Aspects

OZAIR ABAWI

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diagnostic aspects

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Severe Pediatric Obesity: diagnostic aspects
Ernstige obesitas bij kinderen: Diagnostische aspecten

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TABLE OF CONTENTS

1

General introduction

The global pandemic of obesity constitutes one of the most important health challenges of the 21st century.[1] The prevalence of pediatric obesity worldwide has risen dramatically in the past four decades by eightfold in girls and tenfold in boys. As a result, 124 million children and adolescents aged 5-19 years were living with obesity in 2016.[2,3] This number is predicted by the World Obesity Federation (WOF) to further increase to 310 million (16%) by 2030 and 383 million (19%) by 2035.[4] The associated direct and indirect costs of pediatric obesity worldwide are estimated to be US$308 per capita, translating into US$45 billion per year.[5] When focusing on severe pediatric obesity, a 9-fold increase in global prevalence is reported in the past four decades,[6] with prevalence ranging from 1.0% – 6.3% in different countries.[7,8] In the Netherlands, the prevalence of severe pediatric obesity ranges from 0.6% – 2.1% depending on the children's ethnic origins.[9] Severe pediatric obesity is associated with numerous adverse physical and psychosocial health consequences in the short term, e.g. weight stigma, bullying and psychological comorbidities,[10,11] as well as the long term, including type 2 diabetes, cardiovascular disease and many types of cancer.[12] Moreover, pediatric obesity tracks into adulthood in the majority of cases,[13] and even more strongly in severe pediatric obesity: over a mean follow-up interval of 21 years, only 4% of children with severe obesity did not have obesity as adults, and 69% had grade 3 severe obesity as adults.[14] Therefore, severe pediatric obesity does not only lead to a high economic burden,[5] but also to major loss of well-being and productivity both in the short term as well as the long term.[15]

DEFINITIONS OF SEVERE PEDIATRIC OBESITY

Obesity is a complex, relapsing and chronic endocrine disease.[16] It is characterized by an abnormal fat accumulation that impairs health.[16,17] In practice, body mass index (BMI) is used to define obesity (grade 1: BMI ≥30 kg/m^2) and severe obesity (grade 2: BMI ≥35 kg/m^2; grade 3: BMI ≥40 kg/m^2) in adults. Because the relation between BMI and adiposity varies throughout childhood, age- and sex specific BMI standard deviation score (BMI SDS) thresholds that correspond to these adult BMI cut-offs are used to define pediatric obesity and severe pediatric obesity.[18,19] Obesity develops as a result of a caloric imbalance between energy intake and energy output over a prolonged time period and is a multifactorial disease, affected by genetic, environmental, behavioral, socioeconomic and cultural factors.[4,11,20] Although the changes in our modern obesogenic environment are seen as the main driver of the rapidly increasing prevalence of severe pediatric obesity in the past decades, the response to this changed environment varies greatly between individuals and is strongly influenced by underlying genetic factors.[20] Twin and family studies have estimated that

the heritability of BMI is as high as 40-70%.[21] Most children with severe obesity have multifactorial obesity, also called common or polygenic obesity. In these children, the small effects of hundreds or thousands of genetic polymorphisms interact with environmental factors to contribute to their obesity. Currently, over 1000 loci are associated with multifactorial obesity,[20] whereas other loci are exclusively associated with pediatric obesity,[22] or severe pediatric obesity.[23]

DIAGNOSING UNDERLYING MEDICAL CAUSES OF SEVERE PEDIATRIC OBESITY

In a minority of children with severe obesity, a singular underlying medical cause can be identified which causes the individual's obesity.[24] It is crucial for health care professionals to diagnose these underlying medical causes,[24] as differing pathophysiologic mechanisms cause the obesity in these individuals,[25] which therefore required tailored treatments.[26] These underlying causes of severe pediatric obesity and the children harboring them are the main focus of this thesis. Current international guidelines for pediatric obesity identify and define the following underlying medical causes of pediatric obesity: (1) genetic obesity disorders, (2) hypothalamic obesity, (3) endocrine obesity, and (4) medication-induced obesity.[24] In order to provide adequate, patient-tailored treatment for children with severe obesity, it is necessary to provide adequate diagnostics first.[11,24,27] This includes evaluation of the presence or absence of these potential underlying medical causes of obesity or the presence of multifactorial obesity as diagnosis by exclusion.[24] International guidelines, e.g. by the Endocrine Society, as well as national guidelines[28] guide clinicians through the diagnostic process. In short, extensive medical history-taking and physical examination form the basis of the diagnostic approach. Subsequently, additional diagnostic steps are suggested based on the patient's phenotype. Endocrine evaluation is suggested in children with reduced growth velocity. Evaluation of potential hypothalamic obesity is suggested in patients with central nervous system injury. In patients using antipsychotic drugs, re-evaluation of drug choice is recommended. With regard to genetic screening, the guidelines suggest that genetic testing is indicated in children with severe, early-onset obesity (before the age of five years) who have clinical features of genetic obesity disorders and/or a family history of severe obesity. These suggestions form the basis of the systematic diagnostic workup for children and adolescents with severe obesity used in this thesis (Figure 1).

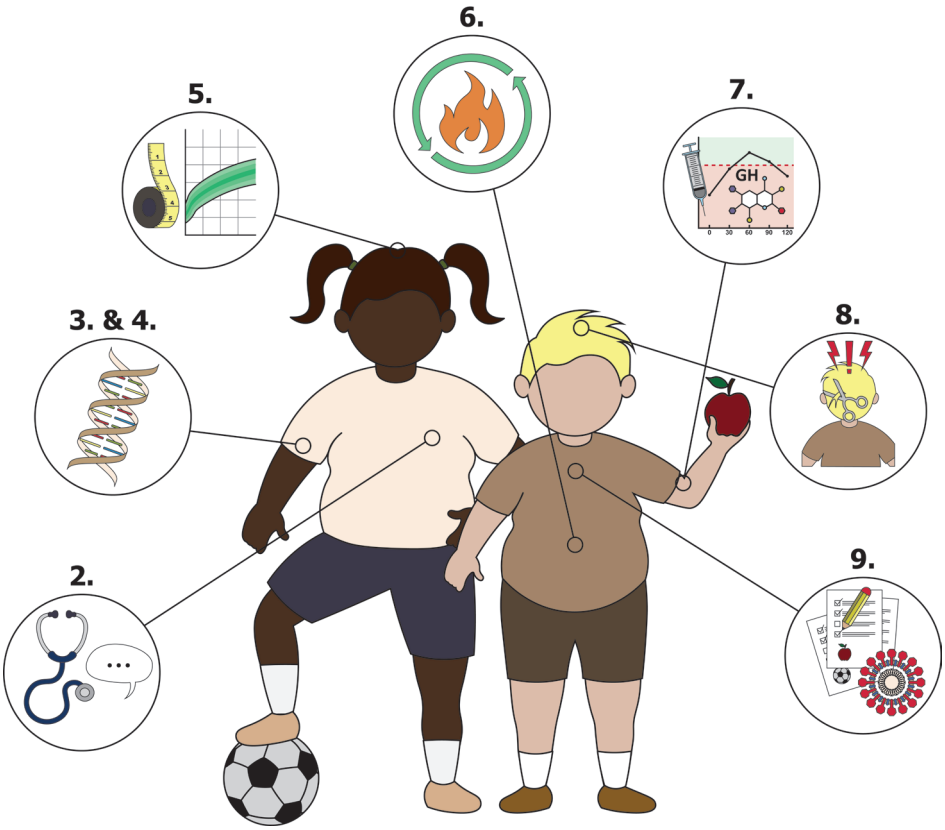

Figure 1. Overview of the diagnostic workup of the pediatric division of Obesity Center CGG.
The numbers refer to the corresponding chapters of this thesis: Chapter 2.: Assessment of children's medical history and comprehensive physical examination and the diagnostic yield of the systematic workup as a whole; Chapters 3. & 4.: Performance of extensive genetic tests; Chapter 5.: detailed growth charts assessments; Chapter 6.: measurement of resting energy expenditure and body composition; Chapter 7.: comprehensive laboratory testing, including endocrine tests; Chapter 8.: measurement of long-term stress hormones (glucocorticoids) in hair; Chapter 9.: assessment of lifestyle behaviors (including physical activity, eating styles and behaviors, sleeping behaviors, perceived stress, and quality of life) and changes therein during the COVID-19 pandemic.

This framework is used for diagnostics and development of personalized treatment algorithms in the pediatric division of Obesity Center CGG (Dutch: *Centrum Gezond Gewicht*), a Dutch reference center for obesity (http://www.centrumgezondgewicht.nl/). This center consists of a collaboration between the departments of Pediatrics and Internal Medicine of three hospitals in Rotterdam, the Netherlands: academic hospital Erasmus MC, and general hospitals Maasstad Ziekenhuis and Franciscus Gasthuis. In short, the diagnostic workup of Obesity Center CGG consists of a systematic assessment of children's medical history (including assessment of family history and growth charts), lifestyle behaviors (including physical activity, eating styles and behaviors, sleeping behaviors, perceived stress, and quality of life), along with com-

prehensive physical examination, laboratory assessments, measurement of resting energy expenditure and body composition, and genetic tests. Obesity-specific genetic tests are performed at the Section Clinical Genetics, department of Human Genetics (Amsterdam UMC, Amsterdam). The diagnostic workup is aimed at diagnosing each of the potential underlying medical causes of obesity mentioned in current international guidelines for pediatric obesity, or, by ruling these causes out, the diagnosis of multifactorial obesity. The systematic diagnostic workup thereby can lead to the development of a personalized, multidisciplinary care plan tailored to the individual's needs. In the following chapters of this thesis, the diagnostic yield of this systematic workup as a whole will be presented, and subsequently more detailed investigation of specific elements of the diagnostic workup will be explored as depicted in Figure 1. In the remainder of this chapter, the different categories of underlying medical causes and the currently unmet needs in daily clinical practice regarding their identification will be addressed.

(1) Genetic obesity disorders

Genetic obesity disorders are caused by rare defects in a single gene or a rare copy number variation involving one or more genes. They are typically inherited in a Mendelian pattern or occur *de novo*. [20] These disorders cause severe pediatric obesity by impairing the function of genes involved in the homeostatic regulation of body weight, appetite, and energy expenditure.[20,29] Most of these disorders have a direct or indirect effect on the leptin-melanocortin pathway, the hypothalamic pathway that regulates satiety and energy expenditure (Figure 2).

Genetic obesity disorders are subdivided into two distinct groups: non-syndromic and syndromic genetic obesity disorders.[20] In non-syndromic genetic obesity, severe obesity is the main phenotypic feature. Typically, obesity onset is early, defined as under the age of 5 years.[24] The obesity is often accompanied by hyperphagia, an extreme and insatiable increase in appetite, even when already having consumed a sufficient amount of food.[11,30] Important examples of these disorders include:

- congenital leptin and leptin receptor deficiency, characterized by severe early-onset obesity, hyperphagia, and pituitary hormone disturbances such as growth hormone deficiency (GHD).[31,32]
- pro-opiomelanocortin (POMC) deficiency, characterized by severe early-onset obesity, hyperphagia, red hair and adrenal insufficiency.[33]
- melanocortin-4-receptor (MC4R) deficiency, the most common non-syndromic genetic obesity disorder, characterized by severe, early-onset obesity, often accompanied by hyperphagia, increased linear growth and increased bone mass.[34]

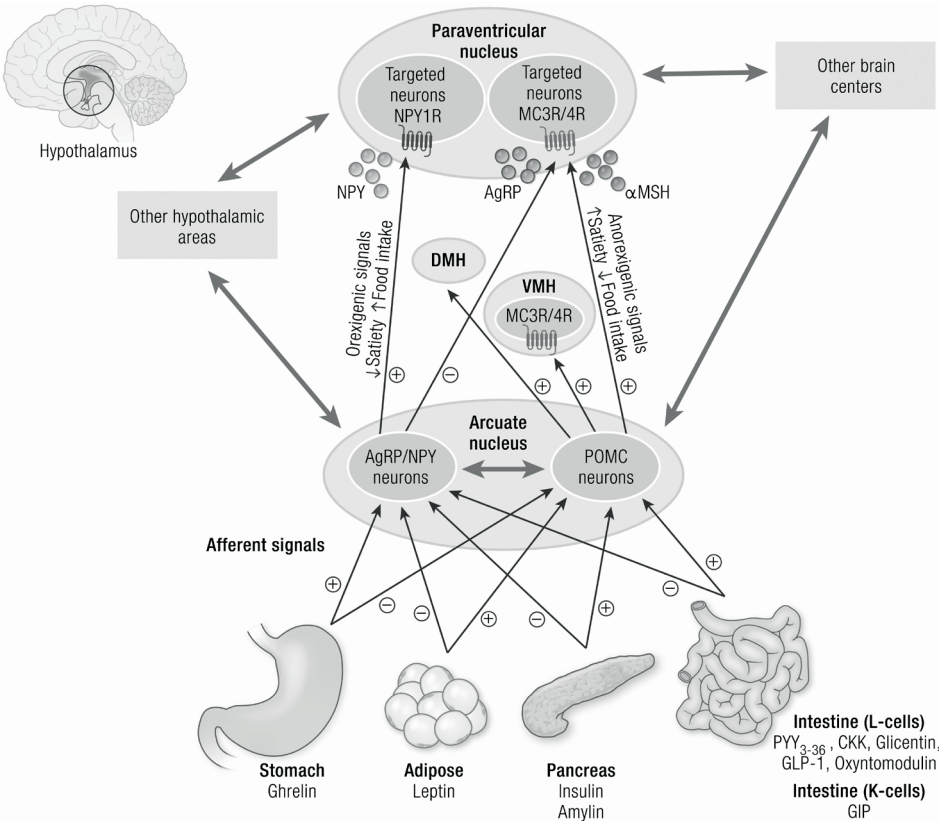

Figure 2. Schematic overview of the hypothalamic leptin-melanocortin pathway and its peripheral afferents. The leptin-melanocortin pathway is the main hypothalamic regulator of homeostatic energy balance, appetite and energy expenditure. It receives peripheral input from the gut, adipose tissue and central nervous system and translates this input into activation of proopiomelanocortin (POMC) neurons (leading to anorexic signalling, decreased appetite and increased energy expenditure) or activation of neuropeptide Y/agouti-related protein (NPY/AgRP) neurons (leading to orexigenic signalling, increased appetite and decreased energy expenditure). The key downstream regulator is the melanocortin 4 receptor (MC4R). Deficiencies in the leptin-melanocortin pathway are associated with severe early-onset obesity, and can be accompanied by disturbances in appetite (hyperphagia) and energy expenditure.[20,29] Abbreviations: aMSH, alpha-melanocyte stimulating hormone; AgRP, agouti-related protein; CCK, cholecystokinin; DMH, dorsomedial nucleus of the hypothalamus; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; MC3R; melanocortin 3 receptor; MC4R, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin; PYY, peptide YY; VMH, ventromedial nucleus of the hypothalamus. Figure reproduced with permission by Oxford University Press on behalf of The Endocrine Society from: Angelidi AM, Belanger MJ, Kokkinos A, Koliaki CS, and Mantzoros CS, Novel Noninvasive Approaches to the Treatment of Obesity: From Pharmacotherapy to Gene Therapy, Endocrine Reviews, 43(3), 2022, Pages 507-557, https://doi.org/10.1210/endrev/bnab034.

By contrast, in syndromic genetic obesity disorders, the obesity is accompanied by intellectual disability, developmental delay, dysmorphic features, congenital anomalies and/or organ dysfunction.[35] Important examples of these disorders include:

- 16p11.2 deletion syndrome, characterized by mild intellectual disability, developmental delay, and autism and/or attention deficit hyperactivity disorder (ADHD).[36]

- Bardet-Biedl syndrome, characterized by intellectual disability, polydactyly, eye, and kidney problems.[37]
- Pseudohypoparathyroidism type 1a, characterized by developmental delay, short stature, skeletal abnormalities, and hormone resistances for e.g. growth hormone (GH), parathyroid hormone, and thyroid hormone.[38]
- Temple syndrome, characterized by neonatal hypotonia and feeding difficulties, developmental delay, and precocious puberty.[39]
- Prader-Willi syndrome, characterized by neonatal hypotonia and feeding difficulties, developmental delay, behavioral problems, and GHD.[40]

Current clinical practice shows that it is difficult to distinguish between children with and without genetic obesity disorders,[41,42] especially since early-onset obesity is becoming more prevalent.[3,4] As an example, recent case series of specific genetic obesity disorders suggest a much earlier onset of obesity than age five years especially in non-syndromic genetic obesity disorders.[41,43] Therefore, more insight is needed into the clinical characteristics and phenotypes of children with genetic obesity disorders in comparison to children without diagnosed genetic obesity disorders to guide clinician's decision who to screen for genetic obesity disorders.

(2) Hypothalamic obesity disorders

Hypothalamic obesity is defined as hypothalamic damage from a tumor, surgery or radiotherapy leading to obesity.[24] Several pathophysiologic mechanisms can ultimately cause severe obesity in these children, namely reduced sympathetic tonus, thyroid metabolism, and brown adipose tissue function, as well as alterations in appetite-regulating hormones leading to hyperphagia.[44,45] Small case series of patients with hypothalamic obesity show that a decreased resting energy expenditure (REE) contributes to these children's obesity, in part owing to differences in body composition.[46,47] However, no studies have compared REE and body composition characteristics of children with hypothalamic obesity to those of children with other underlying medical causes of obesity or multifactorial obesity. Moreover, it is not known whether REE and body composition could potentially distinguish between children with hypothalamic obesity and other underlying medical causes of obesity compared to children with multifactorial obesity. These insights could further guide the diagnostic process of identifying children with underlying medical causes of obesity, as well as lead to more tailored treatment advices, especially in children with decreased REE.

(3) Endocrine obesity disorders

Current international guidelines identify the following endocrine causes of obesity: growth hormone deficiency (GHD), Cushing syndrome, or hypothyroidism.[24] These

diseases are often characterized by by the "endocrine cross": excessive weight gain combined with a decrease in height velocity and/or short stature.[48] As the contribution of hypothyroidism as a singular underlying cause of severe pediatric obesity is subject of ongoing debate,[49] this thesis will primarily focus on the first two endocrine causes of obesity.

(3a) Association of BMI on diagnostics of growth hormone deficiency in severe pediatric obesity

Growth hormone deficiency (GHD) is an important endocrine disorder that should be considered in severe pediatric obesity in combination with short stature and/or decreased height velocity.[24] GHD causes central adiposity and reduced lean body mass through different pathophysiologic mechanisms involving lipid and insulin metabolism, as well as direct effects on adipose cell function and morphology.[50] On its own, GHD can be a rare endocrine cause of obesity which needs specific therapy with recombinant human GH.[51] It can however also prompt investigation into an underlying genetic cause of obesity, e.g. GHD in case of congenital leptin or leptin receptor deficiency,[31,32] GH releasing hormone resistance in case of pseudohypoparathyroidism,[38] or syndromic genetic obesity associated with GHD such as Prader-Willi syndrome.[40] International guidelines recommend to perform two separate growth hormone stimulation tests (GHSTs) in most cases to diagnose GHD.[51] In these tests, a GH secretagogue is administered and GH levels are serially measured; GHD is diagnosed in case of a blunted GH response. It is known that obesity itself can lead to a blunted response to GHSTs that is reversible by weight loss, potentially leading to erroneous diagnoses of GHD in children with obesity.[52] It is however not known how to quantify this effect in children undergoing GHSTs; in fact, this issue has been deemed a topic of high priority by the most recent international guidelines by the Pediatric Endocrine Society.[53] Adjusting for this effect would minimize false-positive diagnoses of GHD and potential unnecessary treatment in children with obesity.

(3b) Association of BMI and cortisol in severe pediatric obesity

It has long been known that exposure to supraphysiologic levels of glucocorticoids, e.g. in Cushing syndrome or due to exogenous glucocorticoid administration, leads to a phenotype characterized by central obesity and metabolic comorbidities such as insulin resistance and dyslipidemia.[54] The pathophysiologic mechanisms leading to obesity involve hepatic and peripheral insulin and lipid metabolism, inflammatory pathways, as well as disrupted signaling of appetite-regulating hormones and increased preference for high-caloric food.[55,56] However, due to the circadian rhythm and acute increases in case of biological or psychological stressors, the measurement of glucocorticoids in serum, urine, or saliva does not reflect the exposure to long-term

glucocorticoid levels.[57] In the past decade, the development of a relatively novel method to measure the glucocorticoids cortisol, the main effector of activation of the hypothalamic-pituitary-adrenal (HPA) axis in relation to physical or psychological stress, and its inactivated form cortisone in hair has gained considerable research interest.[58] Measurement of glucocorticoids in hair, which reflect average glucocorticoid exposure over periods of weeks or months, provide a relatively novel method to investigate the relation between HPA-axis activation and obesity. Previous work and meta-analyses indeed shows that hair cortisol is elevated in children with versus without obesity, but this relationship has not yet been quantified.[59] Quantifying this relationship would improve the understanding of the contribution of chronic HPA-axis activation in children with severe obesity.

(4) Medication-induced obesity

Medication-induced obesity is defined in this thesis as obesity caused by or aggravated by the start or intensification of known weight-inducing medication. The current international guideline for pediatric obesity specifically mentions antipsychotic drugs in this context,[24] but several other classes of medication are known for their effect on body weight. These include antiepileptics, antidepressants, and corticosteroids.[60] Several pathophysiologic mechanisms lead to weight gain, including altered hypothalamic signaling via leptin, neuropeptide Y (an orexigenic neuropeptin), adrenergic and serotonergic pathways.[61,62] The clinical phenotypes of children with medication-induced obesity are scarcely described in current literature and a comparison with children with other underlying medical causes of obesity or multifactorial obesity is currently lacking. Moreover, current guidelines provide little guidance on how to diagnose children with medication-induced obesity.

(5) Multifactorial obesity

As described above, multifactorial obesity is defined in this thesis as obesity caused by a combination of genetic and environmental factors, e.g. lifestyle behaviors, in which the presence of underlying medical causes of obesity is ruled out by a systematic diagnostic workup. These lifestyle behaviors include eating styles and eating behaviors, physical activity, screen time, and wellbeing of children and adolescents. From the first months of 2020 onwards, the coronavirus disease 2019 (COVID-19) pandemic and related lockdown measures had a large negative impact on these lifestyle behaviors.[63] Children and adolescents with severe obesity were even more at risk for these negative mental and physical health consequences,[64] as many of these children already had suboptimal lifestyle behaviors and poorer health-related quality of life (HRQoL) in pre-pandemic circumstances.[65] Therefore, it is important to identify the impact of the COVID-19 pandemic and related lockdown measures and to identify

which subgroups of children and adolescents with obesity are most at risk for these detrimental health consequences. These subgroups can subsequently be targeted and monitored more closely in the current wake of the COVID-19 pandemic to optimize their obesity diagnostics, treatment and monitoring.

UNMET NEEDS IN DIAGNOSTICS OF SEVERE PEDIATRIC OBESITY

Although current international guidelines describe the diagnostic steps in the clinical workup of severe pediatric obesity, little is published about its implementation in practice.[66] The prevalence of the underlying medical causes in severe pediatric obesity is currently unknown. No studies that systematically screened for these underlying causes in severe pediatric obesity have been published as of yet. Only one study evaluated the prevalence of endocrine causes of obesity in a cohort of children and adolescents visiting a specialized endocrinology and obesity clinic and performed genetic testing for *MC4R* deficiency in a subgroup of their cohort. This study found a prevalence of 1.7% of these specific underlying causes.[67] With regard to genetic obesity disorders, several studies investigating cohorts of children with obesity have been published in the past two decades.[66,68,69] Currently, it is widely thought that these disorders can be identified in 2-7% of childhood obesity cases,[29,34] but the exact number is strongly dependent on the characteristics of the studied populations and the genetic tests used. As an example, in a cohort of children with severe obesity from consanguineous parents, a prevalence of 30% of congenital leptin, leptin receptor, and MC4R deficiency was reported,[70] and with broader genetic testing in this cohort it was reported that up to 59% of cases were likely to have a discrete genetic cause for their obesity.[71] Even though the abovementioned underlying medical causes of obesity are considered to be rare to ultra-rare, and diagnostic yield is expected to be low in most clinical settings, they are crucial to identify as they need tailored monitoring and/or a specific targeted therapy.[24,26] Moreover, it is hypothesized that many patients with e.g. specific genetic obesity disorders are currently not identified,[72] and vice versa, that the majority of children in whom genetic screening would be indicated by the international guidelines have not undergone genetic testing.[42] Therefore, more insight is needed into the clinical characteristics of children with severe obesity with and without underlying medical causes. This information can guide clinician's decision who to screen for these underlying medical causes of obesity, and which specific diagnostic instruments should be used for this purpose.

IMPORTANCE OF DIAGNOSING UNDERLYING MEDICAL CAUSES OF OBESITY

As pediatric obesity is a chronic, multifactorial disease, its treatment needs to be multimodal as well.[11,24,28] The cornerstone treatment for every child with severe obesity is a combined lifestyle intervention (CLI), focused on health behaviors including physical activity and diet as well as psychosocial and behavioral interventions. In some individuals however, CLI alone is not enough to reach and/or sustain treatment targets,[73] and additional pharmacotherapy,[74] or in specific cases bariatric surgery,[75] might be needed. Ideally, treatment is age-appropriate, culturally sensitive, family-centered and tailored to the individual patient.[11,24,28] Diagnosing patients with underlying medical causes can end the diagnostic odyssey of patients and their families, *i.e.*, the often long period of time during which patients and their caretakers are in search of an etiologic diagnosis. It can also positively influence the stigma that patients and their families are often confronted with.[76] Moreover, it enables specific tailored treatment options next to CLI. For patients with genetic obesity, this includes genetic and reproductive counseling, organ system surveillance, and tailored advices regarding the expected outcomes of pharmacological treatment and bariatric surgery.[77,78] For specific genetic obesity disorders in the leptin-melanocortin pathway, effective treatment with setmelanotide, an MC4R agonist, has recently become available.[26] For hypothalamic obesity, this includes counseling regarding the possible development of hyperphagia and decreased resting energy expenditure, as well as the expected outcomes of pharmacologic treatment with e.g. central stimulants, setmelanotide, and bariatric surgery.[79] For endocrine disorders causing obesity, this includes therapy aimed at restoring the hormone excess (in Cushing syndrome) or deficiency (in clinical hypothyroidism or growth hormone deficiency) that causes the obesity.[24,48,51] For medication-induced obesities, this includes the consideration of alternative drugs, dosing, and counseling on appetite modulation, e.g. corticosteroid-induced increase of appetite and preference for highly palatable food.[55] For all patients with underlying medical causes of obesity, regular follow-up by experienced specialists is needed both for clinical care as well as for better understanding of the natural history of these conditions and their response to specific treatment options.

THESIS OUTLINE

This thesis focuses on several important diagnostic aspects of severe pediatric obesity as outlined in the systematic diagnostic workup presented in Figure 1. **Chapter 2** describes the overarching systematic diagnostic approach for severe pediatric obesity

used in this thesis to identify underlying medical causes of obesity, evaluates its yield, and provides recommendations for improvement. **Chapter 3** describes the gap between estimated and reported prevalence of a specific rare genetic obesity disorder, leptin receptor deficiency, and provides strategies to improve recognition and diagnosis. In **Chapter 4**, a case series of patients with loss-of-function variants in the *GNB1* gene are presented, which we hypothesize to be a new form of syndromic obesity. In **Chapter 5**, BMI trajectories and age of onset of obesity in rare genetic obesity disorders are presented. The presented data can be used to guide clinician's decision who and when to screen for genetic obesity disorders in children with obesity. **Chapter 6** describes the resting energy expenditure characteristics of children with and without diagnosed underlying medical causes of obesity. This information can be used to guide both diagnostics for underlying medical causes as well as patient-tailored treatment. In **Chapter 7**, the results of a meta-analysis on the quantitative impact of BMI on growth hormone stimulation tests for diagnosing growth hormone deficiency will be discussed. This chapter provides BMI-specific cut-off values to improve diagnosis of GHD in children with overweight and obesity. In **chapter 8**, the results of a meta-analysis on the impact of BMI, BMI SDS and weight circumference on hair glucocorticoids are described. This chapter quantifies the relation between BMI SDS and long-term glucocorticoids. **Chapter 9** describes the influence of the COVID-19 pandemic on the lifestyle behaviors of children with severe obesity. Finally, a general discussion of the studies included in this thesis is provided in **Chapter 10**, including recommendations and perspectives for future research.

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2

Identifying underlying medical causes of pediatric obesity: Results of a systematic diagnostic approach in a pediatric obesity center

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ABSTRACT

Background Underlying medical causes of obesity (endocrine disorders, genetic obesity disorders, cerebral or medication-induced obesities) are thought to be rare. Even in specialized pediatric endocrinology clinics, low diagnostic yield is reported, but evidence is limited. Identifying these causes is vital for patient-tailored treatment.

Objectives To present the results of a systematic diagnostic workup in children and adolescents referred to a specialized pediatric obesity center.

Methods This is a prospective observational study. Prevalence of underlying medical causes was determined after a multidisciplinary, systematic diagnostic workup including growth charts analysis, extensive biochemical and hormonal assessment and genetic testing in all patients.

Results The diagnostic workup was completed in n = 282 patients. Median age was 10.8 years (IQR7.7–14.1); median BMI +3.7SDS (IQR +3.3-+4.3). In 54 (19%) patients, a singular underlying medical cause was identified: in 37 patients genetic obesity, in 8 patients cerebral and in 9 patients medication-induced obesities. In total, thirteen different genetic obesity disorders were diagnosed. Obesity onset <5 years (p = 0.04) and hyperphagia (p = 0.001) were indicators of underlying genetic causes, but only in patients without intellectual disability (ID). Patients with genetic obesity with ID more often had a history of neonatal feeding problems (p = 0.003) and short stature (p = 0.005). BMI-SDS was not higher in patients with genetic obesity disorders (p = 0.52). Patients with cerebral and medication-induced obesities had lower height-SDS than the rest of the cohort.

Conclusions To our knowledge, this is the first study to report the results of a systematic diagnostic workup aimed at identifying endocrine, genetic, cerebral or medication-induced causes of pediatric obesity. We found that a variety of singular underlying causes were identified in 19% of the patients with severe childhood obesity. Because of this heterogeneity, an extensive diagnostic approach is needed to establish the underlying medical causes and to facilitate disease-specific, patient-tailored treatment.

INTRODUCTION

Obesity is a multifactorial disease that has become one of the greatest health challenges of our time.[1] The prevalence of severe obesity in children and adolescents (as defined by the World Health Organization and the International Obesity Task Force (IOTF) was recently shown to range from 1.7% to 6.3% in several countries.[2-4] Body mass index is strongly influenced by genetic susceptibility with an estimated heritability of 40–70%.[5, 6] Most children and adolescents with obesity do not have singular underlying medical disorders causing their obesity, such as endocrine disorders, genetic obesity disorders, cerebral or medication-related causes.[7] The pathophysiologic mechanisms of the underlying medical conditions causing obesity are widely varied, leading to the suggestion to talk about "different diseases causing obesity" or "obesities".[8] Establishing an underlying diagnosis can give insight into the clinical course of the obesity, and lead to tailored monitoring and treatment.[9] In addition, it ends the diagnostic odyssey and can reduce the stigma that patients are confronted with.[10, 11] Since pharmacological treatment for patients with genetic defects affecting the leptin-melanocortin pathway (the hypothalamic system that controls appetite and energy expenditure) is currently being evaluated in clinical trials, identifying these diseases becomes even more relevant.[8, 11, 12]

It is difficult to assess which patients should be evaluated for underlying causes. The current international clinical practice guideline for the evaluation and treatment of pediatric patients with obesity was published in 2017 by the Endocrine Society (ES).[13] In this guideline, clinicians are guided through the diagnostic process. After medical history-taking and physical examination, specific additional diagnostic steps are suggested depending on the findings. In short, endocrine evaluation is recommended in patients with reduced growth velocity; evaluation of hypothalamic obesity in patients with central nervous system (CNS) injury, and re-evaluation of drug choice in patients using antipsychotic drugs. In selected cases, genetic testing is recommended, e.g., in patients displaying extreme early-onset obesity (<5 years) and severe hyperphagia, which are considered cardinal features of genetic obesity disorders. The genetic tests mentioned in the guideline range from karyotyping to DNA diagnostics for deficiencies in the leptin-melanocortin pathway.

As of yet, studies that systematically screen for the underlying medical causes mentioned in the ES guideline in children and adolescents with obesity have not been performed. Previous studies on genetic obesity disorders report an underlying causative genetic defect in 2–5% of non-consanguineous pediatric patients with severe obesity, but prevalence of the other underlying medical causes of obesity has not

been studied.[13-15] Therefore, our primary aim was to analyze the results of a thorough diagnostic workup in a cohort of patients who had been referred to the pediatric division of a specialized tertiary obesity center. Our diagnostic approach included broad evaluation for each patient of all possible underlying medical causes of obesity as mentioned in the ES guideline: endocrine and genetic disorders, as well as cerebral injury and medication use. Moreover, we compared the detailed clinical phenotype of these patients to evaluate whether the patients with underlying medical causes of obesity can be distinguished from those without an underlying medical cause.

METHODS

For this analysis, medical data of children and adolescents aged 0–18 years visiting Obesity Center CGG (Dutch: *Centrum Gezond Gewicht*; English: *Centre for Healthy Weight*) were analyzed. Obesity Center CGG is a Dutch multidisciplinary referral center for obesity consisting of a collaboration between the departments of Pediatrics, Internal Medicine and Surgery of the academic hospital Erasmus MC and collaborating general hospitals Maasstad Ziekenhuis and Franciscus Gasthuis.

In this prospective, observational study, informed consent was obtained at the initial visit according to Dutch law: written informed consent was obtained from parents and children >12 years; for children below age 12 years oral assent was additionally obtained. This also included separate consent forms for genetic testing. The study was approved by the medical ethics committee of the Erasmus MC (MEC-2012-257). Pediatric patients were referred to Obesity Center CGG for diagnostic evaluation (due to suspicion of underlying causes of obesity, severe obesity, or resistance to combined lifestyle intervention), personalized therapeutic advice, or participation in a combined lifestyle intervention (Figure 1).[16] All consecutive patients who provided written informed consent were included at the university medical center Erasmus MC-Sophia Children's Hospital from 2015 to August 2018. From 2016 to August 2018, the collaborating general hospital Maasstad Ziekenhuis also included patients with a suspicion of an underlying medical cause of obesity. Exclusion criteria for this study were inability or refusal to give informed consent, refusal to undergo genetic testing, or not completing the standardized diagnostic approach (Figure 1).

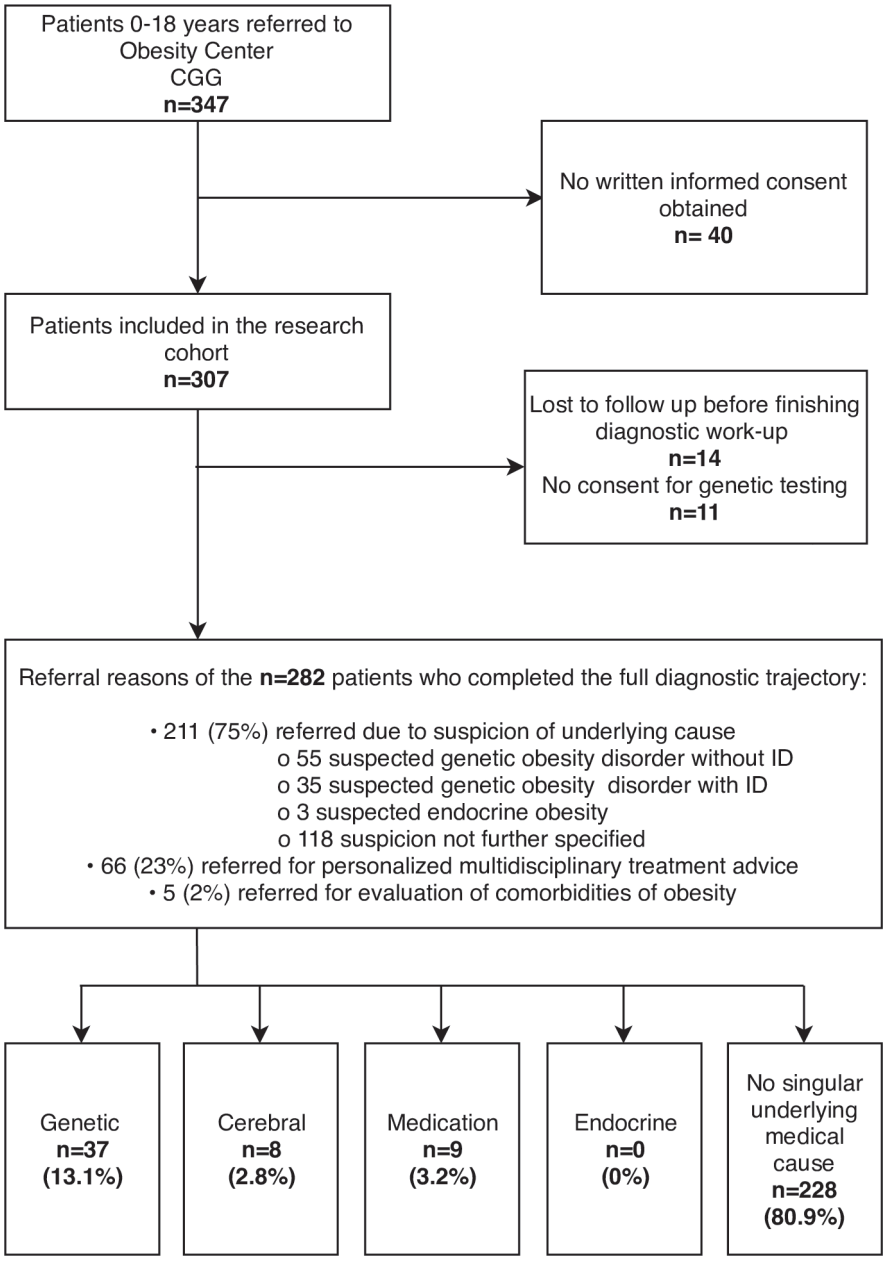

Figure 1. Study flow chart.

Flow chart indicating the inclusion of participants and diagnoses established in our cohort. Abbreviations: CGG, Dutch: Centrum Gezond Gewicht; English: Centre for Healthy Weight; ID, intellectual disability.

A standardized diagnostic approach was applied for all patients (Figure 2), discussed below and in more detail in the S1 Appendix, aimed at identifying underlying endocrine, genetic, cerebral, and medication-induced main causes of obesity. At study entry, medical history-taking, physical examination and extensive assessment of growth charts were performed by a pediatric endocrinologist or pediatrician supervised by a pediatric endocrinologist. A few weeks after the initial visit, patients returned to the outpatient clinic where blood was drawn after an overnight fast for biochemical and hormonal evaluation, and genetic diagnostics. All patients and/or their parents were asked to fill out several questionnaires regarding physical activity, eating behavior, sleeping behavior, stress, and quality of life. Furthermore, all patient records were screened by a clinical geneticist. In case of high suspicion of genetic obesity or abnormal genetic test results, patients were seen by a clinical geneticist at the outpatient clinic. Patients who visited the academic center were also seen by a pediatric physiotherapist, pedagogist, and pediatric dietician. Additional diagnostics (i.e., further genetic testing, neuropsychological or radiologic assessments) were performed when clinically indicated following international clinical guidelines. After the diagnostic procedure, it was assessed for each patient whether an endocrine, genetic, cerebral or medication-induced main underlying cause of obesity could be diagnosed. Contributing factors to weight gain (e.g. sleep deprivation, screen time) were not considered as main underlying causes of obesity. After the diagnostic workup, a patient-tailored treatment plan was designed by the multidisciplinary team in which all relevant findings were incorporated, including advice regarding diet and physical activity, medical treatment (regarding comorbidities) or referral to combined lifestyle intervention, parent support center, psychologist, or psychiatrist. This personalized treatment plan was discussed with the patient and parents and tailored to their personal situation and needs.

Assessments

The features that were assessed during the diagnostic workup are summarized below (details in the S1 Appendix).

Phenotypic features

Clinical history-taking and physical examinations were performed following the Dutch pediatric obesity guideline, including evaluation of neonatal feeding, weight-inducing medication use, development, dysmorphic features, or congenital anomalies.[17] Height, weight and head circumference were measured rounded to the nearest decimal. The Dutch national growth charts, which use the definition of pediatric obesity by Cole *et al.*, were used to calculate standard deviation scores (SDS).[3, 18] Severe obesity was defined by the IOTF definition as a BMI ≥ the age- and sex-specific IOTF BMI-values corresponding to a BMI of 35 kg/m^2 at age 18 years.[3] Each patient's growth charts

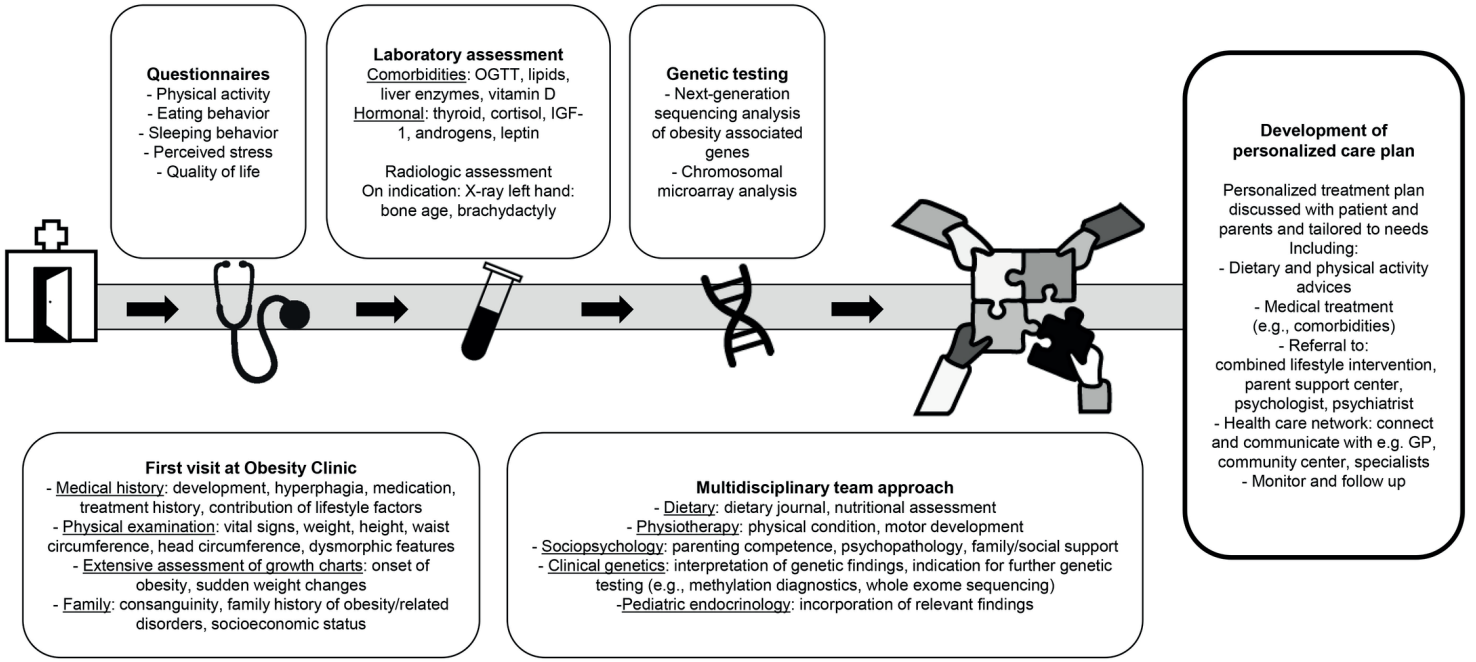

Figure 2. Diagnostic approach

Systematic diagnostic approach for children and adolescents with obesity and a suspicion of an underlying medical cause. Abbreviations: OGTT, oral glucose tolerance test; IGF-1, Insulin-like growth factor 1; GP, general practitioner.

were studied in detail to determine the age of onset of obesity and to evaluate the presence of sudden weight changes. If sudden weight changes were present, it was determined whether these changes were associated with cerebral injury (e.g., tumor in the hypothalamic region) or use of known weight-inducing medication. Short stature was defined as a height-for-age z-score <2 SDS or height-for-age <-1.6 SDS compared to target height; tall stature as a height-for-age z-score >2 SDS or height-for-age >2 SDS compared to target height.[19, 20]

Intellectual disability was determined by the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders 5) definition of intellectual disability or an IQ score ≤70. Family histories of bariatric surgery and extreme obesity (BMI > 40 kg/m^2 for adults, or corresponding pediatric value) were obtained for the past three generations.[3] Information on consanguinity was obtained from questionnaires and additionally from the regions of homozygosity identified by SNP microarray analysis (see below). Presence of hyperphagia was determined by the physician, based on the child's or parents' answers regarding hunger, e.g., satiation and satiety, preoccupation with food, night eating, secret eating, food-seeking behavior, and the distress that accompanies the child's hunger or obsession with food.[21] Patients were considered Dutch if patient and both parents were born in The Netherlands; otherwise, patients were classified as having a migration background.[22] Presence of psychosocial/psychiatric problems was defined as the presence of an established DSM-5 diagnosis (with the exception of intellectual disability) or social problems for which official authorities were involved, such as child protective services. Additionally, Dutch neighborhood socioeconomic status z-scores were calculated. These summarize average income, education and unemployment in postal code areas to provide an estimate of the socioeconomic status of patients.[23] Finally, the contribution of lifestyle factors was assessed. As lifestyle factors play a role in every case of obesity, the multidisciplinary team determined if lifestyle factors were the most important contributor to the obesity for each patient without an underlying medical diagnosis. For example, this label determination was used for patients without an underlying medical diagnosis who reported that obesity started during the divorce of their parents and consequently never resolved. This was subsequently objectified in their growth charts.

Laboratory assessment

Laboratory assessment was performed for all patients. These consisted of screening for comorbidities of obesity, including standard oral glucose tolerance test, lipids, liver enzymes, vitamin D status and hormonal assessment, i.e., thyroid hormones, cortisol, insulin-like growth factor 1, androgens, and leptin. Further details are provided in the S1 Appendix.

Genetic testing

Obesity gene panel sequencing and single nucleotide polymorphism (SNP) microarray analysis were performed in a diagnostic setting for all patients. Three diagnostic obesity gene panel tests successively became available in The Netherlands during the time span of the study (S1 Appendix). All patients were tested at least for the most important genetic obesity disorders mentioned in the ES guideline, such as *GNAS*, *LEP*, *LEPR*, *MC4R*, *PCSK1*, *POMC*, and *SIM1*.[13] Details and complete gene lists are provided in the S1 Appendix. Obesity gene panel sequencing was performed in the ISO 15189 accredited genetic diagnostics laboratories of Amsterdam UMC and UMC Utrecht. Chromosomal microarray analysis and additional diagnostic tests were also performed at the ISO 15189 genetic diagnostics laboratories of other Dutch academic centers. Identified variants were compared with in-house and public databases to exclude common variants. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guideline.[24] Family segregation studies were performed if necessary to clarify the pathogenicity of a variant of uncertain significance (VUS) or copy number variation (CNV). Interpretation of found variants was performed in a diagnostic setting according to the ACMG guideline. Variants of uncertain significance were not classified as genetic obesity disorder, but as a VUS/CNV that possibly explains the obesity phenotype, for which functional studies or other evidence for pathogenicity are necessary. All patients were evaluated by a clinical geneticist specialized in genetic obesity disorders to see whether further genetic testing (e.g., Prader-Willi syndrome (PWS) and Temple syndrome diagnostics, whole exome sequencing) was warranted, for example in case of unexplained intellectual disability, short stature, neonatal hypotonia, multiple congenital anomalies or other signs and symptoms of genetic obesity disorders as mentioned in the ES guideline.[13]

Definition of underlying medical causes of obesity

We used the following definitions of main underlying medical causes of obesity:

Genetic obesity was diagnosed when genotyping revealed known pathogenic variants in obesity-associated genes which matched the clinical phenotype. Likely pathogenic variants, as defined by the American College of Medical Genetics and Genomic (ACMG) guideline were only considered as causative if the clinical phenotype of the patient matched with the found genotype (according to the clinical features mentioned in the ES guideline) and segregation analysis was indicative as well.[24, 13] For genetic obesity disorders not mentioned in the ES guideline, the typical phenotype was based on literature review.[25-32]

Endocrine obesity: Cushing's syndrome and clinical hypothyroidism were considered endocrine causes of obesity. Additional diagnostics for Cushing's syndrome were performed in the presence of impaired growth velocity coinciding with sudden weight gain, Cushingoid phenotype features, and abnormal laboratory results.[13, 33]

Cerebral injury was diagnosed as the cause of obesity in the presence of CNS injury affecting the hypothalamic centers for weight regulation due to craniopharyngioma surgery, meningitis or ischemic damage, coinciding with a sudden progression of obesity (seen as a clear visual slope discontinuity in the growth curve from the time of CNS injury onwards) and the absence of other plausible explanations for the sudden weight gain.

Medication-induced obesity was diagnosed in the presence of start or intensification of known weight-inducing medication (i.e., corticosteroids, anti-epileptic, anti-depressant and anti-psychotic drugs) coinciding with a sudden progression of obesity (seen as a clear visual slope discontinuity in the growth curve) and the absence of other plausible explanations for the sudden weight gain.[34-38]

Analysis

Statistical analysis was performed using SPSS version 24.0 [IBM Corp. Armonk, NY]. Data are presented as median (interquartile range; IQR) and maximum, or mean (standard deviation; SD) and maximum, as appropriate. Differences in features between patients with genetic obesity disorders and patients without a singular underlying medical cause of obesity were analyzed using the chi-squared test, Fisher's exact test, independent sample t-test or Mann-Whitney U test, as appropriate. Two-sided p-values <0.05 were considered statistically significant, as we interpreted these comparisons as hypothesis-generating. For the same reason, we decided not to perform formal statistical testing for comparisons between other patient subgroups due to the small subgroup sizes.

RESULTS

Patient characteristics

In total, 347 patients were referred to Obesity Center CGG during the time span of this study (Figure 1). Of these patients, 282 patients underwent the complete diagnostic workup and were included in these analyses. The majority of these patients presented at the academic hospital (222; 78.7%). Most patients were referred because of suspicion of an underlying cause (Figure 1). All 282 patients underwent the described gene

panel analysis and chromosomal microarray analysis. After consulting with a clinical geneticist, additional genetic diagnostics were performed for 77 patients. The most important modalities were PWS diagnostics in 31 patients; whole exome sequencing in 27 patients; maternal UPD14 diagnostics in 21 patients. Median BMI for age was +3.7 SDS (IQR +3.3-+4.3), indicating severe obesity (Table 1). Most patients were Dutch (183/282, 64.9%); 99/282 (35.1%) had a migration background. In 67/282 (23.8%) of the patients intellectual disability (ID) was present.

Underlying medical causes of obesity

An underlying medical cause of obesity was identified in 54/282 (19.1%) patients in our cohort: 37 genetic obesity disorders, 9 medication-induced obesities, and 8 obesities due to cerebral injury (Table 1). None of the patients' obesity was explained by clinical hypothyroidism or Cushing's disease. In the remaining 228/282 (80.9%) patients no singular underlying medical cause of obesity could be identified. In 17 of these 228 patients a VUS/CNV was identified that possibly explains the obesity phenotype, but this still requires further research, such as functional studies, and therefore falls beyond the scope of this article.[24]

Genetic causes

Of the 37 patients with genetic obesity, 18 patients had a genetic obesity disorder with ID, and 19 without ID. Pathogenic variants in *MC4R* were the most commonly found genetic obesity disorder in our cohort and were found in 9/37 patients, corresponding to 3.2% of the total cohort of 282 patients. The second frequently identified genetic obesity disorders were biallelic *LEPR* pathogenic variants (6/37), followed by *GNAS* pathogenic variants leading to pseudohypoparathyroidism type 1a (5/37). The specific genetic aberrations are presented in Table 2. The clinical phenotypes of all patients with genetic obesity are described in Tables 3 and 4. Although most patients with a genetic obesity disorder had a combination of clinical features typical of their genetic obesity disorder, most patients did not have the *complete* clinical phenotype as mentioned in the ES guideline (Tables 3a and 3b and Table 4). Most notably, 6 out of 18 patients who were diagnosed with a genetic obesity disorder that is typically associated with ID did not have ID or developmental delay (Tables 3a and 3b).

Table 1. Group characteristics of the study population

		All patients	Genetic obesity disorders			Cerebral obesity	Medication-induced obesity	No definite singular underlying medical diagnosis
		Total group	without ID	with ID	Total group	Total group	Total group	Total group
		n = 282	n = 19	n = 18	n = 37	n = 8	n = 9	n = 228
Patient characteristics								
Age at initial visit	Median (IQR) [max]	10.8 (7.7-14.1) [18.0]	10.0 (2.9-14.6) [17.7]	11.2 (7.1-14.7) [16.3]	10.0 (6.0-14.6) [17.7]	11.9 (10.3-16.6) [17.5]	12.3 (9.1-14.8) [17.3]	10.7 (7.7-13.6) [18.0]
Female	n (%)	165 (59%)	14/19 (74%)	12/18 (67%)	26/37 (70%)	5/8 (63%)	5/9 (56%)	129/228 (57%)
Early-onset <5 years	n (%)	182 (65%)	18/19[‡] (95%)	12/18 (67%)	30/37[†] (81%)	4/8 (50%)	4/9 (44%)	146/228 (64%)
Hyperphagia	n (%)	113 (40%)	15/19[‡] (79%)	9/18 (50%)	24/37[‡] (65%)	2/8 (25%)	3/9 (33%)	84/228 (37%)
Anthropometric features								
Height SDS	Mean (SD) [max]	+0.5 (1.3) [+4.2]	+1.1 (1.4) [+4.2]	-0.4[‡] (1.3) [+1.5]	+0.3 (1.5) [+4.2]	-0.3 (0.5) [+0.3]	-0.3 (0.7) [+1.5]	+0.6 (1.3) [+3.7]
Weight SDS	Mean (SD) [max]	+3.7 (1.2) [+7.1]	+4.6[†] (1.5) [+7.0]	+2.3[‡] (1.5) [+5.2]	+3.5 (1.9) [+7.0]	+3.4 (1.0) [+4.7]	+3.4 (0.5) [+4.1]	+3.8 (1.1) [+7.1]
BMI SDS	Median (IQR) [max]	+3.7 (+3.3 - +4.3) [+8.9]	+4.2 (+3.5 - +4.7) [+8.9]	+3.1[‡] (+2.4 - +3.5) [+5.5]	+3.5 (+2.8 - +4.4) [+8.9]	+3.4 (+3.2 - +4.2) [+5.5]	+3.7 (+3.4 - +4.0) [+4.2]	+3.8 (+3.3 - +4.3) [+6.6]
Other clinical features								
Head circumference SDS	Mean (SD) [max]	+1.4 (1.2) [+4.9]	+2.0 (1.2) [+3.9]	+0.9 (1.5) [+3.8]	+1.4 (1.5) [+3.9]	+0.8 (1.0) [+2.1]	+0.2 (1.0) [+0.8]	+1.4 (1.1) [+4.9]
History of neonatal feeding problems	n (%)	17 (6%)	0/19	5/18[‡] (28%)	5/37 (14%)	1/8 (13%)	0/9	11/228 (5%)
Autism	n (%)	37 (13%)	1/19 (5%)	2/18 (11%)	3/37 (8%)	0/8	2/9 (22%)	32/228 (14%)
Parents with obesity	n (%)	190 (67%) of which 68 both	10/19 (53%) of which 1 both	9/18 (50%)	19/37[†] (51%) of which 1 both	3/8 (38%)	7/9 (77%) of which 1 both	161/228 (70%) of which 66 both

Parents with history of bariatric surgery	n (%)	34 (12%) of which 3 both	1/19 (5%) 1 M	1/18 (6%) 1 M	2/37 (5%)	0/8	2/9 (22%)	30/228 (13%) of which 3 both
Consanguinity	n (%)	24 (9%)	2/19 (11%)	0/18	2/37 (5%)	1/8 (13%)	1/9 (11%)	20/228 (9%)
Psychosocial problems	n (%)	130 (46%)	3/19[‡] (16%)	4/18[†] (22%)	7/37[‡] (19%)	3/8 (38%)	5/9 (56%)	115/228 (50%)
Current/past use of weight-inducing medication	n (%)	78 (28%)	5/19 (26%)	2/18 (11%)	7/37 (19%)	3/8 (38%)	9/9 (100%)	59/228 (26%)
Evidently dysmorphic appearance and/or congenital anomaly	n (%)	49 (17%)	1/19 (5%)	12/18[‡] (67%)	13/37[‡] (35%)	1/8 (13%)	3/9 (33%)	32/228 (11%)
Lifestyle factors as most important contributor to obesity	n (%)	75 (27%)	1/19[†] (5%)	0/18[‡]	1/37[‡] (3%)	0/8	2/9 (22%)	72/228 (32%)
Socio-economic status z-score	Median (IQR) [min]	-0.1 (-1.2 - +0.5) [-4.8]	0.0 (-1.0 - +0.5) [-2.6]	-0.3 (-1.2 - +0.3) [-1.8]	0.0 (-1.0 - +0.4) [-2.6]	-0.2 (-1.1 - +1.1) [-3.5]	-0.4 (-1.3 - +0.4) [-3.3]	-0.1 (-1.4 - +0.5) [-4.8]
Short stature	n (%)	11 (4%)	0/19	4/18[‡] (22%)	4/37 (11%)	0/8	0/9	7/228 (3%)
Tall stature	n (%)	60 (21%)	6/19 (32%)	1/18 (6%)	7/37 (19%)	0/8	0/8	53/228 (22%)

Abbreviations: 2ID, intellectual disability; VUS, variant of unknown significance; CNV, copy number variation; VUS, variants of uncertain significance; IQR, interquartile range; max, maximum; SD(S), standard deviation (score); BMI, body mass index; min, minimum.

[†]P<0.05 versus no definite singular underlying medical diagnosis group; [‡] P<0.01 versus no definite singular underlying medical diagnosis group.

Table 2. Overview of genetic alterations in patients diagnosed with a genetic obesity disorder

Genetic obesity disorders without ID				
Pt	**Gene/CNV**	**Reference transcript**	**Genetic alteration**	**Inheritance**
1	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	M
2	*MC4R*	NM_005912.2	Homozygous c.216C>A p.(Asn72Lys)	n.p.
3	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	M
4	*MC4R*	NM_005912.2	Compound heterozygous c.446_450del p.(Phe149Tyrfs*9), c.644T>G p.(Met215Arg)	P and M both heterozygous
5	*MC4R*	NM_005912.2	Homozygous c.779C>A p.(Pro260Gln)	P and M both heterozygous
6	*MC4R*	NM_005912.2	Heterozygous c.913C>T p.(Arg305Trp)	*de novo*
7	*MC4R*	NM_005912.2	Heterozygous c.380C>T p.(Ser127Leu)	P
8	*MC4R*	NM_005912	Heterozygous c.750_751del p.(Ile251Trpfs*34)	n.p.
9	*MC4R*	NM_006147.2	Homozygous c.785del p.(Phe262Serfs*4)	n.p.
10	*LEPR*	NM_001003679.3	Compound heterozygous c.2168c>T p.(Ser723Phe), c.1985T>C p.(Leu662Ser)	P and M both heterozygous
11	*LEPR*	NM_001003679.3	Compound heterozygous c.2051A>C p.(His684Pro), c.2627C>A p.(Pro876Gln)	P and M both heterozygous
12	*LEPR*	NM_002303.5	Compound heterozygous c.1753-1dup p.?, c.2168C>T p.(Ser723Phe)	P and M both heterozygous
13	*LEPR*	NM_002303.5	Homozygous c.1604-8A>G p.? intronic pathogenic variant affecting splicing	P and M both heterozygous
14	*LEPR*	NM_002303.5	Homozygous c.3414dup p.(Ala1139Cysfs*16)	P and M both heterozygous
15	*LEPR*	NM_002303.5	Compound heterozygous c.1835G>A p.(Arg612His), c.2051A>C p.(His684Pro)	P and M both heterozygous
16	*PCSK1*	NM_000439.4	Heterozygous c.541T>C p.(Tyr181His)[a]	M
17	*POMC*	NM_001035256.1	Heterozygous c.706C>G p.(Arg236Gly)[a]	n.p.
18	*SIM1*	n/a	6q16.3 deletion (chr6:100.879.864–102.471.598), disrupting *SIM1*	*de novo*
19	*STX16* (PHP 1b)	NM_003763.5	Heterozygous microdeletion c.331-?_585 + ? p.?	M

Genetic obesity disorders with ID				
Pt	**Gene/CNV**	**Reference transcript**	**Genetic alteration**	**Inheritance**
1	*GNAS* (PHP1a)	NM_001077488	Heterozygous c.85C>T p.(Gln29*)	M
2	*GNAS* (PHP1a)	NM_000516.4	Heterozygous c.794G>A p.(Arg265His)	M
3	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
4	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
5	*GNAS* (PHP1a)	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)[b]	M and PM
6	16p11.2del	n/a	Distal 16p11.2 deletion (chr16:28,825,605–29,043,450, incl. *SH2B1*)	P and MP
7	16p11.2del	n/a	Distal 16p11.2 deletion (chr16:28,819,029–29,043,973,incl. *SH2B1*)	*de novo*
8	16p11.2del	n/a	Proximal 16p11.2 deletion (chr16:29,563,985–30,107,008, not incl. *SH2B1*)	*de novo*
9	mUPD14 (Temple syndrome)	n/a	Temple syndrome (caused by mUPD chromosome 14)	n/a
10	mUPD14 (Temple syndrome)	n/a	Temple syndrome (caused by mUPD chromosome 14)	n/a
11	*Epigenetic error chr14* (Temple syndrome)	n/a	Temple syndrome (caused by imprinting defect on chromosome 14)	n/a
12	*Epigenetic error chr14* (Temple syndrome)	n/a	Temple syndrome (caused by imprinting defect on chromosome 14)	n/a
13	*MKKS* (Bardet-Biedl syndrome)	NM_018848.3	Compound heterozygous c.110A>G p.(Tyr37Cys), c.950_960del p.(Gly317Aspfs*6)	P and M both heterozygous
14	*IFT74* (Bardet-Biedl syndrome)	NM_025103.3	Compound heterozygous c.371_372del p.(Gln124Argfs*9), c.16850-1G>T p.?	P and M both heterozygous
15	*MYT1L*	NM_015025.2	Heterozygous c.808del p.(Gln270Lysfs*11)	*de novo*
16	*POMC*	n/a	2p deletion (chr2:22.791.486–27.942.764), containing *POMC*)	*de novo*
17	*SPG11* (Spastic paraplegia 11)	NM_025137.3	Compound heterozygous c.4534dup p.(Asp1512Glyfs*7), c.5867-?_6477+?del p.? (deletion of exons 31–34	P and M both heterozygous
18	*VPS13B* (Cohen syndrome)	NM_017890.4	Compound heterozygous c.2911C>T p.(Arg971*), c.8697-2A>G p.?	P and M both heterozygous

Abbreviations: CNV, copy number variation; SDS, standard deviation score; BMI, body mass index in kg/m2; ID, intellectual disability; mUPD, maternal uniparental disomy; M, mother; P, father; n.p., segregation analysis not performed; PHP 1b, pseudohypoparathyroidism type 1b; PHP 1a, pseudohypoparathyroidism type 1a; PM, father of mother; MP, mother of father; n/a, not applicable. [a]important genetic risk factor contributing to severe early-onset obesity; [b]siblings.

Table 3a. Clinical characteristics of patients diagnosed with a genetic obesity disorder with ID (part 1)

Gene/ CNV	*GNAS (PHP1a)*	16p11.2 deletion syndrome	Temple syndrome	*MYT1L*
Genetic cause*	Heterozygous disease-associated variant	16p11.2 deletion	Maternal uniparental disomy or imprinting defect of chromosome 14	Heterozygous disease-associated variant
Number of patients	5	3	4	1
Age at diagnosis in years, median (range)	11.6 (3.7-14.8)	6.6 (4.2-15.3)	9.8 (5.0-15.1)	3.3
Clinical features at initial visit				
Age in years, range	3.7-14.8	4.2-15.8	8.1-15.1	5.5
Height SDS, median (range)	-1.0 (-2.2 --0.5)	+0.9 (-2.4 -+1.5)	-1.0 (-2.1 -+1.1)	-0.6
Δ Height SDS vs target height SDS, median (range)	-0.6 (-2.1 -+0.8)	+0.9 (-0.7 -+1.6)	-1.1 (-2.2 -+1.6)	0.0
BMI, median (max)	20.9 (27.1)	29.4 (30.1)	31.2 (33.4)	19.6
BMI SDS, median (max)	+1.8 (+3.6)	+2.8 (+5.3)	+3.3 (+3.5)	+2.5
Early-onset <5 years	5/5	1/3	2/4	Yes
Hyperphagia	1/5	2/3	3/4	Yes
ID	5/5	1/3	1/4	Yes
History of abnormal neonatal feeding behavior	No	No	Hypotonia/feeding problems 4/4	No

Clinical features characteristic of the genetic obesity disorder as mentioned in the Endocrine Society Guideline	Short stature 1/5	Hyperphagia 2/3	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*
	Skeletal defects[b] 4/5	Disproportionate hyperinsulinemia 0/3		
	Impaired olfaction 0/5	Early speech and language delay 2/3 that often resolves 0/3		
	Hormone resistance (e.g. PTH) 5/5	Behavioral problems 0/3		
Additional clinical features characteristic of the genetic obesity syndrome	Subcutaneous calcifications 1/5	N/A	Neonatal hypotonia 4/4	ID 1/1
				Autism 0/1
			Neonatal feeding difficulties 4/4	Behavioral problems 0/1
			Short stature 2/4	
			Precocious puberty 4/4	
	Round facies 3/5		Mild intellectual disability 2/4	
Presence of genetic alteration in parents	All inherited from mother	1 inherited from father, 2 *de novo*	N/A	*De novo*
Presence of obesity in parents who carry the genetic alteration	Obesity *not* present	Obesity *not* present	N/A	N/A

*Abbreviations: CNV, copy number variation; SDS, standard deviation score; BMI, body mass index; ID, intellectual disablility; N/A, not applicable; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone. *exact genetic alterations are listed in Table 2. [a]history of abnormal neonatal feeding behavior, i.e. reduced satiety and/or hypotonia/feeding problems; [b]skeletal defects, i.e. short metacarpalia dig IV and V (hands and/or feet); [c]dysmorphic extremities, e.g. syndactyly/brachydactyly/polydactyly, in our patients polydactyly.*

Table 3b. Clinical characteristics of patients diagnosed with a genetic obesity disorder with ID (part 2)

Gene/ CNV	*MKKS* (Bardet-Biedl syndrome)	*IFT74* (Bardet-Biedl syndrome)	2p-deletion syndrome	*SPG11* (Spastic paraplegia 11)	*VPS13B* (Cohen syndrome)
Genetic cause*	Compound heterozygous disease-associated variants	Compound heterozygous disease-associated variants	2p-deletion syndrome, incl. *POMC*	Compound heterozygous disease-associated variants	Compound heterozygous disease-associated variants
Number of patients	1	1	1	1	1
Age at diagnosis in years, median (range)	1.7	11.2	12.8	14.0	4.4
Clinical features at initial visit					
Age in years, range	4.6	8.9	14.6	11.2	8.5
Height SDS, median (range)	+0.7	+1.5	-1.2	+1.4	-0.7
Δ Height SDS vs target height SDS, median (range)	+0.3	+0.9	0.0	+2.3	-0.7
BMI, median (max)	25.2	24.6	32.5	27.7	20.6
BMI SDS, median (max)	+5.5	+3.0	+3.3	+3.4	+2.6
Early-onset <5 years	Yes	No	Yes	Yes	No
Hyperphagia	No	No	Yes	Yes	No
ID	Not suspected	No	Yes	Yes	Yes
History of abnormal neonatal feeding behavior	Reduced satiety 1/1	Reduced satiety 1/1, resolved after infancy	No	No	Hypotonia/ feeding problems 1/1

Clinical features characteristic of the genetic obesity disorder as mentioned in the Endocrine Society Guideline	Developmen-tal delay 1/1	Developmen-tal delay 0/1	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*
	Dysmorphic extremities[c] 1/1	Dysmorphic extremities[c] 1/1			
	Retinal dystrophy or pigmentary retinopathy 1/1	Retinal dystrophy or pigmentary retinopathy 1/1			
	Hypogonad-ism 0/1	Hypogonad-ism 0/1			
	Renal abnormalities 1/1	Renal abnormalitie 0/1			
Additional clinical features characteristic of the genetic obesity syndrome	N/A	N/A	Hyperphagia (1/1). No POMC deficiency (0/1). Additionally in our patient: ID, coarse facies with large front teeth	Progressive spastic paraplegia 1/1	Failure to thrive in childhood 1/1
				ID 1/1	Hypotonia 1/1
				Peripheral neuropathy 0/1	Microcephaly 1/1
					Visual impairment 1/1
					Neutropenia 1/1
					Prominent central incisors/ uplifted upper lip 1/1
Presence of genetic alteration in parents	Both parents heterozygous	Both parents heterozygous	*De novo*	Both parents heterozygous	Both parents heterozygous
Presence of obesity in parents who carry the genetic alteration	Obesity *not* present (not associated with heterozygosity)	Obesity present in father (not associated with heterozygosity)	N/A	Obesity present in mother (not associated with heterozygosity)	Obesity present in father (not associated with heterozygosity)

*Abbreviations: CNV, copy number variation; SDS, standard deviation score; BMI, body mass index; ID, intellectual disablility; N/A, not applicable; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone. *exact genetic alterations are listed in Table 2. [a]history of abnormal neonatal feeding behavior, i.e. reduced satiety and/or hypotonia/feeding problems; [b]skeletal defects, i.e. short metacarpalia dig IV and V (hands and/or feet); [c]dysmorphic extremities, e.g. syndactyly/brachydactyly/polydactyly, in our patients polydactyly.*

Table 4. Clinical characteristics of patients diagnosed with a genetic obesity disorder without ID

Gene/CNV	*MC4R*		*LEPR*	*POMC*	6q16.3 deletion	*PCSK1*	*STX16* (PHP1b)
Genetic cause*	Homoyzygous/ compound heterozygous disease-associated variants	Heterozygous disease-associated variant	Homoyzygous/ compound heterozygous disease-associated variants	Heterozygous disease-associated variant	6q16.3 deletion incl. part of *SIM1*	Heterozygous disease-associated variant	Heterozygous disease-associated variant
Number of patients	4	5	6	1	1	1	1
Age at diagnosis in years, median (range)	9.2 (1.6-15.4)	7.1 (2.2-15.3)	3.9 (0.7-14.8)	10.0	9.1	11.8	14.8
Clinical features at initial visit							
Age in years, range	6.5-15.4	2.5-15.3	0.7-17.7	10.0	9.1	12.2	17.2
Height SDS, median (range)	+0.8 (+0.7 -+2.2)	+2.1 (0.0 -+4.2)	+1.0 (-1.2 -+2.2)	-0.2	+3.0	-0.2	-0.1
Δ Height SDS vs target height SDS, median (range)	+1.4 (+0.7 -+3.2)	+0.7 (-0.1 -+4.1)	+1.2 (-1.3 -+1.5)	-0.5	+2.4	+1.0	-0.6
BMI, median (max)	34.0 (41.5)	27.9 (38.6)	35.3 (47.5)	28.2	36.8	32.9	31.4
BMI SDS, median (max)	+4.3 (+5.2)	+4.2 (+5.4)	+4.6 (+8.9)	+3.9	+4.4	+3.5	+2.9
Early-onset <5 years	3/4	5/5	6/6	Yes	Yes	Yes	Yes
Hyperphagia	3/4	5/5	5/6	No	Yes	No	Yes
ID	0/4	0/4	0/6	No	No	No	No
History of abnormal neonatal feeding behavior	Reduced satiety 3/4	No	Reduced satiety 4/6	No	No	No	Reduced satiety 1/1
Clinical features characteristic of the genetic obesity disorder as mentioned in the Endocrine Society Guideline	Hyperphagia 4/4	Hyperphagia 4/5	Extreme hyperphagia 5/6	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*	*Genetic obesity syndrome not mentioned in guideline*
	Accelerated linear growth 3/4	Accelerated linear growth 3/5	Frequent infections 0/6				
	Disproportionate hyperinsulinemia 4/4	Disproportionate hyperinsulinemia 1/5	Hypogonadotropic hypogonadism 3/4[c]				
	Low/normal blood pressure 1/4	Low/normal blood pressure 4/4[b]	Mild hypothyroidism 2/6				

Additional clinical features characteristic of the genetic obesity disorder	More severe phenotype than autosomal dominant	N/A	Growth hormone deficiency 1/6	Hyperphagia (less severe than autosomal recessive *POMC* deficiency) 0/1	Characteristics depending on size of deletion: Intellectual disability 0/1	Hyperphagia (less severe than autosomal recessive *PCSK1* deficiency) 0/1	PTH resistance 1/1;
							Occasionally partial TSH resistance 1/1
					Autism 0/1		Enhanced intrauterine growth 1/1
					Behavioral problems 0/1		Occasionally mild brachydactyly 1/1
							Round facies 1/1
Presence of genetic alteration in parents	2/4 both parents heterozygous 2/4 n.p.	3/5 inherited from parent, 1/5 *de novo*, 1/5 n.p.	All parents heterozygous	n.p.	*De novo*	Inherited from mother	Inherited from mother
Presence of obesity in parents who carry the genetic alteration	Obesity present in 1/4 heterozygous parents (known reduced penetrance)	Obesity present in 1 /3 heterozygous parents (known reduced penetrance)	Obesity present in 3/12 heterozygous parents (unclear association with heterozygosity)	N/A	N/A	Obesity present in heterozygous mother	Obesity *not* present in heterozygous mother

*Abbreviations: PHP1b, pseudohypoparathyroidism type 1b; SDS, standard deviation score; BMI, body mass index; ID, intellectual disablility; BP, blood pressure; N/A, not applicable; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; n.p., not performed. *exact genetic alterations are listed in Table 2. [a]history of abnormal neonatal feeding behavior, i.e. reduced satiety and/or hypotonia/feeding problems; [b]In 1 patient, BP could not be measured due to unrest. [c]In 2 prepubertal patients not (yet) detectable.*

In 3/37 cases, a heterozygous mutation/CNV was identified (in 2 patients in *POMC* and in 1 patient in *PCSK1*), which constitutes important genetic risk factors for early-onset obesity as demonstrated in association studies, in contrast to their autosomal recessive forms which cause a more severe clinical phenotype (S1 Appendix).[27, 39]

Cerebral injury as cause of obesity

We identified cerebral injury as the underlying medical cause of obesity in 8/282 (3%) patients. In five patients onset of rapid weight gain, objectified through analysis of their growth charts, coincided with intracranial surgery and/or radiotherapy (two craniopharyngiomas and three malignancies in the hypothalamic region). One patient had congenital anatomic midline defects in the hypothalamic region and clear hyperphagia and excessive weight gain from birth. In the remaining two patients onset of rapid weight gain occurred after meningitis or ischemic infarction, suggesting hypothalamic dysfunction.

Use of known weight-inducing medication as cause of obesity

In 9/282 patients (3%) medication-induced obesity was diagnosed through the combination of extensive evaluation of their growth charts and medication history and exclusion of endocrine, genetic, or cerebral causes of obesity. Of these nine patients, six were chronic users of inhalation corticosteroids (ICS). In 5/6 patients, periods of sudden weight gain, as seen on their growth charts, coincided with intermittent use of oral corticosteroids in the absence of other plausible causes of their sudden weight gain. In the remaining patient periods of intensification of chronic ICS use coincided with sudden weight gain according to the growth chart, without other plausible explanations for the sudden weight gain. In the other three patients the start and restart of antipsychotic drugs in one, and antiepileptic drugs in two patients, coincided with sudden weight gain.

Comparison of phenotype in patients with genetic obesity disorders and patients without a singular underlying medical cause of obesity

Patients with genetic obesity disorders more often had an extreme early-onset of obesity <5 years ($p = 0.04$) and hyperphagia ($p = 0.001$) when compared to patients without a singular underlying medical cause of obesity (Table 1, detailed p-values in S1 Table). Furthermore, the presence of obesity in parents ($p = 0.02$) and psychosocial problems (determined by the involvement of official authorities or DSM-V diagnosis; $p = = 0.001$) were less often present in the genetic obesity group. No significant differences were found with respect to BMI SDS, sex, socio-economic status z-score and family history of consanguinity or bariatric surgery (all $p>0.05$; detailed p-values in S1 Table). When zooming in on patients with genetic obesity with ID, they more

often had short stature (p = 0.005), a history of neonatal feeding problems (p = 0.003), a dysmorphic appearance and/or congenital anomalies (p<0.001), and less severe obesity (lower BMI SDS; p<0.001) than patients without a singular underlying medical cause of obesity. Extreme early-onset obesity <5 years and hyperphagia were not present more often in the patients with genetic obesity disorders with ID (Table 1). With regard to height SDS, patients with genetic obesity without ID had a higher height SDS than patients without a singular underlying medical cause of obesity, although this difference was not statistically significant (p = 0.19). In contrast, patients with genetic obesity with ID had a significantly lower height SDS (p = 0.004).

