Diagnosis and treatment of obese children with insulin resistance

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DIAGNOSIS AND TREATMENT OF OBESE CHILDREN WITH INSULIN RESISTANCE

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Section 1

Introduction and scope of the thesis

Chapter 1

General introduction on diagnosis and treatment of obese children with insulin resistance

General introduction on diagnosis and treatment of obese children with insulin resistance

Nowadays, worldwide more people are overweight or obese than underweight [1]. In 2014, worldwide 10.8% (9.7–12.0) of adult men and 14.9% (13.6-16.1) of adult women were obese, whereas the prevalence of underweight was respectively 8.8% (7.4-10.3) in men and 9.7% (8.3-11.1) in women [1].

Obesity is a condition which is defined as abnormal or excessive body fat accumulation. This condition may impair health, and is of specific concern in view of the increasing prevalence in children [2, 3]. To classify overweight, and thereby obesity, the body mass index (BMI) is used. The BMI is calculated as weight (kg) divided by (height (m))^2, with adult cut-off values for overweight and obesity of respectively ≥25 kg/m^2 and ≥30 kg/m^2 [2]. In children normal growth results in an initial decrease of BMI until the age of 4-5 years, followed by an increase in BMI. Consequently, fixed cut-off values for BMI to classify obesity in children cannot be used, and therefore standard deviation scores (SDS), z-scores, or percentiles are used to the define overweight and obesity [4-6]. These scores are based on the number of standard deviations below or above the median BMI for age and sex of an international population of six large, nationally representative growth studies [4, 5]. Cut-off values used in the Netherlands are BMI-SDS > 1.1 (BMI > p85) for overweight and BMI-SDS > 2.3 (BMI > p95) for obesity [7].

In the 1960's, childhood obesity was very uncommon, with prevalence rates for overweight (BMI > p85) and obesity (BMI > p95) of 4.2-4.6% for children 6-19 years old in the United States [8]. Since that moment, prevalence of childhood obesity is rising. Although prevalence rates from 2007-2012 suggest that the rising prevalence in childhood obesity may have reached a plateau since 2003-2004 [9-11], in 2013-2014 the prevalence was rising again. In 2013-2014 the prevalence rates in children and adolescents 2-17 years old in the US for overweight (BMI > p85) and obesity (BMI > p95) were 33.4 (95%CI 30.9-35.9) % and 17.4 (95%CI 15.2-19.6)%, respectively [3]. In the Netherlands, prevalence of obesity was 0.3% in native boys and 0.5% in native girls aged 2-21 years in 1980. In 2010 however, these prevalence rates for obesity were 1.8% and 2.2% in native boys and girls, respectively, and higher in Moroccan and Turkish descent (6.0% and 8.4% in boys and 7.5% and 8.4% in girls, respectively). These figures should be seen in the context of a prevalence of overweight of 13.3 and 14.9% in native boys and girls, up to 32.5 and 31.7% in boys and girls of Turkish descent [12].

Obesity is most frequently caused by an energy imbalance between intake of calories and calories burned. During the last decades, energy intake shifts towards high-caloric foods containing few vitamins, minerals and other healthy nutrients. At the same time, the energy expended is reduced because of physical activity is reduced

and a sedentary lifestyle is more common [2]. The energy not expended is stored as fat mass. However, there is evidence that more factors contribute to obesity, such as parental obesity, social economic state, maternal nutrition and glucose metabolism during pregnancy and psychological health [13-15]. In 2-4% of the obese children, an endocrine cause (such as hypothyroidism, growth hormone deficiency, Cushing syndrome or hypothalamic obesity), genetic cause (for example leptin deficiency, mutations in POMC or MC4R deficiency) or genetic syndrome (for example Prader-Willi syndrome, Bardet-Biedl and Alstrom syndrome) is found [13, 14, 16-18]. Finally, use of medication, for example anti-epileptic and antipsychotic drugs, can contribute to the development of obesity [18, 19].

Prognosis and consequences of childhood obesity

Childhood obesity is a strong predictor for obesity in adulthood. Whitaker et al. described in 1997 that 79% of children who were obese at age 10-14 years old, were still obese as young adults (21-29 years) [20]. A systematic review by Singh et al, described rates from 47-83% of obese children becoming obese adults [21]. Odds ratios for obese children to become obese adults varied from OR 1.3 for obese children aged 1-2 years to an OR of 22.3 for obese children aged 10-14 years [20, 22]. In adults who were obese during childhood (age 14-19 years) relative risk for all-cause mortality after 31.5 years of follow up was 1.82 (95% CI 1.48–2.43) in men and 2.03 (95% CI 1.51–2.72) in women [23].

There are multiple consequences affecting almost all organ tracts, of which many will be listed here. Consequences of childhood obesity are both psychosocial and somatic. Psychosocial consequences have a high burden on the quality of life on short-term and long-term. Short-term psychosocial consequences are for example poor self-esteem, being bullied, depression and eating disorders [24, 25]. Low self-esteem was found in 34% of obese girls vs 8% in non-obese girls [26]. Long-term psychosocial effects of obesity include higher risk of depression, oppositional defiant disorder and lower incomes [27-29].

Cardiovascular and metabolic consequences are very common, both on short-term and long-term. The cardiovascular short-term consequences are hypertension, dyslipidemia and endothelial dysfunction [16, 24]. In a cohort of 886 obese children, 42% had dyslipidemia and 32% had hypertension [30]. These short-term consequences, which are risk factors for cardiovascular events such as myocardial infarction and stroke, persist in adulthood when obese children become obese adults [31, 32]. BMI during adolescence was associated with death from cardiovascular disease in a follow up of up to 40 years [33]. The metabolic consequences of childhood obesity are insulin resistance (IR), impaired glucose tolerance, and, in a minority of the obese children, type 2 diabetes mellitus (T2DM). In adulthood, developing T2DM is more common.

Pulmonary short-term and long-term consequences are sleep apnoea, asthma and exercise intolerance [34-37]. Regarding the digestive tract, short-term and long-term consequences are equal, these include gall-stones [38, 39], constipation [40-42], hepatic steatosis or non-alcoholic fatty liver disease (NAFLD) [38, 43-45] and gastro-oesophageal reflux [46, 47]. Short-term musculosketelal problems are flat feet [48-50], lower limb pain [51-53], malalignment of knees and fractures of fore-arm, humerus and femur [54-56]. Fifty to seventy percent of the patients with slipped femoral capital epiphysis was obese [56, 57]. Long-term consequences are arthrosis of knee and hip in early adulthood [58]. Childhood obesity has consequences for the urogenital tract in boys as hypogonadism occurs and in girls by the occurrence of PCOS, which may cause an irregular menstrual cycle, and on long-term subfertility [16, 24, 59]. PCOS is associated with IR [59-61].

Insulin resistance in children with obesity

IR is a state in which increased levels of insulin are measured in absence of diabetes mellitus, which results from peripheral tissues being less sensitive to insulin [62]. IR in obesity develops as a result of the excess fat mass. Fat is an endocrine active tissue, which excretes adipocytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and free fatty acids. These adipocytokines induce a chronic inflammatory state [63]. This inflammatory state and the free fatty acid release reduce the muscle glucose uptake, and more insulin is needed to maintain normoglycemia. As a result of this insulin resistance, a compensatory hyperinsulinemia arises. Hyperinsulinemia is correlated with NAFLD [64], hypertriglycidemia [65, 66], hypertension [66] and T2DM [67-69].

Therefore, IR is described to play a key role in the development of metabolic and cardiovascular consequences in obesity [63, 70-72]. Although IR is related to obesity, not all obese children are insulin resistant, and not all insulin resistant children are obese. IR levels increase with the level of overweight [73, 74]. Other factors influencing IR are for example puberty, in which a physiological decrease of ~25-50% in insulin sensitivity was observed, and gender and ethnicity [75-77]. Black, African-American and Mexican American children have higher levels of both fasted insulin and post-glucose load insulin levels than white children, irrespective of their pubertal state [74, 78-81].

As IR is a precursor of T2DM, it can be considered an early marker for those who are at high risk of T2DM. Although the other precursors of T2DM, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are clearly defined by the ADA criteria [82], for IR there is no uniform method and cut off value. As a result, the prevalence of IR reported within the same obese population varies between 40.5% and 80.1% in obese children and adolescents (6-18 years), depending on the definition used [83].

In another study the prevalence of IR in obese children aged 8-10 years was 27.3% or 58.4% depending on the definition used [84].

Treatment of childhood obesity

Lifestyle intervention is the cornerstone of the treatment of obesity, since lifestyle interventions aim to restore the balance between calories ingested and calories burned. The effectivity of lifestyle interventions for the treatment of obesity in children is studied frequently. A Cochrane review and meta-analysis showed a BMI reduction of -3.04 (95% confidence interval (CI) -3.14 to -2.94) and -3.37 (95% CI -3.38 to -3.17) after 6 and 12 months, respectively in children of 12 years and above. The change in BMI-SDS after 6 and 12 months was respectively -0.14 (95%CI -0.17 to -0.12) and -0.14 (-0.18 to -0.10). In younger children (<12 years) after 6 months a small decrease in BMI-SDS was achieved (-0.06 (95%CI -0.12 to -0.01), which declined after 12 months (-0.04 (-0.12 to 0.04) [85]. However, there is large variation in lifestyle programmes, and drop-out rates are high. The effectivity of lifestyle interventions is largely influenced by the motivation of parents. Parent-only interventions for children with obesity showed a reduction in child BMI [86-88]. Finally, to improve the effect of lifestyle interventions for childhood obesity, the use of smartphones and internet-based programmes was studied. Most studies showed an improved compliance and response, and lower drop-out rates. However, no difference in body weight was found between groups using the smartphones or internet-based programmes and the groups receiving standard care [89-91].

In addition to lifestyle intervention, treatment with pharmacological agents, has been explored in obese children and adolescents. The following pharmacological agents have been evaluated in pediatrics [92, 93]: orlistat which is FDA approved for the treatment of obesity, sibutramine (withdrawn in 2010 because of safety reasons [94]), exenatide, and metformin, registered for treatment of T2DM from the age of ten years. According to a Cochrane systematic review, orlistat has shown an additional reducing effect on the absolute BMI in children and adolescents, yet medication related adverse effects such as gastro-intestinal tract symptoms were observed [85]. A review and meta-analysis on the effect of metformin in obese children and adolescents without T2DM concluded that metformin is moderately effective in reducing BMI and IR in hyperinsulinemic obese children and adolescents on short term use (6 months or less): a reduction in mean BMI of 1.42 kg/m^2 and homeostasis model assessment of insulin resistance (HOMA-IR) score by 2.01 [95]. Long-term data are limited to one study of 48 weeks, in which a reduction in BMI of 0.9 kg/m^2 was reported in participants receiving metformin versus an increase of 0.2 kg/m^2 in the placebo group [96].

Finally, the effect of bariatric surgery for severe obese adolescents (BMI of > 40 kg/m^2 or > 35 kg/m^2 with associated co-morbidities) has been studied. In three trials, chil-

dren who failed to achieve weight loss with non-invasive treatments were included. Bariatric surgery (most frequently laparoscopic sleeve gastrectomy) resulted in this selected populations in significant changes in BMI compared to lifestyle intervention groups, with a follow up of two to four years. Complications occurred in 4.1-4.4% of the patients [97-99]. Three-year follow up of adolescents who underwent bariatric surgery, showed improvement in weight, cardiometabolic health and weight-related quality of life. Complications included deficiencies in micronutrients [99].

Objectives of this thesis

Childhood obesity is an increasing problem, with IR as an important consequence. The role of IR in the development of T2DM is clear. Given the lack of a generally accepted definition of IR in children, the exact prevalence and incidence of IR are unclear. Therefore, in **chapter 2**, a literature review on the epidemiology of IR in population based studies in pediatric populations is presented.

In **chapter 3**, the variety in definitions for IR in paediatrics is further investigated. The aim of this study is to review all published definitions for IR in children and to apply these definitions to a population of patients with obesity from a pediatric outpatient clinic. The application of all definitions in this population demonstrates the large heterogeneity in definitions.

The clinical application of IR as a screening measure for children at risk of T2DM is investigated in **chapter 4.** In this study the recommended screening for T2DM using fasted plasma glucose (FPG) is compared to a screening combining FPG with a IR measurement, to investigate whether IR is useful as an additional screening to identify more children at risk for T2DM.

As the recommended screening interval for children at risk for T2DM is 3 years, in **chapter 5**, a follow up study is performed in children at risk for T2DM, to evaluate weight, insulin sensitivity, and progression to T2DM approximately 3 years after being diagnosed with overweight/obesity and IR.

In the second part of this thesis, the effect of long-term treatment with metformin in obese children with IR is presented. **Chapter 6a** presents the study protocol of the randomized controlled double-blind trial (RCT) in which the effect of long-term treatment metformin on BMI and IR was studied. In **Chapter 6b** the results of this RCT are presented.

Since treatment effects from clinical trials might differ from the effects in daily clinical practice, the results of the RCT described in **chapter 6,** are compared to the effects of metformin on BMI in daily clinical practice. The aim of **chapter 7** was to compare the effects of metformin (in addition to a lifestyle intervention programme) on change in

BMI between obese adolescents treated with metformin in daily clinical practice and patients who participated in the above mentioned RCT.

A summary of the conclusions of chapter 2-7 is presented in **chapter 8**. Finally, the future perspectives are discussed.

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Section 2

Prevalence, diagnosis and follow up of children with insulin resistance

Chapter 2

Population-based studies on the epidemiology of insulin resistance in children

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Abstract

Background

In view of the alarming incidence of obesity in children, insight into the epidemiology of the pre-diabetic state insulin resistance (IR) seems important. Therefore, the aim of this systematic review was to give an overview of all population-based studies reporting on the prevalence and incidence rates of IR in childhood.

Methods

PubMed, Embase and Cochrane library were searched in order to find all available population-based studies describing the epidemiology of IR in pediatric populations. Prevalence rates together with methods and cut-off values used to determine IR were extracted and summarized with weight- and sex-specific prevalence rates of IR if available

Results

Eighteen population-based studies were identified, describing prevalence rates varying between 3.1 and 44 %, partly explained by different definitions for IR. Overweight and obese children had higher prevalence rates than normal weight children. In seven out of thirteen studies reporting sex-specific results, girls seemed to be more affected than boys.

Conclusion

Prevalence rates of IR reported in children vary widely which is partly due to the variety of definitions used. Overweight and obese children had higher prevalence and girls were more insulin resistant than boys. Consensus on the definition for IR in children is needed to allow for comparisons between different studies.

Introduction

Nowadays, the body mass index (BMI) is increasing in many populations and childhood obesity is an emerging problem [1-3]. In the United States the prevalence rates of obesity between 1971 and 1974 in 6-11 year old white/black children was 4%. Between 1999 and 2002, these prevalence rates increased to 13% and 20% in white and black children, respectively [4]. In 2012 the overall prevalence rate of obesity in 2-19 year old American children was 17.3% [1]. In developing countries, the prevalence rate of overweight and obesity in preschool children (<5 years old) in 2010 was estimated to be 6.1% and 11.7%, respectively [5]. Moreover, the prevalence of overweight in children <5 years of age raised in the African continent between 2000 and 2013 from 5.1 to 6.2% (+1.1%), while in the American Continents, the prevalence increased with 0.5% (6.9 to 7.4%). (http://apps.who.int/gho/data/view.main.NUTWHOOVERWEIGHTv?lang=en)

The rising prevalence of obesity will cause an increase in obesity related complications such as insulin resistance (IR), hypertension, dyslipidemia and type 2 diabetes mellitus (T2DM) [6, 7]. The energy excess in obesity may result in hyperplasia and hypertrophy of adipocytes, leading to oxidative stress. This oxidative stress of adipocytes induces a chronic low-level inflammation in adipose tissue and production of adipokines, free fatty acids and inflammatory mediators. This inflammation is related to peripheral IR, IR of hepatocytes and impaired insulin secretion by the pancreatic beta-cells. Finally, this process causes dysregulation of glucose homeostasis and development of T2DM [8]. Although obesity plays a key-role in the pathophysiology of IR, IR is an independent risk factor for cardiovascular and metabolic diseases [9-12]. Therefore, it is important to know the extent of IR in pediatric populations. Knowledge on the prevalence rates of IR and its clinical consequences during childhood will increase the awareness of physicians and other health care professionals. Despite the reported association between IR and increased cardiovascular risk in pediatric populations [13], there is no overview of data on the epidemiology of IR in this population. Many studies focus on the extent of IR in overweight and obese populations, but limited studies have a population based study design.

The aim of this study is to systematically review all available population-based studies on the epidemiology of IR in pediatric populations. We will describe the weight and sex specific prevalence and incidence rates of IR in the included studies, together with the study-specific definition used to define IR.

Methods

Systematic search and study selection

This review follows the guidelines of 'Meta-analysis of Observational Studies in Epidemiology (MOOSE) [14]. A systematic search was conducted in PubMed, Embase and the Cochrane library, using the search strategies as displayed in table 1. The search was performed in December 2014, and covered all publications in the time period between the inception of each database and the search date. All articles in English, French, German, Spanish and Dutch languages were included and their title and abstract were screened to find the relevant studies. All results were imported into a RefWorks file (www.refworks.com) and duplicate articles were removed. Subsequently, the title and abstract of all unique results were screened using the exclusion criteria. Articles were excluded if they were review articles, studied a population older than 19 years, or did not report prevalence and/or incidence rates of IR in the abstract. Furthermore, all conference abstracts without a full text publication were excluded. All available full text articles were retrieved and their design was scrutinized to select population-based studies. The reference lists of all included population-based studies were investigated to find relevant articles not included in the original search.

Data extraction and analysis

Data were extracted on the study design, sample size, calendar time of data collection, mean age of participants, ethnicity, criteria used to determine IR (method and cut-off value), prevalence and incidence rates of IR in the complete study population, and if available in subpopulations based on weight category (normal weight, overweight and obesity) and sex. Data were entered in an excel file. Pooling of data was not possible because of the large variability in study design, population and definitions used to determine IR. Data are presented in a descriptive manner.

Table 1. Search strategies

Database	Search strategy
Pubmed	("Insulin Resistance"[Mesh] OR insulin resistan*[tiab] OR insulin sensitivity[tiab] OR (resistan*[tiab] AND insulin*[tiab]) OR metabolic syndr*[tiab]) AND ("Prevalence"[Mesh] OR prevalence*[tiab] OR "Incidence"[Mesh] OR incidence*[tiab]) AND ("Child"[Mesh:noexp] OR "Adolescent"[Mesh] OR "Puberty"[Mesh:noexp] OR "Minors"[Mesh] OR Pediatrics[MeSH:noexp] OR child[tiab] OR children[tiab] OR child care[tiab] OR childhood[tiab] OR child*[tiab] OR childc*[tiab] or childr*[tiab] OR childh*[tiab] OR adoles*[tiab] OR boy[tiab] OR boys[tiab] OR boyhood[tiab] OR girl[tiab] OR girls[tiab] OR girlhood[tiab] OR junior*[tiab] OR juvenile*[tiab] OR kid[tiab] OR kids[tiab] OR minors*[tiab] OR paediatr*[tiab] OR pediatr*[tiab] OR prepubert*[tiab] OR pre-pubert*[tiab] OR prepubesc*[tiab] OR pubert*[tiab] OR pubesc*[tiab] OR school age*[tiab] OR schoolchild*[tiab] OR teen[tiab] OR teens[tiab] OR teenage*[tiab] OR youngster*[tiab] OR youth[tiab] OR youths* OR Primary school*[tiab] OR Secondary school*[tiab] OR Elementary school*[tiab] OR High school*[tiab] OR Highschool*[tiab])
Embase	(prevalence/ or incidence/ or (prevalence* or incidence*).ti,ab.) AND (insulin resistance/ or insulin sensitivity/ or metabolic syndrome X/ or (resistan* and insulin*).ti,ab. or insulin sensitivity.ti,ab. or metabolic syndr*.ti,ab.) AND (child/ or boy/ or girl/ or hospitalized child/ or school child/ or exp adolescent/ or adolescence/ or puberty/ or pediatrics/ or (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth).ti,ab. or youths*.ti,ab. or Primary school*.ti,ab. or Secondary school*.ti,ab. or Elementary school*.ti,ab. or High school*.ti,ab. or Highschool*.ti,ab.)
Cochrane	((prevalence* or incidence*) and ((resistan* and insulin*) or insulin sensitivity or metabolic syndr*) and (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth or youths* or Primary school* or Secondary school* or Elementary school* or High school* or Highschool*)).ti,ab.

Results

Systematic search and study selection

With the search strategy presented in Table 1, in PubMed, Embase and Cochrane 6,788 articles (with 4,596 unique studies) were retrieved. Screening of titles and abstracts led to the exclusion of 4,448 articles (figure 1). The full text of the 148 remaining articles was checked and 76 articles were excluded based on our exclusion criteria. Critical appraisal of the 72 remaining articles resulted in the final inclusion of 18 population-

based studies. All included studies reported prevalence rates of IR and none of them reported incidence rates. An overview of the included studies and extracted data is presented in supplemental table 1.

Study characteristics

The 18 included studies were performed in 13 countries. Except for the African continent, all continents are represented. The studies were performed between 1999 and 2011. Sample sizes varied from 80 to 3,373 children [15, 16]. Most studies recruited their study population at selected schools [15, 17-30]. The New Zealand study population were volunteer adolescents who were recruited by Pacific Island community workers, even though it was not reported where they recruited the participants [16].

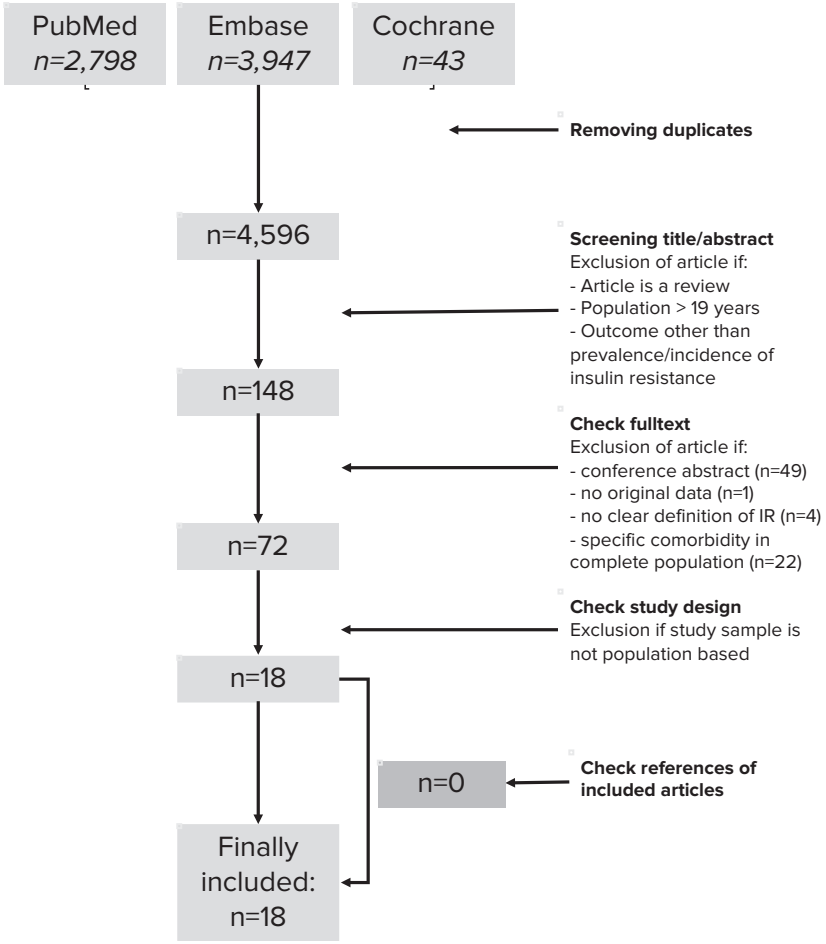

Figure 1. Flowchart of search and included studies

In the majority of the studies (n=14), the age of the study participants was above 10 years [16, 17, 19-24, 26-31]. Four studies included also children younger than 10 years, with ranges that varied between 6-19 years [15, 18, 25, 32]. Ethnicity was not reported in 50% of the studies. All study characteristics are presented in supplemental table 1.

Methods and cut-off values to define IR

In the studies, six different methods were used to determine IR (Table 2). These methods were Homeostasis Model Assessment Insulin Resistance (HOMA-IR), fasted plasma insulin (FPI), Quantitative Insulin sensitivity Check Index (QUICKI), fasted glucose/insulin ratio (FGIR), HOMA2 and the McAuley-index. All these indices are based on FPI; for HOMA-IR, QUICKI, FGIR and HOMA2 fasted plasma glucose (FPG) values are also needed (Table 2). The McAuley index is the only index for which fasted triglycerides are required besides FPG and FPI. None of the above-mentioned equations use anthropometric measurements or values derived from an oral glucose tolerance test. HOMA-IR, FPI and QUICKI were the most frequently used methods to determine IR (HOMA-IR: n=14 [15, 17-22, 24-28, 31, 32]; FPI: n=7 [16, 19, 21-23, 29, 30]; QUICKI n=2 [19, 26], Table 2).

The cut-off values used to define IR for HOMA-IR ranged from 2.1 to 5.56, while for FPI cut-off values varied between 9.85 and 23.7 µU/ml (corresponding with 68.4 and 164.8 pmol/l, respectively) (Table 2). The study of Budak et al. used a cut-off value different from the other studies, as their definition for IR was a HOMA-IR <3.16 which

Table 2. Methods used to calculate insulin resistance

Method	Parameters	Formula	Cut-off values (range)	Studies using the method
HOMA-IR	FPG, FPI	(FPG (mmol/l)*FPI(mU/l))/22.5	2.1 – 4.0	[15, 17-22, 24-28, 31, 32]
FPI	FPI	NA	9.85 – 23.7 µU/ml	[16, 19, 21-23, 29, 30]
QUICKI	FPG, FPI	1/[log (FPI (mU/l))+log (FPG (mg/dl))]	0.33 – 0.35	[19, 26]
FGIR	FPG, FPI	(FPG[mg/dL]/FPI [mU/L])	7	[26]
HOMA2	FPG, FPI	Computer model: HOMA2-calculator: http://www.dtu.ox.ac.uk/homa	2	[16]
McAuley-index	FPI, triglycerides	(2.63 − 0.28 ln[FPI] − 0.31 ln[fasting triglycerides])	6.3	[16]

FPG – Fasted Plasma Glucose; FPI – Fasted plasma insulin; FGIR – Fasted glucose insulin ratio; HOMA(-IR) – Homeostasis model assessment (for Insulin Resistance); QUICKI – Quantitative Insulin Sensitivity check Index)

was in contrast with other studies that defined IR as HOMA-IR greater than a specific value [20]. We did not succeed to contact Budak et al. to verify this cut-off value.

Age and sex specific cut-off values were reported in respectively one [29]. and three studies [22, 27, 29]. Girls had higher cut-off values for FPI and HOMA-IR compared with boys. For both sexes, adolescents aged 14-15 years had the highest cut-off values for FPI [29].