Comparison of patients with cerebral or medication-induced obesities with other subgroups of patients

No assessed phenotype features were specifically present or absent in patients with cerebral or medication-induced obesities (Table 1). However, on a group level, these patients had lower height SDS than patients with genetic obesity disorders without ID or patients without underlying medical causes of the obesity.

DISCUSSION

In this study, an extensive systematic diagnostic approach in a specialized obesity center established an underlying medical cause of obesity in 19% of pediatric patients. These included genetic obesity disorders (13%), medication-induced obesities (3%) and obesities due to cerebral injury (3%). To the best of our knowledge, this is the first study which reports the yield of a broad diagnostic workup in a tertiary pediatric obesity cohort, focusing not only on genetic obesity disorders but also on endocrine, medication-induced, and cerebral causes of obesity. Previously, Reinehr *et al*. assessed the prevalence of endocrine causes and of specific genetic causes, namely clinically identifiable syndromal causes and *MC4R* pathogenic variants in a subgroup of their cohort.[7] Their study, performed in 1405 children and adolescents visiting a specialized clinic for endocrinology and obesity, demonstrated an underlying disorder in 13 (1.7%) patients.

There are some explanations for our high diagnostic yield. First, our patients constitute a tertiary pediatric obesity population with severe obesity who were referred because of a suspicion of an underlying medical cause, or resistance to lifestyle interventions. Thus, we had a higher *a priori* probability of finding underlying medical causes than in an unselected pediatric obesity population. Nevertheless, we show that a broad systematic diagnostic workup is needed to identify these diverse under-

lying causes of obesity. Secondly, medication use and cerebral/hypothalamic injury were not mentioned in the evaluation of other cohorts, although they are part of the recommended diagnostic workup of the ES guideline for pediatric obesity.[13] Furthermore, the guideline mentions only antipsychotics as weight-inducing medication, but we also considered specific antipsychotic or anti-epileptic drugs and prolonged use of corticosteroids as potential cause of obesity in individual patients, but only in the presence of a temporal relationship with onset of obesity, objectified through comprehensive growth chart analysis, and in the absence of other underlying medical causes of obesity or other plausible explanations for the sudden weight gain.[35-38] Comprehensive growth chart analysis was also supportive in the identification of patients with cerebral/hypothalamic injury as the cause of their obesity in our cohort. Thus, future guidelines might benefit from adding growth chart analysis as part of the diagnostic workup of pediatric obesity. Thirdly, intellectual disability was present in 24% of patients, which increased the *a priori* probability of genetic obesity disorders with ID. The last explanation for our high yield is the extensive genetic testing we performed. Pathogenic variants in *MC4R* were the most frequently identified genetic cause of obesity in our cohort (9/282 patients, 3.2%). This number is comparable to previous findings in another Dutch tertiary pediatric cohort (2.1%) and 1.6–2.6% in other non-consanguineous pediatric cohorts screening for genetic obesity.[40-42] However, in many studies, only *MC4R* mutations or a small number of obesity-associated genes are tested.[7,27,40-43] In our cohort, 13 genetic obesity disorders other than *MC4R* were present. Thus, this study shows that extensive genotyping can highly augment the diagnostic yield when performed in similar pediatric obesity cohorts. The extent to which heterozygous mutations/CNV in *PCSK1* and *POMC* are involved in monogenic obesity remains a point of discussion. Association studies clearly demonstrate that these rare variants contribute to a highly increased risk for obesity.[27,39] Moreover, identifying these patients is of clinical importance for patient-tailored treatment as clinical trials with MC4R-agonist setmelanotide will be conducted, as it is hypothesized that these patients will have reduced MC4R functioning.[44]

We did not identify patients with an endocrine disorder as the cause of obesity. None of the patients were diagnosed with Cushing's syndrome. Pediatric Cushing's syndrome is extremely rare, and patients are often referred due to impaired growth velocity and abnormal laboratory results.[13,45] Therefore, in contrast to adults, these patients are not primarily referred to obesity clinics. Retrospective analysis of ICD-10 codes for Cushing's syndrome in the central hospital registries at both participating centers during the entire study period (2015–2018) showed four diagnoses of pediatric Cushing's syndrome in these years; none of these four patients developed severe obesity. Importantly, PWS, the most common genetic obesity disorder with ID, was not identi-

fied in our cohort. This can be explained by the fact that in Dutch pediatric practice, PWS is often diagnosed during the neonatal period due to the typical hypotonia and feeding problems and after diagnosis, clinical care is transferred to specialized PWS expertise centers.

The second aim of our study was to present the phenotype of patients with underlying medical causes and investigate whether they can be distinguished from patients without underlying medical causes. We therefore performed the comprehensive diagnostic workup in all patients. In daily clinical practice with lower *a priori* probability of underlying medical causes, it is complex to determine for whom these diagnostics should be performed. According to literature, one of the most important features to help distinguish these patients is their stature. Reinehr *et al*. reported that short stature had a high sensitivity for underlying causes of obesity in their cohort.[7] In our study, patients with genetic obesity disorders associated with ID, and patients with cerebral and medication-induced obesities in our cohort indeed had lower height SDS than expected based on the fact that obesity is associated with taller stature.[46] However, most of these patients did not fulfill the definition for short stature.[19] Unsurprisingly, cardinal features of genetic obesity disorders, namely early onset of obesity (<5 years) and hyperphagia, were more often present in patients with genetic obesity, but only when ID was not present. On the other hand, patients with genetic obesity disorders with ID more often had a history of neonatal feeding problems and congenital anomalies or dysmorphic features. Thus, presence of these features should lead to consideration to perform additional diagnostics. Contrary to expectations BMI SDS was not significantly higher in patients with genetic obesity compared to patients without underlying medical causes. A possible explanation is that severity of obesity increases the probability of being referred to a pediatric obesity center regardless of whether genetic obesity is diagnosed. Important factors that were more frequently present in the patients without underlying medical causes were psychosocial problems (DSM-5 diagnosis or involvement of authorities such as child protective services). These psychosocial problems might contribute to developing a higher BMI SDS.[47] On group level, we did not find evidence for significant differences in socio-economic status scores between patients with genetic obesity and patients without underlying medical causes, but individual differences in socio-economic factors and obesogenic environments might also play a role. Interestingly, parents of children with a genetic obesity disorder more often had no obesity than parents of children without an underlying cause. This sounds counterintuitive for hereditary obesity disorders, but can be explained by the fact that most of the genetic aberrations in our cohort had occurred *de novo* or had an autosomal recessive inheritance pattern. Thus, negative family history of obesity could therefore suggest a genetic obesity disorder. In conclusion,

we show that several phenotypic features differed significantly between patients with and without underlying medical causes of obesity, but no feature was specific. Thus, a broad diagnostic workup is warranted in patients with a high suspicion of an underlying medical cause of obesity, e.g., in cases with early-onset obesity, hyperphagia, relatively low height SDS (especially in the presence of ID) and presence of sudden weight changes objectified through comprehensive growth chart analysis.

Treatment of multifactorial disorders such as obesity is complex. In our approach, all patients received a multidisciplinary treatment advice tailored to their personal needs, including personalized dietary and physical activity advice (Figure 2). Furthermore, a monitoring and follow-up plan was developed for every patient. Local health care providers, including child health clinic physicians, general practitioners, general pediatricians, and psychologists, were contacted for local implementation of the care plan. In cases with severe hyperphagia, parental support by an educational therapist was offered to cope with the child's behavior. Rehabilitation physicians were consulted when obesity interfered with performance of daily activities such as walking.[10]

Establishing a main underlying cause of obesity can improve personalized treatment.[34] In all our 54 patients with an underlying medical cause, counseling about the diagnosis was given. This included advice pertaining to bariatric surgery, which has unclear long-term success rates for patients with underlying medical causes.[43,48] Patients with genetic obesity were counseled by a clinical geneticist regarding inheritance, associated medical problems and reproductive decisions. Hormonal supplementation was started in case of hormonal deficiencies associated with specific genetic obesity disorders (such as growth hormone treatment in cases with leptin receptor deficiency).[49] In cases of syndromic obesity, the patients were evaluated for associated organ abnormalities or referred for disease-specific surveillance.[13,25-32] In patients with cerebral/hypothalamic injury as cause of obesity and hyperphagia, dexamphetamine treatment was considered.[50] In patients with medication-induced obesity, evaluation of necessity and alternatives for the weight-inducing medication took place in collaboration with the prescribing physician. Follow-up studies are necessary to evaluate the different individual responses to these treatment options. Interesting novel developments are clinical trials with MC4R-agonists in patients with leptin-melanocortin pathway deficiencies, e.g. *POMC* and *LEPR* deficiency, and glucagon-like peptide 1 (GLP-1) agonists for adolescents with obesity.[44,51] These GLP-1 agonists might also be a future treatment option for patients with genetic obesity disorders, as they have been shown to be equally as effective in adults with heterozygous *MC4R* mutations compared to adults without.[52] Recently, it was suggested that a subgroup of patients with severe early-onset obesity might have relative leptin deficiency and therefore might benefit

from recombinant leptin administration.[53] However, the (long-term) effects of these new potential treatment options remain to be investigated.

Strengths and limitations

A major strength of our study is the use of a systematic diagnostic strategy in all patients investigating all medical causes of obesity mentioned in the current international guideline.[13] Moreover, we performed genetic diagnostics in all patients, and further genetic tests when clinically indicated. Furthermore, our relatively high diagnostic yield enabled us to describe the clinical phenotypes of a large number (n = 54) of patients with underlying causes of obesity from a relatively small patient cohort of 282 patients. When performing research in a diagnostic setting, one faces logistical limitations. During our study, three different versions of the diagnostic obesity-associated gene panel test were successively available for clinical use in The Netherlands. Importantly, in all used gene panels at least the most important and well-known obesity-associated genes were tested, including among others *LEP*, *LEPR*, *MC4R*, *POMC*, *PCSK1*, *ALMS1*, *GNAS*, *SH2B1*, and *SIM1*. A strength of our diagnostic setting is that we followed the current ACMG guidelines for variant calling, leading to stringent selection of only pathogenic and likely pathogenic variants for which evidence from validated functional studies and from control populations has already been incorporated.[24] Children and adolescents with a high suspicion of a genetic cause with negative genetic testing results should be viewed as 'unsolved cases', for which current genetic tests are not yet able to pinpoint a diagnosis. As the field of obesity genetics is progressing rapidly, very recently discovered obesity genes were not present in the used diagnostic gene panels.[54] Incorporating these obesity genes might have resulted in an even higher diagnostic yield. Moreover, newer techniques such as whole-genome sequencing will become more easily accessible and affordable in clinical practice and will likely lead to more genetic obesity diagnoses.

We understand that our comprehensive approach is not feasible in every clinical setting, but our data suggest that it has added value for selected patient groups. Prospective studies looking at predictors for underlying medical causes of obesity are necessary but are difficult to establish because of the rarity of these disorders and overlap with common obesity. International collaboration in large multicenter studies using a similar standardized comprehensive approach are required.

Conclusion

In conclusion, we show that a large variety of underlying medical obesity diagnoses can be established in pediatric patients with obesity in tertiary care setting when using a comprehensive diagnostic workup. Investigating endocrine, genetic, cerebral

and medication-induced causes of obesity is needed for these patients to facilitate disease-specific and patient-tailored treatment. Further studies on predictors of underlying medical causes of obesity are needed to improve identification of these patients.

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Author contributions

Literature search was performed by LK, OA, BvdV, BvdZ, MA, EMJB, MMvH, ELTvdA; study design by all authors except MA; data collection by LK, OA, HTMJ, AEB, BvdZ, EMJB, MMvH, ELTvdA; data analysis by LK, OA, BvdZ, MA, EMJB; data interpretation by all authors except HTMJ; generation of figures by LK, OA; writing by LK, OA, MMvH, ELTvdA; critical revision for important intellectual content by all authors.

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SUPPLEMENTARY APPENDIX

1. Protocol Obesity Center CGG pediatric division

Background

Obesity Center CGG (Dutch: '*Centrum Gezond Gewicht*'; English: 'Centre for Healthy Weight') is a Dutch multidisciplinary national referral center for diagnostics and personalized treatment for patients with obesity. Since 2015 children and adolescents visiting the outpatient pediatric CGG clinics of the university medical center Erasmus MC-Sophia Children's Hospital have been included. From 2016 on two collaborating general hospitals (Maasstad Ziekenhuis and Franciscus Gasthuis) have also included patients. In the current study, patients from the general hospital Franciscus Gasthuis were not included in our data analysis, as they did not undergo the complete standardized diagnostic procedure. According to Dutch law, written informed consent was obtained from parents and children >12 years; for children below age 12 years oral assent was obtained. This also included separate consent forms for genetic testing.

Overview of the pathway of the pediatric division of obesity center CGG

1. Review of historical/referral data
2. Intake by pediatric endocrinologist
3. Anthropometric measurements and vital signs
4. Questionnaires
5. Physiotherapist consultation (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)
6. Nutritional assessment (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)
7. Biochemical and hormonal evaluation
8. Genetic testing
9. Development and implementation of the care plan
10. Evaluation of the care plan (follow-up after 1 year)

1. Review of historical/referral data

Based on information provided in the referral letter, the patient is referred to the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital (referral indications: suspicion of an underlying cause of obesity including genetic causes of obesity, complex medical history and obesity) or general hospitals Maasstad Ziekenhuis/Franciscus Gasthuis (referral indications: diagnostic evaluation of possible underlying causes as well as comorbidities of

obesity, personalized therapeutic advice for non-genetic or non-cerebral causes of obesity, or participation in a combined lifestyle program).[1] When a patient referred to a general hospital required specific academic expertise, the protocol is completed at the academic center.

2. Intake by pediatric endocrinologist

All patients are seen by a pediatric endocrinologist or a pediatrician supervised by a pediatric endocrinologist. Extensive phenotyping is performed to identify underlying endocrine, genetic, cerebral, and medication-induced main causes of obesity. A complete medical history is taken according to the Dutch pediatric guideline for evaluation of children and adolescents with obesity, which includes evaluation of neonatal feeding behavior, current and past weight-inducing medication use, motor and intellectual development, dysmorphic features or congenital anomalies.[2] This intake visit is not only focused on possible underlying causes of obesity, but also evaluates general health and well-being, lifestyle factors influencing obesity, possible comorbidities, psychosocial circumstances, and other potential barriers for successful treatment.

3. Anthropometric measurements and vital signs

Physical examination is performed according to the Dutch guidelines on pediatric obesity.[2] A wall-mounted stadiometer is used to measure height in 0.1 cm increments. When a child is under the age of two years, recumbent length is measured using an infantometer. Sitting height is the vertical distance between the sitting surface and the top of the head. It is measured in 0.1 cm increments, using the wall-mounted stadiometer and the sitting surface. Weight is measured using a calibrated scale while the children are lightly clothed and standing without shoes. Body mass index (BMI) is calculated as weight/height in meters squared (kg/m^2). Parental height and weight are also measured when parents are present during the visit at the outpatient clinic; if not present, estimated height and weight of the parents are recorded. Waist circumference in centimeters (0.1 cm increments) is measured between the superior anterior iliac crest and below the lowest rib after normal expiration, with patients standing and unclothed. Occiptofrontal circumference (head circumference; HC) is measured where the largest measurement can be obtained using a flexible tape measure. HC is measured in centimeters (0.1 cm increments). For all measurements, age and sex-specific standard deviation scores (SDS) were calculated using the latest Dutch national growth study as external standard.[3]

Blood pressure is measured on the bared right arm with a digital sphygmomanometer while the patient is seated. Both feet are flat on the floor and the patient is asked not to move or talk during the measurements. Blood pressure is measured twice, the mean is recorded in the patient file. If blood pressure is elevated (>140 mmHg systolic or >90 mmHg diastolic), measurements are repeated twice with short intervals in between. Age, height, and sex-specific standard deviation scores (SDS) are calculated based on the reference values of the American Academy of Pediatrics.[4] Palpated radial pulse is taken while the patient is seated, registering the number of beats in 30 seconds or digitally assessed by the sphygmomanometer.

All measurements are conducted by outpatient clinic assistants who were specially trained.

4. Questionnaires

Patients and/or their parents are asked to fill out the following Dutch questionnaires before or after the visit to the outpatient clinic focusing on physical exercise and fitness, eating behavior, sleep behavior, stress and quality of life:

- Dutch General Obesity Questionnaire[2]
- Dutch Exercise Behavior Questionnaire, in Dutch: '*Basis Vragenlijst Bewegen'*, BVB[5]
- Dutch Eating Behavior Questionnaire, DEBQ[6]
- Sleep Disturbance Scale for Children, SDSC[7]
- Perceived Stress Questionnaire, PSQ[8]
- Pediatric Quality of Life Inventory (PedsQL) 4.0[9]

Data collected through the questionnaires are discussed in the multidisciplinary consultation (see under '9. Development and implementation of the care plan').

5. Physiotherapist consultation (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)

In children and adolescents visiting the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital either the Bruce protocol or the 6-minute walking test (6MWT) is performed under supervision of a pediatric physiotherapist.

The Bruce protocol is a standardized treadmill test with an increasing treadmill speed and incline.[10] Heart rate and perceived exhaustion are monitored. The test is stopped when the child is exhausted; the maximal endurance time (in minutes, one decimal) serves as criterion of exercise capacity. For children who are not able to perform the Bruce protocol, for example due to intellectual disability, the 6MWT is performed. This test measures how far the patient can walk on a flat track in the exercise room when walking as fast as possible for six minutes. The results of both tests are compared to the norms that have been developed for healthy children.[11-13] Findings are discussed in the multidisciplinary consultation (see below).

6. Nutritional assessment (only for patients at the academic center Erasmus MC-Sophia Children's Hospital)

The following nutritional assessment is performed for all children and adolescents visiting the outpatient clinic of the academic center Erasmus MC-Sophia Children's Hospital under supervision of a pediatric dietitian.

- Dietetics: patients or their parents are asked to complete a food diary, recording all foods and drinks consumed over 2 consecutive days. An estimation of the total daily calorie intake is made, as well as an assessment of eating patterns, portion sizes, dietary behavior, and micronutrient intake.
- Resting energy expenditure is measured by indirect calorimetry (Quark RMR, COSMED).
- Body composition (fat mass and fat-free mass) is measured by air displacement plethysmography (BOD POD, COSMED) and/or dual energy x-ray absorptiometry (DEXA).

Findings are discussed in the multidisciplinary consultation (see below).

7. Biochemical and hormonal evaluation

Peripheral blood for biochemical and hormonal evaluation is obtained following overnight fasting. Next, a standard oral glucose tolerance test (OGTT) of 1.75 g of glucose per kg body weight (maximum 75 g glucose in 200 ml water) is performed between 8am and 10am. Plasma glucose and insulin are measured at t=0 and at t=2 hours; insulin at t=2 hours is only measured for patients at the academic hospital. The homeostatic model assessment of insulin resistance (HOMA-IR) value is calculated, using a cut-off for insulin resistance of >3.16.[14] Additionally, at t=0 hemoglobin A1c (HbA1c), total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, alanine transaminase (ALAT), aspartate transaminase (ASAT), Gamma-Glutamyl Transferase (GGT), thyroid hormones (FT4, TSH), cortisol, leptin, insulin-like growth factor 1 (IGF-1), testosterone, anti-Müllerian Hormone (AMH), sex hormone-binding globulin (SHBG), androstenedione, dehydroepiandrosterone sulfate (DHEAS) and 25-hydroxyvitamin D are measured according to local lab standards. All blood analyses are performed at the local medical laboratories of participating hospitals, all of which are ISO 15189 accredited.

8. Genetic testing

The following genetic tests are included in the extensive diagnostic workup:

- Next-generation sequencing analysis of obesity associated gene panel
- SNP-microarray analysis

On clinical suspicion, specific additional diagnostic tests (e.g., Prader-Willi syndrome diagnostics, maternal uniparental disomy (UPD) 14 test, trio whole exome sequencing) are performed.

Further details on the genetic tests can be found in the supplemental paragraph 2 ‘Obesity gene panel sequencing details’.

9. Development and implementation of the care plan

At the academic center Erasmus MC-Sophia Children’s Hospital, all relevant findings of the diagnostic workup are discussed in a multidisciplinary consultation featuring a pediatric dietitian, a pediatric physiotherapist, pedagogue and pediatric endocrinologist. In this multidisciplinary meeting, the patient-tailored care plan is developed. The care plan includes dietary and physical activity advice, medical treatment (e.g. regarding comorbidities) or referral to combined lifestyle intervention, parent support center, psychologist or psychiatrist. Subsequently, patients are invited to the outpatient clinic to discuss the findings and the care plan. Afterwards, the care plan is communicated to the patient’s referrer, who is responsible for implementing the tailored treatment advices locally.

10. Evaluation of the care plan (follow-up after 1 year)

The follow-up visit takes place after at least 1 year and includes evaluation of the patient-tailored care plan during the past year, followed by the same questionnaires, anthropometric

measurements, and biochemical and hormonal evaluations (excl. OGTT) as during the intake visit. The results of genetic testing are discussed at the follow-up visit, or earlier when a relevant genetic alteration is found that requires counseling by a clinical geneticist.

2. Obesity gene panel sequencing details

Obesity gene panel testing is offered to all children who are included in this study. Because of logistic reasons, there were three different tests available in The Netherlands in the time span of this study. The details of the three obesity gene panels are listed below. The identified variants were compared with in-house and public databases, including www.mc4r.org.uk, to exclude common neutral variants. All variants were analyzed using mutation interpretation software to investigate their (possible) clinical relevance . Variants were classified according to the guideline of The American College of Medical Genetics and Genomics (ACMG).[15] If possible, a variant of uncertain significance (VUS) or an unknown copy number variation (CNV) was further investigated by family segregation analysis to clarify the pathogenicity. GRCh37/hg19 was used as reference genome.

Box. Obesity Gene panel UMC Utrecht (Department of Genetics, UMC Utrecht, The Netherlands, ISO15189 accredited). December 2014 – November 2016

Gene	OMIM-entry	Inheritance	Name of associated syndrome or further details about the disease association
ALMS1	606844	Autosomal recessive	Alstrom syndrome
ARL6	608845	Autosomal recessive	Bardet-Biedl syndrome
BBS1, *BBS2*, *BBS4*, *BBS5*, *BBS7*, *BBS9*, *BBS10*, *BBS12*	209901 606151 600374 603650 607590 607968 610148 610683	Autosomal recessive	Bardet-Biedl syndrome
BDNF	113505	Autosomal dominant	Obesity associated gene
CCDC28B	610162	Autosomal recessive	Bardet-Biedl syndrome
CEP290	610142	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
CRHR2	602034	-	Corticotropin-releasing hormone receptor
FLOT1	606998		Link to cholesterol uptake
G6PC	613742	Autosomal recessive	Glycogen storage disease 1a, von Gierke disease
GNAS	139320	Autosomal dominant	Albright hereditary osteodystrophy
IRS1	147545	Autosomal dominant	Comorbidity gene: insulin receptor
IRS2	600797	Autosomal dominant	Comorbidity gene: insulin receptor
IRS4	300904		Comorbidity gene: insulin receptor
KIDINS220	615759	Autosomal dominant	SINO syndrome (spastic paraplegia, intellectual disability, nystagmus, obesity)
LEP	164160	Autosomal recessive	Leptin deficiency
LEPR	601007	Severe: autosomal recessive	Leptin receptor deficiency

LZTFL1	606568	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
MAGEL2	605283	Autosomal dominant	Schaaf-Yang syndrome
MC3R	155540	Autosomal dominant	Obesity associated gene
MC4R	155541	Severe: autosomal recessive Moderate: autosomal dominant	Melanocortin 4 receptor deficiency
MCHR1	601751	-	Obesity associated gene
MKKS	604896	Autosomal recessive	Bardet-Biedl syndrome, McKusick-Kaufman syndrome
MKRN3	603856	Autosomal dominant	Precocious puberty, Prader-Willi region
MKS1	609883	Autosomal recessive	Bardet-Biedl syndrome, Joubert syndrome, Meckel syndrome
MRAP2	615410	Autosomal dominant	Obesity associated gene
NDN	602117	Isolated cases	Prader-Willi region
NTRK2	600456	Autosomal dominant	Obesity associated gene
PAX6	607108	Autosomal dominant	Aniridia and obesity
PCK1	614168	Autosomal recessive	Phosphoenolpyruvate carboxykinase deficiency, cytosolic
PCSK1	162150	Severe: autosomal recessive Moderate: autosomal dominant	Obesity with impaired prohormone processing
PHF6	300414	X-linked recessive	Borjeson-Forssman-Lehmann syndrome
POMC	176830	Severe: autosomal recessive Moderate: autosomal dominant	Obesity, adrenal insufficiency, and red hair due to POMC deficiency
PRKAR1A	188830	Autosomal dominant	Acrodysostosis 1, with or without hormone resistance Carney complex, type 1 Myxoma, intracardiac Pigmented nodular adrenocortical disease
PTEN	601728	Autosomal dominant	PTEN hamartoma tumor syndrome
SIM1	603128	Autosomal dominant	Obesity associated gene
SNRPD2	601061	-	Obesity pathway gene
SNRPN	182279	Autosomal dominant	Prader-Willi region
SPG11	610844	Autosomal recessive	Spastic paraplegia 11
TBX3	601621	Autosomal dominant	Ulnar-mammary syndrome
THRB	190160	Autosomal dominant	Comorbidity gene: thyroid hormone receptor
TMEM67	609884	Autosomal recessive	COACH syndrome, Joubert syndrome Meckel syndrome, Nephronophtisis, modifier of Bardet Biedl syndrome
TRIM32	602290	Autosomal recessive	Bardet Biedl syndrome, Muscular dystrophy, limb girdle, autosomal recessive
TTC8	608132	Autosomal recessive	Bardet Biedl syndrome
TUB	601197	Autosomal recessive	Retinal dystrophy and obesity
WDPCP	613580	Autosomal recessive	Bardet Biedl syndrome

Next Generation Sequencing (NGS) was performed on a SOLiD 5500XL system (Life Technologies). Horizontal coverage of >99% was achieved. Because of low coverage in a part of the *POMC* gene, additional Sanger sequencing was performed for this gene to achieve >99% horizontal coverage. Further details are provided in Kleinendorst et al., 2018.[16]

Obesity Gene Panel VUmc (Department of Genetics, Amsterdam UMC, location VUmc, The Netherlands, ISO15189 accredited). November 2016 – March 2018

Exome sequencing test with a custom filter. Whole-exome capture was performed using SeqCap EZ MedExome (Roche NimbleGen). Sequencing was done on a HiSeq 2500 or Hiseq 4000 sequencer (Illumina) (paired-end 125 bp and 150 bp reads respectively). The analysis was restricted to variants in a predetermined virtual panel of 52 genes associated with obesity and comorbidities. These were the same 52 genes as in the Utrecht obesity gene panel. If the coverage of the *MC4R* gene was less than 30X, additional Sanger sequencing was performed.

Obesity Gene Panel AMC (Department of Genetics, Amsterdam UMC, location AMC, The Netherlands, ISO15189 accredited). March 2018 - present (inclusion for this study: August 2018)

Gene list: *ALMS1, BDNF, CPE, GNAS, LEP, LEPR, MAGEL2, MC3R, MC4R, NPY4R, PCSK1, PHF6, POMC, SH2B1, SIM1, and VPS13B.*

Targeted enrichment was performed with custom in solution captures (SeqCap EZ Choice, Nimblegen). Sequencing was done on a MiSeq sequencer (Illumina) (paired-end 150 bp reads). All genes had a coverage of >30X. The analysis included CNV detection based on the NGS data. Sequences on chromosome 16p11.2 were included on the capture to allow for detection of a 16p11.2 deletion.

3. Supplementary table

S1 Table. P-value table for differences in clinical features between the genetic obesity disorders group and the patients without a singular underlying medical diagnosis

		Genetic obesity disorders without ID n=19	Genetic obesity disorders with ID n=18	Total genetic obesity disorders group n=37	Total no definite singular underlying medical diagnosis group n=228	P-value genetic without ID vs no definite singular underlying medical diagnosis	P-value genetic with ID vs no definite singular underlying medical diagnosis	P-value total genetic vs no definite singular underlying medical diagnosis
Age at initial visit	Median (IQR) [max]	10.0 (2.9-14.6) [17.7]	11.2 (7.1-14.7) [16.3]	10.0 (6.0-14.6) [17.7]	10.7 (7.7-13.6) [18.0]	P=0.32 (3)	P=0.81 (3)	P=0.36 (3)
Female	n (%)	14/19 (74%)	12/18 (67%)	26/37 (70%)	129/228 (57%)	P=0.15 (1)	P=0.41 (1)	P=0.12 (1)
Early-onset <5 years	n (%)	18/19 (95%)	12/18 (67%)	30/37 (81%)	146/228 (64%)	**P=0.006 (1)**	P=0.82 (1)	**P=0.04 (1)**
Hyperphagia	n (%)	15/19 (79%)	9/18 (50%)	24/37 (65%)	84/228 (37%)	**P<0.001 (1)**	P=0.27 (1)	**P=0.001 (1)**
Height SDS	Mean (SD) [max]	+1.1 (1.4) [+4.2]	-0.4 (1.3) [+1.5]	+0.3 (1.5) [+4.2]	+0.6 (1.3) [+3.7]	P=0.19 (4)	**P=0.004 (4)**	P=0.36 (3)
Weight SDS	Mean (SD) [max]	+4.6 (1.5) [+7.0]	+2.3 (1.5) [+5.2]	+3.5 (1.9) [+7.0]	+3.8 (1.1) [+7.1]	**P=0.04 (4)**	**P<0.001 (4)**	P=0.29 (3)
BMI SDS	Median (IQR) [max]	+4.2 (+3.5 - +4.7) [+8.9]	+3.1 (+2.4 - +3.5) [+5.5]	+3.5 (+2.8 - +4.4) [+8.9]	+3.8 (+3.3 - +4.3) [+6.6]	P=0.09 (4)	**P<0.001 (4)**	P=0.52 (3)
Head circumference SDS	Mean (SD) [max]	+2.0 (1.2) [+3.9]	+0.9 (1.5) [+3.8]	+1.4 (1.5) [+3.9]	+1.4 (1.1) [+4.9]	P=0.09 (4)	P=0.20 (4)	P=1.00 (3)
History of neonatal feeding problems	n (%)	0/19	5/18 (28%)	5/37 (14%)	11/228 (5)	P=1.00 (2)	**P=0.003 (3)**	P=0.06 (2)
ID	n (%)	0/19	12/18 (67%)	12/37 (32%)	48/228 (21%)	**P=0.03 (2)**	**P<0.001 (2)**	P=0.13 (1)
Autism	n (%)	1/19 (5%)	2/18 (11%)	3/37 (8%)	32/228 (14%)	P=0.48 (2)	P=1.00 (2)	P=0.44 (2)
Parents with obesity	n (%)	10/19 (53%) of which 1 both	9/18 (50%)	19/37 (51%) of which 1 both	161/228 (70%) of which 66 both	P=0.10 (1)	P=0.07 (1)	**P=0.02 (1)**

Parents with history of bariatric surgery	n (%)	1/19 (5%) 1 M	1/18 (6%) 1 M	2/37 (5%)	30/228 (13%) of which 3 both	P=0.48 (2)	P=0.71 (2)	P=0.28 (2)
Consanguinity	n (%)	2/19 (11%)	0/18	2/37 (5%)	20/228 (9%)	P=0.68 (2)	P=0.38 (2)	P=0.75 (2)
Psychosocial problems	n (%)	3/19 (16%)	4/18 (22%)	7/37 (19%)	115/228 (50%)	**P=0.004 (1)**	**P=0.02 (1)**	**P=0.001 (1)**
Current/past use of weight-inducing medication	n (%)	5/19 (26%)	2/18 (11%)	7/37 (19%)	59/228 (26%)	P=1.00 (2)	P=0.26 (2)	P=0.36 (1)
Evidently dysmorphic appearance and/or congenital anomaly	n (%)	1/19 (5%)	12/18 (67%)	13/37 (35%)	32/228 (11%)	P=0.48 (2)	**P<0.001 (2)**	**P=0.002 (1)**
		Genetic obesity disorders without ID n=19	**Genetic obesity disorders with ID n=18**	**Total genetic obesity disorders group n=37**	**Total no definite singular underlying medical diagnosis group n=228**	**P-value genetic without ID vs no definite singular underlying medical diagnosis**	**P-value genetic with ID vs no definite singular underlying medical diagnosis**	**P-value total genetic vs no definite singular underlying medical diagnosis**
Lifestyle factors as most important contributor to obesity	n (%)	1/19 (5%)	0/18	1/37 (3%)	72/228 (32%)	**P=0.02 (1)**	**P=0.005 (1)**	**P<0.001 (1)**
Socio-economic status z-score	Median (IQR) [min]	0.0 (-1.0 - +0.5) [-2.6]	-0.3 (-1.2 - +0.3) [-1.8]	0.0 (-1.0 - +0.4) [-2.6]	-0.1 (-1.4 - +0.5) [-4.8]	P=0.76 (4)	P=0.95 (4)	P=0.59 (3)
Short stature	n (%)	0/19	4/18 (22%)	4/37 (11%)	7/228 (3%)	P=1.00 (2)	**P=0.005 (2)**	P=0.052 (2)
Tall stature	n (%)	6/19 (32%)	1/18 (6%)	7/37 (19%)	53/228 (22%)	P=0.41 (2)	P=0.13 (2)	P=0.56 (1)

ID, intellectual disability; IQR, interquartile range; max, maximum; SDS, standard deviation score; M, mother. (1) Chi squared test; (2) Fisher's exact test; (3) Independent sample t-test (if necessary, after log transformation). (4) Mann-Whitney U test. Cells in bold indicate a statistically significant difference between the mentioned groups.

4. Supplementary appendix references

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3

Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics

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ABSTRACT

Objective Leptin receptor (LepR) deficiency is an autosomal-recessive endocrine disorder causing early-onset severe obesity, hyperphagia and pituitary hormone deficiencies. As effective pharmacological treatment has recently been developed, diagnosing LepR deficiency is urgent. However, recognition is challenging and prevalence is unknown. We aim to elucidate the clinical spectrum and to estimate the prevalence of LepR deficiency in Europe.

Design Comprehensive epidemiologic analysis and systematic literature review.

Methods We curated a list of *LEPR* variants described in patients and elaborately evaluated their phenotypes. Subsequently, we extracted allele frequencies from the Genome Aggregation Database (gnomAD), consisting of sequencing data of 77 165 European individuals. We then calculated the number of individuals with biallelic disease-causing *LEPR* variants. Results: Worldwide, 86 patients with LepR deficiency are published. We add two new patients, bringing the total of published patients to 88, of which 21 are European. All patients had early-onset obesity; 96% had hyperphagia; 34% had one or more pituitary hormone deficiencies. Our calculation results in 998 predicted patients in Europe, corresponding to a prevalence of 1.34 per 1 million people (95% CI: 0.95–1.72).

Conclusions This study shows that LepR deficiency is more prevalent in Europe ($n = 998$ predicted patients) than currently known ($n = 21$ patients), suggesting that LepR deficiency is underdiagnosed. An important cause for this could be lack of access to genetic testing. Another possible explanation is insufficient recognition, as only one-third of patients has pituitary hormone deficiencies. With novel highly effective treatment emerging, diagnosing LepR deficiency is more important than ever.

INTRODUCTION

Obesity is one of the most urgent health problems of modern times because of its epidemical prevalence, high disease burden, and high mortality.[1] In rare cases, obesity is caused by genetic disorders in the leptin-melanocortin pathway, the hypothalamic system controlling energy expenditure and food intake. The anorexic hormone leptin is mainly secreted by adipose tissue and reflects the body's energy reserves. Hypothalamic leptin signaling leads to activation of the melanocortin-4-receptor (MC4R), resulting in increased energy expenditure and satiety. When this signaling is disturbed, patients develop hyperphagia and early-onset obesity. A recent breakthrough for leptin-melanocortin pathway disorders is treatment with MC4R-agonist setmelanotide, which results in impressive weight loss.[2] One of the endocrine disorders that now can be treated is leptin receptor (LepR) deficiency, a rare autosomal recessive disorder caused by pathogenic variants in the leptin receptor gene (*LEPR*). Adequate functioning of the leptin receptor is essential for maintaining body weight. Moreover, adequate leptin signaling is necessary for onset of puberty, pubertal growth spurt, and production of thyroid-releasing hormone.[3,4] Additionally, LepR-deficient rodents show decreased levels of pituitary growth hormone and stunted growth curves.[5]

When looking at the phenotype of LepR deficiency in humans, patients with LepR deficiency indeed can exhibit hypogonadotropic hypogonadism (HH), hypothyroidism, and/or growth hormone deficiency (GHD) in addition to extreme early-onset obesity and hyperphagia. It remains unclear why some patients only exhibit severe obesity, whereas others also have the associated pituitary hormonal disturbances. Residual receptor activity associated with specific *LEPR* mutations might partially explain this, but has not been investigated systematically.[4] Other features reported in patients with LepR deficiency are frequent infections and hyperinsulinemia, but to what extent they are part of the clinical spectrum of LepR deficiency is unknown.[3,4] In some patients a lower CD4+ T-cell count and a compensatory higher B-cell count has been reported, which is in accordance with known effects of leptin on the immune system.[4] It is hypothesized that this may contribute to early childhood death due to infections.[4] Individuals affected by LepR deficiency have hyperinsulinemia to a degree consistent with the severity of their obesity, although it is suggested that these patients might be predisposed to develop insulin resistance and diabetes at an earlier age.[3,4]

The phenotype variability makes identification of LepR deficiency challenging. Recognition might be further hampered due to lack of awareness of possible rare underlying causes in routine obesity care. In obesity cohort studies, LepR deficiency prevalence of 0–3% is found.[4,6-8] Higher prevalence of up to 10% is reported in cohorts from con-

sanguineous families.[9] However, it is important to realize that these estimations only reflect prevalence of LepR deficiency in selected patient groups. The traditional approach to prevalence estimations of genetic diseases (counting the people diagnosed with the disease) greatly depends on local availability and application of genetic testing. Nowadays, genetic data from large population databases can be used to better estimate general prevalence of genetic disorders.

Aim of this study is to establish the prevalence of LepR deficiency in the general European population. To achieve this, we first performed a systematic literature review to identify all published cases and add unpublished cases from our obesity center. We use the *LEPR* variants from these cases to perform a prevalence estimation based on European allele frequencies. Our second aim is to gather clinical information from published LepR deficiency patients to describe the clinical spectrum.

METHODS

Systematic literature search

A systematic literature search was performed in Embase, Medline (Ovid), Web of Science, Cochrane Library, and Google Scholar to identify all patients with LepR deficiency from its first report in 1998 up to May 2019. The complete search strategy is presented in the supplement (Supplementary file 1, see section on supplementary materialsgiven at the end of this article). In short, the strategy consisted of the themes '*LEPR*'/'LepR deficiency' or 'obesity genetic diagnostics'. We adopted a broad search strategy to not miss studies which sequenced *LEPR* as part of an obesity gene panel. Additionally, we searched for additional cases in ClinVar, the Human Gene Mutation Database, and the Decipher database.[10-12] Finally, we performed a non-systematic search in Researchgate (www.researchgate.net; accessed 24-05-2019; search queries 'LEPR', 'leptin receptor' and 'leptin receptor deficiency') to identify studies and conference abstracts that were not indexed in the mentioned databases.

Title and abstract of all identified studies were screened by two investigators (LK, OA); studies describing patients with LepR deficiency were included; duplicate studies were removed (Fig. 1). In case of disagreement over inclusion, a senior investigator (EvdA/MvH) served as adjudicator. Additionally, reference lists of included studies were screened for relevant articles. Follow-up studies on cases already described in literature were only used for phenotype assessment.

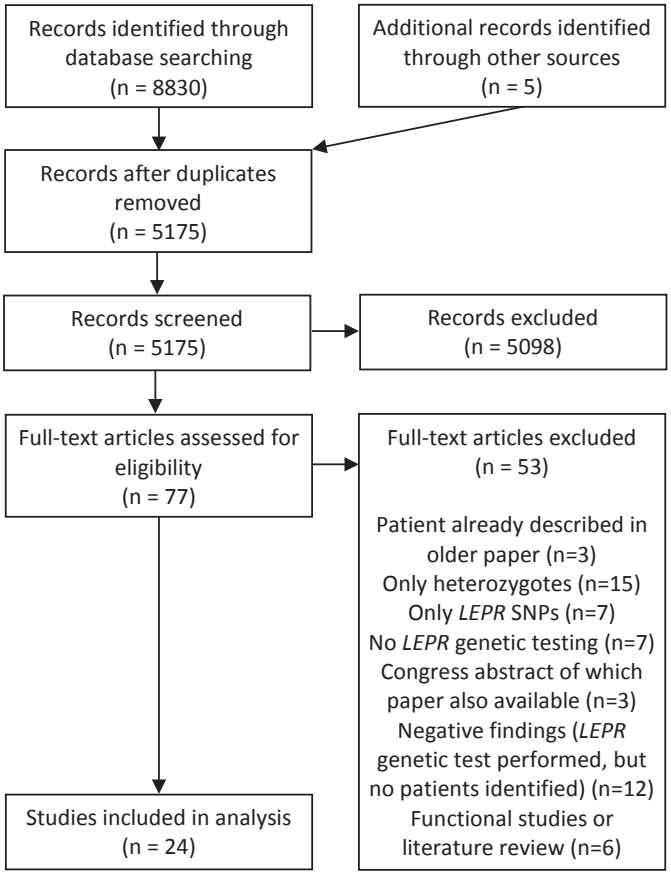

Figure 1. PRISMA flow diagram of systematic literature search
LEPR, leptin receptor gene, SNP, single nucleotide polymorphism.

Data extraction from included articles

An overview of genetic aberrations and phenotype features of patients with LepR deficiency (early-onset obesity, hyperphagia, signs of hypopituitarism and frequent infections) was made. When standard deviation scores (SDS) were not originally reported for anthropometric data, we calculated these using WHO growth charts as external standard.[13] In case insufficient clinical data were reported, we tried to contact corresponding authors to provide additional information.

Case presentation of Dutch patients with LepR deficiency

We present two novel cases of LepR deficiency identified in our obesity center. Written informed consent for publication of their clinical details was obtained from the patient and/or parents. For these two patients' anthropometric data, SDS are presented using Dutch growth charts as external standard.[14]

Selection of variants in LEPR

We extracted disease-associated *LEPR* variants from published cases identified through our systematic literature search and added in-house genetic data (Table 2). Additionally, we curated and added variants with a high likelihood of being pathogenic, that is, loss-of-function (LoF) variants that were proximal to the pathogenic variant p.S1090Wfs*6. This variant is the most distal pathogenic variant reported in a patient with LepR deficiency; hence, LoF variants located more proximally are very likely to cause LepR deficiency. For all selected *LEPR* variants, we extracted allele frequencies from the Genome Aggregation Database (gnomAD). The gnomAD database (https://gnomad.broadinstitute.org/; accessed 06-10-2019) is the largest freely accessible population-based database consisting of sequencing data from 77 165 Europeans. Individuals with known severe pediatric diseases and their first-degree relatives are removed from this database. We excluded variants that did not pass gnomAD's quality control. Because of their distinctive genetic background, Finnish individuals are often omitted from European population studies. However, by performing separate prevalence calculations for Finnish and Non-Finnish cases, we could aggregate the results and provide estimations for the whole European population. All selected *LEPR*variants were evaluated by a clinical laboratory geneticist according to the current international guideline for variant classification.[15] All variants are aligned to the canonical transcript NM_002303.5.

Prevalence calculation

We extracted European population size from the 2019 United Nations World Population Prospects report, which estimates a population size of 747.183 million Europeans, of which 5.532 Finnish Europeans (https://population.un.org/wpp/Download/Standard/Population/; accessed 28-09-2019). We estimated the number of individuals with biallelic (homozygous or compound heterozygous) pathogenic *LEPR* variants by calculating the probability of homozygosity or compound heterozygosity for each possible combination of our selected variants. We assumed that the population was in Hardy-Weinberg equilibrium and that random mating between individuals with and without obesity occurred. We did not correct for specific genetically isolated consanguineous populations in Europe. The CI of our prevalence estimation was calculated using derived variances.[16] We adapted the formulas to allow derivation based on the sum of independent random variables.

RESULTS

Systematic literature search and overview of published cases

In total, 5175 records were screened (Fig. 1), of which 24 records presented unique patients with LepR deficiency and were eligible for inclusion.[2,4,5,7,9,17-35]

From these 24 records, we identified *n* = 86 unique patients with LepR deficiency from 57 different families. We add two new unrelated cases with LepR deficiency (Box 1).

Box 1: Presentation of two new cases with LepR deficiency

The first patient is a 3-year-old boy, referred at age 13 months because of increased linear growth, obesity and hyperphagia. He was born at a gestational age of 36 + 6 weeks with normal birth weight (3840 g, +0.9 SDS). Parents did not report consanguinity, but their families lived in the same small Dutch municipality. There was no history of frequent infections. On presentation at age 13 months, height was 83.2 cm (+1.9 SDS), weight 17 kg (+4.9 SDS), and BMI 24.6 kg/m^2 (+4.4 SDS). Laboratory testing showed a central hypothyroidism. A growth hormone test was performed because of height deceleration, which confirmed GHD. Thyroid and growth hormone supplementation were started. Adrenal insufficiency was excluded by a high-dose ACTH test. MRI cerebrum revealed no anatomic abnormalities in the pituitary region. Obesity gene panel analysis (described in detail elsewhere)[6] revealed a homozygous variant of uncertain significance (VUS) in *LEPR*: c.3414dup p.(Ala1139Cysfs*16). This variant is located in the C terminal domain of the transcript. Since this is a frameshift near the end of the protein, replacing the last 27 amino acids with 15 alternative amino acids, the clinical relevance remains uncertain. However, the typical clinical phenotype (including hormonal disturbances) in the absence of other plausible explanations, makes this homozygous variant the most probable cause of the LepR deficiency phenotype.

The second patient is a 15-year-old girl referred to our obesity center at age 14 years for personalized treatment advice. She was born at a gestational age of 42 weeks with normal birth weight (3400 g, −0.1 SDS). At age 3.5 years, she was referred to a pediatric endocrinologist for evaluation of hyperphagia and obesity. There was no history of frequent infections. Height was 97 cm (−1.2 SDS), weight 23.1 kg (+3.0 SDS), BMI 24.6 kg/m^2 (+4.4 SDS). Laboratory testing showed no signs of hypopituitarism. During clinical follow-up, she had spontaneous start and progression of puberty and menarche at age 12.5 years. Whole-exome sequencing analysis revealed compound heterozygosity for two known pathogenic variants in the *LEPR* gene: c.1835G>A (p.Arg612His), c.2051A>C (p.His684Pro). Previously reported functional studies confirmed impaired functionality of the His684Pro variant, whereas the Arg612His variant has some residual function.[4]

Including these two new cases, 88 patients have now been described worldwide (Table 1), harboring 45 distinct *LEPR* variants (Table 2). Twenty-one of these patients are from European ancestry. To gain more insight in the clinical spectrum of the disease, the phenotypes are summarized in Table 1 and presented on individual level in Supplementary Table 1 (which can be found at website of the European Journal of Endocrinology). Consanguinity was reported in 65/88 (74%) patients. Of the 84 patients in which sex was reported, 42 (50%) were female. Median age at description was 8.0 years (IQR: 3.0–15.2 years). Eighteen (22%) out of the 83 patients in which age was

reported were adults, the three oldest of which were 39, 41, and 55 years old. Median BMI was 39.6 kg/m^2 (IQR: 34.1–49.1 kg/m^2). Mean BMI SDS was +5.2 (SD 2.0) and was not significantly different between males and females (P=0.39). Interestingly, three patients (Dehghani III:9 and III:10, Kakar VII:6) did not have obesity at presentation. A large inter-individual variation was seen with respect to height SDS (mean +0.3 SDS, s.d. 2.1; reported in 49/88 patients): 11/49 (22%) patients had a tall stature (height SDS >2), whereas 8/49 (16%) patients had a short stature (height SDS <−2). Early-onset obesity (<age 5 years) and hyperphagia were the most common phenotypic features (Table 1). In 21 cases, exact age of onset of obesity was reported; when aggregated, median age of onset was 0.3 years (IQR 0.2–0.4). Pituitary hormone disturbances were present in 24 patients (Table 1). In the majority of these patients (15/24, 63%), only one pituitary hormone disturbance was present. Three patients had both HH and GHD; one patient had HH and central hypothyroidism; one patient had GHD and central hypothyroidism. Three patients had HH, GHD as well as central hypothyroidism.

Known and likely pathogenic LEPR variants

Of the 45 distinct variants described in patients with LepR deficiency, only eight variants were present in the global gnomAD population, and seven were present in the European population of the gnomAD database (Table 2). Additionally, 20 LoF variants with a high likelihood of being pathogenic were identified in the European population of the gnomAD database (Supplementary Table 2). As expected, no (likely) pathogenic variants were present in a homozygous state in gnomAD.

Prevalence calculation

The calculated number of individuals with LepR deficiency (caused by biallelic disease-causing variants in the *LEPR* gene) in Europe is 998 patients (95% CI 708–1288). This would indicate that only 21/998 (2.1%) European cases with LepR deficiency are currently described in literature. The prevalence of LepR deficiency based on published European patients would be 0.03 per 1 million people. However, our calculated ‘genetic prevalence’ of LepR deficiency in Europe is 1.34 per 1 million people (95% CI 0.95–1.72 per 1 million people).

Table 1. Summarized overview of clinical characteristics of all 88 currently known patients with LepR deficiency

Features	*n* patients with available data (out of 88)	Interpretation
Early-onset obesity	87	Present in 87 (100%) patients: - 51 (59%) onset before age 2 years - 7 (8%) in (early) infancy - 5 (6%) onset between age 2–6 years - 1 (1%) onset before age 13–14 years - 23 (26%) not further specified
Hyperphagia	84	Present in 81 (96%) patients
Pituitary hormone disturbances	70	Present in 24 (34%) patients
Central hypothyroidism	64	Present in 8 (13%) patients
Growth hormone deficiency*	64	Present in 8 (13%) patients Additionally: - 3 (6%) IGF-1 values below reference range reported - 1 (2%) patients short stature reported
Hypogonadotropic hypogonadism	39	Present in 22 (56%) patients Additionally: - 1 (3%) inconclusive due to young age but low gonadotrophins reported
Hyperinsulinemia	61	Present in 24 (39%) patients Additionally: - 10 (16%) inconclusive because no reference range for insulin values was reported
Frequent infections	44	Present in 23 (52%) patients, of which 3 died due to infections in childhood Additionally: - 2 (5%) lowered CD4+ T cell count reported - 1 (2%) alterations in immune function reported

**Formal diagnosis of growth hormone deficiency by appropriate GH provocation tests. CD4, cluster of differentiation 4; IGF-1, insulin-like growth factor 1.*

DISCUSSION

Leptin receptor deficiency is a rare endocrine disease, but our population genetics-based analysis shows that it is much more prevalent in Europe than expected based on literature. Assuming that most patients with LepR deficiency have been published, as is demonstrated by the ongoing reports of new cases in the past years, this suggests underdiagnosis. This is especially problematic since diagnosing LepR deficiency now has therapeutic consequences: pharmacological treatment aimed at restoring the leptin-melanocortin pathway has recently shown impressive results in terms of weight loss, satiety, and improvement of metabolic parameters.[2]

Table 2. Mutations in the LEPR gene described in patients with LepR deficiency

Reference	*n*	Nationality	Zygosity	Variant in coding DNA	Aberration on protein level (NM_002303.5)	Functional analysis	Allele frequency European non-Finnish population in gnomAD
[35]	1	N.R.	Hom	N.A.	p.M1?	N.R.	8.80E-06
[4]	3	Southern European	Hom	N.A.	p.W31*	N.A.	Not present
[26]	1	Turkish	Hom	c.461dupA	p.N154Kfs*3	*In silico*	Not present
[29]	9	Iranian	Hom	c.464T>G	p.Y155*	*In silico*	Not present
[22]	2	Sudanese	Hom	c.479delA	p.H160Lfs*10	*In silico*	Not present
[22]	1	Guinean	Hom	c.556delT	p.C186Afs*28	*In silico*	Not present
[17]	2	Egyptian		c.946C>A	p.P316T	*In silico*	1.76E-05
[18,26]	2	Turkmen; Turkish	Hom	c.946C>A, c. 1938G>T (both hom)	p.P316T and p.W646C (both hom)	*In silico*	Not present
[4]	1	Turkish	Hom	c.1226C>A	p.A409E	*In vitro*	Not present
[24]	2	French	Comp het	c.1264T>C and c.2131dup	p.Y422H and p.T711Nfs*18	*In silico*	Not present
[32]	1	N.R.	Hom	c.1285+1G>A	p.? (splicing defect)	*In silico*	Not present
[31]	1	Turkish	Hom	c.1603+2T>C	p.? (splicing defect)	*In silico*	Not present
[21]	5	Pakistani	Hom	c.1603+5G>C	p.R468Sfs*33	*In silico*	Not present
[25]	1	Dutch	Hom	c.1604-8A>G	p.K536Sfs*34 and p.V535Dfs*3 (two transcripts)	*In silico*, Sanger, RNA analysis	8.92E-06
[24]	1	French (Reunion)	Comp het	c.1604-1G>A and del exon 6-8	p.? (splicing defect) and p.?	*In silico*	Not present
[23, 9]	2	Pakistani	Hom	c.1675G>A	p.W558*	*In silico*, Sanger	Not present
[25]	1	Dutch	Comp het	c.1753-1dupG and c.2168C>T	p.M585Dfs*2 and p.S723F	*In silico*,Sanger, RNA analysis	Not present
[24]	1	French	Hom	c.1810T>G	p.C604G	*In silico*	Not present
[9]	2	Pakistani	Hom	c.1810T>A	p.C604S	*In silico*	Not present

This publication	1	Dutch	Comp het	c.1835G>A and c.2051A>C	p.R612H and p.H684P	*In silico*	Not present
[4]	1	UK	Comp het	c.N.A. (1-bp deletion in codon 15) and c.1835G>A	p.F15Lfs*4 and p.R612H	*In vitro*(p.R612H)	Not present
[28]	1	Spanish	Hom	c.1835G>A	p.R612H	*In vitro*	4.88E-04
[35]	1	N.R.	Hom	c.1871dupA	p.N624Kfs*21	*In silico*	Not present
[26]	1	German	Comp het	c.1874G>A and c.2051A>C	p.W625* and p.H684P	*In silico*, *In vitro*(p.H684P)	Not present
[34]	3	Middle-eastern	Hom	c.1916C>T	p.P639L	*In silico*	Not present
[27]	1	Dutch	Comp het	c.1985T>C and c.2168C>T	p.L662S and p.S723F	*In silico*	Not present
[4]	1	Norwegian	Hom	N.A.	p.W664R	*In vitro*	5.31E-05
[4, 26]	2	UK; German	Hom	c.2051A>C	p.H684P	*In vitro*	3.87E-05
[7]	1	Dutch	Comp het	c.2051A>C and c.2627C>A	p.H684P and p.P876Q	*In silico*	Not present
[26]	1	German	Comp het	c.2227T>C and c.2598-3_2607delTAGAATGAAAAAG	p.S743P and p.Q865_K870	*In silico*	Not present
[24, 2]	2	Portuguese	Hom	c.2357T>C	p.L786P	*In silico*	8.82E-06
[23, 9]	4	Pakistani	Hom	c.2396-1G>T	p.? (splicing defect)	*In silico*	Not present
[24]	1	Turkish	Hom	c.2491G>A	p.H800_N831del (splicing defect)	*In silico*	Not present
[5]	3	Algerian	Hom	c.2597+1G>A	p.? (splicing defect)	PCR and sequencing	Not present
[30]	1	Pakistani	Hom	c.2675C>G	p.P892R	*In silico*	Not present
[30]	4	Pakistani	Hom	c.3268_3269del	p.S1090Wfs*6	*In silico*	Not present
[33]	5	Indian	Hom	c.3268_3269dup	p.S1090Rfs*6	*In silico*	Not present
This publication	1	Dutch	Hom	c.3414dup	p.A1139Cfs*16	*In silico*	Not present
[19]	1	N.R.	Hom	deletion *DNAJC6* and parts of *LEPR*	p.?	PCR, MPLC	Not present
[9]	1	Pakistani	Hom	1.3 kb and 58.8 kb deletions	p.?	*In silico*	Not present
[26]	1	Turkish	Hom	deletion exon 4–20	p.?	N.A.	Not present

[4]	3	Bangladeshi	Hom	N.A. (4-bp deletion codon 22)	N.A.	*In silico*	Not present
[4]	2	Turkish	Hom	N.A. (11-bp deletion codon 70)	N.A.	*In silico*	Not present
[24]	5	French (Reunion)	Hom	deletion exon 6–8	p.?	*In silico*, PCR	Not present
[4]	1	Iranian	Hom	N.A. (66-bp deletion codon 514)	N.A.	*In silico*	Not present

Abbreviations: bp, base pair; Comp het, compound heterozygous; del, deletion; gnomAD, Genome Aggregation database; Hom, homozygous; MPLC, multiplex polymerase chain reaction/liquid chromatography; n, number of patients; N.A., not applicable; N.R., not reported; PCR, polymerase chain reaction; UK, United Kingdom.

Genetic testing for obesity disorders, including LepR deficiency, is recommended in patients with extreme early-onset (before age 5 years) and clinical features of a genetic obesity disorder and/or a positive family history for extreme obesity.[36] However, a recent review from the United States reports that only 8% of patients in whom genetic testing would be indicated had undergone genetic testing.[37] An important reason for underdiagnosing might be limited access to genetic diagnostics. Although *LEPR* sequencing has become available in clinical practice in the last decade, it is not yet part of routine care in many countries. Indeed, all published European LepR deficiency cases are from high-income countries with well-established diagnostic genetic facilities. Another explanation why patients with LepR deficiency are not identified, is that the clinical phenotype is not sufficiently recognized. Our systematic literature search shows that the majority of patients do not have pituitary hormonal disturbances. It is hypothesized that there might be a genotype–phenotype correlation reflecting residual leptin receptor function in those cases, but the amount of patients is too small to draw conclusions.[3] Thus, LepR deficiency should be suspected in all cases of severe early-onset obesity and hyperphagia, even without signs of hypopituitarism, especially in the case of consanguinity. In the most common monogenic obesity disorder, *MC4R* deficiency, segregation studies have shown incomplete expressivity and penetrance for the obesity phenotype.[38] However, this is not likely for LepR deficiency, as there are no individuals present with biallelic pathogenic *LEPR* variants in gnomAD nor in large control cohorts without obesity.[6,39]

A more daunting possible cause of the discrepancy between amount of described patients versus predicted patients is mortality. Young age of known patients and absence of adult LepR deficiency patients in several large adult cohorts with early-onset obesity could suggest that these patients decease before they are identified.[6-8] This may occur due to the consequences of their severe obesity, but mortality in early childhood due to infections has also been reported.[4,29] Long-term follow-up studies of the clinical course of LepR deficiency have however not yet been performed. These studies are also needed because in some cases, improvement of the endocrine phenotype after puberty has been reported, however, without a clear explanation. Le Beyec *et al*. reported resolving of central hypothyroidism from age 16 years onward and hypogonadism from age 19 years onward in a male patient.[20] Dehghani *et al*. reported that two affected males in a consanguineous family showed BMI normalization from puberty onset onward, in contrast to the affected females in this family who did not show improvement of BMI nor hypogonadotropic hypogonadism, suggesting a sex-specific effect might be present.[29] However, Nizard *et al*. reported resolving of hypogonadotropic hypogonadism in a female patient from age 18 years onward and occurrence of natural pregnancy 2 years after gastric bypass surgery, which challenges

the assumption that hormonal disturbances only resolve in male patients.[40] However, the number of patients is too low to draw conclusions on this phenomenon.

Strengths and limitations

To the best of our knowledge, this is the first systematic literature overview of LepR deficiency cases. We identified 86 published cases, compared to the 57 cases in a previous, non-systematic overview from 2018.[3] A strength of this study is that we could add clinical information from 26/86 (30%) known LepR deficiency cases by contacting authors. Another strength is our stringent variant selection. There is always an insecurity regarding the pathogenicity of variants when functional tests have not been performed. This is even the case for variants identified in patients with clear LepR deficiency phenotypes, such as the male patient described earlier. In 2018, Ayers *et al*. presented a prevalence calculation for LepR deficiency in the United States.[41] However, they estimated prevalence using a far less stringent method by adding variants predicted to be pathogenic solely on the basis of *in silico* prediction tools. It is known that these tools are not specific, leading to high false-positive rates.[42] When we would use their method, this would lead to a prevalence estimation of 8953 patients (95% CI: 7880–10 027 patients). This would be a significant overestimation, whereas our calculation would rather yield an underestimation of actual number of patients. An important limitation of our study is that only 7/45 distinct pathogenic variants identified in patients with LepR deficiency were present in the European gnomAD population. Therefore, when sample size of sequencing data in population databases expands, prevalence calculations might yield a higher number of patients. Another limitation of our calculation is that first-degree relatives from patients with severe pediatric diseases, such as LepR deficiency, are removed from gnomAD, which could have led to a lower allele frequency of pathogenic *LEPR* variants. Moreover, we are aware that it is possible that some diagnosed patients have not been described in literature yet. This could lead to a higher prevalence calculation if these patients have novel *LEPR* variants. Thus, our current prevalence calculation should be seen as a minimum estimation.

Conclusion

LepR deficiency is an endocrine obesity disorder for which encouraging treatment options recently became available. Genetic testing in patients with early-onset obesity, hyperphagia, and/or LepR-associated hormone disturbances is therefore more important than ever. By using large population-based genetic data, we estimated the prevalence of this rare disease in Europe. Our data suggest that the majority of patients with LepR deficiency in Europe are currently not recognized. Improving

awareness and availability of genetic testing for early-onset obesity is needed to help these patients gain access to newly developed effective treatment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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SUPPLEMENTARY APPENDIX

Supplementary file 1. Search strategy for systematic literature search

Date of search: May 17th 2019

Embase – 2822 refs

((('obesity'/exp OR 'body mass'/de OR 'body weight'/exp) AND ('leptin receptor'/de)) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/3 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)):ab,ti) AND ('clinical study'/exp OR (clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*):ab,ti) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT ([animals]/lim NOT [humans]/lim) AND [english]/lim NOT ([Conference Abstract]/lim AND [1800-2016]/py)

Medline – 1990 refs

(((exp Overweight/ OR Body Mass Index/ OR Body Weight/) AND (Receptors, Leptin/)) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) ADJ3 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) ADJ5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)).ab,ti.) AND (exp Clinical Study/ OR (clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*).ab,ti.) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT (exp animals/ NOT humans/) AND english.la. NOT (news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt.

Cochrane (RCTs) – 428 refs

((((obes* OR BMI OR (body NEXT/1 mass*) OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/3 (receptor*)))) OR (((obes* OR BMI OR (body NEXT/1 mass*) OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*)):ab,ti) AND ((clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR woman OR women OR girl* OR boy* OR child*):ab,ti) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*))

Web of Science – 3390 refs

TS=(((((obes* OR BMI OR body-mass* OR weight* OR overweight*) AND (LEPR OR leptinreceptor* OR ((leptin* OR LEP) NEAR/2 (receptor*)))) OR (((obes* OR BMI OR body-mass* OR weight* OR overweight*) NEAR/5 (gene OR genes OR genom* OR mutat* OR genet* OR monogen* OR

nonsyndrom*))) AND (exome* OR sequencing* OR delet* OR mutat* OR variant* OR splic*))) AND ((clinical* OR case OR cases OR patient* OR cohort* OR male* OR female* OR man OR men OR

woman OR women OR girl* OR boy* OR child*)) NOT ((polymorph* OR SNP) NOT (mutation* OR exome* OR delet* OR splic*)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline OR rabbit OR cow OR cows OR bovine OR rodent* OR sheep OR ovine OR pig OR swine OR porcine OR veterinar* OR chick* OR zebrafish* OR baboon* OR nonhuman* OR primate* OR cattle* OR goose OR geese OR duck OR macaque* OR avian* OR bird* OR fish*) NOT (human* OR patient* OR women OR woman OR men OR man))) AND DT=(Article OR Review) AND LA=(English)

Google Scholar – 200 refs (random-top-200)

obese|obesity LEPR|"leptin|LEP receptor"|leptinreceptor clinical -polymorphism|-polymorphisms|-SNP

NB: Studies describing novel heterozygous likely pathogenic variants in patients with obesity were not considered for inclusion as it remains unclear whether homozygosity or compound heterozygosity for these variants would have led to a clinical phenotype of LepR deficiency.

Supplementary Table S1. Overview of clinical characteristics of patients with LepR deficiency

Because this file is less informative in print due to its size and lay-out, the digital file can be accessed via: https://eje.bioscientifica.com/view/journals/eje/182/1/EJE-19-0678.xml?body=supplementaryMaterials-10421

Supplementary table S2. GnomAD allele frequencies

Source of mutation	Genomic position chr1 (gnomAD notation)	Aberration on protein level (gnomAD notation)	Allele Count (AC) European non-Finnish	Allele Number (AN) European non-Finnish	Allele frequency (AF) European non-Finnish	AC Finnish	AN Finnish	AF Finnish
gnomAD database pLoF	66036197	p.Thr29TyrfsTer6	1	113510	8,8098E-06	0	21590	0
gnomAD database pLoF	66036246	p.Tyr46Ter	1	113632	8,80034E-06	0	21630	0
gnomAD database pLoF	66036415	p.Leu101TyrfsTer15	2	15428	0,000129634	0	3476	0
gnomAD database pLoF	66038099	p.Tyr155IlefsTer13	0	111206	0	2	21466	9,31706E-05
gnomAD database pLoF	66058521	p.Met227AsnfsTer12	1	113228	8,83174E-06	0	21638	0
gnomAD database pLoF	66062229	p.Gln268Ter	1	15412	6,48845E-05	0	3468	0
gnomAD database pLoF	66064342	p.? (splicing defect c.850-1G>A)	1	113542	8,80731E-06	0	21628	0
gnomAD database pLoF	66067307	p.Tyr411LeufsTer4	1	113358	8,82161E-06	0	21620	0
gnomAD database pLoF	66067643	p.? (splicing defect c.1403+1_1403+2dupGT)	1	113592	8,80344E-06	0	21648	0
gnomAD database pLoF	66074585	p.? (splicing defect c.1752+1G>A)	3	128788	2,32941E-05	0	25116	0
gnomAD database pLoF	66075790	p.? (splicing defect c.1912+3_1912 +15dupCTGCAGAGATTTT)	1	113744	8,79167E-06	0	21646	0
gnomAD database pLoF	66075910	p.Glu644LeufsTer6	0	128986	0	4	25116	0,000159261
gnomAD database pLoF	66075921	p.Trp646Ter	1	15426	6,48256E-05	0	3476	0
gnomAD database pLoF	66075946	p.Glu657GlyfsTer15	1	113316	8,82488E-06	0	21640	0
gnomAD database pLoF	66083646	p.? (splicing defect c.2213-1G>T)	1	15424	6,4834E-05	0	3456	0
gnomAD database pLoF	66083751	p.Glu773Ter	1	113450	8,81446E-06	0	21466	0
Source of mutation	Variant in coding DNA	Aberration on protein level	Allele Count (AC) European non-Finnish	Allele Number (AN) European non-Finnish	Allele frequency (AF) European non-Finnish	AC Finnish	AN Finnish	AF Finnish

gnomAD database pLoF	66083777	p.Ile783SerfsTer37	1	113420	8,81679E-06	0	21336	0
gnomAD database pLoF	66087142	p.? (splicing defect c.2597+1G>T)	1	113524	8,80871E-06	0	21530	0
gnomAD database pLoF	66102123	p.Glu975Ter	1	15430	6,48088E-05	0	3476	0
gnomAD database pLoF	66102425	p.Tyr1078IlefsTer2	3	113010	2,65463E-05	0	21598	0
Le Beyec *et al.*, 2019 (35)	N/A (start lost)	p.Met1*	1	113632	8,80034E-06	0	21648	0
Mazen *et al.*, 2011 (17)	c.946C>A	p.Pro316Thr	2	113412	1,76348E-05	0	21632	0
Hannema *et al.*, 2016 (25)	c.1604-8A>G	p.Lys536Serfs*34 and p.Val535Aspfs*3 (two transcripts)	1	112058	8,92395E-06	0	21578	0
Albuquerque *et al.*, 2014 (28); Farooqi *et al.*, 2007 (4); This publication	c.1835G>A	p.Arg612His	63	129162	0,00048776	1	25120	3,98089E-05
Farooqi *et al.*, 2007 (4)	N/A	p.Trp664Arg	6	112906	5,31416E-05	1	21630	4,62321E-05
Farooqi *et al.*, 2007 (4); Kohlsdorf *et al.*, 2018 (26); Kleinendorst *et al.*, 2018 (7); This publication	c.2051A>C	p.His684Pro	5	129146	3,87159E-05	0	25122	0
Huvenne *et al.*, 2015 (24)	c.2357T>C	p.Leu786Pro	1	113392	8,81896E-06	0	21206	0

Abbrevations: gnomAD, genome aggregation database; chr, chromosome pLoF, predicted loss-of-function. The numbers in brackets after author name and publication year refer to the reference numbers in the article.

5

Genetic obesity disorders: BMI trajectories and age of onset of obesity compared to children with obesity from the general population

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ABSTRACT

Objectives To offer children with obesity optimal treatment, it is important to identify rare genetic obesity disorders. Early age of onset of obesity (AoO) is a cardinal feature. Current guidelines suggest genetic screening in selected cases with AoO <5y, but this is not validated. We assessed BMI trajectories of children with genetic obesity to identify optimal AoO cut-offs for genetic screening.

Study design This longitudinal, observational study included growth measurements from birth onwards of children with non-syndromic and syndromic genetic obesity and control children with obesity from a population-based cohort. Diagnostic performance of AoO was evaluated.

Results We describe BMI trajectories of 62 children with genetic obesity (29 non-syndromic, 33 syndromic) and 298 controls. Median AoO was 1·2 years in non-syndromic genetic obesity (0.4 and 0.6 years in biallelic *LEPR* and *MC4R*; 1.7 in heterozygous *MC4R*); 2.0 years in syndromic genetic obesity (0.9, 2.3, 4.3, and 6.8 years in pseudohypoparathyroidism, Bardet-Biedl syndrome, 16p11.2del syndrome, and Temple syndrome, respectively); and 3.8 years in controls. Optimal AoO cut-off was ≤3·9 years (sensitivity 0·83, specificity 0·49, AUC 0·79, P<0·001) for non-syndromic and ≤4·7 years (sensitivity 0·82, specificity 0·37, AUC 0·68, P=0·001) for syndromic genetic obesity.

Conclusions This is the largest cohort describing BMI trajectories in genetic obesity to date. Optimal AoO cut-off as single parameter to determine which children should undergo genetic testing was ≤3·9 years. In case of higher AoO, additional features indicative of genetic obesity should be present to warrant genetic testing. Optimal cut-offs might differ across different race and ethnicities.

INTRODUCTION

A more than tenfold increase in pediatric obesity over the last four decades has resulted in 124 million (7%) children with obesity worldwide.[1] The global prevalence of overweight or obesity in children aged <5y is predicted to increase to 11% by 2025.[2] In 2-7% of children with obesity, genetic obesity disorders can be identified.[3-5] Diagnostic yield can increase further by screening high-risk populations using broad genetic tests.[6] Early age of onset of obesity (AoO) is a cardinal feature of genetic obesity.[3,4] Current international guidelines suggests genetic screening in selected cases with age of onset of severe obesity (AoO_{severe}) <5y.[4,7] Clinical practice shows that it can be difficult to distinguish these patients from children with childhood-obesity onset without underlying genetic causes.[8] Prevalence estimations based on population-level genetic data suggest that the majority of patients with genetic obesity are currently not identified.[9] Moreover, only a small minority of children in whom genetic testing is indicated by the guideline actually undergo testing.[10] Diagnosing patients with genetic obesity is vital for patient-tailored treatment, as novel medication has become available for patients with genetic defects in the leptin-melanocortin pathway, the hypothalamic pathway that regulates satiety and energy expenditure.[11,12]

Genetic obesity comprises a heterogeneous group of rare disorders with two distinct subgroups.[4] In non-syndromic genetic obesity, severe early-onset obesity is the main phenotypic feature. In syndromic obesity, developmental delay, intellectual disability, or multiple congenital anomalies are typically present. For the most common syndromic obesity disorder, Prader-Willi syndrome (PWS), it is well-described that the weight increase starts between 2–4·5y;[3] therefore, PWS will not be further discussed in this article. For other genetic obesity disorders however, these trajectories are not yet described in detail. In addition, the guideline cut-off AoO <5y does not distinguish between non-syndromic and syndromic genetic obesity and has not been clinically validated. Several recent pediatric studies report lower AoO, especially in non-syndromic genetic obesity.[8,13,14] Moreover, the 'ideal' cut-off might change as early-onset obesity is becoming more prevalent.[15] Therefore, more insight is needed into the BMI trajectories of children with genetic obesity and optimal cut-offs of AoO and obesity severity to determine the indication for genetic testing.

The primary aim of this study is to present BMI trajectories and AoO of children with genetic obesity. The secondary aim is to identify the optimal diagnostic performance cut-off for BMI trajectory characteristics (AoO, AoO_{severe}, and BMI at yearly age bins) by comparing these characteristics between children diagnosed with genetic obesity

and controls, i.e. children from the general population who developed obesity before age 10 years.

METHODS

For this longitudinal, observational study, we used patient data from the Dutch center of expertise for genetic obesity, a collaboration between the departments of Pediatrics and Internal Medicine of Obesity Center CGG (Erasmus MC, Rotterdam) and the department of Human Genetics (Amsterdam UMC, Amsterdam). For control comparison, data from The Generation R Study (Rotterdam, the Netherlands) were used. [16] All parents/caretakers of children ≤16y gave written informed consent; additionally, children ≥12y gave written informed consent; <12y verbal consent. Both studies were approved by the medical ethics committee of Erasmus MC.

Patients and control population

Patients (0-18y), referred to Obesity Center CGG for diagnostic evaluation and/or multidisciplinary treatment advice, underwent an extensive diagnostic work-up as described in detail previously (https://doi.org/10.1371/journal.pone.0232990.s001).[6] This included extensive genetic testing (gene panel analysis or whole exome sequencing) by ISO15189 accredited academic genetic diagnostics laboratories for the clinically most important genetic obesity disorders as mentioned in the guideline, e.g. *LEP, LEPR, POMC, PCSK1, MC4R, SIM1, ALMS1,* and *GNAS*.[5] Variants were classified following the American College of Medical Genetics and Genomics guideline.[17] Genetic obesity was diagnosed when a pathogenic or likely pathogenic variant or copy number variation (CNV) was identified which matched the patient's clinical phenotype. Genetic diagnosis was confirmed by a clinical geneticist. For this report, we included patients with diagnosed genetic obesity referred from February 2015–March 2020. Exclusion criteria were declining informed consent or genetic testing, or lack of growth measurements (<2 weight/height measurements; Supplementary Figure S1). Patients were subclassified into non-syndromic (including biallelic or heterozygous pathogenic variants) and syndromic genetic obesity. To compare BMI trajectories with a control population of children with multifactorial obesity unlikely to have genetic obesity, we included children from the Generation R Study, a population-based study in the Rotterdam area with follow-up from fetal life onwards.[16] For this report, we selected children who had sustained obesity (≥2 consecutive measurements) to avoid including children in whom obesity was present due to e.g. measurement errors. We also excluded control children with a BMI standard deviation score (SDS) >4SD (n=25), as these children might have other specific underlying causes for their obesity. This

yielded 298/8896 (3·3%) control children with obesity (Supplementary Figure S1), which is in line with obesity prevalence between age 2-12y in the Dutch general population (2·9%).[18]

Assessment of obesity and AoO

For all children, we asked consent to retrieve anthropometric measurements of the Dutch nationwide screening program which all children visit at ages 0.75, 2, 3, 5, 8, 11, 14 months, and 3y. Additionally, for patients with genetic obesity, we collected measurements of all previous contacts with health care professionals before referral, including general practitioners, pediatricians, dieticians, and physical therapists. During follow-up at our center, weight and height were measured in 0.1 cm increments while lightly clothed and standing without shoes. Control subjects were measured similarly at ages 6y and 10y. We calculated BMI and age- and sex-specific SDS using Dutch references.[19] We used *International Obesity Task Force* (IOTF) cut-offs to define obesity and severe obesity (BMI above the age- and sex-specific cut-offs corresponding to adult BMI ≥30 and ≥35 kg/m^2, respectively).[20] Because these cut-offs are only validated for children ≥2y, we used the WHO definition of obesity (weight-for-height-SDS ≥3·0) for children <2y; for this age group, there is no accepted definition of severe obesity.[2] We defined AoO as the age at which the obesity cut-off was first crossed. This was calculated by linear interpolation between the last measurement at which the child did not have obesity and the first measurement at which the child had obesity. We adopted this strategy to mimic daily clinical practice in which individual growth measurements are plotted over reference charts and subsequently connected to yield an individual trajectory.

Statistical analyses

Data are presented as mean±SD or median (interquartile range, IQR). Differences in baseline characteristics and AoO between patients and controls were analyzed using independent sample t-tests, Mann-Whitney tests and chi-squared tests. We used Receiver Operating Characteristics (ROC)-curve analysis to investigate diagnostic performance (sensitivity, specificity, positive likelihood ratio [LR+]) of age of onset of obesity (AoO) and severe obesity (AoO_{severe}). We defined optimal cut-off based on Youden's *J*. Since the aim of using AoO as diagnostic screening tool would be to minimize the number of patients with genetic obesity who would erroneously not be genetically screened (false negatives), we defined optimal cut-off as the value with sensitivity ≥0·80 with the highest Youden's *J*. We calculated posttest probability (PostTP) of genetic obesity and number needed to test to identify one diagnosis based on a pretest genetic obesity prevalence (PreTP) of 2-7%.[3-5] To visualize BMI and BMI SDS trajectories, we categorized measurements analogous to previous studies[8,21] into

age bins: 0y (0·0-0·125), 0·25y (0·125-0·375), 0·5y (0·375-0·625), 1y (0·625-1·25), 1·5y (1·25-1·75), 2y (1·75-2·5), 3y (2·5-3·5), 4y (3·5-4·5), 5y (4·5-5·5), 6y (5·5-7·0), 8y (7·0-9·0), 10y (9·0-11·0), 12y (11·0-13·5), 15y (13·5-16·5), and 18y (16·5-18·5). Furthermore, we calculated ΔBMI and ΔBMI SDS expressed as yearly changes. When a child had multiple measurements available , we calculated mean for that bin. If a child did not have a measurement available for a given age bin, but had measurements available for the previous and following age bin, we calculated the missing data point by linear interpolation. For each age bin, ROC-curve analysis was performed on raw BMI values to evaluate diagnostic performance of obesity severity. We used R version 4·0·0 (R Core Team, 2021) and SPSS version 28 (Armonk, NY: IBM Corp, 2021) with a two-sided α of 0·05.

Role of the funding source

None.

RESULTS

Characteristics of the study populations

We included 62 patients with genetic obesity: 29 non-syndromic (of whom 10 had biallelic and 19 heterozygous variants) and 33 syndromic, and 298 controls with obesity (Supplementary Figure S1). Individual-level clinical and genetic data are presented in Supplementary Table S1. Baseline characteristics are summarized in Table 1. For patients with genetic obesity, mean BMI SDS was +3·1±1·2, indicating severe obesity. A median of 21 BMI measurements (IQR 18-27) per patient were available. For controls, a median of 9 BMI measurements (IQR 7-11) per child were available.

BMI trajectories

BMI trajectories are presented in Figure 1. Patients with non-syndromic genetic obesity had similar weight-for height SDS at birth compared to controls, followed by rapidly increasing BMI within the first two years of life and significantly higher mean BMI SDS from age 0·5y onwards. The rapid increase of BMI was more pronounced in patients with biallelic than heterozygous variants (Figure 1). Patients with syndromic genetic obesity had lower mean weight-for height SDS at birth compared to controls followed by gradually increasing BMI until age 5-6y. Their mean BMI SDS was significantly higher than controls between ages 3-5y only. Disorder-specific BMI trajectories are presented in Figure 2. Notably, a distinction was seen between syndromic genetic obesity disorders with rapid increase in BMI within the first two years of life similar to non-syndromic genetic obesity (e.g. Bardet-Biedl syndrome [BBS], pseudohypo-

parathyroidism type 1a and 1b [PHP]) and syndromes with low weight-for height SDS at birth and gradual BMI increase during childhood (e.g. 16p11.2 deletion syndrome, Temple syndrome). BMI SDS trajectories showed similar patterns and are presented in Supplementary Figures S2 and S3.

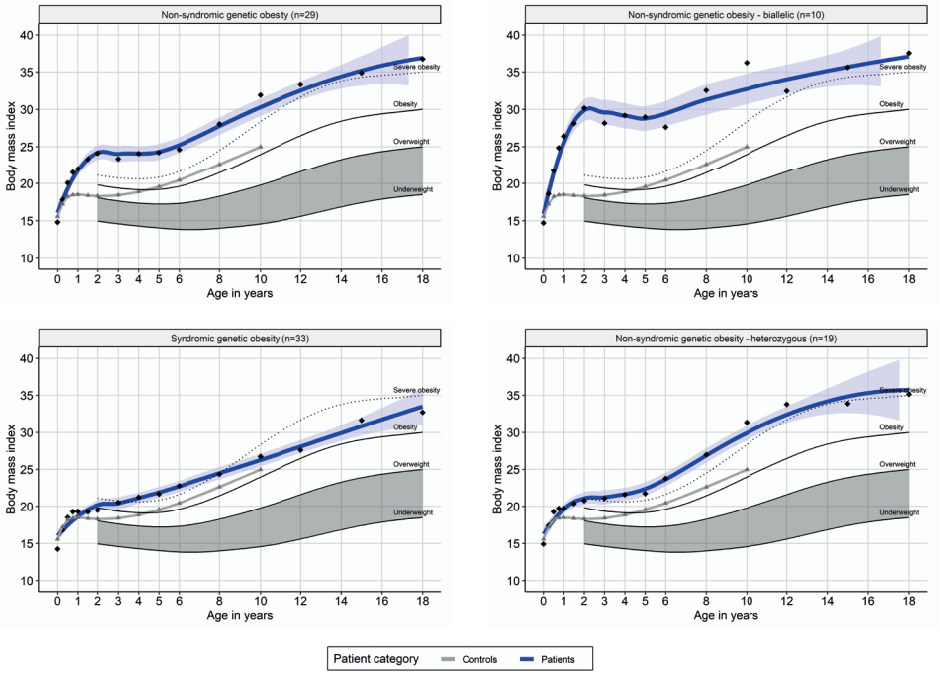

Figure 1. BMI trajectories in patients with and without genetic obesity disorders
Childhood BMI are presented for patients with non-syndromic (upper left panel) and syndromic (lower left panel) genetic obesity disorders, and for biallelic (upper right panel) and heterozygous (lower right panel) non-syndromic genetic obesity separately. The dots indicate the mean values per age bin; the line indicates the locally estimated scatterplot smoothing (LOESS) regression line; the shaded areas around the regression line indicate the 95% CI. The female International Obesity Task Force (IOTF) cut-offs are presented as reference, with the grey shaded area indicating normal weight.

Age of onset of obesity (AoO)

AoO was significantly lower in both non-syndromic and syndromic genetic obesity versus controls (both P<0·01) and was below the guideline cut-off <5y in all subgroups including controls (Table 1). Non-syndromic genetic obesity patients with biallelic variants had lower AoO compared to patients with heterozygous variants (median 0·6y [IQR 0·4-0·7] vs. 2·3y [IQR 1·1-4·3]; P<0.001). Both subgroups had lower AoO compared to controls (both P<0·01). Disorder-specific AoO is presented in Figure 3. The lowest AoO was found in patients with biallelic non-syndromic genetic obesity and PHP. Patients with other syndromic genetic obesity disorders had variable AoO ranging from 1-14y.

Table 1. Group characteristics, anthropometrics and AoO of the study populations

		Patients with genetic obesity			Control population
		All patients n=62	Non-syndromic genetic obesity n=29	Syndromic genetic obesity n=33	Children with obesity before age 11 years from the general population n=298
Characteristics at first visit to Obesity Center CGG (patients) / last Generation R study visit (controls)					
Sex, female	n (%)	39 (63)	18 (62)	21 (64)	173 (58)
Age in years	Median (IQR)	10.5 (6.9; 14.8)	11.5 (6.8; 14.3)	9.3 (6.4; 14.8)	10.5 (9.5; 13.6)
Socio-economic status z-score	Median (IQR)	0.0 (-1.0; +0.6)**	-0.0 (-1.8; +0.6)*	0.2 (-0.5; +0.7)**	-1.2 (-2.5; -0.7)[a]
Height SDS	Mean (SD)	+0.55 (1.46)	+1.26 (1.30)	-0.07 (1.32)	+0.52 (1.01)
Weight SDS	Mean (SD)	+3.17 (1.49)	+4.05 (1.16)	+2.40 (1.32)	+2.42 (1.35)
BMI SDS	Mean (SD)	+3.13 (1.16)	+3.66 (1.13)	+2.68 (0.98)	+2.36 (0.51)
Age of onset of obesity and severe obesity					
AoO, years	Median (IQR)	1.5 (0.7; 3.9)**	1.2 (0.6; 3.8)**	2.0 (0.9; 4.2)**	3.8 (2.3; 6.2)
AoO_{severe}, years	Median (IQR)	1.4 (0.6; 4.4)	1.1 (0.6; 4.9)	1.6 (0.8; 3.0)	2.6 (1.0; 4.1)

[a] *Unknown in n=16 control children.*

*Abbreviations: IQR, interquartile range; SD(S), standard deviation (score); NA, not applicable. AoO, age of onset of obesity grade 1; AoO_{severe}, age of onset of severe obesity. *, P<0.01 compared to control population; **, P<0.001 compared to control population.*

AoO_{severe} was available for n= 28 patients with non-syndromic genetic obesity, n= 25 patients with syndromic genetic obesity and n=157 control subjects without genetic obesity.

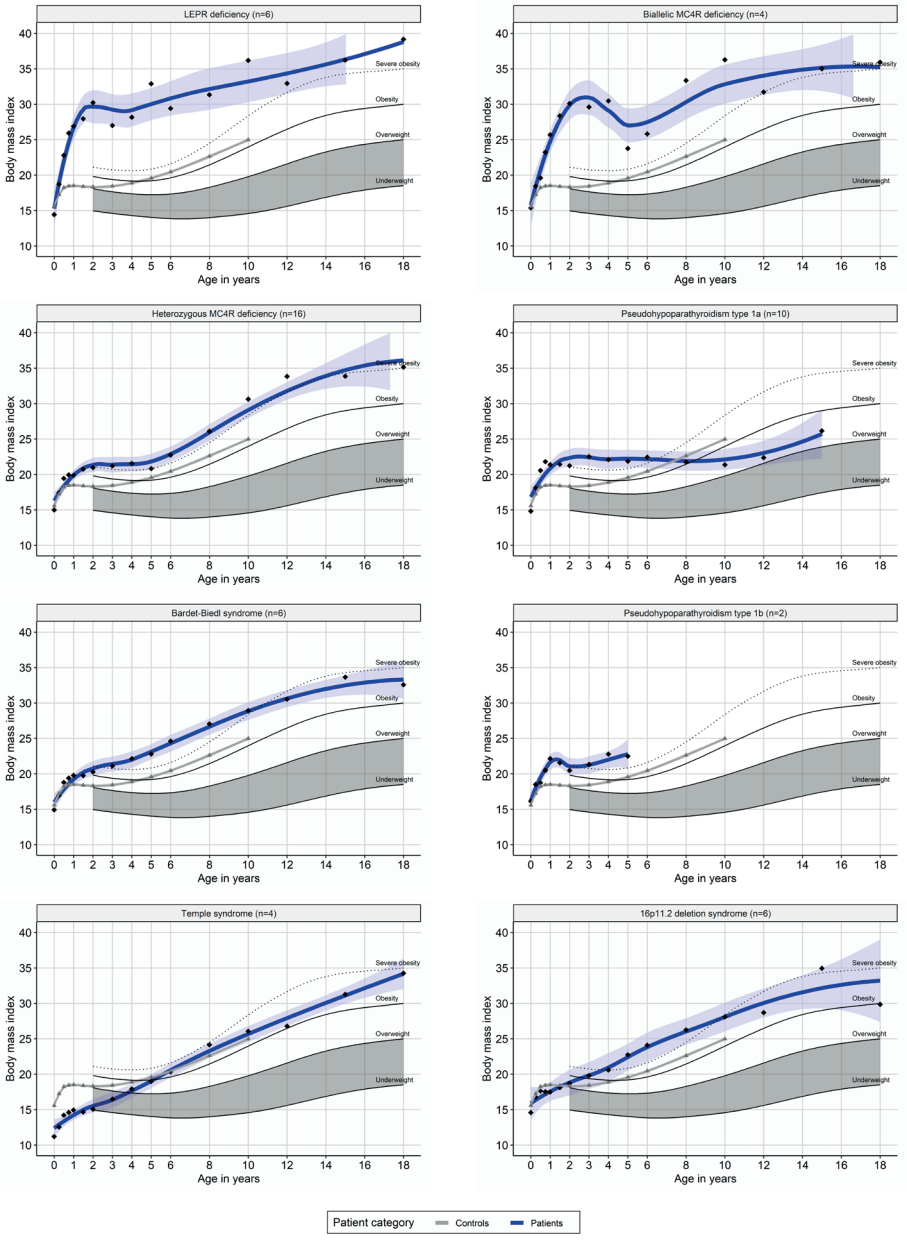

Figure 2. BMI trajectories in specific genetic obesity disorders
Childhood BMI trajectories are presented for patients specific genetic obesity disorders. The dots indicate the mean values per age bin; the line indicates the locally estimated scatterplot smoothing (LOESS) regression line; the shaded areas around the regression line indicate the 95% CI. The female International Obesity Task Force (IOTF) cut-offs are presented as reference, with the grey shaded area indicating normal weight.

Predictive value of BMI trajectory characteristics

Using AoO as single predictor to discriminate between patients versus controls yielded an AUC of 0·79 for non-syndromic (95% CI 0·69–0·88, p<0·001) and 0·68 for syndromic genetic obesity (95% CI 0·56·79, p=0·001, Figure 4). Optimal AoO cut-off for non-syndromic genetic obesity was ≤3·9y. Compared to the guideline cut-off (<5y), this yielded lower sensitivity, but higher specificity and LR+ (Table 2). Optimal AoO cut-off for syndromic genetic obesity was ≤4·7y. Compared to the guideline cut-off (<5y), this yielded the same sensitivity and slightly higher specificity (and LR+ (Table 2). AoO_{severe} showed worse performance (Supplementary results). Severity of obesity using BMI as single predictor yielded good diagnostic performance for non-syndromic genetic obesity from age 0·5y upwards (AUCs 0·73–0·90, all P<0·001) and moderate performance for syndromic genetic obesity between age 1-6y (AUCs 0·61–0·72, P<0·001–0·046, Table 3). Corresponding optimal BMI cut-offs per age bin are presented in Table 3. Changes in growth charts characteristics (ΔBMI, ΔBMI SDS, Δweight-for-height SDS) showed worse performance (Supplementary Tables S2 and S3).

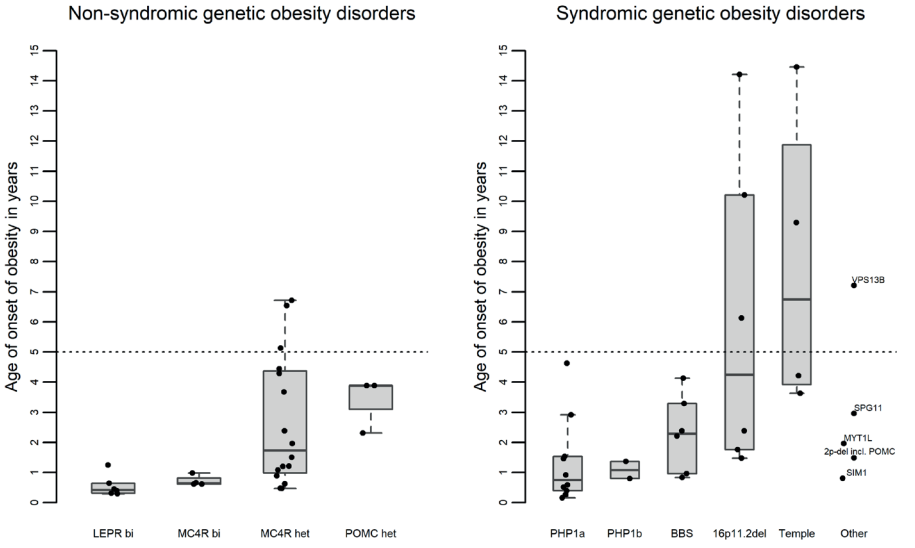

Figure 3. Individual ages of onset of obesity in genetic obesity disorders
Individual age of onset (AoO) of obesity are summarized on individual patient and disorder level. The dots represent the exact AoO of obesity of each patient. The box plot indicates the median and interquartile range of AoO of obesity for the specific genetic obesity disorder. The dotted horizontal line represent the Endocrine Society guideline's cut-off age of 5 years.
Abbreviations: bi, biallelic; het, heterozygous; LEPR, leptin receptor; MC4R, melanocortin 4 receptor; POMC, pro-opiomelanocortin, PCSK1, proprotein convertase subtilisin/kexin type 1; PHP1a, pseudohypoparathyroidism type 1a; BBS, Bardet-Biedl syndrome; 16p11.2del, 16p11.2 deletion syndrome; Temple, Temple syndrome; VPS13B, vacuolar protein sorting 13 homolog b (leading to Cohen syndrome), SPG11, spastic paraplegia 11; MYT1L, myelin transcription factor 1 like; 2p-del incl., deletion of the short arm of chromosome 2 including; SIM1, single-minded homolog 1; PHP1b, pseudohypoparathyroidism type 1b.

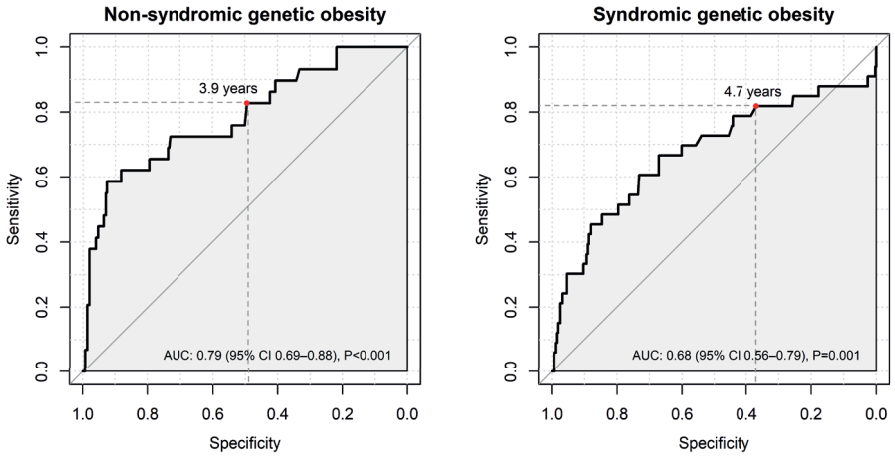

Figure 4. Performance of AoO of severe obesity as diagnostic test in the study population
The left panel depicts the performance of AoO of obesity to distinguish patients with non-syndromic genetic obesity from patients without genetic obesity. The right panel depicts the performance of AoO of obesity to distinguish patients with syndromic genetic obesity from patietns without genetic obesity, Optimal cut-off values (point with highest Youden index and sensitivity of at least 0.80) are marked in red.

Table 2. Overview of diagnostic performance of AoO for non-syndromic and syndromic genetic obesity disorders in patients visiting a pediatric obesity center

Non-syndromic genetic obesity disorders (AUC 0.79, P<0.001)						
	Cut-off value	**Sensitivity**	**Specificity**	**LR+**	**PostTP**	**NNT**
Optimal cut-off value (highest Youden index and sensitivity ≥0.80)	≤3.9 years	0.83	0.49	1.63	3.2-11.0%	9-31
ES guideline cut-off	≤5 years	0.90	0.35	1.37	2.7-9.4%	11-37
Highest Youden index (point of least misclassification)	≤1.25 years	0.59	0.93	7.94	14.0-37.4%	3-7

Syndromic genetic obesity disorders (AUC 0.68, P=0.001)						
	Cut-off value	**Sensitivity**	**Specificity**	**LR+**	**PostTP**	**NNT**
Optimal cut-off value (highest Youden index and sensitivity ≥0.80)	≤4.7 years	0.82	0.37	1.30	2.6-8.9%	11-39
ES guideline cut-off	≤5 years	0.82	0.35	1.25	2.5-8.6%	12-40
Highest Youden index (point of least misclassification)	≤3.0 years	0.67	0.67	2.03	4.0-13.2%	8-25

Abbreviations: AoO, age of onset of obesity grade 1; AUC, area under the receiver-operating characteristics curve; ES, Endocrine Society; LR+, positive likelihood ratio; PostTP, post-test probability (based on a pre-test probability of 2-7%), NNT, number needed to test to diagnose one genetic obesity disorder.

Table 3. Overview of ROC-curve analysis of BMI stratified on age bins

		Non-syndromic genetic obesity						Syndromic genetic obesity					
		AUC (95% CI)	P-value	Optimal BMI cut-off	Sens	Spec	LR+	AUC (95% CI)	P-value	Optimal BMI cut-off	Sens	Spec	LR+
Age bin	**0 years**	0.35 (0.21 - 0.49)	0.019	N/A	N/A	N/A	N/A	0.30 (0.19 - 0.40)	0.001	N/A	N/A	N/A	N/A
	0.5 years	0.73 (0.62 - 0.84)	<0.001	18.3 kg/m^2	0.82	0.45	1.82	0.57 (0.43 - 0.68)	0.317	N/A	N/A	N/A	N/A
	1 years	0.77 (0.66 - 0.89)	<0.001	18.8 kg/m^2	0.82	0.59	1.99	0.61 (0.48 - 0.74)	0.046	16.6 kg/m^2	0.81	0.11	0.90
	1.5 years	0.80 (0.69 - 0.92)	<0.001	18.9 kg/m^2	0.81	0.63	2.21	0.63 (0.50 - 0.76)	0.017	17.3 kg/m^2	0.81	0.21	1.02
	2 years	0.83 (0.72 - 0.94)	<0.001	19.1 kg/m^2	0.82	0.68	2.52	0.66 (0.53 - 0.79)	0.005	16.2 kg/m^2	0.81	0.08	0.88
	3 years	0.81 (0.71 - 0.91)	<0.001	18.9 kg/m^2	0.82	0.61	2.09	0.72 (0.60 - 0.85)	<0.001	17.1 kg/m^2	0.83	0.26	1.12
	4 years	0.77 (0.65 - 0.89)	<0.001	18.5 kg/m^2	0.92	0.39	1.51	0.68 (0.56 - 0.81)	0.001	18.2 kg/m^2	0.83	0.36	1.30
	5 years	0.78 (0.64 - 0.91)	<0.001	19.5 kg/m^2	0.90	0.45	1.64	0.65 (0.52 - 0.79)	0.012	17.9 kg/m^2	0.82	0.17	0.98
	6 years	0.80 (0.68 - 0.91)	<0.001	20.4 kg/m^2	0.85	0.52	1.76	0.65 (0.51 - 0.79)	0.016	18.4 kg/m^2	0.88	0.19	1.09
	8 years	0.88 (0.79 - 0.96)	<0.001	25.3 kg/m^2	0.80	0.90	7.92	0.59 (0.42 - 0.77)	0.148	N/A	N/A	N/A	N/A
	10 years	0.90 (0.80 - 1.00)	<0.001	27.7 kg/m^2	0.87	0.88	7.16	0.63 (0.46 - 0.79)	0.061	N/A	N/A	N/A	N/A

Abbreviations: Sens, sensitivity; spec. specificity; LR+, positive likelihood ratio; SDS, standard deviation score; AUC, area under the ROC curve; ROC, receiver operating characteristic. N/A, optimal cut-off not applicable due to non-significant or inversely significant AUC. Optimal cut-off values defined as cut-offs with highest Youden's index with sensitivity ≥0.80.

DISCUSSION

This study presents childhood BMI trajectories and AoO in 62 pediatric patients with non-syndromic and syndromic genetic obesity disorders compared to 298 children with childhood-onset obesity sampled from the general population. The BMI trajectories show a clear distinction between patients subgroups and controls. Children with bi-allelic non-syndromic genetic obesity showed a rapid increase in BMI and development of severe obesity in the first year of life, while children with heterozygous obesity-associated variants developed obesity after age 1y but well before age 5y. In syndromic genetic obesity, BMI trajectories were more variable and disorder-specific. In children with obesity from the general population, BMI trajectories showed gradually increasing BMI throughout childhood starting from normal birth weight. Our results are in line with recent reports of case series and small patient groups with specific genetic obesity disorders and their BMI trajectories.[8,14,22-24] Median AoO in our study was well before the guideline cut-off <5y[4] in both non-syndromic (1·2y) and syndromic genetic obesity (2·0y), and even in the controls (3·8y). A decreasing AoO in children with obesity is observed worldwide, reflecting the secular trend of increasing obesity prevalence in early childhood.[2,15] Recent longitudinal population-based studies indeed show that the deviation from normal BMI of adolescents with overweight or obesity starts around age 2-3y,[21,25] with BMI acceleration occurring between ages 2-6y.[21] When focussing on children with severe obesity at age 6y, deviation from normal BMI starts as early as age 6 months.[26] Interaction with the obesogenic environment has been hypothesized to shift AoO further downward even in patients with genetic obesity.[27,28] Therefore it is logical that the guideline cut-off <5y needs shifting towards earlier age in the current generation.

Our second aim was to evaluate whether BMI trajectory characteristics can aid clinical decision-making regarding which children should be genetically screened, and what the 'ideal' cut-offs would be. We found between-disorder and interindividual variation of AoO in genetic obesity as well as overlap with controls. The earliest AoO (<1y) was found in biallelic non-syndromic genetic obesity and PHP, in line with a recent study in which 21/22 patients with PHP had AoO <1y.[13] In heterozygous non-syndromic and syndromic genetic obesity disorders, AoO variation between individuals and disorders was large, ranging from <1y–14y. Optimal cut-offs were ≤3·9y for non-syndromic and ≤4·7y for syndromic genetic obesity. Moreover, AoO as single screening parameter performed better for non-syndromic than for syndromic genetic obesity. AoO_{severe} showed worse diagnostic performance than AoO. The current guideline suggests that genetic screening is indicated in cases with AoO_{severe} <5y with additional clinical features suggestive of genetic obesity disorders.[4] However, 10% of patients with

non-syndromic genetic obesity and 18% of patients with syndromic genetic obesity developed obesity after age 5y. Moreover, 24% of patients with syndromic genetic obesity never developed severe obesity and would therefore be missed when using AoO_{severe}. Additionally, we and others found that patients with and without diagnosed underlying causes did not differ in obesity severity,[29] and no accepted definition of severe obesity exists below age 2 years. Therefore, AoO seems to be a more suitable genetic screening parameter than AoO_{severe}. Absolute BMI at pre-specified age bins, showed good performance for non-syndromic genetic obesity from age 0·5y onwards, but less so for syndromic genetic obesity. In 2018, a study suggested absolute BMI cut-offs >27 kg/m^2 at age 2y or >33 kg/m^2 at age 5y to distinguish between biallelic non-syndromic genetic obesity (caused by *LEP* or *LEPR* mutations) and controls with severe obesity.[8] In our cohort, these cut-offs would correctly identify 3/6 patients with biallelic LEPR mutations.

Implications and future directions for clinical practice

As long as genetic testing remains too expensive and challenging to perform in all children with early-onset obesity, clinical criteria are necessary to determine who should be screened. The presented BMI trajectories can aid clinical decision-making. Our data suggest that non-syndromic and syndromic genetic obesity disorders should be viewed separately. AoO can be used as single parameter, even without involving obesity severity or other features like hyperphagia. A cut-off of ≤3·9y performed best in the setting of a pediatric obesity center outpatient clinic. This cut-off identifies most children with non-syndromic genetic obesity (e.g. *LEPR*, *MC4R*) and syndromes BBS and PHP. In case of AoO >3·9y, additional features indicative of genetic obesity disorders, e.g. severe obesity, hyperphagia or family history of severe obesity,[4] should be present to warrant genetic testing to increase specificity due to the overlap with children with obesity in the general population. Moreover, the large AoO variation in syndromic genetic obesity disorders indicates that AoO should not be the main driver for genetic screening. For example, these patients often present with developmental problems at a younger age than their severe obesity, providing an opportunity for earlier diagnosis. If optimal specificity and number needed to screen are required, a more stringent AoO cut-off ≤1·25y showed the best results. Because most genetic obesity disorders are rare, except heterozygous MC4R deficiency,[22] future studies should aim at increasing diagnostic yield by developing evidence-based diagnostic algorithms and disease-specific growth charts by combining data of all known patients with genetic obesity through international collaboration networks. Moreover, our proposed cut-offs should be validated prospectively in unselected cohorts of children referred to pediatricians, and in diverse populations as optimal cut-offs might differ across different race and ethnicities.

Early identification of patients with genetic obesity is crucial for patient-tailored treatment.[6,11,12] Establishing a diagnosis gives the opportunity for genetic counselling, tailored lifestyle interventions and decreases social stigmatization and health risks later in life.[3,8,11] Moreover, effective pharmacologic treatments are available for genetic obesity patients with variants in *LEP, LEPR*, *POMC*, *PCSK1,*[12] and Bardet-Biedl syndrome[30], or show promising results (*MC4R*[31]).

Strengths and limitations

A strength of our study is our unique cohort comprising 13 rare genetic obesity disorders due to extensive genetic testing in our expertise center. Another strength was the large amount of growth measurements per patient, enabling precise estimations of AoO. Previous studies show that it is difficult to find an appropriate control group with childhood-onset obesity for comparing BMI trajectories.[8] In this study, we included controls from a population-based study of children who grew up in the same geographic region and time frame as our patients. Growth data in the controls were available during a long follow-up duration of 10 years, and their median AoO is in line with other recent population-based studies with complete follow-up until adulthood, increasing generalizability of our results.[21] Our study also has its limitations. We did not perform genetic testing in the controls. However, the expected prevalence of mutations is low: 0·3% for pathogenic heterozygous *MC4R* variants whereas other genetic obesity disorders are rare to ultra-rare.[22] Furthermore, we excluded controls with BMI SDS >4. Therefore, we do not expect genetic obesity in the controls. Another limitation is the difference in study design between patients and controls. However, for all subjects, early childhood growth measurements were used from the Dutch nationwide screening program, thereby minimizing between-group heterogeneity. Furthermore, we cannot rule out referral bias as we are a national obesity expertise center. An inherent limitation of childhood obesity research is the lack of a universal obesity definition across childhood: BMI-based definitions are available from age ≥2y, whereas severe obesity is not defined <2y.[2,20] Since current guidelines focus on severe obesity and many children with genetic obesity have AoO <2y, a universally accepted definition of severe obesity <2y is needed.

Conclusion

In conclusion, we present childhood BMI trajectories of patients with non-syndromic and syndromic genetic obesity disorders compared to children with childhood-onset obesity from the general population. We show that AoO can be useful as single parameter to determine which children with early-onset obesity should undergo genetic testing, especially for non-syndromic genetic obesity with optimal cut-off AoO ≤3·9y. In case of later AoO, the decision to perform genetic screening when suspecting syn-

dromic genetic obesity should be guided by the additional clinical features. Identifying genetic obesity is important since new disease-specific treatment modalities are available for specific genetic obesity disorders.

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SUPPLEMENTARY APPENDIX

1. Supplementary results
2. Supplementary Table S1. Overview of clinical and genetic characteristics of patients with genetic obesity disorders
3. Supplementary Table S2. Overview of ROC-curve analysis of delta weight-for-height SDS and delta BMI SDS stratified on age bins
4. Supplementary Table S3. Overview of ROC-curve analysis of delta BMI stratified on age bins
5. Supplementary Figure S1. Study flow diagram
6. Supplementary Figure S2. BMI SDS trajectories in patients with and without genetic obesity disorders
7. Supplementary Figure S3. BMI SDS trajectories in specific genetic obesity disorders
8. Supplementary Figure S4. Performance of AoO of severe obesity as diagnostic test in the study population
9. Supplementary appendix references

Supplementary results

Age of onset of severe obesity (AoO_{severe})

AoO_{severe} was available for 53/62 (85%) patients with genetic obesity (28/29 [97%] non-syndromic and 25/33 [76%] syndromic) and 157 (53%) controls (Table 1). Subjects in whom AoO_{severe} was not available never developed severe obesity (8 [13%] patients and 102 [34%] controls) or did not have growth measurements available before developing severe obesity (39 [13%] controls). Median AoO_{severe} did not differ between patients with non-syndromic or syndromic genetic obesity compared to controls (both $P>0.05$). Patients with non-syndromic genetic obesity due to biallelic variants had lower AoO_{severe} (0.6 years; IQR 0.4–0.7; $P<0.001$) compared to controls whereas patients with non-syndromic genetic obesity due to heterozygous variants had similar AoO_{severe} (3.5 years; IQR 1.0–6.6; $P=0.19$).

Predictive value of AoO_{severe}

AoO_{severe} had worse diagnostic performance compared to AoO of obesity grade 1, both for non-syndromic (AUC 0.58, p=0.20) as well as syndromic obesity (AUC 0.59, p=0.17, Supplementary Figure S4).

Supplementary Table S1. Overview of clinical and genetic characteristics of patients with genetic obesity disorders

Patient	Sex	Gene/CNV	Reference transcript	Genetic alteration	Age of onset of obesity		Characteristics at first visit to Obesity Center CGG	
					AoO	AoO_{severe}	Age in years	BMI SDS
Nonsyndromic genetic obesity disorders - biallelic								
1	female	*LEPR*	NM_001003679.3	Compound heterozygous c.2168c>T p.(Ser723Phe), c.1985T>C p.(Leu662Ser)	0.30	0.30	0.90	6.16
2	female	*LEPR*	NM_001003679.3	Compound heterozygous c.2051A>C p.(His684Pro), c.2627C>A p.(Pro876Gln)	0.31	0.31	0.72	7.73
3	female	*LEPR*	NM_002303.5	Compound heterozygous c.1835G>A p.(Arg612His), c.2051A>C p.(His684Pro)	0.40	0.40	14.55	3.09
4	female	*LEPR*	NM_002303.5	Compound heterozygous c.1753-1dup p.?, c.2168C>T p.(Ser723Phe)	0.45	0.45	10.46	3.41
5	male	*LEPR*	NM_002303.5	Homozygous c.3414dup p.(Ala1139Cysfs*16)	0.64	0.64	1.10	4.52
6	female	*LEPR*	NM_002303.5	Homozygous c.1604-8A>G p.? intronic pathogenic variant affecting splicing	1.25	1.25	17.74	3.62
7	female	*MC4R*	NM_005912.2	Homozygous c.216C>A p.(Asn72Lys)	0.62	0.62	6.47	3.55
8	male	*MC4R*	NM_005912.2	Compound heterozygous c.446_450del p.(Phe149Tyrfs*9), c.644T>G p. (Met215Arg)	0.62	0.62	15.38	3.73
9	female	*MC4R*	NM_006147.2	Homozygous c.785del p.(Phe262Serfs*4)	0.66	0.66	9.11	3.71
10	male	*MC4R*	NM_005912.2	Homozygous c.779C>A p.(Pro260Gln)	0.98	0.98	11.98	1.94
Nonsyndromic genetic obesity disorders - heterozygous								
11[sib]	male	*MC4R*	NM_005912.2	Heterozygous c.493C>T p.(Arg165Trp)	0.47	0.47	17.38	4.31
12	male	*MC4R*	NM_005912.2	Heterozygous c.913C>T p.(Arg305Trp)	0.48	0.48	7.06	3.56
13	female	*MC4R*	NM_005912.2	Heterozygous c.380C>T p.(Ser127Leu)	0.63	0.63	10.49	3.52
14	female	*MC4R*	NM_005912	Heterozygous c.750_751del p.(Ile251Trpfs*34)	0.89	0.89	2.93	5.19

15	male	*MC4R*	NM_005912	Heterozygous c.105C>A p.(Tyr35*)	1.08	1.08	14.08	3.80
16	female	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	1.20	5.96	2.53	2.74
17	male	*MC4R*	NM_005912	Heterozygous c.153del p.(Phe51Leufs*2)	1.21	1.21	2.65	4.11
18[sib]	female	*MC4R*	NM_005912	Heterozygous c.493C>T p.(Arg165Trp)	1.50	1.50	7.58	3.22
19	female	*MC4R*	NM_005912	Heterozygous c.902T>C p.(Ile301Thr)	1.96	n.d.	14.87	2.68
20	male	*MC4R*	NM_005912.2	Heterozygous c.493C>T p.(Arg165Trp)	2.38	2.79	15.08	3.88
21	male	*MC4R*	NM_005912	Heterozygous c.105C>A p.(Tyr35*)	3.67	5.04	11.99	2.63
22	female	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	4.29	6.63	9.97	2.90
23	female	*MC4R*	NM_005912.2	Heterozygous c.785delT p.(Phe262Serfs*4)	4.44	12.79	14.09	3.12
24	male	*MC4R*	NM_005912	Heterozygous c.64A>T p.(Arg22*)	5.13	6.54	12.22	3.52
25	female	*MC4R*	NM_005912	Heterozygous c.105C>A p.(Tyr35*)	6.54	10.04	12.26	3.30
26	female	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	6.71	13.38	15.34	2.60
27	female	*POMC*	NM_001035256.2	Heterozygous c.706C>G p.(Arg236Gly)[a]	2.31	2.54	7.64	3.02
28	female	*POMC*	NM_001035256.1	Heterozygous c.706C>G p.(Arg236Gly)[a]	3.88	4.63	12.57	3.17
29	male	*POMC*	NM_001035256.1	Heterozygous c.706C>G p.(Arg236Gly)[a]	3.88	4.21	11.47	3.31
Syndromic genetic obesity disorders								
30[sib]	male	*GNAS (PHP1a)*	NM_000516.4	Heterozygous c.848G>A p.(Arg283His)	0.16	0.16	9.94	2.85
31	female	*GNAS (PHP1a)*	NM_000516.4	Heterozygous c.1082C>T p.(Pro361Leu)	0.27	0.27	7.64	3.28
32[sib]	male	*GNAS (PHP1a)*	NM_000516.4	Heterozygous c.848G>A p.(Arg283His)	0.40	0.40	8.84	2.80
33	female	*GNAS (PHP1a)*	NM_000516.4	Heterozygous c.794G>A p.(Arg265His)	0.52	0.52	11.64	2.41
34[sib]	female	*GNAS (PHP1a)*	NM_000516.4	Heterozygous c.848G>A p.(Arg283His)	0.59	0.59	2.08	1.81
35[sib]	male	*GNAS (PHP1a)*	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)	0.92	0.92	14.77	.46
36[sib]	female	*GNAS (PHP1a)*	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)	1.46	1.63	12.04	1.55
37	male	*GNAS (PHP1a)*	NM_001077488	Heterozygous c.85C>T p.(Gln29*)	1.53	1.53	3.66	3.19
38[sib]	female	*GNAS (PHP1a)*	NM_018666.2	Heterozygous c.665T>C p.(Met222Thr)	2.91	n.d.	9.27	-.27

39	male	*GNAS (PHP1a)*	N/A	Heterozygous 20q13.32 deletion (chr20:57,427,951_57,589,516) x1,mat incl. *GNAS*	4.63	5.79	5.76	2.56
40	male	*MKKS (BBS)*	NM_018848.3	Compound heterozygous c.110A>G p.(Tyr37Cys), c.950_960del p.(Gly317Aspfs*6)	0.84	0.84	4.56	4.57
41	male	*BBS5 (BBS)*	unknown	Compound heterozygosity for two disease-causing variants in *BBS5*[b]	0.96	0.96	17.77	2.56
42	female	*BBS1 (BBS)*	NM_024649.4	Homozygous c.1169T>G p.(Met390Arg)	2.21	2.88	18.32	3.23
43	male	*BBS10 (BBS)*	NM_024685.4	Homozygous c.271dupT p.(Cys91Leufs*5)	2.38	2.54	14.78	3.37
44	female	*BBS10 (BBS)*	NM_024685.4	Homozygous c.271dupT p.(Cys91Leufs*5)	3.29	n.d.	4.27	2.15
45	female	*IFT74 (BBS)*	NM_025103.3	Compound heterozygous c.371_372del p.(Gln124Argfs*9), c.16850-1G>T p.?	4.13	n.d.	8.85	2.43
46	female	*16p11.2del*	N/A	Heterozygous distal 16p11.2 deletion (chr16:28,843,890-29,044,745), incl. *SH2B1*	1.48	1.72	7.02	3.58
47	female	*16p11.2del*	N/A	Heterozygous distal 16p11.2 deletion (chr16:28,411,104-29,121,815), incl. *SH2B1*	1.76	1.89	14.82	3.33
48	female	*16p11.2del*	N/A	Heterozygous distal 16p11.2 deletion (chr16:28,825,605-29,043,450), incl. *SH2B1*	2.38	2.54	4.20	4.75
49	female	*16p11.2del*	N/A	Heterozygous proximal 16p11.2 deletion of 500kb (chr16: 29,58 - 30,09 MB), not incl. *SH2B1*	6.13	14.29	16.23	3.31
50	female	*16p11.2del*	N/A	Heterozygous distal 16p11.2 deletion (chr16:28,819,029-29,043,973), incl. SH2B1	10.21	n.d.	7.69	1.85
51	female	*16p11.2del*	N/A	Heterozygous proximal 16p11.2 deletion (chr16:29,563,985-30,107,008), not incl. SH2B1	14.21	n.d.	15.84	2.36
52	female	*Temple syndrome*	N/A	Temple syndrome (caused by imprinting defect on chromosome 14)	3.63	4.63	8.13	2.85
53	female	*Temple syndrome*	N/A	Temple syndrome (caused by imprinting defect on chromosome 14)	4.21	5.54	16.33	2.73
54	female	*Temple syndrome*	N/A	Temple syndrome (caused by maternal uniparental disomy chromosome 14)	9.29	14.29	14.09	2.78
55	male	*Temple syndrome*	N/A	Temple syndrome (caused by maternal uniparental disomy chromosome 14)	14.46	n.d.	15.05	2.70
56	female	*STX16 (PHP1b)*	NM_003763.5	Heterozygous microdeletion c.331-?_585 + ? p.?	0.80	0.80	17.19	2.44

57	male	*GNAS (PHP1b)*	N/A	Imprinting defect paternal *GNAS* allele without signs of paternal uniparental disomy 20, leading to sporadic pseudohypoparathyroidism type 1b	1.37	1.37	3.13	3.68
58	female	*SIM1*	N/A	Heterozygous 6q16.3 deletion (chr6:100,879,864–102,471,598), disrupting SIM1	0.81	0.81	9.14	3.56
59	female	*2p-del incl. POMC*	N/A	Heterozygous 2p deletion (chr2:22,791,486–27,942,764), containing POMC	1.49	1.92	14.63	2.75
60	female	*MYT1L*	NM_015025.2	Heterozygous c.808del p.(Gln270Lysfs*11)	1.96	n.d.	5.46	2.02
61	male	*SPG11*	NM_025137.3	Compound heterozygous c.4534dup p.(Asp1512Glyfs*7), c.5867-?_6477+? del p.? (deletion of exons 31–34)	2.96	3.21	11.16	2.75
62	male	*VPS13B (Cohen syndrome)*	NM_017890.4	Compound heterozygous c.2911C>T p.(Arg971*), c.8697-2A>G p.?	7.21	n.d.	8.46	1.88

Legend: [a] risk factor for severe early-onset obesity[1]; [b] Exact variant not reported by the referring pediatrician, sequencing performed in other ISO15189 accredited Dutch academic clinical genetics laboratory

Abbreviations: AoO, age of onset of obesity; AoO_{severe}, age of onset of severe obesity; BBS, Bardet-Biedl syndrome; CNV, copy number variation; n.d., never developed; PHP1a, pseudohypoparathyroidism type 1a; PHP1b, pseudohypoparathyroidism type 1b; SDS, standard deviation score; sib, siblings harboring the same mutation;

Supplementary Table S2. Overview of ROC-curve analysis of delta weight-for-height SDS and delta BMI SDS stratified on age bins.

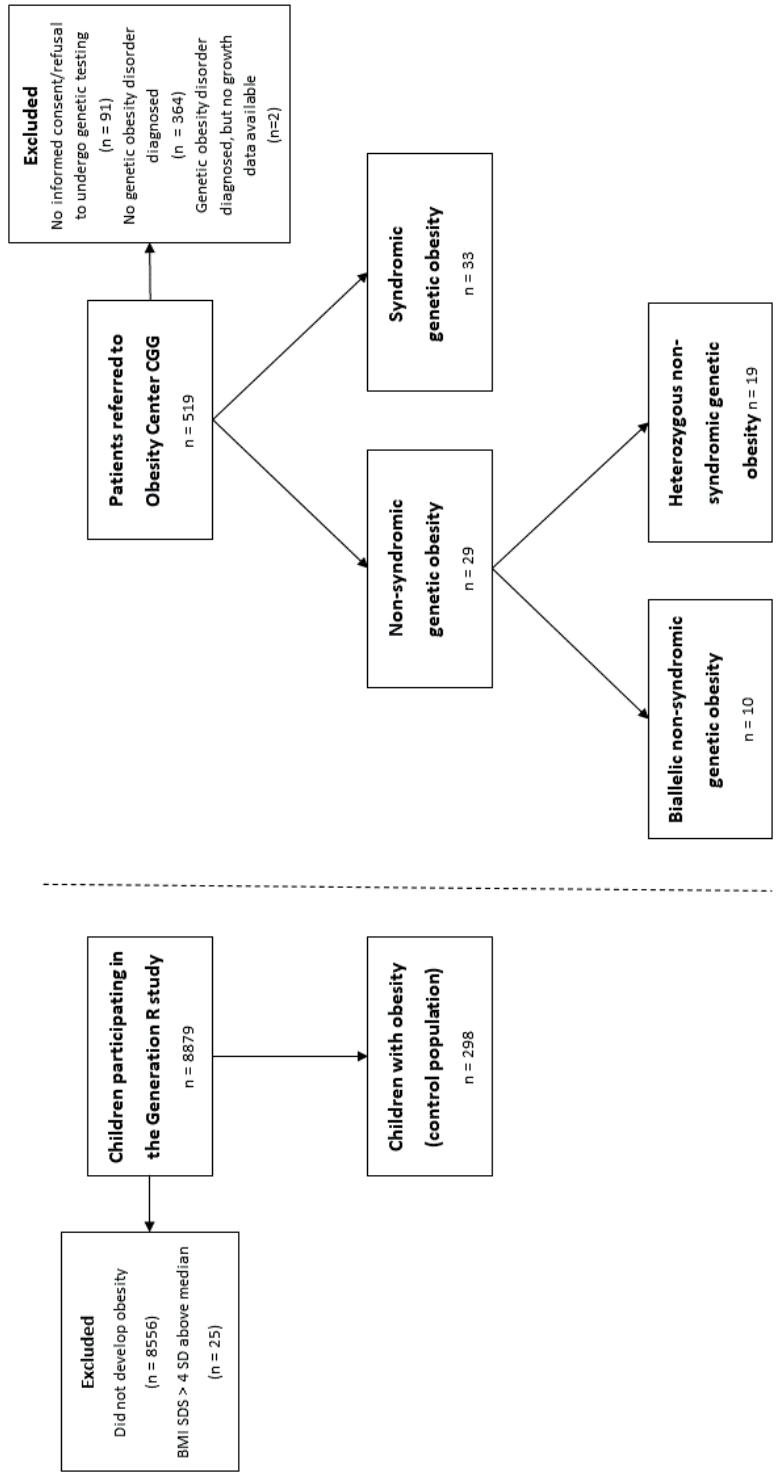
		Non-syndromic genetic		Syndromic genetic	
		AUC (95% CI)	P-value	AUC (95% CI)	P-value
Δ Weight for height SDS (change/year)	0 years	-	-	-	-
	0.5 years	0.71 (0.61 – 0.82)	<0.001	0.59 (0.47 – 0.71)	0.121
	1 years	0.62 (0.50 – 0.74)	0.043	0.44 (0.31 – 0.56)	0.250
	1.5 years	0.62 (0.47 – 0.77)	0.041	0.54 (0.40 – 0.67)	0.510
	2 years	0.50 (0.35 – 0.65)	0.989	0.57 (0.44 – 0.69)	0.239
Δ BMI SDS (change/year)	3 years	0.40 (0.27 – 0.53)	0.090	0.60 (0.47 – 0.72)	0.087
	4 years	0.32 (0.20 – 0.43)	0.002	0.40 (0.28 – 0.51)	0.072
	5 years	0.24 (0.10 – 0.38)	<0.001	0.35 (0.21 – 0.46)	0.008
	6 years	0.28 (0.14 – 0.42)	0.001	0.30 (0.19 – 0.40)	0.001
	8 years	0.25 (0.12 – 0.38)	<0.001	0.25 (0.10 – 0.39)	<0.001
	10 years	0.46 (0.31 – 0.62)	0.629	0.32 (0.19 – 0.45)	0.007

Abbreviations: SDS, standard deviation score; AUC, area under the ROC curve; ROC, receiver operating characteristic.

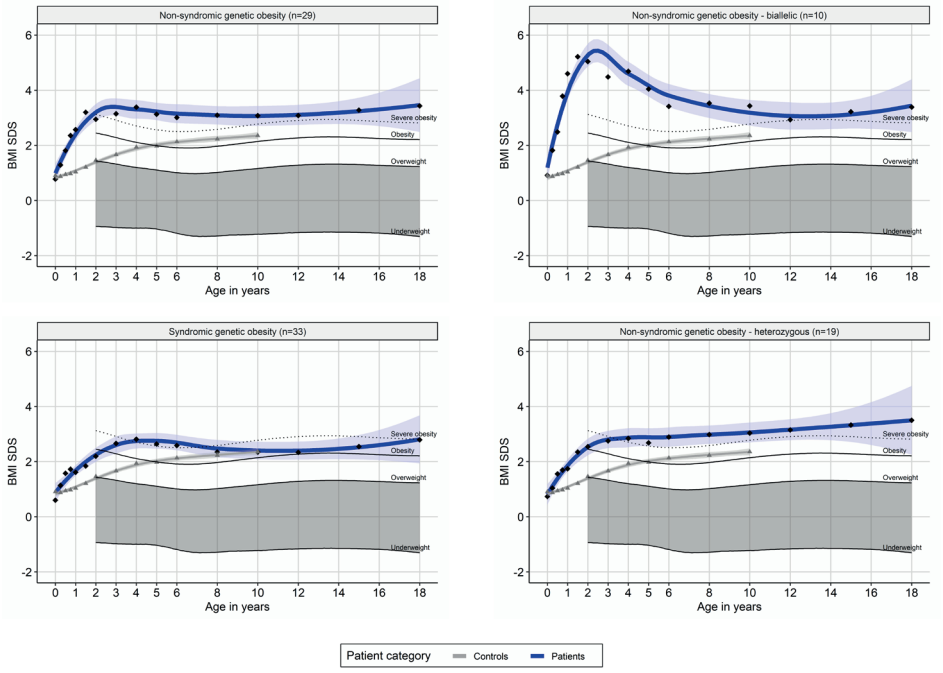
Supplementary Table S3. Overview of ROC-curve analysis of delta BMI stratified on age bins.

		Non-syndromic genetic		Syndromic genetic	
		AUC (95% CI)	P-value	AUC (95% CI)	P-value
Δ BMI (change/year)	0 years	-	-	-	-
	0.5 years	0.63 (0.52 – 0.75)	0.028	0.52 (0.40 – 0.63)	0.788
	1 years	0.60 (0.47 – 0.73)	0.095	0.44 (0.31 – 0.57)	0.272
	1.5 years	0.63 (0.47 – 0.79)	0.035	0.53 (0.40 – 0.66)	0.595
	2 years	0.59 (0.44 – 0.75)	0.126	0.60 (0.48 – 0.73)	0.065
	3 years	0.53 (0.39 – 0.67)	0.599	0.65 (0.52 – 0.77)	0.011
	4 years	0.46 (0.34 – 0.59)	0.543	0.48 (0.35 – 0.60)	0.667
	5 years	0.36 (0.20 – 0.53)	0.053	0.47 (0.33 – 0.60)	0.567
	6 years	0.55 (0.37 – 0.73)	0.462	0.47 (0.32 – 0.62)	0.654
	8 years	0.51 (0.34 – 0.69)	0.843	0.44 (0.28 – 0.61)	0.385
	10 years	0.67 (0.48 – 0.87)	0.026	0.40 (0.25 – 0.56)	0.147

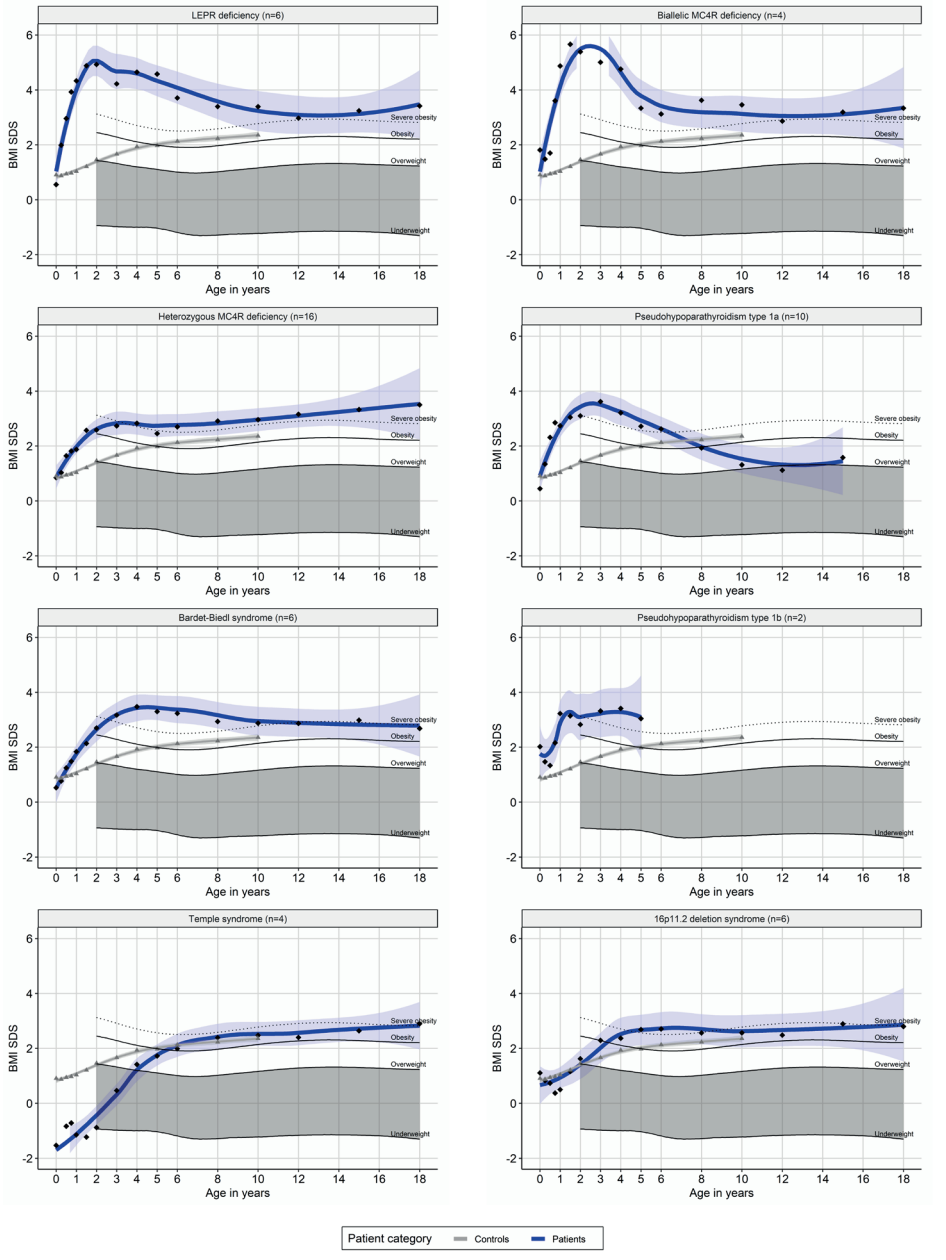
Abbreviations: SDS, standard deviation score; AUC, area under the ROC curve; ROC, receiver operating characteristic.



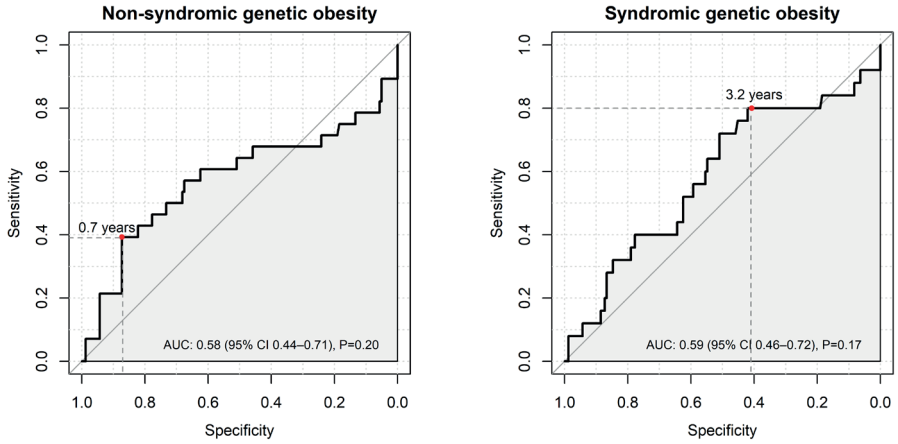
Supplementary Figure S1. Study flow diagram.



Supplementary Figure S2. BMI SDS trajectories in patients with and without genetic obesity disorders
Childhood BMI SDS are presented for patients with non-syndromic (upper left panel) and syndromic (lower left panel) genetic obesity disorders, and for biallelic (upper right panel) and heterozygous (lower right panel) non-syndromic genetic obesity separately. The dots indicate the mean values per age bin; the line indicates the locally estimated scatterplot smoothing (LOESS) regression line; the shaded areas around the regression line indicate the 95% CI. The female International Obesity Task Force (IOTF) cut-offs are presented as reference, with the grey shaded area indicating normal weight.



Supplementary Figure S3. BMI SDS trajectories in specific genetic obesity disorders
Childhood BMI SDS trajectories are presented for patients specific genetic obesity disorders. The dots indicate the mean values per age bin; the line indicates the locally estimated scatterplot smoothing (LOESS) regression line; the shaded areas around the regression line indicate the 95% CI. The female International Obesity Task Force (IOTF) cut-offs are presented as reference, with the grey shaded area indicating normal weight.



Supplementary Figure S4. Performance of AoO of severe obesity as diagnostic test in the study population
The left panel depicts the performance of AoO of severe obesity to distinguish patients with non-syndromic genetic obesity patients from patients without genetic obesity within the patients who developed severe obesity (non-syndromic genetic obesity: 28/29 patients; syndromic genetic obesity: 25/33 patients; controls: 157/298 children). The right panel depicts the performance of AoO of severe obesity to distinguish patients with syndromic genetic obesity patients from patients without genetic obesity. Optimal cut-off values (point with highest Youden's index with sensitivity ≥0.80) are marked in red; for non-syndromic genetic obesity, the restriction on cut-off values with sensitivity ≥0.80 was relieved as this would lead to a negative diagnostic performance (cut-off value of age ≤6.0 years: sensitivity 0.82, specificity 0.06, LR+ 0.87).

Supplementary appendix references

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6

Resting energy expenditure and body composition in children and adolescents with genetic, hypothalamic, medication-induced or multifactorial severe obesity

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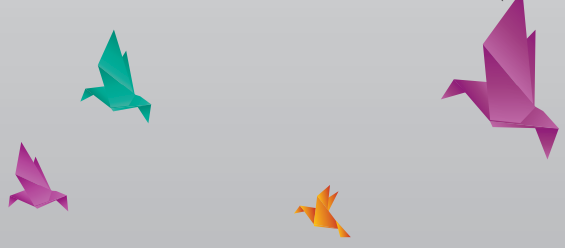


ABSTRACT

Background Pediatric obesity is a multifactorial disease which can be caused by underlying medical disorders arising from disruptions in the hypothalamic leptin-melanocortin pathway, which regulates satiety and energy expenditure.

Aim To investigate and compare resting energy expenditure (REE) and body composition characteristics of children and adolescents with severe obesity with or without underlying medical causes.

Methods This prospective observational study included pediatric patients who underwent an extensive diagnostic workup in our academic centre that evaluated endocrine, non-syndromic and syndromic genetic, hypothalamic, and medication-induced causes of obesity. REE was assessed by indirect calorimetry; body composition by air displacement plethysmography. The ratio between measured REE (mREE) and predicted REE (Schofield equations), REE%, was calculated, with decreased mREE defined as REE% ≤90% and elevated mREE ≥110%. Additionally, the influence of fat-free-mass (FFM) on mREE was evaluated using multiple linear regression.

Results We included 292 patients (146 [50%] with body composition measurements), of which 218 (75%) patients had multifactorial obesity and 74 (25%) an underlying medical cause: non-syndromic and syndromic genetic (n= 29 and 28, respectively), hypothalamic (n= 10), and medication-induced (n= 7) obesity. Mean age was 10.8 ± 4.3 years, 59% were female, mean BMI SDS was 3.8 ± 1.1, indicating severe obesity. Mean REE% was higher in children with non-syndromic genetic obesity (107.4% ± 12.7) and lower in children with hypothalamic obesity (87.6% ± 14.2) compared to multifactorial obesity (100.5% ± 12.6, both $p<0.01$). In 9 children with pseudohypoparathyroidism type 1a, mean REE% was similar (100.4 ± 5.1) Across all patients, mREE was decreased in 60 (21%) patients and elevated in 69 (24%) patients. After adjustment for FFM, mREE did not differ between patients within each of the subgroups of underlying medical causes compared to multifactorial obesity (all $p>0.05$).

Conclusions In this cohort of children with severe obesity due to various etiologies, large inter-individual differences in mREE were found. Consistent with previous studies, almost half of patients had decreased or elevated mREE. This knowledge is important for patient-tailored treatment, e.g. personalized dietary and physical activity interventions and consideration of pharmacotherapy affecting central energy expenditure regulation in children with decreased mREE.

INTRODUCTION

Pediatric obesity has become one of the major global health challenges of our time.[1] Obesity is a complex, multifactorial disease that is caused by a chronic imbalance between energy intake and expenditure.[2] Early-onset severe obesity (defined[3] as an age- and sex-specific BMI corresponding to an adult BMI of ≥35 kg/m^2 with onset before age 5 years) can be caused by underlying medical conditions.[4] These conditions can arise from disruptions in the hypothalamic regulation of hunger, satiety and energy expenditure, e.g. the leptin-melanocortin pathway.[5] The current international guideline for pediatric obesity by the Endocrine Society (ES) distinguishes the following potential underlying medical causes of obesity: endocrine disorders; non-syndromic and syndromic genetic obesity disorders; weight-inducing medication; and hypothalamic dysfunction caused by hypothalamic damage, for example due to a tumor, surgery or irradiation.[6]

Knowledge of an individual's daily caloric needs is an essential part of a patient-tailored obesity management approach which supports long-term weight loss and weight maintenance.[7] Total energy expenditure (TEE) is the amount of energy that individuals use on a daily basis.[8] The most important contributor to TEE is resting energy expenditure (REE), which is defined as the energy required to maintain physiological homeostasis while fasting and accounts for 50-70% of TEE.[7-9] The other main contributors to TEE are physical activity, linear growth and thermic effects of food intake and digestion.[10] TEE can be measured using doubly-labeled water, but as this is expensive and difficult, it is often not feasible in clinical practice[8] Instead, in daily clinical practice, TEE is calculated by assessing REE, after which TEE is calculated by multiplying REE with estimated physical activity level based on the child's age, sex, and physical activities by history taking.[11-13] In practice, REE is often calculated using validated prediction equations based on age, sex, and anthropometrics. However, studies have shown that these prediction equations lack accuracy, which can lead to overestimation or underestimation of daily caloric needs and could hinder adequate obesity treatment.[7] Therefore, indirect calorimetry is the gold standard for measuring REE in clinical practice which then can be used to calculate TEE and to eventually provide a patient-tailored dietary advice.[14-16] Indirect calorimetry measures oxygen consumption and carbon dioxide production using a calibrated and validated metabolic cart under strictly controlled conditions. Subsequently, energy expenditure is calculated based on the individual's oxygen consumption and carbon dioxide production using standard formulas.[17]

In individuals with and without obesity, fat-free mass (FFM) is the most important contributor to REE, accounting for approximately 60-80% of the variation in REE.[8, 9] In line with this, absolute REE (in kcal/day) is increased in children and adolescents with obesity compared to without obesity, but REE adjusted for FFM does not differ.[8,18,19] For children with underlying medical causes of obesity, REE characteristics are less well described. A decreased REE is thought to be the major contributor to obesity in children with pseudohypoparathyroidism type 1a (PHP1a), a syndromic genetic obesity disorder.[20,21] Studies in children with Prader-Willi syndrome (PWS), one of the most common forms of syndromic genetic obesity, show that their reduced REE can be explained by the reduced FFM associated with the syndrome.[22,23] Furthermore, in children with hypothalamic obesity due to hypothalamic lesions or damage after surgery or radiotherapy, REE is lower compared to children with multifactorial obesity even after adjustment for FFM.[24-26] However, differences in REE and body composition characteristics of children and adolescents with early-onset severe obesity with different underlying medical conditions affecting hypothalamic weight regulation have not yet been described within one cohort. As these conditions all affect the hypothalamic pathways that regulate energy expenditure, knowledge of their REE characteristics could improve patient-tailored treatment in these patients.

The aim of this study was to investigate REE in relation to body composition in children and adolescents with early-onset severe obesity with or without the following underlying medical causes: non-syndromic and syndromic genetic obesity disorders, obesity caused by hypothalamic dysfunction after hypothalamic damage, and medication-related obesity.

MATERIALS AND METHODS

For this prospective observational study, we used data of children (up to 19 years) visiting the outpatient clinic of Obesity Center CGG, a Dutch referral center for obesity, at the academic center Erasmus MC-Sophia Children's Hospital (Rotterdam, The Netherlands) between April 2014 and April 2021. Pediatric patients were referred to Obesity Center CGG for diagnostic evaluation of their early-onset severe obesity due to suspicion of underlying medical causes and/or personalized therapeutic advices.[4] All consecutive patients in whom REE was measured using indirect calorimetry as part of the standardized diagnostic workup of Obesity Center CGG were included in this study[4] Exclusion criteria were inability or refusal to give informed consent or not completing the REE measurement (ure 1). This study was approved by the medical ethics committee of the Erasmus MC (MEC-2012-257). All parents/caretakers of children ≤16

years gave written informed consent. Additionally, children aged ≥12 years also gave written informed consent; children aged ≤12 years also gave oral assent.

Assessment of underlying medical causes of obesity

The standardized diagnostic approach of Obesity Center CGG consists of two visits: (1) an initial visit during which patients are screened by a pediatric endocrinologist following Dutch and international guidelines for pediatric obesity. This includes extensive medical history taking, physical examination, and detailed growth charts assessment;[6,27] (2) a subsequent visit where patients return after an overnight fast for indirect calorimetry, body composition assessment and blood sampling including biochemical and hormonal assessment and extensive genetic testing (obesity gene panel, microarray analysis).[4] Height and weight were measured and BMI was calculated rounded to the nearest decimal by trained personnel and converted to age- and sex-specific standard deviation scores (SDS) using Dutch growth charts.[28] The standardized diagnostic approach has previously been described in further detail.[4] After the diagnostic approach was completed, patients were classified in the following groups based on the presence or absence of underlying medical causes of obesity:

- Endocrine disorders: endogenous Cushing's syndrome or clinical hypothyroidism
- Non-syndromic and syndromic genetic obesity disorders: diagnosed when genotyping revealed known (likely) pathogenic variants (as defined by the American College of Medical Genetics and Genomic guideline[29]) in obesity-associated genes which matched the clinical phenotype.[4] Classification of genetic obesity disorders was based on the Endocrine Society's guideline for pediatric obesity[6]
- Medication-related obesity: start or intensification of known weight-inducing medication coinciding with development or progression of obesity in the patient's growth charts in the absence of other plausible explanations for the sudden weight gain[4]
- Hypothalamic obesity: central nervous system (CNS) injury affecting the hypothalamic region that regulates satiety and energy expenditure due to congenital anatomical defects, tumor (e.g. craniopharyngioma), surgery, irradiation, meningitis or ischemic damage, coinciding with development or progression of obesity in the patient's growth charts in the absence of other plausible explanations for the sudden weight gain[4]
- Multifactorial obesity: obesity due to a combination of lifestyle, environmental and genetic background; abovementioned underlying medical causes were excluded in the extensive diagnostic workup.

REE measurement

REE measurements were performed using indirect calorimetry with a metabolic cart (Quark RMR, COSMED, Italy). Patients had fasted overnight (at least 8 hours) and did not perform physical activity prior to the measurement. The Quark RMR was calibrated according to the manufacturer's recommendations. The first 5 minutes of the measurement were excluded from the results to allow acclimation. The aim was to obtain measured REE (mREE) after 15 minutes of measurement in steady state (VCO2 coefficient of variation [CV%] and VO2 CV% both <10).[30] Measured REE was calculated based on VO2 and VCO2 using the Weir equation.[17] If possible, considering the child's age and ability to lie still for at least 20 minutes, the measurement was performed without distraction with a book or screen. For children aged <18 years, the Schofield equations were used to calculate predicted REE (pREE), as a recent systematic review concluded that these provided the most accurate (smallest difference between mREE and pREE) REE predictions in children and adolescents with obesity.[7] The original equations by Schofield were used with application of a conversion factor of 239.006 to transform megajoules to kilocalories.[31] For patients aged ≥18 years at REE measurement, the 1984 Harris & Benedict equations were used as these were shown to be the most accurate in adults with obesity.[32] As a sensitivity analysis, we also calculated pREE based on the equations by Molnár,[33] as a recent large external validation study found that these equations had the best precision (highest proportion of children with pREE within 90-110% of mREE) in children with obesity.[16] Since the Schofield and Molnár equations are based on body weight, we also performed a sensitivity analyses using body composition-based prediction equations specifically designed for children with severe obesity (Lazzer equations).[34]

Body composition measurement

From March 2018 onwards, the standardized diagnostic workup of our obesity center also included body composition measurement using air displacement plethysmography (BOD POD, COSMED, Italy). The BOD POD was warmed up and calibrated according to the manufacturer's instructions. Thoracic volume was predicted by the BOD POD software.[35,36] Patients were instructed to wear swimwear or tight underwear and a swim cap during the measurement. Two-compartment body composition (fat-free mass; FFM and fat mass; FM) was determined from body volume using density model Lohman according to the manufacturer's recommendation for children.[37]

Statistical analyses

Statistical analyses were performed using SPSS version 25.0 (Armonk, NY: IBM Corp.) and GraphPad Prism version 8 (GraphPad Software, Inc.). Data are presented as median (interquartile range; IQR), or mean (standard deviation; SD), as appropriate.

The bias between mREE and pREE (mREE – pREE) in kcal/day and ratio between mREE and pREE (mREE/pREE * 100%; REE%) were calculated, with normal mREE defined as REE% between 90-110% of predicted, decreased mREE defined as REE% ≤90% and elevated mREE defined as REE% ≥110%.[7] Bivariate correlations between mREE and FFM, and REE% and age and BMI SDS were assessed across all patients and in each subgroup of underlying medical causes separately using Pearson's *r* (if sample size ≥25 patients) or Kendall's tau (if sample size between 10-25 patients). The effect of sex and ethnicity on mREE and REE% were assessed using multiple linear regression analyses. For mREE, pairwise comparisons between each of the underlying medical causes versus multifactorial obesity were performed in separate regression analyses (e.g. non-syndromic genetic vs multifactorial, syndromic genetic vs multifactorial, etc.) with adjustments for FFM, FM, and sex. In each regression analysis, the grouping variable was defined as multifactorial obesity =0, underlying cause =1. The difference in slope was tested by including the interaction term underlying cause x FFM. For the regression models with hypothalamic obesity and medication-induced obesity, only the main effect and interaction effect of the underlying cause were entered in the regression models to prevent overfitting. Furthermore, pairwise comparisons were made between REE and body composition characteristics of children with each of the underlying medical causes versus children with multifactorial obesity using unpaired t-tests, Mann-Whitney tests, or chi-squared tests, as appropriate. A Bland-Altman analysis was performed to investigate agreement between mREE and pREE. To investigate proportionality of bias, linear regression analyses with and without adjustment for the presence of underlying medical causes were performed using the bias between mREE and pREE as independent variable and the mean of mREE and pREE as dependent variable. These analyses were performed using the absolute difference between mREE and pREE (mREE – pREE) as well as the relative difference ((mREE – pREE)/(mean of mREE and pREE) * 100%). Finally, since movement and/or agitation during the REE measurement can cause falsely elevated mREE values, we performed sensitivity analyses using only REE measurements in which an optimal steady state was achieved.[30] For these sensitivity analyses, only REE measurements with a fractional concentration of CO2 (FeCO2) >0.5, a measurement duration of at least 5 minutes, and a CV% of <10% for both VO2 and VCO2 were included.[30] For all statistical analyses, two-sided P-values <0.05 were considered statistically significant.

RESULTS

In total, n=292 patients were included (Figure 1), of which 218 (75%) had multifactorial obesity and 74 (25%) had an underlying medical cause (Table 1). This included non-syndromic genetic obesity in 29 (10%) patients, syndromic genetic obesity in 28 (10%) patients, hypothalamic obesity in 10 (3%) patients, and medication-induced obesity in 7 patients (2%; Table 1). The mean age of included patients was 10.8 ± 4.3 years (Table 2). A majority of 172 (59%) patients were female. The mean BMI SDS across all participants was 3.76 ± 1.07, indicating severe obesity. The BOD POD measurement was performed in 146 (50%) patients. Children for whom a BOD POD measurement was available were slightly older than children without a BOD POD measurement, but this group did not differ with regard to other baseline characteristics (Supplementary Table S1).

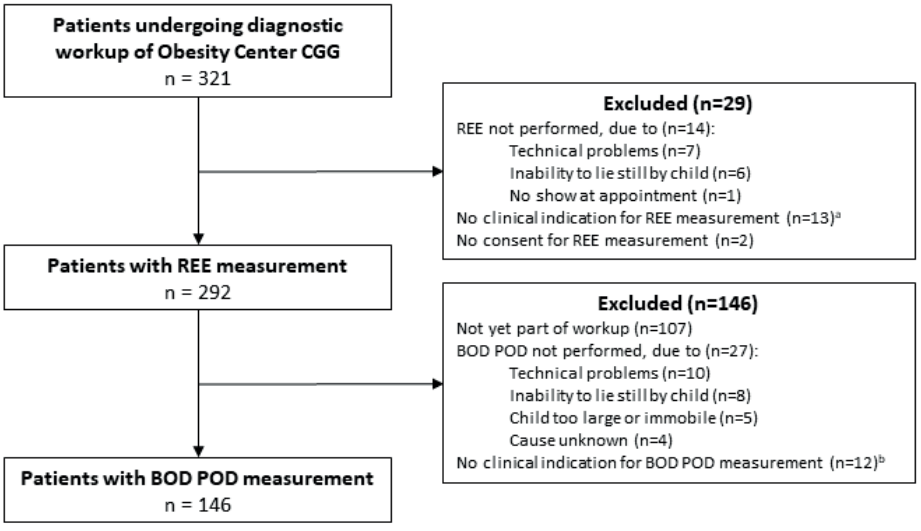


Figure 1. Study flow diagram.
Abbreviations: CGG, 'Centrum Gezond Gewicht' (in English: 'Center for Healthy Weight'); REE, resting energy expenditure. [a]Examples of no clinical indication for REE measurement were: REE already performed elsewhere or patient being too young for reliable measurement; [b]Examples of no clinical indication for BOD POD measurement were: body composition already measured using dual energy X-ray absorptiometry or patient not suitable for reliable measurement e.g. due to severe intellectual disability.

REE and body composition characteristics

The REE and body composition characteristics of the study population are presented in Table 3. Mean mREE was lower in children with syndromic genetic obesity compared to children with multifactorial obesity (1479 ± 360 vs 1719 ± 490 kcal/day, $p<0.05$). The mean percentage of FFM across all patients was 55.2% ± 8.1 and did

not differ between patients with underlying medical causes of obesity and patients with multifactorial obesity (p-values >0.05; Table 3). When expressed in absolute values and adjusted for sex, age, and BMI SDS, FFM was higher compared to multifactorial obesity in children with non-syndromic genetic obesity (adjusted regression coefficient +6.8kg FFM, SE 1.91, p<0.001), but lower in children with syndromic genetic obesity (adjusted regression coefficient -5.3kg FFM, SE 2.23, p=0.02), hypothalamic obesity (adjusted regression coefficient -11.7kg FFM, SE 3.44, p<0.001) and similar in medication-induced obesity (adjusted regression coefficient +2.4kg FFM, SE 5.6, p=0.67). Across all patients, mREE was positively associated to FFM (r = 0.85, p<0.001). REE% was not associated with age (r = -0.06, p=0.26) nor with BMI SDS (r = -0.09, p=0.14; Supplementary Figure S1). Subgroup analyses stratified on underlying medical causes revealed no major differences in the presence or absence and magnitude of these associations (Supplementary Table S2). In linear regression analyses adjusting for FFM and FM, mREE was associated with sex (females vs males -148 kcal/day, SE 36.3, p<0.001), but not ethnicity (non-Dutch vs Dutch -52.3 kcal/day, SE 40.3, p=0.20). After adjustment for body composition, mREE did not differ between patients with each of the underlying medical causes compared to patients with multifactorial obesity (p-values of main effects and interaction effects all >0.05, Table 4; Supplementary Figure S2).

Measured REE vs predicted REE

The mean bias (absolute difference between mREE and pREE) across all patients was -12.0 ± 240 kcal/day, corresponding to a mean REE% of 100.4% ± 12.8 (Table 3). In linear regression analyses, REE% was associated with sex (females vs males +9.4%, SE 1.6, p<0.001) and ethnicity (non-Dutch vs Dutch -5.2%, SE 1.8, p=0.004). This indicates that the Schofield equations tend to underpredict REE in girls compared to boys and overpredict in children with non-Dutch ethnicity compared to Dutch ethnicity. Children with non-syndromic genetic obesity had a positive mean bias and higher REE% compared to children with multifactorial obesity (mean bias +107 ± 231 kcal/day vs -12 ± 236 kcal/day; mean REE% 107.4% ± 12.7 vs 100.5% ± 12.6, both p<0.01, Table 3, Figure 2). On the other hand, children with obesity due to hypothalamic dysfunction showed a negative mean bias and lower REE% compared to children with multifactorial obesity (mean bias -245 ± 270 kcal/day; mean REE% 87.6% ± 14.2, both p<0.01, Figure 2). Similarly, children with medication-induced obesity showed negative mean bias and lower REE% compared to children with multifactorial obesity, although the differences did not reach statistical significance (Table 3). These results remained similar after stratification on sex and ethnicity (Supplementary Figures S3 and S4).