Prevalence of IR

The overall prevalence rates of IR in 17 out of 18 population based studies are presented in figure 2. The study of Ranjani et al. only reported sex specific prevalence rates [32]. The lowest prevalence rate of IR was reported from Greece with 3.1% in children aged 10-12 years (using the cut-off value of HOMA-IR > 3.16 for IR, figure 2) [26]. In the same study population, three other definitions of IR (HOMA-IR > 2.1, QUICKI < 0.35, and FGIR <7) were applied resulting in prevalence rates of 9.2, 12.8 and 17.4%, respectively.

The highest prevalence rate of IR was reported by Grant et al. for the 15-18 year old Pacific Island adolescents in New Zealand [16]. They reported a prevalence rate of 44% with IR defined as FPI > 12 μU/ml. This definition of IR has been used in another study by Bonneau et al. which resulted in a prevalence rate of 11.7% for the 12-18 year old Argentinian adolescents [19].

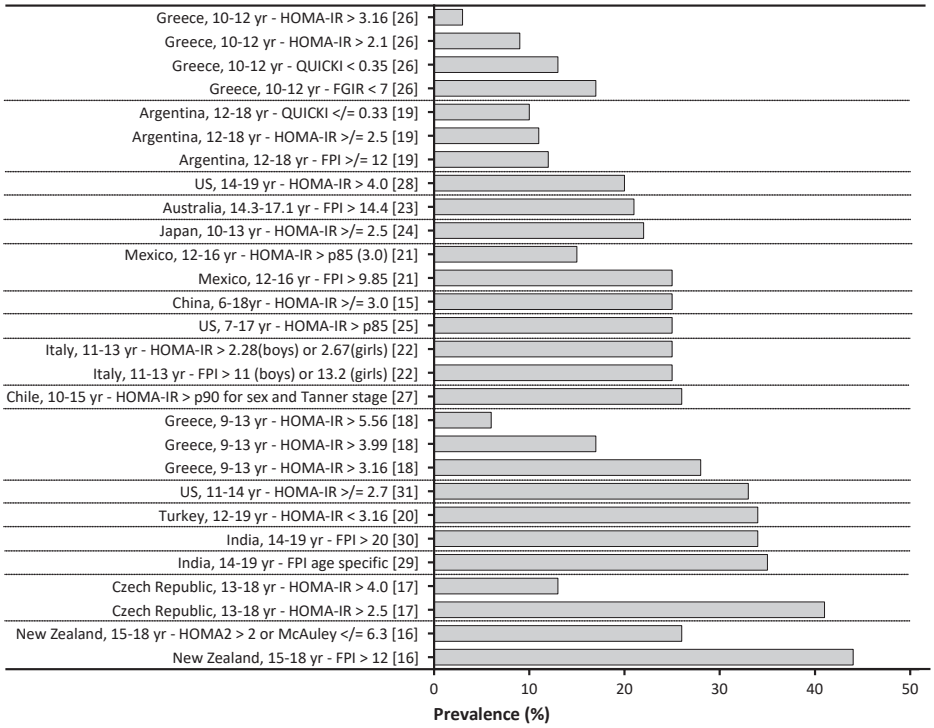

Figure 2. The overall prevalence rates (%) of IR in the included studies

Sex- and weight-specific prevalence of IR

Thirteen studies reported separate prevalence rates for boys and girls (figure 3a). In 7 out of 13 studies, IR was more prevalent in girls [16, 18, 20, 21, 23, 30, 32]. Three studies reported higher prevalence rates for boys [15, 17, 19]. In one study the prevalence rate of IR was similar for boys and girls [22]. In two studies it depended on the criteria used to determine IR whether boys or girls were having the highest prevalence rates [19, 26].

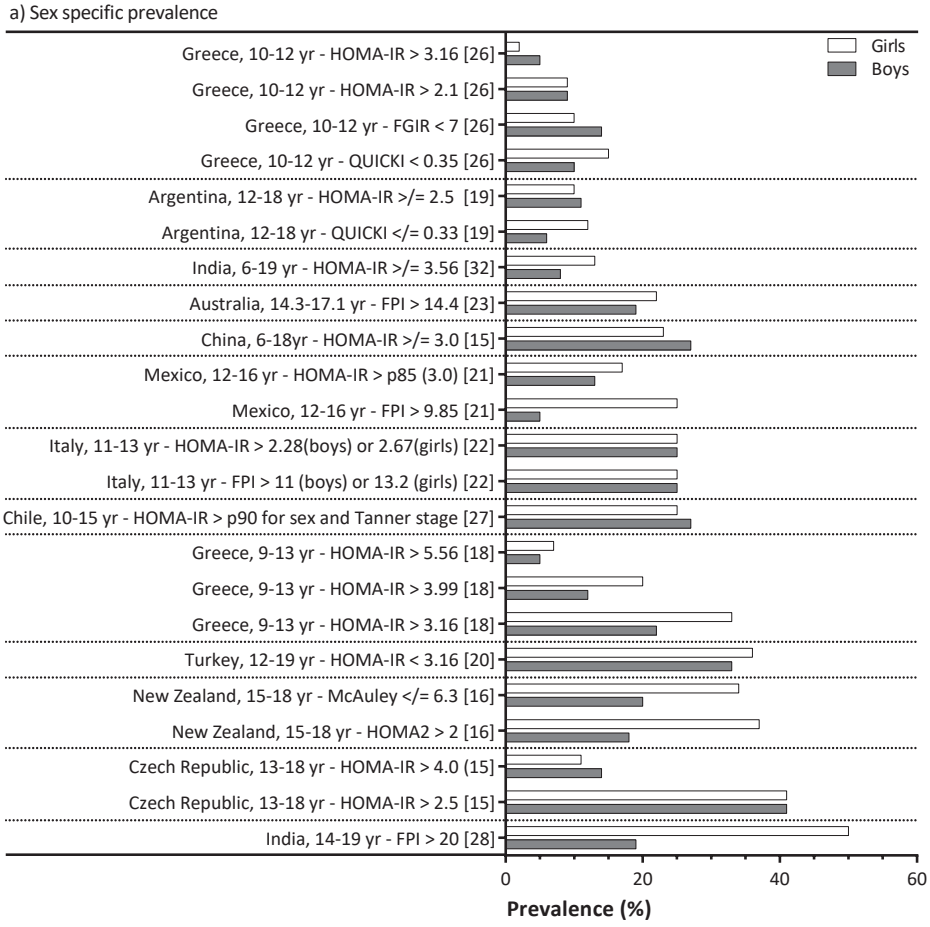

Figure 3b shows the influence of weight (normal, overweight and obesity) on the prevalence of IR. A major difference was observed between normal weight and obese populations. Normal weight populations had substantial lower prevalence rates of IR, irrespective of the used definition for IR. The maximum difference in weight specific prevalence rates of 61.3% was reported in Australian boys, with prevalence rates in normal weight and obese boys of 7.1% and 68.4%, respectively [23].

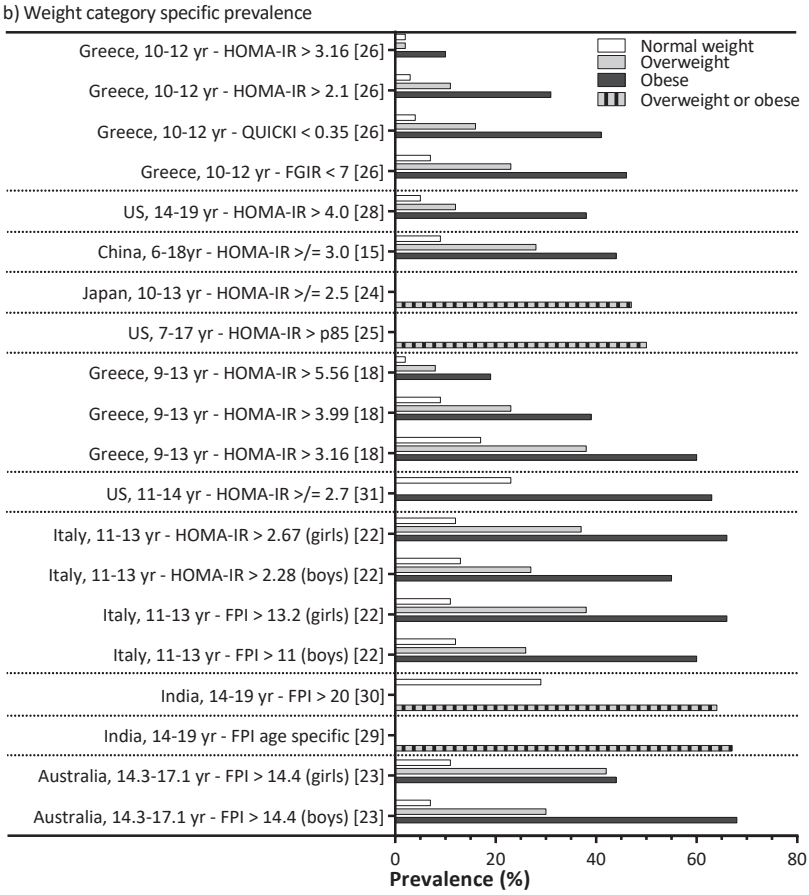

Figure 3. Prevalence of IR by sex (a) and weight category (b)

Discussion

To the best of our knowledge, this is the first systematic review summarizing all available population-based studies on the epidemiology of IR during childhood. While we could not find any population-based study reporting the incidence rate of IR in children, the reported prevalence rates varied between 3.1% in Greek children and 44% in Pacific Island teenagers living in New Zealand. There was not only variation in the prevalence rates of IR, but we also observed that these 18 included studies used 6 different methods combined with diverse cut-off values to determine IR. For instance, the FPI cut-off values varied between 9.85 and 23.7 μU/ml (corresponding with 68.4 and 164.8 pmol/l, respectively) [21, 29] and the HOMA-IR cut-off values ranged between 2.1

and 5.56 [18, 26]. The lack of a uniform definition and cut-off value to determine IR, impedes pooling of data and therefore reporting on overall prevalence rates.

Although substantial variation in the prevalence rates of IR could be partly explained by differences in the study population characteristics (e.g. age, weight, ethnicity, pubertal status, etc.), the use of different methods and cut-off values to determine IR may play an important role as well. As an example, in the study by Manios et al. in 481 Greek school children, different methods resulted in various prevalence rates (i.e. 3.1 versus 12.8 and 17.4 % for HOMA-IR, QUICKI and FGIR, respectively, Figure 2) [26]. Even if studies use the same method to measure IR, different cut-off values impede comparison between studies. Again, in the study by Manios et al., the use of different cut-off values for HOMA-IR method (> 3.16 and > 2.1) in the same study population resulted in prevalence rates of 3.1 and 9.2%, respectively [26]. A lower cut-off value results in a higher prevalence rate of IR and vice versa.

The highest reported prevalence rate for IR was 44% in Pacific Island teenagers (New Zealand) [16]. In that study IR was defined as FPI > 12 μU/ml, which is a relatively low cut-off value that might contribute to the high reported prevalence rate. In another study in Mexico, which used the lowest cut-off value for FPI (FPI > 9.85mU/l) a prevalence rate of 24.8% was reported [21]. When the same cut-off values would have been used in these two studies, the difference in prevalence rates would even have been larger. Even though the difference between these two populations cannot be quantified precisely, not only because of different cut off values, but also because others factors such as age, weight and pubertal stage were not taken into account, this analysis shows that prevalence rates of IR are variable in different populations, which was also observed in other studies.

Overweight or obesity is an important factor influencing the prevalence of IR. The effect of overweight or obesity on IR is clearly observed in all presented studies as prevalence rates in overweight or obese children and adolescents were reported to be higher than in normal weight children and adolescents (Figure 3b). Most studies (7 out 11 studies presenting weight specific prevalence rates) differentiated not only between normal weight and overweight/obesity, but stratified into normal weight, overweight and obese children and adolescents [15, 18, 22, 23, 26, 28, 31]. These studies show an increased prevalence in obese children compared to overweight children. In the study by Caserta et al., odds ratios for IR were calculated for obese and overweight boys and girls comparing to their normal weight peers. The odds ratios of 9.1 (95% confidence interval 4.0-20.4) and 13.2 (4.7-36.9) were reported for obese boys and girls and lower odds ratios of 2.4 (1.2-4.9) and 6.0 (3.1-11.9) were reported for overweight boys and girls, respectively [22]. These results show that with normal weight increasing to obesity the prevalence of IR is rising.

A higher prevalence rate of IR has been observed in girls compared with boys in 7 out of 13 studies reporting sex specific prevalence rates (Figure 3a) [16, 18, 20, 21, 23, 30, 32]. This is in line with the prevalence of T2DM, of which IR is a precursor, as population based studies on the prevalence of T2DM in children and adolescents also show higher prevalence rates in girls [33]. Hirschler et al. found no significant sex-related differences in IR. In their study, IR was associated with BMI and pubertal stage only, and not with gender. Their findings suggested that higher values in IR in girls compared to boys could be due to differences in pubertal development [34]. A study by Moran et al. measured IR using the euglycemic insulin clamp in children at all Tanner stages. At all Tanner stages, girls were more insulin resistant compared to boys. According to Moran et al, this difference in IR between boys and girls could partially be explained by higher levels of adipose tissue in girls compared to boys. However, in an obese subpopulation no difference in IR levels was observed between boys and girls [35]. It is known that pubertal development starts earlier in girls compared to boys (Tanner stage 2 at 11.4-11.9 years vs 11.9-12.3 years, respectively) [36]. Therefore, boys and girls between 10 and 14 years of age might be at another Tanner stage. Since IR is related to pubertal stage [34, 37], a comparison between pubertal girls and boys of same age might result in a higher prevalence rate for IR in girls, because of a higher Tanner stage. The best comparison between boys and girls in pubertal age, would be based on Tanner stages instead of age. Unfortunately, prevalence rates related to Tanner stages were not reported in any of the studies, so we were not able to check the effect of puberty on the prevalence of IR.

Our review has some limitations that should be addressed. At first, we could not compare results and pool the data of different studies, because of the heterogeneity in definition of IR in the presented studies. However, we were able to present an overview of the currently available population based studies, showing higher prevalence rates in girls compared to boys, and in overweight and obese children compared to normal weight children. Another limitation is that all included studies were conducted in recent years. All studies were published between 2004 and 2014 and the data were collected between 2000 and 2011. However, in eight of eighteen studies, the exact period of data collection was not mentioned [15-17, 20, 21, 29, 31, 32]. Therefore, we could not evaluate whether the prevalence of IR is rising along with the increasing prevalence of obesity and T2DM. Finally, as already discussed above, the influence of Tanner stage on prevalence of IR could not be studied because of a lack of data.

Conclusion

In conclusion, the overall prevalence rates of ir in population-based studies of children and adolescents ranged between 3.1 and 44%, which could be partly explained by the use of different methods and cut-off values to determine ir. The prevalence rate of ir was up to 68.4% in obese boys. Girls seemed to have higher prevalence rates of ir than boys, which may however be related to their earlier pubertal development. Consensus on the definition for ir in children is needed to allow for comparisons between different studies, and to assess the value of ir as a screening measure for children and adolescents with an increased risk of cardiometabolic diseases.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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Supplemental table 1. Overview of the included studies and extracted data

Nr	Country, calender time	Methods	Sample representative for / ethnicity	Sample size	Participation degree	Age	Measurements to determine IR	Criteria IR	Prevalence IR (%)					
									Overall	Normal weight	Over-weight	Obese	Boys	Girls
ASIA														
1	China, NR	Cross-sectional population based survey	Chinese children living in Beijing / Chinese	3373	NR	6-18	Fasted blood sample (FPG and FPI)	HOMA-IR ≥ 3,0	25*	8.9*	28.1*	43.8*	26.9*	23.0*
2	India, NR	Randomly selected sample of population based study (Epidemiological Study of Adolescents and Young adults (ESAY))	Adolescents (14-19 yr) in New Delhi region. / Asian Indian	948	NR	14-19	Fasted blood sample (FPI)	14-15 yr: ♂FPI > 128.5 pmol/l, ♀ >164.8 pmol/l; 16-17yr: ♂FPI > 126.1 pmol/l, ♀ >152.6 pmol/l; 18-19 yr: ♂FPI > 121.2 pmol/l, ♀ >162.4 pmol/l	35.4		67.3			
3	India, NR	Door-to-door demographic survey of representive wards of Chennai city.	Children and adolescents in urban South-India / Asian Indian	1519	74%	6-19	Fasted blood sample (FPG and FPI)	HOMA-IR ≥ 3.56					7.8	12.5
4	India, 2000-2003	Randomly selected sample of population based study (ESAY)	Adolescents (14-19 yr) in New Delhi region / Asian Indian	793	NR	14-19	Fasted blood sample (FPI)	FPI> 20 µU/ml	34.2*	29	63.9		18.9*	49.7*
5	Japan, 2009	Cross-sectional study of standard health examination with additional blood sampling in schoolchildren in 5th and 8th grade	Children and adolescents (10-13 yr) in region of Nagano Prefecture / Japanese	310	99,0%	10-13	Fasted blood sample (FPG and FPI)	HOMA-IR ≥ 2.5	21.6		46.8			
AUSTRALIA														
6	Australia, 2004	Cross-sectional population survey of Grade 10 students in NSW School Physical Activity and Nutrition Survey 2004 (SPANS 2004)	Grade 10 students in Sydney metropolitan area (population 4.2 million) / NR	495	NR	14.3-17.1	Fasted blood sample (FPI)	FPI > 100pmol/l	20.6*	♂ 7.1 ♀ 10.9	♂ 29.5 ♀ 41.9	♂ 68.4 ♀ 44.4	19.3	22.4
7	New Zealand, NR	Observational study of pacific island teenagers living in New Zealand, recruited by PI community workers	PI teenagers living in the community in Dunedin, New Zealand / Pacific Island [a]	80	83.0%	15-18	Fasted blood sample (FPG, FPI and fasted triglycerides)	FPI > 12 µIU/ml HOMA2 > 2 or McAuley index ≤ 6.3	44 26.9				17.5 20.0	36.8 34.2

Supplemental table 1. Overview of the included studies and extracted data (continued)

Nr	Country, calender time	Methods	Sample representative for / ethnicity	Sample size	Participation degree	Age	Measurements to determine IR	Criteria IR	Prevalence IR (%)					
									Overall	Normal weight	Over-weight	Obese	Boys	Girls
CARIBBEAN AND CENTRAL AMERICA														
8	Mexico, NR	Cross-sectional survey in subjects randomly selected from public schools in Mexico-City	Adolescents 12-16 yr in Mexico-City / NR	1850	40.1%	12-16	Fasted blood sample (FPG and FPI)	FPI > 9.85 µIU/ml (p75)	24.8*				4.9*	24.7*
								HOMA-IR > p85 (~3.0)	15.3 *				12.7*	17.2*
EUROPE														
9	Czech Republic, NR	Cross-sectional study of a general population cohort	Czech adolescents 13.0-17.9 yr /NR	1518	NR	13.0-17.9	Fasted blood sample (FPG and FPI)	HOMA-IR > 2.5	40.7 *				40.9	40.5
								HOMA-IR > 4.0	13.2 *				14.3	10.8
10	Greece, 2007	Large scale, cross-sectional epidemiological study	Greek schoolchildren 9-13yr / NR	2026	64.1%	9-13	Fasted blood sample (FPG and FPI)	HOMA-IR > 3.16	28.4	16.7	38.0	59.6	22.4	33.2
								HOMA-IR > 3.99	16.6	8.5	22.8	39.1	12.2	20.0
								HOMA-IR > 5.56	6.0	2.4	8.0	19.1	4.5	7.4
11	Greece, 2005-2006	Observational population based study on school children in Crete	Adolescents 10-12yr on Crete / NR	248	NR	10-12	Fasted blood sample (FPG and FPI)	HOMA-IR >2.1	9.2	2.9	10.5	31.0	9.20	9.17
								HOMA-IR > 3.16	3.1	1.9	1.8	10.3	4.60	1.83
								QUICKI < 0.35	12.8	3.9	16.2	41.4	10.34	14.68
								FGIR < 7	17.4	6.8	22.8	45.9	13.79	20.18
12	Italy, 2007-2008	Cross-sectional study of children randomly selected from schools	Adolescents 11-13yr in Southern Italy / NR	575	68.2%	11-13	Fasted blood sample (FPG and FPI)	FPI > p75 (♂ 11.0 pmol/l; ♀ 13.2 mol/l) [b]	25.2 *	♂ 12.4 ♀ 11.2	♂ 25.6 ♀ 38.2	♂ 60.4 ♀ 65.5	25.3*	25.1*
								HOMA-IR > p75 (♂ 2.28, ♀ 2.67) [b]	25.0 *	♂ 13.1 ♀ 11.8	♂ 26.7 ♀ 37.1	♂ 54.7 ♀ 65.5	25.0*	25.1*
13	Turkey, NR	Cross-sectional survey in randomly selected school children, schools stratified for geographical location and socioeconomic levels.	Turkish adolescents 12-19yr living in region of Kayseri / NR	790	97.3%	12-19	Fasted blood sample (FPG and FPI)	HOMA-IR <3.16 [c]	34.7				33.0	36.1

Supplemental table 1. Overview of the included studies and extracted data (continued)

Nr	Country, calender time	Methods	Sample representative for / ethnicity	Sample size	Participation degree	Age	Measurements to determine IR	Criteria IR	Prevalence IR (%)					
									Overall	Normal weight	Over-weight	Obese	Boys	Girls
NORTH-AMERICA														
14	U.S., 2008-2011	Cross-sectional convenience sample of school-based medical screening	Children attending public high school in New York / Hispanic, African-American, other	1185	63%	14-19	Fasted blood sample (FPG and FPI)	HOMA-IR > 4	19.5	4.5	12.4	37.8		
15	U.S., NR	Observational cross-sectional study in middle-school students (TEAMS project)	Adolescents 11-14 yr in inland Pacific Northwest of US / NR [d]	151	NR	11-14	Fasted blood sample (FPG and FPI)	HOMA-IR ≥ 2.7	33.5 *	22.7	62.5			
16	U.S., 2002-2003	Cross-sectional observational study among schoolchildren of schools in Wausau school district	Children and adolescents in urban region of Wausau, U.S. / 13% minority students	535	NR	7-17	Fasted blood sample (FPG and FPI)	HOMA-IR > p85 of normal weight children (BMI <p85) [e]	~25 [e]		50			
SOUTH-AMERICA														
17	Argentina, 2005	Descriptive study of high-school students in the city of Posadas (total amount of adolescents 12-18yr: 35,000)	Adolescents in urban region of Posadas, Argentina / NR	420	60%	12-18	Fasted blood sample (FPG and FPI)	FPI ≥ 12 mU/l	11.7					
								HOMA-IR ≥ 2.5	10.5				10.6	10.4
								QUICKI ≤ 0.33	9.8				6.3	11.9
18	Chile, 2009-2011	Cross-sectional study of children in public schools in Puente Alto County	Chilean children / Chilean	3325	59.2%	10-15	Fasted blood sample (FPG and FPI)	HOMA-IR > p90 for sex and Tanner stage (♂ Tanner I and II: 3.2; Tanner III and IV: 4.2; ♀ Tanner I and II 4.1, Tanner III and IV: 5.0)	25.9				26.9	24.8

Abbreviations: FPG: fasted plasma glucose; FPI: fasted plasma insulin; HOMA-IR: Homeostasis Model assessment Insulin resistance; MA: Mexican-American; NR: not reported; QUICKI: quantitative insulin sensitivity check index

Calculations: HOMA-IR = FPG (mmol/l)*FPI (mU/l) / 22,5 or FPG (mg/dl)*FPI (mU/l) / 405; HOMA2: calculated with HOMA2calculator http://www.dtu.ox.ac.uk/homacalculator/index.php; McAuley index= 2.63 -0.28 ln[fasting insulin] -0.31 ln[fasting triglycerides]; QUICKI= 1/ [log (FPI (mU/l))+ log (FPG (mg/dl))]

Notes: * Calculated by the authors; † extracted from graph. [a] 40% of sample is overweight, 36% is obese; [b] Cut-off point for FPI and HOMA-IR based on p75 of this population; [c] Cut-off point <3.16, while all other studies that use HOMA-IR define IR as HOMA-IR > (...); [d] > 27% of participants BMI > p95; [e] Specific cut-off value for IR not mentioned in the article. Specific prevalence also not mentioned.

REFERENCES SUPPLEMENTARY MATERIAL CHAPTER 2

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Chapter 3

Definition of Insulin Resistance affects prevalence rate in pediatric patients; A systematic review and call for consensus

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Abstract

Background

As a result of the rising prevalence of childhood obesity, there is an increasing interest in the type 2 diabetes mellitus precursor insulin resistance (IR). The aim of this study is to review definitions (methods and cut-off values) to define IR in children and to apply these definitions to a previously described obese pediatric population.

Methods

A systematic literature review on prevalence and/or incidence rates in children was performed. The extracted definitions were applied to an obese pediatric population.

Results

In the 103 identified articles, 146 IR definitions were reported based on 14 different methods. Fasted definitions were used 137 times, whereas oral/intravenous glucose tolerance test derived methods were used 9 times. The homeostasis model for the assessment of insulin resistance (HOMA-IR) and fasted plasma insulin (FPI) were the most frequently used fasted methods (83 and 37 times, respectively). A wide range in cut-off values to define IR was observed, resulting in prevalence rates in the pre-defined obese pediatric population between 5.5% (FPI > 30 mU/l) and 72.3% (Insulin Sensitivity $Index_{Matsuda} \leq 7.2$).

Conclusions

To compare IR incidence and prevalence rates in pediatric populations, a uniform definition of IR should be defined.

Introduction

As the prevalence of childhood obesity and consequently type 2 diabetes mellitus (T2DM) is rising [1-3], there is an increasing interest in Insulin Resistance (IR) as a well-known precursor and risk factor for T2DM [4-7]. The recognition of IR in (obese) children and adolescents at risk for T2DM is important in order to implement preventive measures for T2DM, since T2DM causes major health care costs and burden for the patient [8-11]. Early prevention by recognising IR is therefore important.

The gold standard to determine IR is the euglycemic-hyperinsulinemic clamp study [12,13]. The euglycemic-hyperinsulinemic clamp study measures the glucose uptake, while the subject receives exogenous insulin, resulting in a hyperinsulinemic state. Subjects who are sensitive for insulin will require higher amount of glucose infusion than subjects who are less sensitive for insulin (insulin resistant) to remain euglycemic. This technique requires infusion of both insulin and glucose, and frequent blood sampling to control the hyperinsulinemic and euglycemic state, which is a large burden for the patients. Moreover, expertise in managing the glucose and insulin infusions is essential in order to guarantee patients safety and reliable test results. Because of this invasive and time consuming character, the euglycemic-hyperinsulinemic clamp study is not standard of care in pediatric patients [13].