Table 1. Diagnosed underlying medical causes of obesity in the study population.

Diagnosis category	Number of patients	Details
Non-syndromic genetic obesity	29 (10%)	18 (60%) Heterozygous melanocortin 4 receptor (*MC4R)* deficiency 6 (20%) Biallelic leptin receptor (*LEPR)* deficiency 3 (10%) Heterozygous proopiomelanocortin (*POMC)* deficiency 1 (3%) Heterozygous proprotein convertase subtilisin/kexin type 1 (*PCSK1)* deficiency 1 (3%) Biallelic *MC4R* deficiency
Syndromic genetic obesity	28 (10%)	10 (37%) Pseudohypoparathyroidism type 1a 6 (22%) 16p11.2 deletion syndrome 5 (19%) Bardet-Biedl syndrome 3 (11%) Temple syndrome 1 (4%) Alström syndrome 1 (4%) Cohen syndrome 1 (4%) Pseudohypoparathyroidism type 1b 1 (3%) 6q16.3 deletion including *SIM1*
Hypothalamic obesity	10 (3%)	4 (40%) after surgery and/or radiotherapy for intracranial tumors 2 (20%) in presence of myelomeningocele 1 (10%) after ischemic stroke 1 (10%) after neonatal meningitis 1 (10%) in presence of Chiari I malformation, ectopic neurohypophysis and pituitary hormone deficiencies 1 (10%) in presence of panhypopituitarism, hyperphagia and central precocious puberty, highly suspicious for hypothalamic dysfunction
Medication-induced obesity	7 (2%)	5 (71%) induced by corticosteroids 1 (14%) induced by anti-epileptics 1 (14%) induced by anti-psychotics
Endocrine disorders	0 (0%)	-
Multifactorial obesity	218 (75%)	No singular underlying medical cause of obesity

Table 2. Baseline characteristics of the study population.

	All patients (n=292)	Non-syndromic genetic obesity (n=29)	Syndromic genetic obesity (n=28)	Hypothalamic obesity (n=10)	Medication-induced obesity (n=7)	Multifactorial Obesity (n=218)
Age, years	10.8 (4.3)	10.5 (4.4)	10.8 (4.6)	**14.0 (2.6)***	11.2 (3.5)	10.7 (4.2)
Sex, female, n (%)	172 (59)	19 (66)	19 (68)	7 (70)	3 (43)	124 (57)
Ethnicity, Dutch, n (%)	202 (69)	21 (72)	21 (75)	7 (70)	**1 (14)***	152 (70)
Height, cm	147.4 (23.4)	150.3 (30.6)	142.2 (19.6)	156.2 (11.8)	150.6 (18.3)	147.2 (23.3)
Height SDS	0.33 (1.39)	**1.01 (1.12)***	**-0.32 (1.56)***	**-0.93 (0.87)****	0.20 (0.99)	0.39 (1.37)
Weight, kg	72.6 (33.2)	81.3 (39.2)	59.8 (26.8)	80.7 (17.4)	72.4 (21.6)	72.7 (33.7)
Weight SDS	3.70 (1.53)	4.32 (1.22)*	**2.81 (1.63)****	**2.96 (1.11)***	3.65 (0.39)	3.77 (1.54)
BMI, kg/m^2	31.2 (7.4)	33.1 (6.8)	**28.0 (7.1)***	32.8 (4.4)	31.2 (3.5)	31.3 (7.6)
BMI SDS	3.76 (1.07)	4.12 (1.07)	**3.23 (1.30)***	3.42 (0.57)	3.89 (0.50)	3.79 (1.05)

*Abbreviations: BMI, body mass index; SDS, standard deviation score. Data presented as mean (SD), unless otherwise stated. * P<0.05 ** P<0.01 vs multifactorial obesity.*

Table 3. REE and body composition characteristics of the study population.

	All patients (n=292)	Non-syndromic genetic obesity (n=29)	Syndromic genetic obesity (n=28)	Hypothalamic obesity (n=10)	Medication-induced obesity (n=7)	Multifactorial obesity (n=218)
mREE, kcal/day	1705 (491)	1884 (612)	**1479 (360)***	1535 (236)	1710 (342)	1719 (490)
pREE, kcal/day	1718 (522)	1777 (614)	**1511 (360)***	1780 (324)	1821 (387)	1730 (534)
Mean bias (mREE – pREE), kcal/day	-12 (240)	**107 (231)***	-32 (150)	**-245 (270)****	-111 (365)	-12 (236)
REE%	100.4 (12.8)	**107.4 (12.7)****	99.5 (13.3)	**87.6 (14.2)****	95.5 (17.1)	100.5 (12.6)
Lowered mREE, n (%)	60 (21)	3 (10)	6 (21)	**6 (60)****	2 (29)	41 (19)
Elevated mREE, n (%)	69 (24)	12 (41)	**0 (0)****	1 (10)	2 (29)	54 (25)
FFM, %BW	55.2 (8.1)[a]	56.2 (5.9)[a]	57.1 (8.6)[a]	48.2 (11.2)[a]	56.2 (10.3)[a]	55.1 (8.2)[a]

Abbreviations: mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure (based on Schofield equations); REE%, ratio mREE/pREE; FFM, fat-free mass; %BW, percentage of body weight; kcal, kilocalories.
*Data presented as mean (SD), unless otherwise stated. [a] Available for n=146 patients with available BOD POD measurement (18 non-syndromic, 13 syndromic, 5 hypothalamic, 2 medication-induced, and 108 multifactorial obesities) * P<0.05 ** P<0.01 P<0.001 vs multifactorial obesity.*

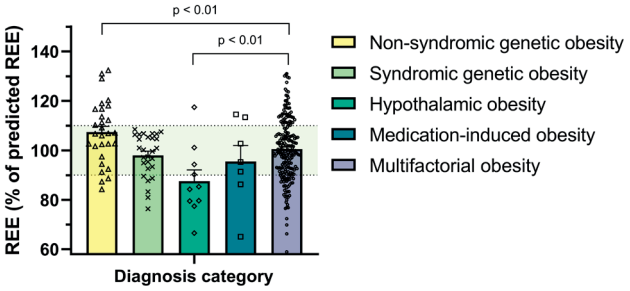


Figure 2. Measured REE expressed as percentage of predicted REE (by Schofield equations) across the study population.
Patients with non-syndromic genetic obesity had higher REE% compared to children with multifactorial obesity whereas children with hypothalamic obesity had lower REE% (both p-values <0.01). The dots represent the individual patients. The bars represent the mean + standard error of the mean. The light green shaded area indicates a REE% between 90 and 110%. Abbreviations: REE, resting energy expenditure.

Decreased mREE

Sixty (21%) patients had a decreased mREE (mREE ≤90% of pREE), of which 3 patients with non-syndromic genetic obesity (a pathogenic heterozygous *MC4R* variant in 2 patients and a heterozygous *PCSK1* variant in one patient; Table 5), 6 patients with syndromic genetic obesity (two 16p11.2 deletion syndrome, 1 Bardet-Biedl syndrome, 1 Cohen syndrome, 1 PHP1b, 1 Temple syndrome; Table 5), 6 patients with obesity caused by hypothalamic dysfunction, 2 patients with medication-induced obesity, and 43 patients with multifactorial obesity. The proportion of children with hypothalamic obesity with decreased mREE was higher than in children with multifactorial obesity (6/10, 60% vs 41/216, 19%; p<0.01). The mean bias between mREE and pREE in the children with decreased mREE was -341 ± 198 kcal/day. This indicates that the Schofield equations would overestimate REE in these children by on average 341 kcal/day compared to mREE.

Elevated mREE

In 69 (24%) patients an elevated mREE (mREE ≥110% of predicted) was found, most of which had multifactorial obesity (n=54) or non-syndromic genetic obesity (n=12); only one patient had hypothalamic obesity and two patients had medication-induced obesity. The highest proportion of elevated mREE was found in children with non-syndromic genetic obesity (12/29 patients, 41%), which was higher than the proportion of children with multifactorial obesity with elevated mREE (54/218, 25%, p<0.05).

REE characteristics in genetic obesity syndromes

When zooming in on the 9 children with PHP1a, a genetic obesity syndrome which has previously been associated with decreased REE, these children showed a mean REE%

Table 4. Results of multiple regression analyses on differences in mREE (kcal/day) between patients with each of the underlying medical causes versus multifactorial obesity.

Non-syndromic genetic vs multifactorial (n=126, R^2 = 0.83)				
	Coefficient	**SE**	**95% CI**	**p-value**
FFM (kg)	13.85	2.13	9.64; 18.06	<0.001
FM (kg)	11.45	1.78	7.93; 14.97	<0.001
Sex, female	-180.90	37.79	-255.71; -106.07	<0.001
Non-syndromic genetic	17.14	158.95	-297.58; 331.85	0.91
Non-syndromic genetic x FFM	3.03	3.41	-3.73; 9.78	0.38
Syndromic genetic vs multifactorial (n=121, R^2 = 0.82)				
	Coefficient	**SE**	**95% CI**	**p-value**
FFM (kg)	14.17	2.15	9.90; 18.43	<0.001
FM (kg)	11.25	1.80	7.69; 14.81	<0.001
Sex, female	-150.81	38.62	-227.32; -74.30	<0.001
Syndromic genetic	-54.38	179.55	-410.04; 301.28	0.76
Syndromic genetic x FFM	-1.47	4.87	-11.12; 8.19	0.76
Hypothalamic vs multifactorial (n=113, R^2 = 0.72)				
	Coefficient	**SE**	**95% CI**	**p-value**
FFM (kg)	25.63	1.58	22.49; 28.76	<0.001
Hypothalamic	-5.37	438.84	-875.15; 864.40	0.99
Hypothalamic x FFM	-5.16	12.11	-29.17; 18.84	0.67
3				
	Coefficient	**SE**	**95% CI**	**p-value**
FFM (kg)	25.63	1.60	22.46; 28.80	<0.001
Medication-induced	-145.81	856.88	-1844.66; 1553.05	0.87
Medication-induced x FFM	3.98	17.67	-31.06; 39.02	0.82

Abbreviations: mREE, measured resting energy expenditure; kcal, kilocalories; CI, confidence interval; FFM, fat-free mass; FM, fat mass.
Data presented as unstandardized regression coefficients (absolute difference in kcal/day adjusted for the other variables in the model). For the regression models with hypothalamic obesity and medication-induced obesity, only the main effect and interaction effect of the underlying cause were entered in the model to prevent overfitting.

of 100.4 ± 5.1 and similar mREE adjusted for FFM (available for 6 patients) compared to children with multifactorial obesity (coefficient -37.5 kcal/day, SE 119.0, p=0.75). Furthermore, none of the children with PHP1a had a decreased mREE (p=0.21 compared to children with multifactorial obesity). In contrast, a decreased REE was found in 2 out of 6 (33%) children with 16p11.2 deletion syndrome and 1 out of 3 (33%) children with Temple syndrome, two genetic obesity syndromes of which REE characteristics have not yet been described. The 6 children with 16p11.2 deletion syndrome had a mean REE% of 99.5 ± 11.4 and similar mREE adjusted for FFM (available for 3 patients) compared to children with multifactorial obesity (coefficient -212.3 kcal/

Table 5. Overview of clinical and REE characteristics of patients with genetic obesity disorders who had a decreased REE

Pt.	Gene/CNV	Reference transcript	Genetic alteration	Age (y)	Sex	BMI SDS	mREE (kcal/day)	REE%[a]	FFM (kg)	FM (kg)
Non-syndromic genetic obesity										
1	*MC4R*	NM_005912.2	Heterozygous c.913C>T p.(Arg305Trp)	7.2	Male	5.15	1371	84.2	-	-
2	*MC4R*	NM_005912.2	Heterozygous c.105C>A p.(Tyr35*)	9.4	Female	3.90	1593	87.3	37.1	33.9
3	*PCSK1*	NM_000439.4	Heterozygous c.541T>C p.(Tyr181His)[b]	12.3	Female	3.55	1409	88.6	-	-
Syndromic genetic obesity										
4	*Epigenetic error chr20* (PHP1b)	n/a	Imprinting defect on paternal allele of chromosome 20 leading to sporadic pseudohypoparathyroidism type 1b	3.2	Male	3.59	842	88.7	13.6	7.1
5	Del16p11.2	n/a	Deletion chromosome 16p11.2 (hg19: 28,843,890_29,044,745)x1	8.1	Female	4.26	1398	89.6	32.4	24.8
6	Del16p11.2	n/a	Deletion chromosome 16p11.2 (hg19: 29,627,349_30,199,713)x1	18.4	Male	3.92	1720	80.9	56.0	44.7
7	*BBS10* (Bardet-Biedl syndrome)	NM_005912	Homozygous c.271dupT p.(C91Leufs*5), leading to Bardet-Biedl syndrome	15.0	Male	3.90	2001	83.3	46.3	54.7
8	*Epigenetic error chr14* (Temple syndrome)	n/a	Imprinting defect on chromosome 14 leading to Temple syndrome	8.2	Female	3.53	1269	87.7	-	-
9	*VPS13B* (Cohen syndrome)	NM_017890.4	Compound heterozygous c.2911C>T p.(Arg971*), c.8697-2A>G p.?, leading to Cohen syndrome	8.6	Male	2.22	968	76.4	-	-

Legend: [a] predicted REE based on Schofield equations (for children <18 years) or 1984 Harris & Benedict equations (adolescents ≥18 years) [b] risk factor for early-onset obesity; n/a, not applicable; -, not available (no BOD POD measurement performed). Abbreviations: CNV, copy number variation; SDS, standard deviation score; REE, resting energy expenditure; kcal, kilocalories; REE%, ratio measured REE/predicted REE; FM, fat mass; FFM, fat-free-mass; PHP1b, pseudohypothyroidism type 1b.

day, SE 152.3, p=0.17). In the 3 children with Temple syndrome, mean REE% was 99.7 ± 10.4 (p>0.05 compared to children with multifactorial obesity); these 3 children did not have a BOD POD measurement available. In the five children with Bardet-Biedl syndrome, mean REE% was 96.5 ± 8.6 and mREE adjusted for FFM (available for 2 patients) was similar compared to children with multifactorial obesity (coefficient 28.4 kcal/day, SE 151.4, p=0.85).

Bland-Altman analyses

The Bland-Altman plot of mREE vs pREE is presented in Figure 3. When expressing the bias in absolute numbers (mREE – pREE in kcal/day), the limits of agreement were -482 kcal to +457 kcal/day. A statistically significant negative relation was found between the mean of mREE and pREE and the absolute bias between mREE and pREE (unstandardized regression coefficient -0.066 kcal/day, SE=0.028, p=0.02, Figure 3a). This indicates that with increasing values for the mean of mREE and pREE, the absolute negative bias between mREE and pREE becomes larger. This negative relationship remained similar after adjustment for presence of underlying causes (unstandardized regression coefficient -0.076, SE=0.028, p=0.007). However, when expressing the bias in relative difference, this negative relationship was no longer present (unstandardized regression coefficient -0.0021%, SE 0.0016, p=0.17, Figure 3b), also after adjustment for presence of underlying causes (unstandardized regression coefficient -0.0028%, SE 0.0015, p=0.07). The mean relative bias was -0.42% with limits of agreement of –26% to +25%.

Sensitivity analyses

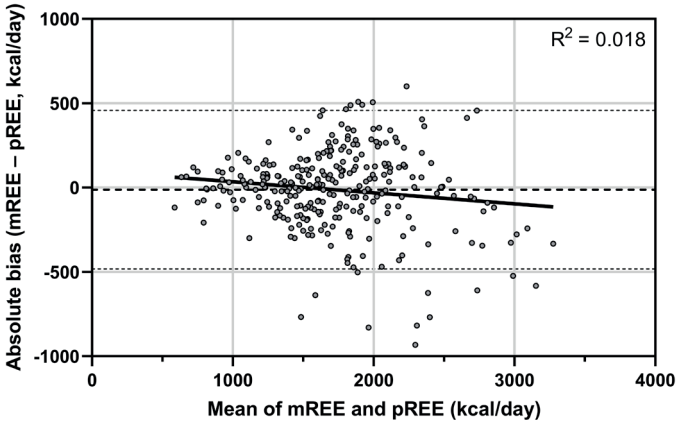
Sensitivity analyses using only REE measurements in which an optimal steady state was achieved (n=172 measurements) showed similar numerical results with regard to REE and BOD POD characteristics. Most differences between the subgroups were no longer statistically significant, probably due to the smaller sample sizes (Supplementary Table S3). When restricting these analysis to patients in whom body composition was measured, again similar numerical results were found without statistically significant differences (Supplementary Table S4).

Sensitivity analyses using the Molnár equations to calculate pREE ($pREE_{Molnár}$) showed similar results with regard to differences in REE characteristics between patients with underlying medical causes of obesity and patients with multifactorial obesity (Supplementary Table S5). Interestingly, $pREE_{Molnár}$ underestimated mREE in almost all patient subgroups with an average mean bias ranging between +55 and +131 kcal/day across the patient subgroups, except for patients with hypothalamic obesity, who had a mean bias of -116 ± 201 kcal/day (p<0.01 vs multifactorial obesity). This resulted in

a mean REE% of 105.1% ± 13.6 in the total study population and a higher proportion of patients with an elevated mREE (37% vs. 24%) and a lower proportion of patients with a decreased mREE (12% vs. 21%) when compared to the results using the Schofield equations to calculate pREE (Supplementary Table S5). $REE\%_{Molnár}$ was associated with sex (females vs males -5.6%, SE 1.6, p=0.001) but not with ethnicity (non-Dutch vs Dutch -0.1%, SE 1.8, p=0.95), indicating that the Molnár equations tend to overpredict REE in girls compared to boys. Bland-Altman analyses using the Molnár equations showed a statistically significant positive relation between the mean of mREE and $pREE_{Molnár}$ and the absolute bias between mREE and $pREE_{Molnár}$ (unstandardized regression coefficient 0.14 kcal/day, SE 0.026, p<0.001; Supplementary Figure S5a). This indicates that with increasing values for the mean of mREE and pREE, the absolute positive bias between mREE and pREE becomes larger. Adjustment for underlying causes showed similar results (p<0.001). The relative bias also showed a small but statistically significant positive association with the mean of mREE and $pREE_{Molnár}$ (unstandardized regression coefficient 0.007%, SE 0.0017, p<0.001; Supplementary Figure S5b), which remained similar after adjustment for underlying causes (p<0.001).

Sensitivity analyses using the body-composition based Lazzer equations to calculate pREE ($pREE_{Lazzer}$) also showed similar results (Supplementary Table S6). On group level, the mean absolute bias between mREE and $pREE_{Lazzer}$ was -21 kcal, resulting in an average REE% of 98.5% ± 12.1. Moreover, similar results were found with regard to differences in REE characteristics between patients with underlying medical causes of obesity and patients with multifactorial obesity: patients with non-syndromic genetic obesity had higher REE% (105.0% ± 9.4) than children with multifactorial obesity (98.7% ± 12.0) whereas children with hypothalamic obesity had lower REE% (86.6% ± 3.7, both p<0.05). $REE\%_{Lazzer}$ was associated with sex (females vs males +5.3%, SE 2.0, p=0.008) but not with ethnicity (non-Dutch vs Dutch -3.1%, SE 2.3, p=0.18), indicating that the Lazzer equations tend to underpredict REE in girls compared to boys. Bland-Altman analyses using the Lazzer equations showed a statistically significant positive relation between the mean of mREE and $pREE_{Lazzer}$ and the absolute bias between mREE and $pREE_{Lazzer}$ (unstandardized regression coefficient 0.15 kcal/day, SE 0.038, p<0.001; Supplementary Figure S6a). This indicates that with increasing values for the mean of mREE and pREE, the absolute positive bias between mREE and pREE becomes larger. Adjustment for underlying causes showed similar results (p<0.001). The relative bias also showed a small but statistically significant positive association with the mean of mREE and $pREE_{Lazzer}$ (unstandardized regression coefficient 0.011%, SE 0.0022, p<0.001; Supplementary Figure S6b), which remained similar after adjustment for underlying causes (p<0.001).

(a)



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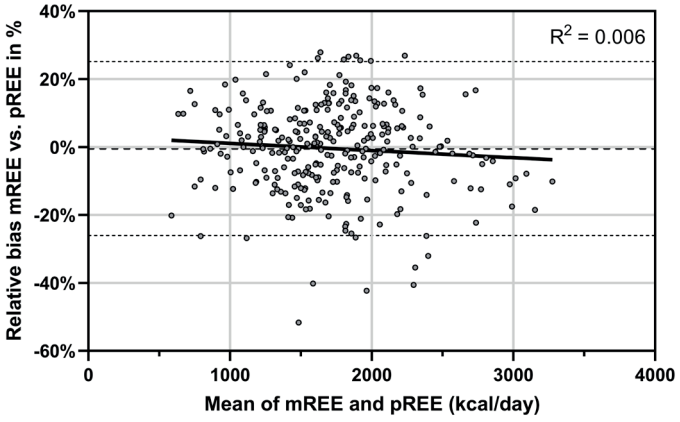

Figure 3. Bland-Altman plot for the agreement between mREE and pREE (by Schofield equations).
The dots represent the individual patients. The middle dashed line represents the mean absolute (a) or relative bias (b) across the study population. The upper and lower dashed lines represent the upper and lower limits of agreement (mean bias ± 1.96 SD) of mREE and pREE. The solid line represents the linear regression fit line.
Abbreviations: mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure (using the Schofield equations).

DISCUSSION

This study presents the REE and body composition characteristics of a cohort of children with early-onset severe obesity with and without a diagnosis of underlying medical disorders that affect the hypothalamic regulation of satiety and energy expenditure. On a group level, measured REE seems to match predicted REE quite

accurately, with a mean bias across the study population of -12 kcal/day and a mean measured REE of 100.5% of predicted values. However, our main finding is that large inter-individual and between-disorder differences between measured and predicted REE were found across all subgroups of patients. Almost half of the patients showed measured REE that was ≥10% decreased or elevated compared to predicted REE. In the 21% of patients with a decreased measured REE, the mean difference between measured and predicted REE was -341 kcal/day. The highest proportion of decreased REE was found in children with hypothalamic obesity, who on average had a measured REE of 87.6% of predicted values. The strong association between measured REE and FFM (available in 50% of patients) was similar across all patient groups with and without underlying causes. Moreover, no differences were found in measured REE adjusted for FFM between children with underlying medical causes of obesity compared to children with multifactorial obesity. Thus, our study underlines the importance of measuring REE and relating the values to body composition in all children with early-onset severe obesity with or without a diagnosis of underlying medical causes that affect hypothalamic weight regulation.

In the past decades, several studies that concomitantly measured both TEE as well as REE in children with obesity concluded that reduced REE on its own is not the major cause of common obesity.[8,38,39] Although some studies have investigated REE in specific patient subgroups with underlying medical causes of obesity, our study is to our knowledge the first to investigate REE and body composition characteristics in a relatively large cohort of children with early-onset severe obesity due to various underlying medical causes that can affect the central homeostatic maintenance of energy balance. The hypothalamic leptin-melanocortin system is a key element of the regulation of hunger, satiety and energy balance.[5] The main downstream effector is the melanocortin-4 receptor (MC4R), which upon stimulation by its endogenous ligand α-MSH promotes satiety and increases energy expenditure, whereas antagonism of MC4R action increases food intake and energy conservation.[40] In the current report, we studied children with non-syndromic and syndromic genetic obesity disorders, hypothalamic damage and weight-inducing medication as models of hypothalamic obesity and investigated REE and body composition characteristics compared to children with multifactorial early-onset severe obesity.

Multifactorial obesity

In our cohort, REE% in children with multifactorial obesity on group level matched predicted values, with a mean bias of only -12 kcal/day, corresponding to a mean REE% of 100.5%. However, the large standard deviation of REE% of 12.8% indicates that the inter-individual differences in measured versus predicted REE were considerable.

Furthermore, over half of our patients with multifactorial obesity had a REE% between 90-110%. These results are in line with previous general pediatric obesity cohort studies where mean REE% ranged between 90-111% and the proportion of patients with predicted REE within 10% of measured REE using the Schofield equations ranged from 21-61%.[16,41-43] Furthermore, our study confirms that the strong association between FFM and mREE is also observed in children with severe obesity.[44]

Non-syndromic genetic obesity disorders

Our results showed that measured REE in these patients is on average +107 kcal higher than predicted REE in children with non-syndromic genetic obesity disorders. This result can be explained by the fact that these patients had more severe obesity than children with multifactorial obesity, and since BMI z-score is positively associated with FFM,[45] a relatively higher FFM. Indeed, patients with non-syndromic genetic obesity had +6.8 kg higher FFM than children with multifactorial obesity after adjustment for age, sex and BMI SDS, and their mREE adjusted for FFM did not differ from children with multifactorial obesity. Thus, measuring body composition in these patients is necessary to correctly interpret their REE. Although the genetic defects of these patients interfere with hypothalamic leptin-melanocortin signalling,[5] and *Mc4r* knockout mice correspondingly show reduced basal oxygen consumption,[40] most studies investigating REE in humans with these rare, non-syndromic genetic obesity disorders did not find evidence for decreased REE. These studies, performed in 29 patients with MC4R deficiency,[46] two[47] and eight[48] patients with LEPR deficiency and one patient with PCSK1 deficiency[49] report a normal REE. In contrast, the first two children ever to be described with biallelic *POMC* variants were found to have a decreased REE ranging between -17% and -27% compared to the Schofield equations.[50] Another study in eight adult Pima Indians with heterozygous pathogenic *MC4R* variants showed on average -140 kcal/day lower REE compared to non-genetic obesity controls.[51] Whether this finding, which has not been replicated in other patients with *MC4R* deficiency, is related to the specific ethnic background of these patients or unidentified factors affecting REE remains to be investigated. In our study, two patients with heterozygous pathogenic *MC4R* variants and one patient with a heterozygous *PCSK1* variant that is a risk factor for early-onset obesity[4] had a decreased REE, but the proportion of patients with decreased REE did not differ between the non-syndromic genetic obesity disorders (3/29; 10%) and the multifactorial obesity group (41/218; 19%). Together, this suggests that REE can be decreased in non-syndromic genetic obesity disorders, but not more or less often than in children with early-onset severe multifactorial obesity. Therefore, it remains important to measure REE in these patients and to not rely on predicted REE only.

Syndromic genetic obesity disorders

Contrary to our expectations, we did not find major differences in REE characteristics in syndromic genetic obesity disorders compared to patients with multifactorial obesity. Various syndromic disorders in this patient group are associated with lower lean body mass and/or muscle hypotonia.[23,52-55] Yet, it seems that the Schofield equations can accurately predict REE in these patients on group level, as these patients had an average REE% of 99.5%. Moreover, we did not find differences in mREE adjusted for FFM compared to children with multifactorial obesity, which is in line with previous studies performed in children and/or adults with Prader-Willi syndrome,[22,23,56] Alström syndrome,[52] and Bardet-Biedl syndrome.[53] For other syndromic obesity disorders in our study population, namely Temple syndrome, 16p11.2 deletion syndrome and Cohen syndrome, REE characteristics have not yet been described in literature. Although we found no evidence for a decreased REE% in patients with these rare syndromic obesity disorders, it should be noted that the small sizes of these subgroups in our study population warrant further studies before any conclusions regarding REE characteristics can be made. In contrast, for patients with pseudohypoparathyrodism type 1A (PHP1a), a genetic obesity syndrome caused by the loss of the maternal allele of the imprinted *GNAS* locus leading to disturbed MC4R signalling,[5,57,58] decreased REE compared to multifactorial obesity,[20,59,60] and compared to prediction equations has been described.[21] In line with this, brain-specific *Gnas* knockout mice show reduced REE and increased feed efficacy (weight gain per kcal consumed).[58] Therefore, a decreased REE rather than hyperphagia is assumed to underlie the obesity associated with this syndrome. At present, REE measurements of 45 patients with PHP1a and 3 siblings with PHP1b have been described in literature,[20,21,57,59,60] and both reduced[20,21,59] as well as normal[60] mREE adjusted for FFM compared to controls are reported in these studies. In our current study, we add REE data on 9 PHP1a and 2 PHP1b patients. Interestingly, we did not find evidence for a decreased REE except for one of our PHP1b patients with a REE% of 88.7%, even in our sensitivity analyses using only REE measurements in which an optimal steady state was achieved. Furthermore, mREE did not differ from children with multifactorial obesity after adjustment for FFM. Whether this arises from differing patient characteristics such as age, sex, and ethnic background, or REE and FFM measurement methods, remains to be investigated. Another possible explanation is that the specific gene variants in our patients and the previously described patients show differing residual *GNAS* activity *in vivo*. Our results regarding normal REE in PHP1a are in line with a recent report in patients with obesity caused by heterozygous pathogenic *GNAS* variants, where hyperphagia was reported for 11/22 patients and decreased REE compared to prediction equations were found in only 2/6 patients and were hypothesized to be associated with partial thyrotropin resistance.[57] However, this effect can be excluded in our study as the PHP1a patients

that had biochemical signs of hormone deficiencies were adequately supplemented at the time of the REE and body composition measurements. Together, our results suggest that the obesity phenotype of patients with PHP1a can be more variable than currently assumed and might not necessarily be driven by a decreased REE only.

Hypothalamic obesity

Our results confirm the decreased measured REE versus prediction equations in patients with hypothalamic obesity due to hypothalamic damage.[24-26] The pathophysiologic mechanisms involved in these patients include reduced sympathetic tonus, thyroid metabolism, and brown fat activity as well as leptin and insulin resistance. Moreover, altered levels of α-MSH and satiety-regulating gut hormones can be seen, ultimately interfering with leptin-melanocortin signalling.[61,62] In previous studies, decreased mREE after adjustment for FFM compared to multifactorial obesity has been reported, namely in 18 children with hypothalamic obesity due to a hypothalamic lesion or damage,[26] and in 8 patients with hypothalamic obesity after treatment for craniopharyngioma.[24] In contrast, other studies report a similar ratio of mREE per kg of FFM compared to controls, namely in 23 children after treatment for craniopharyngeoma[25] and 15 adults with various hypothalamic lesions.[56] In our study, we did not find statistically significant differences in mREE adjusted for FFM between the patients with hypothalamic obesity compared to children with multifactorial obesity, although visual comparison of the regression fit lines (Supplementary Figure S2) shows a downward shift in hypothalamic obesity indicative of a lower mREE adjusted for FFM, in line with previous studies. The lack of statistical significance can probably be explained due to the small sample size of patients with hypothalamic obesity with available body composition measurements in our cohort. Altogether, our results suggest that their relatively low FFM (on average -11.7 kg compared to multifactorial obesity adjusted for age, sex, and BMI SDS) is an important driver of the lower REE compared to prediction equations in these patients. Interestingly, in two previous studies, the relationship between mREE and FFM was less strong or did not reach statistical significance in the subgroups of patients with hypothalamic obesity.[24,56] This suggests that, in contrast to multifactorial obesity, FFM might not be the most important factor determining REE in hypothalamic obesity. Another potential explanation for the differences between studies might be the different degrees and types of hypothalamic damage. As an example, our hypothalamic obesity group included two patients with meningomyelocele, both of which had a decreased REE% of 79.7% and 84.3%. This is in line with a recent study in 31 children with obesity with meningomyelocele where an average REE of 82% of predicted values was found.[63] Importantly, a head-to-head comparison of these studies is hampered by the use of different meth-

ods to assess body composition (bioimpedance analysis [BIA].[25,63] or dual energy x-ray absorptiometry [DXA][24,26,56]) and different indirect calorimetry systems.

Medication-induced obesity

We found that mREE in patients with medication-induced obesity is highly variable, yielding on average a slightly lower REE% of 95.5% and overestimation of +111 kcal/day versus predicted values. However, these differences were not statistically significant, probably due to the small sample size of this subgroup. The weight-inducing effects of most antipsychotic drugs, several antiepileptic drugs, and all corticosteroids are well-described.[64,65] Several mechanisms for inducing weight gain are proposed, such as central effects on the hypothalamus via leptin, neuropeptide Y (an orexigenic neuropeptide), serotonin, and adrenergic signalling.[66-68] Although it can be hypothesized that these mechanisms could lead to a decreased REE, findings from clinical studies have not been consistent. In a prospective study of 54 adolescents who started a second-generation antipsychotic, mREE did not change after 1 year of treatment despite an average weight gain of +10.8kg, leading to a decrease in REE%.[69] In contrast, other studies, e.g. in children on long-term treatment with valproic acid for epilepsy,[70] did not detect differences in mREE adjusted for body weight versus healthy control children. For corticosteroids, the weight-inducing effects are most likely mediated through increased intake and central fat deposition,[68] as both experimental administration of potent glucocorticoids as well as cortisol antagonists do not lead to altered REE.[68, 71] Furthermore, REE adjusted for FFM is not altered in patients with Cushing's syndrome, a disease characterized by highly elevated systemic cortisol levels.[72] As the majority of our patients with medication-induced obesity used corticosteroids, this could explain the normal REE in this subgroup. Moreover, some of our patients with medication-induced obesity were not using this medication anymore at the time of REE measurement, which might explain the normal REE in this subgroup. Taken together, more research is needed to characterize the effects of weight-inducing medication on REE.

Use of prediction equations in children with early-onset severe obesity

Our main study finding was that a high variability in REE measurements compared to prediction equations were found across the entire study population. This is reflected by the large limits of agreement in our Bland-Altman analyses. An important reason for this variability is the inherent limitation of using REE prediction equations, which do not account for physiologic variability between patients with the same age, sex, and anthropometric characteristics that are used in the prediction equations. Other reasons for this variability might be related to patient characteristics such as

variation in linear growth, pubertal stage, body composition (extremely low FFM), ethnic background, currently unidentified (poly)genetic risk factors affecting central energy expenditure regulation, or acute weight gain or loss, e.g. due to ongoing lifestyle interventions during REE measurement. Moreover, we cannot rule out that the lowered REE% in a subgroup of the patients with multifactorial obesity might be caused by underlying medical causes that we currently cannot diagnose with available techniques. We expected a high prevalence of decreased REE values in our study population based on the various underlying causes of our patients, but the Schofield equation on average predicted REE accurately in our population with a mean bias of only -12 kcal/day. The majority (56%) of our patients had a measured REE between 90-110% of predicted, and 21% and 24% of patients showed a decreased or elevated REE, respectively. In fact, the performance of the Schofield equation in our cohort was better than in most previous reported studies of pediatric patients with obesity. In these studies, higher mean biases and lower proportions of 21-61% of patients with predicted REE between 90-110% of measured REE were found.[7,16,41-44,73] An important drawback of the Schofield equations is that they are based on age categories (0-<3 years, 3-<10 years and 10-<18 years). Using the adjacent age category for patients at the limits of these categories would have explained the decreased REE of 1/60 patients and elevated REE of 12/69 patients. Thus, caution is warranted in the interpretation of the Schofield equations around the limits of the age categories, especially in case of elevated REE. To overcome this limitation of the Schofield equations, we performed sensitivity analyses using the Molnár equations. The largest external validation study to date recently showed that these have the highest "correct classification fraction", that is, pREE within 90-110% of measured values, in Caucasian children with obesity.[16] In these sensitivity analyses, we found similar results as in our analyses using the Schofield equations, which further strengthens our conclusions. It is important to realize that over the past years, several studies have investigated which prediction equations perform best in children with obesity. These studies show conflicting results varying from the Molnár equations,[42] Schofield equations for height and weight,[74,75] Lazzer equations,[43,76] Mifflin equations,[44] and WHO[77] equations. This variability might be related to different characteristics of the studied populations, such as age, sex, ethnic background and obesity severity, as well as differences in indirect calorimeters and test procedures and protocols. Hence, direct translation from any prediction equation into treatment advice in pediatric patients with severe obesity should be performed with caution. Additionally, measured REE should be related to body composition measures for correct interpretation. Our Bland-Altman analyses showed signs of proportionality of bias with increasing mean of mREE and pREE using both the Schofield (increasing underprediction) and Molnár (increasing overprediction) equations. Furthermore, sex differences were seen with regard to REE%, namely un-

derprediction in girls relative to boys using the Schofield equations and overprediction using the Molnár equations. This should be taken into account when trying to interpret measured REE of older children and/or those with the most severe obesities.

Implications for clinical practice

Our study underlines that measurement of REE can aid in developing a patient-tailored obesity approach in children with early-onset severe obesity. To estimate daily caloric needs in current clinical practice, TEE is calculated based on REE and child characteristics such as age, sex, and physical activity level.[11-13,16] Our results show that relying on predicted REE, whilst keeping all child characteristics such as physical activity level constant, would potentially overestimate or underestimate daily caloric needs by ≥10% in almost half of the children in our study population. As an example, this would translate into a significant average overestimation of daily caloric needs by 341 kcal/day in the 21% of patients with a decreased measured REE. Furthermore, specific therapeutic options can be considered in children with decreased measured REE, such as exercise training programs aimed at increasing or preserving lean body mass during weight loss.[9] In adults, a recent non-randomized study showed that extensive phenotyping, including assessment of reduced energy expenditure, followed by a phenotype-tailored treatment approach, showed higher weight loss than standard-of-care treatment.[78] Moreover, pharmacotherapy affecting central energy regulation can be considered in specific cases of children with severe obesity and reduced REE. Examples are dextroamphetamine or methylphenidate, which are centrally acting stimulants that increase serotonin, dopamine, and/or norepinephrine signalling. These drugs have shown promising results in smaller case series with non-syndromic genetic obesity and acquired hypothalamic obesity due to hypothalamic damage.[79,80] Furthermore, in patients with specific non-syndromic genetic obesity disorders such as POMC, LEPR and PCSK1 deficiency, the MC4R agonist setmelanotide has shown impressive results in terms of weight loss and increased satiety.[81] This might be partially explained by increased energy expenditure.[82] Finally, recent studies show favourable effects of glucagon-like peptide 1 (GLP-1) agonists, an anorexigenic gut hormone, both in adolescents with multifactorial obesity,[83] as well as in adults with heterozygous *MC4R* variants and 16p11.2deletion syndrome.[84, 85] Whether this is mediated through changes in REE is currently unclear.[85] Future studies should investigate whether children with severe obesity with decreased REE can benefit from these treatments.

Strengths and limitations

A major strength of our study is our relatively large cohort of patients with various rare, underlying medical disorders that lead to obesity. Our study expands knowledge

of REE characteristics in hypothalamic obesity due to genetic disorders or hypothalamic damage. We are the first to describe REE and body composition characteristics in Temple syndrome and 16p11.2 deletion syndrome. Moreover, we describe REE characteristics of patients with all underlying medical causes that are described in current international pediatric obesity guidelines within one cohort.[6] Another strength of our study is the standardized protocol in which all anthropometric, REE, and body composition measurements were collected. This was reflected by the fact that the sensitivity analysis using only REE measurements in which an optimal steady state was achieved showed similar numerical results as our main analyses. Furthermore, many studies that investigated REE in children with underlying medical causes of obesity only evaluated measured and predicted REE, and did not take body composition into account. By assessing body composition and comparing our results to children with multifactorial obesity, we could show that the differences between the patient subgroups disappeared when adjusting measured REE to FFM.

An inherent limitation of our study is that measured REE values were compared to predicted values. These are known to be inaccurate,[7,16,42] despite being the only available external standard. We specifically chose to use the Schofield equations for our analyses based on the most recent systematic review,[7] and performed additional sensitivity analyses using the Molnár equations based on the most recent and largest external validation study to date.[16] This sensitivity analysis showed consistent outcomes, strengthening the generalizability of our study results. Furthermore, the use of 10% deviation from predicted values is an arbitrary cut-off, and we chose this cut-off because it is used in the large majority of studies comparing mREE with pREE.[7,16,41-44,73] Another limitation pertaining to the translation of our results into implications for clinical practice is that we did not measure physical activity level in this study. Ideally, a personalized dietary requirement advice would rely on direct measurement of TEE (using doubly labelled water) or measurement of REE (by indirect calorimetry) multiplied by an objectively measured physical activity level (by accelerometer). However, even if an objective estimate of TEE would have been achieved, compliance according to energy requirements is often an important issue to address during follow-up. It is important to realize that currently available techniques to diagnose and understand underlying medical causes of pediatric obesity have limitations, and some of our patients might have underlying polygenetic or epigenetic vulnerabilities or a combination of factors which we cannot currently classify into a separate subgroup of underlying medical cause. Moreover, as this study was performed in an academic obesity center, we cannot exclude the possibility that in a subgroup of our patients with multifactorial obesity, a singular underlying medical (e.g. genetic) cause might be present which we cannot detect with current knowledge and technologies. Nota-

bly, we measured body composition using air displacement plethysmography, which should be taken into account when comparing our results to studies that used BIA or DXA. As our study was cross-sectional, we cannot assess whether the decreased REE in our patients might have contributed to the development or clinical course of their obesity. Longitudinal studies investigating REE and TEE have scarcely been performed in children with multifactorial obesity.[86,87] These studies are yet to be performed in children with underlying medical causes of obesity to investigate the role of energy expenditure in the natural course of their obesity and response to treatment.

Conclusion

In conclusion, we here show that resting energy expenditure in children with early-onset severe obesity due to multifactorial obesity or various underlying medical disorders that affect hypothalamic weight regulation demonstrates a large between-individual and between-disorder heterogeneity. A substantial number of patients have decreased or elevated values compared to prediction equations, corresponding to underprediction or overprediction of daily caloric needs of hundreds of calories. In half of our population, body composition data were available. Subgroup analyses in this group showed that children with hypothalamic obesity had a significantly lower measured REE than predicted and a lower FFM, whereas children with non-syndromic genetic obesity showed a significantly higher measured REE than predicted and a higher FFM. No differences in measured REE were found after adjustment for FFM between the patients with vs. without underlying medical causes. Thus, our study underlines the importance of measuring REE and body composition in children with early-onset severe obesity with or without underlying medical causes that affect hypothalamic weight regulation. This knowledge can aid in developing patient-tailored treatment approaches, such as personalized dietary interventions or physical activity interventions aimed at increasing lean body mass. Furthermore, pharmacologic treatment affecting central energy expenditure regulation could be considered in children with decreased measured REE.

Data Availability Statement

The datasets presented in this article are not readily available because they contain information that may compromise participants' anonymity, but can be made available upon reasonable request. Requests to access the datasets should be directed to the CGG Steering Committee (Prof. Erica L.T. van den Akker, centrumgezondgewicht@erasmusmc.nl).

Ethics Statement

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam, The Netherlands. Written informed consent to participate in this study was provided by the participants' legal guardian/ next of kin.

Author Contributions

OA, EK: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, validation, visualisation, writing – original draft, verifying the underlying data. MW: data curation, formal analysis, investigation, methodology, project administration, validation, visualisation, writing – review & editing. SB: data curation, investigation, methodology, project administration, validation, writing – review & editing, verifying the underlying data. ER, MH: conceptualisation, investigation, methodology, resources, supervision, validation, visualisation, writing – review & editing. BV, CG: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualisation, writing – review & editing, verifying the underlying data. EA: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualisation, writing – review & editing, verifying the underlying data. All authors contributed to the article and approved the submitted version.

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Conflict of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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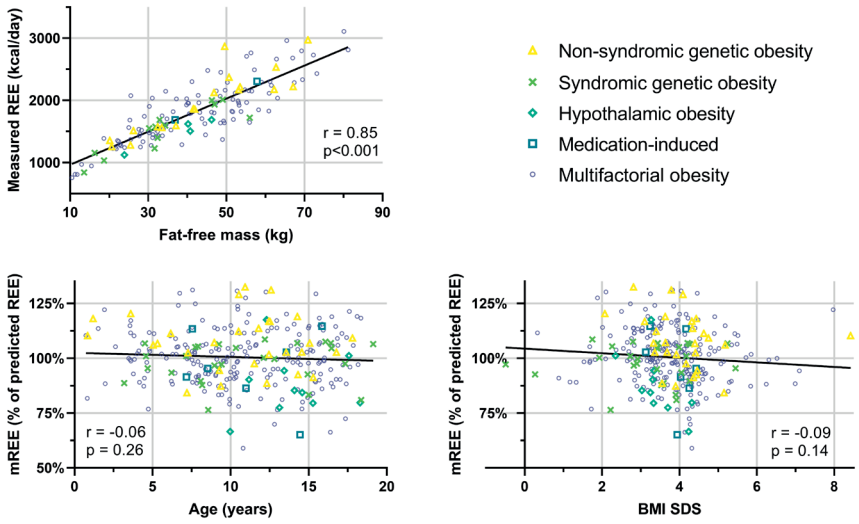
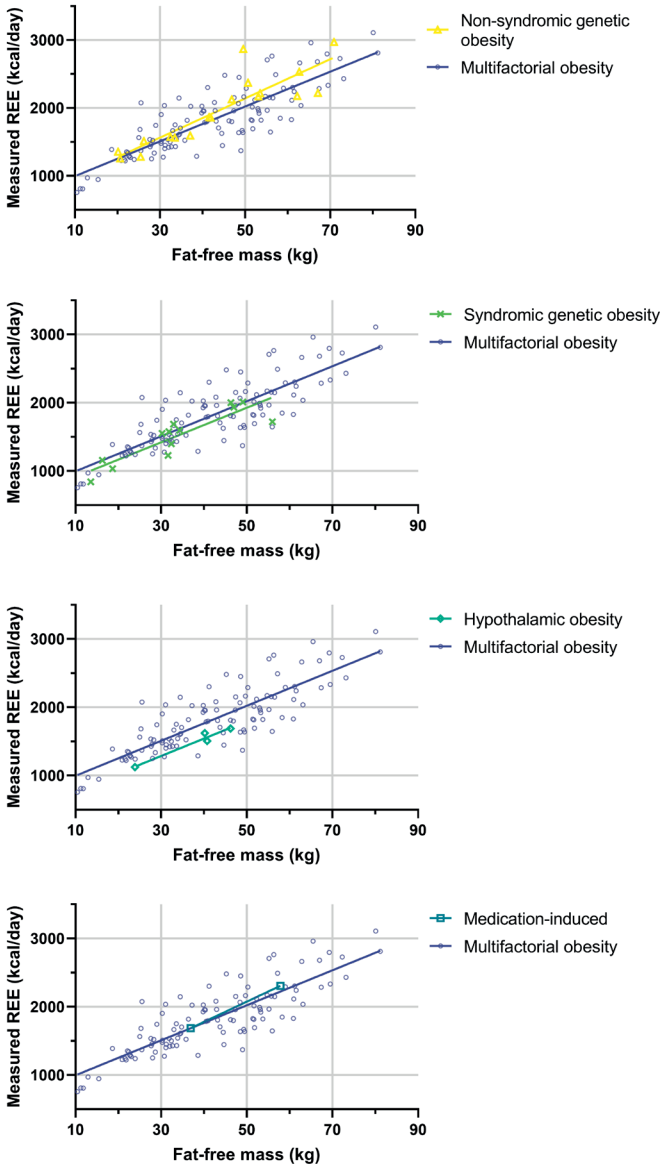
SUPPLEMENTARY APPENDIX

Supplementary figures



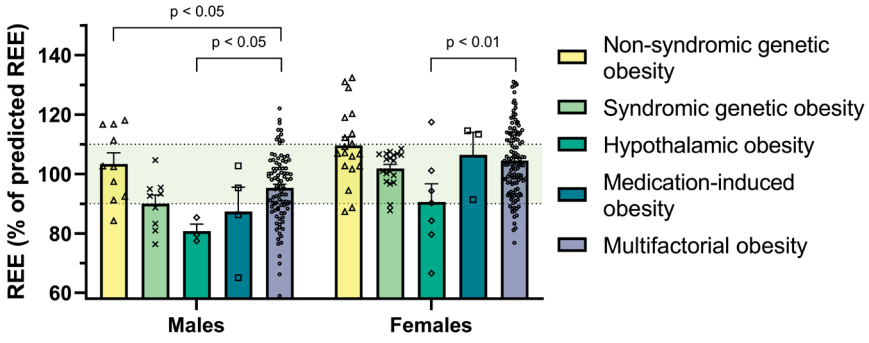
Supplementary Figure S1. Scatter plots showing the relations between measured REE and FFM, and REE% and age and BMI SDS.
The dots represent the individual patients. The line represents the linear regression fit line across the study population
Abbreviations: (m)REE, (measured) resting energy expenditure; FFM, fat-free mass; kcal, kilocalories; SDS, standard deviation score.



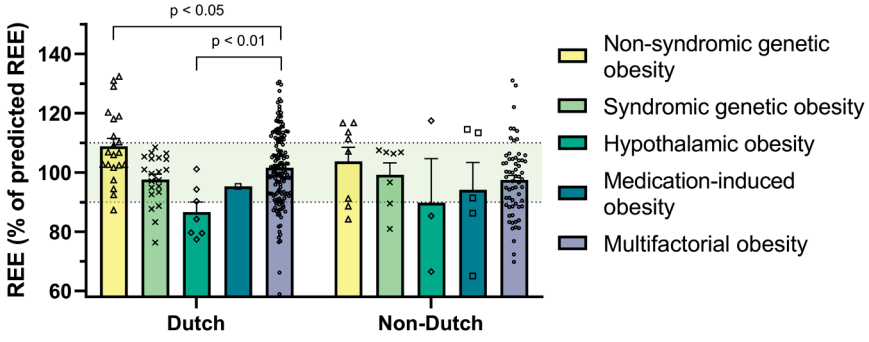
Supplementary Figure S2. Scatter plots showing the relations between measured REE and FFM stratified on underlying medical causes.

*No differences were found between the intercept nor slope for each the underlying medical causes compared to multifactorial obesity. The dots represent the individual patients. The line represents the linear regression fit line for each underlying medical cause: non-syndromic genetic obesity: REE = 28.9*FFM + 697.9, R^2=0.78; syndromic genetic obesity: REE = 25.3*FFM + 660.4, R^2=0.79; hypothalamic obesity: REE = 25.5*FFM + 520.5, R^2=0.95; medication-induced obesity: REE = 29.6*FFM + 594.4, R^2=1.00; multifactorial obesity: REE = 25.7*FFM + 739.3.9, R^2=0.71.*

Abbreviations: REE, resting energy expenditure; FFM, fat-free mass; kcal, kilocalories; SDS, standard deviation score.

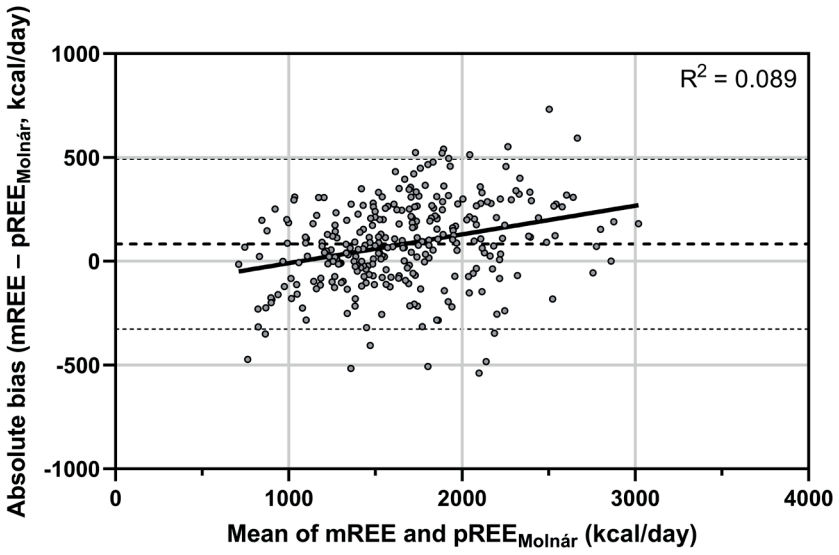


Supplementary Figure S3. Measured REE expressed as percentage of predicted REE (by Schofield equations) stratified on sex.
Male patients with non-syndromic genetic obesity had higher REE% compared to children with multifactorial obesity (p<0.05) whereas both male as well as female children with hypothalamic obesity had lower REE% (p<0.05 and p <0.01, respectively). The dots represent the individual patients. The bars represent the mean + standard error of the mean. The light green shaded area indicates a REE% between 90 and 110%.
Abbreviations: REE, resting energy expenditure.

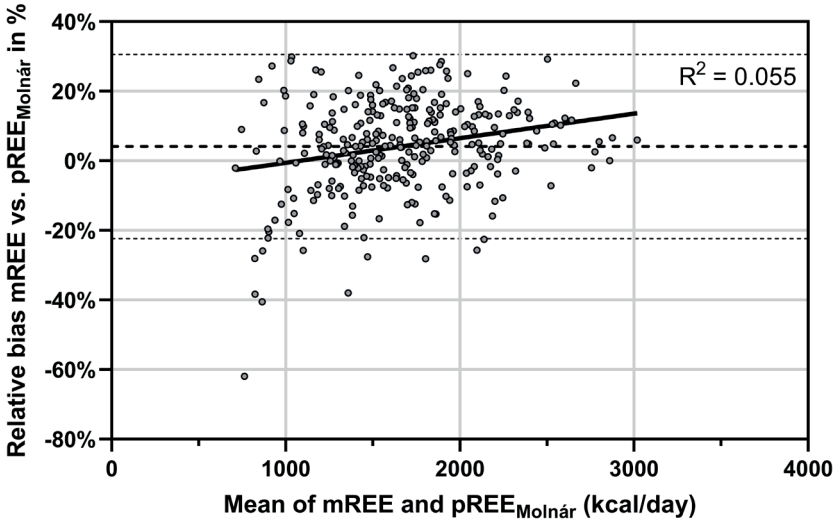


Supplementary Figure S4. Measured REE expressed as percentage of predicted REE stratified on ethnicity.
Dutch patients with non-syndromic genetic obesity had higher REE% compared to children with multifactorial obesity (p<0.05) whereas Dutch children with hypothalamic obesity had lower REE% (p<0.05 and p <0.01, respectively). The dots represent the individual patients. The bars represent the mean + standard error of the mean. The light green shaded area indicates a REE% between 90 and 110%.
Abbreviations: REE, resting energy expenditure.

(a)

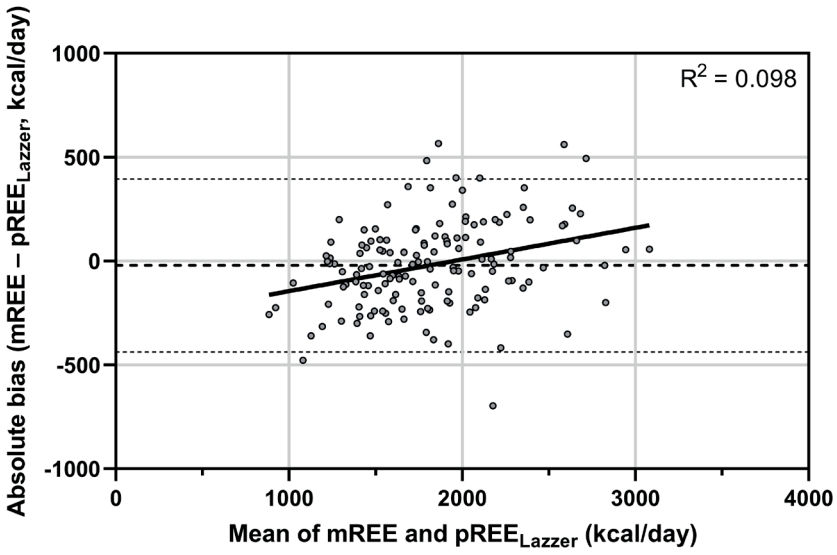


(b)



Supplementary Figure S5. Bland-Altman plot for the agreement between mREE and pREE$_{Molnar}$ (by Molnár equations).
The dots represent the individual patients. The middle dashed line represents the mean absolute (A) or relative bias (B) across the study population. The upper and lower dashed lines represent the upper and lower limits of agreement (mean bias ± 1.96 SD) of mREE and pREE$_{Molnár}$. The solid line represents the linear regression fit line. Abbreviations: mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure (using the Molnár equations).

(a)



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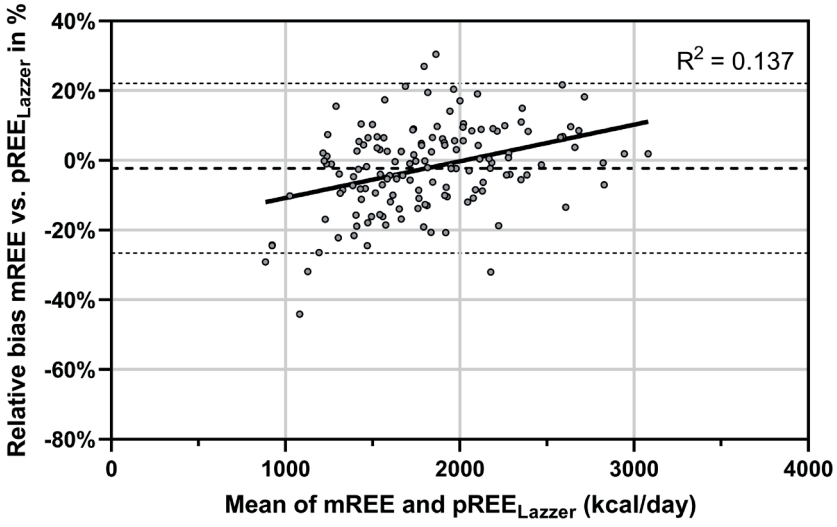

Supplementary Figure S6. Bland-Altman plot for the agreement between mREE and pREE$_{Lazzer}$ (by Lazzer equations).
The dots represent the individual patients. The middle dashed line represents the mean absolute (A) or relative bias (B) across the study population. The upper and lower dashed lines represent the upper and lower limits of agreement (mean bias ± 1.96 SD) of mREE and pREE$_{Lazzer}$. The solid line represents the linear regression fit line. Abbreviations: mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure (using the Lazzer equations).

Supplementary Tables

Supplementary Table S1. Comparison of baseline characteristics between patients with and without BOD POD measurement.

	No BOD POD measurement (n=146)	BOD POD measurement available (n=146)	P-value
Age, years	10.1 (4.4)	11.6 (4.0)	0.002
Sex, female, n (%)	92 (63)	80 (55)	0.15
Ethnicity, Dutch, n (%)	100 (69)	102 (70)	0.48
Height, cm	143.0 (24.8)	151.9 (21.1)	0.001
Height SDS	0.37 (1.46)	0.29 (1.31)	0.63
Weight, kg	67.0 (33.1)	78.1 (32.5)	0.004
Weight SDS	3.64 (1.70)	3.77 (1.34)	0.50
BMI, kg/m^2	30.4 (7.8)	32.0 (6.9)	0.07
BMI SDS	3.73 (1.20)	3.78 (0.92)	0.70

Abbreviations: BMI, body mass index; SDS, standard deviation score. Data presented as mean (SD), unless otherwise stated.

Supplementary Table S2. Correlation coefficients between REE and patient characteristics.

Parameter	Correlation with	All patients (n=292)	Non-syndromic genetic obesity (n=29)	Syndromic genetic obesity (n=28)	Hypothalamic obesity (n=10)	Medication-induced obesity (n=7)	Multifactorial obesity (n=218)
REE%	Age	-0.06	-0.12	0.11	0.02	-	-0.04
REE%	BMI SDS	-0.09	-0.21	0.06	-0.47	-	-0.13*
mREE	FFM[a]	0.85[a]***	.79[a]***	0.77[a]***	-	-	0.84[a]***

Abbreviations: mREE, measured resting energy expenditure; REE%, ratio mREE/predicted REE (based on Schofield equations); FFM, fat-free mass; SDS, standard deviation score; -, correlation not assessed due to small sample size. The presented correlation coefficients are Pearson's r (in case of n ≥ 25) or Kendall's τ (in case of n between 10 and 25).

[a] Available for n=146 patients with available BOD POD measurement (18 non-syndromic, 13 syndromic, 5 hypothalamic, 2 medication-induced, and 108 multifactorial obesities)

** P<0.05 ** P<0.01 *** P<0.001*

Supplementary Table S3. Sensitivity analysis of REE and body composition characteristics including only REE measurements in which an optimal steady state was achieved

	All patients (n=172)	Non-syndromic genetic obesity (n=17)	Syndromic genetic obesity (n=12)	Hypothalamic obesity (n=5)	Medication-induced obesity (n=3)	Multifactorial obesity (n=135)
mREE, kcal/day	1752 (468)	1904 (495)	1517 (313)	1507 (287)	1854 (396)	1760 (477)
pREE, kcal/day	1748 (501)	1799 (494)	1527 (326)	1666 (330)	1949 (300)	1760 (521)
Mean bias (mREE – pREE), kcal/day	4 (241)	106 (238)	-10 (142)	-159 (261)	-94.8 (165)	0 (247)
REE%	101.3 (12.7)	106.7 (13.7)	99.6 (7.4)	91.4 (15.0)	94.8 (8.2)	101.3 (12.7)
Lowered mREE, n (%)	32 (19)	3 (18)	1 (8)	**3 (60)***	1 (33)	24 (18)
Elevated mREE, n (%)	47 (27)	7 (41)	**0 (0)***	1 (20)	0 (0)	39 (29)
FFM, %BW	55.1 (8.4)[a]	56.3 (5.8)[a]	59.8 (8.1)[a]	47.0 (15.6)[a]	56.2 (10.3)[a]	54.7 (8.3)[a]

Abbreviations: mREE, measured resting energy expenditure; pREE, predicted resting energy expenditure (based on Schofield equations); REE%, ratio mREE/pREE; FFM, fat-free mass; %BW, percentage of body weight; kcal, kilocalories.
*Data presented as mean (SD), unless otherwise stated. [a] Available for n=103 patients with available BOD POD measurement (13 non-syndromic, 8 syndromic, 3 hypothalamic, 2 medication-induced, and 77 multifactorial obesities) * P<0.05 vs multifactorial obesity.*

Supplementary Table S4. Sensitivity analysis of REE characteristics using the Lazzer equations to calculate predicted REE in the subgroup of measurements in which an optimal steady-state was achieved (n=103 [71%] of patients in whom measured body composition data were available)

	All patients (n=103)	Non-syndromic genetic obesity (n=13)	Syndromic genetic obesity (n=8)	Hypothalamic obesity (n=3)	Medication-induced obesity (n=2)	Multifactorial obesity (n=77)
mREE, kcal/day	1797 (476)	1908 (489)	1541 (284)	1369 (289)	1997 (438)	1816 (486)
$pREE_{Molnár}$, kcal/day	1805 (415)	1839 (468)	1619 (298)	1562 (257)	2018 (146)	1823 (422)
Mean bias (mREE – $pREE_{Lazzer}$), kcal/day	-8 (211)	70 (145)	-79 (182)	-193 (36)	-21 (293)	-6 (222)
REE%	99.3 (12.0)	103.9 (8.7)	95.5 (10.9)	87.2 (4.2)	98.4 (14.6)	99.4 (12.0)
Lowered mREE, n (%)	22 (21)	0 (0)	2 (25)	2 (67)	1 (50)	17 (22)
Elevated mREE, n (%)	17 (17)	4 (31)	0 (0)	0 (0)	0 (0)	13 (17)

Abbreviations: mREE, measured resting energy expenditure; $pREE_{Lazzer}$, predicted resting energy expenditure (based on Lazzer equations); REE%, ratio mREE/ $pREE_{Lazzer}$; kcal, kilocalories.
Data presented as mean (SD), unless otherwise stated. No statistically significant differences were observed in pairwise comparisons for each of the underlying medical causes compared to multifactorial obesity (all P>0.05)

Supplementary Table S5. Sensitivity analysis of REE characteristics using the Molnár equations to calculate predicted REE

	All patients (n=292)	Non-syndromic genetic obesity (n=29)	Syndromic genetic obesity (n=28)	Hypothalamic obesity (n=10)	Medication-induced obesity (n=7)	Multifactorial obesity (n=218)
mREE, kcal/day	1705 (491)	1884 (612)	**1479 (360)***	1535 (236)	1710 (342)	1719 (490)
$pREE_{Molnár}$, kcal/day	1623 (430)	1753 (517)	**1423 (316)***	1651 (241)	1654 (296)	1629 (434)
Mean bias (mREE – $pREE_{Molnár}$), kcal/day	83 (209)	131 (228)	56 (143)	**-116 (201)****	55 (293)	89 (207)
REE%	105.1 (13.6)	107.0 (13.8)	104 (9.3)	**93.5 (12.3)****	104.1 (16.1)	105.6 (13.9)
Lowered mREE, n (%)	36 (12)	3 (10)	3 (11)	**4 (40)****	1 (14)	25 (12)
Elevated mREE, n (%)	108 (37)	11 (38)	7 (25)	2 (20)	4 (57)	84 (39)

Abbreviations: mREE, measured resting energy expenditure; $pREE_{Molnár}$, predicted resting energy expenditure (based on Molnár equations); REE%, ratio mREE/$pREE_{Molnár}$; kcal, kilocalories.

Data presented as mean (SD), unless otherwise stated.

* P<0.05 ** P<0.01 vs multifactorial obesity.

Supplementary Table S6. Sensitivity analysis of REE characteristics using the Lazzer equations to calculate predicted REE

	All patients (n=146)	Non-syndromic genetic obesity (n=18)	Syndromic genetic obesity (n=13)	Hypothalamic obesity (n=5)	Medication-induced obesity (n=2)	Multifactorial obesity (n=108)
mREE, kcal/day	1801 (481)	1973 (519)	**1518 (370)***	1446 (234)	1997 (438)	1819 (480)
$pREE_{Molnár}$, kcal/day	1822 (416)	1872 (441)	1643 (312)	1669 (252)	2018 (146)	1838 (429)
Mean bias (mREE – $pREE_{Lazzer}$), kcal/day	-21 (213)	**101 (184)***	-125 (191)	**-223 (72)***	-21 (293)	-20 (212)
REE%	98.5 (12.1)	**105.0 (9.4)***	91.9 (12.9)	**86.6 (3.7)***	98.4 (14.6)	98.7 (12.0)
Lowered mREE, n (%)	33 (23)	**0 (0)***	4 (31)	**4 (80)***	1 (50)	24 (22)
Elevated mREE, n (%)	20 (14)	**6 (33)***	0 (0)	0 (0)	0 (0)	14 (13)

Abbreviations: mREE, measured resting energy expenditure; $pREE_{Lazzer}$, predicted resting energy expenditure (based on Lazzer equations); REE%, ratio mREE/ $pREE_{Lazzer}$; kcal, kilocalories.

Data presented as mean (SD), unless otherwise stated.

* P<0.05 ** P<0.01 vs multifactorial obesity.

7

Impact of BMI on growth hormone stimulation tests in children and adolescents: a systematic review and meta-analysis

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ABSTRACT

Background Peak stimulated growth hormone (GH) levels are known to decrease with increasing BMI, possibly leading to overdiagnosis of GH deficiency (GHD) in children with overweight and obesity. However, current guidelines do not provide guidance how to interpret peak GH values of these children. The aim of this systematic review and meta-analysis was to study the effect of BMI standard deviation score (SDS) on stimulated peak GH values in children, to identify potential moderators of this association, and to quantify to which extent peak GH values in children with obesity are decreased.

Methods This systematic review was performed in accordance with the PRISMA guidelines. Medline, Embase, Cochrane, Web of Science, and Google Scholar databases were searched for studies reporting impact of weight status on peak GH in children. Where possible, individual participant data was extracted and/or obtained from authors. Quality and risk of bias were evaluated using the Scottish Intercollegiate Guidelines Network (SIGN) checklists. Primary outcome was the association between peak GH values and BMI SDS. The pooled correlation coefficient *r*, 95% confidence interval (CI) and heterogeneity statistic I^2 were calculated under a multilevel, random effects model. In addition, exploratory moderator analyses and meta-regressions were performed to investigate the effects of sex, pubertal status, presence of syndromic obesity, mean age and mean BMI SDS on study level. For the individual participant data set, linear mixed-models regression analysis was performed with BMI SDS as predictor and ln(peak GH) as outcome, accounting for used GH stimulation agent and study.

Results In total, 58 studies were included, providing data on n=5135 children (576 with individual participant data). Thirty-six (62%) of studies had high, 19 (33%) medium and 3 (5%) low risk of bias. Across all studies, a pooled *r* of -0.32 (95% CI -0.41 to -0.23, n=2434 patients from k=29 subcohorts, I^2=75.2%) was found. In meta-regressions, larger proportions of males included were associated with weaker negative correlations (p=0.04). Pubertal status, presence of syndromic obesity, mean age and BMI SDS did not moderate the pooled *r* (all p>0.05). Individual participant data analysis revealed a beta of -0.123 (95% CI -0.160 to -0.086, p<0.0001), *i.e.*, per 1 point increase in BMI SDS, peak GH decreases by 11.6% (95% CI 8.3 to 14.8%).

Conclusions To our knowledge, this is the first systematic review and meta-analysis to investigate the impact of BMI SDS on peak GH values in children, showing a significant negative relation. Importantly, this relation is already present in the normal range

of BMI SDS and could lead to overdiagnosis of GHD in children with overweight and obesity. All in all, with ever-rising prevalence of pediatric obesity, there is a need for BMI (SDS)-specific cut-off values for GH stimulation tests in children. Based on the evidence from this meta-analysis, we suggest the following weight status-adjusted cut-offs for GH stimulation tests with cut-offs for children with normal weight of 5, 7, 10, and 20 µg/L: for children with overweight: 4.6, 6.5, 9.3, and 18.6 µg/L; for children with obesity: 4.3, 6.0, 8.6, and 17.3 µg/L.

INTRODUCTION

The prevalence of pediatric obesity has increased dramatically in the past decades, resulting in over 124 million (7%) children and adolescents living with obesity worldwide.[1] Obesity is a multifactorial disease caused by an imbalance between energy intake and expenditure. Endocrine conditions such as growth hormone deficiency (GHD), hypothyroidism or hypercortisolism can lead to obesity, but are considered rare in children and adolescents.[2] According to current international guidelines for pediatric obesity, endocrine testing is only recommended in children who are short relative to their genetic potential or have decreased growth velocity in combination with weight gain.[2] However, obesity itself is known to influence growth hormone diagnostics.[3,4] This systematic review focuses on the interpretation of growth hormone (GH) stimulation tests in children (up to age 18 years) with obesity. Growth hormone is an anterior pituitary hormone, secreted in a pulsatile pattern mostly during deep sleep.[5] The main effects of GH are exerted in the liver, where it stimulates the production of insulin-like growth factor-1 (IGF-1). IGF-1 is an anabolic hormone which plays a key role in linear growth.[6] Plasma levels of GH are regulated by negative feedback loops mainly involving two hypothalamic hormones, growth hormone-releasing hormone (GHRH) and somatostatin, as well as direct negative feedback of IGF-1 on GH secretion (Figure 1). GHD is a disease characterized in children by decreased linear growth, increased central adiposity, decreased fat-free-mass, and metabolic derangements including insulin resistance.[7] Treatment with recombinant GH is indicated to normalize linear growth and improve body composition.[4,7] GHD can occur isolated or as part of a syndrome associated with short stature, such as Prader-Willi syndrome (PWS) or Turner syndrome.[8]

The diagnosis of GHD is based on clinical criteria, which incorporate, among others, auxologic parameters (e.g., short stature), radiologic parameters (e.g., bone age), laboratory findings (e.g., plasma IGF-1 values) and clinical signs and symptoms indicative of syndromes associated with poor growth (e.g., disproportionate stature). Due to the short half-life of GH, its direct measurement is not helpful in the diagnosis of GHD. Instead, dynamic GH stimulation tests are a key element in the diagnosis of GHD. These tests involve administration of a GH secretagogue and subsequent serial measurement of plasma GH values (Figure 1).

Current international guidelines by the Pediatric Endocrine Society and the Growth Hormone Research Society require an inadequate response in two separate GH stimulation tests to diagnose GHD.[3,4] In these guidelines, it is mentioned that the peak GH levels decrease with increasing BMI. The pathophysiologic mechanisms that

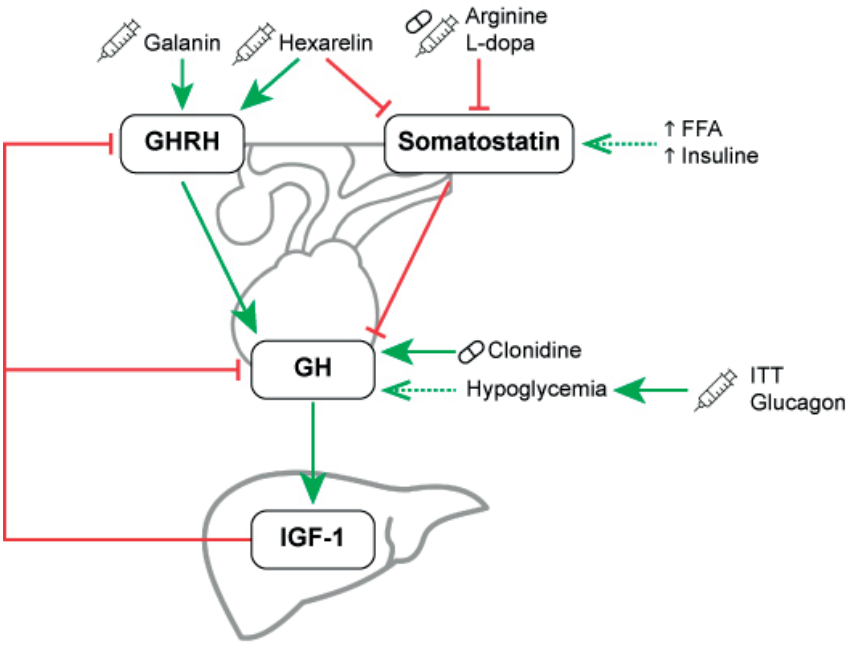

Figure 1. Schematic representation of the hypothalamic-pituitary-somatotropic axis and the effect of several GH secretagogues used in GH stimulation tests
GH secretagogues can be administered orally (indicated by the tablet icon), intramuscularly or intravenously (indicated by the syringe icon). Clonidine and hypoglycemia, either introduced by insulin in the insulin tolerance test (ITT) or by glucagon administration, directly stimulate pituitary secretion of GH. Beta-adrenergic receptor agonists, such as arginine and L-dopa, exert their GH stimulating effect by lowering the chronic inhibitory somatostatinergic tone. On a hypothalamic level, the neuropeptide galanin stimulates the release of GHRH. The synthetic growth hormone-releasing peptide hexarelin is a ligand for the growth hormone secretagogue receptor which stimulates the production of GHRH and inhibits the release of somatostatin.[5]
Abbreviations: GHRH, growth hormone-releasing hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1; ITT, insulin tolerance test.

are suggested to underlie this association include altered GH secretory bursts and increased GH clearance, inhibition of GH synthesis by increased insulin and/or free fatty acids levels, and increased somatostatinergic tonus.[7] Consequently, the negative association of peak GH levels with BMI could lead to overdiagnosis of GHD in children with overweight or obesity. However, these current guidelines state that there is insufficient evidence to use BMI-adjusted cut-offs in children and thus do not provide guidance how to interpret the peak GH levels of children with overweight or obesity.[3,4] In adults, BMI-adjusted cut-off values for defining positive GH stimulation tests have been proposed for the glucagon stimulation test and the GHRH+arginine test.[9,10] Obesity-adjusted diagnostics are not available yet for children. The 2019 guideline by the Pediatric Endocrine Society emphasizes that further research in the impact of obesity on the diagnosis of GHD in children is a topic considered with high priority by the expert group.[3] But so far, the extent to which body composition impacts the clinical

value of GH stimulation tests has not yet been assessed systematically. Therefore, the aim of this systematic review and meta-analysis was to study the effect of BMI on peak GH values after stimulation tests in children, to identify potential moderators of this association, and to quantify to which extent peak GH values after stimulation tests in children with obesity are decreased. Based on this information, we propose age,-, sex-, and weight status-adjusted cut-offs for peak GH to help clinicians and clinical chemists in interpreting peak GH values in children with overweight or obesity.

MATERIALS AND METHODS

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and Meta-analysis of Observational Studies in Epidemiology (MOOSE) checklist.[11,12]

Search strategy and selection criteria

We conducted a systematic literature search to identify all published studies reporting data on GH stimulation tests in children (including adolescents) and the possible impact of weight status. A medical information specialist designed a search strategy for the Embase, Medline (Ovid), Web of Science, Cochrane Library and Google Scholar databases from inception up to 18 March 2021. In short, the search strategy combined the keywords “weight/obesity”, “growth hormone”, “stimulation test” and “children/adolescents”. In addition, reference lists of all included studies as well as all identified international guidelines were systematically screened for potentially relevant articles.[13] The complete search strategy

is presented in the Supplementary Information 1. Inclusion criteria were: (1) performance of a standard GH stimulation test; (2) inclusion of a pediatric (sub)population (aged 0-18 years); (3) peak GH analyzed on individual level; and (4) peak GH analysis stratified on weight status on a continuous and/or categorical scale. Exclusion criteria were: (1) case reports; (2) review articles; (3) studies in which stimulated GH was only analyzed on group level per time point; (4) studies in which weight status was not taken into account in the analysis of peak GH; and (5) studies which only included children with other diseases that are likely to influence the GH/IGF-1 axis, e.g., central precocious puberty. The search results were exported to reference management software (Endnote version X9, Clarivate Analytics) and duplicates were removed. Afterwards, two researchers, one physician with a background in pediatric endocrinology (OA) and one clinical chemist (DA), screened all 1862 studies independently in two stages (Figure 2).

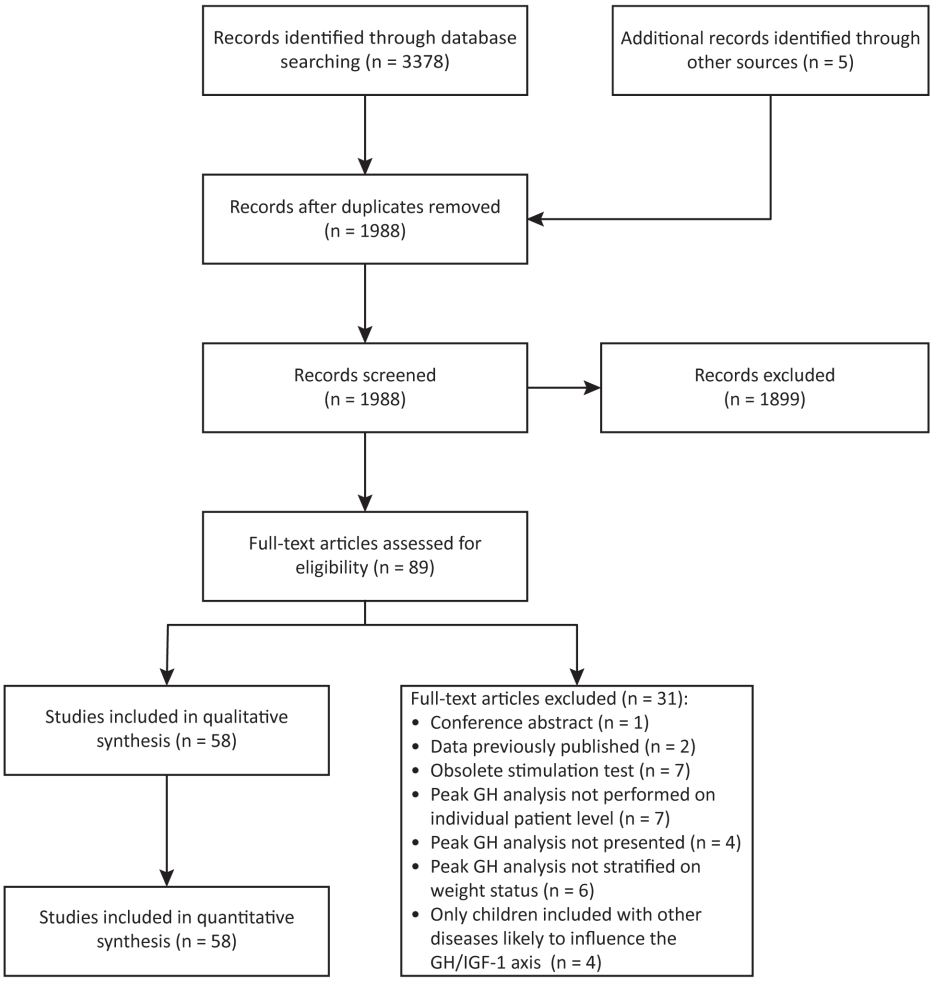

Figure 2. The PRISMA flow diagram for the systematic review.
Abbreviations: GH, growth hormone; IGF-1, insulin-like growth factor-1.

First, titles and abstracts were screened independently by both investigators, blinded for each other's screening decisions. Subsequently, the full text of all identified articles was screened by both researchers independently. In both screening stages, discrepancies between the two researchers were discussed until consensus was reached; in case of disagreement, a third, senior investigator (EvdA or YdR) served as adjudicator.

Data extraction

Descriptive, methodological and outcome data from the included studies were extracted using a predesigned data extraction sheet. All data were extracted by one of the two first authors (OA, DA) and were subsequently verified by the other researcher

to ensure accuracy. The following data were extracted: study characteristics (sample size, in- and exclusion criteria, design), study population characteristics (syndromic or non-syndromic obesity, normal or short stature, pubertal stage, age, weight status, peak GH and IGF-1 SDS), applied definitions (for obesity, for inadequate response to the used GH stimulation tests, and for GHD), details regarding the used GH stimulation test and GH assay characteristics (including calibration of assay against WHO standard), and the number of children with and without obesity who showed an inadequate response to the GH stimulation test. All studies reported peak GH either in µg/L or ng/mL; in this systematic review, all values are expressed in the SI-units µg/L. In case insufficient data were reported to include studies in quantitative analyses, corresponding authors of studies published from 2010 onwards were contacted twice in a two-week time frame to obtain the missing data. For all qualitative and quantitative analyses, patients were divided into three categories: patients with GHD (GHD+) and patients without GHD with/without obesity (No GHD, OB+, No GHD, OB-). Furthermore, we separately analyzed patients with syndromic obesity, *i.e.,* Turner syndrome; Prader-Willi syndrome (PWS), Bardet-Biedl syndrome (BBS), pseudohypoparathyroidism type 1a (PHP1a), and Kabuki syndrome.

Individual participant dataset

We curated a data set containing individual participant data for meta-analyses. When tables with data on individual level were given, these data were extracted manually from the individual studies. When studies presented a scatterplot for the relation between a weight parameter (e.g., BMI) and peak GH, all individual data points from the scatterplot were extracted using an online tool (WebPlotDigitizer version 4.3, url: https://apps.automeris.io/wpd/). All data extractions were performed by one of the first authors (OA, DA) and thoroughly double-checked by the other first author to ensure accuracy. In total, individual participant data were available for n=1738 stimulation tests in 1474 children from 27 included studies, of which n=726 GH stimulation tests in 576 children from 22 studies with data on BMI standard deviation score (SDS) and peak GH values (individual participant dataset is provided in the supplement). When individual participant data were available for a study that reported a weight parameter other than BMI, e.g., ideal body weight percentage (IBW%), we transformed the reported weight parameter to BMI using the growth reference charts mentioned in the study. We used the McLaren method for these transformations since this was the recognized method to calculate ideal body weight at the time of publication of most of these studies (1960s to 1980s).[14] When no external growth reference standard was specified, we used the growth reference charts of Tanner[15] as these were the most widely used external growth standards during that time span. We calculated BMI SDS for all studies with individual participant data available that did not report a BMI SDS

using the 2000 Centers for Disease Control and Prevention growth charts (for American studies from 2000 onwards) or the 2006 World Health Organization (WHO) growth charts (for all other studies).[16,17]

Study quality and risk of bias assessment

Quality and risk of bias of the included studies were evaluated using the Scottish Intercollegiate Guidelines Network (SIGN) checklists for cohort studies, case-control studies and diagnostic accuracy studies.[18] Because most studies contained elements of several of these different study designs, we compiled all relevant domains across the three SIGN checklists to enhance the relevance of the risk of bias assessment. All SIGN checklists contain the same conclusion domain where studies are ultimately considered to have low risk of bias (SIGN: "high quality"), medium risk of bias (SIGN: "acceptable") or high risk of bias (SIGN: "unacceptable"). All risk of bias assessments were performed independently by two researchers (DA, OA) blinded for each other's decisions; inconsistencies were settled by discussion until consensus was reached.

Statistical analysis

All meta-analyses were conducted in R version 3.6.3 using the packages *metafor* and *lme4* with a two-sided α of 0.05. Prior to analyses, medians and interquartile ranges were converted to means and standard deviations.[19] Furthermore, peak GH values were multiplied by a correction factor when authors of a study stated that this was necessary to compare their peak GH values with literature data. Where needed, subgroup means were pooled.[20] The overall weighted mean and standard deviation stratified on type of GH stimulation test were calculated using the same formulas. For analytical purposes, we divided studies that performed stratified analysis of separate stimulation tests in each individual patient into separate subcohorts, whilst accounting for the possible non-independence of observations between subcohorts in all subsequent analyses. We aimed to perform four complementary quantitative analyses: (1) a meta-analysis of correlation coefficients between peak GH and BMI SDS, (2) linear mixed-models regression analysis on the individual participant data; (3) a meta-analysis of the relative risk (RR) of a diagnosis of GHD in children referred for short stature with obesity versus without obesity; (4) a comparison of the proportion of children without GHD with obesity versus without obesity who remained below the pre-specified peak GH cut-off value.

For the meta-analysis of correlation coefficients, we calculated the bivariate correlation (Pearson's r for normally distributed data and Spearman's ρ otherwise) between BMI SDS and peak GH for all subcohorts of studies with individual participant data available that did not report a correlation coefficient if the sample size was $\geq$25

patients. For studies without available individual participant data, correlation coefficients were calculated for each subcohort using the standardized mean difference of peak GH between patients without GHD with obesity versus without obesity.[21] Subsequently, Fisher's *r*-to-*z* transformation was applied to all individual correlation coefficients. Finally, the estimated pooled correlation coefficient, 95% confidence interval (CI) and prediction interval (PI) were computed using a multilevel random effects model accounting for possible within-study (*i.e.* subcohort) correlation.[22] Between-study heterogeneity was assessed using the I^2 statistic and Cochrane's *Q* test, with I^2 >25% and p-value for Cochrane's *Q* test < 0.05 indicating heterogeneity. The possible presence of publication bias was assessed using contour-enhanced funnel plots and Egger's regression test (p-value <0.05 indicating publication bias) with addition of sampling variance as moderator in our multilevel model to account for within-study correlation.[22,23] Exploratory moderator analyses were performed with mixed-effect models for categorical parameters (e.g., type of GH stimulation test) and meta-regression with random-effects models for continuous parameters (e.g., mean age of the study participants).

Secondly, we performed linear mixed-models regression analysis on the individual participant dataset with outcome ln(peak GH) and predictor BMI SDS, accounting for used GH stimulation agent (fixed effect), study (random effect), and number of separate GH stimulation tests performed in an individual patient (random effect). Natural splines with 2 or 3 degrees of freedom were added to the model to investigate possible non-linearity, but comparison of models revealed a better fit (lowest Akaike Information Criterion and Bayesian Information Criterion) in the linear model, *i.e.*, without natural splines. Addition of interaction terms between BMI SDS and used GH stimulation agent revealed no interaction of used GH stimulation agent on the effect of BMI SDS on ln(peak GH). Therefore, these interaction terms were omitted from the final models.

Thirdly, we aimed to perform a meta-analysis on the risk ratios (RRs) for a diagnosis of GHD in children with obesity versus without obesity under a random effects model.

Finally, the proportion of patients without GHD with obesity versus without obesity that remained below the pre-specified study-specific peak GH cut-off value were compared using x^2-tests, both across all studies as well as stratified per type of GH stimulation agent.

RESULTS

Characteristics of the included studies

The search strategy identified 1988 articles in the selected databases after deduplication (Figure 2). In total, 58 articles describing 104 subcohorts of patients met inclusion criteria and were included in this study.[24-81] The main characteristics of included studies are summarized in Table 1 and Supplementary Table S1. Forty-eight studies were published between 1967-2010; ten studies were published in the past decade. In total, n=5135 children were included (median per study 30; IQR 14-77), of which 633 children (12.3%) had obesity without GHD (No GHD, OB+) and 2006 children (39.1%) had GHD. The mean age of children on subcohort level ranged from 7.4-15.9 years, with a weighted mean of 10.2 ± 3.6 years (available for 47 studies, n=4318 children). The mean BMI SDS on subcohort level ranged from -0.8 until +4.3, with a weighted mean of 0.13 ± 1.54 (available for 25 studies, n=2081 children). Out of the 3713 children with available information on pubertal status, 2669 (71.9%) were pre-pubertal. Sex steroid priming was either not performed or not mentioned in all studies except for one in which a subgroup of 5 boys with constitutional growth delay received an intramuscular testosterone injection before GH stimulation testing.[46]

Across all studies, 15 different stimulation tests were used, most importantly the arginine (12 studies), clonidine (15 studies), dopamine (7 studies), GHRH (17 studies), GHRH+arginine (5 studies) tests and the insulin tolerance test (13 studies). Most studies made use of a radioimmunoassay (RIA) to measure GH in plasma or serum.[26-30,32-34,37-43,45-48,50-52,54-57,60-66,69,71-77,79] In more recent studies, chemiluminescence or enzyme linked immunometric assays were used.[24,25,35,36,44,49,67,70,80,81] Five studies mentioned the use of calibrated GH assays.[24,33,57,61,62] Thirty-two studies pre-specified a cut-off value for inadequate peak GH response. The majority of these studies (18/32, 56%) used a cut-off value of 10 µg/L (range 5-10 µg/L). For the GHRH+arginine test, a cut-off value of 20 µg/L was used. None of the included studies used or proposed BMI-specific cut-off values for their GH stimulation tests.

Weighted mean peak GH values for the most frequently used stimulation tests in non-syndromic children are presented in Figure 3. In 16 studies, children with syndromic obesity were included: in 6 studies Turner syndrome (n=470 children), in 6 studies PWS (n=54 children), in 2 studies PHP1a (n=18 children), in 1 study Kabuki syndrome (n=18 children), and 1 study BBS (n=5 children). Weighted mean peak GH values for the most frequently used stimulation tests for these studies are presented in Supplementary Figure S1.

Table 1. Overview of baseline characteristics of included studies. In total, 58 studies were included describing n=5135 children.

Study	n pt.	IPD available	Stature	Sex	Pubertal status	Age in years M ± SD or range (min - max)			Weight status M ± SD			
			Short or normal	% male	% pre-pubertal	GHD+	No GHD, OB+	No GHD, OB-	Type of weight measure*	GHD+	No GHD, OB+	No GHD, OB-
Referred for short stature												
Patel *et al.*, 1994[57]	176	Yes	Short	61%	66%	-	NM	NM	BMI%	-	143.4 ± 16.0	93.6 ± 10.6
Stanley *et al.*, 2009[67]	116	Yes	Short	68%	72%	-	NM	NM	BMI SDS	-	1.9 ± 0.4	-0.1 ± 0.9
Lee *et al.*, 2011[42]	187	No	Short	66%	93%	8.3 ± 2.9	-	8.6 ± 2.9	BMI SDS	-0.5 ± 1.1	-	-0.9 ± 1.0
Loche *et al.*, 2011[49]	199	No GHD, OB+ only	Short	67%	73%	10.1 ± 3.1	11.0 ± 4.6	10.7 ± 3.3	BMI SDS	-0.1 ± 1.5	2.3 ± 0.3	-0.6 ± 1.1
Lee *et al.*, 2013[43]	88	Yes	Short	58%	83%	-	NM	NM	BMI SDS	-	1.7 ± 0.2	-0.9 ± 0.9
Barrett *et al.*, 2014[24]	67	No	Short	67%	100%	11.8 ± 2.6	NM	NM	BMI SDS	0.2 ± 1.0	NM	NM
Yang *et al.*, 2019[78]	460	Yes	Short	39%	100%	7.4 ± 3.1	-	-	BMI SDS	0.3 ± 1.1	-	-
Yau *et al.*, 2019[79]	315	Yes	Short	70%	54%	11.5 ± 2.2	NM	NM	BMI SDS	0.0 ± 1.1	2.0	-0.5 ± 0.9
Case-control design												
Wegienka *et al.*, 1967[77]	2	No	NM	0%	-	-	14.0 ± 0.0	-	-	-	NM	-
Croughs *et al.*, 1968[34]	27	No	Normal and short	52%	-	9.9 ± 3.1	9.5 ± 2.7	6.3 ± 3.4	-	NM	NM	NM
Kaplan *et al.*, 1968[40]	49	No	Normal and short	61%	100%	10.1 ± 3.7	4.0	7.5 ± 3.0	-	NM	NM	NM
Carnelutti *et al.*, 1970[28]	27	Yes	Normal	48%	-	-	7.6 ± 2.7	7.6 ± 1.4	IBW%	-	153.4 ± 11.4	NM
Weber *et al.*, 1970[76]	35	Yes	Normal and short	46%	-	-	13.0 ± 2.7	10.6 ± 2.8	BMI SDS	-	2.7 ± 0.4	-0.4 ± 2.1
Parra *et al.*, 1971[55]	25	No	Normal	44%	-	-	12.4 ± 3.0	14.1 ± 3.2	BMI SDS	-	3.3 ± 0.6	0.2 ± 0.7

Girard *et al.*, 1972[38]	80	Yes	Normal and short	-	-	-	10.5 ± 2.9	10.5 ± 1.3	BMI SDS	-	2.7 ± 0.6	1.5 ± 0.4
Komatsu *et al.*, 1973[41]	9	No	NM	78%	-	16.0	8.1 ± 3.3	-	-	NM	NM	-
Vanderschueren *et al.*, 1974[74]	43	No	Normal	-	100%	NM	NM	NM	-	NM	NM	NM
Josefsberg *et al.*, 1976[39]	717	No	Normal and short	72%	-	-	13.4 ± 3.2	12.1 ± 3.9	Weight SDS	-	1.3 ± 1.5	-2.0
Topper *et al.*, 1984[71]	19	Yes	Short	53%	47%	-	10.0 ± 3.7	11.8 ± 2.4	Skinfold mm	-	29.4 ± 6.6	6.2 ± 1.2
Pertzelan *et al.*, 1986[59]	9	Yes	NM	58%	22%	-	11.5 ± 3.3	-	Weight SDS - Height SDS	-	4.0 ± 1.7	-
Pintor *et al.*, 1986[60]	37	No GHD, OB+ only	Normal and short	79%	64%	10.3 ± 2.5	8.7 ± 1.5	10.7 ± 3.9	BMI SDS	NM	2.7 ± 0.4	NM
Ranke *et al.*, 1986[62]	68	No	Normal and short	-	62%	12.2 ± 4.4	NM	10.7	-	NM	NM	NM
Van Vliet *et al.*, 1986[73]	34	Yes	Short	62%	88%	11.3 ± 5.3	10.9 ± 4.0	10.6 ± 2.5	BMI SDS	0.7 ± 1.1	2.6 ± 0.4	0.1 ± 1.0
Loche *et al.*, 1987[47]	30	No GHD, OB+ only	Normal	57%	100%	NM	9.3 ± 2.2	NM	BMI SDS	NM	3.1 ± 0.8	NM
Rosskamp *et al.*, 1987[64]	40	No	Normal	50%	58%	-	10.6 ± 3.3	11.1 ± 3.4	-	-	NM	NM
Cordido *et al.*, 1989[30]	2	Yes	Normal	50%	-	-	14.0 ± 2.8	-	BMI SDS	-	2.5 ± 0.5	-
Ghigo *et al.*, 1989[37]	17	No	Normal	71%	100%	-	8.7 ± 0.9	9 - 14	BMI	-	26.7 ± 1.5	NM
Loche *et al.*, 1989[50]	19	No	Short	68%	100%	-	5.2 - 13.0	6.8 - 11.3	IBW%	-	145.8 - 198.2	NM
Cordido *et al.*, 1990[31]	4	Yes	Normal	0%	-	-	13.0 ± 0.8	-	BMI SDS	-	2.7 ± 0.4	-
Loche *et al.*, 1990[52]	12	No	Short	75%	100%	-	8.8 - 10.0	7.7 - 12.8	-	-	NM	NM
Reiter *et al.*, 1991[63]	12	No	Short	0%	100%	-	-	10.6 ± 2.8	IBW%	-	-	93 ± 3.5
Singh *et al.*, 1991[65]	40	No	Normal	60%	-	-	9.0 – 14.0	9.0 - 16.0	-	-	NM	NM
Tanaka *et al.*, 1991[68]	6	No	NM	33%	0%	-	17.2 ± 2.4	-	BMI	-	34.7 ± 4.7	-
Tanaka *et al.*, 1991[69]	942	No	NM	65%	84%	9.8 ± 3.5	-	-	-	NM	-	-

Loche *et al.*, 1992[51]	17	No	Short	59%	-	-	5.3 - 10.7	6.8 - 11.3	IBW%	-	148 - 200	90 - 110
Loche *et al.*, 1992[45]	13	No	Short	69%	100%	-	6.4 - 10.6	6.8 - 11.0	IBW%	-	141 - 186	90 - 110
Cappa *et al.*, 1993[27]	17	No GHD, OB+ only	Normal and short	65%	100%	-	9.4 ± 2.4	10.4 ± 1.6	IBW%	-	NM	90 - 110
Loche *et al.*, 1993[48]	14	No	Short	64%	-	-	5.3 - 12.8	6.8 - 11.3	IBW%	-	142 - 225	90 - 110
Martul *et al.*, 1993[53]	106	No	Normal	-	100%	NM	NM	NM	-	NM	NM	NM
Loche *et al.*, 1995[46]	60	No	Short	58%	-	8.4 - 21	7.5 - 12.0	5.9 - 14	BMI	NM	23.0 - 30.5	NM
Vaccaro *et al.*, 1995[72]	24	No	Normal and short	67%	100%	-	9.6 ± 1.3	10.7 ± 2.5	IBW%	-	131.5 ± 15.8	98.5 ± 9.2
Volta *et al.*, 1995[75]	53	No	Normal and short	58%	55%	-	10.9 ± 2.5	10.2 ± 3.1	BMI	-	28.4 ± 3.6	NM
Bideci *et al.*, 1997[26]	82	No	Normal	51%	49%	-	10.5 ± 3.0	10.3 ± 2.9	BMI	-	27.6 ± 4.1	18.8 ± 2.0
Coutant *et al.*, 1998[33]	42	No	NM	60%	100%	-	9.6 ± 1.9	-	BMI	-	25.6 ± 2.6	-
Pirazzoli *et al.*, 1999[61]	56	No	Short	0%	100%	NM	NM	NM	-	NM	NM	NM
Misra *et al.*, 2008[54]	30	No	Normal	0%	0%	-	13.7 ± 1.7	16.1 ± 1.6	BMI SDS	-	3.7 ± 1.5	0.0 ± 0.5
Perotti *et al.*, 2013[58]	30	No	Normal	47%	0%	-	-	15.9 ± 1.4	BMI	-	-	21.4 ± 2.9
Liang *et al.*, 2018[44]	108	No	Normal	84%	68%	-	12.2 ± 1.4	11.7 ± 2.0	BMI SDS	-	2.5 ± 0.9	-0.1 ± 0.9
Syndromic obesity - PWS												
Pertzelan *et al.*, 1986[59]	3	Yes	NM	58%	100%	10.9 ± 1.8	-	-	Weight SDS - Height SDS	5.9 ± 2.7	-	-
Costeff *et al.*, 1990[32]	6	Yes	Normal and short	17%	83%	8.8 ± 0.6	-	10.4 ± 1.4	BMI SDS	1.5 ± 1.4	-	0.4 ± 0.7
Cappa *et al.*, 1993[27]	9	Yes	Normal and short	56%	-	12.8	10.5 ± 3.1	-	BMI SDS	4.3	4.3 ± 1.0	-
Beccaria *et al.*, 1996[25]	11	Yes	Normal and short	55%	18%	12.7 ± 1.2	11.8 ± 3.1	13.6 ± 1.9	BMI SDS	2.2 ± 0.9	2.1 ± 0.0	1.2 ± 0.8
Thacker *et al.*, 1998[70]	18	Yes	Normal and short	63%	-	8.1 ± 4.4	6.5 ± 0.5	11.6	BMI SDS	2.6 ± 1.3	3.9 ± 1.1	1.3

Casamitjana *et al*, 2021[80]	4	Yes	Normal	25%	0%	-	17.0	16.0 ± 1.0	BMI SDS	-	2.3	0.9 ± 0.1
Syndromic obesity - Turner syndrome												
Reiter *et al.*, 1991[63]	17	Yes	Short	0%	100%	-	12.2 ± 2.3	10.6 ± 3.1	BMI SDS	-	2.5 ± 0.3	0.7 ± 0.8
Pasquino *et al.*, 1992[56]	15	Yes	Short	0%	100%	13.5 ± 1.9	10.9	12.2 ± 3.0	BMI SDS	0.8 ± 1.1	2.4	0.2 ± 1.4
Patel *et al.*, 1994[57]	48	No	Short	0%	87.5%	-	NM	NM		-	NM	NM
Vaccaro *et al.*, 1995[72]	15	No	Normal and short	0%	100%	-	NM	NM	-	-	NM	NM
Cavallo *et al.*, 1999[29]	300	No	NM	0%	0%	10.2 ± 3.0	NM	NM	BMI SDS	1.5 ± 1.5	NM	NM
Pirazzoli *et al.*, 1999[61]	75	No	Short	0%	100%	NM	NM	NM	-	NM	NM	NM
Syndromic obesity - BBS												
Soliman *et al.*, 1996[66]	5	Yes	Normal and short	100%	100%	7.0	9.8 ± 3.5	-	BMI SDS	4.3	3.7 ± 0.3	-
Syndromic obesity - PHP1a												
Germain-Lee *et al.*, 2003[36]	8	Yes	Normal and short	38%	63%	8.9 ± 2.3	-	6.8 ± 4.8	BMI	24.4 ± 2.5	-	15.6 ± 0.2
de Sanctis *et al.*, 2007[35]	10	Yes	Normal and short	20%	-	12.6 ± 0.8	4.9 ± 1.6	11.7 ± 3.2	BMI SDS	2.1 ± 0.1	3.5 ± 0.9	1.2 ± 1.2
Syndromic obesity - Kabuki syndrome												
Schott *et al.*, 2016[81]	26	Yes	Normal and short	44%	100%	5.9 ± 2.2	6.8 ± 2.2	7.0 ± 2.0	BMI SDS	1.1 ± 1.6	3.1 ± 1.0	-0.2 ± 1.2

*Abbreviations: M, mean; SD(S), standard deviation (score); GHD+, patients with growth hormone deficiency; No GHD, OB+, patients without growth hormone deficiency with obesity; No GHD, OB-, patients without growth hormone deficiency without obesity; IBW%, ideal body weight %; NM, not mentioned; PWS, Prader-Willi syndrome; BBS, Bardet-Biedl syndrome; PHP1a, pseudohypoparathyroidism type 1a. * In case several weight measures were reported, BMI SDS was the preferred outcome that we report, followed by BMI and IBW%.*

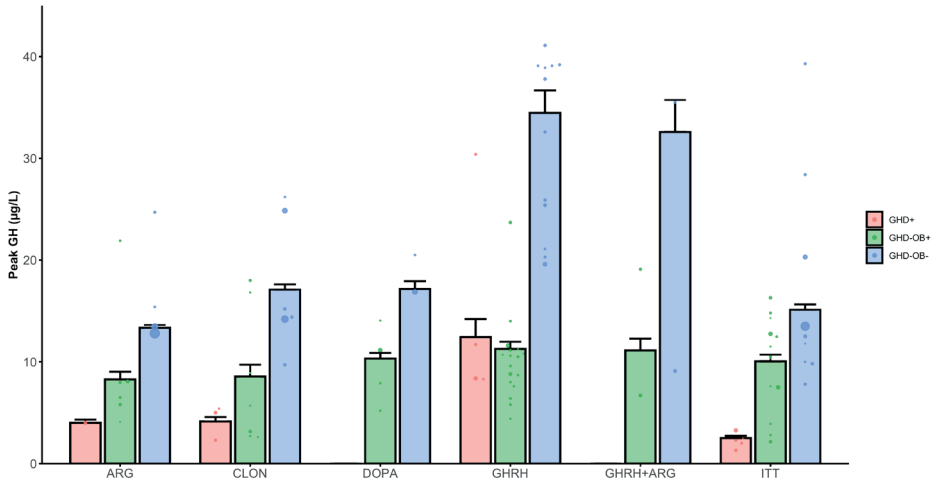

Figure 3. Weighted mean peak GH values in the studies of children with non-syndromic obesity, stratified on GHD status and weight status.
Data were available for n=2518 children from k=51 subcohorts. The dots represent the mean of individual subcohorts and the barplot represents the weighted mean peak GH ± SEM. In the case of the DOPA, GHRH and GHRH+ARG tests, no studies with results on children with GHD were identified.
Legend: GHD+: children with growth hormone deficiency; No GHD, OB+: children with obesity without growth hormone deficiency; No GHD, OB-: children without obesity without growth hormone deficiency; ARG, arginine test; CLON, clonidine test; DOPA, dopamine test; GHRH, growth hormone-releasing hormone test; GHRH+ARG, combined growth hormone-releasing hormone + arginine test; ITT, insulin tolerance test.
Abbreviations: GH, growth hormone; GHD, growth hormone deficiency; SEM, standard error of the mean.

Risk of bias

Out of the 58 studies, 3 were rated as having high quality (low risk of bias) and 19 as having acceptable quality (medium risk of bias), whereas the remaining 36 studies were rated as having high risk of bias (Supplementary Table S2). The most important reasons for risk of bias were: (1) unclear patient selection procedures; (2) no pre-defined peak GH threshold for the used stimulation test, and/or no clear definition of GHD; (3) not using calibrated GH assays; (4) use of IBW% or other currently abandoned anthropometric measurements to classify weight status of patients instead of BMI/BMI SDS; (5) comparison of patients with obesity with normal stature to patients without obesity with idiopathic/familial short stature (in some studies defined by peak GH values above a pre-specified threshold without other endocrine abnormalities).

Qualitative synthesis

In general, three subtypes of studies were recognized (Table 1): (1) a case-control design of children without GHD with obesity compared to children without obesity and/or children with GHD in 40 studies (n=2945 children);[26-28,30,31,33,34,37-40,44-48,50-52,54,55,58-65,68,69,71-77] (2) an observational cohort design investigating the impact of BMI SDS on a continuous scale in children referred to a pediatric endocrinology center for analysis of short

stature in 8 studies (n=1608 children);[24,42,43,49,57,67,78,79] (3) syndromic obesity (with or without a control group) in 16 studies (n=569 children).[25,27,29,32,35,36,56,57,59,61,63,66,70,72,80,81]

The first category of studies generally aimed at comparing peak GH values in otherwise healthy children with and without obesity, with some studies additionally comparing to children with GHD. In general, children with obesity were found to have mean peak GH values in between those of children with normal weight and children with GHD (Figure 3), irrespective of the stimulation agent. In several studies, addition of a cholinergic agent such as pyridostigmine or a beta blocker such as atenolol led to a partial reversal of GH responsiveness.[31,37,45,50,75] One study found that peak GH levels in children with obesity after hexarelin, a synthetic neuropeptide with strong GH-stimulating effects, were similar to the levels found in children without obesity after GHRH.[46] In 14 studies, IGF-1 levels were additionally measur ed,[26,27,33,44,47,50,52,54,58,62-64,72,73] and were found to be in the normal range or even higher in children with versus without obesity Perotti *et al.* found that fat mass index on DXA-scan correlated more strongly to peak GH than BMI.[58]

The second category of studies investigated the impact of BMI on peak GH values in children referred for short stature to pediatric endocrinology clinics.[24,42,43,49,57,67,78,79] These cohorts predominantly included pre-pubertal children, with more than 70% pre-pubertal participants in 7 out of 8 studies (range 54-100%). In 7 out of 8 studies, a majority of boys were included (range 58-70%). In 2/8 studies, only children without GHD were included.[43,57] whereas one study included only children with GHD.[78] On a continuous scale, all 8 studies reported statistically significant negative correlation coefficients ranging from -0.08 to -0.29 for the relation between BMI SDS (7 studies) or BMI% (1 study) with peak GH values in children without GHD.[43,49,57,67,79] In two studies, the negative association between BMI and peak GH remained significant after correction for age, gender and pubertal status[79] and additionally IGF-1 values.[42] In contrast, Stanley *et al.*[67] and Lee *et al.*[43] reported that the association was no longer statistically significant in pubertal children or in both pre-pubertal and pubertal children after stratification on pubertal status.

When focusing on children with GHD, Yang *et al.* found a negative correlation between BMI SDS and peak GH of -0.10.[78] This phenomenon was also observed by Tanaka *et al.*, who reported a correlation coefficient of -0.25 for IBW% versus peak GH in a sample of 789 pre-pubertal children with GHD from the Pfizer International Growth (KIGS) Database, an international registry for children treated with GH analogues.[69] By contrast however, two studies reported no association between BMI and peak GH within children with GHD.[49,79]

The third category of studies investigated the presence of GHD in the context of genetic obesity syndromes associated with short stature and found GHD in a median of 25.8% (IQR 8.3-38.3%) of the study participants.[25,27,29,32,35,36,56,57,59,61,63,66,70,72,80,81] Pertzelan *et al.* suggested that patients with syndromic obesity have even lower peak GH responses than patients with non-syndromic obesity, even when degree of obesity is taken into account.[59]

Side effects of GH stimulation tests were mentioned in 20 studies. For the ITT and glucagon tests, symptoms of hypoglycemia such as nausea and vomiting were recorded,[28,74,76] which led to discontinuation of the test in one study in 2/13 children.[28] In case of clonidine testing, a transient decrease of blood pressure and drowsiness were recorded.[25,49,71] In tests investigating GHRH alone, no side effects were mentioned,[47,48,50-52] whereas mild abdominal discomfort, borborygmi and facial flushing were recorded as side-effects when GHRH was combined with cholinergic agents or beta blockers.[25,27,30,31,47,48,50,51] For galanin, the only side effect recorded was a temporary bad taste,[52,53] whereas hexarelin did not induce any side effects.[46]

Quantitative syntheses

Correlation between peak GH and BMI SDS in patients without GHD

For 10 studies (11 subcohorts), correlation coefficients between peak GH and BMI SDS were provided in the original publications for patients without GHD or calculated using individual participant data. For an additional 11 studies (18 subcohorts), correlation coefficients were calculated using the standardized mean difference of peak GH between patients without GHD with obesity versus without obesity. All subcohorts for which correlation coefficients were available concerned non-syndromic children. When pooled, BMI SDS showed a moderate, statistically significant negative correlation with peak GH (pooled r = -0.32, 95% CI -0.41 to -0.23, 95% PI -0.62 to 0.07, n=2434 patients from k=29 subcohorts; ure 4). Study heterogeneity was large (I^2 = 75.2%, Cochrane's Q-test p<0.0001) and was fully explained by between-study heterogeneity; within-study (*i.e.*, subcohort) heterogeneity was found to account for $1.4*10^{-8}$% of total variance. In exploratory moderator analysis, larger proportion of males included was associated with weaker negative correlations (Table 2). Furthermore, studies investigating cohorts referred for short stature showed weaker negative correlations than studies with case-control designs. The proportion of pre-pubertal patients, mean age and BMI SDS of the populations and type of GH stimulation agent that was used did not significantly moderate the pooled r (Table 2). No clear evidence for publication bias was found through visual inspection of the funnel plot (Supplementary Figure S2), which was supported by the results of Egger's regression test (p=0.10), although the

funnel plot confirms the pattern of cohort studies reporting weaker negative correlations than studies with case-control design (Supplementary Figure S2). In sensitivity analyses, correlation origin (provided by authors or calculated for this meta-analysis) and correlation calculation method did not moderate the pooled correlation coefficient (Table 2).

Individual participant data analysis

Data on peak GH values and BMI SDS on individual level were available for n=726 GH stimulation tests from 576 children from 22 studies. Linear mixed-models analysis yielded a beta coefficient of -0.123 (95% CI -0.160 to -0.086, p<0.0001) for ln(peak GH) per one point increase in BMI SDS. This corresponds to a decrease in peak GH by 11.6% (95% CI 8.3 to 14.8%) per 1 point increase in BMI SDS. When focusing on the 8 studies with children referred for short stature to a pediatric endocrinology clinic, data was available from 4/8 studies (n=457 stimulation tests from 369 children). These 4 studies showed a beta coefficient of -0.079 (95% CI -0.118 to -0.028, p=0.0017) for ln(peak GH) per one point increase in BMI SDS. This corresponds to a decrease in peak GH by 7.1% (95% CI 2.7 to 11.2%) per 1 point increase in BMI SDS. In both analyses, used GH stimulation agent did not moderate the association between ln(peak GH) and BMI SDS (p-values >0.05).

Proportion of patients referred for short stature with GHD with/without obesity

In only one of the 8 studies that included children referred to pediatric endocrinology clinics due to short stature, presented data allowed calculation of the RR of a diagnosis of GHD in children with obesity versus without obesity,[79] making a formal meta-analysis impossible. In this study, 1 out of 160 (0.6%) children without GHD were classified as having obesity versus 8 out of 155 (5.2%) children who received a diagnosis of GHD. This would correspond to a RR of 1.85 (95% CI 1.43-2.40; p<0.0001) for a diagnosis of GHD in children referred for short stature with obesity compared to without obesity. When, as an alternative to a formal meta-analysis, all available data of these 8 cohort studies is pooled across all studies, data on weight category were available for n=1508 children. Of these children, 27 out of 893 (3.0%) children without GHD were classified as having obesity versus 36 out of 615 (5.9%) children who received a diagnosis of GHD (p=0.007). This would correspond to a RR of 1.43 (95% CI 1.14-1.78; p=0.002) for a diagnosis of GHD in children referred for short stature with obesity compared to children referred for short stature without obesity.

Table 2. Results of meta-regressions and subgroup analyses for the meta-analysis of correlation coefficients between peak GH and BMI SDS. Data were available for k=29 sub-cohorts.

Continuous moderators (meta-regression)	***k* cohorts**	**% Between-study heterogeneity explained**	**Effect size (slope)**	**95% CI**	**Q_m**	**P-value**
Years since publication	29	0.6	-0.001	-0.007; 0.006	0.04	0.85
% males	**25**	**17.4**	**0.591**	**0.028; 1.154**	**4.24**	**0.04**
% prepubertal	19	3.4	-0.108	-0.255; 0.471	0.34	0.56
Mean age	21	0.8	-0.030	-0.105; 0.045	0.62	0.43
Mean BMI SDS	13	4.0	-0.067	-0.200; 0.065	0.99	0.32
Categorical moderators (subgroup analysis)	***k* cohorts**	**Between-study heterogeneity (I^2)**	**Effect size (pooled *r*)**	**95% CI**	**Q_m**	**P-value**
Type of (non-syndromic) study					**7.37**	**0.007**
Cohort referred for short stature	**6**	**25.7**	**-0.18**	**-0.26; -0.10**		
Case-control	**23**	**75.1**	**-0.38**	**-0.48; -0.26**		
Used stimulation agent					4.79	0.31
Arginine and/or dopamine	7	85.4	-0.27	-0.44; -0.09		
Clonidine	3	53.0	-0.34	-0.49; -0.17		
GHRH	6	34.4	-0.44	-0.56; -0.29		
GHRH + second agent	4	32.7	-0.43	-0.57; -0.26		
Hexarelin	1	-	-0.20	-		
Insulin and/or glucagon	7	74.0	-0.23	-0.41; -0.04		
Risk of bias					0.62	0.73
Low	3	82.6	-0.25	-0.49; 0.02		
Moderate	7	73.7	-0.37	-0.54; -0.18		
High	19	76.2	-0.32	-0.43; -0.19		
Correlation origin					0.04	0.84
Originally provided by authors	7	57.4	-0.31	-0.42; -0.18		
Calculated for this meta-analysis	22	79.8	-0.32	-0.44; -0.19		
Correlation calculation method					0.22	0.64
Standardized mean difference	18	53.3	-0.35	-0.44; -0.25		
Pearson/Spearman	4	92.8	-0.24	-0.62; 0.23		

Abbreviations: CI, confidence interval; SDS, standard deviation score.
Legend: k, *number of cohorts;* r, *correlation coefficient;* Q_m, *Cochrane's Q for the moderator variable.*

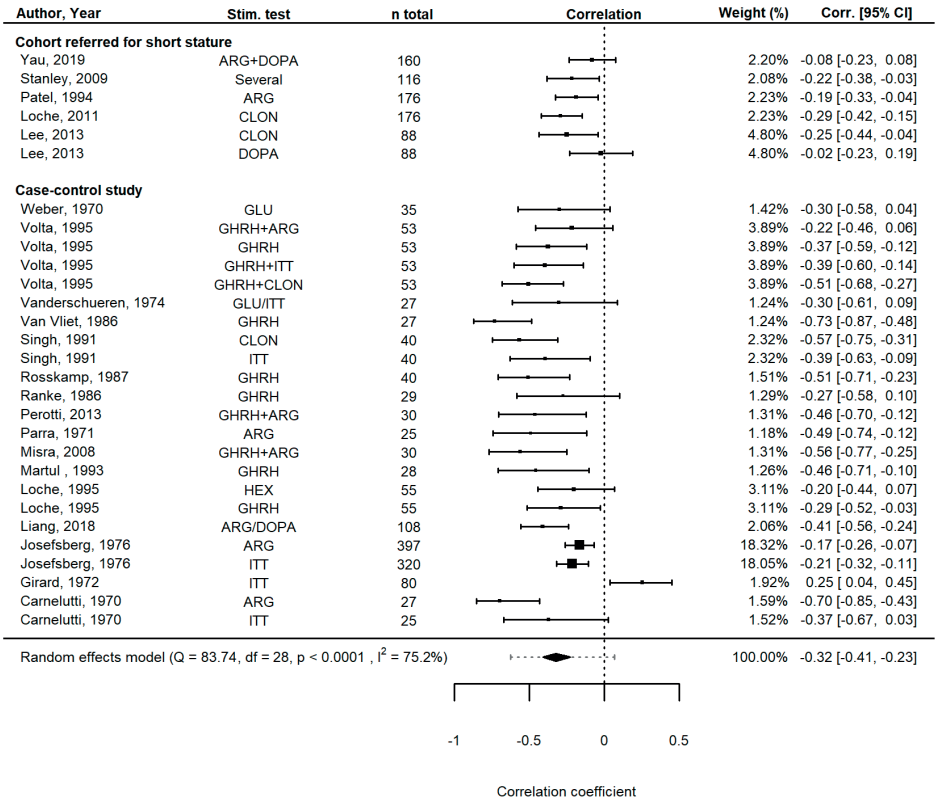

Figure 4. Forest plot showing the meta-analysis of correlation coefficients between peak GH and BMI SDS in children without GHD.
Data were available for n=2434 patients from k=29 subcohorts.
Legend: CLON, clonidine; GHRH, growth hormone-releasing hormone; PD, pyridostigmine; ITT, insulin tolerance test; ARG, arginine; DOPA, dopamine; GAL, galanine; HEX, hexareline; GHRP-6, growth hormone-releasing peptide-6.
Abbreviations: Corr, correlations; CI, confidence interval; RE, random effects; df, degrees of freedom.

Proportion of non-syndromic patients without GHD with/without obesity remaining below the pre-specified peak GH threshold

For 20 studies (30 subcohorts, n=2034 GH stimulation tests in non-syndromic children), data was available on the proportion of children without GHD with obesity versus without obesity who showed an inadequate response to the GH stimulation test, *i.e.*, remained below the pre-specified peak GH cut-off value. Across all studies, in 213/391 (54.5%) GH stimulation tests in children with obesity and 260/1643 (15.8%) GH stimulation tests in children without obesity, peak GH remained below the pre-specified cut-off value ($p<0.0001$). This corresponds to an overall RR of 3.44 (95% CI 2.98 - 3.97; $p<0.0001$) for an inadequate response to the GH stimulation test in children without GHD with obesity compared to children without obesity. When stratifying the results on the used stimulation agent, no large differences were found between the

stimulation agents (Table 3). The lowest proportions of inadequate responses, both in children with and without obesity, were observed using the GHRH+arginine test. The insulin tolerance test, which is considered to be the gold standard test in the existing literature, did not perform better than other GH stimulation tests, with over half of the children with obesity showing an inadequate response in the test (Table 3).

Table 3. Overview of non-syndromic patients without GHD with obesity and without obesity who showed an inadequate response in the GH stimulation test based on a pre-specified peak GH cut-off values. Data were available from n=2034 children from k=30 subcohorts.

	k cohorts	n No GHD, OB+ below cut-off/total (%)	n No GHD, OB- below cut-off/total (%)	P-value	RR[a] (95% CI)
All tests	30	213/391 (55)	260/1643 (16)	<0.0001	3.44 (2.98 – 3.97)
Stimulation agent					
ARG	4	20/65 (31)	84/543 (16)	0.003	1.98 (1.31 – 3.01)
CLON	6	32/51 (63)	52/318 (16)	<0.0001	3.84 (2.77 – 5.32)
DOPA	2	4/9 (44)	25/85 (29)	0.45	1.51 (0.68 – 3.37)
GHRH	6	26/59 (44)	0/95	<0.0001	NA
GHRH+ARG	2	3/15 (20)	3/45 (7)	0.32	3.00 (0.68 – 13.31)
GHRH+PD	1	3/8 (38)	0/9	0.08	NA
ITT	7	53/104 (51)	64/404 (16)	<0.0001	3.22 (2.40 – 4.31)
Various stimulation agents					
ARG/DOPA	1	70/78 (90)	0/30	<0.001	NA
ARG+CLON/DOPA+PROP/ CLON+DOPA+PROP/ ARG+DOPA	1	2/2 (100)	32/114 (28)	0.15	3.56 (2.66 – 4.78)

Abbreviations: k, number of cohorts; n, number of patients; No GHD, OB+, patients without GHD with obesity; No GHD, OB-, patients without GHD without obesity; ARG, arginine; CLON, clonidine; GHRH, growth hormone-releasing hormone; PD, pyridostigmine; ITT, insulin tolerance test; PROP, propranolol; NA, not applicable.
Legend: [a]relative risk for showing an inadequate response in the GH stimulation test based on a pre-specified peak GH cut-off value for patients without GHD with obesity compared to patients without GHD without obesity.

DISCUSSION

To our knowledge, this is the first systematic review and meta-analysis to investigate and quantify the impact of weight status on peak GH values after GH stimulation tests in children. Our results show a significant overlap between mean peak GH values of children with GHD and children with obesity without GHD. Furthermore, a moderate, negative pooled correlation of -0.32 between BMI SDS and peak GH values was found. Studies that included a larger proportion of males and studies with cohort designs

showed slightly weaker negative correlations. Individual participant data analysis showed an 11.6% decrease in peak GH values per 1 point increase in BMI SDS across all studies. Importantly, the negative association between BMI SDS and peak GH is already occurring within the normal range of BMI SDS and is independent of the used stimulation agent. This could ultimately lead to overdiagnosis of GHD in children with overweight or obesity.

The diagnosis of GHD in children is challenging due to the pulsatile secretion of GH, lack of anatomical substrate or concomitant hormone deficiencies in the case of idiopathic GHD and lack of an established threshold for GH stimulation test results to distinguish partial GHD from variation in the normal range.[3,4] Shortly following the first publication in 1963 of a method to measure stimulated GH after insulin-induced hypoglycemia in healthy adults,[82] several studies reported blunted responses in children and adults with obesity, although the exact pathophysiology was not yet understood.[83] In the following decades, several mechanisms were identified that are currently thought to at least largely explain the blunted GH response to stimulation tests in obesity. First, increased fat mass is associated with a decrease of both the frequency as well as the amplitude of GH secretory bursts and with increased GH clearance, leading to decreased GH half-life.[84,85] Second, increased insulin levels are thought to play an important role, either via direct inhibition of pituitary GH synthesis and release,[7] or via peripheral inhibition of the production of IGF binding protein 1 by the liver, leading to increased IGF-1 levels.[86]. Third, increased levels of free fatty acids (FFA) in obesity are thought to inhibit pituitary growth hormone release either directly or at least partly via an increase in somatostatinergic tone.[87] Fourth, it is well known that in obesity, growth hormone-binding protein (GHBP) is secreted in an increased amount and serum levels in children are strongly correlated with BMI.[88] Growth hormone immunoassays may be affected by high plasma concentrations of GHBP,[89] and this could lead to a potential negative bias in peak GH values, especially when using modern assays with monoclonal antibodies and shorter incubation time.[90] Finally, both a chronic increase in somatostatinergic tone as well as a direct inhibitory effect of increased free IGF-1 levels caused by decreased levels of IGF-binding proteins 1 and 2 have been hypothesized by various studies both in humans as well as in animal models, but their contribution to the hyporesponsiveness of GH to stimulation tests in obesity has been disputed.[7] Importantly, the blunted GH response to GH stimulation is shown to be reversible through weight loss in both adults[91] as well as children.[53,92] Several studies investigated the addition of pharmaceutical agents to GH stimulation agents in obesity. Addition of acipimox, a nicotinic acid analogue which causes an acute reduction of FFA levels through direct inhibition of FFA production by the liver, was shown to reverse the blunted GH response to arginine testing in adults.[87]

Other studies in children found partial reversal of hyporesponsiveness to GHRH in obesity with addition of either pyridostigmine, an acetylcholinesterase inhibitor,[30,37,50] galanin, a neuropeptide widely expressed in the central nervous system and gut,[52] or atenolol, a selective β_1-blocker.[51] As all these agents exert their effect through inhibition of somatostatin, these clinical findings strengthen the hypothesis of an increased somatostatinergic tonus in obesity. Of note, all studies investigating the addition of pharmaceutical agents in GH stimulation tests had a case-control design comparing individuals with obesity versus without obesity. Their usefulness in cohort studies of children referred for short stature has not yet been investigated, and current clinical guidelines do not mention their potential use.[3,4] Therefore, addition of these agents to GH stimulation tests in current clinical practice of children referred for short stature is probably limited until more data becomes available.

Our meta-regression results show that the negative correlation between BMI SDS and peak GH values in children without GHD were significantly moderated by study design. This finding is of particular clinical importance, since most identified studies investigating this relation were small case-control studies, comparing children with obesity versus without obesity. When focusing only on cohort studies performed in children referred for short stature without GHD, BMI SDS showed a more modest negative correlation with peak GH of -0.18 and a 7.1% decrease in peak GH values per 1 point increase in BMI SDS. Furthermore, meta-regression showed that the proportion of males included was associated with weaker negative correlations. Current pediatric guidelines do not mention sex in the interpretation of GH stimulation test results of children referred for short stature.[3,4] Moreover, recently published studies in children with short stature do not report sex differences in results of GH stimulation tests,[93] although these sex differences have been reported in adults undergoing GHRH+arginine tests.[94] Given that the weighted mean age of participants was 10.2 years, *i.e.* around the "pre-pubertal dip" of growth velocity, our finding may be explained by the lack of sex steroid priming in all but one of the included studies. Sex steroid priming is known to increase specificity of GH stimulation tests and can prevent inappropriate diagnosis of GHD and subsequent need for GH treatment in children with constitutional delay of growth and puberty.[4,95] As such, the 2016 guideline by the Pediatric Endocrine Society advocates the use of sex steroid priming in all pre-pubertal children from age 11 years (boys) or 10 years (girls) onwards.[4] In contrast however, the 2019 guideline from the Growth Hormone Research Society states that the efficacy of priming for improving the diagnostic performance of GH stimulation testing in general is unclear.[3] It could be argued that especially in children with overweight or obesity, who are already at risk of showing blunted peak GH responses, sex steroid priming before GH stimulation tests could have additional benefits to reduce false positive test results, but this

remains to be investigated. Moreover, it is important to standardize the stimulation testing procedure itself, among which the route of administration, quantity of stimulation agents and timing of blood draws.[96]

All in all, the negative association between peak GH values and BMI SDS, which is already present in the normal range of BMI SDS, could lead to overdiagnosis of GHD in children with overweight or obesity. A formal meta-analysis of the relative risk of a diagnosis of GHD in children with short stature with obesity compared to without obesity was not possible due to a lack of reported data stratified on both weight status and GHD status. Our analyses when pooling available data across all these studies hint toward an increased risk of a diagnosis of GHD in children with obesity, as can be expected since peak GH values were used to define GHD in most of the studies that pre-defined GHD. On the other hand, GH treatment registry studies investigating response to GH treatment found no difference in delta growth velocity or delta height SDS in children with overweight and obesity compared to children with normal weight.[97] This would suggest that children with overweight and obesity are not more often misclassified as GH deficient than children with normal weight. It is important to realize that the combination of short stature and obesity is rare, and in our meta-analysis, only 63/1508 (4.2%) children referred for short stature with available data on weight status had obesity. Obesity itself is characterized by slightly increased linear growth during childhood and normal adult height.[98] The combination of short stature or decreased growth velocity and obesity or unexplained weight gain should therefore prompt the clinician's attention to a potential underlying medical cause for the child's obesity, e.g. hypercortisolism or genetic obesity syndromes.[2,4] A recent study investigating underlying medical causes of obesity indeed found that lower height SDS was one of the most important predictors of genetic obesity syndromes (mean height SDS -0.4 vs +0.6 in children with obesity without an underlying medical cause), although only a minority of children with genetic obesity syndromes in this study (4/18, 22%) had short stature.[99] In our meta-analysis, a diagnosis of GHD was made in a median of 25% of children with syndromes associated with short stature, most of which are also associated with obesity. Therefore, clinicians should be aware of the relatively high likelihood of GHD in children with obesity, short stature, and features indicative of an underlying syndrome such as congenital anomalies, dysmorphic features, or developmental delays.

Importantly, GHD is a clinical diagnosis relying on a combination of auxologic, radiologic, and clinical findings besides growth hormone stimulation tests. An ideal GH stimulation test would aid in the diagnosis of GHD by distinguishing healthy children from children with GHD with minimal side effects, be easy to perform, and show re-

producibility of the test results.[100-102] However, none of the currently used stimulation tests in children fulfil these criteria, and it has even been argued that GH stimulation tests should not be used in the diagnostic workup of GHD in children.[103] Furthermore, based on our current analyses, a pattern favoring a singular stimulation agent could not be observed, as 16% of children without GHD without obesity and 55% of children with obesity across all studies showed a peak GH value below the pre-specified cut-off of the study. Even in the case of the insulin tolerance test (ITT), which has been considered the gold standard test to identify GHD.[7,102] even though it is rarely performed due to the risks associated with insulin-induced severe hypoglycemia,[3,4,7,104] over half of children with obesity remained below the peak GH threshold after ITT. This highlights the need for novel, more potent stimulation agents. Synthetic neuropeptides such as hexarelin and macimorelin are examples of these stronger stimulation agents acting through the growth hormone secretagogue receptor (GHSR),[105] with the latter already included in the 2019 American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for adult GHD.[46,104] Final results from pediatric studies investigating macimorelin are expected in the near future,[106] but the impact of BMI on the results of GH stimulation tests with these agents as well as their performance in case of hypothalamic dysfunction as cause of GHD (rather than pituitary dysfunction) remains to be investigated both in adults as well as children.[3,104] Besides innovations in GH stimulation testing, stratification of patients based on pre-test likelihood estimated from auxologic, radiologic and anatomic data with subsequent calculation of post-test likelihood based on IGF-1 SDS, as recently proposed, could further aid clinical decision-making with regard to GH stimulation tests.[107]

Important for a good interpretation of the GH stimulation tests is the standardization of the used GH assays. The current immunoassays are more specific for GH, especially when a monoclonal antibody is used.[102,108,109] Growth hormone has a wide variety of molecular isoforms which are picked up differently by the used antibodies in the assays, especially when using polyclonal antibodies. The first standardization of GH took place in 1969 with the IRP 66/127, which contained a variety of GH isoforms. Nowadays, calibration takes place on the 22-kD GH isoform (IS 98/574 or IS 88/624).[90] Regardless, there is a need for universal harmonization of GH assays.[102] As an example, in the Netherlands, growth hormone assay harmonization took place in the early 2010's and resulted in a decrease of imprecision from 22% to 6.7% using the IS 88/624 calibrator.[110,111] Most of the used cut-offs for GH stimulation tests are determined on older studies using radioimmunoassay with polyclonal antibodies. In addition to known variation between assays and laboratories, cut-offs need to be revised when using the new more specific immunoassays.[3,4,102] More far-reaching adjustments are needed when mass spectrometry is used in practice for a GH assay.[112]

What peak GH cut-off values should be used for children referred for short stature with overweight and obesity? Our individual participant data analysis showed a decrease in peak GH values of 7.1% per 1 point increase in BMI SDS in these children. To calculate the corresponding weight-status adjusted cut-offs, the following equation can be used:

$$cut\text{-}off_{adjusted}\ (\mu g/L) = cut\text{-}off_{normal\ weight} * 0.929^{BMI\ SDS}$$

When, in accordance with WHO definitions, overweight and obesity in children ≥2 years are defined as a BMI SDS ≥1 (85th percentile) and ≥2 (97.5th percentile),[1,17] this would translate into a peak GH cut-off value of 9.3 µg/L for overweight and 8.6 µg/L for obesity if the cut-off value for normal weight is set at 10 µg/L (Figure 5). If the cut-off value for normal weight is set at 7 µg/L, the proposed cut-off values would be 6.5 µg/L and 6.0 µg/L for overweight and obesity, respectively. For the GHRH+arginine test, in which a cut-off of 20 µg/L is used, the cut-off for overweight would be 18.6 µg/L and for obesity 17.3 µg/L. Importantly, these cut-offs need to be validated prospectively. Gender and puberty status should preferably always be included in future studies so that it is possible to investigate the effect of sex and puberty status on these weight-status adjusted cut-offs.

Normal weight	Overweight	Obesity
		
7 µg/L	6.5 µg/L	6.0 µg/L
10 µg/L	9.3 µg/L	8.6 µg/L
20 µg/L	18.6 µg/L	17.3 µg/L

Figure 5. Weight status-adjusted cut-offs for children with overweight and obesity based on our meta-analysis results.
Adjusted cut-offs based on BMI SDS (BMI adjusted for age and sex) are provided for stimulation tests with cut-offs for children with normal weight of 5, 7, 10, or 20 µg/L.

Strengths and limitations

A strength of our systematic review and meta-analysis is its elaborate design including rigorous extraction of individual participant data in a large subgroup of patients. Where possible, we contacted corresponding authors for additional information. Furthermore, we applied several complementary meta-analytic methods which showed consistent outcomes, improving the scientific rigor.

One of the limitations of this systematic review is that most included studies had a small sample size. To overcome this, we extracted individual participant data where possible and adopted a minimal group size of 25 patients in our meta-analysis of correlations to minimize the risk of small sample bias. Unfortunately, individual participant data was not available for all studies and since most studies were performed decades ago, data requests were not always possible. Therefore, we used validated statistical methods to obtain the required data for our meta-analyses from the originally reported data, such as the calculation of correlation coefficients via the standardized mean difference,[21] and performed sensitivity analyses to confirm that these different statistical methods did not moderate our meta-analytic findings. Another limitation was that most of the included publications were case-control studies. These studies often included children with and without obesity with normal stature, and GH stimulation tests would normally not be performed in these populations. Furthermore, risk of bias assessment showed that the majority of included studies had a high risk bias. To overcome these issues, we performed sensitivity analyses restricted to cohort studies with children referred for short stature. These sensitivity analyses showed similar results although the effect sizes were slightly smaller and likely less biased. Another limitation was that many studies used radioimmunoassay to determine GH concentrations, which are known to be less specific for the 22-kD growth hormone isoform than current assays with monoclonal antibodies. Therefore, we used the peak GH threshold provided by the authors for our analyses of the proportions of children with and without obesity that failed the GH stimulation tests.

Conclusion

In conclusion, we systematically reviewed the current literature on the effect of weight status on GH stimulation test results in children. Our meta-analyses showed a significant negative correlation between BMI SDS and peak GH concentration in children, with 1 point increase in BMI SDS corresponding to an 11.6% decrease in peak GH values. Given the increasing prevalence of pediatric obesity, our study highlights the need for BMI SDS-specific cut-off values for GH stimulation tests in children with short stature. Based on the results of the current meta-analysis, we propose weight-status adjusted cut-offs for GH stimulation tests and provide a general equation to calculate weight status-adjusted cut-offs for GH stimulation tests in children using age- and sex-adjusted BMI SDS. Future studies should prospectively validate these cut-offs in children with short stature.

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SUPPLEMENTARY APPENDIX

Overview of contents:

1. Supplementary information 1: Search strategy
2. Supplementary table S1: Growth hormone stimulation test characteristics and outcomes of cohorts from all included studies
3. Supplementary table S2: Risk of bias assessment of included studies
4. Supplementary figure S1: Weighted mean peak GH for children with syndromic obesity
5. Supplementary figure S2: Funnel plot
6. Supplementary appendix references

Individual participant dataset Because this file is less informative in print due to its size and lay-out, the digital file can be accessed via: https://doi.org/10.6084/m9.figshare.16437915.v1

Supplementary information 1. Search strategy for systematic literature search.

Date of search: July 13th 2020

Embase – 1183 refs

('obesity'/exp OR 'obese patient' OR (obese OR obesity OR adiposit* OR overweight*):ab,ti,kw) **AND** ('growth hormone deficiency'/de OR 'growth hormone'/de OR 'somatomedin C'/de OR (hyposomatotropinis* OR growth-hormon* OR GH OR somatotropin* OR somatomedin-C OR growth-factor-1 OR growth-factor-I OR IGF-1 OR IGF1 OR IGF-I OR IGFI):ab,ti,kw) **AND** ('diagnostic test'/exp OR 'glucagon'/de OR 'arginine'/de OR 'clonidine'/de OR 'dopamine'/de OR 'insulin tolerance test'/de OR 'growth hormone releasing factor'/de OR (GHRH OR GHRF OR GRF OR IIH OR ITT OR L-dopa OR dopamin* OR clonidin* OR arginin* OR glucagon* OR glukagon OR range* OR value* OR interval* OR ((diagnos* OR blood* OR function* OR lab* OR stimulat* OR provocat* OR toleranc* OR insulin* OR growth-hormon* OR GH OR somatotropin*) NEAR/3 (test OR tested OR tests OR testing OR result* OR research*)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/3 (peak* OR stimul* OR provocat* OR increas* OR enhanc* OR respons* OR induc*)) OR ((insulin*) NEAR/3 (hypoglycem* OR status)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/3 (releas*-hormone* OR releas*-factor*))):ab,ti,kw) **AND** (child/exp OR adolescent/exp OR adolescence/exp OR 'child behavior'/de OR 'child parent relation'/de OR pediatrics/exp OR childhood/exp OR 'child nutrition'/de OR 'infant nutrition'/exp OR 'child welfare'/de OR 'child abuse'/de OR 'child advocacy'/de OR 'child development'/de OR 'child growth'/de OR 'child health'/de OR 'child health care'/exp OR 'child care'/exp OR 'childhood disease'/exp OR 'child death'/de OR 'child psychiatry'/de OR 'child psychology'/de OR 'pediatric ward'/de OR 'pediatric hospital'/de OR 'pediatric anesthesia'/de OR 'pediatric intensive care unit'/de OR 'neonatal intensive care unit'/de OR 'prematurity'/de OR (adolescen* OR preadolescen* OR infan* OR newborn* OR (new NEXT/1 born*) OR baby OR babies OR neonat* OR prematur* OR pre-matur* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR

underag* OR (under NEXT/1 (age* OR aging OR ageing)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR suckling* OR PICU OR NICU OR PICUs OR NICUs):ab,ti,kw) *NOT ([animals]/lim NOT [humans]/lim)* NOT ([Conference Abstract]/lim)

Medline – 968 refs

(exp Obesity/ OR obese patient OR (obese OR obesity OR adiposit* OR overweight*).ab,ti,kf.) **AND** (exp Growth Hormone/ OR Insulin-Like Growth Factor I/ OR (hyposomatotropinis* OR growth-hormon* OR GH OR somatotropin* OR somatomedin-C OR growth-factor-1 OR growth-factor-I OR IGF-1 OR IGF1 OR IGF-I OR IGFI).ab,ti,kf.) **AND** (Diagnostic Tests, Routine/ OR Glucagon/ OR Arginine/ OR Clonidine/ OR Dopamine/ OR exp Growth Hormone-Releasing Hormone/ OR (GHRH OR GHRF OR GRF OR IIH OR ITT OR L-dopa OR dopamin* OR clonidin* OR arginin* OR glucagon* OR glukagon OR range* OR value* OR interval* OR ((diagnos* OR blood* OR function* OR lab* OR stimulat* OR provocat* OR toleranc* OR insulin* OR growth-hormon* OR GH OR somatotropin*) ADJ3 (test OR tested OR tests OR testing OR result* OR research*)) OR ((growth-hormon* OR GH OR somatotropin*) ADJ3 (peak* OR stimul* OR provocat* OR increas* OR enhanc* OR respons* OR induc*)) OR ((insulin*) ADJ3 (hypoglycem* OR status)) OR ((growth-hormon* OR GH OR somatotropin*) ADJ3 (releas*-hormone* OR releas*-factor*))).ab,ti,kf.) **AND** (exp Child/ OR exp Infant/ OR exp Adolescent/ OR exp "Child Behavior"/ OR exp "Parent Child Relations"/ OR exp "Pediatrics"/ OR "Child Nutrition Sciences"/ OR "Infant nutritional physiological phenomena"/ OR exp "Child Welfare"/ OR "Child Development"/ OR exp "Child Health Services"/ OR exp "Child Care"/ OR "Child Rearing"/ OR exp "Child development Disorders, Pervasive"/ OR "Child Psychiatry"/ OR "Child Psychology"/ OR "Hospitals, Pediatric"/ OR exp "Intensive Care Units, Pediatric"/ OR (adolescen* OR infan* OR newborn* OR (new ADJ born*) OR baby OR babies OR neonat* OR prematur* OR pre-matur* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under ADJ1 (age* OR aging OR ageing)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR suckling* OR PICU OR NICU OR PICUs OR NICUs). ab,ti,kf) *NOT (exp animals/ NOT humans/)* NOT (news OR congres* OR abstract* OR book* OR chapter* OR dissertation abstract*).pt.

Cochrane (RCTs) – 58 refs

((obese OR obesity OR adiposit* OR overweight*):ab,ti) **AND** ((hyposomatotropinis* OR growth-hormon* OR GH OR somatotropin* OR somatomedin-C OR growth-factor-1 OR growth-factor-I OR IGF-1 OR IGF1 OR IGF-I OR IGFI):ab,ti) **AND** ((GHRH OR GHRF OR GRF OR IIH OR ITT OR L-dopa OR dopamin* OR clonidin* OR arginin* OR glucagon* OR glukagon OR range* OR value* OR interval* OR ((diagnos* OR blood* OR function* OR lab* OR stimulat* OR provocat* OR toleranc* OR insulin* OR growth-hormon* OR GH OR somatotropin*) NEAR/3 (test OR tested OR tests OR testing OR result* OR research*)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/3 (peak* OR stimul* OR provocat* OR increas* OR enhanc* OR respons* OR induc*)) OR ((insulin*) NEAR/3 (hypoglycem* OR status)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/3 (releasing-hormone* OR releasing-factor*))):ab,ti) **AND** ((adolescen* OR preadolescen* OR infan* OR newborn* OR (new

NEXT/1 born*) OR baby OR babies OR neonat* OR prematur* OR pre-matur* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under NEXT/1 (age* OR aging OR ageing)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR suckling* OR PICU OR NICU OR PICUs OR NICUs):ab,ti)

Web of Science – 917 refs

TS=(((obese OR obesity OR adiposit* OR overweight*)) AND ((hyposomatotropinis* OR growth-hormon* OR GH OR somatotropin* OR somatomedin-C OR growth-factor-1 OR growth-factor-I OR IGF-1 OR IGF1 OR IGF-I OR IGFI)) AND ((GHRH OR GHRF OR GRF OR IIH OR ITT OR L-dopa OR dopamin* OR clonidin* OR arginin* OR glucagon* OR glukagon OR range* OR value* OR interval* OR ((diagnos* OR blood* OR function* OR lab* OR stimulat* OR provocat* OR toleranc* OR insulin* OR growth-hormon* OR GH OR somatotropin*) NEAR/2 (test OR tested OR tests OR testing OR result* OR research*)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/2 (peak* OR stimul* OR provocat* OR increas* OR enhanc* OR respons* OR induc*)) OR ((insulin*) NEAR/2 (hypoglycem* OR status)) OR ((growth-hormon* OR GH OR somatotropin*) NEAR/2 (releas*-hormone* OR releas*-factor*)))) AND ((adolescen* OR preadolescen* OR infan* OR newborn* OR (new NEAR/1 born*) OR baby OR babies OR neonat* OR prematur* OR pre-matur* OR child* OR kid OR kids OR toddler* OR teen* OR boy* OR girl* OR minors OR underag* OR (under NEAR/1 (age* OR aging OR ageing)) OR juvenil* OR youth* OR kindergar* OR puber* OR pubescen* OR prepubescen* OR prepubert* OR pediatric* OR paediatric* OR school* OR preschool* OR highschool* OR suckling* OR PICU OR NICU OR PICUs OR NICUs)) NOT ((animal* OR rat OR rats OR mouse OR mice OR murine OR dog OR dogs OR canine OR cat OR cats OR feline OR rabbit OR cow OR cows OR bovine OR rodent* OR sheep OR ovine OR pig OR swine OR porcine OR veterinar* OR chick* OR zebrafish* OR baboon* OR nonhuman* OR primate* OR cattle* OR goose OR geese OR duck OR macaque* OR avian* OR bird* OR fish*) NOT (human* OR patient* OR women OR woman OR men OR man))) AND DT=(Article OR Review)

Google Scholar (random top-100)

obese|obesity|adipositas hyposomatotropinism|”growth hormone”|”somatotropin C”|”somatomedin C”|”growth factor 1|I” clonidin|arginin|glucagon|glukagon|”diagnosis|blood|function|lab|stimulation|provocation|tolerance|insulin test|tests” child|children|adolescent

Supplementary table S1. Growth hormone stimulation test characteristics and outcomes of cohorts from all included studies. In total, 58 studies were included providing data on n=5135 children.

Study (first author)	Stimulation agents	n patients			Mean peak GH M ± SD µg/L			Peak GH cut-off µg/L	No GHD patients failing GHST n (%)	
		GHD+	No GHD, OB+	No GHD OB-	GHD+	No GHD, OB+	No GHD, OB-		No GHD, OB+	No GHD, OB-
Referred for short stature										
Patel *et al.*, 1994[1]	ARG	-	16	160	-	8.0 ± 4.3	13.4 ± 8.6	<7.5	3 (19)	15 (9)
Stanley *et al.*, 2009[2]	Several[b]	-	2	114	-	6.4 ± 2.7	15.5 ± 7.9	<5, <7, <10	1 (50) 1 (50) 2 (100)	8 (7) 14 (12) 32 (28)
Lee *et al.*, 2011[3]	CLON/DOPA/ITT	66	-	121	8.0 ± 3.2	-	21.3 ± 9.5	<10	-	NM
Loche *et al.*, 2011[4]	CLON	23	5	171	5.0 ± 2.9	8.9 ± 5.5	14.2 ± 3.4	<10	3 (60)	47 (27)
Lee *et al.*, 2013[5]	CLON	-	3	85	-	16.8 ± 5.3	24.9 ± 10.1	<10	0	2 (2)
	DOPA				-	14.1 ± 7.2	16.9 ± 9.3	<10	1 (33)	25 (29)
Barrett *et al.*, 2014[6]	ARG/CLON	11	NM	NM	NM[a]	NM[a]	NM[a]	<5, <7, <10	All NM	All NM
Yang *et al.*, 2019[7]	CLON/DOPA/ITT	460	-	-	6.5 ± 2.5	-	-	<10	-	-
Yau *et al.*, 2019[8]	ARG+DOPA	155	1	159	7.6 ± 2.1	16.9	16.2 ± 5.7	<10	NM	NM
Case-control design										
Wegienka *et al.*, 1967[9]	ITT	-	2	-	-	11.5 ± 1.5	-	NM	-	-
Croughs *et al.*, 1968[10]	ITT	9	5	13	2.0 ± 2.4	2.8 ± 1.9	39.3 ± 33.6	<5	4 (80)	0
Kaplan *et al.*, 1968[11]	ITT	18	1	30	1.3 ± 1	7.6	12.5 ± 6.6	NM	-	-
Carnelutti *et al.*, 1970[12]	ITT	-	11*	14	-	14.8 ± 20.0	28.4 ± 14.0	NM	-	-
	ARG		13*		-	6.5 ± 6.5	24.7 ± 11.4	NM	-	-
Weber *et al.*, 1970[13]	GLU	-	7	28	-	5.1 ± 2.7	10.4 ± 5.7	NM	-	-
Parra *et al.*, 1971[14]	ARG	-	17	8	-	5.8 ± 6.6	15.4 ± 10.5	NM	-	-

Girard *et al.*, 1972[15]	ITT	-	21	59	-	16.3 ± 9.8	20.3 ± 15.0	<9	6 (29)	NM
Komatsu *et al.*, 1973[16]	ITT	1	8		2.5	12.5 ± 5.0	-	NM	-	-
Vanderschueren *et al.*, 1974[17]	GLU/ITT	16	1	26	0.3 ± 0.4	1	34.3 ± 19.8	NM	-	-
Josefsberg *et al.*, 1976[18]	ITT	-	42	278	-	7.5 ± 6.3	13.5 ± 6.5	<6	15 (36)	57 (21)
	ARG	-	40	357	-	8.1 ± 6.0	12.8 ± 5.5	<6	16 (40)	69 (19)
Topper *et al.*, 1984[19]	CLON	-	13	6	-	18.0 ± 7.4	26.2 ± 12.4	<10	3 (23)	0
	ITT				-	10.5 ± 5.0	10.0 ± 5.5	<10	7 (54)	3 (50)
Pertzelan *et al.*, 1986[20]	GHRH	-	9	-	-	9.6 ± 6.7	-	NM	-	-
Pintor *et al.*, 1986[21]	GHRH	14*	4	18*	11.7 ± 11.7	4.4 ± 3.1	25.9 ± 14.9	<5	3 (75)	NM
	CLON	7*		10*	2.3 ± 0.8	2.7 ± 0.8	9.7 ± 3.6	<5	4 (100)	NM
	ITT	14*		19*	2.3 ± 0.8	3.9 ± 1.5	7.8 ± 2.1	<5	3 (75)	NM
Ranke *et al.*, 1986[22]	GHRH	39	3	26	8.4 ± 9.4	10.8 ± 6.2	72.4 ± 68.4	<10	1	0
	ITT		-	-	3.3 ± 2.2	-	-	<10	-	-
	ARG		-	-	4.0 ± 2.6	-	-	<10	-	-
Van Vliet *et al.*, 1986[23]	GHRH	7	11	16	8.3 ± 7.7	10.5 ± 8.9	32.6 ± 19.6	<5	3 (27)	0
Loche *et al.*, 1987[24]	GHRH	-	15	15	-	11.2 ± 7.6	NM	<10	10 (67)	NM
Rosskamp *et al.*, 1987[25]	GHRH	-	20	20	-	23.7 ± 16.1	41.1 ± 13.4	<10	7 (35)	0
Cordido *et al.*, 1989[26]	GHRH	-	2	-	-	10.7 ± 1.1	-	NM	-	-
	GHRH+PD				-	42.5 ± 31.8	-	NM	-	-
Ghigo *et al.*, 1989[27]	GHRH	-	6	11	-	8.0 ± 2.0	20.3 ± 15.3	NM	-	-
	GHRH+PD				-	28.3 ± 11.0	58.2 ± 25.5	NM	-	-
Loche *et al.*, 1989[28]	GHRH	-	11	8	-	6.4 ± 5.0	21.1 ± 11.0	NM	-	-
	GHRH+PD				-	25.9 ± 14.6	44.4 ± 11.3	NM	-	-

Cordido *et al.*, 1990[29]	CLON	-	2	-	-	5.7 ± 0.6	-	NM	-	-
	CLON+PD				-	14.5 ± 0.1	-	NM	-	-
	ARG	-	1	-	-	4.1	-	NM	-	-
	ARG+PD				-	15.2	-	NM	-	-
	ITT	-	1	-	-	14.3	-	NM	-	-
	ITT+PD				-	14.8	-	NM	-	-
Loche *et al.*, 1990[30]	GHRH	-	5	7	-	7.6 ± 3.6	38.9 ± 28.6	NM	-	-
	GAL				-	6.0 ± 2.7	8.8 ± 3.71	NM	-	-
	GHRH+GAL				-	30.2 ± 7.2	73.2 ± 11.1	NM	-	-
Reiter *et al.*, 1991[31]	GHRH	-		12	-		39.2 ± 17.7	NM	-	-
Singh *et al.*, 1991[32]	ITT	-	20	20	-	2.2 ± 1.6	9.8 ± 12.6	<7	20 (100)	4 (20)
	CLON				-	3.2 ± 5.0	15.2 ± 11.4	<7	18 (90)	3 (15)
Tanaka *et al.*, 1991[33]	ARG	-	6	-	-	21.9 ± 10.8	-	<10	0	-
	DOPA				-	5.2 ± 0.7	-	<5	3 (50)	-
	GHRH				-	11.3 ± 5.1	-	<10	2 (33)	-
Tanaka *et al.*, 1991[34]	Several[c]	942	-	-	NM[a]	-	-	<10	-	-
Loche *et al.*, 1992[35]	GHRH	-	9	8	-	14.0 ± 8.4	39.1 ± 23.2	NM	-	-
	GHRH+ATEN				-	26.2 ± 12.9	65.8 ± 34.2	NM	-	-
Loche *et al.*, 1992[36]	DOPA	-	6	7	-	7.9 ± 4.6	20.5 ± 13.0	NM	-	-
Cappa *et al.*, 1993[37]	GHRH+PD	-	8	9	-	14.3 ± 9.0	52.2 ± 27.1	<10	3 (38)	0
Loche *et al.*, 1993[38]	GHRH	-	6	8	-	10.6 ± 4.9	39.1 ± 23.2	NM	-	-
Martul *et al.*, 1993[39]	CLON	5	2	17	5.4 ± 3.4	2.6 ± 0.6	14.4 ± 4.5	<10	NM	NM
	GAL	2	5	10	2.1 ± 1.3	3.0 ± 1.3	7.1 ± 2.2	<10	NM	NM
	GHRH	11	6	22	30.4 ± 24.9	8.7 ± 5.1	37.8 ± 25.8	<10	NM	NM
	GHRP6	9	6	11	12.5 ± 14.4	14.5 ± 6.4	25.3 ± 11.3	<10	NM	NM

Loche *et al.*, 1995[40]	GHRH	5	10	45	NM	5.8 ± 2.5	19.6 ± 19.3	NM	-	-
	HEX				NM	19.7 ± 13.9	52.3 ± 67.0	NM	-	-
Vaccaro *et al.*, 1995[41]	CLON	-	6	18	-	NM	NM	<10	4 (67)	0
Volta *et al.*, 1995[42]	GHRH	-	27	26	-	8.8 ± 6.5	25.4 ± 28.6	NM	-	-
	GHRH+ITT				-	5.9 ± 8.9	17.5 ± 17.0	NM	-	-
	GHRH+CLON				-	4.7 ± 4.0	22.4 ± 21.2	NM	-	-
	GHRH+ARG				-	6.7 ± 5.4	9.1 ± 5.5	NM	-	-
Bideci *et al.*, 1997[43]	ITT	-	42	40	-	12.8 ± 2.5	NM	NM	-	-
	DOPA				-	11.2 ± 3.2	NM	NM	-	-
Coutant *et al.*, 1998[44]	GHRH	-	42	-	-	11.6 ± 9.7	-	NM	-	-
Pirazzoli *et al.*, 1999[45]	ARG	29		27	NM[a]	-	NM[a]	<8	NM	NM
	DOPA				NM[a]	-	NM[a]	<8	NM	NM
Misra *et al.*, 2008[46]	GHRH+ARG	-	15	15	-	19.1 ± 2.1	35.5 ± 1.9	<9	3 (20)	1 (7)
Perotti *et al.*, 2013[47]	GHRH+ARG	-	-	30*	-	-	51.5 ± 28.1	<19	-	2 (7)
	ITT	-	-	2*	-	-	11.8 ± 0.5	<6.1	-	0
Liang *et al.*, 2018[48]	ARG/DOPA	-	78	30	-	4.1 ± 3.9	16.3 ± 4.4	<10	70 (90)	NM
Syndromic obesity										
Pertzelan *et al.*, 1986[20]	GHRH	3	-	-	2.6 ± 0.9	-	-	NM	-	-
Costeff *et al.*, 1990[49]	CLON	4	-	2	0.6 ± 0.1	-	7.7 ± 0.1	<5	-	0
Cappa *et al.*, 1993[37]	GHRH+PD	1	8	-	0.7	17.1 ± 8.6	-	<10	2 (25)	
Beccaria *et al.*, 1996[50]	CLON	4	2	5	1.4 ± 0.8	5.4 ± 4.5	10.2 ± 7.4	<7	1 (50)	2 (40)
	GHRH+PD				13.0 ± 4.5	17.3 ± 3.9	24.2 ± 12.3	<20	1 (50)	1 (20)
Thacker *et al.*, 1998[51]	ARG	12*	3*	1*	4.3 ± 2.7	14.4 ± 2.3	36	<10	0	0
	CLON	2	-	-	2.9 ± 1.2	-	-	<10	-	-
	DOPA	12*	3*	1*	3.5 ± 2.4	14.0 ± 10.7	14.9	<10	1 (33)	0

Casamitjana *et al.*, 2021[52]	GHRH+ARG	-	1	3	-	11.4	18.1 ± 6.7	BMIadjusted[e]	0	0
	GLU				-	4.6	9.3 ± 7.1	<1, <3	0 0	0 1 (33)
Reiter *et al.*, 1991[31]	GHRH	-	6	11	-	6.9 ± 1.9	22.6 ± 16.2	NM	-	-
Pasquino *et al.*, 1992[53]	CLON	4	1	10	3.3 ± 1.7	7.3	8.6 ± 7.0	<10	1 (100)	7 (70)
	GHRH				5.2 ± 2.4	14.5	22.3 ± 7.0	<10	1 (100)	7 (70)
Patel *et al.*, 1994[1]	ARG	-	10	38	-	NM	NM	<7.5	NM	NM
Vaccaro *et al.*, 1995[41]	CLON	-	8	7	-	NM	NM	<10	NM	NM
Cavallo *et al.*, 1999[54]	Several[d]	109	13	178	NM[a]	NM[a]	NM[a]	<10	-	-
Pirazzoli *et al.*, 1999[45]	ARG DOPA	33	15	27	NM[a]	NM[a]	NM[a]	<8	NM	NM
Soliman *et al.*, 1996[55]	CLON	1	4	-	2.1	7.1 ± 3.9	-	<5	2 (50)	-
Germain-Lee *et al.*, 2003[56]	ARG+DOPA	6	-	2*	4.9 ± 2.7	-	36.5 ± 32.5	<10	-	0
	GHRH+ARG			1*	13.5 ± 7.1	-	39.5	<20	-	0
de Sanctis *et al.*, 2007[57]	GHRH+ARG	2	4	4	7.3 ± 0.9	12.3 ± 6.5	31.1 ± 10.1	<20	3 (75)	0
Schott *et al.* 2016[58]	ARG	5	12*	9*	4.5 ± 1.5	7.4 ± 1.1	10.9 ± 2.8	<7	1 (33)	1 (13)
	CLON		11*	8*	5.4 ± 0.9	9.3 ± 4.3	12.3 ± 9.0	<7	1 (33)	3 (33)

Abbreviations: GHST, growth hormone stimulation test; GH(D), growth hormone (deficiency); No GHD, OB+, children without GHD with obesity; No GHD, OB-: children without GHD without obesity; GHD+, children with GHD; M, mean; SD, standard deviation; ARG, arginine; CLON, clonidine; DOPA, dopamine; PROP, propranolol; ITT, insulin tolerance test; GHRH, growth hormone-releasing hormone; GLU, glucagon; GAL, galanin; ATEN, atenolol; GHRP-6, growth hormone-releasing peptide 6; HEX, hexarelin; NM, not mentioned, PWS, Prader-Willi syndrome; BBS, Bardet-Biedl syndrome; PHP1a, pseudohypoparathyroidism type 1a.