As alternatives, many less invasive methods have been developed to establish IR in daily clinical practice [14-17]. These methods vary in terms of parameters that are needed to calculate IR and in invasiveness. Some methods are based on measurements in fasted blood samples, whereas others require measurements derived from an oral glucose tolerance test (OGTT), which is used in daily practice or a (frequently sampled) intravenous glucose tolerance test ((FS)IVGTT), which is not suitable for daily practice. Most frequently used methods based on fasted blood samples are the homeostasis model for the assessment of insulin resistance (HOMA-IR), the quantitative insulin-sensitivity check index (QUICKI) and the fasted glucose/insulin ratio (FGIR). The use of fasted plasma insulin as measure for IR has been described frequently as well [18]. Most often used methods based on OGTT or (FS)IVGTT are the Insulin sensitivity indexes of Cederholm, Belfiore or Stumvoll (based on OGTT) or the Minimal model analysis of frequently sampled intravenous glucose tolerance test [13,19].

However, there seems no consensus yet on which method and cut-off value is the preferable one [12,18,20]. Therefore, all methods are being used concurrently, which impedes comparison of incidence and prevalence rates of IR between populations and countries and to study these rates over time. Therefore, the aim of this study is to review the different methods and definitions of IR as used to estimate prevalence rates of IR in pediatric populations. First, we present an overview of the definitions and cut-off values used to determine IR in publications describing the prevalence of IR in

children and adolescents. Secondly, to illustrate the impact of the definition on the prevalence of IR, we calculated the prevalence of IR using the different definitions in a previously described population of obese children and adolescents from a pediatric obesity outpatient clinic [21].

Methods

Systematic review of definitions of IR

A systematic review of available literature in The Cochrane library, PubMed and Embase was performed in December 2014. The search strategy is displayed in Appendix 1. After importing the results into Refworks (www.refworks.com) and removing duplicates, abstracts were screened for title and abstract. The exclusion criteria were: language (other than English, French, German, Spanish or Dutch); review articles; study population > 19 years of age; and the lack of reporting on the prevalence of IR in the aim or results part of the abstract. Publications were checked for full text availability. Conference abstracts without a full text publication were excluded, as well as articles not clearly describing a definition for IR. From the articles that fulfilled the criteria, methods defining IR (including mathematical formula), parameters used in the method and the used cut-off values were extracted.

Application of reported definitions to a previously described population of obese children

The definitions reported in the above-described publications were applied to a previously reported population of 311 obese children and adolescents from a pediatric obesity outpatient clinic [21]. As part of standard of care, all these children underwent an OGTT. Data were collected retrospectively. Collected data were anthropometric measurements, fasted plasma glucose (FPG), fasted plasma insulin (FPI) and 2-hour plasma glucose measured during an OGTT. A detailed description of the data collection is provided in a previously published study [21]. The characteristics of the population of obese children are displayed in table 1.

If the same cut-off values were reported in different studies as less or greater than (< or >) and less or greater than or equal to (≤ or ≥), we only calculated the prevalence of IR with the definition using less or greater than (< or >).

Table 1. Characteristics of the population of obese children visiting a pediatric obesity outpatient clinic between January 2006 and December 2009 (n=311) [21]

	Mean	Range
Age	10.83 (3.20)	2.4 – 17.7
Male (%)	50.5	-
Height, cm	149.4 (18.5)	90.5 – 185.8
Weight, kg	66.7 (25.9)	20.7 – 153.9
BMI, kg/m^2	28.71 (5.23)	20.24 – 47.83
BMI-SDS	2.93 (.49)	2.31 – 5.52
FPG, mmol/l	5.0 (0.5)	3.4 – 8.6
FPI, µU/l	12.7 (10.0)	2 – 61
2hr-PG, mmol/l	6.4 (1.5)	3.3 – 20.3
T2DM, n (%)	5 (1.6)	-

Abbreviations: BMI – Body mass index; BMI-SDS – Body mass index standard deviation score

Data analysis

IBM-SPSS version 21.0 was used to calculate IR according to the different definitions, and to calculate the percentage of the population being insulin resistant according to the different definitions.

Results

Searching the three databases yielded 4.596 unique results. Screening of title and abstract led to exclusion of 4430 articles. Of the remaining 166 articles, 103 articles could be included for data extraction (figure 1). Study characteristics of all included studies are summarized in Supplementary Table 1.

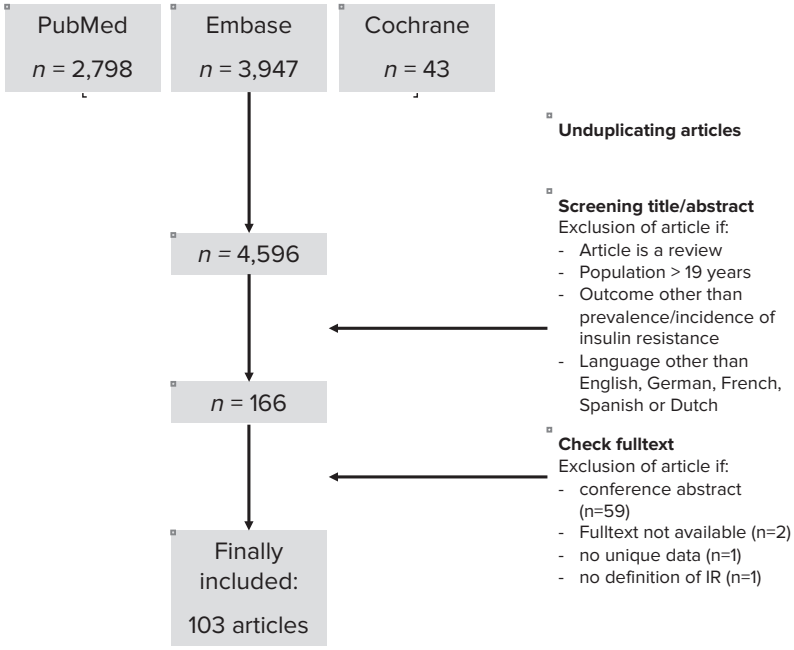

Figure 1. Flowchart of literature search

Methods to determine IR

Table 2 gives an overview of the reported methods to determine IR extracted from the 103 articles. These articles were reporting on 146 definitions. Fasted definitions were used 137 times, whereas OGTT/IVGTT derived methods were used 9 times.

Overall we identified 14 methods to determine IR. Seven (50%) methods are based on parameters derived exclusively from fasted blood samples, the other seven use parameters of fasted blood samples combined with parameters obtained from an OGTT or IVGTT. Out of the fasted methods, HOMA-IR and FPI were the most frequently used methods to determine IR: these were reported 83 and 37 times, respectively. The other five fasted methods were each used one to nine times, and the seven OGTT/IVGTT based methods were used one or two times.

FPI was used as parameter in 11 out of 14 methods. In two methods, the insulin concentration derived from the OGTT was used; one definition defined IR based on the insulin value after 120 minutes and the other method used the maximum concentration during the OGTT. The only method not using insulin was the definition based on C-peptide (Table 2).

Table 2. Overview of reported methods and range of used cut-off values to determine IR in children.

Method	Parameters	Formula	Range of used cut-off values	Number of studies using method*
Based on fasted samples				
HOMA-IR	FPG, FPI	(FPG (mmol/l)*FPI(mU/l))/22.5	> 1.14 – 5.56	83
FPI	FPI	NA	7.34 – 30 mU/l	37
QUICKI	FPG, FPI	1/[log (FPI (mU/ml))+log (FPG (mg/dl))]	0.300 – 0.360	9
FGIR	FPG, FPI	(FPG[mg/dL]/FPI [mIU/L])	< 6 - 7	4
HOMA2	FPG, FPI	Computer model: HOMA2-calculator: http://www.dtu.ox.ac.uk/homa	> 1.53 - 2	2
McAuley-index	FPI, triglycerides	(2.63 − 0.28 ln[FPI] − 0.31 ln[fasted triglycerides])	≤ 6.3	1
C-peptide	C-peptide	NA	≥4.4 ng/ml	1
Based OGTT/IVGTT derived samples				
Insulin during OGTT	Insulin at 120’	NA	>45-75 mU/l	2
OGIS	Glucose at 0’, 90’ and 120’. Insulin at 0’ and 90’	Webcalculator: http://webmet.pd.cnr.it/ogis/ogis.php	<400 -436	2
Maximum insulin during OGTT	Insulin max	NA	>150 mU/l	1
$ISI_{Matsuda}$	FPG, FPI, Glucose and insulin during OGTT at 30’, 60’, 90’ and 120’	10.000/ √((FPG (mg/dl) × FPI (µU/ml) × (Mean OGTT Glucose (mg/dl) × Mean OGTT Insulin (mU/l))	≤ 7.2	1
Si(IVGTT)	Glucose and insulin during IVGTT at -5’, -1’, 2’, 4’, 8’, 10’, 19’, 22’, 30’, 40’, 50’, 60’, 70’, 90’, 180’ and 240’.	Computerized model, using the program MINMOD.[19]	$4.5x10^4$ µU/ml/min	1
$IRI_{Belfiore}$	Glucose and insulin during OGTT at 0’, 60’ and 120’.	2/[[1/(GLYp x INSp)]+1]	> 1.27	1
Σ insulin during OGTT	Insulin during OGTT at 0’, 30’, 60’, 90’ and 120’	$Insulin_0$ + $insulin_{30}$ + $insulin_{60}$ + $insulin_{90}$ + $insulin_{120}$	> 300 µU/ml	1

* Some studies used more than one definition.

Abbreviations: *FGIR – Fasted glucose to insulin ratio; FPG – fasted plasma glucose; FPI – Fasted plasma insulin; HOMA(-IR) – Homeostasis Model Assessment (for Insulin Resistance); IRI – insulin resistance index; ISI – Insulin sensitivity index; NA – not applicable; OGIS – oral glucose insulin sensitivity; OGTT – oral glucose tolerance test; Si(IVGTT) – insulin sensitivity from intravenous glucose tolerance test; QUICKI – quantitative insulin-sensitivity check index.*

Cut off values

Table 2 provides for each of the methods to determine IR, the range in reported cut-off values. For the fasted methods, typically wide ranges in cut-off values were observed: for the commonly used method HOMA-IR, cut-off values ranged from 1.14 to 5.56. The same was observed for FPI with cut-off values ranging from 7.34 to 30 mU/l. In the less frequently used OGTT derived methods, a wide range in cut-off values was reported as well: for insulin at 120 minutes during the OGTT this range varied between 45-75 mU/l (Table 2).

In addition, some studies used separate cut-off values for boys and girls, for example for HOMA-IR 2.28 and 2.67, respectively, and for prepubertal and pubertal children, for example QUICKI <0.33 for prepubertal and <0.36 for pubertal children.

Application of definitions for IR to a population of obese children and adolescents

Figure 2 shows the results of the application of the different definitions to the available clinical data of a population of 311 obese children and adolescents from our pediatric obesity outpatient clinic [21].

All fasted methods except C-peptide could be applied as well as prevalence rates based on different cut-off values per pubertal stage. For the OGTT/IVGTT based methods, results of Si(IVGTT)and $IRI_{Belfiore}$ could not be presented from the available data.

Comparing the prevalence rates of definitions based on fasted blood samples only, the lowest prevalence was 5.5% (FPI > 30 mU/l) and the highest prevalence was 64.0% (FPI > 7.34 mU/l). For the definitions based on OGTT/IVGTT derived values, the lowest prevalence was 18.8%, based on oral glucose insulin sensitivity (OGIS) < 400, and the highest prevalence was 72.3% ($ISI_{Matsuda} \leq 7.2$).

For the HOMA-IR and the QUICKI, the range in prevalence due to the variation in cut-off values was 10.0-62.0% and 10.9-65% respectively. For FPI this range was even wider: 5.5-64.0%. For the OGTT derived definition based on insulin at 120' the prevalence rates were 34.5-63.2%.

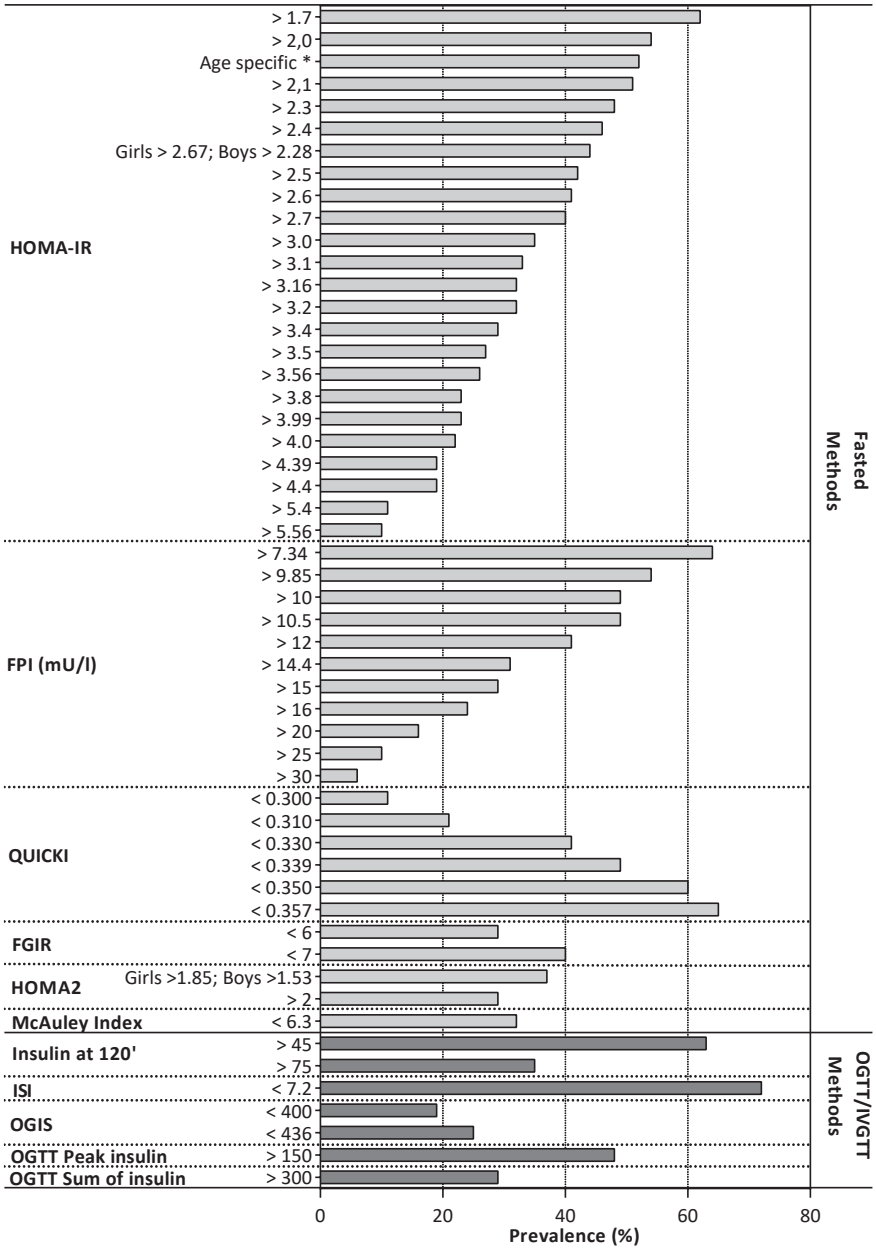

Figure 2. Prevalence of IR in a pediatric population visiting an obesity outpatient clinic (n=311) using different methods and cut-off values of IR.

Abbreviations: *FGIR – Fasted glucose to insulin ratio; FPI – Fasted plasma insulin; HOMA(-IR) – Homeostasis Model Assessment (for Insulin Resistance); ISI – Insulin sensitivity index; OGIS – oral glucose insulin sensitivity; OGTT – oral glucose tolerance test; QUICKI – quantitative insulin-sensitivity check index.*

** Age-specific cut-off values for HOMA-IR: 2-5 yr > 1.14; 5.1-10 yr > 1.67 ; 10.1-15 yr > 2.53; 15.1-19yr > 2.52*

Discussion

The current review of the pediatric literature shows that many different methods and cut-off values are used to determine IR in children and adolescents. The impact of these different definitions on prevalence rates is demonstrated by applying the various definitions to a given dataset of obese children and adolescents, which resulted in a wide range of prevalence rates (i.e. 5.5 – 72.3%). This finding emphasizes the need for a standard definition to be able to compare incidence and prevalence rates of IR between populations and countries and particularly to study these rates over time.

The gold standard test for IR is the euglycemic-hyperinsulinemic clamp. However, this test is not useful for screening purposes in clinical practice because of the expertise needed to perform the test on one hand, and the invasive and time-consuming character of the test, resulting in high burden for the patient on the other hand. As a result, the euglycemic-hyperinsulinemic clamp is only used in experimental settings. Due to the invasive character of the gold standard test for IR, many surrogate methods have been developed. Different studies have been performed to determine the correlations of the methods with the euglycemic-hyperinsulinemic clamp. However, most of these studies were performed in adults, and few of them in pediatric populations. In pediatric populations, the methods based on fasted blood samples, i.e. HOMA-IR, QUICKI and FGIR, have moderate to strong correlations with IR assessed with the euglycemic-hyperinsulinemic clamp, respectively 0.51-0.81, 0.43-0.91 and 0.25-0.92 [6,12,13,22-24]. For the OGTT derived methods, the $ISI_{Matsuda}$ index has a moderate to good correlation as well (0.74-0.78). No data are available for the correlation between the euglycemic-hyperinsulinenic clamp and the $IRI_{Belfiore}$ index in pediatric populations [13]. Since all indices have moderate to good correlations, this criterion does not distinguish in which method would be the best to use.

The optimal test to define IR in children and adolescents should be in our opinion minimally invasive and pose a minimal burden to the child, in order to be widely applicable in the growing population of obese children and adolescents. Therefore, methods based on fasted blood samples have an advantage over methods using blood samples obtained during an OGTT or IVGTT. Although an OGTT or IVGTT is less invasive than the euglycemic-hyperinsulinemic clamp, repetitive vena punctures or a venous cannula over 120 minutes are necessary for collecting blood samples, while fasted methods only require one vena puncture to collect the blood sample.

Another criterion for the preferred method is the reproducibility. The test has to be reliable in repeated measurements, as it will be used for the follow up of children with IR. As described previously, many studies in pediatric populations focus on the correlation of surrogate methods with a gold standard test, unfortunately they do not describe the reproducibility. The available data for reproducibility for the methods to

determine IR are from adult studies. Henriquez et al studied in 78 adults without T2DM the reproducibility of HOMA-IR, QUICKI and FPI. Fasted blood samples were taken twice from each participant within 30 minutes on the same day. This resulted in a coefficient of variation (CV) for HOMA-IR of 11.8% (7.8-11.9), for QUICKI 1.8% (1.1 – 2.9) and for FPI 13.4% (8.8 – 21.9) [25]. The low CV reported for the QUICKI was however debated by Antuna et al. because this measure is composed of log transformed values of FPG and FPI [26]. When the CV of log transformed HOMA-IR values are compared to the CV of the QUICKI, similar, low CV's were found for both measures. Since all of these formulas are based on the same measurements of glucose and insulin, the CV is not discriminating between HOMA-IR and QUICKI either.

Finally, the method should preferably be easy to use in daily clinical practice. HOMA-IR is easier to calculate than QUICKI, because the QUICKI uses log-transformed glucose and insulin values (Table 2), even though in this era of apps this may be debated. While there seems not much difference between the HOMA-IR and the QUICKI, we propose to use the HOMA-IR because its ease of use and because our study shows that HOMA-IR is already the most frequently used method to determine IR in pediatric study populations

In addition to the different methods described, we observed a wide range in cut-off values within the different methods. This wide range of cut-off values leads to a large variation in the prevalence of IR even when one method (e.g. HOMA-IR) is used (Figure 2). The definition of a cut-off value for IR with clinical relevance to identify children and adolescents at risk for T2DM, will help the clinician to select the patients who require lifestyle intervention to prevent or delay the onset of T2DM.

In this study, more than 25 cut-off values for HOMA-IR have been described, and still it is not clear which cut-off value is the best to define IR. To date, studies are available on the use of HOMA-IR as screening measure to identify children and adolescents with impaired glucose tolerance and T2DM during an OGTT. To identify T2DM in a population of obese children and adolescents, Shah et al. reported a HOMA-IR value of 7.9 as the best critical value with a sensitivity of 62% and specificity of 70%. Unfortunately, they did not report on the best value to identify impaired glucose tolerance in their study population [27]. The study of Brar et al, who studied the optimal threshold for impaired glucose tolerance or T2DM, identified a cut-off value of 3.4 in a population of obese pediatric patients [28]. This cut-off value resulted in a sensitivity of 72.2% (46.4-89.3) and a specificity of 60.7% (50.8-69.9%) for impaired glucose tolerance or T2DM during an OGTT. Other cut-off values studied were 2.7, 3.1 and 4.0, resulting in lower sensitivity and specificity [28]. In a study from our own group in overweight and obese children screening with FPG and HOMA-IR of 3.4 identified all cases of T2DM and up to 64% of cases of impaired glucose tolerance [21]. The use of HOMA-IR with cut-off value of 3.4 resulted in sensitivity of 70% and specificity 72.6%, with a positive predic-

tive value of 21.4% and a negative predictive value of 95.7%. However, to properly define the cut off value for the HOMA-IR and use it as a screening measure in obese children to predict impaired glucose tolerance and T2DM in the future, longitudinal epidemiological studies of a cohort of obese children and adolescents should be performed, with regular checks of their insulin sensitivity state and glucose metabolism including an eventual diagnosis of T2DM. Future studies should also focus on the need for age, sex and pubertal stage specific cut-off values, since studies providing data on HOMA-IR in large study populations, found differences in IR values for different age, sex and Tanner stages [29, 30]. In our opinion, until further evidence becomes available, the lowest reported HOMA-IR value from the above reported studies (i.e. 3.4) improving detection of T2DM in obese children and adolescents could be used as additional screening measure. This screening should be used in addition to the ADA recommended three-yearly screening with FPG [31].

To our best knowledge, our report is the first to show the large variety in prevalence rates of IR in a given obese pediatric population caused by the heterogeneity of the different definitions. A strength of our study is the availability of data from a previously described population of 311 obese children and adolescents, who underwent an OGTT for clinical reasons. We were able to calculate all fasted methods except C-peptide. As C-peptide has been described to be a measure of insulin secretion and is produced in equal amounts along with insulin, it is possible to use it as a measure for endogenous insulin production. Especially in patients using exogenous insulin, C-peptide was reported useful to establish endogenous insulin production [32]. In order to define IR in a non-diabetic population, we think that C-peptide does not have any advantage over insulin. Moreover, from the OGTT/IVGTT based methods, we were not able to calculate Si(IVGTT) and $IRI_{Belfiore}$. Finally, a comparison with the gold standard method was not possible, as we do not use the euglycemic-hyperinsulinemic clamp test as part of standard of care in our clinic.

Conclusion

In conclusion, we reported in this study all published methods and cut off values used to define IR in pediatric populations. When these definitions were applied to a known population of 311 obese children and adolescents, a large variety of prevalence rates of IR was found. As a result, we conclude that a uniform definition for IR is needed to allow comparison between studies and populations and to be able to follow trends in incidence and prevalence rates over time. Longitudinal, epidemiological studies are necessary to investigate which level of IR is clinically relevant, and will help the

clinician to select the patients who require lifestyle intervention to prevent or delay the development of T2DM.

Conflicts of interest

None of the authors reports a conflict of interest regarding publication of this paper.

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M.A. performed the literature review and data analysis and wrote a first version of the manuscript. All authors discussed study design, data and interpreted the results. C.K., A.B. and M.V. reviewed and edited the manuscript. All authors take full responsibility for the contents of the manuscript, M.V. is the guarantor of this work.

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SUPPLEMENTARY MATERIAL TO CHAPTER 3

Appendix 1. Search strategies of literature search

Database	Search strategy
Pubmed	(("Insulin Resistance"[Mesh] OR insulin resistan*[tiab] OR insulin sensitivity[tiab] OR (resistan*[tiab] AND insulin*[tiab]) OR metabolic syndr*[tiab]) AND ("Prevalence"[Mesh] OR prevalence*[tiab] OR "Incidence"[Mesh] OR incidence*[tiab]) AND ("Child"[Mesh:noexp] OR "Adolescent"[Mesh] OR "Puberty"[Mesh:noexp] OR "Minors"[Mesh] OR Pediatrics[MeSH:noexp] OR child[tiab] OR children[tiab] OR child care[tiab] OR childhood[tiab] OR child*[tiab] OR childc*[tiab] or childr*[tiab] OR childh*[tiab] OR adoles*[tiab] OR boy[tiab] OR boys[tiab] OR boyhood[tiab] OR girl[tiab] OR girls[tiab] OR girlhood[tiab] OR junior*[tiab] OR juvenile*[tiab] OR kid[tiab] OR kids[tiab] OR minors*[tiab] OR paediatr*[tiab] OR pediatr*[tiab] OR prepubert*[tiab] OR pre-pubert*[tiab] OR prepubesc*[tiab] OR pubert*[tiab] OR pubesc*[tiab] OR school age*[tiab] OR schoolchild*[tiab] OR teen[tiab] OR teens[tiab] OR teenage*[tiab] OR youngster*[tiab] OR youth[tiab] OR youths* OR Primary school*[tiab] OR Secondary school*[tiab] OR Elementary school*[tiab] OR High school*[tiab] OR Highschool*[tiab])
Embase	(prevalence/ or incidence/ or (prevalence* or incidence*).ti,ab.) AND (insulin resistance/ or insulin sensitivity/ or metabolic syndrome X/ or (resistan* and insulin*).ti,ab. or insulin sensitivity.ti,ab. or metabolic syndr*.ti,ab.) AND (child/ or boy/ or girl/ or hospitalized child/ or school child/ or exp adolescent/ or adolescence/ or puberty/ or pediatrics/ or (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth).ti,ab. or youths*.ti,ab. or Primary school*.ti,ab. or Secondary school*.ti,ab. or Elementary school*.ti,ab. or High school*.ti,ab. or Highschool*.ti,ab.)
Cochrane	((prevalence* or incidence*) and ((resistan* and insulin*) or insulin sensitivity or metabolic syndr*) and (child or children or child care or childhood or child* or childc* or childr* or childh* or adoles* or boy or boys or boyhood or girl or girls or girlhood or junior* or juvenile* or kid or kids or minors* or paediatr* or pediatr* or prepubert* or pre-pubert* or prepubesc* or pubert* or pubesc* or school age* or schoolchild* or teen or teens or teenage* or youngster* or youth or youths* or Primary school* or Secondary school* or Elementary school* or High school* or Highschool*)).ti,ab.

Supplementary table 1. Study characteristics of all included studies

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
AFRICA															
1	Egypt, 2015	Cross-sectional study in overweight and obese children referred from a Pediatric Endocrinology to a Pediatric Hepatology unit	76	2008-2009	2-15	Overweight and obese	NR	HOMA-IR ≥ 3.5	34.2						
								QUICKI < 0,33	43.4						
2	Egypt, 2011	Observational study of patients referred because of hepatomegaly or elevated ALT	33	NR	2-13	Overweight and obese	NR	HOMA-IR ≥ 3.5	48.5						
ASIA															
3	China, 2013	Cross-sectional population based survey	3373	NR	6-18	All	Chinese	HOMA-IR ≥ 3,0	25*	8.9*	28.1*	43.8*	26.9*	23.0*	
4	China, 2013	Cross-sectional study of high risk participants of a population based study and of a group of schoolchildren	3203	April - October 2004	6-18	All	Chinese	HOMA-IR > 1.7		33.6	68.9	80.1			
								HOMA-IR > 2.3		17.9	47.7	63.2			
								HOMA-IR > 2.6		12.9	38.5	55.4			
								HOMA-IR > 3.0		8.9	28.6	44.3			
								HOMA-IR > 3.2		7.1	24.5	40.5			
5	China, 2010	Cross-sectional case-control study of patients with PCOS	128	2004-2009	19.0	All	Asian	HOMA-IR > p95							PCOS: 46.9, control 17.5
								FPI > p95							PCOS: 29.7 control 7.5

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
6	India, 2013	Door-to-door demographic survey of representive wards of Chennai city.	1519		6-19	All	Indian	HOMA-IR ≥ 3.56					7.8	12.5	
7	India, 2013	Cross-sectional analysis of school going adolescents in a South-Indian population	120	NR	11-18	All	NR	HOMA-IR > 3.16		0	26	64			
8	India, 2011	Cross-sectional, case-control study	94	2006-2007	6-11	All	Bengali	FPI > 15 µIU/ml	40.4	18	61				
								HOMA-IR > 2.5	41.5	18	63				
9	India, 2010	Case series of patients with PCOS	49	2006-2008	12-19	All	NR	Glucose/insulin ratio < 7.0	69.4						
10	India, 2008	Cross-sectional population based study	948	NR	14-19	All	NR	FPI > 128.5 pmol/l (14-15yr); >126.1 pmol/l (16-17yr); >162.4 pmol/l (18-19yr)	35.4	29.3	67.3				14-15 yr: 32.6; 16-17 yr: 39.1; 18-19 yr: 32.7
11	India, 2006	Randomly selected sample of population based study	793	2000-2003	14-19	All	NR	FPI> 20 µU/l		29	63.9				
12	Iran, 2010	Retrospective study	110	2006-2008	4-18	Obese	NR	HOMA-IR > 4.0	28.2*				36.7	26.1	<10 yr: 23.8, > 10 yr 31.8
13	Iran, 2009	Cross-sectional study among survivors of childhood ALL	55	2003-2007	6-19	All	NR	FPI > 24 mU/l]	16						
14	Israel, 2005	Retrospective review of medical records	256	1997-2003	Mean: 13	Overweight obese	Jewish, Arabs	HOMA-IR >2	81.2				39.2	60.8	Tanner stage I: 63.2; II-III: 82.1; IV-V: 88.7
								QUICKI <0.339	77.7						
15	Japan, 2012	Cross-sectional study in schoolchildren	310	2009	10-13	All	Japanese	HOMA-IR ≥ 2.5	21.6		46.8				

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
16	Korea 2009	Review of medical records of children with NAFLD	80	1995-2008	12.0 (2.8)	Overweight and obese	NR	HOMA-IR > 2.0	96						
17	Kuwait, 2014	Baseline analysis of data from an intervention study	80		10-14	Obese	Kuwaiti	HOMA-IR > 3.16	67.5						
18	Lebanon, 2010	Cross-sectional survey in subjects selected from private and public schools, exclusion of children with chronic illness, antihypertensive, antihyperglycemic or lipid metabolism drugs.	140	2007-2008	~10	All	NR	FPI ≥ 15 mIU/l		28.6	56.0	67.8			
								HOMA-IR > 3.16		25.0	56.0	70.1			
19	Middle East, 2010	Cross-sectional study in children with impaired glucose tolerance	31	NR	13.2 (3.5)	Overweight, obese	Iranian	HOMA-IR > 3.0	19.4 *						
20	Thailand, 2011	Cross-sectional study in obese children of a nutrition clinic	89	2007	4-18	Obese	Thai	HOMA-IR > 3.16				58.4			
								FPI > 25 µIU/ml				27.3			
21	Thailand, 2010	Retrospective review of medical records of children surviving ALL	131	1997-2004	Apr-20	All	NR	FPI ≥ 20 µU/ml WBISI < 5 Insulinogenic index	30.5						
22	Thailand, 2009	Cross-sectional substudy of a longitudinal cohort study among HIV-infected children	54	NR	9.8 (2.5)	"Small and thin", (weight-for age Z score -1.91 (1.03))	NR	HOMA-IR ≥ 3.16	6.5						<10yr: 3.7; >10yr: 10.5
								c-peptide ≥ 4.40 ng/ml	0						<10yr: 0; >10yr: 0

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
								FPI ≥ 25.0 µU/ml	2.0						<10yr: 0; >10yr: 4.5
AUSTRALIA															
23	Australia, 2010	Cross-sectional study of Grade 10 students	495	2004	14.3-17.1	All	NR	FPI > 100pmol/l	20.6*	♂ 7.1 ♀ 10.9	♂ 29.5 ♀ 41.9	♂ 68.4 ♀ 44.4	19.3	22.4	
24	Australia, 2006	Cross-sectional, baseline analysis of randomized controlled trial	99	NR	6-9	Overweight obese	NR	FPI > 51 pmol/l	74						
								FPI ♀ > 35 pmol/l (~5 mU/l), ♂ > 40.6 pmol/l (~6 mU/l)	85						
25	New Zealand, 2008	Observational study of pacific island teenagers living in New Zealand	80	NR	15-18	All	NR	FPI > 12 µIU/ml	44						
								HOMA2 > 2 or	26.9				17.5	36.8	
								McAuley index ≤ 6.3					20.0	34.2	
CARIBBEAN AND CENTRAL AMERICA															
26	Costa Rica, 2009	Cross-sectional survey among overweight and obese schoolchildren	214	NR	8-10	Overweight and obese	NR	HOMA-IR ≥ 2.4	55.1				50.0	60.6	
								FPI >10.5 µU/l	59.8				51.8	68.3	
27	Costa Rica, 2008	Observational study of prepubertal overweight or obese children	214	NR	8-10	Overweight and obese	Tri-ethnic heritage (Spanish, indige-nous, Africans)	FPI > 20 mIU/l	20.6		2.8	17.8			
								HOMA-IR > 5.4	10.7		1.9	8.8			

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
								QUICKI < 0.300	11.7		1.9	9.8			
								FGIR < 7	46.7		10.8	35.9			
28	Cuba, 2010	Cross-sectional observational study in first degree family of T1DM patients	193	NR	2-19	All	146 white	HOMA-IR >1.14 (2-5yr); > 1.67 (5.1-10yr); >2.53 (10.1-15yr); >2.52 (15.1-19yr)	24.9						
29	Mexico, 2013	Cross-sectional study in children recruited from primary schools	174	NR	6-13	Normal weight and obese	NR	HOMA-IR ≥ 2.4	32.75	16.85		49.41			
30	Mexico, 2012	Cross-sectional analysis of baseline data from children participating in Health Workers Cohort Study	916	NR	7-18	All	NR	HOMA-IR ≥ 3.5	20.3				17.1	23.4	
31	Mexico, 2010	Cross-sectional survey in subjects randomly selected from public schools	1850	NR	12-16	All	NR	FPI > p75 (~9.85 µIU/ml)	24.8 *				4.9*	24.7*	
								HOMA-IR > p85 (~3.0)	15.3 *				12.7*	17.2*	
32	Mexico, 2010	Cross-sectional observational study among obese schoolchildren	466	NR	11-13	Obese	NR	FPI≥ 15 µU/ml	56				45	71	
								HOMA-IR ≥ 3.4 (=p90)	51				43	63	
33	Mexico, 2007	Comparative, observational study in obese and non-obese subjects	240	NR	10-19	All	NR	FPI > 16 µU/ml	27.1*	4		50			

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
34	Mexico, 2006	Cross-sectional observational study among randomly selected schoolchildren	317	NR	10-14	All	NR	FPI> 16 µU/l	15.1						Family history T2DM: positive 72.9; negative 27.1
EUROPE															
35	Austria, 2007	Case control study of NAFLD patients with age and sex matched controls	40	NR	5-18	Obese	NR	HOMA-IR > 3.2 or OGIS < 436 ml/min/m^2	72*						With NAFLD: 81%; Without NAFLD: 63%
36	Czech Republic, 2014	Cross-sectional study of a general population cohort	1518	NR	13.0-17.9	All	NR	HOMA-IR > 2.5	40.7 *				40.9	40.5	
								HOMA-IR > 4.0	13.2 *				14.3	10.8	
37	Czech Republic, 2013	Cross-sectional study in obese children referred to a obesitology department by their pediatrician	274	NR	9-17	Obese	NR	HOMA-IR > 3.16	53						With metabolic syndrome: 70%, without metabolic syndrome: 43%
								QUICKI <0.357	86						
38	Finland, 2009	Cross-sectional study among survivors of childhood brain tumors	52	NR	14.2 (3.8-28.7)	All	NR	FPI > 20 mU/l	4						No cranial irradiation: 3%; Cranial irradiation: 5%
39	France, 2009	Retrospective study of medical records of children visiting an obesity clinic	244	2003-2006	0-18	Obese	NR	HOMA-IR>75th percentile	61.4				75.7	53.1	
40	France, 2009	Observational study in children visiting an obesity clinic	50	NR	6-16	Overweight and obese	NR	HOMA-IR >75th percentile	68						

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
41	Germany, 2011	Retrospective chart review of children visiting an obesity clinic	1053	2001-2008	1-17	Overweight, obese, extremely obese	German, Turkish, other	Elevated HOMA-IR according to Allard et al. [43]	40.3						
42	Germany, 2005	Cross-sectional observational study in children with normal glucose tolerance	90	NR	3-16	Obese	NR	HOMA-IR ≥ 2.0, ISI Matsuda < 7.2	68						
43	Greece, 2008	Observational population based study on school children in Crete	522	2005-2006	10-12	All	NR	HOMA-IR >2.1	9.2	2.9	10.5	31.0	9.20	9.17	
								HOMA-IR > 3.16	3.1	1.9	1.8	10.3	4.60	1.83	
								QUICKI < 0.35	12.8	3.9	16.2	41.4	10.34	14.68	
								FGIR < 7	17.4	6.8	22.8	45.9	13.79	20.18	
44	Greece, 2014	Large scale, cross-sectional epidemiological study	2026	2007	9-13	All	NR	HOMA-IR > 3.16	28.4	16.7	38.0	59.6	22.4	33.2	
								HOMA-IR > 3.99	16.6	8.5	22.8	39.1	12.2	20.0	
								HOMA-IR > 5.56	6.0	2.4	8.0	19.1	4.5	7.4	
45	Greece, 2007	Observational study among young ALL survivors	80	NR	5.2-24.1	All	NR	FPI > 28.7 µU/ml	8						
46	Hungary, 2009	Cross-sectional study of children visiting an obesity clinic	113	NR	13.1 (2.4)	Obese	Caucasian European	FPI > 25 mU/l	73						
								HOMA-IR > 4.0	84.1						
								Insulin at 120' > 45 mU/l	89.3						

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
47	Hungary, 2008	Cross-sectional observational study of children visiting an obesity clinic	250	NR	13.0 (6.9)	Obese	Caucasian European	FPI > 25 mU/l	70.0						
								HOMA-IR > 4.0	77.6						
								Insulin at 120' > 45 mU/l	88.0						
48	Hungary, 2008	Baseline analysis of data from an intervention study	114	NR	5-17	Overweight and obese	NR	HOMA-IR > 4.4	32.5 *						
								OGIS < 400	37.7 *						
49	Italy, 2010	Cross-sectional study of children randomly selected from schools	575	2007-2008	11-13	All	NR	FPI > p75 (♂ 11.0 pmol/l; ♀ 13.2 mol/l)	25.2 *	♂ 12.4 ♀ 11.2	♂ 25.6 ♀ 38.2	♂ 60.4 ♀ 65.5	25.3*	25.1*	
								HOMA-IR > p75 (♂ 2.28, ♀ 2.67)	25.0 *	♂ 13.1 ♀ 11.8	♂ 26.7 ♀ 37.1	♂ 54.7 ♀ 65.5	25.0*	25.1*	
50	Italy, 2008	Cross-sectional, case-control study	191 cases, 76 controls	2003-2006	Cases: 11.15 (3.4) Control: 10.69 (3.3)	Overweight and obese	Caucasian	HOMA-IR >2.5 (prepubertal), > 4 (pubertal)	42.7*	3.2	33.3	43.6			Severe obese: 63.5 %
51	Italy, 2006	Cross-sectional, case-control, observational study	100 cases, 50 controls	NR	3-16	Normal and obese	NR	HOMA-IR >2.5 prepubertal, >4 pubertal	28*						Normal weight: children 3.0; adolescents 0; Obese: children 40.8; adolescents 41.2
52	Italy, 2001	Observational study in children with IUGR	49	NR	9.1 (3.3)	All	NR	Glucose/insulin ratio < 6	22			42.9*			

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
53	Nether-lands, 2013	Baseline analysis of data from an intervention study			3-5	Overweight and obese		HOMA2 > 1,53 (boys) or > 1.85 (girls)	7.7		4.0	10.0			
54	Nether-lands, 2011	Analysis of a paediatric obese cohort	1007	2004-2009	3-18	Overweight and obese	Dutch, Turkish, Moroccan, other	HOMA-IR ≥ 3.5	54.1						Prepubertal 34.6; pubertal 69.7
															Dutch 56.6; Turkish 59.7; Moroccan: 45.2
55	Nether-lands, 2009	Cross-sectional study of cohort of patients referred for obesity	528	2004-2007	3-16	Overweight and obese	Dutch, Turkish, Moroccan, Surinamese, other	HOMA-IR ≥ 3.5	47.7						< 10 yr 21.8, > 10 yr 60.1
56	Nether-lands 2009	Cross-sectional study of cohort of patients referred for obesity	516	2004-2008	3-18	Overweight and obese	Dutch, Turkish, Moroccan, other	HOMA-IR ≥ 3.5	48.8						Prepubertal 26.1; pubertal 62.3
															Turkish 54.9, Moroccan 37.4, Dutch: 46.8, other: 49.4
57	Neth-erlands 2008	Observational pilot study	155	2005-2007	2-18	Obese	Caucasian, others	HOMA-IR >3.1	60						
58	Poland, 2010	Evaluation of children born SGA	91	NR	4.78-9.75	All	NR	HOMA-IR > 4.0	0.0						
								$IRI_{Belfiore}$>1.27	14.3						

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
59	Portugal, 2010	Cross-sectional study in a population based sample of obese children	82	NR	7-9	Overweight and obese	NR	FPI ≥ 15 µU/ml	7.3						
								HOMA-IR ...	8.5						
60	Slovakia, 2010	Cross-sectional study of children visiting an obesity clinic	98	NR	5.2-16	Obese	NR	HOMA-IR > 3.16							<10 yr 4.8; >10yr 37.7
61	Spain, 2011	Retrospective review of obese children	100	2008	6-14	Overweight and obese	Caucasian, hispanic	FPI > 2SD	32						
								HOMA-IR >2SD	29						
								Insulin in OGTT >150 µU/ml	31						
								Insulin in OGTT >75 µU/ml at 120'	45						
								QUICKI < 2SD	93						
62	Spain, 2007	Observational study	97	NR	6-14	Obese	NR	HOMA-IR ≥ 3.8	45.4						
63	Spain, 2005	Cross-sectional observational study in children born SGA	46	NR	6-9	Normal	NR	Insulin > 10 IU/ml	11						
64	Spain, 2003	Cross-sectional study	95	NR	4-16	Obese	Spanish-Caucasian, Hispanic, Gypsie	HOMA-IR >4		4.1		35.8			
65	Turkey, 2014	Cross-sectional study of cohort of patients referred for obesity	451	2008-2012	8-18	Obese	NR	HOMA-IR > 3.16	54.8 *						NAFLD: 72%; without NAFLD: 39%

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
66	Turkey, 2011	Cross-sectional study in children with chronic kidney disease	66	2007	5-21	Normal weight	Turkish	HOMA-IR >p95	15.6 *						Predialysis: 7/20 (35 %); Dialysis: 3/46 (6.5%)
67	Turkey, 2010	Cross-sectional survey in randomly selected school children	790	NR	12-19	All	NR	HOMA-IR <3.16	34.7				33.0	36.1	
68	Turkey 2008	Retrospective, cross-sectional study of children visiting an obesity out-patient clinic	112	2002-2004	2-18	Obese	NR	FPI ≥15 mU/l (TS I), ≥30 mU/l (TS II-IV), ≥20 mU/l (TS V)	20.5						
								Peak insulin during OGTT ≥ 150 mIU/	34.8						
69	Turkey, 2008	Observational, cross-sectional, case-control study of children referred for obesity	169	2003-2005	Mean: ~10	Normal and obese	Turkish	HOMA-IR > 2.5	39.6	10.3		59.4*			
70	Turkey, 2007	Cross-sectional observational study	148	NR	8-18	Obese	NR	Σ insulin during OGTT > 300 µU/ml	37.1						
71	Turkey, 2007	Cross-sectional cohort study	196	2000-2005	7-18	Obese	Turkish	HOMA-IR >3.16	43						Prepubertal 34; pubertal 45
72	Turkey, 2006	Observational, cohort study	169	NR	7-18	Obese	Turkish	HOMA-IR > 3.16	40.2						Prepubertal 20; pubertal 43.7
								FPI > 15 mIU/l (prepubertal), > 30 mIU/l (TS II-IV)	29.5						Prepubertal 29; pubertal 56.5
73	Turkey, 2006	Multicenter cross-sectional, observational study	105	NR	10-18	All	NR	HOMA-IR > 2.5	63.8						

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
								FPI > 20 µU/ml	57.1						
74	UK, 2005	Cross-sectional observational study of clinical sample	103	1999-2002	2-18	Obese	White, black, South Asian, mixed	FPI ≥15 mU/l (TS I), ≥30 mU/l (TS II-IV), ≥20 mU/l (TS V)	40				36	42	
NORTH-AMERICA															
75	Canada, 2010	Cross-sectional analysis of children with fatty liver detected with ultrasound	38	2005-2006	5.5-19.9	All	Caucasian, Hispanic, Asian, other	HOMA-IR > 3.0	66						
								QUICKI < 0.31	50						
76	Canada, 2008	Follow-up study of cohort of children born from women with GDM	68	NR	7-11	All	Caucasian	HOMA-IR >2.5	15	4	38	45			
77	USA, 2013	Cross-sectional data analysis of a sample of participants of the NHANES study	766	2003-2008	12-19	All	White, black, MA, other race	HOMA-IR > 4.39	17.6						
78	USA, 2012	Cross-sectional convenience sample of school-based medical screening	1185	2008-2011	14-19	All	Hispanic, African-American, other	HOMA-IR > 4	19.5	4.5	12.4	37.8			
79	USA, 2012	Observational cross-sectional study in middle-school students	151	NR	11-14	All	NR	HOMA-IR ≥ 2.7	33.8 *	22.7		62.5			
80	USA, 2011	Cross-sectional analysis of fasting laboratory data of HIV-infected patients	402	2007-2009	7-16	All	NR	HOMA-IR >2.5 prepubertal, >4 pubertal	15.2						Prepubertal: 12.5%, pubertal 15.9%
81	USA, 2011	Population based sample (NHANES)	1571	1999-2002	12-18	All	White, black, MA, other race	HOMA-IR > 4.39	11.8						Black: 16.8, MA 16.9, white 9.3

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
82	USA, 2011	Cross-sectional data analysis of a sample of participants of a community based lifestyle program	105	2007	9-13	Overweight and obese	AA	HOMA-IR > 2.5	38.1*						
83	USA, 2007	Cross-sectional observational study in children with chronic kidney disease	43	NR	6-21	Normal and overweight	White, AA	FPI > p95	33						
								HOMA-IR > 4.39	16						
84	USA, 2007	Cross-sectional study	86	2001-2003	8-20	Obese	Caucasian, African, Hispanic, Asian	Si(IVGTT) < $4.5x10^4$ µU/ml/min	70.9						
85	USA, 2006	Cross-sectional observational study among schoolchildren	716	2002-2003	7-17	All	13% minority students	HOMA-IR > p85	~25		50				
86	USA, 2006	Population based study	1802	1999-2002	12-19	All	NHW, black, MA	HOMA-IR > 4.39				52.1			
87	USA, 2006	Observational cross-sectional study among schoolchildren	247	NR	7-17	All	89% Caucasian	QUICKI < p15 (~0.33 prepubertal, ~0.36 postpubertal)			2^{nd} grade: 47 11^{th} grade: 51				Prepubertal 24.5; postpubertal 26.2
88	USA, 2006	Observational study among schoolchildren	1740	2003	13.6 (0.6)	All	Hispanic, AA, Caucasian, Native American, other.	FPI ≥ 30 µU/ml	36.2	16.0	36.2	72.3			Hispanic 44.3; AA 29.3; Caucasian 20.5; Native American 36.4

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
89	USA, 2004	Cross-sectional observational study in children previously identified at risk for T2DM.	139	NR	10-12	All	MA	FPI > 15 µU/ml	59.7						
SOUTH-AMERICA															
90	Argentina, 2013	Cross-sectional, descriptional study.	75	2011-2012	2-14	Overweight and obese	NR	FPI > 15 µIU/ml	60				64	57.9	
								HOMA-IR > 3	66.6						
91	Argentina, 2011	Descriptive study of high-school students	420	2005	12-18	All	NR	Insulin ≥ 12 mU/l	11.7						
								HOMA-IR ≥ 2.5	10.5				10.6	10.4	
								QUICKI ≤ 0.33	9.8				6.3	11.9	
92	Bolivia, 2008	Cross-sectional observational study of patients with obesity	61	2006-2007	5-18	Obese	Bolivian	HOMA-IR >3.5	39.4				50	29	
93	Brazil, 2014	Cross-sectional study in adolescents visiting an obesity outpatient clinic	79	2011-2012	10-18	Obese	NR	HOMA-IR > 3.16	29.1				43.5	56.5	
94	Brazil, 2011	Cross-sectional descriptive study in vertically HIV-infected children	119	2007	6-19	All	NR	HOMA-IR ≥ 2.5	16.7						
95	Brazil, 2009	Cross-sectional, population based epidemiological study	109	NR	7-11	All	NR	HOMA-IR > p90	10*	0	0	20.0			
								FPI ≥ 20 µU/ml	11*	0	0	21.8			
96	Brazil, 2009	Transversal observational study of students with central obesity	175	2005-2006	11-18	Overweight and obese	71.4% nonwhite	HOMA-IR ≥ 3.16	22.9						
97	Brazil, 2008	Two stage cross-sectional observational study among schoolchildren with obesity, low birth-weight, positive FH on CVD	205	NR	6-10	All	NR	HOMA-IR > 3.1	8.6*	0.9	6.8	10.3			

Supplementary table 1. Study characteristics of all included studies (continued)

Nr.	Country, year of publication	Methods	Sample size	Calender time	Age range	Weight category	Ethnicity	Criteria IR	Prevalence IR (%)						
									Overall	Normal weight	Over-weight	Obese	Boys	Girls	Other subpopulations
98	Brazil, 2005	Cross-sectional analysis of adolescents with family history of T2DM	99	NR	10-19	All	Brazilian	HOMA-IR > 2.5	22.2	10.9	23	43.5			
99	Brazil/Italy 2008	Cross-sectional study among obese adolescents	509	NR	15-19	Obese	Brazilian, Italian	FPI ≥ 20 µIU/ml	0.2*						
								HOMA-IR >3.16	30.5*						
100	Chile, 2014	Retrospective cohort study of children from public schools linking present data to perinatal records	3290		10-15	All	Chilean	HOMA-IR > p90 for sex and TS (♂ TS I-II: 3.2; TS III-IV: 4.2; ♀ TS I-II 4.1, TS III-IV: 5.0)	25.5				26.4	25.2	
101	Chile, 2013	Cross-sectional study of children in public schools in Puente Alto County	3325	2009-2011	10-15	All	Chilean	HOMA-IR > p90 for sex and TS (♂ TS I-II: 3.2; TS III-IV: 4.2; ♀ TS I-II 4.1, TS III-IV: 5.0)	25.9				26.9	24.8	
102	Chile, 2010	Cross-sectional analysis from a cohort study, childen with negative family history of T1DM, astma and no steroid use.	324	2006	~4.0	All	Chilean	HOMA-IR ≥ 3.2	1.54 *				1.1	2.1	
103	Chile, 2003	Cross-sectional study in children from an obesity clinic	88	NR	12 (2.4)	All	NR	HOMA-IR >3.8				79			

Abrreviations: FPG: fasted plasma glucose; FPI: fasted plasma insulin; HOMA-IR: Homeostasis Model assessment Insulin resistance; MA: Mexican-American; NR: not reported; QUICKI: quantitative insulin sensitivity check index; TS: Tanner Stage

Calculations: HOMA-IR = FPG (mmol/l)*FPI (mU/l) / 22,5 or FPG (mg/dl)*FPI (mU/l) / 405; HOMA2: calculated with HOMA2calculator http://www.dtu.ox.ac.uk/homacalculator/index.php; McAuley index= 2.63 -0.28 ln[fasting insulin] -0.31 ln[fasting triglycerides]; QUICKI= 1/ [(log (FPI (mU/l))+ log (FPG (mg/dl))]

Notes: * Calculated by the authors; † extracted from graph

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Chapter 4

How to screen obese children at risk for type 2 diabetes mellitus?

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Abstract

Background

Recommended screening to identify children at risk for diabetes and its precursors impaired glucose tolerance (IGT) and insulin resistance (IR) is fasted plasma glucose (FPG). This study evaluates the added value of fasted plasma insulin (FPI).

Methods

This study analyzed routinely collected data of an oral glucose tolerance test (OGTT) of 311 obese children (10.8±3.2 years). Diabetes and IGT were defined according to the American Diabetes Association criteria, IR as HOMA-IR ≥ 3.4.

Results

Cases diagnosed with an OGTT if FPG ≥ 5.6 mmol/l, compared to an OGTT performed if FPG ≥ 5.6 mmol/l or HOMA-IR ≥ 3.4, were respectively four (80%) vs five (100%) with diabetes, 7 (28%) vs 16 (64%) with IGT and 0 (0%) vs 93 (100%) with IR.

Conclusions

Screening with FPG and FPI has equal burden compared to screening with FPG alone, identifies all patients with diabetes, and more patients with precursors of diabetes.

Introduction

Childhood obesity is an increasing public health problem, although some studies suggest that the prevalence may have reached a plateau [1,2]. Over the last decades, there was a worldwide increase in prevalence of overweight and obesity among children, with an obesity prevalence of 2-3% worldwide [1-5]. Childhood obesity leads to many complications such as type 2 diabetes mellitus, hypertension, dyslipidemia, and the metabolic syndrome [6].