Legend: [a] Study did not stratify outcomes on GHD and weight status; [b] Used stimulation agents: ARG+CLON/DOPA+PROP/CLON+DOPA+PROP/ARG+DOPA; [c] Used stimulation agents: ARG/CLON/DOPA/GHRH/GLU/GLU+PROP/ITT; [d] Used stimulation agents: ARG/CLON/DOPA/ITT. [e]BMI-adjusted cut-offs according to Corneli et al.[59]

In the columns with patient numbers, a '' sign indicates ≥1 separate GHST performed in the same patient.*

In the column regarding type of GHST, a '+' sign indicates a combined test, whereas a '/' sign indicates separate tests, but test results were not presented stratified on type of GHST. E.g., "GHRH+ARG" indicates a single test consisting of administration of both GHRH and arginine, whereas "CLON/ARG" indicates that patients either received a clonidine test or an arginine test, but results were not stratified on stimulation agent.

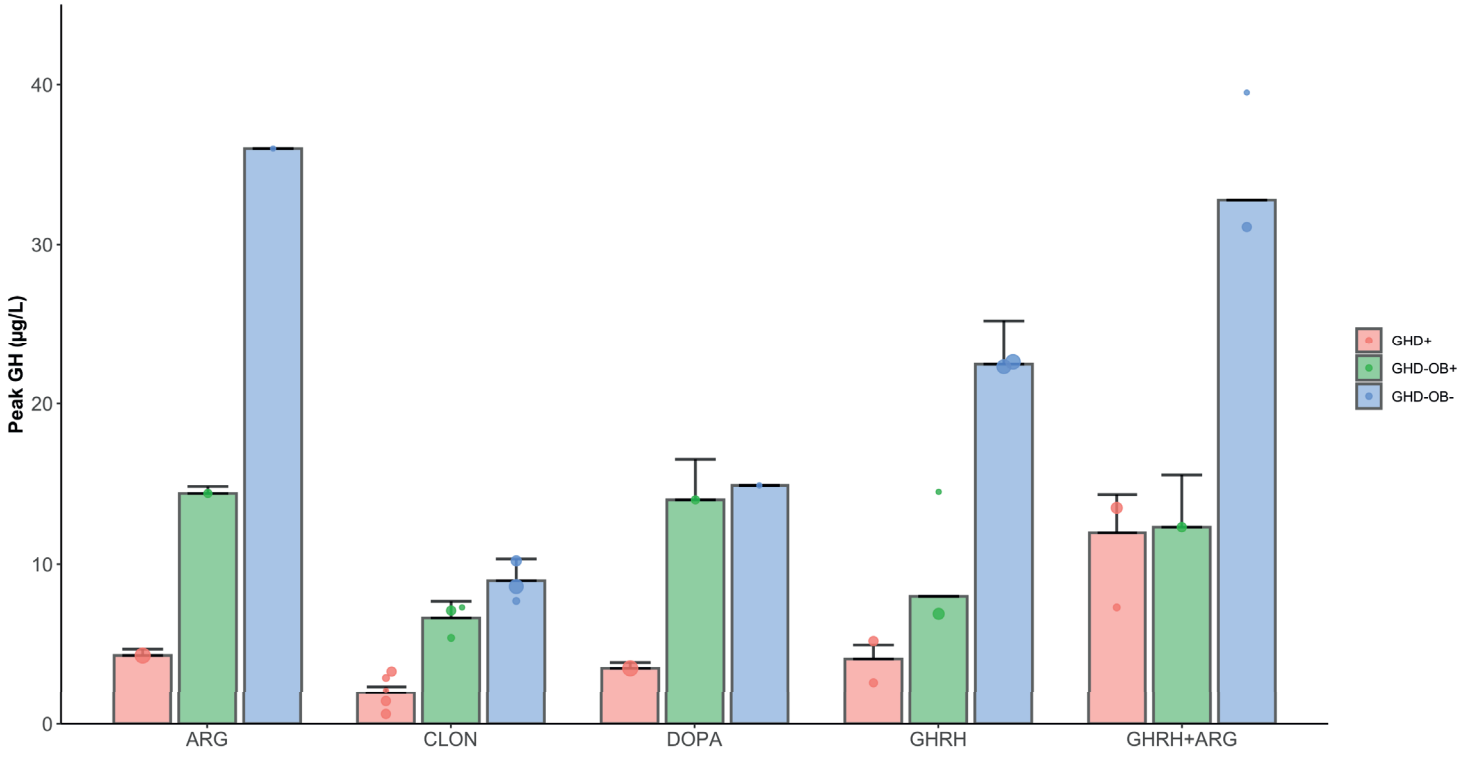
Supplementary table S2. Risk of bias assessment of included studies.

Study	SIGN cohort question 1.1	SIGN cohort question 1.2	SIGN cohort question 1.3	SIGN cohort question 1.7.	SIGN cohort question 1.10	SIGN Case-control question 1.3	SIGN Case-control question 1.7	SIGN diagnostic accuracy question 1.1	SIGN diagnostic accuracy question 1.4	SIGN diagnostic accuracy question 2.2	SIGN Risk of Bias assessment
Cohort referred for short stature											
Patel *et al.*, 1994[1]	Yes	Yes	No	CS	No	Yes	Yes	CS	Yes	Yes	Low
Stanley *et al.*, 2009[2]	Yes	Yes	No	CS	CS	Yes	No	Yes	Yes	No	High
Lee *et al.*, 2011[3]	Yes	NA	No	Yes	CS	Yes	Yes	Yes	Yes	Yes	Medium
Loche *et al.*, 2011[4]	Yes	Yes	No	Yes	No	CS	No	Yes	Yes	Yes	Medium
Lee *et al.*, 2013[5]	Yes	Yes	Yes	CS	Yes	Yes	Yes	Yes	Yes	Yes	Medium
Barrett *et al.*, 2014[6]	Yes	Yes	No	CS	No	Yes	No	Yes	Yes	Yes	High
Yang *et al.*, 2019[7]	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes	No	Yes	Medium
Yau *et al.*, 2019[8]	Yes	Yes	Yes	Yes	CS	Yes	Yes	Yes	Yes	Yes	Low
Case-control design											
Wegienka *et al.*, 1967[9]	Yes	NA	No	CS	No	Yes	Yes	CS	No	No	High
Croughs *et al.*, 1968[10]	Yes	CS	No	CS	CS	CS	CS	CS	CS	No	High
Kaplan *et al.*, 1968[11]	Yes	No	No	CS	CS	CS	Yes	CS	No	No	High
Carnelutti *et al.*, 1970[12]	Yes	Yes	No	CS	CS	Yes	Yes	CS	Yes	No	High
Weber *et al.*, 1970[13]	Yes	Yes	No	CS	No	CS	CS	CS	Yes	No	High
Parra *et al.*, 1971[14]	Yes	Yes	No	CS	CS	CS	Yes	CS	Yes	No	High
Girard *et al.*, 1972[15]	No	CS	No	CS	No	CS	CS	CS	CS	Yes	High
Komatsu *et al.*, 1973[16]	Yes	CS	No	CS	No	CS	CS	CS	CS	No	High
Vanderschueren *et al.*, 1974[17]	Yes	CS	No	No	CS	CS	CS	CS	CS	No	High
Josefsberg *et al.*, 1976[18]	Yes	Yes	No	CS	No	CS	Yes	CS	Yes	Yes	High
Topper *et al.*, 1984[19]	Yes	No	No	CS	No	CS	Yes	CS	No	Yes	High
Pintor *et al.*, 1986[21]	Yes	CS	No	Yes	CS	CS	Yes	CS	CS	Yes	Medium
Ranke *et al.*, 1986[22]	Yes	CS	No	Yes	CS	CS	Yes	CS	No	Yes	Medium
Van Vliet *et al.*, 1986[23]	Yes	No	No	Yes	CS	CS	Yes	CS	No	Yes	Medium
Loche *et al.*, 1987[24]	Yes	Yes	No	CS	CS	CS	Yes	CS	No	No	High
Rosskamp *et al.*, 1987[25]	Yes	Yes	No	CS	No	CS	Yes	CS	No	No	High
Cordido *et al.*, 1989[26]	Yes	Yes	No	CS	No	CS	Yes	CS	Yes	No	High
Ghigo *et al.*, 1989[27]	Yes	No	No	CS	CS	Yes	Yes	CS	Yes	No	High
Loche *et al.*, 1989[28]	Yes	No	No	CS	CS	CS	Yes	CS	CS	No	High

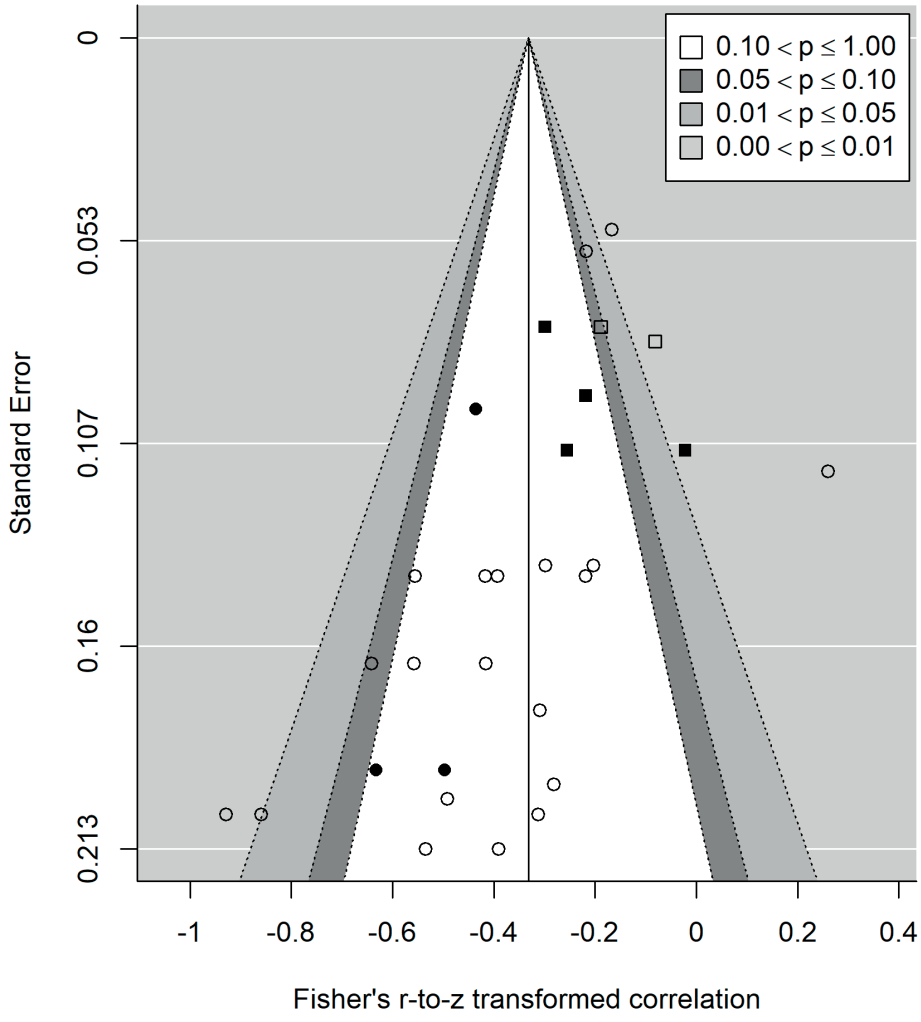
Cordido *et al.*, 1990[29]	Yes	Yes	No	CS	No	CS	CS	CS	Yes	No	Medium
Loche *et al.*, 1990[30]	Yes	CS	No	CS	No	CS	Yes	CS	No	No	Medium
Singh *et al.*, 1991[32]	Yes	Yes	No	CS	Yes	CS	CS	CS	Yes	Yes	High
Tanaka *et al.*, 1991[33]	Yes	NA	No	CS	Yes	CS	CS	CS	No	Yes	Medium
Tanaka *et al.*, 1991[34]	Yes	Yes	Yes	Yes	No	CS	NA	Yes	No	Yes	High
Loche *et al.*, 1992[24]	Yes	CS	No	CS	CS	CS	CS	CS	CS	No	High
Loche *et al.*, 1992[35]	Yes	CS	No	CS	CS	CS	CS	CS	CS	No	High
Loche *et al.*, 1993[38]	Yes	No	No	CS	CS	CS	Yes	CS	No	No	High
Martul *et al.*, 1993[39]	Yes	Yes	No	Yes	No	CS	Yes	CS	Yes	Yes	Medium
Loche *et al.*, 1995[40]	Yes	No	No	CS	CS	CS	Yes	CS	CS	No	High
Volta *et al.*, 1995[42]	Yes	No	No	CS	CS	CS	Yes	CS	No	No	High
Bideci *et al.*, 1997[43]	Yes	Yes	No	CS	Yes	CS	Yes	CS	Yes	No	High
Coutant *et al.*, 1998[44]	Yes	Yes	No	CS	No	CS	Yes	CS	Yes	No	High
Misra *et al.*, 2008[46]	Yes	Yes	Yes	CS	Yes	Yes	Yes	CS	Yes	Yes	Low
Perotti *et al.*, 2013[47]	Yes	NA	No	CS	NA	Yes	Yes	CS	CS	Yes	Medium
Liang *et al.*, 2018[48]	Yes	Yes	No	CS	Yes	Yes	Yes	CS	Yes	Yes	High
Syndromic obesity											
Pertzelan *et al.*, 1986[20]	Yes	NA	No	CS	CS	CS	CS	CS	No	No	High
Costeff *et al.*, 1990[49]	Yes	Yes	No	Yes	CS	CS	No	CS	No	Yes	Medium
Reiter *et al.*, 1991[31]	Yes	No	No	CS	CS	CS	Yes	CS	No	No	High
Pasquino *et al.*, 1992[53]	Yes	Yes	No	Yes	No	CS	CS	CS	Yes	Yes	High
Cappa *et al.*, 1993[37]	Yes	No	No	CS	CS	CS	Yes	CS	No	No	High
Vaccaro *et al.*, 1995[41]	Yes	No	No	CS	No	Yes	Yes	CS	No	Yes	High
Beccaria *et al.*, 1996[50]	Yes	Yes	No	CS	CS	CS	CS	CS	No	Yes	High
Soliman *et al.*, 1996[55]	Yes	Yes	No	CS	Yes	CS	No	CS	No	No	High
Thacker *et al.*, 1998[51]	Yes	Yes	No	Yes	No	CS	CS	Yes	No	Yes	Medium
Cavallo *et al.*, 1999[54]	Yes	Yes	No	Yes	No	CS	CS	CS	No	Yes	High
Pirazzoli *et al.*, 1999[45]	Yes	No	No	Yes	No	CS	Yes	CS	Yes	Yes	Medium
Germain-Lee *et al.*, 2003[56]	Yes	Yes	No	Yes	Yes	CS	No	CS	No	Yes	Medium
de Sanctis *et al.*, 2007[57]	Yes	Yes	No	Yes	Yes	CS	CS	CS	No	Yes	Medium
Schott *et al.*, 2016[58]	Yes	Yes	No	Yes	CS	CS	CS	CS	Yes	Yes	Medium
Casamitjana *et al.*, 2021[52]	Yes	Yes	No	Yes	Yes	CS	CS	Yes	Yes	Yes	Medium

Abbreviations: SIGN, Scottish Intercollegiate Guidelines Network; CS, Can't say; NA, not applicable.
Legend:
SIGN cohort question 1.1: "The study addresses an appropriate and clearly focused question."
SIGN cohort question 1.2: "The two groups being studied are selected from source populations that are comparable in all respects other than the factor under investigation." Interpretation: did the patients with and without obesity both have short stature, normal stature or differing stature?
SIGN cohort question 1.3: "The study indicates how many of the people asked to take part did so, in each of the groups being studied."
SIGN cohort question 1.10: "The method of assessment of exposure is reliable." Interpretation: was the definition of obesity adequate?
SIGN Case-control question 1.3: "The same exclusion criteria are used for both cases and controls."
SIGN Case-control question 1.7: "It is clearly established that controls are non-cases."
SIGN diagnostic accuracy question 1.1: "A consecutive sequence or random selection of patients is enrolled."

SIGN diagnostic accuracy question 1.4: "The included patients and settings match the key question" Interpretation: do both patients with and without obesity have short stature and are they tested in a clinical setting for the possible presence of growth hormone deficiency?"
SIGN diagnostic accuracy question 2.2: "If a threshold is used, it is pre-specified." Interpretation: was the threshold that was used to indicate a failed growth hormone stimulation test pre-specified?
SIGN Risk of Bias assessment: Ultimate risk of bias assessment: high (SIGN: "unacceptable – reject"), medium (SIGN: acceptable), or low (SIGN: high quality).



Supplementary figure S1. Weighted mean peak GH for children with syndromic obesity.
Data were available from n=117 children from k=12 subcohorts. The dots represent the mean of individual studies and the barplot represents the weighted mean peak GH ± SEM. Legend: GHD+: children with growth hormone deficiency; No GHD, OB+: children with obesity without growth hormone deficiency; No GHD, OB-: children without obesity without growth hormone deficiency; ARG, arginine test; CLON, clonidine test; DOPA, dopamine test; GHRH, growth hormone-releasing hormone test; GHRH+ARG, combined growth hormone-releasing hormone + arginine test; ITT, insulin tolerance test.
Abbreviations: GH, growth hormone; GHD, growth hormone deficiency; SEM, standard error of the mean.



Supplementary figure S2. Funnel plot
Contour-enhanced funnel plot. The squares indicate cohort studies, whereas the dots indicate studies with a case-control design. The black squares/dots represent studies with correlation coefficients originally provided by the authors. The open squares/dots represent studies for which correlation coefficients were calculated for this meta-analysis.

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8

Cross-sectional relation of long-term glucocorticoids in hair with anthropometric measurements and their possible determinants: a systematic review and meta-analysis

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ABSTRACT

Background Long-term glucocorticoids (HairGC) measured in scalp hair have been associated with body mass index (BMI), waist circumference (WC), and waist-hip-ratio (WHR) in several cross-sectional studies. We aimed to investigate the magnitude, strength, and clinical relevance of these relations across all ages.

Methods We performed a systematic review and meta-analysis (PROSPERO registration CRD42020205187) searching for articles relating HairGC to measures of obesity. Main outcomes were bivariate correlation coefficients and unadjusted simple linear regression coefficients relating hair cortisol (HairF) and hair cortisone (HairE) to BMI, WC, and WHR.

Results We included k=146 cohorts (n=34,342 individuals). HairGC were positively related to all anthropometric measurements. The strongest correlation and largest effect size were seen for HairE-WC: pooled correlation 0.18 (95%CI 0.11-0.24; k=7; n=3,158; I^2=45.7%), pooled regression coefficient 11.0cm increase in WC per point increase in 10-log-transformed HairE (pg/mg) on liquid-chromatography-(tandem) mass spectrometry (LC-MS) (95%CI 10.1-11.9cm; k=6; n=3,102). Pooled correlation for HairF-BMI was 0.10 (95%CI 0.08-0.13; k=122; n=26,527; I^2=51.2%) and pooled regression coefficient 0.049kg/m^2 per point increase in 10-log-transformed HairF (pg/mg) on LC-MS(95%CI 0.045-0.054 kg/m^2; k=26; n=11,635).

Discussion There is a consistent positive association between HairGC and BMI, WC, and WHR, most prominently and clinically relevant for HairE-WC. These findings overall suggest an altered setpoint of the hypothalamic-pituitary-adrenal axis with increasing central adiposity.

BACKGROUND

The prevalence of obesity, defined in adults as a body mass index (BMI; weight in kg divided by height in meters squared) ≥30 kg/m^2, has increased dramatically worldwide over the past decades[1]. An imbalance between energy intake and expenditure is regarded as the major cause of obesity. Numerous distinct characteristics and conditions can contribute to obesity within an individual[2]. One important contributing factor may be chronic exposure to the stress hormone cortisol, the major end-product of the hypothalamic-pituitary-adrenal (HPA) axis. In healthy individuals, cortisol secretion and metabolism are closely linked and tightly regulated. Cortisol is converted by 11-beta-hydroxysteroid dehydrogenase type 2 (11ß-HSD-2) to the biologically inactive cortisone in end-organ tissues, but can be converted back to cortisol by 11-beta-hydroxysteroid dehydrogenase type 1 (11ß-HSD-1) on tissue-level[3]. Exposure to very high levels of endogenous or exogenous glucocorticoids (GC), such as in Cushing's syndrome, leads to a phenotype characterized by abdominal obesity and other features of the metabolic syndrome[4,5]. It is hypothesized that even a chronic mild increase of GC, *i.e.,* in the high-physiological range, can contribute to overweight and obesity in the general population[2]. Despite many efforts over the last decades to explore this relation in different matrices such as blood, saliva and urine, conflicting results were found[6]. This may be due to cortisol's circadian rhythm, its pulsatile secretion and the daily variation following changing circumstances such as acute stress. Hence, measurements that reflect a shorter term (minutes or hours for serum and saliva, days for urine) seem less suitable to investigate this association in the general population[7].

In the past decennium, a relatively novel technique has allowed researchers to study long-term levels of GC by measuring cortisol and cortisone levels in scalp hair (HairF and HairE, respectively). Every centimeter of scalp hair is believed to represent the cumulative GC exposure of one month[8]. HairGC measurements are now considered an easily applicable, non-invasive and reproducible method for assessing long-term GC exposure[8]. A systematic review and meta-analysis by Stalder *et al*. that was conducted in September 2015 (when the number of studies that used HairGC started to increase rapidly) identified several possible influencers of HairF levels. The authors concluded that variation in HairF levels on study level could be related, among other factors, to differences in mean BMI of the study populations[9]. Gray *et al*. and Ling *et al*. also reported that BMI and BMI standard deviation score (SDS), *i.e.*, BMI z-scores adjusted for age and sex that are most often used in pediatric studies[10], were important determinants of HairF levels in children[11,12]. However, in the last years, many new large-scale studies in various age categories have been published that have investigated the relation between HairGC and anthropometric features. Some of

these studies showed a positive relation[13,14], while other studies showed no relation between HairGC and anthropometric measurements[15,16]. It is unclear whether these conflicting results can be explained by differing population characteristics such as mean age, sex, and prevalence of obesity, use of corticosteroids, handling of outliers, or the various laboratory methods that were used.

Moreover, other anthropometric measurements than BMI are considered equally or even more relevant to cardiometabolic health, such as waist circumference (WC) and waist-hip-ratio (WHR), which both are markers of central adiposity[17]. These deserve specific attention as GC are known to particularly induce abdominal obesity[18]. Likewise, there are suggestions that hair cortisone might correlate stronger to obesity than cortisol itself[19]. However, a meta-analysis that summarizes all evidence considering different anthropometric parameters in association with both HairF and HairE as well as relevant moderators of these relationships is missing.

Therefore, the aim of the current systematic review and meta-analysis was to investigate the cross-sectional relations between HairGC levels (HairF and HairE), and anthropometric measurements (BMI, BMI SDS, WC and WHR), and to explore the possible influence of relevant characteristics of the population and laboratory methods.

METHODS

We performed this systematic review and meta-analysis in concordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and Meta-analysis of Observational Studies in Epidemiology (MOOSE) checklist[20,21]. This systematic review was registered at the PROSPERO database (Registration number CRD42020205187 December 7th 2020)[22].

Search strategy and selection criteria

A university health sciences librarian designed a comprehensive search to identify studies and conference abstracts concerning hair cortisol and/or hair cortisone and measurements of obesity. To avoid missing potentially relevant papers we designed a broad search strategy combining the elements ‘hair’, ‘cortisol/cortisone’, and ‘BMI/WC/WHR/anthropometrics’, including their synonyms without any restrictions other than ‘studies in humans’. The search was conducted in the following databases from inception up to 16 November 2020: Medline (Ovid), Embase, Cochrane, Web of Science, Scopus, Cinahl, PsycInfo, and Google Scholar. The complete search strategy is provided in the Supplementary Appendix. Search results were exported to reference

management software (EndNote version X9, Clarivate Analytics) and duplicates were removed prior to screening.

All identified studies were independently screened in two stages by two physicians (EV, OA, or MM) with a background in adult (EV, MM) and pediatric (OA) endocrinology. All studies that reported original HairGC data in humans were included in the title/abstract screening stage and were subsequently assessed full-text. Disagreements were solved by discussion among the first authors (EV, OA, MM) and the senior author (EvR) until consensus was reached. Additionally, reference lists of all included studies and relevant reviews were screened systematically for potentially relevant articles[23]. We included studies that reported cross-sectional associations between HairGC and measurements of obesity. We excluded case-reports, animal studies, review articles, non-English or non-peer reviewed studies, and studies in which hair sampling and weight measurements were not performed simultaneously (ure 1). Pediatric studies that only included children younger than age 2 years were also excluded because BMI-based definitions of obesity are not available for this age group[10]. We contacted all corresponding authors of articles that reported both HairGC and anthropometric data but did not report an association between these two outcomes to ask if they could provide us with an association measure. Of articles that also included patients with mental or physical diseases that are known to influence the relation between GCs and obesity, we only included the separate analyses of healthy controls if available. When data of the same participants were reported in several studies, we included the study that reported a bivariate association (correlation coefficient or unstandardized simple linear regression coefficient) between HairGC and measurements of obesity. If more than one article reported a bivariate association, we included the study with the largest sample size.

Data Extraction

Descriptive, methodological and outcome data were extracted from all included studies by two researchers independently (EV, OA, or MM) using a predesigned standardized data extraction sheet. Discrepancies were resolved by discussion among the first authors (EV, OA, MM) and the senior author (EvR). The following descriptive data were extracted: study population characteristics (sample size and cohort characteristics: age, sex, prevalence of obesity, mean levels of HairF and HairE in pg/mg) and laboratory methods: liquid chromatography-(tandem) mass spectrometry based measurements (LC-MS or LC-MS/MS, in this review further collectively abbreviated as LC-MS), enzyme-linked immunosorbent assays (ELISA), or chemiluminescent immunoassays (CLIA). The reported outcomes of interest were any cross-sectional associations between HairGC (HairF, HairE) and measurements of obesity, *i.e.*, BMI, BMI SDS, WC, and

WHR. In studies presenting multiple data points of the same participants (e.g. before and after an intervention), only baseline associations were extracted. When insufficient data were reported for meta-analysis, corresponding authors were contacted twice in a two-week time frame. In case of non-response, data were extracted from previous meta-analyses where possible[9,12].

Risk of bias assessment

Risk of bias was assessed by two researchers independently (EV, OA, or MM) using the Quality In Prognostic Studies (QUIPS) tool[24]. In short, the QUIPS tool aids in the assessment of potential bias sources from the following study domains: study participation, study attrition, prognostic factor measurement, outcome measurement, confounding measurement, and statistical analysis. The subdomains on which risk of bias was assessed were: population selection criteria (QUIPS 1; study participation), the used laboratory methods (QUIPS 3; prognostic factor measurement), whether or not anthropometric measurements were objectively measured (QUIPS 4; outcome measurement), whether or not corticosteroid use was taken into account and whether any consideration was given to handling outliers in HairGC values (QUIPS 5; study confounding), and reporting of relevant statistics (QUIPS 6; statistical analysis and reporting). All subdomains were scored as 'low', 'moderate', or 'high' risk of bias on individual cohort level. We omitted the study attrition domain of the QUIPS tool (QUIPS 2) since it was not applicable to our cross-sectional research question. Discrepancies between the researchers were solved by discussion among the first authors (EV, OA, MM) and the senior author (EvR).

Qualitative synthesis

For the qualitative synthesis, we summarized all authors' conclusions regarding cross-sectional associations between HairGC levels and obesity measurements, *i.e.*, correlation coefficients, regression coefficients, or comparison of HairGC levels and obesity measurements across categories.

Statistical analysis

All meta-analyses were conducted in R version 3.6.3 with an α of 0.05[25]. For all descriptive data, median and (interquartile) range were converted to means and standard deviations prior to analyses[26]. Furthermore, subgroup means from individual studies as well as the pooled means across all studies were pooled[27]. When not originally reported, standard errors were calculated based on reported confidence intervals or p-values and degrees of freedom using the T-distribution.

Meta-analysis of correlation coefficients

For all studies reporting bivariate correlations (correlation coefficients), Fisher's r-to-z transformation was applied to transform individual correlations stratified on all combinations of HairGC (HairF, HairE) and obesity measurements (BMI/BMI SDS, WC, WHR). As several studies reported correlations within distinct subgroups, we calculated the pooled correlation coefficients, 95% confidence intervals (CIs) and prediction intervals (PIs) using multilevel random effects models[28,29]. One study was excluded for all meta-analyses, as the reported correlation coefficient for BMI vs. HairF of the total cohort was 0.91. We assume this is a typographic error, as the authors state that they only found a statistically significant correlation in the highest tertile of the polygenic susceptibility score (which was reported to be 0.269, making a correlation of 0.91 for the total cohort impossible)[30]. These authors did not respond to our contact attempts.

The I^2 statistic and Cochrane's Q test were used for the assessment of between-study heterogeneity, with I^2 >25% and p-value for Cochrane's Q test <0.05 indicating heterogeneity. For all meta-analyses with data from at least 10 cohorts, exploratory moderator analyses were performed using mixed-effect models for categorical parameters (e.g., used laboratory method) and random-effects models for continuous parameters (e.g., mean age of the study participants). Publication bias was assessed using contour-enhanced funnel plots.

Meta-analysis of unstandardized simple linear regression coefficient

For all studies reporting unstandardized simple linear regression coefficient between 10-log transformed HairGC (HairF or HairE) in pg/mg as independent variable and untransformed obesity measurements (BMI, BMI SDS, WC, WHR) as dependent variable, pooled regression coefficients and 95% CIs were calculated using the statistical approach described by Bini *et al.* and Becker & Wu[31,32]. In short, this approach allows pooling of linear regression coefficients using weighted least squares provided that the independent and dependent variable have been measured in the same manner across all studies. Therefore, we calculated pooled regression coefficients of 10-log transformed HairGC on untransformed obesity measurements, stratified on laboratory method. Between-study heterogeneity was assessed using the Q_w-statistic described by Bini *et al.*[31].

RESULTS

The literature search identified 1017 unique citation titles of which a total of 120 studies[5,13,14,16,19,30,33-146] comprising 146 separate cohorts were included (Figure 1). This corresponds to a total of 34,342 included participants of which 15,698 (46%) were sampled from general population-based studies (Table 1). The remaining 18,644 (54%) participants were sampled from studies where study inclusion was based on medical criteria (e.g. individuals with obesity), occupational characteristics (e.g. health-care workers), or socio-economic characteristics (e.g. children from low-income parents). The majority of participants (24,004; 70%) were sampled from studies in adults (mean age ≥18 years). Most studies analyzed participants living in Germany (32/146 cohorts, 22%), The Netherlands (23/146 cohorts, 16%) and Canada (18/146 cohorts, 12%). For 70/146 cohorts (48%), correlation coefficients and/or regression coefficients that were not reported in original papers were obtained by contacting authors.

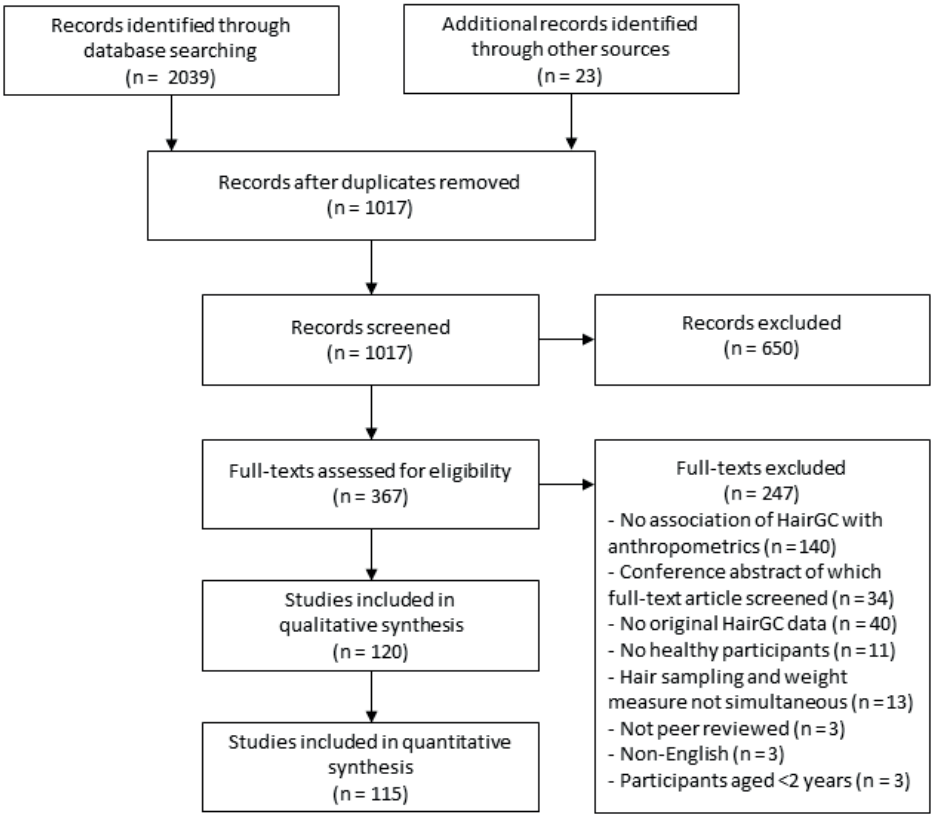

Figure 1. PRISMA flow diagram.
Abbreviations: HairGC, hair glucocorticoids.

Table 1. Overview of included cohorts.

Study	n	Age in years M ± SD	BMI in kg/m^2 or BMI SDS M ± SD	% Male	% Obesity	HairF in pg/mg M ± SD	HairE in g/mg M ± SD	HairGC analysis	Risk of Bias*	Reported bivariate correlations	Reported regression coefficients
Abdulateef *et al*. 2019	65	33.1 ± 10.4	26.4 ± 5.7	9.8	28.1	17.2		ELISA	23311	**AC**	AC
Abell *et al*. 2016	3634	69.8 ± 5.8	26.7 ± 4.5	68.4	19.8	12.6 ± 46.4		LC-MS	21121	**ACD**	**A**
Aguilo *et al*. 2018	53	56.7 ± 12.5	25.1 ± 3.9	30.2	9.4	14.0 ± 9.0		ELISA	23321	**A**	**A**
Berger *et al*. 2019 - Cohort WPHC	207	40.3 ± 16.9	31.5 ± 7.2	44.4		14.2 ± 27.8		ELISA	23331	A	
Berger *et al*. 2019 - Cohort YPC	122	19.4 ± 3.1	25.2 ± 6.9	43.4		7.8 ± 9.3		ELISA	23331	A	
Boesch *et al*. 2014	177	20.1 ± 1.1	23.6 ± 3.1	100		358.8 ± 159.1		ELISA	23131	A	
Bossé *et al*. 2018	598	64.9 ± 6.8	29.3 ± 6.5	80.6	36.9	11.9 ± 26.7		CLIA	32121	**AC**	**AC**
Brianda *et al*. 2020	134		24.6 ± 4.4	7.1		82.3 ± 94.3		ELISA	23311	**A**	**A**
Castro-Vale *et al*. 2020	128	49.1 ± 15.5	27.0 ± 3.9	68		4.7 ± 3.7		LC-MS	21331	A	A
Cedillo *et al*. 2020	62	29.2 ± 7.5†	30.0 ± 7.7†	0	27.0	130.7 ± 124.5†		ELISA	23131		A
Chan *et al*. 2014	57	44.5 ± 12.5†‡	27.6 ± 6.8†‡	45.6	33.3	98.8 ± 74.8†‡		ELISA	33121	AC	
Chen *et al*. 2013	53	40.7 ± 6.6	22.4 ± 2.9	98.11		18.9 ± 13.6		LC-MS	22331	A	
Chen *et al*. 2015 - female adults	75	43.3 ± 8.9	30.2 ± 5.5	0		4.6 ± 3.4		LC-MS	21121	AD	
Chen *et al*. 2015 - male adults	10	41.6 ± 9.2	29.4 ± 1.9	100		3.1 ± 1.5		LC-MS	21121	AD	
Davison *et al*. 2019	344	25.4 ± 1.5†‡	23.7 ± 6.3	43		4.3 ± 4.9	6.3 ± 5.8	LC-MS	11121	AE	
Dettenborn *et al*. 2010 - employed	28	32.6 ± 9.3	22.6 ± 3.8	42.9		7.1 ± 3.0		CLIA	22321	A	
Dettenborn *et al*. 2010 - unemployed	31	36.7 ± 11.0	24.6 ± 6.3	3.2		10.2 ± 7.2		CLIA	22321	**A**	
Diebig *et al*. 2016	129	32.3 ± 12.1	24.4 ± 4.3	24		11.6 ± 13.2		CLIA	22331	A	A
Dowlati *et al*. 2010 - controls	87	65.7 ± 11.1	27.5 ± 4.9	80.5		185.3 ± 131.6		ELISA	33321	A	
Enge *et al*. 2020	470	38.6 ± 8.9	24.6 ± 4.9	34	8.3	6.1 ± 7.4		LC-MS	11311	**A**	**A**
Engert *et al*. 2018	332	40.7 ± 9.2	23.6 ± 3.3	40.7		1.6 ± 1.0	2.5 ± 0.7	LC-MS	11131	AE	
Etwel *et al*. 2014	39	23.8 ± 6.2	23.2 ± 4.8	0		257.2 ± 101.8		ELISA	23121	A	

Feeney *et al.* 2020	1876	66.4 ± 8.7		25.6	31.5	18.8 ± 48.1	12.4 ± 10.3	LC-MS	11111	**AE**	**AE**
Feller *et al.* 2014	654	65.8 ± 8.4	27.5 ± 4.4	46		35.1 ± 32.8		CLIA	12111	**ACD**	
Fischer *et al.* 2017	139	50.6 ± 14.6	27.5 ± 6.0	28	28			ELISA	13311	A	
Gao *et al.* 2014 - adult control	23	41.5 ± 12.8	22.9 ± 2.7	61	0	4.3 ± 3.9		LC-MS	22321	A	
Gao *et al.* 2014- adult earthquake survivor	20	45.5 ± 14.2	23.4 ± 2.1	60	0	46.3 ± 48.4		LC-MS	22321	A	
Garcia-Leon *et al.* 2018	62	33.0 ± 3.7	22.8 ± 2.9	0		127.9 ± 111.5		ELISA	13313	A	
Gidlow *et al.* 2016	132	41.4 ± 11.4	25.1 ± 4.8	28.9		10.8 ± 9.4		ELISA	13321	A	A
Grass *et al.* 2015 - study I	42	24.8 ± 5.7	21.3 ± 2.9	52.4		3.5 ± 2.3		LC-MS	11311	A	
Grass *et al.* 2015 - study II	52	25.0 ± 4.9	22.8 ± 3.2	57.7		3.2 ± 3.8		LC-MS	11311	A	
Henley *et al.* 2014	109			29.8	16.2	592.2 ± 304.8†		ELISA	13123	A	A
Hollenbach *et al.* 2018	59	36 ± 6	32.2±9.8	3.4		27.8 ± 30.8		ELISA	13321	**A**	
Hunter *et al.* 2020	140	22.8 ± 6.0	27.2 ± 6.6	0	29	11.2 ± 23.5		LC-MS	21321	A	A
Jackson *et al.* 2017	2527	67.9 ± 7.3	28.2 ± 5.2	41	30.5	30.5 ± 76.7		LC-MS	11131		
Janssens *et al.* 2017	111	43.4 ± 10.4	24.4 ± 3.8	60	10.8	14.9 ± 9.4‡		LC-MS	21111	AD	AD
Kozik *et al.* 2015	66	71.9 ± 5.8	25.0 ± 4.0	33.3		25.8 ± 17.2		ELISA	13321	AC	
Kuehl *et al.* 2015	41	41.2	23.3 ± 3.6†	36.6	14.1	4.3 ± 4.2†	19.8 ±21.5†	CLIA	22121	ACE**F**	**ACEG**
Lanfear *et al.* 2020	41	68.1 ± 5.3		48		10.5 ± 13.6		LC-MS	11321	D	
Larsen *et al.* 2016 - fathers	231	40.3 ± 5.4	26.2 ± 3.7	100		177.4 ± 119.2		ELISA	23331	**A**	**A**
Larsen *et al.* 2016 - mothers	301	38.0 ± 4.3	26.6 ± 5.4	0		146.1 ± 102.3		ELISA	23331	**A**	**A**
Lehrer *et al.* 2020	141	45.8 ± 15.2		32.6				ELISA	13122	**D**	D
Ling *et al.* 2020 - mothers	35	29.7 ± 5.6	32.4 ± 7.0	0	58.1	7.0 ± 8.1		ELISA	23111	**A**	A
Manenschijn *et al.* 2013	283	74.8 ± 7.1‡	27.4 ± 4.0‡	33.9		23.2 ± 10.1‡		ELISA	13121	AC	
Manenschijn, Koper *et al.* 2011	46							ELISA	23321	**CD**	
Mazgelyte *et al.* 2019	163	38.5 ± 9.3	26.6 ± 5.3‡	100		237.8 ± 160.8‡		LC-MS	21131	**AC**	**AC**
McLennan *et al.* 2016	246	42.0 ± 11.2	26.4 ± 5.3	10.2	23.8	15.1 ± 14.6		CLIA	22311	**A**	**A**

Menning *et al*. 2015 - breast cancer no chemotherapy	33	52.4 ± 7.3	24.0 ± 3.8	0		23.8 ± 16.6		ELISA	33331	A	
Menning *et al*. 2015 - controls	38	50.1 ± 8.7	24.5 ± 3.5	0		27.0 ± 13.7		ELISA	23331	A	
Menning *et al*. 2015- breast cancer chemotherapy	32	50.2 ± 9.2	25.8 ± 4.5	0		33.4 ± 26.2		ELISA	33331	A	
Michaud *et al*. 2016	675	52.0 ± 15.2	28.2 ± 5.8	36.1		278.2 ± 553.8		ELISA	13331	A	A
Mwanza *et al*. 2016	473	19.3 ± 1.4	31.4 ± 3.8†	61.3	7	11.4 ± 3.9‡	35.0 ±15.8‡	LC-MS	12333		
Nery *et al*. 2018	16	37.5 ± 5.9	31.1 ± 6.1	0	50			ELISA	33331	A	
O'Brien *et al*. 2013	135	30.3 ± 12.8		35		14.5 ± 19.1		ELISA	23131	D	
Olstad *et al*. 2016 - women	70	43.4 ± 7.2	26.2 ± 6.0	0	18.6	123.7 ± 71.2		ELISA	23321	A	A
Ouellette *et al*. 2015 - high stress mothers	30	38.2 ± 3.2	25.3 ± 6.0	0		244.6 ± 449.5		ELISA	23321	A	
Ouellette *et al*. 2015 - low stress mothers	30	37.5 ± 5.2	29.9 ± 8.3	0		126.7 ± 165.4		ELISA	23321	A	
Pickett *et al*. 2020	91	24.6 ± 6.5	30.1 ± 7.7	0	42	68.0 ± 161.9		ELISA	13131	AC	AC
Pittner *et al*. 2020 - adults	171	44.5 ± 14.8	25.8 ± 4.9	25.1	19.3	3.5 ± 5.7	8.6 ± 6.9	LC-MS	21321	AE	AE
Pulopulos *et al*. 2014	54	64.8 ± 4.2	26.3 ± 3.5†	24.6	11.1	2.4 ± 2.2†		LC-MS	21111	A	A
Qi *et al*. 2014	39	30.2 ± 6.1‡	21.5 ± 2.4	0		24.9 ± 20.0‡		LC-MS	31311	A	
Radin *et al*. 2019	166	42.4 ± 5.1	25.5 ± 5.2	0	17.1	52.9 ± 24.3†		ELISA	23121	ACD	ACD
Saleem *et al*. 2013 - completers	56	66 ± 11	27.3 ± 4.2	85.7		233.2 ± 173.0		ELISA	33111	A	
Saleem *et al*. 2013 - non-completers	43	61 ± 11	28.5 ± 5.0	70		153.5 ± 110.5		ELISA	33111	A	
Schalinski *et al*. 2015 - healthy controls	12	31.9 ± 7.5	22.5 ± 4.1	0	9.1	12.6 ± 11.0		CLIA	12321	A	A
Schalinski *et al*. 2019 - healthy controls	75	25.4 ± 6.7	23.4 ± 3.6	54.7	5.3	7.3 ± 5.4†		CLIA	22111	A	A
Serwinski *et al*. 2016	164	43.6 ± 9.8	24.1 ± 4.4	0	10.8	8.4 ± 6.3		LC-MS	21111	A	A
Skoluda *et al*. 2012 - controls	70	36.6 ± 11.5	23.0 ± 2.5	17.1				CLIA	22321	A	

Skoluda *et al*. 2012 - endurance athletes	304	38.3 ± 11.6	22.7 ± 2.3	41.1				CLIA	22321	A	
Smith, L. *et al*. 2019	3741	68.4 ± 8.0	28.3 ± 5.3	33.6		26.2 ± 68.8		LC-MS	21131		**A**
Stalder *et al*. 2010 - non-alcoholic controls	20	43.7 ± 11.2	26.5 ± 3.6	80				CLIA	32331	A	
Stalder *et al*. 2013	1258	39.6 ± 7.3‡	27.1 ± 3.5‡	84.8		22.5 ± 11.7‡	38.5 ± 16.3‡	LC-MS	21111	**ACDEFG**	
Stalder *et al*. 2014 - caregivers	20	71.2 ± 6.1	26.7 ± 3.8	5				CLIA	22311	A	
Stalder *et al*. 2014 - controls	20	72.2 ± 6.4	25.1 ± 3.9	15				CLIA	12311	A	
Stalder, Steudte *et al*. 2012 - study I	155	24.1 ± 4.2	22.2 ± 3.4	26.5	3.9	17.7 ± 10.6		CLIA	22311	**A**	
Stalder, Steudte *et al*. 2012 - study II	58	30.5 ± 12.1	24.0 ± 4.9	32.8	12.1	21.6 ± 16.0		CLIA	22311	**A**	
Staufenbiel *et al*. 2015	1425	45.9 ± 13.8		28.2		3.6 ± 2.5‡	11.1 ± 5.8‡	LC-MS	11111	**ACEF**	**ACEG**
Steudte *et al*. 2013 - non-traumatized controls	28	37.6 ± 14.1	23.4 ± 3.05	10.7				LC-MS	11311	A	
Steudte *et al*. 2013 - traumatized controls	25	41.7 ± 12.3	23.8 ± 3.9	8				LC-MS	21311	A	
Steudte, Kolassa *et al*. 2011	17	20.1 ± 5.7	21.4 ± 2.3	64.7				CLIA	22331	A	
Steudte, Stalder *et al*. 2011	15	35.7 ± 9.3	22.9 ± 3.5	13.3				CLIA	12311	A	
Steudte-Schmiedgen *et al*. 2015 - non-traumatized soldiers	129	26.2 ± 5.2	24.6 ± 2.7	100				LC-MS	21311	A	
Steudte-Schmiedgen *et al*. 2017	17	31.3 ± 9.4†	25.4 ± 5.0	11.8		14.1 ± 16.3	19.9 ± 11.3	LC-MS	21321	AE	
Suijker *et al*. 2018	15	45.2 ± 15.4	24.9 ± 4.7	43.8	12.5	21.7 ± 14.9		ELISA	31321	A	A
Van Aken *et al*. 2018	61	34.8 ± 6.7†	25.3 ± 4.6†	0		44.4 ± 36.2†		ELISA	23321	A	
Van den Heuvel, Stalder *et al*. 2020	216	43.8 ± 13.3†	31.6 ± 8.1†	0	53.7	6.3 ± 5.2†‡		LC-MS	21111	ACD	ACD
Van den Heuvel, Acker *et al*. 2020	164	46.5 ± 15.0	30.5 ± 7.3‡	0	50.9	6.2 ± 6.4		LC-MS	11111	ACD	ACD
Van den Heuvel, Du Plessis *et al*. 2020	56	59.6 ± 8.7	29.5 ± 5.9	0	46.4	5.0 ± 4.5†	8.5 ± 6.2	LC-MS	31111	ACDEF**G**	ACDEG
Van der Valk *et al*. 2020	51	40.7 ± 12.6	39.7±5.6	27.5	100	5.8 ± 5.3	17.8 ±13.8	LC-MS	31121	ACEF	ACE**G**

Van Holland *et al*. 2012	27	46.2 ± 10.6	26 ± 4	81				ELISA	23331	A	
Van Manen *et al*. 2019	32	47.8 ± 8.5‡	27.8 ± 4.6	43.8	28.1	10.9 ± 11.7	23.9 ± 15.9	LC-MS	31121	ACEF	ACEG
Walther *et al*. 2016	271	57.1 ± 10.7	25.4 ± 3.4	100		8.0 ± 6.3	24.6 ± 16.4	LC-MS	11333	**ADEG**	
Walton *et al*. 2013	10	28 ± 13	27.1 ± 3.6	30				ELISA	33311	A	
Wang *et al*. 2019	68	32.5 ± 6.1		0	15	6.3 ± 6.5‡		LC-MS	21323	A	
Wells *et al*. 2014	324	41.9 ± 15.8	27.0 ± 6.5	28.1	24.7	274.4 ± 222.0		ELISA	23311	**A**	**A**
Wester *et al*. 2014	47	45 ± 11.3‡		23.4	100			ELISA	33123	AC	
Wester *et al*. 2017	295	46.8 ± 11.7‡	25.9 ± 4.3	25.4	19.32			LC-MS	11131	AC	ACEG
Wester *et al*. 2017 - healthy controls	174	36.3 ± 8.4‡	26.8 ± 4.9	42.5				ELISA	23331	A	
Wu *et al*. 2019	160	45.7 ± 9.8	26.6 ± 3.1	55.4	31	23.4 ± 30.5		ELISA	33121	A	A
Younge *et al*. 2015	151	41.3 ± 14.2	25.5 ± 4.9	37.1				ELISA	33111	**A**	**A**
Zai *et al*. 2017	248								13333	**A**	
Zekas *et al*. 2019	81	36.5 ± 6.2		100				LC-MS	21131	**C**	
PEDIATRIC COHORTS											
Bryson *et al*. 2020	297	3.1 ± 0.1	16.8 ± 1.8	39.4	22.9	8.5 ± 7.8		ELISA	23131	A	A
Chen *et al*. 2015 - female adolescents	47	15.8 ± 3.1	24.3 ± 5.2	0		3.4 ± 1.9		LC-MS	21121	AD	
Chen *et al*. 2015 - male adolescents	32	15.0 ± 2.1	21.7 ± 4.5	100		4.0 ± 2.5		LC-MS	21121	AD	
Condon *et al*. 2019	45	6.8 ± 2.1	0.7 ± 1.2		22.2	57.3 ± 112.7		ELISA	23131	B	B
De Kruijff *et al*. 2020	278	10.8 ± 4.6	-0.1 ± 1.0	51.1	0.8	3.1 ± 3.1		LC-MS	21311	**AB**	**AB**
Distel *et al*. 2019	52	8.4 ± 1.3	20.8 ± 4.4	39	29.3	20.6 ± 63.4		ELISA	23121	**A**	**A**
Evans *et al*. 2019	92	10.1 ± 0.3	17.3 ± 2.1	34.8		3.0 ± 4.5	10.1 ± 12.0	LC-MS	11121	AE	A
Föcker *et al*. 2016	20	17.3 ± 1.0	-0.3 ± 1.1	0		12.6 ± 9.7		CLIA	12121	B	
Frisch *et al*. 2020	18	7.4 ± 1.0	15.8 ± 2.4	44	0	2.8 ± 2.4		ELISA	33321	A	
Gao *et al*. 2014 - young male control	29	16.7 ± 0.6	21.4 ± 2.4	100	0	13.9 ± 10.9		LC-MS	22321	A	

Gao *et al*. 2014 - young male earthquake survivor	20	16.8 ± 0.8	21.7 ± 2.4	100	0	25.3 ± 17.1		LC-MS	22321	A	
Genitsaridi *et al*. 2019	300	10.5 ± 2.6	25.7 ± 5.4†	25.3	46.7	8.9 ± 1.0†		CLIA	32131	ACD	
Gerber *et al*. 2017	318	7.3 ± 3.5	16.3 ± 2.2	46.9	8	12.2 ± 9.7		CLIA	12111	**AC**	**AC**
Golub *et al*. 2019	137	7.6 ± 0.6	16.1 ± 1.8	47.5	0			ELISA	13113	A	
Grunau *et al*. 2013 - full term	42	7.8 ± 0.8	16.8 ± 3.2	35.7		416.2 ± 873.0		ELISA	33311	A	
Grunau *et al*. 2013 - pre-term	91	7.7 ± 0.3	15.7 ± 2.4	46.2		301.2 ± 560.8		ELISA	33311	A	
Hu *et al*. 2017	1263	8.0 ± 0.8		47.3		11.8 ± 1.9†		ELISA	13123	**A**	
Ilg *et al*. 2020	134	12.0 ± 4.0	18.6 ± 3.7	57		3.7 ± 2.3	13.4 ± 7.2	LC-MS	31323	**AE**	
Ince-Askan *et al*. 2019	117	9.8 ± 2.4†	0.4 ± 1.1†	59.8	7.7	1.3 ± 1.0†	7.4 ± 3.5†	LC-MS	21131	AE	ABCDEFG
Kamps *et al*. 2014	10	10.5 ± 1.3	0.1 ± 1.0	50	10	4.8 ± 4.0		LC-MS	11121	AB	AB
Larsen *et al*. 2016 - children	363	5.4 ± 1.07	16.1 ± 1.2	55		146.5 ± 179.0		ELISA	23131	A	BCD
Lehto *et al*. 2018	599	4.7 ± 0.9	15.9 ± 1.4	52	2.1	41 ± 77		CLIA	12131	C	C
Ling *et al*. 2020 - children	35	4.7 ± 0.8	0.7 ± 1.0	51.4	20	32.0 ± 45.4		ELISA	23111	B	AB
Michels *et al*. 2017	81	12.7 ± 1.7	-0.03 ± 0.9	53.6	0	25 ± 5		LC-MS	11121	A	AB
Murray *et al*. 2016	54	9.5 ± 0.3	17.6 ± 2.3	20.3	0	3.2 ± 2.9†		ELISA	33131	A	A
Olstad *et al*. 2016 - children	30	14.3 ± 3.9	0.3 ± 1.1	56.7	10	96.6 ± 49.6		ELISA	23121	B	B
Ouellet-Morin *et al*. 2016	34	17		26.5		33.0 ± 24.5		ELISA	13331	A	A
Ouellette *et al*. 2015 - high stress daughters	30	7.5 ± 0.7	15.3 ± 2.2	0		89.9 ± 235.1		ELISA	23321	A	
Ouellette *et al*. 2015 - low stress daughters	30	7.7 ± 0.7	15.6 ± 2.8	0		104.4 ± 218.3		ELISA	23321	A	
Panter-Brick *et al*. 2019	203	14.4 ± 1.7	0.0 ± 1.0	56.9	5.3	9.5 ± 10.0		ELISA	23121	**A**	**A**
Papafotiou *et al*. 2017 - normal weight	25	7.8 ± 1.2	-0.03 ± 0.6	0		1.2 ± 0.6		LC-MS	11121	AB	A
Papafotiou *et al*. 2017 - obesity	25	7.4 ± 1.3	2.9 ± 1.4	0	100	4.1 ± 5.0		LC-MS	31121	**A**	A**B**
Petimar *et al*. 2020 - mid-childhood	599	7.9 ± 0.8	0.3 ± 1.0	45.9	8.8	1.3 ± 1.5‡		LC-MS	11121	**AC**	**ABC**

Pittner *et al.* 2020 - children	61	12.4 ± 3.2	0.2 ± 1.0	42.6	3.3	1.6 ± 1.5	5.7 ± 2.9	LC-MS	21321	BE	BE
Pyle Hennessey *et al.* 2020	100	5.8 ± 0.3	15.6 ± 1.7	48	3.1	6.8 ± 6.9		ELISA	23121	A	A
Schloss *et al.* 2018	75	4.6 ± 0.3		41.3				ELISA	23312	A	
Slopen *et al.* 2018	344	2.1 ± 0.1	17.5 ± 1.7	43.2	11.1	19.0 ± 42.4		ELISA	13121	A	A
Smith, J. *et al.* 2019	114	8.5 ± 0.3	17.0 ± 2.6	42.1		4.2 ± 4.1		ELISA	23311	AC	
Sun *et al.* 2018	1000	9.0 ± 0.9	18.6 ± 3.2	42.1		12.0 ± 2.0[‡]		ELISA	13131	**A**	**A**
Van Dammen *et al.* 2020	181	15.7 ± 2.0	20.3 ± 3.2	38.9	1.6	3.5 ± 2.1		LC-MS	12331	**AB**	**AB**
Vehmeijer *et al.* 2020	2042	6.1 ± 0.6	0.2 ± 0.9	47.5	3.6	1.9 ± 1.4	9.6 ± 7.4	LC-MS	11121	**AE**	**A**
Vepsäläinen *et al.* 2021	565	4.8 ± 0.9	15.9 ± 1.5	37.9	2.1	40.9 ± 77.1		CLIA	12131	A	A
Wagner *et al.* 2019	434	12.0		38.5	9.9			LC-MS	11112		B
White *et al.* 2017	537	10.0 ± 3.1[†]	0.0 ± 0.8	49.3				CLIA	22322	**B**	

**Risk of bias: 1, low risk of bias; 2, moderate risk of bias; 3, high risk of bias. Each number represents the assessed QUIPS domains. The following definitions were used for low, moderate of high risk of bias: QUIPS 1 (study participation), 1: population-based sampling, 2: population selection on non-medical or social conditions, 3: population selection on medical conditions not evidently related to disturbances of the HPA-axis; QUIPS 2 (study attrition), not applicable and therefore not scored; QUIPS 3 (prognostic factor measurement), 1: HairGC analysis using LC-MS, 2: HairGC analysis using CLIA, 3: HairGC analysis using ELISA; QUIPS 4 (outcome measurement), 1: anthropometric measurements objectively measured, 2: anthropometric measurements self-reported; QUIPS 5 (study confounding), 1: both outliers and corticosteroid use taken into account, 2: only outliers or only corticosteroid use taken into account, 3: outliers and corticosteroid use both not taken into account; QUIPS 6 (statistical analysis and reporting), 1: relevant statistics fully reported, 2: relevant statistics partly reported, 3: relevant statistics not reported*

[†]Pooled means.

[‡]Means calculated from either median and interquartile range, or from median and range.

Reported bivariate correlation/regression coefficient: A, HairF vs BMI; B, HairF vs BMI SDS; C, HairF vs WC; D, HairF vs WHR; E, HairE vs BMI; F, HairE vs WC; G, HairE vs WHR. Significant associations are represented in bold.

Abbreviations: CI, confidence interval; HairF, hair cortisol; HairE, hair cortisone; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio; LC-MS, liquid chromatography-(tandem) mass spectrometry; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescent immunoassay.

Description of study characteristics

The weighted mean age of cohorts involving adults (available for n=23,467) was 53.3 ± 18.4 years and weighted mean BMI (n=19,653) was 27.0 ± 5.4 kg/m^2. For studies involving children, weighted mean age (n=9,904) was 7.8 ± 3.3 years and weighted mean BMI SDS (n=4,108) was 0.2 ± 1.0. Forty-three of the 146 cohorts (29%) included children (mean age <18 years). The majority of the cohorts had a population that was predominantly female (104 cohorts had >50% females), although the proportion of females within all included subjects was 44%. Of the 43 pediatric cohorts, two specifically included only children with obesity[63,99], whereas the other 41 cohorts either had no criteria regarding weight status or included only children with normal weight. In adults, two of the 103 cohorts exclusively included adults with obesity (BMI ≥30 kg/m^2)[131,141], whereas the other 101 cohorts either had no criteria regarding weight status or included only adults with normal weight or overweight. In twelve of the 103 adult cohorts (12%), the mean BMI of the included population was 30 kg/m^2 or higher. Details on the mean BMI of the studies can be found in Table 1.

BMI was the most commonly reported obesity measurement in 138/146 cohorts (95%), followed by WC in 30/146 cohorts (21%), WHR in 20/146 cohorts (14%), and BMI SDS in 16/43 pediatric cohorts (37%). For 145 cohorts (99%) the used laboratory method was reported, which were ELISA (63/145 cohorts, 43%), LC-MS or LC-MS/MS (56/145 cohorts, 39%), or CLIA (26/145 cohorts, 18%). In all cohorts HairF was reported, whereas HairE was additionally reported in 19/146 cohorts (13%).

Mean crude HairGC concentrations across the studies varied widely with reported means ranging from 1.2 - 592.2 pg/mg for HairF and 2.45 - 38.48 pg/mg for HairE. Mean HairF concentrations were higher in studies that used an ELISA (weighted mean 95.6 ± 236.4 pg/mg) compared to studies that used CLIA (24.0 ± 45.1 pg/mg) or LC-MS (mean 13.36 ± 13.39 pg/mg and mean 12.2 ± 39.5 pg/mg in a sensitivity analysis without Mazgelyte *et al.*[86], which was a significant outlier in mean HairF level). All HairE analyses except for one[78] were performed using LC-MS. In the studies that reported both HairE and HairF concentrations, HairE levels in most cases were higher than HairF levels (Table 1).

Risk of bias

Risk of bias assessments on cohort level are presented in Table 1. With respect to the selection of the population domain (QUIPS 1), 25 (17%) cohorts had a high, 75 (52%) medium, and 46 (31%) low risk of bias. Regarding the prognostic factor (HairGC) measurement domain (QUIPS 3), 65 (45%) cohorts had a high, 31 (21%) medium, and 50 (34%) low risk of bias. For the outcome measurement domain (QUIPS 4), 75 (51%)

cohorts had a moderate and 71 (49%) a low risk of bias. In the domain of accounting for possible confounders (QUIPS 5), 37 (25%) cohorts had a high, 64 (44%) medium, and 45 (31%) low risk of bias. With regard to the statistical domain (QUIPS 6), 10 (7%) cohorts had a high, 4 (3%) medium, and 132 (89%) low risk of bias.

Qualitative synthesis

An overview of all outcomes reporting any relation between HairGC and obesity measurements is shown in Supplementary Table S1.

Quantitative synthesis

Meta-analysis of correlation coefficients

In total, 140/146 cohorts (96%) from 115 unique studies were included in the meta-analyses of correlations, comprising data of 28,830 participants. The pooled correlation coefficients ranged from 0.10-0.18 (all p<0.0001). The strongest pooled correlation was found for HairE vs. WC (pooled *r* = 0.18; Table 2; Supplementary Figures S1-S6). Meta-regressions and subgroup analyses were possible for the associations between HairF vs. BMI, BMI SDS, WC, and WHR; and HairE vs. BMI. In subgroup analyses, neither applied laboratory methods nor population-based sampling moderated the correlations between HairGC and obesity measurements (all p-values >0.05, Table 3). Subgroup analyses on all QUIPS domains showed no moderation by risk of bias categories except for QUIPS domain 4 (assessment of outcome, *i.e.*, self-reported BMI vs. measured): studies with self-reported BMI showed stronger correlations with HairF than studies with measured BMI (pooled *r* of 0.15 vs. 0.07, respectively; Q=14.34, p<0.0001).

Table 2. Pooled correlation coefficients.

	k cohorts	n participants	Pooled *r*	95% CI	95 % PI	P value	Between-study heterogeneity		
							I^2 (%)	Q	P value
HairF vs. BMI	122	26,527	0.10	0.08; 0.13	-0.04; 0.24	<0.0001	51.2	221.4	<0.0001
HairF vs. BMI SDS	11	1,247	0.12	0.06; 0.18	0.06; 0.18	<0.0001	0.0	11.8	0.30
HairF vs. WC	24	11,006	0.11	0.07; 0.15	-0.03; 0.26	<0.0001	68.3	59.7	<0.0001
HairF vs. WHR	16	6,786	0.11	0.07; 0.15	0.03; 0.19	<0.0001	28.4	22.3	0.10
HairE vs. BMI	16	8,210	0.11	0.07; 0.15	0.00; 0.21	<0.0001	52.7	31.0	0.01
HairE vs. WC	7	3,158	0.18	0.11; 0.24	0.06; 0.29	<0.0001	45.7	9.6	0.14
HairE vs. WHR	2	1,314	NA*	NA	NA	NA	NA	NA	NA

Abbreviations: CI, confidence interval; HairF, hair cortisol; HairE, hair cortisone; NA, not applicable; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio
**meta-analysis not performed due to small number of cohorts*

Table 3. Results of subgroup analyses in the meta-analyses of correlation coefficients.

	Moderator	k cohorts	I^2 (%)	Pooled *r*	95% CI	$Q_{between}$	P-value
HairF vs BMI	QUIPS 1: Study participation (population-based sampling)					0.34	0.55
	Yes	34	51	0.10	0.07; 0.13		
	No	88	51	0.11	0.08; 0.14		
	QUIPS 3: Prognostic factor measurement (HairGC analysis method)					0.05	0.98
	LC-MS	47	51	0.10	0.07; 0.14		
	ELISA	52	35	0.10	0.07; 0.14		
	CLIA	21	66	0.11	0.05; 0.17		
	QUIPS 4: Outcome (anthropometric) measurement					**14.34**	**<0.001**
	Self-reported	**67**	**22**	**0.15**	**0.12; 0.18**		
	Objectively measured	**55**	**62**	**0.07**	**0.04; 0.10**		
	QUIPS 5: Study confounding					2.74	0.43
	CS use and outliers handled	39	62	0.13	0.09; 0.17		
	Only outliers handled	22	29	0.09	0.05; 0.13		
	Only CS use handled	33	30	0.10	0.06; 0.15		
	Neither handled	28	51	0.08	0.03; 0.12		
	QUIPS 6: Statistical analysis (Relevant statistics fully reported)					0.01	0.93
	Yes	118	50	0.10	0.08; 0.13		
	No	4	65	0.10	-0.04; 0.23		
HairF vs BMI SDS	Population-based sampling (QUIPS 1)					0.12	0.73
	Yes	4	0	0.14	0.01; 0.27		
	No	7	0	0.12	0.05; 0.18		
	QUIPS 3: Prognostic factor measurement (HairGC analysis method)					0.63	0.73
	LC-MS	6	70.7	0.06	-0.13; 0.25		
	ELISA	3	0	0.07	-0.13; 0.26		
	CLIA	2	0	0.13	0.04; 0.21		
	QUIPS 4: Outcome (anthropometric) measurement					2.11	0.15
	Self-reported	4	0	0.14	0.08; 0.20		
	Objectively measured	7	32.1	-0.01	-0.19; 0.18		
	QUIPS 5: Study confounding					0.86	0.83
	Both handled	2	0	0.13	0.03; 0.24		
	Only outliers handled	2	0	0.13	0.04; 0.21		
	Only CS use handled	5	60.8	-0.01	-0.31; 0.28		
	Neither handled	2	0	0.13	0.00; 0.26		
HairF vs WC	Population-based sampling (QUIPS 1)					**3.95**	**0.05**
	Yes	9	65	0.07	0.02; 0.13		
	No	15	60	0.15	0.09; 0.20		
	QUIPS 3: Prognostic factor measurement (HairGC analysis method)					0.17	0.92
	LC-MS	12	78	0.11	0.05; 0.18		
	ELISA	7	4	0.10	0.02; 0.17		

	CLIA	5	77	0.11	0.02; 0.21		
	QUIPS 4: Outcome (anthropometric) measurement					0.67	0.41
	Self-reported	3	40	0.18	0.01; 0.35		
	Objectively measured	21	71	0.11	0.06; 0.15		
	QUIPS 5: Study confounding					5.90	0.12
	Both handled	9	68	0.08	0.02; 0.15		
	Only outliers handled	3	0	0.16	0.13; 0.19		
	Only CS use handled	7	33	0.13	0.05; 0.21		
	Neither handled	5	77	0.10	-0.03; 0.23		
HairF vs WHR	Population-based sampling (QUIPS 1)					0.56	0.46
	Yes	4	57	0.15	0.03; 0.26		
	No	12	36	0.10	0.04; 0.15		
	QUIPS 3: Prognostic factor measurement (HairGC analysis method)					0.34	0.56
	LC-MS	11	33	0.10	0.05; 0.15		
	ELISA	4	76	0.16	-0.03; 0.34		
	QUIPS 4: Outcome (anthropometric) measurement					**5.79**	**0.02**
	Self-reported	**2**	**0**	**0.36**	**0.16; 0.53**		
	Objectively measured	**14**	**36**	**0.10**	**0.06; 0.14**		
	QUIPS 5: Study confounding					2.85	0.24
	Both handled	6	53	0.09	0.02; 0.15		
	Only outliers handled	5	0	0.13	0.10; 0.16		
	Only CS use handled	4	57	0.23	0.06; 0.38		
	Neither handled	1	NA	NA	NA		
HairE vs BMI	QUIPS 1: Study participation (population-based sampling)					0.02	0.89
	Yes	6	40	0.11	0.07; 0.15		
	No	9	46	0.12	0.02; 0.21		
	QUIPS 4: Outcome (anthropometric) measurement					0.24	0.62
	Self-reported	3	78	0.22	-0.20; 0.57		
	Objectively measured	12	59	0.12	0.07; 0.16		
	QUIPS 5: Study confounding					**8.08**	**0.04**
	Both handled	4	55	0.16	0.11; 0.21		
	Only outliers handled	4	0	0.07	0.04; 0.11		
	Only CS use handled	5	0	0.09	-0.02; 0.20		
	Neither handled	2	61	0.05	-0.12; 0.21		

Abbreviations: CI, confidence interval; HairF, hair cortisol; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio; LC-MS, liquid chromatography-(tandem) mass spectrometry; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescent immunoassay. NB: subgroup analyses were only performed when data of at least 2 cohorts were available within a subgroup and 10 cohorts across all subgroups.

In meta-regressions, we found that studies that included larger proportions of males showed stronger correlations between HairF and WC (estimated slope 0.0022 per percentage point increase in proportion of males, 95% CI 0.0010 to 0.0033, p= 0.0002) and HairF and WHR (estimated slope 0.0011 per percentage point increase in proportion of males, 95% CI 0.0001 to 0.0021, p= 0.02; Table 4; Supplementary Figures S7-S8). Furthermore, studies including more participants with obesity showed weaker correlations between HairF and BMI (estimated slope -0.0029 per percentage point increase in proportion of participants with obesity, 95% CI -0.0049 to -0.0010, p= 0.0028), and studies with higher BMI SDS showed weaker correlations between HairF and BMI SDS (Table 4, Supplementary Figure S9). Mean age and mean HairF concentration of the study population did not moderate the correlations between HairGC and obesity measurements (all p-values >0.05, Table 4). In contrast, higher mean HairE was associated with stronger positive correlations (estimated slope 0.0046 per point increase in mean HairE on study level, 95% CI 0.0025-0.0068, p<0.0001). Visual inspection of the funnel plots showed no evidence for publication bias, *i.e.*, no systematic trends were found between standard error (as proxy for study sample size) and magnitude and direction of the reported correlation coefficients (Supplementary Figures S10-S15).

Meta-analysis of regression coefficients

The pooled regression coefficients stratified on analysis method are presented in Table 5. The pooled regression coefficient for 10-log transformed HairF as independent variable on BMI as dependent variable measured for LC-MS-based measurements was based on the largest number of cohorts (k=26 cohorts comprising 11,635 individuals). The pooled regression coefficient for LC-MS-based measurements was 0.049 kg/m^2 (95% CI 0.045-0.054; Table 5). This indicates that for LC-MS-based measurements, 1 point increase in 10-log HairF was associated with 0.049 kg/m^2 higher BMI. One point increase in 10-log HairE was associated with 1.15 kg/m^2 higher BMI (95% CI 0.987-1.310 kg/m^2). The highest pooled regression coefficient was found for HairE on dependent variable WC, where 1 point increase in 10-log HairE was associated with 11.0 cm larger WC (95% CI 10.1-11.9 cm) on LC-MS. There was no significant between-study heterogeneity (all p-values >0.05, Table 5).