The American Diabetes Association (ADA) advises to measure fasted plasma glucose (FPG) every 2 years in children at risk for type 2 diabetes mellitus [7, 8]. Obesity is defined as one of the risk factors for which screening is necessary. Furthermore, the guideline differentiates between FPG as a screening measure, and the oral glucose tolerance test (OGTT) as a diagnostic tool. If FPG is impaired (FPG ≥ 5.6 mmol/l), additional diagnostic testing with an OGTT is recommended.

In order to improve the identification of children at risk for type 2 diabetes mellitus or metabolic complications, additional screening methods have been analysed. Five studies described FPG as an insufficient predictor for impaired glucose tolerance (IGT) [9-13]. Two studies concluded that FPG alone is insufficient to detect all cases of IGT and recommended to add serum triglyceride concentrations to the screening [12, 14] .Studies on the use of HbA1c disagree with each other on the use of HbA1c for identifying children with IGT or type 2 diabetes mellitus; two studies conclude that HbA1c is a good predictor for IGT and type 2 diabetes mellitus [15, 16], while others conclude that HbA1c is an insufficient predictor [17, 18]. No studies describing the additional value of fasted plasma insulin (FPI) and type 2 diabetes mellitus precursor insulin resistance (IR) in screening for type 2 diabetes mellitus were found.

Hyperinsulinemia or insulin resistance (IR) has been identified as independent precursor for impaired glucose tolerance (IGT) or type 2 diabetes mellitus [19-22]. The prevalence of IR and IGT is higher in obese children than in normal weight children, which implicates a relation between IR and IGT and obesity [23]. Furthermore, studies in obese children have shown more children to have IGT than impaired FPG, which implicates that screening with FPG (eventually followed by an OGTT if FPG ≥ 5.6 mmol/l) will not identify all children with IGT [10, 11, 13, 24, 25]. Another important limitation of the recommendations of the guidelines is that although both FPG and OGTT provide information on glucose homeostasis, they fail to identify IR. For the diagnosis of IR measurement of insulin is required. Several methods are available to determine insulin resistance. The gold standard, is the hyperinsulinemic-euglycemic clamp-study, which is invasive and time-consuming. A simple method to determine insulin resistance is the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). This model uses FPG and FPI to calculate IR. Since IR is related to IGT and type 2 diabetes mellitus,

the use of FPI as an additional screening tool might distinguish more precisely which children should undergo an OGTT to diagnose IGT or diabetes.

The aim of this study was to evaluate in patients from a pediatric obesity out-patient clinic the percentages with type 2 diabetes mellitus, IGT and IR identified using FPG and an OGTT if FPG ≥ 5.6 mmol/l (according to current obesity guidelines), versus the percentages diagnosed when FPI is considered in addition to FPG, followed by an OGTT if FPG ≥ 5.6 mmol/l or HOMA-IR ≥ 3.4.

Research design and methods

A retrospective chart review was performed using routinely collected information from children who visited the pediatric obesity out-patient clinic in an 850 bed hospital in the Netherlands, between the January 2006 and December 2009. During that period an OGTT was part of standard care.

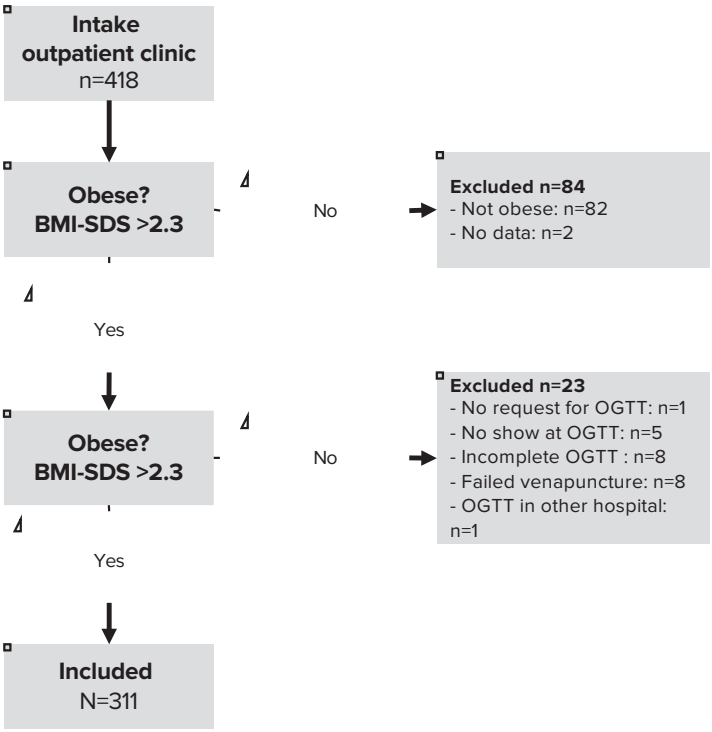
Children were selected for inclusion if there were data available on anthropometric measurements (height and weight), and an OGTT including FPG and FPI (Figure 1). Children who were not obese, did not have complete data on the OGTT or did not have an OGTT within 3 months before or after anthropometric measurements were excluded. Three hundred eleven children were included (Figure 1).

The study protocol was approved by the Medical Ethical Committee of the St Antonius hospital. As only routinely collected information was used and analysed anonymously, the need for written informed consent of the children and their parents was waived.

Data collection

All data were collected from the medical records. Age of the child at the day of anthropometric measurements was recorded. Height was measured with a precision of 0.1 cm, using a digital stadiometer (De Grood, DGI 250D) and weight to 0.05 kg accuracy using a digital scale (Seca). Body Mass Index (BMI) was calculated as kg/(height in meters)^2. BMI-standard deviation score (BMI-SDS) was calculated using a web application of TNO (Dutch organisation for applied scientific research) prevention and healthcare: 'the TNO growth calculator for professionals' (http://groeiweb.pgdata.nl/calculator.asp). Obesity was defined as BMI-SDS > 2.3 [26].

The OGTT was performed after an overnight fast (at least eight hours prior to the test), with 1.75 gram glucose per kilogram bodyweight (maximum 75 gram glucose in 300 ml water). A baseline blood sample for FPG and FPI was drawn. A second blood sample for plasma glucose was drawn 120 minutes after glucose intake (2-hr PG).



Abbreviations: BMI-SDS, Body mass index-standard deviation score; OGTT, oral glucose tolerance test

Figure 1. Flowchart of study population

Definitions

For the interpretation of plasma glucose levels the criteria of the ADA were used: impaired FPG when FPG ≥5.6 mmol/l, IGT when 2-hr PG ≥7.8 and < 11.1 mmol/l, and diabetes when FPG ≥7.0 mmol/l and/or 2-hr PG ≥11.1 mmol/l [27]. IR was calculated using HOMA-IR. HOMA-IR is defined as fasting plasma glucose (mmol/L) x fasting plasma insulin (mU/L) / 22.5. The cut-off point for IR was defined as HOMA-IR ≥ 3.4, based on the mean value for 95th percentile in two studies in normal weight children [28, 29].

Statistical analysis

All data were reported as mean ±SD. The percentages of patients with type 2 diabetes mellitus, IGT and IR identified with different screening strategies were calculated using IBM-SPSS statistics version 19.0. No additional statistical tests were performed.

Results

Patients

Four hundred eighteen (418) children visited the outpatient clinic between 2006 and 2009. (Figure 1) Eighty-four (84) children were excluded because they were not obese (n=82) or no anthropometric data were available (n=2) and 23 children were excluded because no OGTT was performed (n=15), or the OGTT results were not reliable, because of vomiting after drinking the glucose solution, or failed blood sampling at 2 hour blood sampling (n=8). Three hundred eleven (311) children could be included in the analysis. Patient characteristics are shown in table 1.

Table 1. Population characteristics, n=311

	Mean (SD)	Range
Anthropometrics		
Age	10.8 (3.3)	2.4 – 17.7
Female,n (%)	153 (49.2)	-
Height, cm	149.4 (44.6)	90.5 – 185.8
Weight, kg	67.0 (25.7)	20.7 – 153.9
BMI, kg/m^2	28.9 (5.2)	20.8 – 47.8
BMI-SDS	2.93 (0.49)	2.31 – 5.52
Biochemical parameters		
FPG, mmol/l	5.0 (0.5)	3.4 – 8.6
FPI, µU/l	12.7 (10.0)	2 – 61
2hr-PG, mmol/l	6.4 (1.5)	3.3 – 20.3
HOMA-IR	2.89 (2.48)	0.30 – 19.88

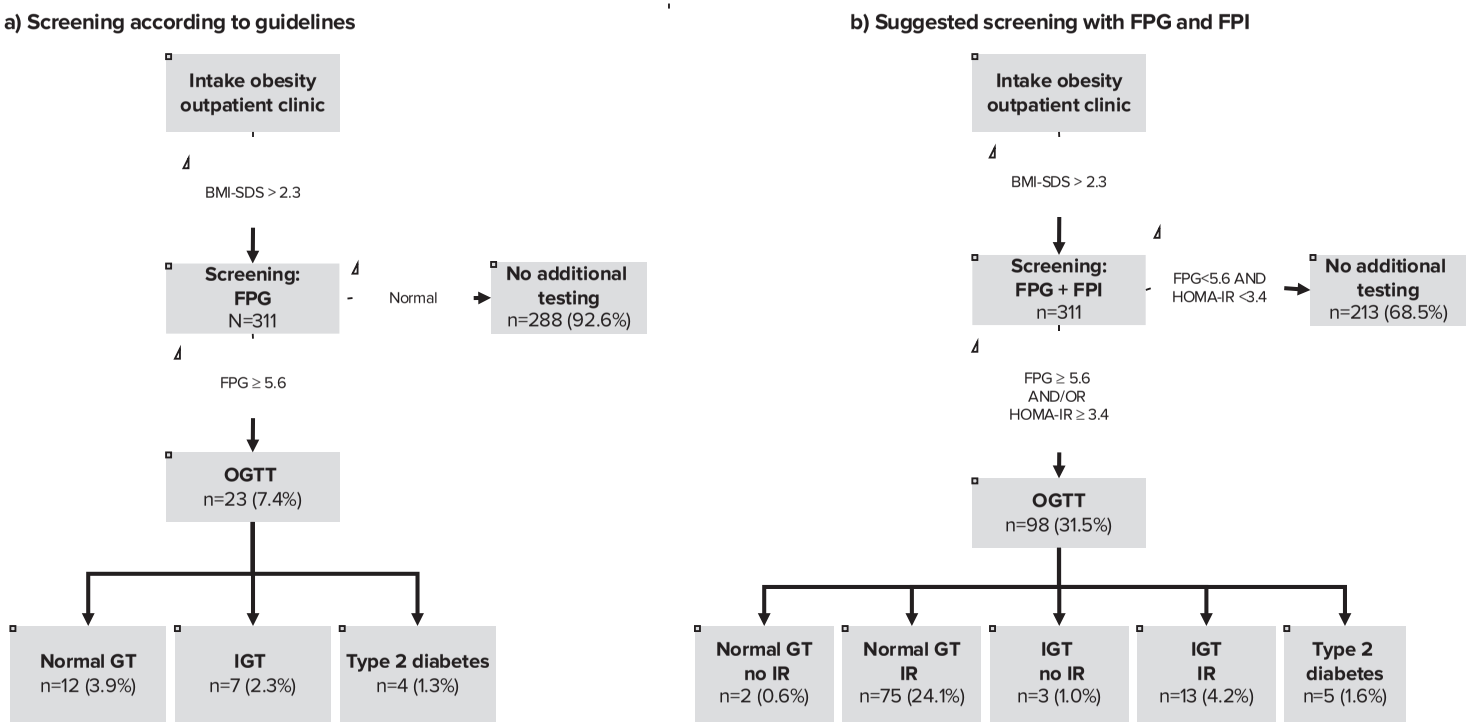
***Abbreviations**: BMI, body mass index; SDS, standard deviation score; FPG, fasted plasma glucose; FPI, fasted plasma insulin; PG,plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance.*

Percentages of diabetes, IGT and IR

If screening would be performed with FPG alone, according to the guidelines, in 23 children (7.4%) an OGTT should be performed, because of FPG ≥ 5.6 mmol/l [7, 8, 30]. The additional OGTT would result in diabetes diagnosed in four patients, and IGT in seven patients (Figure 2a), while IR could not be identified.

If FPI would be added to screening with FPG, 98 children (31.5%) would undergo an OGTT, because of FPG ≥ 5.6 mmol/l (n=22) and/or HOMA-IR ≥ 3.4 (n=93) (Figure 2b). This OGTT would result in identifying five cases of diabetes, 16 cases of IGT, and 93 cases of IR.

Overall, screening with FPG compared to screening with FPI in addition to FPG would result in 23 vs. 98 OGTTs performed, identification of four vs. five cases of diabetes, seven vs. 16 cases of IGT and zero vs. 93 cases of IR.



BMI-SDS, Body mass index-standard deviation score; FPG, fasted plasma glucose; OGTT, oral glucose tolerance test; (I)GT, (impaired) glucose tolerance; HOMA-IR, Homeostasis Model Assessment – Insulin Resistance; IR, Insulin resistance

Figure 2. Results of screening according to the guidelines (a) and screening with FPG and FPI (b)

Compared to performing an OGTT in all cases (n=311), screening with FPG alone (followed by and OGTT if FPG ≥ 5.6 mmol/l) identifies 4 out of 5 patients (80%) with diabetes, 7 out of 25 patients (28%) with IGT, and 0 out of 93 patients (0%) with IR, while screening with FPG and FPI (followed by an OGTT if FPG ≥ 5.6 mmol/l and/or HOMA-IR ≥ 3.4) identifies 5 out of 5 patients (100%) with T2DM, 16 out of 25 patients (64%) with IGT and 93 out of 93 patients (100%) with IR.

Discussion

Identification of obese children at risk for type 2 diabetes mellitus as early as possible is of utmost importance in order to be able to prevent or delay diabetes and other metabolic and cardiovascular diseases. For early identification, it is essential to have a screening tool which is very sensitive. In this study we evaluated the use of FPI in addition to FPG for screening obese children at risk for type 2 diabetes mellitus. Our data suggest that screening with FPI in addition to FPG (to calculate HOMA-IR) identifies more children with diabetes and the precursors IGT and IR than screening with FPG alone. As FPG and FPI can be measured from the same blood sample there is no extra burden for the patient for this additional screening with FPI.

Other studies compared the use of FPI or HOMA-IR in addition to FPG in obese children to diagnose the metabolic syndrome and concluded that for screening purposes, HOMA-IR is preferred over FPG because IR has a stronger relation with the other components of the metabolic syndrome [31, 32]. Golley et al. evaluated different definitions of the metabolic syndrome and also concluded that more patients were identified with the metabolic syndrome if insulin is part of the definition [33]. Our study supports as well the use FPI in addition to FPG to identify to children at risk for type 2 diabetes mellitus, because the number of patients with diabetes identified increases up to 100%, while the number of patients identified with IGT are more than doubled from 28 to 64%. For IR, the percentage of patients identified increase from 0 to 100% by adding FPI to the screening.

Although another 36% of children with IGT are not identified if screening with FPI in addition to FPG is used, results largely improve compared to screening with FPG alone (72% of children with IGT not identified). The only possibility to identify IR, IGT and diabetes in all patients is to perform an OGTT including FPI in all patients. However, performing an OGTT in all patients, as suggested by Felszeghy et al [34], leads to substantially higher burden for these patients, as two out of three OGTTs will have a normal result.

Our study shows that a cut-off value of 3.4 for HOMA-IR, is suitable in identifying children at risk of type 2 diabetes mellitus. Although there is much discussion about

the cut-off value for the HOMA-IR, the use of this cut-off value for HOMA-IR combined with FPG ≥ 5.6 mmol/l yields no false negative test results for diabetes, and up to 64% of children with IGT is identified. A lower cut-off value of HOMA-IR may decrease this number of false negatives even more, while this potentially may increase the number of false positives. An increased number of false positive leads to more additional testing with the OGTT, which leads to a higher burden for the patients with associated higher healthcare costs.

There is evidence that insulin resistance, and therefore HOMA-IR, is influenced by puberty and ethnicity [28, 29]. We did not consider these factors in our definition of insulin resistance, which is based on 95thpercentile values in the study of d'Annunzio et al. [29]. The use of specific cut-off values for HOMA-IR for pubertal stage might result in even better identification of children at risk for IGT and type 2 diabetes mellitus.

In conclusion, screening with FPG and FPI identifies all patients with type 2 diabetes mellitus, and significantly more patients with precursors of type 2 diabetes mellitus (IGT (28 to 64%) and IR (0 to 100%)), while the burden for the children is equal to screening with FPG alone.

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M.A. performed data analysis and rewrote the manuscript after a first draft was made by L.K. L.K. performed data collection and wrote the first draft version of the manuscript. All authors discussed study design, data and interpreted the results. S.F, A.B, C.K. and M.V. reviewed and edited the manuscript. All authors take full responsibility for the contents of the manuscript, M.V. is the guarantor of this work. None of the authors reports a conflict of interest.

An abstract of preliminary data regarding prevalence of insulin resistance and the relation with family history has been presented at the Dutch Medicine Days 2011, and has been published in the British Journal of Clinical Pharmacology 2013; 75: 561-562.

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Chapter 5

A follow up study on BMI-SDS and Insulin Resistance in overweight and obese children at risk for type 2 diabetes mellitus

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Abstract

Objectives

To evaluate BMI-SDS, insulin sensitivity, and progression to type 2 diabetes mellitus (T2DM) in children at risk for T2DM approximately 3 years after being diagnosed with overweight/obesity and insulin resistance (measured by Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)).

Methods

Out of 86 invited children, 44 (mean age 15.4 ± 3.6 years) participated. Medical history, physical examination, and laboratory workup were performed.

Results

While the mean BMI-SDS significantly increased from 2.9 to 3.4, the mean HOMA-IR significantly decreased from 5.5 to 4.6 (baseline vs follow up visit). Change in HOMA-IR was only due to a decrease in mean fasting plasma insulin (FPI) (24.1vs 21.1, p =0.073).

Conclusions

Although increase in BMI-SDS in these children is worrisome, the American Diabetes Association recommended screening interval of three years for children at risk for T2DM is not too long based on the fact that none of our study participants developed T2DM.

Introduction

The increasing incidence and prevalence of overweight and obesity in children during the last decades is one of the most important public health concerns because it results in metabolic disturbances like hypertension, dyslipidemia, insulin resistance (IR) and impaired glucose tolerance (IGT), all clustered in the metabolic syndrome [1-5]. In its turn, the metabolic syndrome may lead to micro-and macro-vascular complications and type 2 diabetes mellitus (T2DM). In addition, overweight and obese children are also at increased risk of respiratory, musculoskeletal, and psychological disorders [6].

According to the guidelines of the American Diabetes Association (ADA), children who are overweight and obese and have two or more additional risk factors for diabetes (including family history of T2DM, ethnicity, signs of IR, and maternal history of gestational diabetes) should be screened every three years by measuring fasting plasma glucose (FPG) to identify T2DM [7]. Although it is clear that IR is the most powerful predictor of future development of T2DM [8], little is known about the time interval between the onset of IR and progression to T2DM in overweight and obese children. Therefore, the aim of the present study is to evaluate the body mass index standard deviation score (BMI-SDS), insulin sensitivity, and progression to T2DM in children at risk approximately 3 years after being diagnosed with overweight/obesity and IR.

Research design and methods

Study Participants

Participants for the current study were recruited out of a cohort of overweight (1.1 < BMI-SDS ≤ 2.3) or obese (BMI-SDS > 2.3) children who visited the pediatric obesity outpatient clinic of St. Antonius hospital (Nieuwegein/Utrecht, The Netherlands) between January 2006 and December 2009 [9, 10]. At the pediatric obesity outpatient clinic, children are screened for underlying medical conditions leading to overweight and obesity and they are referred to a lifestyle intervention program outside the hospital. The lifestyle intervention program consists of weekly supervised physical training, behavioural therapy and several sessions with a dietician over an 18 week period. Although all children are referred to the lifestyle intervention program, there was no exact information on participation or completion of the program.

In total 86 overweight and obese children were identified with IR, defined as Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) ≥ 3.4 [10]. These children with IR and additional risk factors of T2DM (including family history of T2DM, ethnicity, and maternal history of gestational diabetes) [8] were invited approximately 3 years

after their initial visit to the pediatric obesity outpatient clinic, to evaluate their current health status (BMI-SDS, insulin sensitivity, and progression to T2DM) and to participate in this observational study.

Medical history and physical examination (baseline and follow up)

During both (baseline and follow up) visits a medical history was taken, including a family history of obesity, diabetes mellitus, cardiovascular diseases, hypertension and hypercholesterolemia. In addition, the use of medication, especially metformin or other glucose lowering medications was evaluated. Physician performed a physical examination including determining the Tanner stage and special attention was paid on signs of acanthosis nigricans [11, 12]. Anthropometric measurements were taken including height (cm), weight (kg). Standing height (cm) was measured to the nearest 0.1 cm with a digital stadiometer (De Grood, DGI 250D) and body weight (kg) was measured on a digital scale (Seca) to the nearest 0.05 kg, with each subject dressed in light clothes and without shoes [11]. Length-standard deviation score (length-SDS) and BMI-SDS were calculated using a web application of the Dutch organization for applied scientific research prevention and healthcare (Toegepast Natuurwetenschappelijk Onderzoek (TNO): “the TNO growth calculator for professionals” (http://groei-web.pgdata.nl/calculator.asp)).

Laboratory investigations (baseline and follow up)

At baseline, all participants underwent an oral glucose tolerance test (OGTT) after an overnight fast with 1.75 gram glucose per kilogram bodyweight with a maximum of 75 gram glucose, according to the hospital protocol. This OGTT included a fasting plasma insulin (FPI) measurement. HOMA-IR was used as a surrogate measure for insulin sensitivity and was calculated as: FPG (mmol/L) * FPI (mU/mL) / 22.5 [13, 14]. The cut off value of HOMA-IR ≥ 3.4 was used to diagnose IR [10]. The OGTT results were interpreted according to the ADA guidelines: impaired FPG defined as FPG ≥ 5.6 mmol/l, IGT defined as 7.8 ≤ 2-hr plasma glucose (PG) < 11.1 mmol/l, and T2DM as FPG ≥ 7.0 mmol/l or 2-hr PG ≥ 11.1 mmol/l [7, 10].

During the follow up visit, fasting blood samples (5 mL) were drawn according to standard practice, and used for the analysis of FPG and FPI. HOMA-IR was calculated to define the current status of insulin sensitivity in the study participants. If FPG was ≥ 5.6 mmol/l and/or HOMA-IR ≥ 3.4, an additional OGTT was advised to evaluate glucose tolerance [10].

Ethical approval

The study was performed at the pediatric department of the St. Antonius hospital, Nieuwegein/Utrecht, The Netherlands. The study protocol was approved by the eth-

ics committee (Verenigde Commissies Mensgebonden Onderzoek (VCMO)) of the St. Antonius hospital, Nieuwegein/Utrecht, the Netherlands (21/11/2011).

Statistical analysis

Data were recorded and entered into a computer system for subsequent tabulation and statistical analysis. Data are presented as mean ± standard deviation (SD) for the continuous variables. The accordance with a normal distribution was confirmed by the Kolmogorov-Smirnov test. Paired sample t-test was used to compare baseline and follow up values of BMI-SDS and HOMA-IR. For FPG and FPI the difference between the values at the baseline and follow up visit was not normally distributed, and the non-parametric Signed Rank test was used to compare baseline and follow up values. Furthermore, baseline characteristics of all children with IR were evaluated to compare study participants and non-participants. The number of study participants was large enough to report a statistical power of > 80% for the used tests by applying a level of significance of 5% [15, 16]. All tests were two tailed, and p-values below 0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 20 (SPSS Inc. Chicago, Illinois, USA).

Results

At baseline, a total of 86 overweight and obese children (40 boys) with IR were identified with mean age of 12.6 [SD ± 2.7] years and mean BMI-SDS of 3.0 [SD ± 0.5]. Out of these 86 children who were diagnosed with IR at baseline, 44 (51%) children (24 boys) agreed to participate in the follow up study. Detailed anthropometric characteristics and laboratory results of all patients with IR at the baseline visit are depicted in table 1. The table shows that the mean age, BMI-SDS, FPG, FPI, and HOMA-IR values of the participants and non-participants were not significantly different.

Table 1. Anthropometry criteria and laboratory findings of all patients with IR at baseline (N = 86) stratified as study participants and non-participants

	Study participants			Non-participants		
Characteristics	Boys (n=24)	Girls (n=20)	Total (n=44)	Boys (n=16)	Girls (n=26)	Total (n=42)
Age (years)						
Mean (±SD)	12.2 (±2.2)	11.6 (±3.3)	11.9 (±2.7)	13.8 (±2.4)	12.9 (±2.8)	13.2 (±2.6)
Median	11.9	12.8	12.2	14.5	12.6	13.9
Range	6.7 – 15.6	5.3 – 16.8	5.3 – 16.8	7.4 – 16.6	3.9 – 16.4	3.9 – 16.6
Weight (kg)						
Mean (±SD)	80.8 (±18.5)	75.3 (±28.1)	78.3 (±23.3)	100.6 (±27.6)	85.7 (±22.0)	91.4 (±25.1)
Length (cm)						
Mean (±SD)	159.6 (±13.1)	153.5 (±16.1)	156.8 (±14.7)	168.0 (±12.8)	160.3 (±15.0)	163.2 (±14.6)
Length-SDS						
Mean (±SD)	0.5 (±0.9)	0.5 (±1.3)	0.5 (±1.1)	0.2 (±0.8)	0.4 (±0.8)	0.4 (±0.8)
BMI						
Mean (±SD)	31.3 (±3.7)	30.9 (±7.5)	31.1 (±5.7)	35.0 (±6.0)	32.7 (±4.8)	33.6 (±5.3)
BMI-SDS						
Mean (±SD)	3.0 (±0.3)	2.8 (±0.7)	2.9 (±0.5)	3.3 (±0.6)	2.9 (±0.4)	3.1 (±0.5)
Median	3.0	2.9	3.0	3.4	2.9	3.1
Range	2.3 – 3.6	1.6 – 3.7	1.6 – 3.7	1.7 – 4.1	2.4 – 4.0	1.7 – 4.1
FPG (mmol/L)						
Mean (±SD)	5.2 (±0.5)	5.1 (±0.5)	5.2 (±0.5)	5.2 (±0.4)	5.2 (±0.5)	5.2 (±0.4)
Median	5.2	5.1	5.1	5.1	5.1	5.2
Range	4.2 – 6.2	4.3 – 6.0	4.2 – 6.2	4.5 – 6.0	4.5 – 6.0	4.5 – 6.0
FPI (mU/mL)						
Mean (±SD)	25.9 (±12.4)	21.9 (±6.0)	24.1 (±10.1)	22.1 (±6.7)	24.9 (±8.7)	23.8 (±8.1)
Median	22.0	19.0	20.5	21.0	22.0	21.5
Range	16.0 – 61.0	15.0 – 31.0	15.0 – 61.0	16.0 – 41.0	16.0 – 48.0	16.0 – 48.0
HOMA-IR						
Mean (±SD)	6.0 (±2.9)	5.0 (±1.5)	5.5 (±2.4)	5.1 (±1.6)	5.7 (±1.9)	5.4 (±1.8)
Median	4.9	4.3	4.7	4.9	4.9	4.9
Range	3.5 – 14.6	3.4 – 8.1	3.4 – 14.6	3.4 – 9.8	3.5 – 10.9	3.4 – 10.9

***Abbreviations**: SDS, standard deviation score; BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.*

Anthropometric characteristics of the study participants at baseline and follow up visits are presented in table 2. The median follow up was 3.3 (range: 1.3-5.8) years. It is apparent from this table that the mean BMI-SDS increased significantly from 2.9 at baseline to 3.4 at follow up ($p < 0.001$). Only 7/44 (16%) children had a BMI-SDS ≤ 2.3 at the follow up visit of whom 3 had a BMI-SDS ≤ 2.3 at baseline as well. The mean

BMI-SDS did not differ significantly between boys and girls at the follow up visit. While Tanner stage was not recorded in 17/44 (39%) participants at baseline, of the recorded Tanner stages, 16/44 (36%) were pre-pubertal (T1) and 11/44 (25%) were in Tanner stage 2, 3 and 4. At the follow up visit the Tanner stage recording of the study participants was complete, showing that 3/44 (7%) were pre-pubertal (T1), 17/44 (39%) in Tanner stage 2-4, and 23/44 (52 %) of the participants had reached the final Tanner stage.

Table 2. Anthropometry criteria of the study participants at the baseline and follow up visits

	Baseline visit			Follow up visit		
Characteristics	Boys (n=24)	Girls (n=20)	Total (n=44)	Boys (n=24)	Girls (n=20)	Total (n=44)
Age (years)						
Mean (±SD)	12.2 (±2.2)	11.6 (±3.3)	11.9 (±2.7)	15.6 (±1.8)	15.1 (±3.3)	15.4 (±2.6)
Median	11.9	12.8	12.2	16.0	15.7	15.9
Range	6.7 – 15.6	5.3 – 16.8	5.3 – 16.8	9.7 – 18.9	8.9 – 19.6	8.9 – 19.6
Weight (kg)						
Mean (±SD)	80.8 (±18.5)	75.3 (±28.1)	78.3 (±23.3)	102.6 (±17.9)	92.1 (±20.6)	97.8 (±19.7)
Length (cm)						
Mean (±SD)	159.6 (±13.1)	153.5 (±16.1)	156.8 (±14.7)	175.5 (±9.3)	166.1 (±10.3)	171.2 (±10.8)
Length-SDS						
Mean (±SD)	0.5 (±0.9)	0.5 (±1.3)	0.5 (±1.1)	0.2 (±0.5)	0.4 (±1.8)	0.2 (±1.0)
BMI						
Mean (±SD)	31.3 (±3.7)	30.9 (±7.5)	31.1 (±5.7)	33.3 (±5.5)	33.4 (±7.0)	33.4 (±6.2)
BMI-SDS						
Mean (±SD)	3.0 (±0.3)	2.8 (±0.7)	2.9 (±0.5) *	3.4 (±0.7)	3.3 (±0.9)	3.4 (±0.8) *
Median	3.0	2.9	3.0	3.5	3.4	3.5
Range	2.3 – 3.6	1.6 – 3.7	1.6 – 3.7	1.8 – 4.5	1.9 – 4.9	1.8 – 4.9

Abbreviations: BMI; Body Mass Index; BMI-SDS: BMI-Standard Deviation Score;
* There is a significant difference between baseline and follow up values ($p < 0.05$).

Laboratory results of baseline and follow up visits are presented in table 3. There was no significant difference between mean baseline (5.2 mmol/l) and follow up (5.1 mmol/l) FPG values ($p = 0.808$). At follow up, impaired FPG was observed in 8 children (7 boys). Four out of these eight children were already diagnosed with impaired FPG at baseline. For FPI, there was a decrease from 24.1 to 20.1 mU/mL, even though the difference was not statistically significant ($p = 0.073$).

Table 3. Laboratory findings of the study participants at the baseline and follow up visits

	Baseline visit			Follow up visit		
	Boys (n=24)	Girls (n=20)	Total (n=44)	Boys (n=24)	Girls (n=20)	Total (n=44)
FPG (mmol/L)						
Mean (±SD)	5.2 (±0.5)	5.1 (±0.5)	5.2 (±0.5)	5.2 (±0.5)	5.0 (±0.4)	5.1 (±0.5)
Median	5.2	5.1	5.1	5.1	5.1	5.1
Range	4.2 – 6.2	4.3 – 6.0	4.2 – 6.2	4.4 – 6.5	4.3 – 5.7	4.3 – 6.5
FPI (mU/mL)						
Mean (±SD)	25.9 (±12.4)	21.9 (±6.0)	24.1 (±10.1)	16.5 (±10.1)	23.8 (±14.6)	20.1 (±12.7)
Median	22.0	19.0	20.5	16.5	18.0	17.0
Range	16.0 – 61.0	15.0 – 31.0	15.0 – 61.0	2.0 – 41.0	8.0 – 62.0	2.0 – 62.0
HOMA-IR						
Mean (±SD)	6.0 (±2.9)	5.0 (±1.5)	5.5 (±2.4) *	4.0 (±2.7)	5.2 (±3.1)	4.6 (±2.9) *
Median	4.9	4.3	4.7	3.6	3.9	3.7
Range	3.5 – 14.6	3.4 – 8.1	3.4 – 14.6	0.4 – 10.6	1.9 – 12.1	0.4 – 12.1

Abbreviations: FPG: Fasting Plasma Glucose; FPI: Fasting Plasma Insulin; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; SD: Standard Deviation

* There is a significant difference between baseline and follow up values ($p < 0.05$).

Concerning the HOMA-IR which was higher than 3.4 by definition at baseline in all participants, the mean value decreased significantly by 16% from 5.5 at baseline to 4.6 at follow up ($p = 0.05$, table 3). At follow up, 26 out of 44 (59%) study participants (13 boys) remained insulin resistant (HOMA-IR ≥ 3.4). In the participants (18/44) with normal insulin sensitivity (HOMA-IR < 3.4) at follow up, mean BMI-SDS was significantly lower than in the insulin resistant group (mean BMI-SDS 2.9 vs. 3.7, respectively, $p = 0.001$). However, both groups with and without IR, had an increased BMI-SDS at the follow up visit compared with baseline. The 7 overweight children (BMI-SDS ≤ 2.3) had a lower mean HOMA-IR than the children with BMI-SDS > 2.3 (mean HOMA-IR 3.3 and 4.8, respectively).

Nine out of 44 study participants used metformin (55.5 % boys, aged 10.6 to 19 years). Although mean BMI-SDS in metformin users was lower than non-users (3.1 versus 3.4), mean HOMA-IR in metformin users was higher than the other group (6.2 versus 4.1).

Mean FPG, FPI and HOMA-IR did not differ significantly between boys and girls at the follow up visit, however the mean FPI was considerably higher in girls compared to boys ($P = 0.072$) (table 3). Although mean BMI-SDS increased significantly in boys ($p = 0.001$), there was a significant decrease in the mean FPI ($p = 0.028$) and HOMA-IR ($p = 0.05$) from the baseline visit to follow up. In girls BMI-SDS, FPI, and HOMA-IR values increased during the study period but the increase was only significant for BMI-SDS ($p = 0.001$). None of the study participants were diagnosed with T2DM after a median follow up of 3.3 years.

Discussion

In this study, we evaluated BMI-SDS, insulin sensitivity, and progression to T2DM in children at risk for T2DM approximately 3 years after being diagnosed with overweight or obesity and IR.

We observed a significant increase of 17.0% in mean BMI-SDS from baseline to the follow up visit ($p < 0.001$). However, the HOMA-IR (as surrogate measure for IR) decreased significantly by 16.0%, even though the mean HOMA-IR remained above the cutoff value for IR (≥ 3.4). This change in HOMA-IR can be mainly explained by the substantial reduction in mean FPI from the baseline to the follow up visit ($P = 0.073$) since the mean FPG did not change significantly (table 3). This reduction in FPI is probably due to progression to the final Tanner stage of the participants (23/44 Tanner stage 5) at the follow up visit, because insulin sensitivity increases at the end of puberty (T5) to pre-pubertal levels (T1) [17]. Particularly in boys a substantially lower FPI was observed compared to the values in girls ($P=0.072$), which is in line with the findings of Moran et al, who studied the effects of Tanner stage on insulin resistance in children who underwent an euglycemic clamp study [17].

At follow up, participants were screened for the development of T2DM according to the ADA recommendation in which FPG was used for screening and an OGTT was performed if FPG ≥ 5.6 mmol/l [7]. Eight out of forty-four (18%) participants were diagnosed with impaired FPG and out of them 4 were already diagnosed at baseline with impaired FPG. In those 4 children who agreed to have the recommended OGTT, no signs of IGT or T2DM were observed. An intriguing question is whether we may have missed children with T2DM based on screening on FPG alone [10]. In our previous project of evaluating a cohort of 311 overweight and obese children, in all of whom an OGTT was performed, we observed that screening according to the ADA recommendation on the basis of using FPG test and an additional OGTT if FPG ≥ 5.6 mmol/l would have resulted in 1 child unidentified as T2DM patient and 7 children with glucose tolerance, because in those 8 cases FPG was < 5.6 [10]. Although in the current study no children were diagnosed with T2DM, 4 OGTT were not performed and the diagnoses might have been missed in children with normal FPG as explained above.

We could not confirm the findings of Reinehr et al. who concluded that failure to achieve weight loss in obese children is associated with a decrease in insulin sensitivity [18]. Reinehr et al measured the effect of weight reduction on the improvement of insulin sensitivity (measured as any change in HOMA-IR and quantitative insulin sensitivity check index (QUICKI)) in obese children after one year. Their study population consisted of 57 obese children and adolescents (46% boys, with a median age of 10 (range 6-14) years) and 60% of them were in pre-pubertal stage defined as Tanner stage 1. In our study the median age of the participants at baseline was 12.2 (range 5.3

– 16.8) years which was substantially higher than children in Reinehr's study. Reinehr et al. studied the effect of weight reduction on the improvement of insulin sensitivity after one year while we studied BMI-SDS and insulin sensitivity after a median time interval of 3.3 years. The consequence of this time difference is that more than 50% of our children progressed to the final stage of puberty (T5) in which insulin sensitivity returns to pre-pubertal levels, explaining the increase in insulin sensitivity despite increase in BMI-SDS [18].

This study has some limitations that must be addressed. Our main limitation is that only 51% of children at risk for T2DM agreed to participate in the study which resulted in the limited number of participants, however there was sufficient statistical power to detect the differences between studied values but might not be enough to detect small differences. We didn't find any significant difference between study participants and non-participants on mean age, BMI-SDS, FPG, FPI and HOMA-IR values at baseline. Therefore, there was no selection of the participants and probably they are a good reflection of the entire group of children at risk for T2DM at baseline. We decided to assess the medical records of the non-participants to check for any evidence on the development of T2DM at the pediatric or internal diabetic out-patient clinic. From these records at the diabetic out-patient clinic in our hospital, there was no evidence for T2DM for the non-participant group. Patients ≤ 18 years old will always be seen at the pediatric diabetic out-patient clinic, whereas patients > 18 years old might be followed up for T2DM by the general practitioners. Since the mean age of the non-participants at baseline was 13.2 (±2.6) years, the vast majority of the non-participants were registered at the pediatric diabetic out-patient clinic.

Since data on Tanner stages at baseline were only available in 39% of the participants, we could not take into account if the observed IR at baseline was related to the Tanner stage [17]. However, data on Tanner stages were recorded in all participants at the follow visit, showing that 52% of the participants reached the final Tanner stage. Consequently, the decrease in IR observed at follow up is probably due to the effect of increased insulin sensitivity at the final Tanner stage.

Our variable follow up time of 1.3 to 5.8 years is a limitation of our study (16 children had follow up time of less than 3 years), because it is possible that these children (with follow up time of less than 3 years) develop T2DM if the follow up time for them was longer. Additionally, children < 10 years were included, despite the fact that the ADA recommendation is meant for children ≥ 10 years of age. At baseline 11 participants were < 10 years of age, and at follow up still 3 participants were < 10 years of age. It is known that development of T2DM increases with age and therefore the priori chance to develop T2DM is lower in these children aged less than 10 years.

In conclusion, the current study in children at risk for T2DM showed that after a follow up of approximately 3 years, insulin sensitivity increased significantly and none of

the children developed T2DM. While the steady increase in BMI-SDS in these children is worrisome, it seems that the ADA recommended screening interval of three years for T2DM in children at risk is not too long based on the fact that none of our study participants developed T2DM.

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Section 3

Treatment of obese children with insulin resistance

Chapter 6

Metformin study

Chapter 6a

METFORMIN: an efficacy, safety and pharmacokinetic study on the short-term and long-term use in obese children and adolescents

Study protocol of a randomized controlled study

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ABSTRACT

Background

The prevalence of childhood obesity is rising, as well as insulin resistance, increasing the risk of diabetes mellitus type 2. To prevent these complications, lifestyle intervention is the corner stone in treatment. However, long-term efficacy of lifestyle intervention is questionable. In addition to lifestyle intervention, pharmacological treatments have been explored. Metformin has been shown to be moderately effective to reduce BMI in obese adolescents with hyperinsulinemia. However, data on pharmacokinetics and long-term efficacy and safety are lacking as well as an evidence based dosing regimen for this age group. The primary objective of the Metformin study is to determine the effect of adding Metformin treatment to lifestyle-intervention in reducing BMI in obese adolescents with insulin resistance. In addition, pharmacokinetics of Metformin in obese adolescents will be studied.

Methods/Design

The Metformin study is a multi-centre prospective study, that consists of two parts of each 18 months: a double-blind randomized placebo-controlled trial (part1) and an open-label follow up study (part 2). During part 1 the participants will be given Metformin 1000 mg or placebo twice daily and will be offered a lifestyle intervention programme. One hundred forty-four participants will be included, 72 in each arm. Primary endpoints are reduction in body mass index, insulin resistance and percentage body fat.

Discussion

This study will provide data on short and long-term efficacy and safety of Metformin and on the pharmacokinetics of Metformin in obese adolescents.

Trial registration

ClinicalTrials.gov number NCT01487993; EudraCT nr. 2010-023980-17.

Background

The prevalence of obesity in adolescents is increasing rapidly, having a significant impact on both physical and psychosocial health [1]. Currently, the worldwide prevalence of obesity in children and adolescents is 2% to 3%, using the International Obesity Taskforce standard definition for paediatric obesity in children and adolescents 5 to 17 years of age [2]. In the Netherlands, the prevalence of obesity in children and adolescents (4–15 years) of Dutch descent is 1.8% in boys and 2.2% in girls. In Turkish boys and girls, these numbers are higher, 8.4% and 8.0%, respectively [3].

In obese children and adolescents, insulin resistance, impaired fasting glucose, impaired glucose tolerance, dyslipidaemia, and hypertension occur with increased frequency [4-6]. In addition, several medical conditions, such as poor pulmonary function, hepatic steatosis, sleep apnoea, and orthopaedic complications, are associated with obesity [1,5,7-8]. These medical conditions often persist into adulthood and will result in substantial psychosocial and somatic morbidity, with loss of school or working days [9].

Current treatments for obesity are lifestyle, drug, and surgical interventions [10-12]. Behavioural lifestyle intervention can produce significant reduction of obesity in children and adolescents [13]. However, the efficacy of lifestyle intervention programs on body mass index (BMI) and all related complications on the long-term are questionable, taking into account the high drop out and the frequent relapse of obesity in this group of patients. Therefore, in clinical practice, adding a pharmacological agent to conventional treatment is often considered. Three agents have been studied: orlistat, a gastrointestinal lipase inhibitor, sibutramine, a serotonin and noradrenalin re-uptake inhibitor, and metformin, an insulin sensitizing agent [10-12, 14]. Both orlistat and sibutramine have been shown to have an additional reducing effect on the absolute BMI in children and adolescents, yet medication-related adverse effects, such as tachycardia, hypertension, arrhythmia, and gastro-intestinal tract symptoms, were frequently reported [11, 15-16]. Efficacy of metformin was investigated in hyperinsulinemic, obese adolescents by Park et al. in a systematic review [12]; they concluded that metformin is moderately efficacious in reducing BMI and insulin resistance in the short term (less than 6 months). The authors stated that large long-term studies are needed to establish the role of metformin in the treatment of obese adolescents. This conclusion is based on studies in obese adults without Type 2 diabetes mellitus (T2DM), in which metformin has been shown to prevent progression from impaired fasting glucose and impaired glucose tolerance to T2DM [17, 18]. Although results in obese children and adolescents are sparse, these results, and the observed benefits in adults, have led to increased off-label use of metformin in obese children and adolescents with, and even without insulin resistance [19-26].

In conclusion, obesity in adolescence is increasing rapidly, with large medical and psychosocial sequelae. While standard treatment is lifestyle intervention, metformin is often added to this treatment, despite the lack of proper randomised trials on efficacy and safety, particularly upon prolonged treatment [12, 27-31]. While metformin is licensed in adolescents for the treatment of T2DM, it is not yet licensed for obese children with and without insulin resistance.

Methods/design

Objectives

This study has four main objectives: The primary objective of the METFORMIN study is to determine the efficacy of metformin in combination with lifestyle intervention in obese adolescents with insulin resistance versus placebo with lifestyle intervention after 18 months in reducing BMI and insulin resistance. The secondary objective is to determine the safety and tolerability of metformin in obese adolescents with insulin resistance. The tertiary objective is to study the population pharmacokinetics (PK) of metformin in obese adolescents. Finally, the quaternary objective is to determine the long-term (36 months) efficacy and long-term safety and tolerability of metformin in obese adolescents with insulin resistance.

Other objectives are to compare values of body fat measured using bio-impedance with values of body fat measured using dual energy X-ray absorptiometry (DEXA scan), and to compare insulin sensitivity measured by the Whole Body Insulin Sensitivity Index with insulin sensitivity calculated by Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) in obese children and adolescents. Furthermore, arterial stiffness will be measured and evaluated over time.

Design

The metformin study is a multicentre study, divided in two parts, both of 18 months. The first part is a randomized, double-blind placebo controlled trial, with two parallel groups. At study entry, participants are randomized to metformin or placebo for 18 months. All participants will be offered a lifestyle intervention program. This program consists of supervised physical training twice weekly, and individual dietary advice during hospital visits. Participants will visit the hospital 9 times during this part of the study. Between these visits, monthly telephone calls are made. The second part of the study is an open label, follow up study. Participants who remain obese and insulin-resistant at entrance of the second part, can choose between metformin treatment and no medication. Participants who do not meet these criteria, do not use medication in this part of the study. Therefore, after the follow up study there are four arms:

participants with metformin in both parts, metformin in part 1 and no medication in part 2, placebo in part 1 and metformin in part 2, and placebo in part 1 and no medication in part 2 (Figure 1).

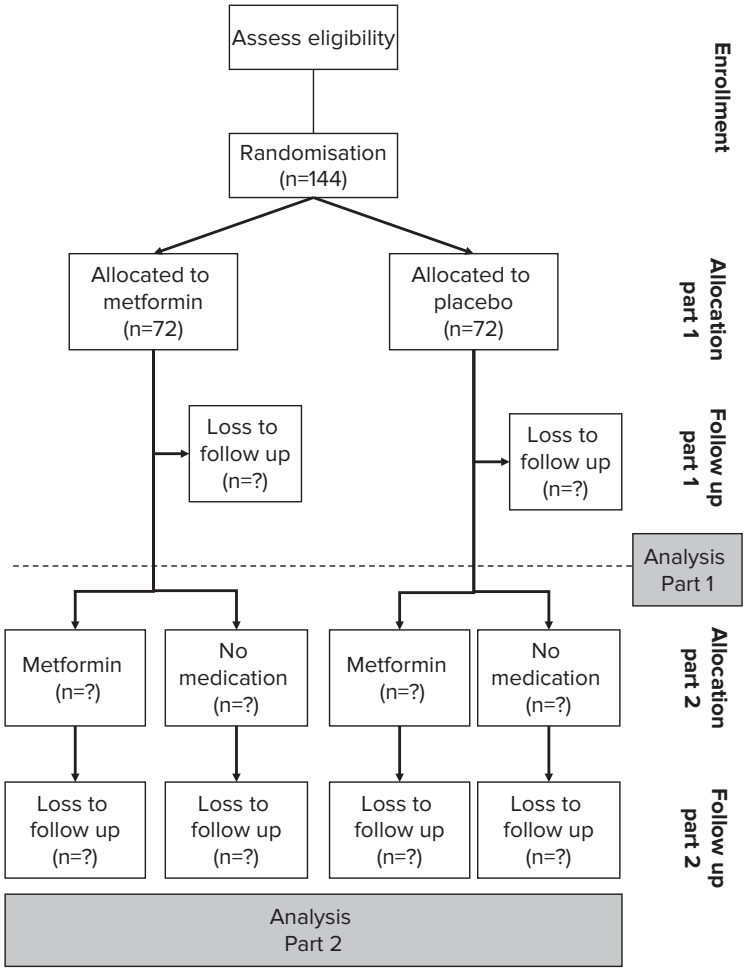

Figure 1. Flowchart Metformin study

During part 2, no supervised physical training is offered. Participants using Metformin will visit the hospital six times, participants not using medication will have three hospital visits and three phone calls. Participants using Metformin are seen more frequently to monitor potential adverse events.

The study protocol has been approved by the Medical Ethical Committee of the St Antonius Hospital, Nieuwegein, the Netherlands. The study is registered in the Clinical Trials register (ClinicalTrials.gov numberNCT01487993).

Participants

Recruitment of participants takes place at the paediatric outpatient clinics of the study centres. Patients are eligible for this study if they meet the inclusion and exclusion criteria as listed in Table 1. Informed consent will be obtained from all participants/ parents or caretakers.

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age: 10-16 years	Presence of T2DM, PCOS or endocrine disorders treated with corticosteroids
Obesity defined as BMI-SDS >2.3	Height <-1.3 SD of target height
Insulin resistance defined as HOMA-IR ≥ 3.4	Syndromal disorders with or without mental retardation
Caucasian descent	Pregnancy
Informed consent signed by parents and participant	Use of antihyperglycaemic drugs, ritonavir or ACE-inhibitors
	(History of) alcohol abuse
	Impaired renal or hepatic function (Renal function defined as GFR < 80 ml/min; GFR= 40 x length (cm) / serumcreatinin (µmol/l). Hepatic function: ALAT >150% of normal value for age)
	Insufficient knowledge of Dutch language

Sample size

A power analysis for reduction in BMI (primary endpoint) and for reduction of insulin resistance calculated by the HOMA-IR has been performed. For BMI, a sample size of 47 participants per group (metformin and placebo) is sufficient to detect a change in BMI of 2.94% with 90% power. Sample size for HOMA-IR was calculated using a simulation based on retrospective data available from our obesity out-patient clinic. Group sample sizes of 60 patients in both groups were found sufficient for the detection of a difference of 1.6 with a significance level of 0.05. To prevent inadequate power due to drop out of participants, 20% more patients will be included. This means a total amount of 144 children and adolescents have to be included in the study.

Randomisation

Subjects will be assigned to metformin or placebo in accordance with a randomisation schedule generated by the department of Clinical Pharmacy of the St Antonius Hospital, using PASW Statistics 18.0. Randomisation will be done in blocks of 20 subjects assuring a balanced study after each 20 consecutive inclusions per research site. All research staff is blinded for treatment allocation during the time of the study. Randomisation lists will be kept under secured access in the clinical pharmacy department of both participating hospitals.

The research physician assigns included participants to consecutive study numbers, which correlate with the randomisation and medication number.

Breaking of the study blind

The study blind will be broken after all participants have finished part 1 of the study. In case of emergencies (serious adverse events, suspected unexpected serious adverse reactions), the blind will be broken after consultation of the principal investigator. Subsequently, these events will be reported to the Medical Ethical Committee.

Intervention

Metformin

After randomisation, all participants will receive either metformin 500 mg tablets or identical placebo tablets. Medication is given according to an increasing dosage regimen. In week 1, participants take 1 tablet daily. Every week, dosage increases with 1 tablet, reaching a maximum of 4 tablets in week 4. This maximum dose will be administered till the end of part 1.

In case the participant develops gastro-intestinal symptoms, dosage will be reduced to the last well-tolerated dosage. Participants will be asked to return remaining study medication every visit. Pill counts will be performed by a research assistant. During the follow up study, metformin will be administered according to the dosage regimen of part 1.

Physical training

During part 1 of the study, physical training will be offered in groups, supervised by a physiotherapist. Training sessions will be twice weekly, and last for one hour. The main goals are creating pleasure in physical exercise, improvement of endurance and coordination. Attendance at the training will be recorded. All participants will perform a standardised fitness test at study entry, halfway part 1 and at the end of parts 1 and 2.

Statistical analysis

Data will be analysed using IBM SPSS Statistics. Baseline data will be reported as descriptive statistics. Normally distributed data will be reported as mean±SD and nonparametric data as median (range).

Efficacy of metformin

To assess the effect of metformin versus placebo, the Students T test will be used to compare means of normally distributed data and the Mann-Whitney U test to compare nonparametric data. The χ^2 test will be used for dichotomous outcomes (the development of impaired fasted glucose, impaired glucose tolerance, T2DM, and the presence of micro- and macro- vascular complications versus the study groups). General linear

models (analysis of repeated measures) or mixed models (if too much data is missing) will be used to determine the therapeutic effect of the drug. After part 2 of the study, comparisons will be made between the four subgroups (metformin in part 1 and part 2; metformin in part 1 and no medication in part 2; placebo in part 1 and metformin in part 2; placebo in part 1 and no medication in part 2) (Figure **1**).

Safety and tolerability

Safety will be reported as the amount of cases in which hepatic and renal functions exceed safety limits. Tolerability will be reported as descriptive statistics of adverse effects in relation with the achieved dosage level.

Pharmacokinetics

All observed metformin plasma concentrations will be analysed using nonlinear mixed effects modelling to develop a population pharmacokinetics/pharmacodynamics (PK/PD) model. Using this modelling approach, infrequently obtained samples and observations in the clinical situation can be utilized to analyse determinants of variability in drug response [32-34]. In both the population PK (drug concentrations) and PD (efficacy and safety endpoints) models, the influence of age, bodyweight, BMI, percentage of body fat, gender, Tanner stage, and genetic constitution will be evaluated resulting in individualized dosing regimens. In addition to demographic parameters, the influence of genetic variation in the SLC47A1 gene, which may play an important role in the pharmacokinetics of metformin, is studied [35-37]. If other relevant genes are discovered during the metformin study, these genes will also be determined.

General procedures and measurements

Table 2 shows which measurements are performed during parts 1 and 2 of the study. In Table 3, blood sampling per visit is specified. Participants in both the metformin and placebo group undergo the same procedures and measurements. Two additional measurements, namely indirect calorimetry, a DEXA scan, and an additional physical test, are performed in study participants included at the Jeroen Bosch hospital study site.