DISCUSSION

In the current systematic review including 34,342 unique subjects, HairGC levels showed a significant positive relation with anthropometric measurements. In the meta-analyses, pooled correlation coefficients ranged between 0.10 for hair cortisol vs. BMI and 0.18 for hair cortisone vs. WC. The largest effect size was found for

Table 4. Results of meta-regressions in the meta-analyses of correlation coefficients.

	Moderator	*k* cohorts	% Between-study heterogeneity explained	Estimate (slope)	95% CI	Q_m	P value
HairF vs BMI	Mean age	120	0.3	0.0006	-0.0005; 0.0017	1.32	0.25
	Mean BMI	113	0.7	0.0003	-0.0050; 0.0057	0.01	0.90
	Adults only	84	0.7	-0.0082	-0.0180; 0.0016	2.70	0.1003
	Mean HairF	115	0.002	0.0000	-0.0002; 0.0003	0.10	0.76
	LC-MS	44	2.1	0.0008	-0.0042; 0.0057	0.09	0.76
	CLIA	23	7.4	-0.0025	-0.0092; 0.0041	0.55	0.46
	ELISA	47	0.03	0.0000	-0.0003; 0.0003	0.02	0.88
	% obesity	**57**	**11.9**	**-0.0029**	**-0.0049; -0.0010**	**8.95**	**0.0028**
	% males	122	2.5	0.0003	-0.0006; 0.0011	0.38	0.54
HairF vs BMI SDS	Mean age	11	11.0	0.0127	-0.0091; 0.0344	1.30	0.25
	% males	10	18.6	0.0037	-0.0012; 0.0087	2.18	0.14
	Mean BMI SDS	**10**	**86.4**	**-0.2108**	**-0.3408; -0.0807**	**10.09**	**0.0015**
	Mean HairF	10	1.03	-0.0006	-0.0040; 0.0028	0.12	0.73
HairF vs WC	Mean age	23	21.9	0.0011	-0.0007; 0.0028	1.46	0.23
	Mean BMI	20	9.3	0.0013	-0.0081; 0.0106	0.07	0.79
	Adults only	17	18.3	-0.0080	-0.0267; 0.0108	0.69	0.41
	Mean HairF	21	0.002	0.0003	-0.0006; 0.0012	0.46	0.50
	% obesity	16	0.03	-0.0002	-0.0030; 0.0027	0.02	0.89
	% males	**23**	**39.5**	**0.0022**	**0.0010; 0.0033**	**14.29**	**0.0002**
HairF vs WHR	Mean age	15	10.7	0.0024	-0.0006; 0.0055	2.10	0.12
	Mean BMI	12	13.3	-0.0120	-0.0315; 0.0074	1.47	0.23
	Adults only	10	54.4	-0.0170	-0.0377; 0.0037	2.59	0.11
	Mean HairF	14	4.0	0.0014	-0.0020; 0.0047	0.65	0.42
	LC-MS	11	25.2	0.0056	-0.0013; 0.0126	2.53	0.11
	% males	**15**	**28.7**	**0.0011**	**0.0001; 0.0021**	**5.07**	**0.02**
HairE vs BMI	Mean age	15	27.9	0.0016	-0.0004; 0.0035	2.56	0.11
	Mean BMI	12	47.2	0.0096	-0.0006; 0.0197	3.41	0.0649
	Mean HairE	**13**	**65.2**	**0.0046**	**0.0025; 0.0068**	**17.96**	**<.0001**
	% males	15	12.2	0.0010	-0.0010; 0.0030	0.99	0.32

Abbreviations: CI, confidence interval; HairF, hair cortisol; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio; LC-MS, liquid chromatography-(tandem) mass spectrometry; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescent immunoassay; NA, not available or not applicable.

the relation between hair cortisone and waist circumference: one point increase in 10-log-transformed hair cortisone concentration (e.g. an increase from 1 pg/mg to 10 pg/mg) on LC-MS-based assays was associated with 11 cm larger waist circumference. For the outcome BMI, an increase of 1.15 kg/m^2 per one point increase in 10-log transformed hair cortisone on LC-MS-based assays was found. Moderator analysis in

Table 5. Pooled regression coefficients.
NB: meta-regressions were only performed when data of at least 10 cohorts were available.

	k cohorts	n participants	Analysis method	Pooled beta	95% CI	Between-study heterogeneity	
						Q_w	P value
HairF independent - BMI dependent	8	1,984	**CLIA**	**0.02**	**0.016; 0.03**	0.26	>0.05
	26	11,635	**LC-MS**	**0.05**	**0.045; 0.054**	0.50	>0.05
HairF independent - BMI SDS dependent	-	-	CLIA	-	-		
	6	998	**LC-MS**	**0.20**	**0.14; 0.27**	0.11	>0.05
HairF independent - WC dependent	4	1,556	**CLIA**	**0.02**	**0.02; 0.03**	0.13	>0.05
	10	4,259	**LC-MS**	**1.26**	**1.08; 1.44**	0.15	>0.05
HairF independent - WHR dependent	-	-	CLIA	-	-		
	5	1,805	LC-MS	-0.01	-0.01; -0.00	0.00	>0.05
HairE independent - BMI dependent			CLIA	-	-		
	9	5,266	**LC-MS**	**1.15**	**0.98; 1.31**	0.08	>0.05
HairE independent - WC dependent			CLIA	-	-		
	6	3,102	**LC-MS**	**11.0**	**10.1; 11.9**	0.05	>0.05

Abbreviations: CI, confidence interval; NS, not significant; HairF, hair cortisol; HairE, hair cortisone; NA, not applicable; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio
-, meta-analysis not performed due to insufficient number of cohorts

the meta-analyses of correlation coefficients showed that a higher percentage of male participants was associated with stronger correlations in the relations between hair cortisol vs. WC and hair cortisol vs. WHR. A higher percentage of participants with obesity of the included cohorts was associated with less strong correlations in the relation hair cortisol vs. BMI. Interestingly, no evidence was found for a moderating influence on study level of other important covariates that are known to influence either HairGC or obesity measurements in individual persons, namely age, laboratory methods, and handling of outliers and exogenous corticosteroid use.

In the largest of our meta-analyses, for HairF vs. BMI (n=26,527 participants), we confirmed the modest positive relations in exploratory analyses of Stalder *et al*. and Ling *et al*. between HairF and BMI/BMI SDS[9,12]. Evidently, there is a relation between measures of obesity and long-term glucocorticoid levels, a relation that has been controversial for measurement of GC levels in other matrices that reflect shorter time periods[6]. As GC are known to contribute to central adiposity, e.g. in Cushing's syndrome, it might be possible that in the study of a gradually developing disease such as obesity, long-term GC measurements offer a different and perhaps more appropriate perspective to the role of the HPA-axis.

The current study indicates that this relation is strongest (*i.e.*, the highest correlation coefficient and the largest effect size) for cortisone, the inactive form of cortisol, and waist circumference. Although the pooled correlation coefficients and pooled regression coefficients for the most frequently studied outcome HairF vs. BMI were statistically significant (pooled correlation coefficient 0.10, pooled regression coefficient 0.049 kg/m^2 increase in BMI per 1 point increase in 10-log transformed HairF on LC-MS), the small effect size here seems to have less clinical relevance compared to the large effect size we found for the relation HairE vs.WC. We believe that the consistency of our findings across all studied outcomes is indicative of an altered setpoint of the HPA-axis in obesity. This may induce or aggravate obesity, although causality cannot be proven by our study because of its limitation to cross-sectional associations. Yet, the fact that HairGC apparently relate strongly to measures of abdominal obesity matches the paradigm that chronic exposure to higher levels of GCs specifically induce abdominal obesity[18]. Importantly, specifically abdominal obesity increases mortality, e.g. by compromising cardiometabolic health and increasing the risk of many chronic diseases[147].

Previous meta-analyses already demonstrated an overall relation between HairF and BMI. However, this was investigated in smaller groups that also included individuals with psychosocial or biological factors affecting the HPA-axis such as post-traumatic stress disorder[9], or limited to children only[12]. Therefore, another important aim of our study was to identify moderators and subgroups within this relation on study level. This could improve the eventual applicability of HairGC measurements in the context of weight variability and additionally increase our understanding of the underlying biological mechanisms.

Strikingly, the pooled correlations between parameters of obesity and cortisone, the inactive form of cortisol, tended to be stronger than the relations with cortisol itself. The equilibrium between cortisol and cortisone is controlled by the enzymes 11ß-hydroxysteroid dehydrogenase type 1 and 2 both in the circulation (which is mostly determined by hepatic enzyme activity) as well as at tissue level, differing per tissue type[148]. With regard to scalp hair, it has been suggested that human hair follicles display a functional equivalent of the HPA-axis and can synthesize cortisol[149], although this finding has until now not been confirmed by others. However, there are currently no reports regarding balance between cortisol and cortisone at the shaft level. Therefore, it is believed that at least HairF represents cumulative circulating levels of cortisol[150], which presumably also holds true for HairE and cortisone. Perhaps this more stable circulating 'reservoir' of inactive cortisol can be seen as a better indicator of chronic hypercortisolism related to adiposity, considering the stronger

relations that we found for HairE. Moreover, this matches previous findings that HairE has a better diagnostic efficacy than HairF in the diagnostic screening for endogenous hypercortisolism[4].

Furthermore, in contrast to Ling *et* al[12], our meta-analyses did not indicate that LC-MS based cortisol measurements had a stronger relation to obesity than ELISA or CLIA-based measurements. In principle, the LC-MS-based method has a higher specificity than the ELISA method because it mostly lacks the interference from other steroid compounds[151]. The finding that LC-MS-based studies did not show a higher correlation for cortisol and obesity measurements than ELISA-based studies could also point towards an actual biological effect that in obesity, there is a more general activation of the HPA-axis. This general activation could lead to increased levels of other steroid hormones such as cortisone, which could potentially reduce issues associated with cross-reactivity in this context.

The percentage of males was a significant influencer of the relation between WC and HairF, with a similar trend for WHR and HairF, but not for HairF and BMI. For both WC and WHR, cut-off values are sex-specific, with males generally having a larger WC and WHR than females. This might contribute to the stronger associations between HairGC and anthropometric measurements in studies that contain more males. Unfortunately, lack of raw data hampered stratification for sex.

We also observed that studies that had a high percentage of participants with obesity found less strong associations between HairF and BMI. Although HairGC levels may explain less of the weight variability in cohorts with individuals with obesity compared to cohorts that include wider weight ranges, it has clearly been established that individuals with obesity in general have higher HairGC than individuals without obesity[14,141,152], an observation that is confirmed by our current analyses. It might be possible that within individuals with obesity, HairGC relate more to metabolic health than to anthropometrics per se. Another explanation could be the presence of a certain 'tipping point', perhaps the development of hepatic steatosis, that may interfere with cortisol-metabolizing enzymes, leading to or maintaining the state of hypercortisolism.

In contrast to our expectations, we found that studies using self-reported BMI reported stronger correlations to HairGC levels than studies using objective anthropometric features (r=0.15 and r=0.07 respectively for HairF-BMI). One possible explanation for this finding could include higher perceived weight stigma in individuals with obesity. Weight stigma is associated with adverse psychological consequences, such as anxiety,

lower self-esteem, poor quality of life, as well as with higher HairF levels[153]. When perceived weight stigma would cause individuals with obesity to overestimate their own weight, this could result in stronger correlations between BMI and HairGC levels, although this is highly speculative. Other possible areas of bias, e.g. the selection of participants (whether or not the participant selection was population-based or based on medical, occupational or socio-economic characteristics), the consideration of possible confounders (outliers of HairGC measurements and corticosteroid use), and the statistical reporting all did not affect the outcomes.

As expected given the large number of included studies, we observed a relatively high between-study heterogeneity in our meta-analyses of correlation coefficients, up to an I^2 of 68% for HairF vs. WC. Although some of our studied moderators could explain part of this heterogeneity, the majority is still unexplained. Hence, there may be a role for other factors that are known to influence HairGC levels and/or obesity that we did not account for in the current report. For example, a recent meta-analysis demonstrated that adversity also relates to long-term GC levels, although this relation is complex and depends on the type and timing of adversity and on the studied population[154]. Adversity and stressful conditions can have similar complex relations to obesity[155]. We did not include these factors as possible moderators in our analyses due to a lack of universally accepted definitions that we could apply to all studies. However, we do not suspect a major influence of stressful conditions on our results as sensitivity analyses focusing on population-based cohorts were comparable to the analyses based on all data.

A major strength of the current study was our comprehensive search in which we included all studies that reported any association between measures of adiposity and HairGC levels, including studies that did not primarily aim to investigate these associations. To minimize the risk of publication bias due to incomplete reporting of results based on statistical significance, we contacted corresponding authors of all included studies for additional information. In addition, we contacted all corresponding authors of studies that reported anthropometric measurements and HairGC but not an association. This yielded additional information for 70 cohorts (48%). This limits the risk of publication bias, which was also confirmed by our funnel plots (Supplementary Figures S10-S15). Moreover, an important addition of our work compared to the two systematic reviews and meta-analyses that have already been published on this topic was that we studied both the active form cortisol and the inactive form cortisone, their relations to different measures of adiposity, and also investigated effect sizes complementary to correlations. This has yielded the valuable conclusion that both the strongest correlation as well as the strongest, clinically relevant effect size are

actually seen for HairE vs. WC, instead of the most commonly studied association HairF vs. BMI. Another strength of our study is that we focused on studies that did not include participants with severe diseases affecting GC levels, which have therefore not disturbed our findings.

A limitation of our study was that we obtained data that related to full cohorts instead of individual person-data. This restricts our conclusions to comparisons across cohorts instead of across individuals. However, by pooling regression coefficients we could provide an effect size that is applicable on individual level. Other limitations relate to the lack of standardization of HairGC analysis methods and the usefulness of HairGC itself, as there are still numerous issues unsolved. For example, the ubiquitously reported growth speed of scalp hair, 1 cm per month, may vary considerably by ethnicity and season[8]. Other issues represent the high prevalence of overall CS use (which may influence basal cortisol levels and were found to be used by 11% of the Dutch population, a number that may be even higher in other countries[140,156]), hair characteristics such as color, treatment and washing frequency[157], and the unresolved issue of how to handle HairGC outliers[158,159]. These characteristics were often not reported in the included studies, which prevented comparison across studies. Then again, the results of our analyses in the subgroup of studies that accounted for outliers and corticosteroid use, the two issues that are most likely related to obesity, did not differ significantly from the results in the subgroup of studies that did not account for outliers, corticosteroid use, or neither. It should however be noted that we only assessed whether studies handled outliers at all, and that the exact manner of handling outliers in (psycho)endocrine research is still a separate topic of discussion[159]. Lastly, this review only included cross-sectional associations while any conclusion on the prognostic or predictive value of HairGC for future obesity should come from studies investigating longitudinal relations, which have however until now only been performed scarcely[134,160].

Altogether, we confirmed a consistent positive association between anthropometric measurements and hair glucocorticoids. This relation was most often studied for hair cortisol and BMI, but showed the strongest correlation and largest effect size for hair cortisone and waist circumference. These relations were not influenced by mean age, mean BMI or mean HairGC levels, nor by the used laboratory methods of the studies. However, the percentage of males, the percentage of participants with obesity and objective measurement of weight instead of self-reported weight represented important features to take into account when assessing hair glucocorticoids in cohorts. Although causality is not yet proven, our results suggest that higher long-term glucocorticoid levels measured in scalp hair, especially cortisone, may contribute to

or reflect the state of specifically central adiposity. Future longitudinal studies should investigate whether higher hair glucocorticoid levels can have clinical relevance in predicting the development or deterioration of obesity. Our results emphasize the importance of accounting for BMI and/or waist circumference or waist-hip-ratio when interpreting hair glucocorticoid levels in individuals or on a group level.

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Conflict of interest

The authors declare that there are no conflicts of interest for all authors.

Author contributions

EvdV, OA, and MM: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, and writing-original draft. AA: data curation, formal analysis, investigation, visualization, and writing-review and editing. VW, AI, EvdA, YdR, and BvdV: formal analysis, investigation, methodology, supervision, validation, and writing-review and editing. TS: data curation, formal analysis, investigation, supervision, validation, and writing-review and editing. SH: conceptualization, formal analysis, investigation, methodology, supervision, validation, visualization, writing-review and editing. EvR: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing-review and editing.

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SUPPLEMENTARY APPENDIX

Appendix 1. Search strategy.

Search date: 16 November 2020

Embase

('hydrocortisone'/exp OR (cortisol* OR cortison* OR hypocortisol* OR hypercortisol*):ab,ti,kw) AND (hair/de OR 'scalp hair'/de OR 'hair level'/exp OR 'hair analysis'/exp OR (hair OR

hairs):ab,ti,kw) AND ('body mass'/exp OR 'waist circumference'/de OR 'waist hip ratio'/exp OR 'body weight'/exp OR obesity/exp OR 'anthropometric parameters'/de OR anthropometry/de OR 'birth weight'/exp OR weight/de OR 'cardiometabolic risk'/exp OR 'skinfold thickness'/de OR 'body fat'/de OR 'health status'/de OR 'general health status assessment'/exp OR ((body NEAR/3 mass*) OR weight OR 'birth weight' OR birthweight OR bmi OR (waist NEAR/3 (circumferen* OR hip)) OR obes* OR (metabol* NEAR/3 syndrom*) OR overweight* OR anthropometr* OR (physiological* NEAR/3 measure*) OR (cardiometabol* NEAR/3 risk) OR 'body fat' OR (fat NEAR/3 percentage*) OR ((health OR functional*) NEAR/3 (measure* OR status* OR state OR general*))):ab,ti,kw) NOT ([animals]/lim NOT [humans]/lim)

Medline Ovid

("hydrocortisone"/ OR (cortisol* OR cortison* OR hypocortisol* OR hypercortisol*).ab,ti,kf.) AND (hair/ OR (hair OR hairs).ab,ti,kf.) AND ("Body Weights and Measures"/ OR "Body Mass Index"/ OR exp "Body Weight"/ OR exp "waist circumference"/ OR "Waist-Hip Ratio"/ OR "Skinfold Thickness"/ OR "body weight"/ OR obesity/ OR Anthropometry/ OR exp "birth weight"/ OR exp "Health Status"/ OR ((body ADJ3 mass*) OR weight OR "birth weight" OR birthweight OR bmi OR (waist ADJ3 (circumferen* OR hip)) OR obes* OR (metabol* ADJ3 syndrom*) OR overweight* OR anthropometr* OR (physiological* ADJ3 measure*) OR (cardiometabol* ADJ3 risk) OR "body fat" OR (fat ADJ3 percentage*) OR ((health OR functional*) ADJ3 (measure* OR status* OR state OR general*))).ab,ti,kf.) NOT (exp animals/ NOT humans/)

Cochrane

((cortisol* OR cortison* OR hypocortisol* OR hypercortisol*):ab,ti) AND ((hair OR hairs):ab,ti) AND ((body NEAR/3 mass*) OR weight OR 'birth weight' OR birthweight OR bmi OR (waist NEAR/3 (circumferen* OR hip)) OR obes* OR (metabol* NEAR/3 syndrom*) OR overweight* OR anthropometr* OR (physiological* NEAR/3 measure*) OR (cardiometabol* NEAR/3 risk) OR 'body fat' OR (fat NEAR/3 percentage*) OR ((health OR functional*) NEAR/3 (measure* OR status* OR state OR general*))):ab,ti

Web of science

TS=(((cortisol* OR cortison* OR hypocortisol* OR hypercortisol*)) AND ((hair OR hairs)) AND ((((body NEAR/2 (mass*)) OR weight OR "birth weight" OR birthweight OR bmi OR (waist NEAR/2 (circumferen* OR hip)) OR obes* OR (metabol* NEAR/2 syndrom*) OR overweight* OR anthropometr* OR (physiological* NEAR/2 measure*) OR (cardiometabol* NEAR/2 risk)) OR "body fat" OR (fat NEAR/2 percentage*)) OR ((health OR functional*) NEAR/2 (measure* OR status* OR state OR general*))))

Scopus

TITLE-ABS-KEY(((cortisol* OR cortison* OR hypocortisol* OR hypercortisol*)) AND ((hair OR hairs)) AND ((((body W/2 (mass*)) OR weight OR "birth weight" OR birthweight OR bmi OR (waist W/2 (circumferen* OR hip)) OR obes* OR (metabol* W/2 syndrom*) OR overweight* OR anthropometr* OR (physiological* W/2 measure*) OR (cardiometabol* W/2 risk)) OR "body fat" OR (fat W/2 percentage*)) OR ((health OR functional*) W/2 (measure* OR status* OR state OR general*))))

Google scholar

First 100:

Cortisol hair|hairs “body mass |weight”|”birth weight”|birthweight|bmi|”waist circumferen|hip”|obesity|obese|”metabolic syndrome”|overweight|anthropometric|anthropometry|”body fat”|”fat percentage”|”health status”

allintitle: 21

Cortisol hair|hairs “body mass |weight”|”birth weight”|birthweight|bmi|”waist circumferen|hip”|obesity|obese|”metabolic syndrome”|overweight|anthropometric|anthropometry|”body fat”|”fat percentage”|”health status”

Cinahl

(MH “hydrocortisone” OR TI(cortisol* OR cortison* OR hypocortisol* OR hypercortisol*) OR AB(cortisol* OR cortison* OR hypocortisol* OR hypercortisol*)) AND (MH “Hair+” OR TI(hair OR hairs) OR AB(hair OR hairs)) AND (MH “Body Weights and Measures” OR MH “Body Mass Index” OR MH “Body Weight+” OR MH “waist circumference+” OR MH “Waist-Hip Ratio” OR MH “Skinfold Thickness” OR MH “obesity” OR MH “Anthropometry” OR MH “birth weight+” OR MH “Health Status+” OR TI((body N2 mass*) OR weight OR “birth weight” OR birthweight OR bmi OR (waist N2 (circumferen* OR hip)) OR obes* OR (metabol* N2 syndrom*) OR overweight* OR anthropometr* OR (physiological* N2 measure*) OR (cardiometabol* N2 risk) OR “body fat” OR (fat N2 percentage*) OR ((health OR functional*) N2 (measure* OR status* OR state OR general*))) OR AB((body N2 mass*) OR weight OR “birth weight” OR birthweight OR bmi OR (waist N2 (circumferen* OR hip)) OR obes* OR (metabol* N2 syndrom*) OR overweight* OR anthropometr* OR (physiological* N2 measure*) OR (cardiometabol* N2 risk) OR “body fat” OR (fat N2 percentage*) OR ((health OR functional*) N2 (measure* OR status* OR state OR general*)))) NOT (MH “animals+” NOT MH “human”)

PsycInfo

(exp hydrocortisone/ OR (cortisol* OR cortison* OR hypocortisol* OR hypercortisol*).ab,ti.) AND (hair/ OR (hair OR hairs).ab,ti.) AND (exp Body Weight/ OR

Body Mass Index/ OR Obesity/ OR Anthropometry/ OR Health Status/ OR ((body ADJ3 mass*) OR weight OR “birth weight” OR birthweight OR bmi OR (waist ADJ3 (circumferen* OR hip)) OR obes* OR (metabol* ADJ3 syndrom*) OR overweight* OR anthropometr* OR (physiological* ADJ3 measure*) OR (cardiometabol* ADJ3 risk) OR “body fat” OR (fat ADJ3 percentage*) OR ((health OR functional*) ADJ3 (measure* OR status* OR state OR general*))).ab,ti.) NOT (exp animals/ NOT humans/)

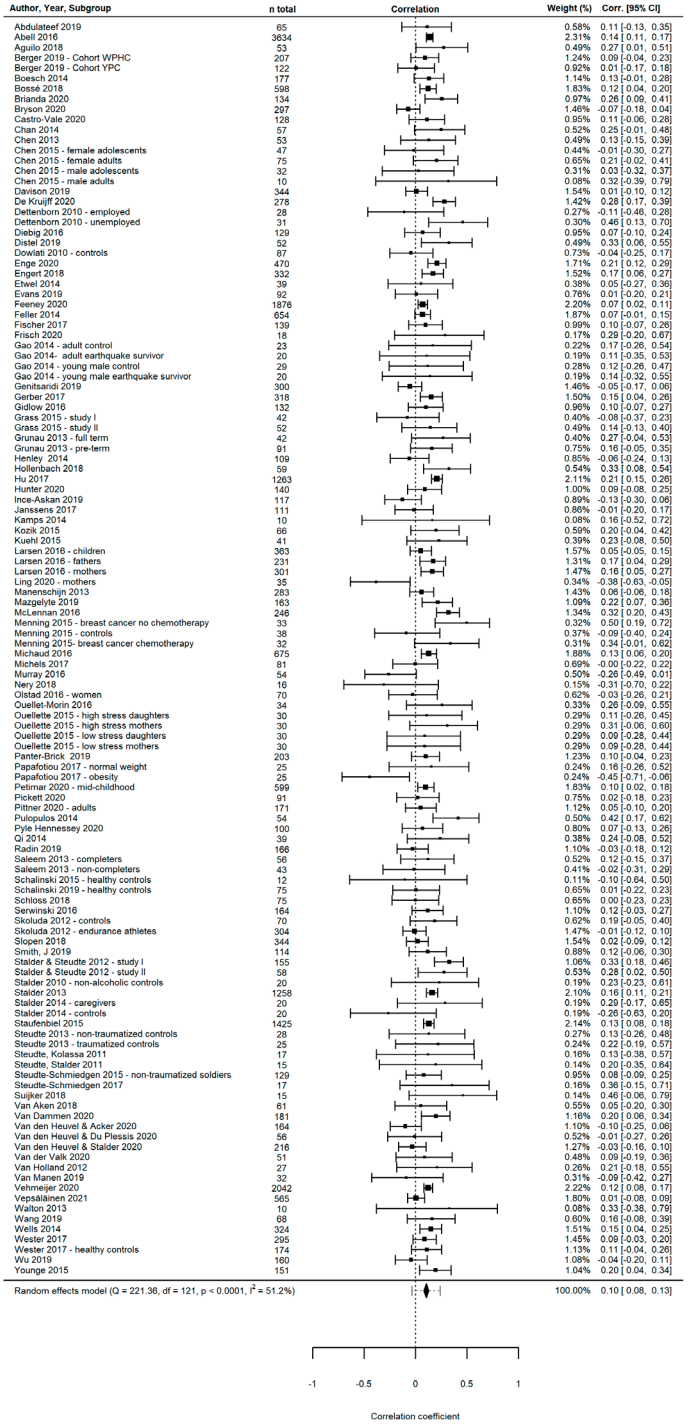
Supplementary Table S1. Qualitative overview of reported associations between obesity measurements and HairGC

	BMI	WC	WHR	BMI SDS
HairF- categorical	n= 13,209. 15/26 cohorts show positive relation, i.e., higher HairF levels in individuals with obesity/ overweight (1-15); 3 cohorts negative relation, i.e., lower HairF levels in individuals with obesity/overweight (16-18); 1 cohort shows lower BMI in individuals with high HairF levels (19); 7 cohorts found no relation between HairF and BMI (20-26)	n=2,778 2/4 cohorts show higher HairF in individuals with higher WC levels (9, 10); 1 cohort found higher WC in the high HairF group (22); 1 cohort found no relation between HairF and WC (24)	n=271 0/1 cohorts show relation (13)	n=50 1/1 cohort show higher HairF in children with obesity versus those without obesity (12)
HairF- bivariate correlation	n=27,861 34/129 cohorts show positive relation (1, 4, 7, 8, 10, 11, 13, 27-50), 2 cohorts show negative relation (12, 19), 93 cohorts show no relation (2, 3, 5, 6, 12, 14-18, 20, 22-24, 26, 31, 36, 38, 51-108).	n=11,419 11/27 cohorts show positive relation (1, 10, 28, 34, 40, 43, 62, 107, 109-111); 16 cohorts show no relation (3, 15, 16, 18, 24, 51, 72-74, 81, 85, 92, 101-103, 112)	n=7,357 5/18 cohorts show positive relation (1, 62, 109, 110, 113); 13 cohorts show no relation (13, 16-18, 55, 85, 101, 102, 114, 115)	n=1,247 3/11 cohorts show positive relation (30, 116); 1 cohort shows negative relation (12); 8 cohorts show no relation (12, 19, 45, 71, 78, 82, 117, 118)
HairF- partial correlation	n=2,527 1/1 cohort shows positive relation (9)	n=2,527 0/1 cohorts show relation (9)	NA	NA

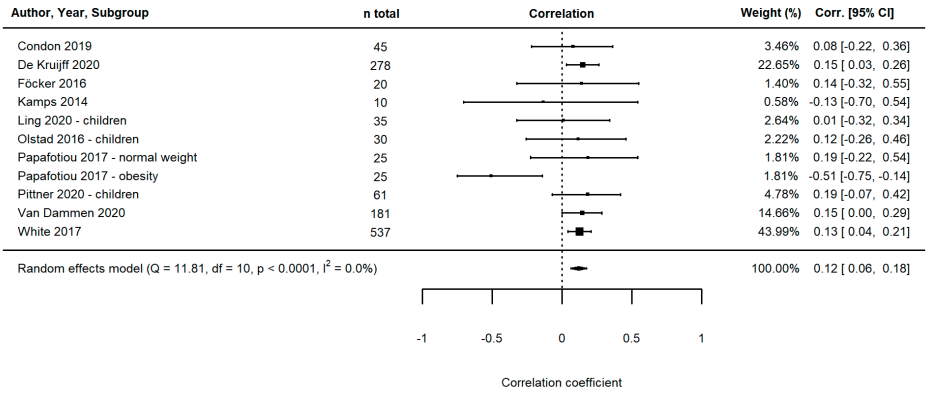
HairF independent - simple regression	n=18,953 21/54 cohorts show positive relation (1, 4, 10, 11, 27, 28, 30, 32-34, 36, 37, 39, 41, 43, 45-47, 73, 110); 1 cohort shows negative relation (40); 32 cohorts show no relation (2, 12, 17-20, 24, 51, 54, 58, 61, 70, 71, 75, 76, 78, 81-83, 85-87, 89, 91, 99, 101-103, 105, 107)	n=6,500 7/18 cohorts show positive relation (10, 28, 34, 40, 43, 107, 110); 11 cohorts show no relation (18, 24, 36, 51, 73, 81, 85, 101-103, 112)	n=2,334 1/7 cohorts show relation (110); 6 cohorts show no relation (17, 18, 36, 85, 101, 102)	n=1,734 4/11 cohorts show relation (30, 40, 82, 117); 1 cohort shows negative relation (12); 6 cohorts show no relation (12, 20, 36, 45, 71, 78)
HairF dependent - simple regression	n=2,729 5/20 cohorts show positive relation (29, 30, 33, 39, 45); 15 cohorts show no relation (18, 24, 26, 51, 58, 61, 66, 71, 79, 86, 99, 101-103, 107)	n=715 1/6 cohorts show positive relation (107); 5 cohorts show no relation (24, 51, 101-103)	n=389 0/3 cohorts show relation (20, 101, 102)	n=984 2/5 cohorts show positive relation (25, 30); 3 cohorts show no relation (45, 71, 75)
HairF independent - multiple regression	n=1,109 1/2 cohorts show negative relation (44); 1 cohort shows no relation (6)	NA	NA	n=35 0/1 cohorts show relation (19)
HairF dependent - multiple regression	n=3,803 1/2 cohorts show positive relation (48, 119); 1 cohort shows no relation (120)	n=117 0/1 cohorts show relation (20)	n=141 0/1 cohorts show relation (113)	n=117 0/1 cohorts show relation (20)
HairE- categorical	n=2,769 3/5 cohorts show positive relation between HairE and BMI, i.e., higher hairE in individuals with obesity (4, 13, 21); 2 cohorts show no relation between HairE and BMI (20, 24)	n=32 0/1 cohorts show relation (24)	n=271 1/1 cohort shows positive relation (13)	NA

HairE- bivariate correlation	n=8,615 5/18 cohorts show positive relation (4, 13, 43, 46, 110); 13 cohorts show no relation (8, 20, 23, 24, 50, 57, 61, 73, 82, 101, 103, 107)	n=3,158 4/7 cohorts show positive relation (43, 73, 103, 110); 3 cohorts show no relation (24, 101, 107)	n=1,585 3/3 cohorts show positive relation (13, 101, 110)	NA
HairE- partial correlation	NA	NA	NA	NA
HairE independent- simple regression	n=5,327 3/10 cohorts show positive relation (4, 43, 110); 7 cohorts show no relation (20, 24, 73, 82, 103, 107)	n=3,102 4/6 cohorts show positive relation (43, 73, 103, 110); 2 cohorts show no relation (24, 107)	NA	NA
HairE dependent- simple regression	n=434 0/4 cohorts show relation (24, 101, 103, 107)	n=434 1/4 cohorts show positive relation (103); 3 cohorts show no relation (24, 101, 107)	NA	NA
HairE independent- multiple regression	NA	NA	NA	n=117 0/1 cohorts show relation (20)
HairE dependent- multiple regression	NA	n=117 0/1 cohorts show relation (20)	NA	

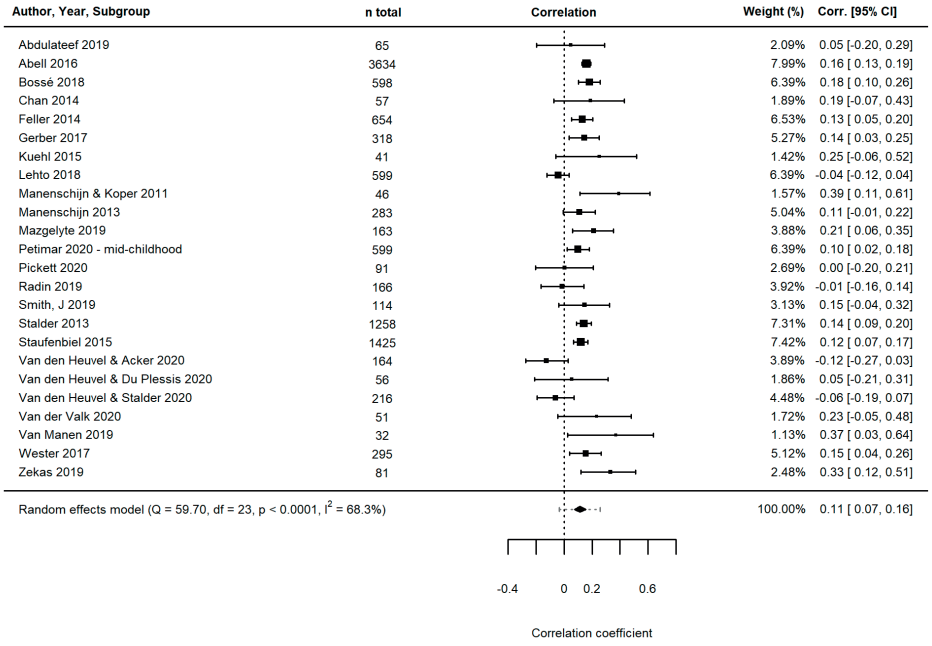
Abbreviations: CI, confidence interval; HairF, hair cortisol; HairE, hair cortisone; SDS, standard deviation score; WC, waist circumference; WHR, waist-to-hip ratio; NA, not available



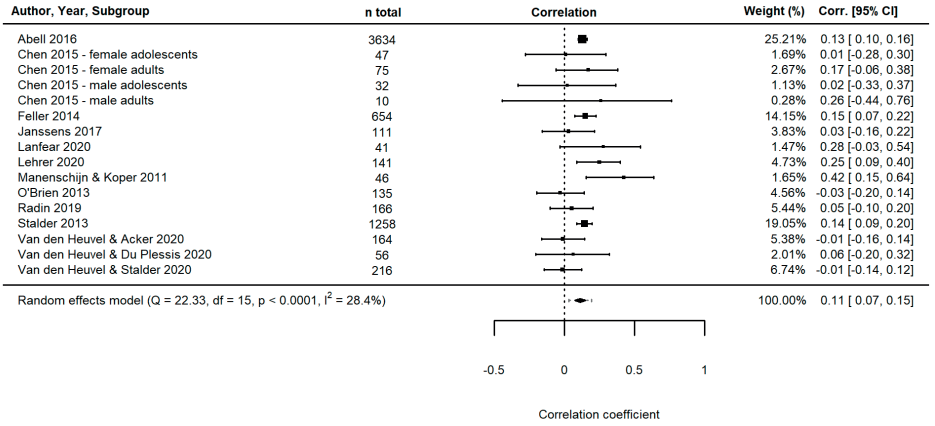
*Supplemental Figure S1. Forest plot for the meta-analysis of correlation coefficients between **HairF and BMI**.*



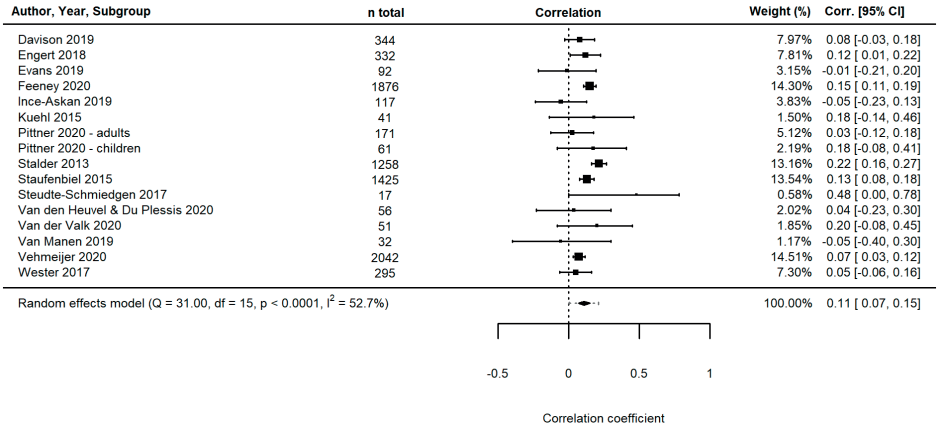
*Supplemental Figure S2. Forest plot for the meta-analysis of correlation coefficients between **HairF and BMI SDS**.vv*



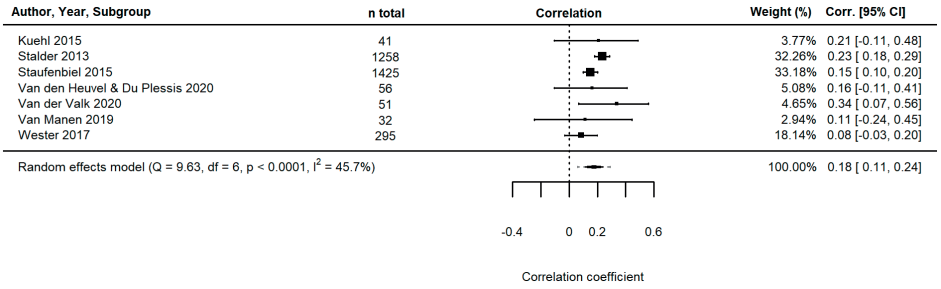
*Supplemental Figure S3. Forest plot for the meta-analysis of correlation coefficients between **HairF and WC**.*



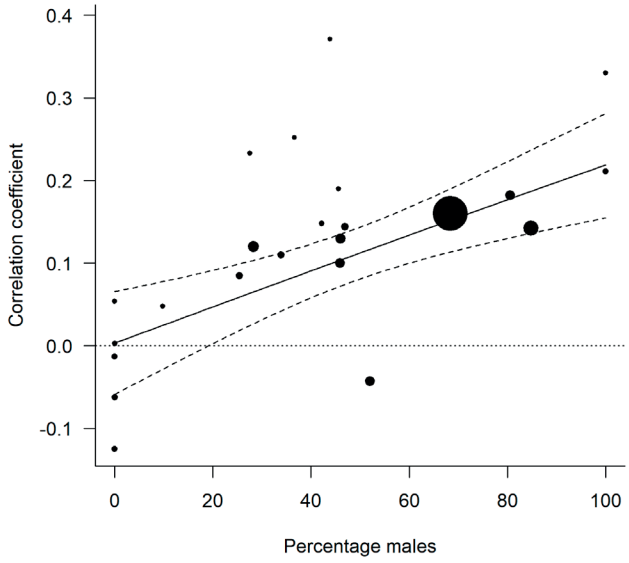
*Supplemental Figure S4. Forest plot for the meta-analysis of correlation coefficients between **HairF and WHR**.*



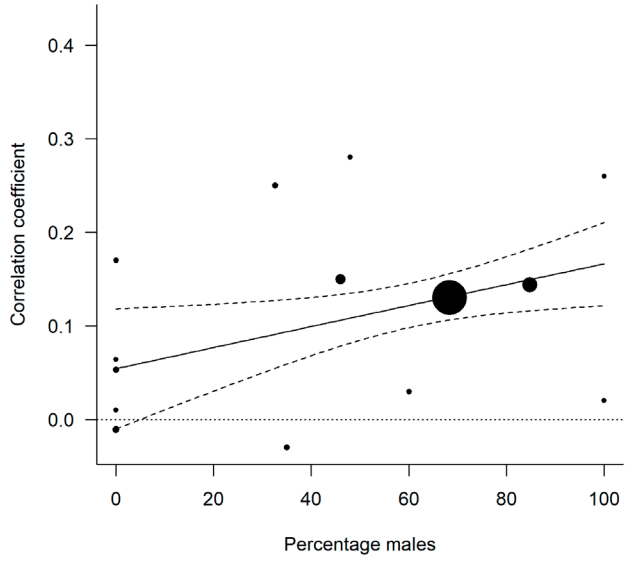
*Supplemental Figure S5. Forest plot for the meta-analysis of correlation coefficients between **HairE and BMI**.*



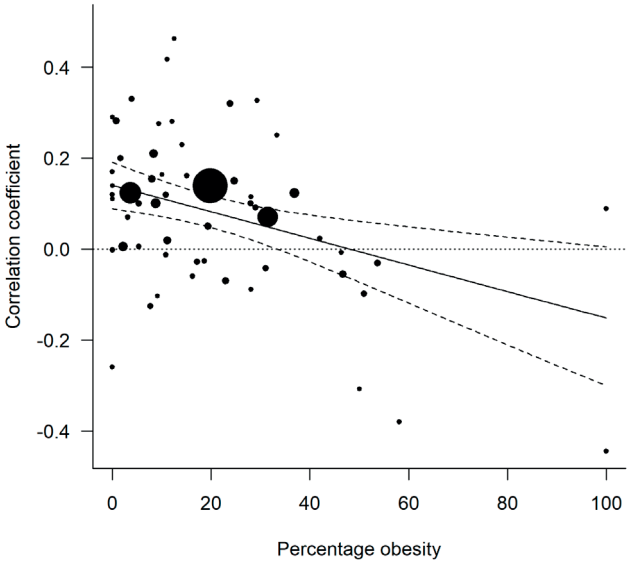
*Supplemental Figure S6. Forest plot for the meta-analysis of correlation coefficients between **HairE and WC**.*



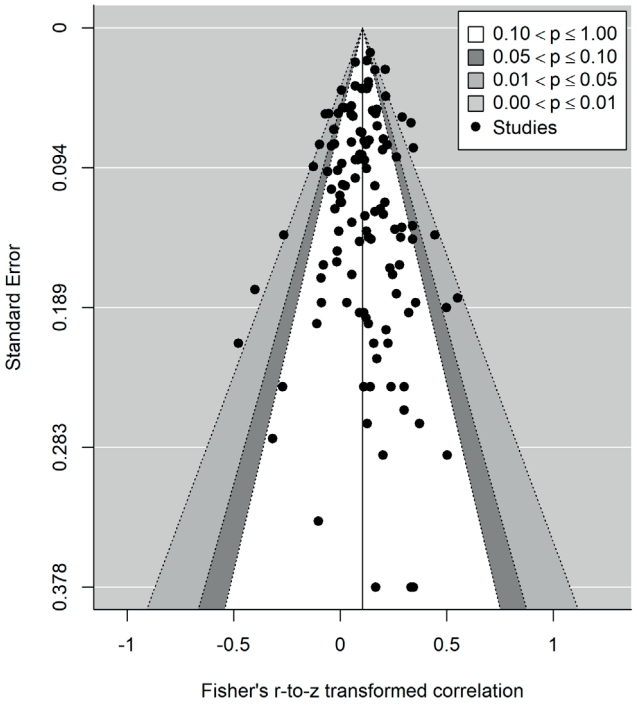
*Supplemental Figure S7. Bubble plot for the meta-regression on proportion of males in the meta-analysis of correlation coefficients between **HairF and WC**. The size of the dots represents the study sample size. The dashed lines represent the 95% confidence interval.*



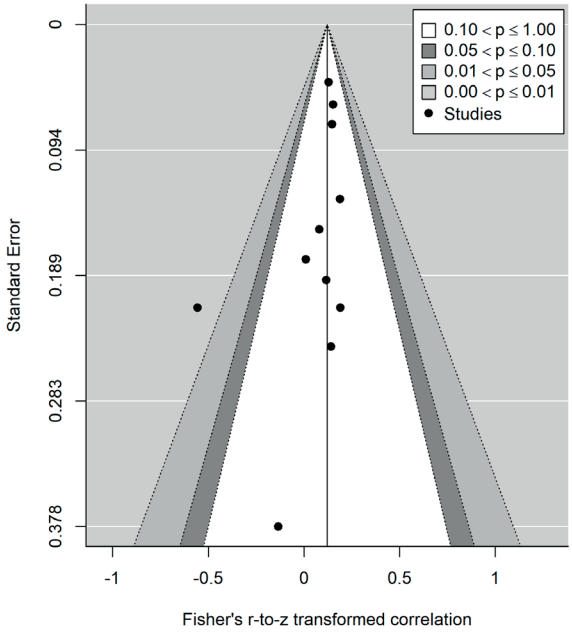
*Supplemental Figure S8. Bubble plot for the meta-regression on proportion of males in the meta-analysis of correlations between **HairF and WHR**. The size of the dots represents the study sample size. The dashed lines represent the 95% confidence interval.*



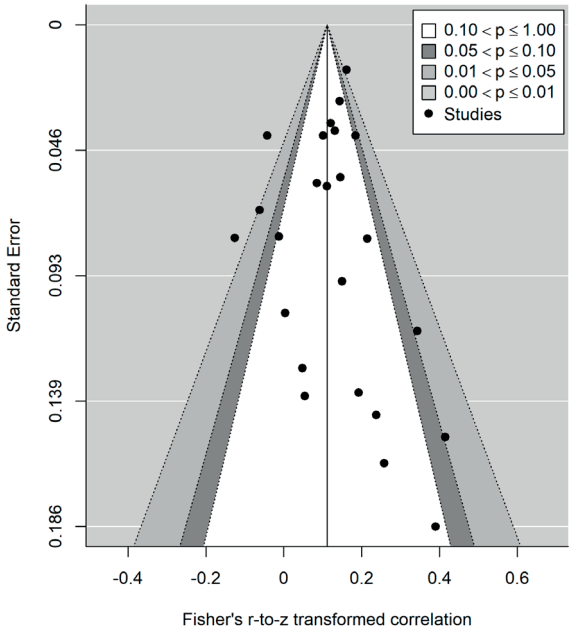
*Supplemental Figure S9. Bubble plot for the meta-regression on proportion of individuals with obesity in the meta-analysis of correlations between **HairF and BMI**. The size of the dots represents the study sample size. The dashed lines represent the 95% confidence interval.*



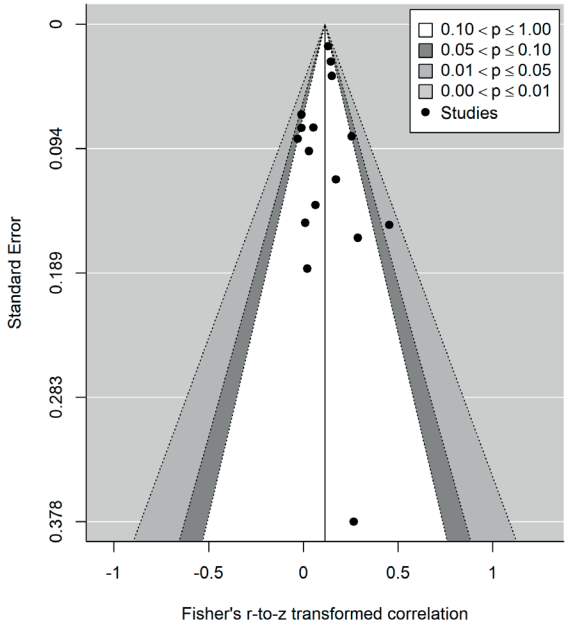
*Supplemental Figure S10. Funnel plot for the meta-analysis of correlation coefficients between **HairF and BMI**.*



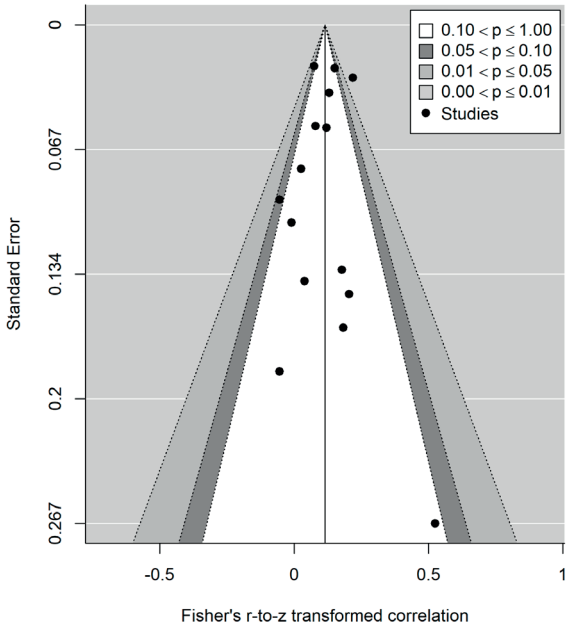
Supplemental Figure S11. Funnel plot for the meta-analysis of correlation coefficients between ***HairF and BMI SDS****.*



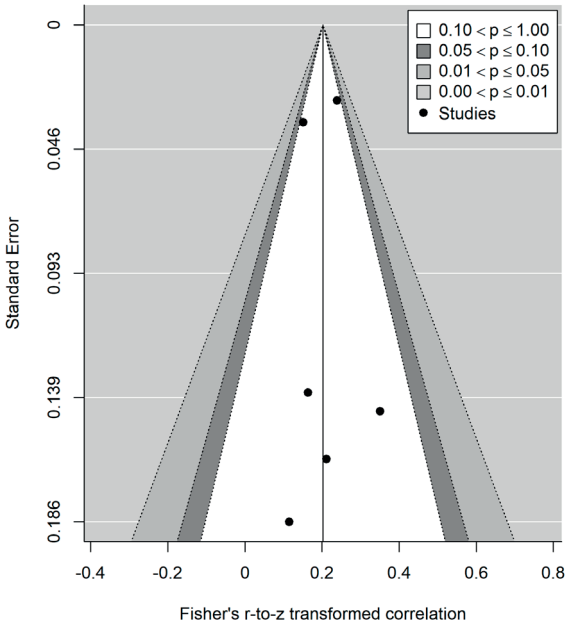
Supplemental Figure S12. Funnel plot for the meta-analysis of correlation coefficients between ***HairF and WC****.*



*Supplemental Figure S13. Funnel plot for the meta-analysis of correlation coefficients between **HairF and WHR**.*



*Supplemental Figure S14. Funnel plot for the meta-analysis of correlation coefficients between **HairE and BMI**.*



*Supplemental Figure S15. Funnel plot for the meta-analysis of correlation coefficients between **HairE and WC**.*

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COVID-19 related anxiety in children and adolescents with severe obesity: a mixed-methods study

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ABSTRACT

Recent studies report negative mental health effects of the COVID-19-related lockdown measures in general pediatric cohorts. Since obesity is a risk factor for COVID-19 in adults, children (including adolescents) with obesity might perceive themselves to be vulnerable. Using a combined quantitative and qualitative approach, we explored COVID-19-related anxiety in pediatric patients with severe obesity in the Netherlands using semi-structured telephone interviews and the Pediatric Quality of Life Inventory (PedsQL) questionnaire, which had also been completed by the study population at baseline in the year prior to the COVID-19 outbreak. In total, 75 families participated in the semi-structured telephone interviews during the lockdown, April 2020. Characteristics of included patients were: median age 10.5 years (IQR 7.6–15.2); 52% female; mean BMI SDS 3.8 (SD 1.0).

COVID-19-related anxiety was reported for 24/75 (32%) children. The mean decrease in PedsQL score between baseline visit and COVID-19 outbreak did not differ between children for whom anxiety was reported versus those for whom it was not (mean change -10.3 ± 36.5 vs. -3.3 ± 24.4, p=0.54). Self-imposed strict quarantine measures were taken by 19/75 (25%) families. During follow-up, several families reported that the previous contact alleviated their anxiety. In conclusion, health care professionals should address possible COVID-19-related anxiety in children with severe obesity. Addressing COVID-19-related anxiety could mitigate its potential negative effects.

INTRODUCTION

During the current coronavirus disease 2019 (COVID-19) pandemic, governments across the world have used differential lockdown and quarantine measures to mitigate the spread of the virus. Recent studies report how this situation affected the psychological wellbeing of children (including adolescents).[1-9] These studies report several adverse effects on psychological wellbeing such as anxiety, worrying, irritability, depressive symptoms, and even posttraumatic stress disorder symptoms in 18.9–43.7% of children sampled from the general population in Asian, European or American countries. Moreover, a recent study in Italian children and adolescents with obesity, showed unfavorable changes in eating, sleeping, and activity behaviors during COVID-19 quarantaine.[10]

Obesity is regarded as a risk factor for COVID-19 in adults.[11] Consequently, children with obesity might perceive themselves to be vulnerable. Moreover, we noticed COVID-19 related concerns during our regular contacts with children and their parents at the outpatient clinic of our pediatric obesity center when the governmental lockdown measures in The Netherlands were effectuated. On top of that, quality of life is already known to be diminished in children with severe obesity in comparison to the general population.[12,13] However, no studies have assessed such psychological aspects of the COVID-19 outbreak in children and adolescents with obesity. Therefore, we designed a combined quantitative and qualitative study to explore the psychological impact of the COVID-19 outbreak and related lockdown measures in children (including adolescents) with severe obesity and their potential effects on lifestyle behavior. When conducting this study, COVID-19 related anxiety appeared to be an important theme, similar to results from the previously mentioned literature from general populations. Accordingly, we want to present our in-depth findings regarding COVID-19 related anxiety in children with severe obesity and their parents.

METHODS

This study was approved by the ethics committee of the Erasmus MC. All data were collected for health care purposes and filed in the patient's medical records. Written informed consent was obtained from all patients and/or their caregivers to use their health data for research purposes after pseudonymization.

Study participants

In the Netherlands, selective lockdown measures including school closures were established from 16 March 2020 onwards. During the first month, between 2-23 April 2020, when these measures were in full effect, we contacted all parents of children currently under treatment at Obesity Center CGG (Erasmus MC-Sophia Children's Hospital), a national referral center for obesity. Patients are referred to Obesity Center CGG for diagnostic evaluation and/or personalized therapeutic advice.[14] We approached parents of all patients who had completed the diagnostic workup of our obesity center and whose last visit to the outpatient clinic was in 2019 or 2020. We did not approach parents of children who have severe intellectual disability or severe behavioral problems, as we expected that their families' experiences during the lockdown period would not be representative. Because this study was conducted in the context of patient care, we included all eligible study participants even after data saturation for qualitative analyses had been achieved.

Telephone interviews

A semi-structured telephone interview lasting 20-30 minutes, was conducted by a treating physician (OA, BVDV, MW) to explore the impact of the COVID-19 outbreak and related measures on the children's lifestyle behavior and quality of life. In most cases, parents were interviewed as proxy for their children, and children were invited to actively participate in the interviews if verbal communication skills allowed it. All parents of eligible patients were contacted in a three-week time frame, during which the treating physicians had weekly meetings to discuss the previous' weeks findings and gain insights from each other's experiences. The physicians used a structured interview format with 37 predefined variables for categorical data and 20 predefined open-ended questions to comprehensively document the telephone interviews in the patients' medical records. Additionally, field notes were collected during the interviews and qualitative analyses. The predefined interview question related to anxiety was: "*Does your child experience stress or anxiety due to the Corona outbreak?*". The predefined interview questions related to lockdown measures was: *"What kind of lockdown measures did your family take, especially regarding: school? Day-care attendance? Work? Social contacts? Hobbies?"*. Based on the answers on these questions, additional questions were asked in the context of patient care to further explore thoughts and reasons behind anxiety and imposed lockdown measures, and if present, whether our proactive support was necessary to minimize the impact on weight-related health. After all interviews had been conducted, the comprehensive records were exported from the patient's medical records for analyses.

Quantitative assessments and analysis

Height and weight were measured during the previous hospital visit within the past year by trained outpatient clinic assistants and BMI was converted to age- and sex-specific standard deviation scores (SDS) using Dutch reference charts.[15] Both at the baseline visit prior to the COVID-19 pandemic as well as during the lockdown measures, the 23-item Pediatric Quality of Life inventory™ (PedsQL™) 4.0 (parents proxy-report version) was completed. We assessed the total score and the subscore for emotional functioning, ranging from 0–100 with higher scores indicating better quality of life.[16] Quantitative data were analyzed using SPSS version 25.0 [IBM]. Differences in patient characteristics between patients for whom anxiety was reported compared to those for whom anxiety was not reported in the abovementioned question were analyzed using (paired samples) t-tests or Mann-Whitney tests with an α of 0.05.

Qualitative analysis

Qualitative data were analyzed using MAXQDA 2018 [VERBI Software] following best practice methods for qualitative studies and were reported following the Consolidated Criteria for Reporting Qualitative Research (COREQ) checklist.[17,18] Two physicians (OA, MW) independently coded all interviews according to the Grounded Theory after all telephone interviews had been conducted.[19] According to this theory, first a deductive, theory-driven approach was used, followed by an inductive, data-driven approach, by two of the three interviewing physicians. The two physicians started by open coding of interview data independently. The applied codes were then compared and differences were solved by consensus. Subsequently, a code tree was developed in a meeting with the study team using axial coding. To minimize the possibility of structural differences between the three physicians who conducted the interviews, the code tree was developed based on interviews from a subset of 24 patients, 8 patients per interviewing physician. Finally, selective coding was used to identify the code categories that were most relevant to our research question. The axial and selective coding steps were also performed independently by both physicians and differences were solved by consensus. During the entire qualitative analysis process, a study log was kept by the two physicians and memos were used to carefully note emerging ideas about the data analysis which were discussed during weekly meetings with the study team, to further ensure rigor.

RESULTS

In total, 90 families were approached. Seventy-five participated in the telephone interviews, of which 40 also completed the PedsQL questionnaire. Table 1 shows the baseline characteristics of the patients.

Anxiety related to the COVID-19 outbreak and related measures was reported for 24/75 (32%) children. Baseline characteristics and quality of life did not differ significantly between patients for whom anxiety was reported versus not reported (Table 1 and 2). The mean PedsQL total score between baseline visit and COVID-19 outbreak slightly decreased in the study population, although not statistically significant (mean change -6.3 ± 29.9. P = 0.26). A bigger decrease was seen in the children for whom anxiety was reported versus those who did not (mean change -10.3 ± 36.5 vs. -3.3 ± 24.4), but this was also not statistically significant (Table 2).

Table 3 reports the identified reasons behind this anxiety and the behavioral consequences. Most of the children with reported anxiety were afraid to be at increased risk for COVID-19 infection. No children and only two parents specifically mentioned obesity as reason for their anxiety. In total, 19 families, either with children with reported anxiety (6/24; 25%) or without (13/51; 25%), took self-imposed quarantine measures additional to governmental lockdown measures, such as total home confinement (Table 3). In five families with severe anxiety leading to negative lifestyle consequences telephone follow-up in the following weeks was deemed necessary in the context of patient care by the treating physician. During this follow-up, 3/5 families reported that their concerns had been alleviated by information offered in the previous contact with the physician (Table 3).

DISCUSSION

In this Dutch study, COVID-19 related anxiety was reported for a considerable proportion (32%) of children with severe obesity under treatment at a tertiary center. To our knowledge, this is the first study to investigate COVID-19 related anxiety in children and adolescents with obesity, and only few studies explored similar psychological effects in children with other chronic diseases. A recent study in children with type 1 diabetes in India reported that moderate or severe stress was present in nearly 60% of their patients during the COVID-19 pandemic, but this did not differ from age- and gender-matched controls.[20] Another study in children with cystic fibrosis in Turkey also did not find a difference in anxiety scores between their patients and age-matched

Table 1. Baseline characteristics of the study population.

Characteristic	All patients (n=75)	Children for whom anxiety was reported (n=24)	Children for whom anxiety was not reported (n=51)	P-value
Age in years, median (IQR)	10.5 (7.6 – 15.2)	11.0 (8.7 – 15.9)	10.2 (6.8 – 15.2)	0.74
Sex, female (%)	39 (52%)	15 (63%)	24 (47%)	0.21
Ethnicity, Dutch (%)	50 (67%)	17 (71%)	33 (65%)	0.42
Socioeconomic status score, median (IQR)	0.0 (-0.7 – +0.7)	0.0 (-0.6 – +0.7)	0.0 (-1.2 – +0.7)	0.87
Body mass index SDS at last visit to hospital, mean (SD)	3.8 (1.0)	3.7 (0.9)	3.8 (1.0)	0.87

Legend: IQR, interquartile range; SD(S), standard deviation (score); COVID-19, coronavirus disease 2019.

Table 2. Quality of life during COVID-19 related lockdown measures.

Characteristic		All patients (n=40)	Children for whom anxiety was reported (n=18)	Children for whom anxiety was not reported (n=22)	P-value[a]
PedsQL score on emotional functioning, mean (SD)	During COVID-19	59.4 (21.8)	57.5 (24.0)	60.9 (20.3)	0.63
	Delta baseline vs COVID-19	-3.5 (35.2)	-5.0 (40.7)	-2.2 (30.7)	0.82
PedsQL total score, mean (SD)	During COVID-19	66.2(17.7)	65.9 (20.0)	66.5 (16.2)	0.93
	Delta baseline vs COVID-19	-6.3 (29.9)	-10.3 (36.5)	-3.3 (24.4)	0.54

[a]P-value for the difference between children for whom anxiety was reported versus those who did not. SD(S), standard deviation (score); COVID-19, coronavirus disease 2019; baseline, measured at the outpatient visit in the year prior to the COVID-19 outbreak.

Table 3. Identified themes regarding COVID-19 related anxiety and lockdown measures and relevant passages from the documentation of the telephone interviews

Themes	Relevant passages
Theme 1: reasons for anxiety in children	
Theme 1.1: anxious for being at risk for COVID-19	- Child (17y, F) is afraid that she is more likely to get ill due to Corona because of her health problems. - Child (10y, M) is afraid he will get more ill than others from Corona.
Theme 1.2: anxious for health of family members at risk for COVID-19 due to perceived vulnerability	- Child (11y, M) is concerned for his mother. He always wants to join her during her weekly visits to the supermarket. If it was up to him, she would stay home all the time. - Child (9y, M) is afraid his father might get ill, because his father has heart failure and COPD.
Theme 2: reasons for anxiety in parents	
Theme 2.1: anxious for child being at risk for COVID-19 due to perceived vulnerability	- Mother is afraid that her child (5y, F) is at increased risk because of her obesity. Therefore, they already confined themselves to home before governmental lockdown measures were taken. - Father is not sure if he will let his son (11y, M) go to school after school reopenings due to his asthma.
Theme 2.2 : anxious for transmitting COVID-19 to family members at risk	- Child (15y, F) is not allowed to have contact with friends, because parents fear she will transmit Corona to their 75 year old grandfather who lives with them. - Child (11y, M) is not allowed to play with friends, because of his mother's asthma. He's also not allowed to visit his grandparents.
Theme 3: Behavioral consequences of anxiety	
Theme 3.1: additional restrictions imposed by parents regarding home confinement and social contacts	- Parents cancelled all support and care from health care professionals on their own initiative because parents perceive their child (16y, F) to be vulnerable. - Initially, the family was anxious and stayed at home all the time. Yesterday mother and child (5y, F) went outside for the first time since three weeks. - Child (11y, F) is not allowed to play with friends anymore.
Theme 3.2: additional restrictions self-imposed by child only	- Child (11y, M) is afraid to play outside. Even before the national lockdown measures were issued, he declined to go outside when his parents asked him to. In the past 1.5 month, he only went outside three times. - Child (9y, M) doesn't want to meet with friends anymore, because he thinks his father is at increased risk for COVID-19.
Theme 3.2: concerns alleviated by health care professional	- In the beginning, the child (11y, F) was afraid to be at risk because of her obesity. After the talk with health care professional X her concerns were relieved. - Quote by mother of child (5y, F): *"For my own peace of mind, I will discuss my concerns with my general practitioner. I don't want to be afraid."*

controls.[21] In the general population, severe stress and traumatizing symptoms in children have been reported in a qualitative study from India and COVID-19-related restrictions seemed to be the primary cause.[22] This is in line with a previous qualitative report on the 2003 SARS and 2009 H1N1 pandemics, which showed that 30% of children who had been isolated or quarantined met the clinical cut-off score for post-traumatic stress disorder.[23] These studies cannot be directly compared with ours due to differences in study population, design and sociocultural contexts. However, these studies together with ours imply that COVID-19 related psychological distress such as stress and anxiety might be experienced by a significant minority of children and adolescents, both with and without obesity.

Recent reports show that lifestyle behaviors including physical activity and screen time are negatively impacted by the COVID-19 outbreak and related lockdown measures in Chinese school children and Italian children with obesity.[10,24] In a significant proportion of the families (25%) in our study, self-imposed quarantine measures were taken, even though measures advised by our national authorities did not differentiate between children with obesity or other chronic diseases and healthy children. These strict self-imposed measures are a concern because they can add to the known negative effects of the COVID-19 pandemic on lifestyle behavior. The anxiety that potentially underlies these self-imposed measures seems to be modifiable. In the families for whom short-term follow-up was necessary, we experienced that discussing this emotion with patients and parents and educating them can relieve concerns and make them lift their strict self-imposed measures. Topics that can be discussed with parents and children, using age-appropriate language, are: reassurance that children with obesity are currently perceived to be at low risk; reduction of exposure to COVID-19 related (social) media outlets; maintaining daily life routines as much as possible given governmental measures; encourage children to maintain social contacts, e.g., via the internet; and stimulating parents to promote positive mental and social wellbeing in their families and involving their children in the process.[25] Our qualitative analysis indicated that two important reasons behind the anxiety were the child's fear of being at risk for COVID-19 and the fear of infecting family members who are perceived to be vulnerable for COVID-19. In addition, the recent report on patients with cystic fibrosis found, similar to us, that anxiety could be alleviated in 84% of mothers by the health care professional during a telephone interview.[21] It is known that worrying of children for their parents can put a heavy burden on them, and effective communication with children can protect their psychological health.[26,27] We did not find differences in baseline characteristics nor in quality of life assessed by the PedsQL questionnaire or obesity severity between patients with and without COVID-19 related anxiety. This underscores that health care professionals should be

aware of the possible presence of COVID-19 related anxiety during all contacts with children and adolescents with severe obesity, not only in specific subgroups.

Strengths and limitations

A strength of our study is our qualitative approach which enabled us to explore possible arguments behind COVID-19 related anxiety and its potential modifiability. Moreover, our relatively large sample size allowed us to reach data saturation. A strength of our quantitative analyses is the comparison of PedsQL scores before and during the COVID-19 outbreak, as it is known that quality of life is already compromised in children with severe obesity.[12,13] A limitation of this study is its cross-sectional analysis; follow-up studies are needed to evaluate the course and effect of COVID-19 related anxiety on weight-related health and will be performed for our patient group. We did not consider including a control group without obesity because our study was designed to explore the impact of the COVID-19 outbreak and its consequences on lifestyle behaviors specifically in children with severe obesity. Accordingly, our patients served as their own control for the quantitative analyses. This should be kept in mind when attempting to extrapolate our findings.

In conclusion, health care professionals should be aware of the possible presence of COVID-19 related anxiety and its behavioral consequences, especially in children with severe obesity. Addressing this anxiety could mitigate its potential negative effects on the psychological wellbeing and lifestyle behaviors of these children.

Conflicts of interests statement

The authors declare no conflicts of interests.

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OA, MW, EvdE, EvdA, and BvdV conceived the study. OA, MW, and BvdV collected the data and performed the literature search. All authors were involved in data analysis, data interpretation, writing and editing of the manuscript and had final approval of the submitted and published versions,

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9b

Impact of the COVID-19 pandemic and related lockdown measures on lifestyle behaviors and wellbeing in children and adolescents with severe obesity

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ABSTRACT

Introduction COVID-19 lockdown measures have large impact on lifestyle behaviors and wellbeing of children. The aim of this mixed-methods study was to investigate the impact of COVID-19 lockdown measures on eating styles and behaviors, physical activity (PA), screen time, and health-related quality of life (HRQoL) in children (0-18 years) with severe obesity.

Methods During the first COVID-19 wave (April 2020), validated questionnaires were completed and semi-structured telephone interviews were conducted with parents of children with severe obesity (adult BMI-equivalent ≥35kg/m^2) and/or with the children themselves. Changes in pre-pandemic versus lockdown scores of the Dutch Eating Behavior Questionnaire Children (DEBQ-C), Pediatric Quality of Life Inventory (PedsQL^{TM}), and Dutch PA Questionnaire were assessed. Qualitative analyses were performed according to the Grounded Theory.

Results Ninety families were approached of which 83 families were included. Characteristics of the included children were: mean age 11.2 ± 4.6 years, 52% female, mean BMI SD-score +3.8 ± 1.0. Emotional, restrained, and external eating styles, HRQoL, and (non-educational) screen time did not change on group level (all $p>0.05$). However, weekly PA decreased (mean difference -1.9 hours/week, $p=0.02$), mostly in adolescents. In the majority of children, mean weekly PA decreased to ≤2 hours/week. Children with high emotional and external eating scores during lockdown or pre-existent psychosocial problems had the lowest HRQoL ($p<0.01$). Qualitative analyses revealed an increased demand for food in a significant proportion of children (n=21), mostly in children <10 years (19/21). This was often attributed to loss of daily structure and perceived stress. Families who reported no changes (n=15) or improved eating behaviors (n=11) attributed this to already existing strict eating schemes that they kept adhering to during lockdown.

Conclusion This study shows differing responses to COVID-19 lockdown measures in children with severe obesity. On group level, PA significantly decreased and in substantial minorities eating styles and HRQoL deteriorated. Children with pre-existent psychosocial problems or pre-pandemic high external or emotional eating scores were most at risk. These children and their families should be targeted by health care professionals to minimize negative physical and mental health consequences.

INTRODUCTION

It has been suggested that the impact of the coronavirus disease 2019 (COVID-19) lockdown measures on lifestyle behaviors and general wellbeing of children and adolescents is larger than that of the infection itself.[1] In most countries, lockdown measures of varying duration and stringency included closing of schools and sports clubs and social distancing measures. Population-based studies in children and adolescents across the world have shown overall decreases in physical activity (PA) and increases in screen time and sedentary behavior.[2-7] Moreover, equivocal changes in food choices are described, with both increased intake of healthy foods such as fruit and vegetables as well as increased intake of unhealthy food categories reported.[6,8-10] Children and adolescents with obesity are thought to be at even larger risk for lifestyle changes and weight gain due to lockdown measures.[11]

In pre-pandemic circumstances, children and adolescents with obesity already are found to have differing scores for restrained, emotional and external eating and poorer health-related quality of life (HRQoL) than children and adolescents without obesity.[12-14] Moreover, we recently reported our first findings during the COVID-19 pandemic in children and adolescents with severe obesity, which revealed the presence of COVID-19-related anxiety in a significant minority of families, resulting in additional self-imposed quarantine measures.[15] This might further exacerbate the negative impact of COVID-19 lockdown in this patient population.

To date, few studies investigated the impact of COVID-19-related lockdown measures on lifestyle factors in pediatric patients with obesity, reporting similar results as the abovementioned studies with regard to PA, screen time, and consumption of unhealthy foods.[16-18] It is unknown whether this is caused by changed eating styles, such as external or emotional eating. For example, external eating could be affected by the presence of food stimuli at home or the closure of food establishments, while emotional eating could be increased by negative emotions during lockdown.

Therefore, the aim of this study was to investigate the impact of COVID-19-related lockdown measures on eating styles and behaviors, PA, screen time, and HRQoL in children (including adolescents up to 18 years) with severe obesity, using a combined quantitative and qualitative approach. This information can help caregivers in minimizing the short- and long-term negative consequences of these COVID-19-related lockdown measures.

MATERIALS AND METHODS

This mixed-methods study was performed within a larger observational study[19] investigating diagnostic and therapeutic aspects of severe pediatric obesity (defined by a BMI above the age- and sex-specific *International Obesity Task Force* cut-off values that correspond to a BMI of ≥35 kg/m^2 at age 18 years).[20] The presented data were prospectively collected for health care purposes according to standardized protocols and were recorded in the patient's medical records.

Study setting

In the Netherlands, school closures were established from 16 March 2020 onwards as part of selective lockdown measures including closings of e.g. sports clubs and food establishments, followed by urgent governmental advices on 23 March 2020 to stay at home.

Study participants

During the first month of the lockdown (2-23 April 2020), we contacted all parents of children (including adolescents up to 18 years) that were under treatment at Obesity Center CGG at the academic center Erasmus MC-Sophia Children's Hospital (Supplementary Fig. 1). Children are referred for diagnostics, e.g. due to early-onset obesity or signs of insatiable behavior for multidisciplinary treatment advices.[19] We approached parents of children who had completed our diagnostic workup and whose last visit was in 2019 or early 2020 (pre-pandemic). We did not approach parents of children with severe intellectual disabilities or children who lived in residential care settings, as their families' experiences during the lockdown might not be representative for a patient population with severe obesity. Twenty children were lost to follow-up, *i.e.*, did not continue their treatment at our obesity center (Supplementary Fig. 1).

Telephone interviews

A treating physician (OA, BVDV, MW) conducted a semi-structured telephone interview to evaluate and explore the effects of the lockdown measures on the children's lifestyle behaviors and HRQoL. Parents were interviewed as proxy for their children in most cases, depending on their age and cognitive abilities. None of the included children were siblings within the same family. A structured format with 37 predefined multiple-choice and 20 open-ended questions was used. After conducting the interviews, the comprehensive physicians' records were used for qualitative analyses. Additionally, in-depth semi-structured interviews were performed with 8 children between ages 10-14 years using video-calls (details in Supplementary Material).

Qualitative analysis

All interviews were independently coded by two physicians (OA, MW) according to the Grounded Theory using a deductive, theory-driven approach followed by an inductive, data-driven approach.[21] Further details are provided in the Supplementary Material. As this study was conducted in the context of patient care, all eligible study participants were included even after we had achieved data saturation. Importantly, the qualitative analyses were conducted before the quantitative analyses to avoid any biases through prior knowledge of the quantitative outcomes. Qualitative data were analyzed using MAXQDA 2018 (VERBI Software) following best practice methods and reported following the Consolidated Criteria for Reporting Qualitative Research (COREQ) checklist.[22,23]

Quantitative assessments and analysis

Pre-pandemic height and weight were measured by trained personnel. BMI was converted to age- and sex-specific standard deviation scores (SDS) using Dutch reference charts.[24] Ethnicity, socio-economic status (SES) z-score and whether subjects lived in urban or rural areas (both based on postal code), signs of insatiable behavior, autism (DSM-V diagnosis), intellectual disability/developmental delay (DSM-V diagnosis), and/or psychosocial problems (DSM-V diagnosis or involvement of psychosocial health care professionals) were assessed pre-pandemic; exact definitions are presented in the Supplementary Methods. Three validated questionnaires were completed by the children and/or their parents both at baseline as well as during lockdown:

- The Dutch Eating Behavior Questionnaire - Child (DEBQ-C) assesses three eating styles: restrained eating (eating less than desired to lose or maintain body weight), emotional eating (eating in response to negative emotions), and external eating (eating in response to food cues). Percentile scores ranging from 0-100 were calculated based on population norms,[25] and were recoded into low (<p20), average (p20 – p80), or high (>p80) scores.
- The Dutch PA Questionnaire assesses weekly time spent on PA, including school transfers, sports at school or sport clubs, and playing outside.[26] Furthermore, it was assessed whether the child fulfills the WHO Global Recommendations on Physical Activity for Health criterion of ≥1h of moderate- to high-intensity daily PA.[27] We compared the proportion of children fulfilling these recommendations pre-pandemic and during lockdown to the general Dutch population, adjusting for age categories and year of assessment.[28] Furthermore, daily sedentary screen time (excluding digital education) was assessed. From this, the proportion of children adhering to the 2016 American Academy of Pediatrics (AAP) recommendations for screen time, *i.e.*, <1h/day for children aged 2-5 years and <2h/day for children aged ≥6 years, was calculated.[29]

- The Pediatric Quality of Life inventory™ 4.0 (parents proxy-report version) (PedsQL) questionnaire assesses HRQoL on four domains: physical, emotional, social, and educational functioning. Sub- and total scores are converted to percentile scores ranging from 0–100, with higher scores indicating better HRQoL.[30] In our center, we use the cut-off value <p60 to identify clinically relevant low scores, based on a large study in children with obesity in which this percentile reflects approximately mean -1SD.[31]

Quantitative data are given as mean (SD) or number (percentage). For our primary quantitative analyses, we compared differences between questionnaire outcomes during lockdown versus pre-pandemic. Additionally, we performed drop-out analyses in which we analyzed differences in baseline characteristics between included and excluded patients, as well as patients who participated in the telephone interviews or completed each of the questionnaires vs. those who did not. The following statistical tests were used: for unpaired data, t-tests for normally distributed continuous variables, Mann-Whitney tests for non-normally distributed continuous data, and chi-squared tests/Fisher's exact tests for categorical data, as appropriate. For paired analyses, paired samples t-tests were used for normally distributed continuous variables, Wilcoxon signed-rank tests for non-normally distributed continuous variables, and McNemar tests for categorical variables. Furthermore, it was evaluated whether baseline characteristics (i.e. age, sex, ethnicity, SES z-score, living in urban vs rural areas, signs of insatiable behavior, autism, intellectual disability/developmental delay, or psychosocial problems) influenced the results of our qualitative and quantitative analyses using chi-squared tests or linear regression. Finally, we examined whether scores on the DEBQ-C and Dutch PA questionnaire influenced PedsQL scores during lockdown using linear regression analyses. In the qualitative data analyses, we categorized children based on qualitative outcomes (e.g. increased demand for food of the child reported by parents) and quantitatively evaluated differences in baseline characteristics using the appropriate statistical tests. Quantitative data were analyzed using SPSS version 25.0 (IBM Statistics) with a two-sided α of 0.05.