Adverse events and co-medication

During scheduled phone calls and hospital visits, participants and/or their parents will be asked about adverse events (AEs) and use of co-medication during the past week(s). Collected data for AEs are: start date, stop date, description of AE, kind of action taken regarding study medication (continued, dose adjusted or interrupted, permanently discontinued), therapy for AE, severity of AE (mild, moderate, severe, life threatening, death), whether the AE was expected, whether the AE was serious,

Table 2. Measurements during part 1 and 2 of the study

Visit	Measurements														
	Adverse Events	Co-medication	History	Physical examination	Blood sampling	Urine sampling	OGTT	Metformin daycurve	Bio-impedance	PWV	Fitness test	IWQOL-questionnaire	Dietary diary	Calorimetry[c]	DEXA-scan[c]
Part 1															
0			x	x[d]	x	x	x		x	x	x	x	x	x	x
1[a]	x	x													
3	x	x			x										
5	x	x			x										
9[a]	x	x													
13	x	x		x	x										
17[a]	x	x													
21[a]	x	x													
25	x	x		x	x										
29[a]	x	x													
33[a]	x	x													
37	x	x		x[d]	x		x	x	x		x	x	x		x
41[a]	x	x													
45[a]	x	x													
49	x	x		x	x										
53[a]	x	x													
57[a]	x	x													
61	x	x		x	x										
65[a]	x	x													
69[a]	x	x													
73	x	x		x[d]	x	x	x		x	x	x	x	x	x	x
Part 2															
86[b]	x	x													
98	x	x		x	x										
110[b]	x	x													
122	x	x		x	x										
134[b]	x	x													
146	x	x		x	x	x	x		x	x	x	x	x		x

[a] phone calls; [b] visit for Metformin user, phone call for non-user; [c] performed in subsample in one of the study centres; [d] extensive examination.

and whether the AE occurred during study treatment. Collected data for concomitant medication are: start date, stop date, type, and dose of medication, and duration of use (single dose, intermittent dosage, chronic use). For chronically used medication, changes in prescription, i.e., dose and frequency, will be checked.

History

At the first visit, an extensive history will be taken. Duration of pregnancy, birth weight, neonatal feeding, and the presence of diabetes gravidarum during pregnancy of the participants is questioned. Further, information on diseases, hospital admissions, and use of medication, alcohol, and tobacco is collected. Regarding family history, data on hypertension, obesity, hypercholesterolemia, cardiovascular disease (myocardial infarction, stroke, transient ischemic attack, peripheral arterial occlusions), and diabetes mellitus in first (parents) and second degree (grandparents, brothers, sisters) family members are collected. Level of education of participants and both parents is recorded, as well as height and weight of both parents. Girls will be asked whether and when they had their menarche.

Physical examination and anthropometrics

Every visit, except at week 3 and 5, anthropometric measurements are performed. These include: height, weight, and waist, hip, neck, and right wrist circumference.

Height will be measured with a digital stadiometer in the St Antonius Hospital, and with an analogue stadiometer at the Jeroen Bosch hospital. Height will be recorded to the nearest 0.1 cm.

Weight is recorded on a digital scale in all study centres, and is recorded to the nearest 0.05 kg. Waist, hip, neck, and right wrist circumferences are measured with the same tape-measure in both centres, by the research physician. All measurements are recorded to the nearest 0.1 cm. Waist circumference is measured at the level of the navel and hip circumference at the level of the anterior superior iliac spine. Neck circumference is measured three times, the smallest circumference is recorded. Circumference of the right wrist is measured at the level of Lister's tubercle.

Blood pressure and heart rate are measured with subjects in a seated position using a cuff appropriate for the participants' arm circumference. In both study centres, blood pressure will be measured electronically.

During the visits in weeks 0, 37, 73, and 146, an extended physical examination is performed by the research physician. This examination includes auscultation of heart, lungs, and abdomen, and abdomen palpation. Abnormal findings will be recorded. The skin will be examined for the presence of acanthosis nigricans, striae, acne, and, in girls, hirsutism. For all participants, pubertal stage according to the classification of

Tanner will be recorded. In girls, this includes development of the breasts and pubic hair; in boys, stage of pubic hair and testicular volume is estimated.

Blood sampling

Blood samples will be collected by venapuncture or, in case of an OGTT, by venous cannula during scheduled hospital visits (Table 2). Before venapuncture or insertion of the venous cannula, local anaesthetics will be applied to the skin (Xylocaine spray, 100 mg/mL, AstraZeneca bv). The specification of measurements per blood sample is shown in Table 3. All samples will be collected by research staff and analyzed in the clinical laboratory of the St Antonius Hospital.

Urine sampling

Urine samples will be collected at three time points. The sample will be analysed for the concentration of creatinine and micro-albumin, and tested for protein. Additionally, in the first urine sample of female participants, a pregnancy test is performed.

Oral glucose tolerance test

Oral glucose tolerance tests (OGTT) will be performed at four time points. Participants will come to the hospital after an overnight fast. After insertion of a venous cannula and after obtaining the baseline blood sample (t=0), participants will receive a solution of glucose: 1.75 g/kg body weight with a maximum of 75 g, dissolved in 200 to 300 mL of water. Blood samples will be taken for glucose and insulin concentrations at 30, 60, 90, and 120 minutes after ingestion of the glucose solution.

Table 3. Specification of measurements per blood sample.

Visit	Measurements											
	Fasted glucose	Fasted insulin	OGTT[a]	HbA1c	Blood count, indices	Urea, creatinin	Alat	Lipid profile	Vitamin B12	CRP	DNA	Metformin
Part 1												
0			x	x	x	x	x	x	x	x	x	x
3												x
5												x
13	x	x				x	x					x
25	x	x		x	x	x	x		x			x
37			x			x	x					x[b]
49	x	x		x	x	x	x		x			x
61	x	x				x	x					
73			x	x	x	x	x	x	x	x		x
Part 2												
98	x	x		x	x	x	x		x			
122	x	x		x	x	x	x		x			
146			x	x	x	x	x	x	x	x		

[a] 'Glucose and insulin at t=0, 30, 60, 90 and 120' [b] Metformin daycurve (baseline sample and five subsequent samples)

Metformin day-curve

During the OGTT of week 37, a metformin day-curve will be performed. A baseline sample is collected after insertion of the venous cannula (t=0). Participants will take the oral study medication after ingestion of the glucose solution for the OGTT. Afterwards, samples for serum concentration of metformin are taken at 60, 120, 240, 360, and 480 minutes. Samples will be stored in the toxicological laboratory of the St Antonius Hospital at -20°C until analysis is to be performed.

Body composition

In order to obtain data on body composition, bio-impedance analysis is performed at regular time-points. In all participating hospitals the same leg-to-leg bio-impedance analysis measurement will be performed using a Tanita BC-420MA body composition analyser (Tanita Corporation, Tokyo, Japan). Participants will stand barefoot on the metal plates of the scale. A correction for weight of clothes (1 kg), sex, age, and height are entered manually into the Tanita system. After analysis, a print out with body weight, estimated percentage of body fat, fat free mass, and total body water is made.

In study participants of the Jeroen Bosch Hospital, a DEXA scan will be performed additionally. The DEXA scan will show the fat distribution and will give the amount of lean and total body mass.

Arterial stiffness

Arterial stiffness is assessed by pulse wave velocity and augmentation index. The pulse wave velocity measurement is a non-invasive measurement using ultrasound and is measured using the SphygmoCor (Model SCOR-Px, Software version, 7.01; AtCor Medical Pvt. Ltd, Sydney, Australia). Both pulse wave velocity, which correlates with stiffness of the aorta, and augmentation index, reflecting endothelial function, will be used to monitor the development of vascular complications. The measurements will be performed with participants in a supine position. Before and after the measurement, blood pressure will be recorded. All pulse wave velocity measurements will be performed twice. The augmentation index will be calculated in a pulse wave analysis measurement from the right radial artery. For calculation of the pulse wave velocity, the distance between the sternal notch and the expected site for recording at the right carotid artery and right femoral artery will be measured with a tape measure to the nearest 0.1 cm. This distance is entered into the computer before measurements are performed.

Fitness test

Physical fitness of the participants will be monitored using the modified Shuttle walking test for endurance, while static and dynamic balance tests, according to Movement-ABC will be used to test coordination and strength. All tests will be performed by trained physiotherapists.

Quality of life

Body weight-related quality of life is measured using the Impact of Weight on Quality of Life-Kids (IWQOL-Kids) questionnaire [38]. This questionnaire consists of 27 items in four domains: physical comfort, body esteem, social life, and family relations; a validated Dutch translation is used [39]. The questionnaire will be handed out during the OGTT, or sent to the participants' home address to be filled in advance. Participants are free to choose whether they answer the questionnaire in the hospital or at home.

Diet

Participants will be asked to complete a 3-day dietary diary three times during part 1 of the study, and once during the second part of the study. During the following visit, the diary will be discussed with participants. Advice on a healthy diet is given by the research physician based on this diary. If diet advice is not sufficient a dietician will be consulted.

Measurements performed in a subsample

Indirect calorimetry

This additional test will be performed in one of the participating study centres (Jeroen Bosch Hospital). All of the metabolic processes that occur in the body result ultimately in the production of heat. Direct calorimetry measures the heat production directly; indirect calorimetry makes use of the assumption that all energy-releasing reactions in the body ultimately depend on the utilization of oxygen. Indirect calorimetry, using the ventilated hood principle, is a measurement in which the subject has to lay down in a thermoneutral environment after an overnight fast. In a time span of 15 min, the subject needs to lay still while oxygen uptake and dioxide production of the body are measured. This measurement is an estimate of basal metabolic rate. Calorimetry will be performed when the subject is in fastened condition.

DEXA scan

In participants included at the Jeroen Bosch hospital, an additional DEXA scan will be performed. This scan will show fat distribution and will accurately determine lean and total body mass.

VO2-max test

Maximal oxygen uptake will be measured using a standardized test for maximal aerobic power: the VO2 max test. The VO2 max test is a single continuous 3- to 5-minute submaximal effort test on a stationary bicycle. It consists of progressive increments in effort (graded exercise) to the point at which the subject will no longer continue to exercise.

Outcome measures

Along with the multiple objectives in this study, there are multiple outcome measures.

Primary endpoints and measures

Primary study endpoint for efficacy of metformin is the reduction in BMI standard deviation score, which is calculated from the anthropometric measurements, and the reduction of insulin resistance calculated by the HOMA-IR.

Secondary outcome measures

Secondary outcome parameters for safety of metformin treatment are hepatic and renal function tests, and concentration of vitamin B12. For tolerability, the number of adverse events (in relation to the achieved dose level) is the outcome parameter.

Tertiary outcome measures

Tertiary outcome parameters are the PK parameters of metformin in obese children and adolescents. These parameters are estimated using population PK-PD modelling techniques in which a comprehensive covariate analysis will be performed allowing to account for variability in PK parameters on the basis of individual characteristics such as age, bodyweight, BMI, percentage of body fat, gender, Tanner stage, and genetic constitution.

Quaternary outcome measures

Quaternary outcome parameters for long-term efficacy and long-term safety and tolerability (36 months) of metformin are similar to the primary and secondary outcome measures. In addition, the percentage of patients that has developed impaired fasting glucose, impaired glucose tolerance, and T2DM is evaluated. Furthermore, the development of micro- and macro-vascular complications is assessed, by measuring micro-albuminuria and arterial stiffness.

Other outcome measures

Other outcome parameters are values of body fat measured by bio-impedance compared to values of body fat measured using DEXA scan, insulin sensitivity measured by the whole body insulin sensitivity index compared with insulin sensitivity calculated by HOMA-IR, HbA1c, β-cell function calculated by HOMA-β%, oral disposition index, insulin secretion calculated by the insulinogenic index, physical fitness measured by validated fitness tests, basal metabolic rate measured by calorimetry, and quality of life measured by validated quality of life questionnaire.

Limitations of the study

The major limitation of our study is in the open-label, second part of the study. In this part, participants who are still eligible for receiving metformin, i.e., who remain insulin resistant and obese, may choose between taking the drug or not. This will provide a bias in the second part of the study, as participants motivated to lose weight are more likely to choose metformin compared to non-motivated participants. Possibly, this will cause an overestimation of the efficacy of metformin during the second part off the study. This will be taken into account when analysing the results of the second part of the study. The open label construction will not influence adverse reactions to the drug, therefore making it possible to draw firm conclusions about the 36-month safety and tolerability of metformin.

Discussion and trial status

This article provides the detailed study protocol of our metformin study, including all objectives and outcome measures, a description of the intervention and all study procedures. After completion of the study, the gap in knowledge of long-term effects of metformin on body weight can be filled. Safety and tolerability of metformin use up to 36 months will be investigated. Furthermore, pharmacokinetics of metformin in obese children and adolescents will be known.

Currently, the trial is ongoing and recruitment of participants continues. To date, 60 participants have been included, of whom 13 finished the first part of the trial. Results on efficacy, safety, and tolerability of 18 months of metformin treatment in obese children and adolescents with insulin resistance are expected in summer 2015. First results of PK analysis are expected in autumn 2014. Long-term efficacy, safety, and tolerability results are expected early 2017. These findings will be published in international peer reviewed journals.

List of abbreviations

AE(s) – Adverse event(s); BMI(-SDS) – Body mass index (-standard deviation score); DEXA – Dual-energy X-ray Absorptiometry; HOMA-β% - Homeostasis Model Assessment for Beta cell function; HOMA-IR - Homeostasis Model Assessment for Insulin Resistance; IWQOL – Impact of Weight on Quality of Life; OGTT – Oral Glucose Tolerance Test; PD – Pharmacodynamics; PK – Pharmacokinetics; T2DM – Type 2 Diabetes Mellitus

Competing interests

None of the authors reports a conflict of interest.

Authors' contributions

MV, CK and EM designed the study and secured the funding.

MA and ME implemented the study and are responsible for data collection. Both MA and ME contributed equally in drafting the manuscript. MV, CK and EM reviewed and edited the manuscript. All authors take full responsibility for the contents of the manuscript. All authors read and approved the final manuscript.

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Chapter 6b

Long-term treatment with metformin in obese, insulin resistant adolescents

Results of a randomized double blinded placebo-controlled trial

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Abstract

Background

As adolescents with obesity and insulin resistance may be refractory to lifestyle intervention therapy alone, additional off-label metformin therapy is often used. In this study, the long-term efficacy and safety of metformin *versus* placebo in adolescents with obesity and insulin resistance is studied.

Methods

In a randomized placebo controlled double blinded trial, 62 adolescents with obesity aged 10-16 years old with insulin resistance received 2000mg of metformin or placebo daily and physical training twice weekly over 18 months. Primary endpoints were change in BMI and insulin resistance measured by the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR). Secondary endpoints were safety and tolerability of metformin. Other endpoints were body fat percentage and HbA1c.

Results

Forty-two participants completed the 18 month-study (66% girls, median age 13 (12-15) years, BMI 30.0 (28.3-35.0) kg/m^2 and HOMA-IR 4.08 (2.40-5.88)). Median ΔBMI was +0.2 (-2.9-1.3) kg/m^2 (metformin) versus +1.2 (-0.3-2.4) kg/m^2 (placebo) (p=0.015). No significant difference was observed for HOMA-IR. No serious adverse events were reported. Median change in fat percentage was -3.1 (-4.8-0.3) vs -0.8 (-3.2-1.6)% (p=0.150), in fat mass -0.2 (-5.2-2.1) vs +2.0 (1.2-6.4) kg (p=0.007), in fat free mass +2.0 (-0.1-4.0) vs +4.5 (1.3-11.6) kg (p=0.047), and in ΔHbA1c +1.0 (-1.0-2.3) vs +3.0 (0.0-5.0) mmol/mol (p=0.020) (metformin vs placebo).

Conclusions

Long-term treatment with metformin in adolescents with obesity and insulin resistance results in stabilisation of BMI and improved body composition compared to placebo. Therefore, metformin may be useful as additional therapy in combination with lifestyle intervention in adolescents with obesity and insulin resistance.

Introduction

Childhood obesity is an important pediatric health issue, with rising prevalence rates in almost all European countries, the United States and Canada [1]. While it was recently reported that the prevalence of overweight and obesity may be stabilizing, percentages are still high, i.e. 37.8 and 36.6% for 11-15 years old boys and girls respectively [2].

Obesity increases the risk of insulin resistance (IR). Children with obesity with IR have a higher risk of developing type 2 diabetes mellitus (T2DM) [3], metabolic syndrome [3,4] and cardiovascular diseases [5,6]. Reduction of body mass index (BMI) is known to reduce the risk of developing these diseases [7-9]. However, IR might be a limiting factor in losing weight in children and adolescents with obesity. In a study by Chiavaroli et al. the efficacy of a one-year weight loss intervention programme in children with and without IR, was evaluated. Children without IR achieved a reduction in BMI-standard deviation score (BMI-SDS) following a weight loss intervention programme, whereas children with IR did not [10]. Therefore, for children with obesity and IR additional (pharmacotherapeutic) therapies to lose weight are often considered.

Metformin is an antidiabetic drug, which reduces peripheral insulin resistance, increases the peripheral glucose uptake and decreases gluconeogenesis of the liver [11]. Although metformin is approved for the treatment of T2DM from the age of 10 years onwards, there is an increasing number of prescriptions for off-label indications such as obesity, polycystic ovarian syndrome (PCOS) and type 1 diabetes mellitus, with percentages reported between 8-20% [12-14].

The evidence for the efficacy of metformin in the treatment of obesity and IR in children is however scarce. A systematic review and meta-analysis of five randomized trials with a trial duration of 6 months (n=320) showed a moderate reduction in BMI (-1.42 kg/m^2 (95%CI 0.83-2.02)) and in IR measured by the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) (-2.01 (95%CI 0.75-3.26)) [15]. Long-term data on the efficacy of metformin is limited to one trial, which studied the efficacy of metformin over 48 weeks, and reported a small reduction in BMI [16]. No other studies with treatment duration more than 6 months were identified.

Therefore, the aim of this randomized, double blinded, placebo controlled trial (RCT) in adolescents with obesity with IR is to study the effect of metformin versus placebo on the change in BMI and in IR (measured by HOMA-IR) after 18 months. Secondary objectives included safety and tolerability, as well as change in body fat percentage, HbA1c, quality of life, and physical fitness after 18 months.

Methods

A brief description of the methods is provided here, since the study protocol has been published elsewhere [17].

Study design and participants

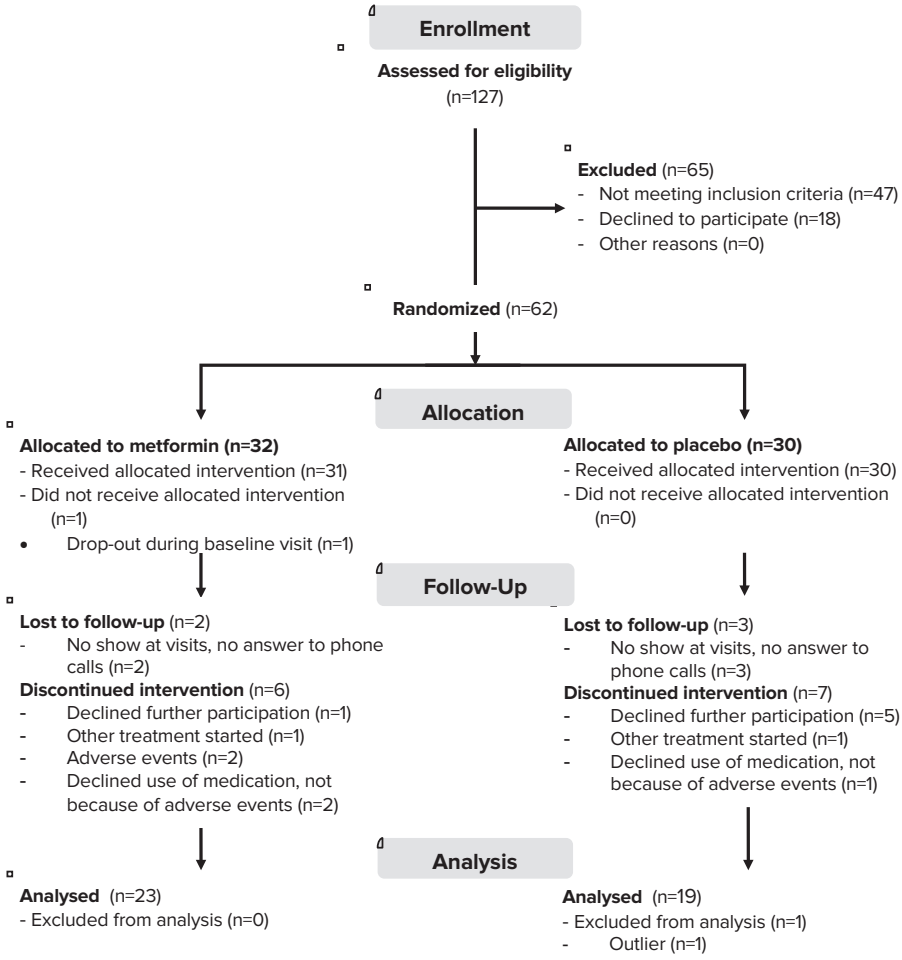
In this 18 months multicentre RCT (ClinicalTrials.gov number NCT01487993), participants were recruited at the pediatric outpatient clinics of the participating study centres (St. Antonius Hospital in Nieuwegein/Utrecht (July 2011-March 2014) and Jeroen Bosch Hospital, 's Hertogenbosch (November 2012-March 2014), The Netherlands). For inclusion and exclusion criteria see Figure 1. All clinical measurements were performed in the pediatric outpatient clinics or day-care wards of these hospitals; the fitness tests were performed at the physical therapy outpatient clinic of the St. Antonius Hospital and the Sports Medical Centre of the Jeroen Bosch Hospital. The study protocol was approved by the Medical Ethical Committee of the St. Antonius Hospital, Nieuwegein/Utrecht, the Netherlands and written informed consent was obtained from the parents and if applicable from the children (aged ≥ 12 years). From the younger children, oral consent was obtained. All procedures were in accordance with the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO) of the Netherlands.

Randomisation and blinding

Consecutive study numbers for eligible participants corresponding with the randomisation code and medication number (e.g. Study number 1, corresponds with randomisation number 1 and medication number 1) were allocated. The randomisation schedule (in blocks of 20 per study centre) was generated by the department of Clinical Pharmacy of the St. Antonius Hospital, using PASW Statistics 18.0. Both subjects and study staff were blinded during the 18-month treatment period. The randomisation code was kept secured in the department of Clinical Pharmacy. The blind was not broken for any of the participants.

Sample size

For ΔBMI, a group sample size of 47 per group was calculated to have 90% power with a significance level of 5% to detect a difference of 2.94% in BMI (ΔBMI ±1 kg/m^2). For ΔHOMA-IR, a group sample size of 60 participants per group was found to detect a difference of 1.6 in HOMA-IR with a significance level of 5% and 90% power. Taking a drop-out of 20% into account, the sample size was set at 144 participants.



Inclusion criteria: Age 10-16 years; BMI-SDS > 2.3; HOMA-IR ≥ 3.4; Caucasian descent; Written informed consent

Exclusion criteria: T2DM; PCOS; Endocrine disorders treated with corticosteroids; Height < 1.3 SD from target height; Syndromal disorders; Pregnancy; (History of) alcohol abuse; Impaired renal function (GFR <80ml/min); Impaired hepatic function (ALT > 150% of normal value for age); Insufficient knowledge of Dutch language

Figure 1. Consort Flow Diagram

Interventions

Medication

All participants received either immediate-release metformin 500 mg tablets (Centrapharm, Etten-Leur, The Netherlands or identical placebo tablets (Apotheek Haagse Ziekenhuizen, Den Haag, The Netherlands) in an increasing dosing regimen, with a maximum dose of two tablets twice daily in the fourth study week. Subjects were

advised to take the medication during or after breakfast and dinner. In case of gastro-intestinal complaints, the dosage was reduced to the last well-tolerated dose. After symptoms had disappeared, the dosage was again increased to the maximum of two tablets twice daily, if tolerated. To estimate medication compliance, pill counts were performed on returned medication packages during each hospital visit (every 3 months).

Physical training

Physical training by a physical therapist was offered twice weekly to all participants. During the monthly phone calls and three monthly visits participants were encouraged to attend these trainings.

Outcomes

Primary outcome measures

Primary endpoint was the change in BMI after 18 months (ΔBMI). BMI was calculated as weight (kg)/((height (m))^2, and was assessed every 3 months. The corresponding age and sex adjusted BMI, the BMI-SDS, was calculated by the "TNO Groeicalculator voor professionals" (https://groeiweb.pgdata.nl/calculator.asp). As second primary outcome, ΔHOMA-IR over 18 months (HOMA-IR = fasting plasma glucose(FPG)(mmol/L) x fasting plasma insulin (FPI)(mU/L) / 22.5) was evaluated [18]. FPG and FPI were measured and HOMA-IR was calculated every 3 months.

Secondary outcome measures

Secondary endpoints were safety and tolerability of metformin. Safety outcome measures were renal and hepatic function tests, measured at baseline and every 3 months during treatment. Vitamin B12 levels were measured, and levels <140 pmol/l were defined as abnormal. Tolerability was assessed by the amount of observed adverse events, and the achieved maximum dosage levels. The reasons for drop out of participants were registered.

Other outcome measures

Other endpoints were change in body fat percentage measured by bio-impedance analysis (BIA) using a leg-to-leg BIA measurement, and HbA1c after 18 months. Furthermore, change in quality of life assessed using a validated Dutch translation of the Impact of Weight on Quality of Life-Kids (IWQOL-kids) questionnaire [19,20] and change in physical fitness assessed during validated fitness tests after 18 months were

analysed. Participants were asked to fulfil a dietary diary at baseline, 9 months and 18 months to calculate caloric intake.

Statistical analysis

All participants who started treatment (i.e. they used at least 1 tablet of metformin or placebo) and finished follow up of 18 months were analysed. Since most parameters were not normally distributed, data are reported as medians with interquartile ranges. To assess the effect of metformin versus placebo after 18 months of treatment on the continuous scales, the Mann Whitney U test was applied. To compare the frequencies of categorical, dichotomous data, a chi-squared test was used. All analyses have been conducted with SPSS for Windows version 22.0.

Results

Participants

One hundred twenty-seven (127) participants were assessed for eligibility (Figure 1). Sixty-two (62) participants were allocated to metformin (n=32) or placebo (n=30), of which 42 could be included in the final analysis (Figure 1). One patient in the placebo group was excluded from the analysis, because this patient was an outlier with a change in BMI-SDS of -4.47. There was no difference in baseline age, sex, BMI, HbA1c and HOMA-IR between the participants lost to follow up and participants who completed the 18-month treatment period (supplemental table 1 and 2).

Baseline characteristics

Baseline characteristics of the analysed participants are presented in table 1. Overall more girls than boys were included. In both groups, most participants were early pubertal and family history positive for obesity and diabetes mellitus was frequently reported. Median BMI at baseline was 29.8 (28.1-34.5) kg/m^2 for the metformin and 30.5 (28.7-38.6) kg/m^2 for the placebo group, corresponding with an age and sex specific BMI-SDS of 3.10 (2.72-3.52) and 3.38 (3.10-4.20) respectively (Table 1).