RESULTS

In total, 116 patients visited Obesity Center CGG during the study period, of which 90 families were approached (exclusion criteria presented in Supplementary Fig. 1). Of these families, 83 participated in the quantitative analyses and 75 in the telephone interviews. The mean age of the 83 included children was 11.2 ± 4.6 years; 43 (52%) were females; and mean BMI SDS was 3.8 ± 1.0, indicating severe obesity (Table 1).

Baseline characteristics did not differ between children who were included in this study (n=83) vs. those who were not (n=33, all p-values >0.05, Supplementary Table S1). Similarly, baseline characteristics did not differ between children who participated in the telephone interviews (n=75) vs. those who did not (n=8, all p-values >0.05, Supplementary Table S2). A thematic summary of main findings and illustrative quotes are presented in Table 2.

Table 1. Characteristics of the study population at their most recent visit to the hospital pre-pandemic.

Characteristic	All patients (n=83)
Age in years, mean (SD)	11.2 (4.6)
Sex, female (%)	43 (52)
Ethnicity, Dutch (%)	56 (68)
Socioeconomic status z-score, mean (SD)	-0.1 (1.2)
Living conditions, urban, n (%)	65 (78)
BMI SDS, mean (SD)	+3.8 (1.0)
Signs of insatiable behavior, n (%)	38 (46)
Intellectual disability/developmental delay, n (%)	26 (31)
Autism, n (%)	14 (17)
Psychosocial problems, n (%)	46 (55)

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

Table 2. Identified themes and illustrative quotes from the qualitative analysis

Theme 1: changes in eating styles and behaviors during lockdown	
Theme 1.1 – increased demand for food	*R1, girl, 10y: "Well, I am craving pancakes way more, because the pancake-mix is standing there [in the kitchen]. (…) I want those the whole time, for breakfast or for lunch, I think: I want pancakes."*
Theme 1.2 – no changes in eating behaviors in families who already had strict schedules regarding food	*Mother of R4, boy, 13y: "Well, I try, we try together to keep the daily structure. We start with school on time and eat normal snacks, so it won't become a feeding frenzy. Which actually does happen in the weekends a bit."*
Theme 1.3 – positive changes in eating behaviors due to decreased external eating stimuli	*R4, boy, 13y: "Actually, yes, it is easier. Because my mother is at home the whole time. Sometimes you think, I can take something and then… Yes, so it is easier to eat healthy." Father of R6, boy, 10y: "He really is managing very good. He indicates well when he is full. I think it is even better than when he's at school.*

Theme 2: changes in physical activities during lockdown	
Theme 2.1 – decreased physical activities related to lockdown measures and/or anxiety	*R3, girl, 10y: "Sometimes it is difficult, if we are playing tag and we can't touch each other."* *R1, girl, 10y: "We bike less, we almost never walk and we watch a lot more movies, well, I watch a lot more movies. I watched a whole series in two days."*
Theme 2.2 – important role of parents and peers in motivating children to engage in physical activity	*R3, girl, 10y: Before COVID we had an exercise club, with two other girls. (…) It's a pity that stopped, because those girls were fun to exercise with."* *Father of R7, boy, 11y: "I take him outside sometimes, I say to him: Come on, go outside for an hour or half an hour. (…) But I can't take him to the park every day, because sometimes he is scared. Then he says, he doesn't want to, because he'll get COVID."*
Theme 3: changes in emotional wellbeing of child and family dynamics during lockdown	
Theme 3.1 – deteriorated emotional wellbeing of child and worsened family dynamics	*R4, boy, 13y: "Well, [I miss] my grandma, we do see her but only outside and on 1,5 meter distance."* *R7, boy, 11y: "I find it hard that I can't talk with my friends or play outside. We can't do that. We play video games and talk on the phone, but that's boring to do the whole time."*
Theme 3.2 – increased demands on parents due to different parenting roles	*Father of R7, boy, 11y: "It is really tough, it is very boring now. Life went almost down the drain because of that disease. Not just mine, but of the whole of humanity. (…) I find it very difficult; I can't see my colleagues; I can't do anything, you know. I can't go outside, I can't see my friends, for me it is also tough. But I can handle it, I can cope with it, but for children, it is difficult."*
Theme 3.3 – improved family dynamics due to increased family time and space for children's emotions	*Mother of R4, boy, 13y: We are doing quite well, we can just endure each other well."*
Theme 4: Impact of lockdown on daily structure of children	
Theme 4.1 – difficulties in adapting to changes in daily structure	*R8, boy, 10y: "Today I woke up at 11am and yesterday I also woke up at 11am."*

Theme 1: changes in eating styles and behaviors during lockdown

Dutch Eating Behavior Questionnaire – Child version (DEBQ-C)

The DEBQ-C was completed in 59/83 (71%) families during lockdown. Their children's baseline characteristics did not differ from those that did not complete the questionnaire (all p-values >0.05, Supplementary Table S3). On group level, all scores remained unchanged over time (all p-values >0.05, Table 3). No effect of sex was found on changes in restrained,emotional, or external eating (all p-values >0.05). The majority of children with high scores on restrained eating (21/29, 72%), emotional eating (15/27, 56%), and external eating (24/26, 92%) during lockdown already had high scores pre-pandemic.

When looking into subgroups, 20 (34%) children reported an increase of ≥10 percentiles in restrained eating versus 10 (17%) a decrease (p=0.07). Baseline characteristics were not associated with changes in restrained eating (all p-values >0.05). Fifteen (26%) children reported an increase of ≥10 percentiles in emotional eating versus 10 (18%) a decrease (p=0.32). Children for whom ≥10 percentiles increase in emotional eating was reported more often had pre-existent psychosocial problems (73% vs 30%, p=0.049) and on average were older, although this was not statistically significant (11.3 vs 9.1 years, p=0.32). Fourteen (24%) children reported an increase of ≥10 percentiles in external eating versus 19 (32%) a decrease (p=0.38). Children for whom ≥10 percentiles increase was reported were younger, although this was not statistically significant (9.7 vs 11.3 years, p=0.42).

Table 3. Dutch Eating Behavior Questionnaire for Children (DEBQ-C) scores pre-pandemic and during lockdown.

	Pre-pandemic Mean ± SD scores or n (%)	During lockdown Mean ± SD scores or n (%)	Δ	P-value
Restrained eating				
All patients (n=59)	59.5 ± 32.6	63.4 ± 33.8	+3.9	0.39
High scores Average scores Low scores	24 (41%) 23 (39%) 12 (20%)	29 (49%) 21 (36%) 9 (15%)		0.38
Emotional eating				
All patients (n=57*)	58.0 ± 32.8	67.2 ± 32.9	+9.2	0.11
High scores Average scores Low scores	20 (35%) 27 (47%) 10 (18%)	27 (47%) 24 (41%) 6 (10%)		0.20
External eating				
All patients (n=59)	68.2 ± 31.5	68.5 ± 28.4	+0.3	0.57
High scores, n (%) Average scores, n (%) Low scores, n (%)	31 (53%) 24 (41%) 4 (7%)	26 (44%) 29 (49%) 4 (7%)		0.36

Abbreviations: SD, standard deviation
**Subscore missing at baseline for n=2 patients.*

Qualitative results - eating behaviors

An increased demand for food by the child was reported for 21/75 (28%) children. Most of these children lived in urban areas (20/21, 95%, p=0.033), were <10 years old (19/21, 90%, p<0.001) and showed signs of insatiable behavior (17/21, 81%, p<0.001). These children on average had a slightly lower SES z-score, although this was not statistically significant (mean -0.4 SDS, p=0.24). An increased demand for food was associated with higher external eating scores (mean 85.7 vs 62.6, p<0.001) during lockdown. Most parents attributed the increased demand to loss of daily structure

and loss of delimited lunch box portion sizes due to school closings. Other reported reasons were increased stress, eating out of boredom, and food-seeking behavior. Consequently, many parents had to put more effort to maintain control over their child's eating behavior. In some families this led to increased conflicts.

Fifteen (20%) families reported no changes in eating behaviors, mostly because they already had strict eating schemes due to previous dietary and/or pedagogic support. Moreover, eleven families reported improved eating behavior during lockdown, mostly due to decreased external eating stimuli, although their external eating scores did not differ significantly (mean 75.4 vs 67.3, p=0.43).

Theme 2: changes in physical activities and screen time during lockdown

Dutch PA questionnaire

The PA questionnaire was completed by 55/83 (66%) families during lockdown. Their children's baseline characteristics did not differ from those who did not complete the questionnaire (all p-values >0.05, Supplementary Table S4). On group level, mean weekly PA time decreased significantly and mean weekly (non-educational) screen time did not change (p-values 0.02 and 0.65, respectively, Table 4). No effect of sex was found on changes in weekly PA time (p=0.66). With regard to weekly screen time, girls showed an increase from 15.2 ± 9.9 hours to 18.6 ± 11.9 hours during lockdown, whereas boys showed a decrease from 20.9 ± 12.6 hours to 17.3 ± 11.7 hours during lockdown (p=0.003). Thirty-two (58%) children fulfilled the WHO recommendations pre-pandemic (Table 4), similar to 49% of children in the Dutch general population (p=0.33). This did not change significantly during lockdown (27/55, 49%, p=0.33 vs. pre-pandemic). Children who fulfilled WHO recommendations during lockdown were younger (9.2 vs 13.2 years, p=0.002) and more often (21/27, 78%, p=0.004) already fulfilled the recommendations pre-pandemic. During lockdown, 19/55 (35%) children adhered to the AAP screen time recommendations, similar to 22/55 (40%) pre-pandemic (p=0.65).

Qualitative results - physical activity

Many families (42/75, 56%) reported a decrease of their child's PA during lockdown. Often (36/75, 48%), family members tried to motivate their children into PA, which succeeded in two-third of families. Reasons for not succeeding were anxiety for COVID-19 infection in children and/or parents to leave the house and preference of child to perform PA with peers rather than parents. Reasons for succeeding were use of online videos, performing PA together with family members, parents having more

Table 4. Time spent on physical activities and screen time pre-pandemic and during lockdown

		Pre-pandemic Mean ± SD	During lockdown Mean ± SD	Δ	P-value
Physical activity (h/wk)	All patients (n = 55)	9.1 ± 6.7	7.2 ± 7.6	-1.9	**0.02**
	Patients who fulfil Dutch physical activity guidelines:				
	Pre-pandemic and during lockdown (n = 21)	14.2 ± 5.8	13.3 ± 5.6	-0.9	0.42
	Neither pre-pandemic nor during lockdown (n = 17)	2.8 ± 1.7	0.7 ± 0.9	-2.1	**0.001**
	Pre-pandemic but not during lockdown (n = 11)	12.6 ± 4.0	2.0 ± 2.4	-10.6	**0.003**
	During lockdown but not pre-pandemic (n = 6)	3.3 ± 1.2	14.0 ± 8.5	+10.7	**0.03**
		Pre-pandemic Mean ± SD	**During lockdown Mean ± SD**	**Δ**	**P-value**
Screen time (h/wk)	All patients (n = 54)	18.2 ± 12.9	18.0 ± 11.7	-0.2	0.65
	Patients who fulfil AAP recommendations for screen time:				
	Pre-pandemic and during lockdown (n = 11)	8.0 ± 4.0	6.5 ± 3.9	-1.5	0.33
	Neither pre-pandemic nor during lockdown (n = 24)	26.4 ± 12.5	24.4 ± 9.7	-2.0	0.42
	Pre-pandemic but not during lockdown (n = 11)	7.0 ± 3.63	20.7 ± 10.0	+13.7	**0.003**
	During lockdown but not pre-pandemic (n = 8)	23.0 ± 10.1	7.8 ± 4.7	-15.2	**0.01**

Abbreviations: SD, standard deviation

time to spend on PA with their children, and parents arranging outside play dates with peers.

A minority of children (11/75, 15%) reported no change in PA during lockdown. Another subgroup (7/75, 9%) reported increased PA due to playing outside more often. Some families bought sports equipment to enhance possibilities, such as a punching ball or trampoline.

Theme 3 - Changes in emotional wellbeing and family dynamics during lockdown

Pediatric Quality of Life questionnaire (PedsQL)

The PedsQL was completed by 49/83 (59%) families during lockdown, which included more often families with a child with psychosocial problems (67% vs 38%, p=0.009) or autism (24% vs 6%, p=0.026, Supplementary Table S5). On group level, mean sub- and total scores improved slightly during lockdown, although not statistically significant (all p-values >0.05, Table 5). No effect of sex was found on changes in mean sub- and total scores (all p-values >0.05). Most children with low total scores during lockdown had low scores pre-pandemic (17/20, 85%). The children with low scores during lockdown more often had pre-existent psychosocial problems (85% vs 54%, p=0.023) and

autism (45% vs 11%, p = 0.007). Eleven (23%) children reported an increase of ≥10 percentiles of total score versus six (13%) a decrease of ≥10 percentiles (p=0.23). This was unrelated to baseline characteristics (all p-values >0.05). During lockdown, total scores were not associated with time spent on PA, screen time, or restrained eating (all p-values >0.05), but were negatively associated with emotional eating (ß=-0.28, SE=0.72, p<0.001) and external eating (ß=-0.29, SE=0.90, p=0.002).

Table 5. Pediatric Quality of Life Inventory (PedsQL) scores pre-pandemic and during lockdown

	Pre-pandemic Mean ± SD scores or n (%)	During lockdown Mean ± SD scores or n (%)	Δ	P-value
Physical functioning				
All patients (n=49)	63.5 ± 24.8	66.3 ± 23.1	+2.8	0.12
Low scores (<p60)	24 (49%)	21 (43%)		0.45
Emotional functioning				
All patients (n=49)	58.4 ± 20.6	60.1 ± 22.3	+1.7	0.45
Low scores (<p60)	23 (47%)	26 (53%)		0.55
Social functioning				
All patients (n=49)	63.9 ± 22.9	67.7 ± 23.7	+3.8	0.12
Low scores (<p60)	20 (41%)	15 (31%)		0.18
Educational functioning				
All patients (n=48)	62.7 ± 18.3	66.1 ± 21.9	+3.4	0.32
Low scores (<p60)	18 (38%)	18 (38%)		1.00
Total scores				
All patients (n=48)	62.4 ± 18.3	65.4 ± 18.6	+3.0	0.06
Low scores (<p60)	23 (49%)	20 (42%)		0.51

Abbreviations: SD, standard deviation

Qualitative results - emotional wellbeing and family dynamics

During lockdown, 46/75 (61%) parents reported deteriorated emotional wellbeing of their child and worsened family dynamics. The most frequently experienced negative emotions were anger (n=27, 36%), boredom (n=25, 33%), and anxiety (n=24, 32%), mostly related to conflicts due to being at home together all the time. Other reasons were increased conflicts regarding eating behavior, loss of predictability of daily structure, missing social contacts with friends, family and/or teachers, and the limited possibilities in daily activities. Several parents reported difficulties with the increased demand of combining working from home themselves with all different parenting roles: having to organize home schooling, motivate their children to engage in PA, and control their eating behavior. These pedagogical demands compromised their adherence to the lifestyle advices that they had received from health care professionals pre-pandemic.

Fourteen (19%) families reported positive changes in family dynamics. The increased family time, with more space for their children's emotions and needs, led to better understanding of each other. Two families mentioned that the temporary pause of therapies with health care professionals enabled them to unwind and four families (5%) reported less stress due to school closures.

Theme 4 – Impact of lockdown on daily structure of children

Qualitative results – daily structure of children

All children had to cope with changes in daily structure, and 33/75 (44%) had difficulties adapting. Most frequently, sleeping patterns were disturbed. Families that experienced no difficulties in adapting had pre-existent or newly implemented strict daily schedules in place to help their children to keep the normal structure of school weeks as much as possible.

DISCUSSION

To our knowledge, this is the first study reporting the impact of COVID-19-related lockdown measures on eating styles and behaviors, physical activity, screen time, and health-related quality of life in children and adolescents with severe obesity. Our quantitative analyses showed that on group level, time spent on PA decreased significantly. In half of the population, mean time spent on PA decreased to ≤2 hours/week. When zooming in on subgroups, children with pre-existent psychosocial problems more often showed increased emotional eating. In addition, the lowest health-related quality of life scores during lockdown were seen in children with pre-pandemic high scores on external and emotional eating or pre-existent psychosocial problems. Our qualitative analyses revealed an increased demand for food by predominantly younger children with signs of insatiable behavior and/or higher external eating scores. Moreover, a majority of parents reported deteriorated emotional wellbeing of their child and worsened family dynamics during the lockdown.

To date, one Italian study in 41 children with obesity investigated the impact of COVID-19 lockdown on time spent on physical activity (as reported by parents during a telephone interview) and found a decreased PA (-2.3 hrs/week), which is similar to the -1.9 hrs/week decrease in our study.[16] When zooming in on our study population during lockdown, children who managed to adhere to PA guidelines during lockdown were significantly younger (9.2 vs. 13.2 years) and more often adhered to PA guidelines pre-pandemic. In line with recent findings, encouragement from parents or peers seemed

important.[2,4,5,32] Moreover, in half of our population mean time spent on PA decreased dramatically to ≤2hr/wk, which was often attributed to COVID-19-related anxiety, as we ourselves as well as a recent US study reported recently.[15,13] This alarming lack of PA puts these children at risk for negative mental health effects and weight gain.[33-35]

Contrary to our expectations, we did not identify statistically significant changes in emotional eating or external eating on group level during lockdown. Moreover, most children with high scores during lockdown already had high scores pre-pandemic. Notably, our study population has higher DEBQ-C scores pre-pandemic as can be expected in a population with severe obesity.[12] These pre-pandemic eating styles as well as pre-existent insatiable behavior seemed the most important predictors of high emotional and external eating scores during lockdown. Of note, we did not investigate whether eating styles correlate directly to food intake, but high scores on external or emotional eating may put children at risk for weight gain. To date, one Saudi-Arabian study reported prevalence of high emotional eating in healthy young women (12% vs. 47% in our population) and found a positive association with BMI and perceived stress.[36] In our study, children with increased emotional eating scores during lockdown significantly more often had pre-existent psychosocial problems. Moreover, adhering to pre-pandemic strict daily schedules was reported to help in minimizing the experienced impact of COVID-19 lockdown on children's eating behaviors.

HRQoL in children with obesity is known to be diminished and is associated with severity of the obesity and older age.[13,14] In our study, only 13% reported a decrease vs. 23% an increase of ≥10 percentiles in PedsQL scores. However, PedsQL scores were considerably lower compared to another cohort of children with obesity pre-pandemic (mean total score 65.4 versus 75.5, respectively).[14] We identified one other study that measured HRQoL using the PedsQL during lockdown in children from the general population, which reported an almost 15 points higher mean total score compared to our population.[37] Accordingly, the absence of a further decline in mean total PedsQL score in our population could be explained by a 'ceiling' effect. The lower PedsQL scores in our study might also have been caused by the characteristics of our academic patient population, which included a relatively large proportion of children with intellectual disability, autism, and/or psychosocial problems. Indeed, our drop-out analyses revealed that the PedsQL questionnaire was more often completed by families whose children had autism and/or pre-existent psychosocial problems and these children significantly more often showed low HRQoL scores during lockdown compared to children without these characteristics. Interestingly, we did not find an association between HRQoL and PA or screen time, although other studies have suggested a protective effect of PA on the mental health impact of the COVID-19 pandemic in children.[33,35,37-39]

We did find a strong negative association between HRQoL scores and emotional and external eating during lockdown.

Several studies have underlined the importance of healthy family dynamics during lockdown.[5,39-41] In our population, families who reported improved dynamics attributed this to increased family time and more space for each other's emotions. Moreover, having enough physical space at home and having the financial possibility to buy for example sports equipment was beneficent. A substantial part of families reported increased tensions and difficulties with juggling between competing parenting roles during lockdown. In our clinical experience pre-pandemic, parents of children with obesity already have to put substantial effort in managing healthy lifestyle choices for their children. The additional parenting roles, remote working and possible job insecurities associated with the COVID-19 pandemic can therefore put an extra strain on them parents of children with severe obesity. Broadly in line with our results, recent general population studies found similar mental and social health complaints in families during lockdown. These were associated with family characteristics such as living in single-parent families, having less space at home, having multiple siblings, having pre-existent medical problems in the family, and changes in parental working conditions.[40,42,43] Moreover, increased parental COVID-19-related stress was found to be associated with non-nutritive use of food and snacks, such as emotional and instrumental feeding.[44] These studies together with ours, highlight the importance of evaluating the need for parental support, especially in families with the abovementioned risk factors. Although we and others did not find a statistically significant effect of SES z-score on our outcomes on group level,[7] our qualitative data suggest that children from families with lower SES might have more challenges to face. Moreover, the COVID-19 lockdown measures, especially school closures, have been shown to exacerbate existing inequalities, e.g. children's risk of psychosocial or mental problems,[37] or food insecurity.[45]

Based on our study, we recommend a pro-active approach in specific patient subgroups to minimize negative effects of lockdown, e.g. by offering individualized adjustments to patient- and family-specific medical support, together with other involved health care professionals. First, children who already were at risk pre-pandemic, e.g. due to psychosocial problems, insatiable behavior, high emotional and external eating, and not fulfilling WHO PA recommendations, show the worst outcomes during lockdown. Second, COVID-19-related anxiety, when present, seems to influence PA.[15] Third, adolescents seem to be at risk for increased emotional eating and decreased PA, whereas younger, *i.e.*, prepubertal, children more often show increased external eating.

Strengths and limitations

A strength of our study is the evaluation of multiple lifestyle behaviors and wellbeing that are known to have reciprocal interactions, in a unique population of children with severe obesity. Furthermore, we compared validated questionnaire data longitudinally, enabling us to identify the children who improved or deteriorated during lockdown. Our mixed-methods design provided insights in the reasons why children succeeded or failed in maintaining a healthy lifestyle. It should be noted that we did not use transcriptions of the telephone interviews. However, all relevant information was documented comprehensively in the medical records using an extensive pre-defined format. This study was performed within the first two months of the first COVID-19-related lockdown in the Netherlands, providing us the unique opportunity to investigate the acute impact of these unforeseen circumstances. As children's and families' lifestyle behaviors, wellbeing, and attitudes toward the lockdown measures may have changed since, follow-up studies are needed. Another limitation is that we did not record whether questionnaires were completed by children or their parents, which might have influenced reported behaviors. Our study was designed to compare lifestyle factors and wellbeing in children with severe obesity pre-pandemic and during COVID-19 lockdown. Therefore, we did not include an additional control group of children without obesity.

Conclusion

In conclusion, our mixed-methods study shows differing responses to COVID-19-related lockdown measures in children and adolescents with severe obesity. Quantitative analyses revealed that on group level, physical activity declined, whereas non-educational screen time, eating styles, and health-related quality of life did not change significantly. Qualitative analyses showed that a minority of families kept adhering to strict schedules and reported no changes or improved lifestyle behaviors, whereas a substantial part of families reported a deterioration in physical activity, eating behaviors and health-related quality of life. Children with pre-existent psychosocial problems, insatiable behavior, or pre-existent high external or emotional eating were most at risk for the negative effects on lifestyle behaviors and wellbeing. These children need to be targeted by health care professionals to minimize short- and long-term negative physical and mental health consequences.

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Statement of Ethics

This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center (Erasmus MC), Rotterdam, The Netherlands, approval number MEC-2012-257. In accordance with Dutch law, all caregivers of children ≤16 years gave written informed consent; additionally, children aged ≥12 years gave their written informed consent and children aged ≤12 years gave their oral assent.

Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

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Author contributions

MW, OA: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, validation, visualisation, writing – original draft, verifying the underlying data. EvdE: conceptualisation, data curation, formal analysis, investigation, methodology, validation, visualisation, writing – review & editing, verifying the underlying data. JH, AB, LK: conceptualisation, investigation, methodology, writing – review & editing. EvR, EvdA: conceptualisation, funding acquisition, investigation, methodology, resources, software, supervision, validation, visualisation, writing – review & editing. BvdV: conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualisation, writing – review & editing, verifying the underlying data.

Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the Data sharing committee (CGG Steering Committee, Dr. E.L.T. van den Akker, centrumgezondgewicht@erasmusmc.nl) upon reasonable request.

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SUPPLEMENTARY APPENDIX

1. Supplementary Methods
2. Supplementary Tables
3. Table S1. Characteristics of the study population vs. patients who were excluded at their most recent visit to the hospital pre-pandemic.
4. Table S2. Characteristics of the patients who participated in the telephone interviews vs. those that did not at their most recent visit to the hospital pre-pandemic.
5. Table S3. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Dutch Eating Behavior-Child version (DEBQ-C) questionnaire vs. those that did not.
6. Table S4. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Dutch Physical Activity (PA) questionnaire vs. those that did not.
7. Table S5. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Pediatric Quality of Life Inventory (PedsQL) vs. those that did not.

1. Supplementary Methods

Qualitative analysis

Regular semi-structured interviews

All interviews were independently coded by two physicians (OA, MW) according to the Grounded Theory,[1] using a deductive, theory-driven approach followed by an inductive, data-driven approach. The two physicians commenced by open coding of the interviews independently. Afterwards, the coded segments were compared; differences were solved through discussion. Following this, the study team developed a code tree using axial coding based on interviews from a subset of 24 patients (8 patients per interviewing physician). After all remaining interviews were coded using the final code tree, selective coding was performed to identify the code categories most relevant to the research aims. These code categories were finally summarized into four themes: changes in eating styles and behaviors, changes in physical activities, changes in emotional wellbeing of child and family dynamics and impact on daily structure of children. The axial and selective coding steps were also performed independently by both physicians; differences were solved through discussion. To further ensure rigor, a study log was kept during this entire process and memos were used to carefully note emerging ideas about the data analysis which were discussed during weekly meetings of the study team. Importantly, the qualitative data analyses were performed after all interviews were conducted.

In-depth semi-structured interviews

Because most regular semi-structured interviews were either conducted with parents alone or together with their children, we performed additional in-depth semi-structured interviews with a subset of eight of our included children within a two-week timeframe after the regular telephone interview. For these interviews we approached children aged 10-14 years We did not approach children with syndromic obesity, mental disorders, developmental delay or severe

behavioral problems, as we expected their experiences during the lockdown would not be representative for our patient population and we expected difficulties for them to participate in an interview by video-call. The interviews focused on environmental factors influencing the lifestyle behaviors of children and adolescents before and during COVID-19 lockdown. Three girls and 5 boys consented to participate. At one interview, a mother was present and at two interviews, a father. Deductive exploratory analyses, based on the code tree that we had developed for the qualitative analyses of the regular telephone interviews, were performed on the full transcripts of the in-depth interviews. Our aim for these analyses was to collect insightful quotes related to the qualitative analyses of the regular telephone interviews.

Quantitative analysis

The following definitions were used for the assessed baseline characteristics presented in this study and previous studies of Obesity Center CGG.[2]

Ethnicity was defined according to the definition of the Dutch Central Agency for statistics as Dutch if patient and both parents were born in The Netherlands; otherwise, patients were classified as having a migration background.[3]

Socioeconomic status z-scores were retrieved from the Netherlands Institute for Social Research. These z-scores summarizing average income, education and unemployment in postal code areas to provide an estimate of the socioeconomic status of patients.[4]

Whether subjects lived in urban or rural areas was determined using the 2020 data on urbanization from the Dutch Central Bureau for Statistics (CBS). According to CBS definitions, Dutch living areas are classified into five categories of urbanization based on postal code area: 'no', 'small', 'moderate', 'strong' or 'very strong' degrees of urbanization.[5] Accordingly, we dichotomized patients into living in rural (CBS: 'no' or 'small' degree of urbanization) or urban (CBS: 'moderate', 'strong' or 'very strong' degree of urbanization) areas.

Presence of *insatiable behavior* was determined by the physician, based on the child's or parents' answers regarding hunger, e.g., satiation and satiety, preoccupation with food, night eating, secret eating, food-seeking behavior, and the distress that accompanies the child's hunger or obsession with food.[6]

Intellectual disability/developmental delay was determined by the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders 5) definition of intellectual disability or an IQ score ≤70.[2]

Psychosocial problems was defined as the presence of an established DSM-5 diagnosis (with the exception of intellectual disability) such as major depressive disorder, or social problems for which official authorities were involved, such as child protective services.[2]

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Supplementary Tables

Table S1. Characteristics of the study population vs. patients who were excluded at their most recent visit to the hospital pre-pandemic.

Characteristic	All excluded patients (n=33)	All included patients (n=83)	P-value
Age in years, mean (SD)	12.2 (3.9)	11.2 (4.6)	0.24
Sex, female (%)	20 (61)	43 (52)	0.39
Ethnicity, Dutch (%)	22 (67)	56 (68)	0.80
Socioeconomic status z-score, mean (SD)	-0.1 (1.3)	-0.1 (1.2)	1.00
Living conditions, urban, n (%)	28 (85)	65 (78)	0.43
BMI SDS, mean (SD)	+3.8 (1.0)	+3.8 (1.0)	0.65
Signs of insatiable behavior, n (%)	13 (39)	38 (46)	0.53
Intellectual disability/developmental delay, n (%)	9 (27)	26 (31)	0.67
Autism, n (%)	4 (12)	14 (17)	0.52
Psychosocial problems, n (%)	15 (46)	46 (55)	0.33

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

Table S2. Characteristics of the patients who participated in the telephone interviews vs. those that did not at their most recent visit to the hospital pre-pandemic.

Characteristic	Patients who participated in the telephone interviews (n=75)	Patients who did not participate in the telephone interviews (n=8)	P-value
Age in years, median (IQR)	10.5 (7.6 – 15.2)	11.0 (6.5 – 15.7)	0.99
Sex, female (%)	39 (52)	4 (50)	1.00
Ethnicity, Dutch (%)	50 (69)	6 (75)	1.00
Socioeconomic status z-score, median (IQR)	+0.0 (-0.7 – +0.7)	+0.4 (-0.1 – +1.1)	0.17
Living conditions, urban, n (%)	59 (79)	6 (75)	1.00
BMI SDS, median (IQR)	+4.0 (+3.2 – +4.4)	+3.6 (+3.0 – +3.9)	0.40
Signs of insatiable behavior, n (%)	34 (45)	4 (50)	1.00
Intellectual disability/developmental delay, n (%)	23 (31)	3 (38)	0.70
Autism, n (%)	13 (17)	1 (13)	1.00
Psychosocial problems, n (%)	40 (53)	6 (75)	0.29

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

Table S3. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Dutch Eating Behavior-Child version (DEBQ-C) questionnaire vs. those that did not.

Characteristic	Patients who filled out the DEBQ-C (n=59)	Patients who did not fill out the DEBQ-C (n=24)	P-value
Age in years, median (IQR)	10.2 (7.6 – 15.5)	11.5 (7.1 – 15.0)	0.80
Sex, female (%)	32 (54)	11 (46)	0.49
Ethnicity, Dutch (%)	43 (75)	13 (54)	0.06
Socioeconomic status z-score, median (IQR)	+0.1 (-0.5 – +0.8)	+0.0 (-1.1 – +0.4)	0.20
Living conditions, urban, n (%)	47 (80)	18 (75)	0.64
BMI SDS, median (IQR)	+3.8 (+3.0 – +4.4)	+3.9 (+3.3 – +4.5)	0.42
Signs of insatiable behavior, n (%)	31 (53)	7 (29)	0.053
Intellectual disability/developmental delay, n (%)	20 (34)	6 (25)	0.43
Autism, n (%)	12 (20)	2 (8)	0.33
Psychosocial problems, n (%)	36 (61)	10 (42)	0.11

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

Table S4. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Dutch Physical Activity (PA) questionnaire vs. those that did not.

Characteristic	Patients who filled out the Dutch PA questionnaire (n=55)	Patients who did not fill out the Dutch PA questionnaire (n=28)	P-value
Age in years, median (IQR)	10.2 (7.6 – 15.5)	11.5 (7.1 – 15.0)	0.88
Sex, female (%)	29 (53)	14 (50)	1.00
Ethnicity, Dutch (%)	40 (76)	16 (57)	0.09
Socioeconomic status z-score, median (IQR)	+0.0 (-0.6 – +0.8)	+0.1 (-1.0 – +0.6)	0.52
Living conditions, urban, n (%)	44 (80)	21 (75)	0.60
BMI SDS, median (IQR)	+3.9 (+3.0 – +4.4)	+3.8 (+3.3 – +4.4)	0.84
Signs of insatiable behavior, n (%)	28 (51)	10 (36)	0.19
Intellectual disability/ developmental delay, n (%)	19 (35)	7 (25)	0.38
Autism, n (%)	12 (22)	2 (7)	0.13
Psychosocial problems, n (%)	34 (62)	12 (43)	0.10

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

Table S5. Characteristics at their most recent visit to the hospital pre-pandemic of the patients who filled out the Pediatric Quality of Life Inventory (PedsQL) vs. those that did not.

Characteristic	Patients who filled out the PedsQL (n=49)	Patients who did not fill out the PedsQL (n=34)	P-value
Age in years, median (IQR)	11.2 (8.1 – 16.1)	10.3 (4.9 – 15.0)	0.06
Sex, female (%)	24 (49)	19 (54)	1.00
Ethnicity, Dutch (%)	36 (77)	20 (59)	0.09
Socioeconomic status z-score, median (IQR)	-0.0 (-0.6 – +0.7)	+0.1 (-1.0 – +0.7)	0.73
Living conditions, urban, n (%)	38 (78)	27 (79)	0.84
BMI SDS, median (IQR)	+4.0 (+3.2 – +4.4)	+3.6 (+2.7 – +4.2)	0.11
Signs of insatiable behavior, n (%)	28 (51)	10 (36)	0.80
Intellectual disability/ developmental delay, n (%)	19 (39)	7 (21)	0.08
Autism, n (%)	12 (24)	2 (6)	0.03
Psychosocial problems, n (%)	33 (67)	13 (28)	0.009

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score; COVID-19, coronavirus disease 2019

10

General discussion

GENERAL DISCUSSION

The unprecedented rise in severe pediatric obesity that we are currently facing poses an extraordinary challenge that our society must tackle.[1] Obesity is a complex, relapsing and chronic endocrine disease and is defined as such both internationally by the World Health Organization (WHO) as well as in The Netherlands by the Health Council of the Netherlands (*Gezondheidsraad*).[2-4] Although changes in obesogenic environments and lifestyle behaviors are unequivocally the main culprit, it is the interaction between these factors and our biological background and genetic predisposition that ultimately drives the increased prevalence of severe pediatric obesity.[5] Therefore, as with other chronic, multifactorial diseases, effective treatment of severe pediatric obesity is only possible if the contributing biological, psychological and social factors within a patient are identified.[6-8] This is not only vital for patients, caregivers, and their social environment to understand their disease and reduce stigma, but it also enables tailored treatment: counseling about the natural history and expected clinical course of the disease, associated medical problems, advices regarding different treatment modalities such as pharmacotherapy and bariatric surgery, as well as genetic counseling including inheritance and reproductive decisions. This thesis investigated several important aspects of severe pediatric obesity in a selected cohort of children with diagnosed or suspected underlying medical causes of obesity referred to an academic obesity center. The findings of this thesis can improve diagnostics for underlying medical causes of severe pediatric obesity: genetic obesity disorders, hypothalamic obesity, endocrine obesity disorders, medication-induced obesity, and multifactorial obesity. The results of the individual chapters and implications for clinical care and future research will be discussed.

Diagnosing underlying medical causes

In **chapter 2**, we have shown that a systematic diagnostic workup can lead to a high yield of diagnosed underlying medical causes of obesity of 19% in a selected cohort of children with severe obesity referred to a specialized obesity center. This was the first study aimed at evaluating all categories of potential underlying medical causes of pediatric obesity as mentioned in current international guidelines,[6,8] showing a higher yield than reported in literature due to the selection of the study cohort and comprehensive genetic testing strategy.[9] This study provides a framework for a systematic diagnostic approach that can be used in different centers and settings. It shows that a broad workup is needed. Important additions over the diagnostic suggestions of current guidelines[6-8] are the use of comprehensive growth charts analysis to diagnose medication-induced obesity and hypothalamic obesity, as well as the several classes of weight-inducing medication other than antipsychotics, e.g. corticosteroids and

antidepressants, that can cause medication-induced obesity.[10] Moreover, a negative rather than positive family history of severe obesity predicted genetic obesity disorders due to the occurrence of recessive disorders. In the remainder of this chapter, the specific findings of this thesis for each category of underlying medical causes will be addressed.

Genetic obesity disorders and improvement of diagnostic strategies

The results of **chapter 2** and other recent studies allude to the fact that, despite diagnostic suggestions of current international guidelines, many children with underlying medical causes of obesity, especially genetic obesity disorders, currently remain undiagnosed.[11-13] In **chapter 3**, we calculated this large gap between reported versus expected patients for a hallmark non-syndromic genetic obesity disorder: leptin receptor (LepR) deficiency. In the systematic review in **chapter 3** we show that the majority (66%) of reported patients with LepR deficiency do not have pituitary hormone disturbances; hence, severe early-onset obesity and hyperphagia can be the only symptoms of LepR deficiency. Moreover, only 2% of expected patients based on allele frequencies were actually reported in literature, and most of the reported patients were children or young adults. This diagnostic gap suggests underreporting, underdiagnosis, early mortality, or a combination of these factors. Based on other recent studies and the overlap in pathophysiology, it can be expected that these observations also hold true for similar genetic obesity disorders, e.g. LEP, POMC, and PCSK1 deficiency.[13-15] Moreover, even the most common genetic obesity disorder, MC4R deficiency, which can hardly be regarded as a rare disease given its reported prevalence of 2-5% in children with obesity,[16,17] might be more prevalent than expected. Recent population-based data from the UK shows that 1 in 330 individuals within the population, regardless of weight status, had loss of function (LoF) variants in MC4R.[18] Taken together, ours and these recent studies implicates that genetic screening for leptin-melanocortin pathway deficiencies should be performed in all cases with early-onset severe obesity and hyperphagia, even without the classically associated hormone disturbances or associated signs and symptoms.

For this purpose, it is essential to know which cut-off value of age of onset of obesity has optimal performance in distinguishing between children with and without genetic obesity disorders. Current guidelines define early-onset obesity as an onset before the age of 5 years,[6-8] but this cut-off is not based on clinical studies and is not validated. Therefore, in **chapter 5**, we presented the BMI trajectories of the largest cohort to date of children with non-syndromic and syndromic genetic obesity disorders compared to children from the general population who develop obesity before the age of 10 years. Moreover, we show that age of onset of obesity can guide the decision

which children with early-onset obesity to screen for genetic obesity even as single screening parameter. Optimal diagnostic performance was seen for a more stringent age of onset of obesity cut-off of ≤3.9 years compared to current guidelines' cut-off of ≤5 years. Of note, specific syndromic genetic obesity disorders (i.e. PHP and BBS) showed BMI trajectories similar to those of non-syndromic genetic obesity. Another important finding is related to the comparison with children from the general population who developed obesity before the age of 10. On average their age of onset of obesity was 3.8 years. This young age probably reflects the secular trend of increasing prevalence of early-onset obesity worldwide.[19] To keep specificity high, cut-offs for genetic screening needs to be adjusted to this secular trend. The BMI trajectories we presented can aid clinicians' decision who to screen for genetic obesity, and which genetic obesity disorders to suspect based on the individual's trajectory. Future studies should prospectively assess the yield of these proposed cut-offs in different clinical settings such as the general pediatric practice or community centers. Moreover, studies are needed to see how the diagnostic value of the age of onset of obesity (AoO) can be increased when combined with other features indicative of genetic obesity, e.g. hyperphagia, into prediction models that can be prospectively assessed in clinical practice.

Regardless of exact cut-offs for indication of genetic screening, our systematic review in **chapter 3** shows that more awareness from health care providers and better access to genetic testing facilities is needed. Furthermore, ongoing reporting of cases is essential to gain more insights into the clinical phenotypes. It is likely that patients with milder phenotypes are less likely to have been reported, because patients with the most severe phenotypes typically undergo genetic testing first, which can lead to ascertainment bias and overestimation of genetic risks.[20] As an example, it has been shown for MC4R deficiency that carriers of LoF variants identified through population-based cohorts did not always have an obesity phenotype, as opposed to cohorts of patients who were selected to undergo genetic testing due to their obesity phenotype.[18,21] Moreover, genotype-phenotype correlations, which have been suggested by some studies,[22,23] are difficult to establish as of yet due to the small number of patients currently reported in literature and the paucity of available in-depth phenotype data. For this, international registries and collaborations are needed. As an example, we have recently established a European collaboration to gain insight into the natural history of the height and weight trajectories associated with leptin-melanocortin pathway deficiencies.[24] The rarity of many genetic obesity disorders presented in **chapter 5** shows the necessity to compile growth data of patients to establish disease-specific growth charts, as has been established in other syndromic disorders such as Prader-Willi syndrome and Turner syndrome.[25-27]

As we show in **chapter 2**, it is important that patients with the clinical phenotype of a genetic obesity disorder (e.g. severe early-onset obesity with or without hyperphagia) without a diagnosis should be seen as currently unsolved cases from a genetic standpoint. It is also important to realize that both a positive family history of severe obesity in case of autosomal dominant disorders, which is mentioned in current guidelines, as well as negative family history in case of autosomal recessive disorders can hint towards a genetic obesity disorder. One could argue that these children could benefit similarly as children with genetic obesity disorders from tailored treatment and closure of their diagnostic odyssey when being diagnosed as having a "genetic obesity"-like disorder. Since the field of obesity genetics is rapidly evolving, diagnostics should be repeated over time, and registries should be seen as living databases where children without a current diagnosis with high suspicion of underlying causes might receive a diagnosis in the future. An example are the 6% of children described in **chapter 2** in whom we found variants of uncertain clinical significance (VUS) for which functional studies are necessary to establish causality with regard to their obesity. Moreover, it is to be expected that new advances in genetic diagnostics can further increase diagnostic yield. For example, novel genes have been associated in recent years with genetic obesity disorders. Examples in this thesis include the patients with loss-of-function variants in *GNB1* that we described in **chapter 4**. Examples from recent literature include *KSR2*, *ADCY3*, and *ASIP*, which have not yet been part of currently used obesity gene panels in routine clinical care.[5,21,28,29] Diagnosing these disorders enables tailored treatment with e.g. MC4R-agonists, which have been approved by regulation bodies in the US and Europe for several leptin-melanocortin pathway deficiencies including POMC, PCSK1, and LEPR deficiency as well as BBS, while the effect on several other genetic obesity disorders is currently being investigated in clinical trials.[30-32] In the future, innovative genetic tests such as global methylation studies as well as the inclusion of oligogenic genetic obesities and polygenic risk scores might further narrow the missing heritability observed in research into the genetics of obesity.[5,33,34] As genetic testing will become increasingly available in clinical practice with reduced associated costs, this will likely further increase the yield of systematic diagnostic workups.

Hypothalamic obesity disorders and measurement of resting energy expenditure in severe pediatric obesity

Apart from direct effects on satiety and appetite, gene expression in the hypothalamic leptin-melanocortin pathway also influences body weight homeostasis via changes in the hypothalamic setpoint for resting energy expenditure (REE).[17] Previous literature had linked decreases in REE to hypothalamic damage causing obesity, but REE characteristics across children with various underlying medical causes of obesity had not

been studied. In **chapter 6**, we found large inter-individual differences between measured REE vs predicted REE in children with and without underlying medical causes, but the between-group differences were found to be due to differences in fat-free-mass (FFM). Moreover, we confirmed that children with hypothalamic obesity have a decreased measured REE compared to predicted REE, which again can be explained by a decreased FFM. Notably, despite previous suggestions in smaller case series, decreased REE does not seem to explain obesity in non-syndromic and syndromic genetic obesity, even in PHP1a, a syndromic genetic obesity disorder that had been associated with decreased REE in earlier studies.[35-37] Our study shows that measuring REE does not directly contribute to the diagnostic workup of children with early-onset severe obesity on group level, except for children with suspected hypothalamic obesity in whom a decreased measured REE is more likely to be found. On an individual basis however, measurement of REE can have therapeutic consequences regarding dietary and physical activity advice as well as specific pharmacotherapy in children with decreased measured REE, e.g. central stimulants.[38,39] Therefore, we recommend measuring REE and body composition in selected children with hypothalamic obesity, genetic obesity or severe early-onset obesity with unexplained therapy resistance to guide patient-tailored treatment. In future research, repeated measurements of REE during combined lifestyle treatment and/or pharmacologic treatment could further improve our understanding of differences in treatment response, especially in children with underlying medical causes with decreased REE. Objective measurement of total energy expenditure and/or physical activity could further increase our understanding of the contribution of the different categories of energy balance metabolism to treatment response. Moreover, consensus regarding optimal prediction of REE to compare measured REE values[40] and its relation to body composition using different methods (e.g. dual x-ray absorptiometry and air displacement plethysmography) is needed.

Endocrine obesity disorders and associations of BMI SDS with stimulated growth hormone and long-term glucocorticoid levels

Endocrine diagnostics are indicated in children with obesity with decreased height velocity or short stature. This includes endocrine function tests aimed to exclude growth hormone deficiency, hypercortisolism or hypothyroidism.[8] This thesis focused on two specific research questions related to the normal reference ranges of endocrine tests in children with obesity: (1) the quantitative impact of BMI SDS on stimulated growth hormone (GH) levels in the diagnostic workup of children with growth hormone deficiency (GHD); (2) the quantitative relation between BMI SDS and long-term glucocorticoid levels. In **chapter 7**, we quantified the effect of increasing BMI on peak GH levels after a growth hormone stimulation test (GHST) by performing a systematic review and meta-analysis. Our study yields BMI SDS-adjusted cut-offs

that can be used to interpret GHST results in children with overweight and obesity. Moreover, we show that obesity is rare in children with suspected GHD in general. However, children with syndromic disorders seem to have a higher prevalence of GHD and obesity that pediatricians need to be aware of. The use of BMI SDS-adjusted cut-offs in clinical practice can lead to less overdiagnosis, and possible overtreatment, of GHD in children with obesity. Future studies should prospectively assess the merit of these BMI SDS-adjusted cut-offs in clinical practice. In **chapter 8**, we confirm the strong association between anthropometric measures of adiposity, such as BMI, and long-term glucocorticoids both in children as well as in adults. The strongest association was found for waist circumference and hair cortisone. Through our meta-analysis, we quantified the effect of BMI SDS on hair cortisol in children. Our results suggest an altered set point of the HPA-axis with increasing adiposity, especially with central obesity. This raises the question whether reference ranges for cortisol measurements in blood, saliva or urine for the diagnosis of pediatric Cushing's syndrome should be adjusted similarly for BMI SDS as we have shown in **chapter 6** for peak GH and GHD.[41,42] Moreover, measuring long-term glucocorticoids in hair shows promise as non-invasive tool in the diagnostics of Cushing's syndrome in adults,[43] but data in children are still lacking. However, there are many unresolved issues that need to be addressed before implementation in clinical practice. These include the direction of causality between increased long-term glucocorticoids in hair and obesity, which has been scarcely studied in longitudinal studies,[44,45] and the influence of lifestyle interventions on this relationship, for which an ongoing study is being performed at Obesity Center CGG. Furthermore, there are unresolved issues relating to the measurement technique itself, e.g. standardization, influence of hair growth speed, and influence of corticosteroid use.[46,47] Future studies evaluating longitudinal trajectories of hair glucocorticoids in children with severe with or without underlying medical causes and their metabolic profiles are needed. Moreover, the influence of combined lifestyle intervention on the association between hair glucocorticoids and obesity and the predictive value of hair glucocorticoids for explaining the large interindividual variation in treatment response are topics of interest for future studies.

Multifactorial obesity and impact of COVID-19 and lockdown measures on lifestyle behaviors

In children with multifactorial obesity, genetic polymorphisms and other biologic factors interact with environmental factors and lifestyle behaviors, ultimately leading to obesity.[5] During the research period of this thesis, the COVID-19 pandemic led to dramatic changes in these environmental factors and lifestyle behaviors and were therefore subject of our investigations in **chapter 9**. We show that the pandemic-related lockdown measures led to a reduction of weekly physical activity time from 9

to 7 hours, with only 49% of children with severe obesity achieving WHO recommendations of at least 1 hour of daily physical activity.[48] Moreover, in subgroups we found distinct effects with regard to eating styles or behaviors and health-related quality of life. The most important finding was that children who were already vulnerable before the pandemic due to psychosocial problems deteriorated further in weight and health-related quality of life. Indeed, several studies showed aggravation of pediatric obesity prevalence in the general population, as well as a further increased BMI in children who were already living with obesity.[49-51] Our study highlights the need to identify the subgroups who are most at risk for the negative effects of the COVID-19 pandemic on lifestyle behaviors. These subgroup of patients may benefit from proactive clinical monitoring and evaluation of the need of organizing additional medical and paramedical support. Moreover, we show that the application of strict schedules or schemes, e.g. for eating behaviors, can have a protective effect. Furthermore, we showed that addressing COVID-19 related anxiety could alleviate its negative effects on lifestyle behaviors. Therefore, the collateral damage caused by lockdown measures in these and other vulnerable subgroups of children should be weighed by policy makers.

Future perspectives

The research presented in this thesis was conducted mostly in children with an obesity severity on the tip of the pediatric obesity iceberg: children with severe obesity, many of which with underlying medical causes or a suspicion thereof. In order to tackle the pediatric obesity epidemic and change our modern obesogenic environment however, we need to make the necessary societal changes from the very basis of the obesity pyramid. Universal prevention aimed at preventing overweight and obesity through promotion of healthy food and physical environments are direly needed, especially for children with vulnerable socioeconomic positions.[6,8] Individual prevention, including access to and reimbursement of combined lifestyle interventions are necessary to prevent aggravation in children with obesity. These interventions need to be integrative, delivered as locally as possible as part of a family-centered approach, and monitored in collaboration between generalists and specialists.[6] For children with severe obesity preventive measures alone are not sufficient and additional treatment interventions will often be required. As with any other chronic, multifactorial disease, a holistic diagnostic workup is needed that assesses lifestyle, psychosocial and biomedical factors that facilitate patient-tailored treatment rather than a one-size-fits-all referral to lifestyle intervention. This thesis shows that, especially in children with severe obesity, a broad, systematic diagnostic approach is needed to identify potential underlying medical causes of obesity first in order to tailor treatment to the individual patient. These underlying causes have

characterized features, such as early-onset of obesity, hyperphagia, family history of extreme obesity, decreased energy expenditure, organ-specific abnormalities and/or associated hormonal disturbances. However, many patients are currently not recognized, and it is not feasible to perform such a workup in all children currently, due to high costs, limited space in secondary and tertiary care of these referrals, and limited yield in settings with lower a priori risk of finding underlying medical causes. Thus, improvement of diagnostic strategies are needed by establishing predictors of underlying medical causes and improving selection of children with the highest risk. This includes, among others, thorough medical history taking including evaluation of both positive as well as negative family history of severe obesity; thorough physical examination including evaluation of specific signs and symptoms associated with underlying medical causes; prospective evaluation of growth charts trajectories and evaluation of cut-offs for diagnostic yield of underlying medical causes and cost effectivity; consensus on hyperphagia definitions, as currently used questionnaires were designed for Prader-Willi syndrome and show overlapping scores in children with and without underlying medical causes;[52,53] better understanding of the contribution of measuring REE; and guidance on when to perform which genetic tests. At Obesity Center CGG, a web-based algorithm is currently being developed for health care professionals that integrates the abovementioned factors into recommendations for diagnostics for underlying medical causes, comorbidities and tailored treatment. This will allow a dynamic evaluation of the 'ideal' threshold for performing diagnostics for underlying medical causes with regard to the balance between sensitivity vs. specificity, which will be different depending on the setting and population characteristics. It can be expected that improved phenotyping will lead to better tailored treatment and improvement of treatment outcomes, although this has only been reported scarcely in literature, e.g. in individual and case series,[38,39,54,55] and in an observational study in adults.[56] Thus, international collaboration, establishment of registries and multicenter cohorts are the way forward to better understand between-disorder and within-disorder heterogeneity regarding underlying medical causes of severe pediatric obesity and response to treatment outcomes.

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Appendix

SUMMARY

Severe pediatric obesity is a complex, relapsing and chronic endocrine disease. It is associated with various adverse physical and psychosocial health sequelae in the short and long term, leading to a high burden on well-being and productivity. Obesity is a multifactorial disease caused by genetic, environmental, behavioral, socioeconomic and cultural factors. In a minority of children with severe obesity, the obesity phenotype is caused by a singular underlying medical cause interfering with the function of the hypothalamic leptin-melanocortin pathway, which regulates satiety and energy expenditure. Current international guidelines define the following categories of underlying medical causes: (1) genetic obesity disorders, (2) hypothalamic obesity, (3) endocrine obesity, and (4) medication-induced obesity. This thesis investigated several important diagnostic aspects of severe pediatric obesity

Chapter 1 describes the different categories of underlying medical causes and their pathophysiology. Moreover, an overview is given of the systematic diagnostic workup of the pediatric division of Obesity Center CGG, which forms the basis of the diagnostic aspects investigated in this thesis.

In **chapter 2**, the yield of the systematic diagnostic workup is described. A singular underlying medical cause was identified in 19% of patients, most of which were genetic obesity disorders (13% of patients). This chapter shows that an extensive diagnostic approach is needed to identify the underlying medical causes. Moreover, in all patients with an underlying medical cause, the diagnosis facilitated disease-specific, patient-tailored treatment.

Chapter 3 describes the results of a comprehensive systematic literature review and epidemiologic analysis on the prevalence of leptin receptor (LepR) deficiency. By using data of over 77,000 European individuals, we showed that the reported prevalence of LepR deficiency (based on case reports and case series) in Europe is only 2% of predicted prevalence, suggesting underreporting, underdiagnosis, early mortality, or a combination of these factors. Moreover, the majority of patients did not have the pituitary hormone disturbances (central hypothyroidism, growth hormone deficiency, and/or hypogonadotropic hypogonadism) that are typically associated with LepR deficiency. This suggests that genetic screening for leptin-melanocortin pathway deficiencies should be performed in all cases with early-onset severe obesity and hyperphagia, even without hormone disturbances or associated signs and symptoms.

In **chapter 4**, a case series of patients with loss-of-function variants in the *GNB1* gene is presented. By compiling all available data from the literature and our patients, we show that obesity is significantly overrepresented in patients with loss-of-function variants. Thus, *GNB1* should be considered in the differential diagnosis of syndromic genetic obesity.

Chapter 5 describes the BMI trajectories of patients with non-syndromic genetic obesity, syndromic genetic obesity, and controls with obesity from a population-based cohort study. Distinct trajectory patterns were seen for each of the subgroups. The presented BMI trajectories can thereby guide clinicians' decision to perform genetic testing. Moreover, we show that the optimal cut-off value of age of onset of obesity when used as a screening parameter to decide whether genetic testing is indicated, is ≤3.9 years. This is lower than current international guidelines suggest, reflecting the secular trend of increasing early-onset obesity worldwide.

In **chapter 6**, the resting energy expenditure characteristics of children with and without diagnosed underlying medical causes of obesity are described. Resting energy expenditure was higher in patients with non-syndromic genetic obesity and lower in patients with hypothalamic obesity compared with patients with multifactorial obesity, but the differences were no longer statistically significant after adjustment for fat-free mass. The large between-disorder and inter-individual variation shows that measuring resting energy expenditure and body composition do not directly contribute to diagnosing underlying medical causes, but can improve patient-tailored treatment interventions in children with severe obesity.

In **chapter 7**, we have quantified the negative association between BMI and peak stimulated growth hormone values for the diagnosis of growth hormone deficiency (GHD). By compiling available studies over the past six decades in a systematic review and meta-analysis using individual participant data, we calculated BMI-specific cut-off values to improve diagnosis of GHD in children with overweight and obesity.

In **chapter 8**, we have similarly quantified the positive association of BMI, BMI standard deviation score and weight circumference on scalp hair glucocorticoids in both children and adults. Our findings suggest an altered setpoint of the hypothalamic-pituitary-adrenal axis with increasing central adiposity. Moreover, we provide pooled regression coefficients for the associations between anthropometrics and scalp hair glucocorticoids that can be applied on the individual level.

Chapter 9 describes the influence of the first lockdown and associated measures of the COVID-19 pandemic on the lifestyle behaviors of children with severe obesity using both quantitative as well as qualitative research methods. We showed that weekly physical activity decreased significantly on group level to ≤2 hours/week in the majority of patients. Moreover, eating styles and health-related quality of life deteriorated in subgroups of patients with high emotional and external eating scores or pre-existing psychosocial problems. This chapter identifies the subgroups of patients and their families that should be proactively targeted by health care professionals to mitigate negative physical and mental health consequences.

Finally, a general discussion in the context of current literature is provided in **Chapter 10**, including recommendations, future perspectives and implications.

SAMENVATTING

Ernstige obesitas bij kinderen is een complexe, chronische endocriene ziekte. Het is geassocieerd met verscheidene negatieve gevolgen voor fysieke en psychosociale gezondheid, zowel op de korte als lange termijn. Dit leidt tot een hoge last op welzijn en productiviteit. Obesitas is een multifactoriële ziekte die wordt veroorzaakt door verschillende factoren: genetische, omgevings-, socio-economische en culturele factoren. In een minderheid van kinderen met ernstige obesitas wordt het obesitasbeeld veroorzaakt door een onderliggende medische oorzaak. Deze oorzaak verstoort de functie van het leptine-melanocortinesysteem in de hypothalamus. Dit systeem reguleert de verzadiging en verbranding. Huidige internationale richtlijnen onderscheiden de volgende categorieën van onderliggende medische oorzaken: (1) genetische obesitasaandoeningen; (2) hypothalame obesitas; (3) endocriene obesitas; en (4) medicatie-geïnduceerde obesitas. Dit proefschrift onderzocht verschillende belangrijke diagnostische aspecten van ernstige obesitas bij kinderen.

Hoofdstuk 1 beschrijft de verschillende categorieën van onderliggende medische oorzaken en hun pathofysiologie. Bovendien is een overzicht gegeven van het systematische diagnostische zorgpad van het Centrum Gezond Gewicht (Engels: *Obesity Center CGG*). Dit vormt de basis van de diagnostische aspecten die in dit proefschrift zijn onderzocht.

In **hoofdstuk 2** is de opbrengst van het systematische diagnostische zorgpad beschreven. Een specifieke onderliggende medische oorzaak werd gevonden in 19% van de patiënten, waarvan de meeste genetische obesitasaandoeningen (13% van de patiënten). Dit hoofdstuk toont aan dat uitgebreide diagnostiek nodig is om de onderliggende medische oorzaken aan te tonen. Bovendien leidde het stellen van de diagnose in alle patiënten met een onderliggende medische oorzaak tot ziekte-specifieke behandeling op maat.

Hoofdstuk 3 beschrijft de resultaten van een uitgebreide systematische literatuurreview en epidemiologische analyse van de prevalentie van leptinereceptordeficiëntie (LepR-deficiëntie). Door gebruik te maken van de gegevens van meer dan 77.000 Europeanen, toonden wij dat de beschreven prevalentie van LepR-deficiëntie in de literatuur (op basis van studies die één of enkele patiënten beschrijven) in Europa slechts 2% van de voorspelde prevalentie is. Dit suggereert dat er sprake is van onderrapportage, onderdiagnose, vroege mortaliteit of een combinatie van deze factoren. Bovendien had de meerderheid van de patiënten geen hypofysehormoonstoornissen die typisch geassocieerd zijn met LepR-deficiëntie: centrale hypothyreoïdie,

groeihormoondeficiëntie en/of hypogonadotroop hypogonadisme. Dit suggereert dat genetische screening voor deficiënties in het leptine-melanocortinesysteem moeten worden verricht in alle gevallen van vroeg ontstane ernstige obesitas met hyperfagie, zelfs als er geen symptomen van de geassocieerde hypofysehormoonstoornissen zijn.

In **hoofdstuk 4** worden enkele patiënten gepresenteerd met loss-of-function varianten in het *GNB1*-gen. Door alle beschikbare gegevens uit de literatuur en onze patiënten samen te voegen, konden wij aantonen dat obesitas significant vaker voorkomt in patiënten met loss-of-function varianten. Daarom zouden afwijkingen in het *GNB1* moeten worden overwogen in de differentiaaldiagnose van syndromale genetische obesitas.

Hoofdstuk 5 beschrijft de BMI-trajecten van patiënten met niet-syndromale genetische obesitas, syndromale genetische obesitas, en controlekinderen met obesitas uit een populatiestudie. In iedere subgroep werd een verschillend BMI-traject gezien. De gepresenteerde BMI-trajecten kunnen daarom de klinische besluitvorming over het verrichten van genetische diagnostiek ondersteunen. Bovendien tonen wij aan dat de optimale afkapwaarde voor de ontstaansleeftijd van obesitas als screeningsparameter voor de beslissing of er genetisch onderzoek moet worden verricht of niet ≤3,9 jaar is. Dit is lager dan de suggesties van de huidige internationale richtlijnen en reflecteert de gestage trend van toenemende obesitas op de vroege kinderleeftijd die wereldwijd gezien wordt.

In **hoofdstuk 6** worden de rustverbrandingskarakteristieken van kinderen met en zonder gediagnosticeerde onderliggende medische oorzaak beschreven. Kinderen met niet-syndromale genetische obesitas hadden een hogere rustverbranding dan kinderen met multifactoriële obesitas, terwijl kinderen met hypothalame obesitas juist een lagere rustverbranding hadden dan kinderen met multifactoriële obesitas. De verschillen waren echter niet meer statistisch significant na correctie voor vetvrije massa. De grote verschillen tussen aandoeningen en individuen reflecteert dat het meten van rustverbranding en lichaamssamenstelling niet direct bijdraagt aan het diagnosticeren van onderliggende medische oorzaken, maar wel aan de patiënt-specifieke behandeling op maat in kinderen met ernstige obesitas.

In **hoofdstuk 7** hebben wij de negatieve associatie tussen BMI en piek groeihormoonwaarden in stimulatietesten gekwantificeerd voor de diagnose van groeihormoondeficiëntie. Wij hebben de beschikbare studies van de afgelopen 60 jaar samengevoegd in een systematische review en meta-analyse op individueel patiëntniveau. Hiermee

hebben we BMI-specifieke afkapwaarden berekend om de diagnose van groeihormoondeficiëntie in kinderen met overgewicht en obesitas te verbeteren.

In **hoofdstuk 8** hebben wij een vergelijkbare kwantificatie uitgevoerd voor de positieve relatie tussen BMI, BMI standaarddeviatiescore en buikomtrek op glucocorticoïden in hoofdhaar voor zowel kinderen als volwassenen. Onze bevindingen suggereren dat er een veranderd ‘setpoint’ van de hypothalamus-hypofyse-bijnieras is bij toenemende centrale adipositas. Bovendien presenteren we gepoolde regressiecoëfficiënten voor de associatie tussen antropometrische parameters en glucocorticoïden in hoofdhaar die kunnen worden gebruikt op individueel patiëntniveau.

Hoofdstuk 9 beschrijft de invloed van de eerste lockdown en geassocieerde maatregelen van de COVID-19 pandemie op leefstijlgedragingen van kinderen met ernstige obesitas. Hierbij werd zowel gebruik gemaakt van kwantitatieve als kwalitatieve onderzoeksmethoden. We zagen dat op groepsniveau fysieke activiteit statistisch significant afnam tot ≤2 uur per week in de meerderheid van de patiënten. Bovendien werd er een negatief effect op eetstijlen en gezondheidsgerelateerde kwaliteit van leven gezien in subgroepen van patiënten met hoge scores op emotioneel of extern eten en patiënten met pre-existente psychosociale problemen. In dit hoofdstuk worden de subgroepen van patiënten en hun families beschreven die daarom proactief moeten worden benaderd door zorgprofessionals om de negatieve gevolgen op fysieke en mentale gezondheid te verminderen.

Tot slot wordt een algemene discussie in de context van de huidige wetenschappelijke literatuur gepresenteerd in **hoofdstuk 10**, inclusief aanbevelingen, toekomstige perspectieven en implicaties.

LIST OF PUBLICATIONS

Included in this thesis

Abawi O*, Kleinendorst L*, *et al*. Identifying underlying medical causes of pediatric obesity: results of a systematic diagnostic approach in a tertiary obesity center. *PloS One* 2020;15(5):e0232990.

Kleinendorst L*, **Abawi O***, *et al*. Leptin receptor deficiency: a systematic literature review and prevalence estimation based on population genetics. *Eur J Endocrinol* 2020;182(1):47-56, doi: 10.1530/EJE-19-0678.

Kleinendorst L, **Abawi O**, *et al*. Obesity and loss of function GNB1 variants - A new form of syndromic obesity? *(Under review)*

Abawi O, *et al*. Genetic obesity disorders: BMI trajectories and age of onset of obesity compared to children with obesity from the general population. *J Pediatr 2023;262:113619, doi: 10.1016/j.jpeds.2023.113619.*

Abawi O*, *et al*. Resting energy expenditure and body composition in children and adolescents with genetic, hypothalamic, medication-induced or multifactorial severe obesity. *Front Endocrinol*. 2022;13:862817.

Abawi O*, Augustijn D*, *et al*. Impact of body mass index on growth hormone stimulation tests in children and adolescents: a systematic review and meta-analysis. *Crit Rev Clin Lab Sci*. 2021;58(8):576-595.

van der Valk ES*, **Abawi O***, *et al*. Cross-sectional relation of long-term glucocorticoids in hair with anthropometric measurements and their possible determinants: a systematic review and meta-analysis. *Obes Rev*. 2022;23(3):e13376.

Abawi O*, Welling MS*, *et al*. COVID-19 related anxiety in children and adolescents with severe obesity: a mixed-methods study. *Clin Obes* 2020;10(6):e12412.

Welling MS*, **Abawi O***, *et al*. Impact of the COVID-19 pandemic and related lockdown measures on lifestyle behaviors and wellbeing in children and adolescents with severe obesity. *Obes Facts*. 2021;430-440.

Not included in this thesis

Abawi O, *et al*. 11-oxygenated androgens are strongly associated with treatment quality in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency *(Manuscript in preparation)*

Abawi O, *et al*. Predicting treatment quality assessment of children with congenital adrenal hyperplasia using 24h urine metabolomics profiling and a machine learning-assisted approach *(Manuscript in preparation)*

Raftopoulou C*, **Abawi O*,** *et al*. Leukocyte telomere length in children with congenital adrenal hyperplasia. *J Clin Endocrinol Metab*. 2023;108:443-452.

van Rossum EFC, Welling MS, van der Voorn B, van der Valk ES, **Abawi O**, van den Akker ELT. Pharmacotherapy for obesity. Ned Tijdschr Geneeskd. 2021 Jan 19;165:D4907.

Clément K*, van den Akker ELT*, ... **Abawi O,** *et al*. Efficacy and safety of setmelanotide, an MC4R agonist, in individuals with severe obesity due to LEPR or POMC deficiency: single-arm, open-label, multicentre, phase 3 trials. *Lancet Diabetes Endocrinol* 2020;8(12):960-970.

Kleinendorst L, Alsters SM, **Abawi O,** *et al*. Second case of Bardet-Biedl syndrome caused by biallelic variants in IFT74. *Eur J Hum Genet* 2020; 28(7):943-46.

Abawi O, *et al*. Evaluation of multiple referral strategies for axial spondyloarthritis in the SPondyloArthritis Caught Early (SPACE) cohort. RMD Open 2017;3(1):e000389, doi: 10.1136/rmdopen-2016-000389.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: **Ozair Abawi**	PhD period: **2018 - 2022**
Erasmus MC Department: **Pediatrics, division of Endocrinology**	Promotor(s): **Prof. Dr. Erica L.T. van den Akker; Prof. Dr. Elisabeth F.C. van Rossum**
Research School: **Molecular Medicine**	Supervisor: **Prof. Dr. Erica L.T. van den Akker**

1. PhD training

	Year	Workload (Hours/ECTS)
General courses		
- Systematic literature search 1 (Embase) course	2018	0.4
- Systematic literature search 2 (Pubmed) course	2018	0.2
- Endnote course (Medical Library)	2018	0.2
- BROK	2018	1.5
- CC02 Biostatistical Methods I	2018	5.7
- Basic course on R	2019	2
- Scientific Integrity	2019	0.3
- EP03 Biostatistical Methods II	2019	4.3
- Biomedical English Writing	2020	2
- Personal Leadership & Communication	2021	1
Specific courses (e.g. Research school, Medical Training)		
- LUMC Basic Methods and Reasoning in Biostatistics	2018	1.5
- Genetics for Dummies	2018	0.6
- Basic and Translational Endocrinology	2019	2.2
- Excel 2010 Advanced	2020	0.4
- Photoshop & Illustrator course	2021	0.3
- Indesign course	2021	0.15
Seminars and workshops		
- Nationaal Obesitas Symposium	2018	0.3
- EASO COM Summit Meeting	2019	0.6
- Nationaal Obesitas Symposium	2020	0.3
- ESPE Connect Online	2020	1
- Dutch Endocrine Meeting	2021	0.3
- ESPE Science Symposium	2021	0.6
Presentations		
- Webinar – COVID-19 and obesity in children	2020	0.3

International conferences		
- International Obesity Genetics Collaboration meeting AMC (Oral presentation)	2018	0.5
- ECO congress (Poster presentation)	2018	1
- International Obesity Genetics Collaboration meeting AMC (Oral presentation)	2019	0.5
- EASO NIU autumn school (Poster presentation)	2019	1
- ESPE congress (Poster presentation x2)	2020	1
- International Obesity Genetics Collaboration meeting AMC (Oral presentation)	2020	0.5
- ECO/ICO congress (Poster presentation x2)	2020	1
- e-ECE congress	2020	1
- ENDO congress (Poster presentation x2)	2021	1
- ECO congress (Oral presentation, poster presentation)	2021	1
- e-ECE congress (Poster presentation)	2021	1
- ESPE congress (Oral presentation, poster presentation)	2021	1
- ECO/IFSO congress (Oral presentation x2, poster presentation)	2022	1
- ENDO congress + Early Career Forum (Oral presentation x2, poster presentation)	2022	1.3
- I-DSD symposium	2022	0.8
National conferences		
- NASO spring meeting	2018	0.3
- Dutch Endocrine Meeting (Oral presentation)	2019	0.8
- NASO spring meeting	2019	0.3
- Sophia Research Days (Oral presentation)	2019	0.5
- Dutch Endocrine Meeting (Poster presentation)	2020	0.8
- NASO spring meeting	2020	0.3
- NASO spring meeting (Oral presentation)	2021	0.5
- Sophia Research Days	2021	0.3
- NVK congress (Oral presentation)	2021	0.5
- JNVE congress (Oral presentation)	2021	0.8
- Dutch Endocrine Meeting	2022	0.6
- Sophia Research Days (Poster presentation)	2022	0.5
- NASO spring meeting (Oral presentation)	2022	0.5
Other		
- Peer reviewer international scientific journals (*Nat Rev Endocrinol, Obes Rev, Front Endocrinol, Front Nutr, Humanit Soc Sci, Child Obes, Horm Res Paediatr, Moll Cell Pediatr, PLOS ONE*)	2020 – 2022	2.0

2. Teaching

	Year	**Workload (Hours/ECTS)**
Lecturing		
- MEDILEX Nascholing Obesitas bij kinderen	2020	0.3
- MEDILEX Nascholing Obesitas bij kinderen	2021	0.3
- Recording highlight videos for ECO congress 2021 and 2022 for Obesitas Platform	2021 – 2022	0.3
Supervising practicals and excursions, Tutoring		
- Student coach – Bachelor students Medicine (EUR)	2019 – 2022	2.0

Supervising Master's theses		
- Supervisor Master's thesis Medicine student (2x 16 weeks) + Bachelor student University College (26 weeks)	2019 – 2020	3.0
3. Other		
	Year	**Workload (Hours/ECTS)**
- Organisation pediatric endo research meeting 1x/2wks + multiple oral presentations	2018 – 2022	2.0
- CGG research meeting 1x/mo + multiple oral presentations	2018 – 2022	2.0
- International Genetic Obesity Club meeting 1x/mo		
- Organising & presenting on symposium for CAH patients & parents	2022 2019	0.5 0.3
- Organising & presenting on symposium for CGG patients & parents	2020	0.3
- Tulips Young Investigators Day		
- TULIPS Grant writing & Presenting Day	2018	0.3
- Organising TULIPS PhD weekend 2022	2019	0.3
- TULIPS PhD curriculum	2022	0.5
- Committee member Green Team Biomedical Research Erasmus MC	2020 – 2022 2021 – 2022	4.0 1.0
- Research visit Inselspital, Bern (Switzerland) – Department of Pediatric Endocrinology, project "Novel CAH monitoring tools using machine learning" May – August 2022	2022	
Total ECTS *ECTS, European Credit Transfer and Accumulation System* *1 ECTS represents 28 hours*		65.55
4. Awards and Grants		
	Year	**Workload (Hours/ECTS)**
- Travel grant Erasmus Trust Fonds (€150,-)	2018	
- Sophia Research Days top 3 best abstracts	2019	
- ESPE registration grant (€100,-)	2021	
- JNVE Young Talent Award (€250,-)	2021	
- NASO travel award 2022 (€150,-)	2022	
- ENDO Early Career Forum 2022 ($400,-)	2022	
- ENDO Outstanding abstract award ($750)	2022	
- Ter Meulen Grant (€7800,-)	2022	
- SNSF Scientific Exchange grant (CHF 9500,-)	2022	
- ENDO Outstanding abstract award ($750)	2023	
5. Selection of media performances		
- TV interview on Chapter 2 (*Jeugdjournaal 19-5-2020*)	2020	
- Dutch general national newsarticles on Chapter 2 (e.g. Algemeen Dagblad, Trouw, De Telegraaf, NOS.nl, NU.nl)	2020	
- Interview NVE magazine 'Endocrinologie' on Chapter 7	2021	
- Interview NVKC magazine 'Laboratoriumgeneeskunde' on Chapter 7	2021	
- Dutch medical journal 'Medisch Contact' news articles on Chapters 2 and 7	2020, 2021	
- TV interview on Chapter 8 (*TV Rijnmond 28-9-2021*)	2021	

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CURRICULUM VITAE

Ozair Abawi was born in Kabul, Afghanistan and moved to Amsterdam with his family at age 1 years. He obtained his VWO diploma at the Vossius Gymnasium Amsterdam (summa cum laude) in 2011. During his high school years, he attended the Pre-University College at Leiden University, graduating cum laude in 2011. He continued to study Medicine at the Leiden University Medical Center (LUMC), and obtained his medical degree in 2019 (summa cum laude). As part of his clinical rotations, he visited the Gynecology department of the Diakonessenhuis in Paramaribo, Surinam. His interest for pediatrics was sparked during his senior rotation at the department of General Pediatrics of the Willem-Alexander Kinderziekenhuis (LUMC), Leiden. During his research internship, he got involved at the Obesity Center CGG (Dutch: “Centrum Gezond Gewicht”) of the Erasmus MC in Rotterdam.

After obtaining his medical degree, he started his PhD at Obesity Center CGG and the department of Pediatric Endocrinology (Erasmus MC, Rotterdam) under the supervision of prof. dr. Erica van den Akker and prof. dr. Liesbeth van Rossum. Moreover, Ozair followed the PhD curriculum of Stichting TULIPS (Training Upcoming Leaders in Pediatric Science). His research received media attention several times varying from articles in general news outlets (e.g. Algemeen Dagblad, De Telegraaf, Trouw, Jeugdjournaal, NOS), as well as medical journals (e.g. Medisch Contact, Endocrinologie, Tijdschrift voor Laboratoriumgeneeskunde).

Ozair was also involved in an international multicenter project regarding congenital adrenal hyperplasia, for which he attended the department of Pediatric Endocrinology and Diabetology at Inselspital, Bern, Switzerland, as a visiting researcher in the summer of 2022 and returned as a research fellow in the fall of 2022.

Starting from January 2023, Ozair started his clinical career as a pediatric resident (ANIOS Kindergeneeskunde) at the Reinier de Graaf Gasthuis in Delft. He has continued his clinical work as a pediatric resident (AIOS Kindergeneeskunde) starting from January 2024 at the Franciscus Gasthuis & Vlietland Ziekenhuis, Rotterdam. Moreover, he will continue his research at Obesity Center CGG as a postdoctoral researcher.