Table 1. Baseline characteristics of the analysed participants

		Metformin (n= 23)	Placebo (n=19)
Clinical measurements			
Age (yr)		13.6 (12.6-15.3)	12.0 (11.3-14.0)
Gender, n (%)			
- Boys		6 (26.1)	8 (42.1)
- Girls		17 (73.9)	11 (57.9)
Height (cm)		162.9 (159.0-168.0)	162.0 (160.0-166.0)
Height-SDS		-0.08 (-0.65-0.71)	0.51 (-0.15-1.34)
Weight (kg)		82.2 (75.4-92.7)	86.1 (74.0-103.0)
BMI (kg/m^2)		29.8 (28.1-34.5)	30.5 (28.7-38.6)
BMI-SDS		3.10 (2.72-3.52)	3.38 (3.10-4.20)
Hip circumference (cm)		101.0 (93.0-107.8)	100.8 (96.9-112.3)[#]
Waist circumference (cm)		97.0 (94.0-106.0)	103.8 (100.0-119.4)[#]
Waist-to hip ratio		1.00 (0.95-1.04)	1.05 (0.96-1.10)[#]
Systolic blood pressure (mmHg)		118 (115-124)	119 (113-126)
Diastolic blood pressure (mmHg)		69 (61-72)	67 (57-77)
Tanner stage, n (%)			
- Prepubertal (Tanner stage 1)		3 (13.0)	3 (16.7)
- Pubertal (Tanner stage 2-4)		17 (74.0)	12 (66.6)
- Postpubertal (Tanner stage 5)		3 (13.0)	3 (16.7)
Family-history, first and/or second degree, n (%)			
Obesity		20 (86.9)	16 (84.2)
Diabetes Mellitus		15 (65.2)	9 (47.4)
Hypercholesterolemia		14 (60.9)	9 (47.4)
Hypertension		16 (69.5)	13 (68.4)
Cardiovascular disease		14 (60.9)	14 (73.7)
Highest level of education, n (%)			
Participant	*Lowest*	4 (17.4)	8 (42.1)
	Low	16 (69.6)	7 (36.8)
	Middle	3 (13.0)	4 (21.1)
	High	0 (0)	0 (0)
Father	*Lowest*	2 (8.7)	1 (5.3)
	Low	9 (39.1)	5 (26.3)
	Middle	7 (30.4)	9 (47.4)
	High	3 (13.0)	2 (10.5)
	Unknown	2 (8.7)	2 (10.5)
Mother	*Lowest*	0 (0)	3 (15.8)
	Low	10 (43.5)	10 (52.6)
	Middle	8 (34.8)	4 (21.1)
	High	4 (17.4)	0 (0)
	Unknown	1 (4.3)	2 (10.5)

Table 1. Baseline characteristics of the analysed participants (continued)

	Metformin (n= 23)	Placebo (n=19)
Biochemical measurements		
Glucose 0' (mmol/l)	4.8 (4.7-5.0)	4.8 (4.5-5.0)
Glucose 120' (mmol/l)	6.0 (5.6-6.6)	5.9 (4.8-7.2)
Insulin 0' (mU/l)	18.0 (11.0-27.0)	23.0 (12.0-26.0)
Insulin 120' (mU/l)	94.0 (63.0-138.0)	90.0 (54.0-128.0)
HOMA-IR	4.00 (2.30-6.36)	4.85 (2.40-5.78)
HbA1c (mmol/mol)	33 (31-34)	32 (31-34)
Cholesterol (mmol/l)	4.8 (3.9-5.3)	4.4 (4.1-5.0)
HDL (mmol/l)	1.10 (1.02-1.22)	1.16 (1.03-1.36)
LDL (mmol/l)	2.9 (2.3-3.3)	2.4 (2.1-3.2)
TG (mmol/l)	1.4 (0.9-1.6)	1.4 (1.0-1.8)
Total cholesterol/HDL-ratio	4.3 (3.4-4.7)	3.8 (2.7-5.0)
ALT (U/l)	19 (15-26)	21.5 (14.5-28.5)
Creatinine (µmol/l)	51 (48-55)	50 (47-56)
Vitamin B12 (pmol/l)	365 (267-420)	336 (280-492)
Bio-impedance		
Body fat (%)	38.6 (36.5-43.2)	41.2 (36.9-44.1) #
Fat mass (kg)	31.8 (25.0-39.4)	33.6 (27.6-48.5) #
Fat free mass (kg)	48.9 (45.4-53.0)	50.4 (42.6-54.5) #
Quality of Life by IWQOL-Kids	*n=22*	*n=15*
Section 1, Physical comfort	83.3 (72.5-94.2)	73.3 (63.3-86.7)
Section 2, Body esteem	75.6 (63.3-88.3)	68.9 (55.6-84.4)
Section 3, Social life	86.7 (76.7-93.3)	86.7 (80.0-90.0)
Section 4, Family relations	100 (95.8-100)	100 (90.0-100)
Physical fitness	*n=18*	*n=13*
Shuttle walk test, distance in m	1500 (1138-1500)	1500 (955-1500)
9 meter sprint test (sec)	2.47 (2.35-2.60)	2.70 (2.05-3.00)
10x5 m sprint test (sec)	20.63 (19.21-23.45)	20.28 (19.15-22.10)
Situps in 30 seconds (n)	21 (17-30)	17 (14-19)
Time to stand up from supine position	2.10 (1.80-2.84)	2.40 (1.90-2.96)

Reported values are median (Interquartile range) or numbers (%). # n=18
Abbreviations: SDS – standard deviation score; BMI – body mass index; HOMA-IR - homeostasis model assessment for insulin resistance; HDL – high density lipoprotein; LDL – low density lipoprotein; ALT – alanine aminotransferase; IWQOL – impact of weight on quality of life.

Medication compliance

Two participants did not return any medication packages during the study. In the metformin group 74% (17/22 participants) returned their medication boxes at least 4 times, versus 69% in the placebo group (13/18 participants). The returned boxes contained on average 28% and 24% of its content for the metformin and placebo groups,

respectively. In case of full compliance, the remaining content should be 7% because all medication boxes had a surplus when dispensed.

Effect on BMI and HOMA-IR

Table 2 presents the 18 months treatment results of metformin versus placebo; the absolute values for BMI and other parameters, as well as changes over 0-18 months are displayed. After 18 months, median ΔBMI was +0.2 (-2.9-1.3) kg/m^2 in the metformin group versus + 1.2 (-0.3-2.4) kg/m^2 in the placebo group (p=0.015). Figure 2 shows that this difference between the two groups can be explained by a decrease in ΔBMI in the metformin group during the first 6-9 months of treatment and subsequent return to baseline values, which was not observed in the placebo group.

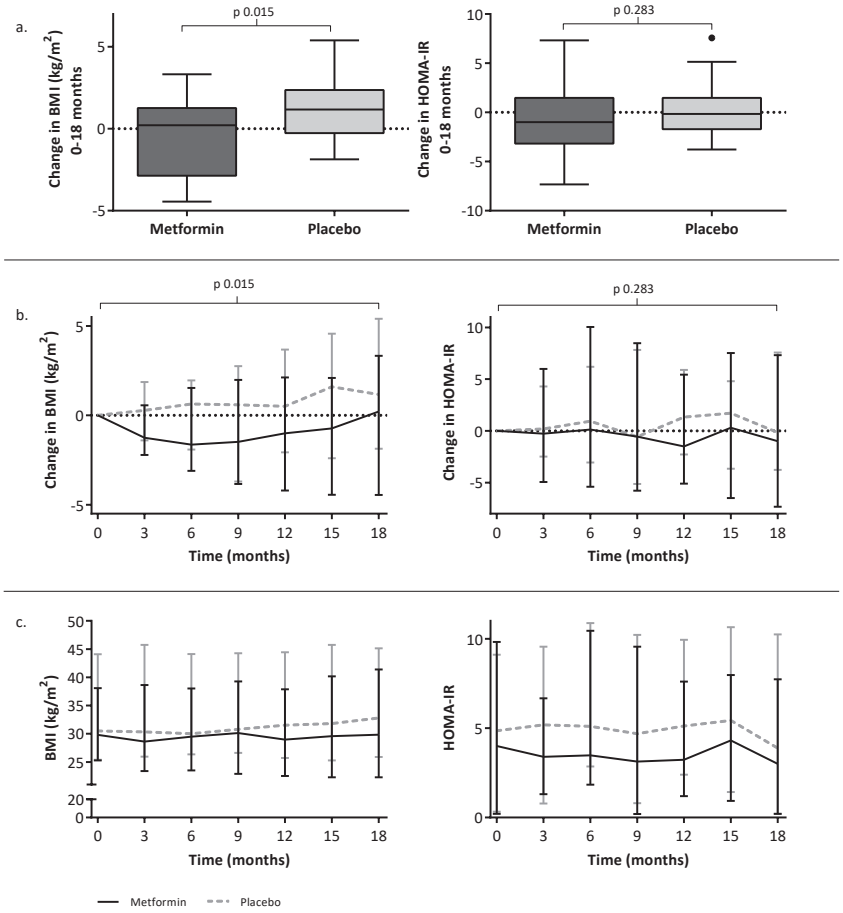


Figure 2. Effect of metformin on primary endpoints BMI and HOMA-IR after 18 months
a. Change in BMI and HOMA-IR between t=0 and t=18 months; b. Median ΔBMI and ΔHOMA-IR over time; c. Median BMI and HOMA-IR over time.

Table 2. Treatment effects of metformin versus placebo after 18 months

	Metformin (n= 23)			Placebo (n=19)			Metformin vs placebo, p-value
	T=0	T=18	Δ T=18 – T=0	T=0	T=18	Δ T=18 – T=0	Δ T=18 – T=0
Primary outcomes							
BMI	29.8 (28.1-34.5)	29.9 (26.3-33.6)	0.2 (-2.9-1.3)	30.5 (28.7-38.6)	32.8 (29.3-40.4)	1.2 (-0.3-2.4)	**0.02**
BMI-SDS	3.10 (2.72-3.52)	2.90 (2.34-3.39)	-0.12 (-.50-0.08)	3.38 (3.10-4.20)	3.29 (3.02-4.18)	0.04 (-.24-0.10)	0.08
HOMA-IR	4.00 (2.30-6.36)	3.00 (2.00-4.29)	-1.00 (-3.17-2.25)	4.85 (2.40-5.78)	3.88 (2.86-5.56)	-0.16 (-1.71-1.48)	0.28
Other outcomes							
HbA1c	33.0 (31.0-34.0)	33.5 (30.8-34.3)	1.0 (-1.0-2.3)	32.0 (31.0-34.0)	36.0 (33.0-37.0)	3.0 (0.0-5.0)	**0.02**
Height (cm)	162.9 (159.0-168.0)	166.5 (160.3-171.0)	2.1 (0.5-6.6)	162.0 (160.0-166.0)	168.3 (163.7-171.3)	6.2 (2.5-8.7)	
Weight (kg)	82.2 (75.4-92.7)	83.4 (76.6-94.2)	1.6 (-4.2-5.9)	86.1 (74.0-103)	96.7 (79.0-111.0)	12.0 (2.7-17.0)	
Bio-impedance		*n=21*			*n=17*		
Body fat %	38.6 (36.5-43.2)	37.6 (30.9-40.9)	-3.1 (-4.8-0.3)	41.2 (36.9-44.1)	41.2 (37.7-46.9)	-0.8 (-3.2-1.6)	0.15
Fat mass (kg)	31.8 (25.0-39.4)	31.1 (22.6-37.6)	-0.2 (-5.2-2.1)	33.6 (27.6-48.5)	34.9 (29.5-53.1)	2.0 (1.2-6.4)	**0.007**
Fat free mass (kg)	48.9 (45.4-53.0)	49.4 (46.7-55.4)	2.0 (-0.1-4.0)	50.4 (42.6-54.5)	52.0 (48.9-65.1)	4.5 (1.3-11.6)	**0.05**
IWQOL-kids		*n=17*			*n=12*		
Total score	83.0 (76.7-91.9)	90.0 (81.5-98.1)	2.6 (0.2-5.7)	80.0 (72.6-85.2)	84.8 (77.8-90.0)	5.2 (-2.2-9.6)	0.94
Section 1	83.3 (72.5-94.2)	91.7 (79.2-100)	6.7 (0-13.3)	73.3 (63.3-86.7)	90.0 (76.7-93.3)	6.7 (3.3-21.7)	0.41
Section 2	75.6 (63.3-88.3)	82.2 (66.1-94.4)	2.2 (-2.2-5.6)	68.9 (55.6-84.4)	73.3 (67.8-87.8)	5.6 (-3.9-10.6)	0.66
Section 3	86.7 (76.7-93.3)	95.0 (86.7-99.2)	3.3 (0-6.7)	86.7 (80.0-90.0)	90.0 (86.7-95.0)	0 (-6.7-6.7)	0.25
Section 4	100 (95.8-100)	100 (95.8-100)	0 (0-3.3)	100 (90.0-100)	100 (95.0-100)	0 (0-5.8)	0.86

Table 2. Treatment effects of metformin versus placebo after 18 months (continued)

	Metformin (n= 23)			Placebo (n=19)			Metformin vs placebo, p-value
	T=0	T=18	Δ T=18 – T=0	T=0	T=18	Δ T=18 – T=0	Δ T=18 – T=0
Fitness test		*n=15*			*n=7*		
Shuttle walk test (m)	1500 (1163-1500)	1500 (1415-1500)	0 (0-120)	1180 (850-1500)	1360 (1170-1500)	270 (-100-320)	0.52
9 meter sprint (sec)	2.48 (2.40-2.65)	2.53 (2.38-2.71)	0.01 (-0.14-0.12)	2.70 (2.00-3.09)	2.60 (2.47-3.04)	0.08 (-0.37-0.58)	0.46
10x5 m sprint (sec)	20.63 (19.21-23.44)	20.58 (19.14-21.46)	-0.40 (-2.30-0.60)	21.00 (20.00-25.53)	21.10 (20.53-24.03)	-0.10 (-0.60-0.53)	0.46
Situps in 30 seconds (n)	21 (17-30)	24 (19-30)	0 (-11-8)	17 (14-19)	23 (19-25)	5 (4-11)	0.33
Time to stand up from supine position (sec)	2.10 (1.80-2.84)	2.31 (2.13-2.58)	-0.05 (-0.33-0.61)	2.40 (1.90-2.96)	2.88 (2.26-2.93)	-0.03 (-0.32-1.24)	0.49

All values are median (IQR). *Abbreviations: BMI – body mass index; SDS – standard deviation score; HOMA-IR - homeostasis model assessment for insulin resistance; IWQOL – impact of weight on quality of life*

No significant difference was observed for ΔHOMA-IR after 18 months between both groups (Table 2). Figure 2 shows that in accordance with this lack of difference between the groups at 18 months, there is also no evidence for a difference in profile of ΔHOMA-IR over time during the study.

Secondary endpoints safety and tolerability

Safety

No severe adverse advents occurred in either group. There were no derangements of renal or hepatic function (Table 3). In three participants of the metformin group, vitamin B12 levels below the threshold of 140 pmol/l were measured at 18 months (136, 117 and 108 pmol/l respectively).

Table 3. Safety and tolerability of metformin versus placebo

	Metformin (n= 23)	Placebo (n=19)	p-value
Safety, n(%)			
ALT > 69 U/l (girls) or >78 U/l (boys)	0	0	NA
GFR < 60 ml/min	0	0	NA
Vitamin B12 <140 pmol/l	3 (13.0)	0	NA
Tolerability *Adverse events, n(%)*			
Nausea	17 (73.9)	8 (42.1)	**0.04**
Diarrhoea	14 (60.9)	9 (47.4)	0.38

Abbreviations: ALT – alanine aminotransferase; GFR – glomerular filtration rate; NA – not applicable

Tolerability

Two out of nine participants lost to follow up in the metformin group discontinued treatment because of adverse events. One patient had severe nausea despite dosage reductions. The other patient suffering from abdominal pain and discomfort, was not willing to try dosage reductions and terminated study participation. Four participants in the metformin group did not tolerate the maximum dose of 2000mg daily because of adverse events; these participants used 1000 mg daily (n=3) or 1500 mg daily (n=1). In the placebo group, no participants dropped out because of adverse events.

Well-known side effects of metformin, nausea and diarrhoea, were reported in both groups during the study, but participants using metformin suffered significantly more from nausea (73.9%, n=17) than the participants receiving placebo (42.1%, n=8) (p=0.037). Diarrhoea occurred in 60.9 %(n=14) of the metformin users and 47.4% (n=9) of the placebo users (p=0.38) (Table 3).

Effect of metformin on HbA1c and body composition

Table 2 and Figure 3 show that HbA1c increased in both groups, with a significantly larger increase in the placebo group (p=0.02). None of the participants had HbA1c values above the normal threshold after 18 months.

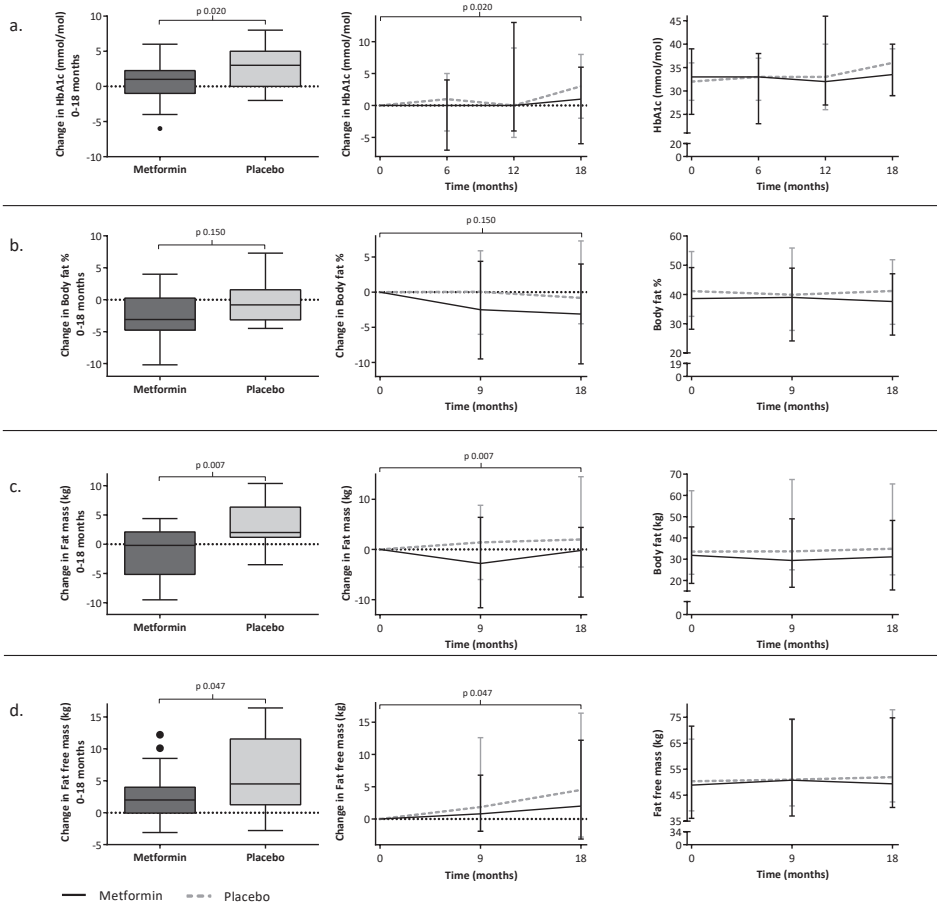


Figure 3. Effect of metformin on HbA1c and body composition after 18 months
Boxplots represent the Δ-values between t=0 and t=18 months. Graphs represent median values.

In the metformin group, fat mass decreased versus an increase in the placebo group (p=0.007) (Table 2, figure 3). Concerning fat free mass, in the metformin group the increase was + 2.0 (-0.1-4.0) kg versus + 4.5 (1.3-11.6) kg in the placebo group (p=0.047). There was no significant change in body fat percentage (Figure 3).

Effect of metformin on quality of life and physical fitness

Table 2 shows results for quality of life measured by IWQOL-kids. For all sections and the total score, there was no difference in quality of life. Due to a poor attendance at the physical tests after 18 months, physical fitness tests could only be analysed in a small subgroup (metformin n=15, placebo n=7) (Table 2). At baseline, more than 50% of the participants completed the shuttle walk test, therefore the median score of the shuttle walk test was similar to the maximum score and no significant differences were observed in this small subgroup. Dietary diaries were not completed and returned adequately, and therefore the caloric intake could not be calculated and analysed.

Discussion

In this RCT in adolescents with obesity and insulin resistance, we found that assignment to the metformin group was associated with an initial decrease in BMI over the first 6-9 months of treatment after which BMI returned to baseline level, whereas BMI increased in placebo users. Changes in body composition and HbA1c over 18 months were also in favour of metformin. In contrast, in the placebo group, a steady increase in BMI was observed over 18 months. No serious adverse events were reported and most participants tolerated metformin up to 1000mg twice daily, only two participants discontinued treatment because of adverse events.

Our study is the first study in an obese non-diabetic pediatric population reporting on the long term effect (> 1 year of treatment) of metformin on BMI. Beneficial effects on BMI upon short term treatment with metformin have been reported before by Burgert et al, who reported upon 4-month treatment a reduction in BMI of -0.9 kg/m^2 (95%CI -2.0-0.3 kg/m^2) versus an increase in BMI of +1.2kg/m^2 (-0.1-2.4 kg/m^2) in placebo [21]. Upon 48 weeks of treatment, Wilson et al. reported a significant reduction in BMI of -0.9±0.5 kg/m^2 for the metformin group versus +0.2±0.5 kg/m^2 in the placebo group (p=0.03) [16]. Comparing our results with these previous short term results, it seems that our results after 12 months closely resemble them (i.e. median ΔBMI -1.0 (-3.4-0.6) kg/m^2 for metformin versus +0.6 (-0.2- 2.1) kg/m^2 for placebo, Figure 2)). In our study, where we report on treatment effects after 18 months, the difference between metformin and placebo remained significant even though it seems that BMI values return to baseline in the metformin group. However, in the placebo group there was no evidence of a decrease in BMI (Figure 2). An intriguing question is therefore how BMI will change over time after these 18 months. Lavine et al. treated children with obesity and non-alcoholic fatty liver disease for 96 weeks with metformin [22]. However, the treatment in this study was not primarily focused on weight loss, and participants did not receive lifestyle intervention. They reported changes in BMI after 96 weeks of +1.3

(0.6-2.0) kg/m^2 for metformin versus +1.9 (1.1-2.7) kg/m^2 for placebo (p=0.25) [22]. This finding illustrates that metformin without lifestyle intervention may not be effective in changing BMI. As a consequence, follow up results of our study (open label results) upon 36 months treatment with metformin with lifestyle intervention will need to be awaited [17]. Until then, in our opinion, lifestyle intervention remains an important part of obesity treatment to which metformin therapy over 18 months seems to be of added value to reduce BMI.

In our study, two participants discontinued treatment and four participants received a reduced dosage because of adverse events, even though there were no serious adverse events or derangements in hepatic and renal function tests. These findings are comparable to the study of Wilson et al. of 48 weeks, where 1 patient dropped out because of nausea [16]. In studies where metformin was administered over 2-6 months, no severe adverse events, elevated hepatic or renal function tests, or decreased vitamin B12 were reported. Concerning vitamin B12, in our study, 3 participants in the metformin group had decreased vitamin B12 levels and therefore monitoring of vitamin B12 levels upon long-term use of metformin should be considered. In all studies nausea and diarrhoea were the most frequently reported side-effects [21,23-29]. Even though these side effects of metformin are mostly mild and self-limiting, a small number of participants (6%) did not tolerate metformin because of these side-effects [28]. It is known that the incidence of gastro-intestinal side-effects is higher in patients using immediate release metformin compared to extended release metformin [30-32]. Therefore, the use of extended release metformin could be considered in the small number of patients with serious gastro-intestinal side-effects. From this study, it seems that safety and tolerability of long term metformin treatment is comparable to short-term treatment (6 months to 48 weeks), with no serious adverse events and only a small percentage of participants who do not tolerate metformin.

In the current study, participants treated with metformin were found to have an improved body composition measured by BIA after 18 months, with a decrease in fat mass and increase in fat free mass compared to placebo. In the placebo group, the change in fat free mass was larger than the change in fat mass. The placebo group has a larger increase in height (table 2) during the 18 months; the increase in fat free mass might be related to this increase in height. We assume that this increase in height, and therewith in fat free mass, is caused by a difference in pubertal stage during the study. At t=18months, in the metformin group 38.1% was pubertal (Tanner Stage 2-4) and 57.1% postpubertal (Tanner stage 5), compared to 64.7% pubertal and 35.3% postpubertal in the placebo group. In the metformin group an increase in fat free mass in accordance with their increase in height over 18 months was found, without an increase in fat mass, resulting in a stable BMI. Also in other studies, a favourable effect of metformin on body composition (measured by DEXA or BIA) after 2-11 months of

treatment, compared to placebo was reported [16,21,24,29]. In adults, a decrease in body fat percentage was related to a decrease in systolic and diastolic blood pressure and cholesterol levels [33]. Therefore, a change in body composition during metformin treatment might have a positive influence on cardiovascular risk factors.

A limitation of our study is the number of included participants. For the primary endpoint (ΔBMI), 66% of the targeted number of participants was included, while for the change in HOMA-IR this percentage was only 43%, despite a prolongation of the inclusion period by 1.5 years. Furthermore, the dropout rate was 32%, whereas a dropout rate of 20% was anticipated. This high dropout rate illustrates the difficulties in motivating adolescents with obesity for long-term treatment and follow up. This difficulty is underlined by the poor attendance at physical fitness tests and by the dietary diaries, which had limited completeness and reliability. Frequent phone calls and written reminders by the study staff did not improve the compliance. The low number of included participants and high dropout rate could have resulted in insufficient power to statistically test our hypotheses. However, although our study has less power than anticipated, we were able to detect a significant effect of metformin on the primary outcome measure (ΔBMI). For comparison, with respect to the IR outcome, other studies with sufficient power did not find an effect on IR after 6 months and 48 weeks either [16,25,28]. Another limitation is the measurement of IR. All participants had HOMA-IR ≥ 3.4 during the screening for eligibility. At baseline, which was planned within a few weeks from screening, some participants had HOMA-IR values <3.4, while still being obese (BMI-SDS > 2.3). As a possible explanation for this finding, participants may not tell the truth about their fasting state during the screening. Another reason may be the large coefficient of variation that has been reported for fasting insulin [34,35]. Since HOMA-IR is based on fasting insulin, HOMA-IR will vary as well resulting in HOMA-IR < 3.4, thereby explaining the lack of difference in this parameter.

Conclusion

In conclusion, long-term treatment with metformin in adolescents with obesity and insulin resistance results in a stabilisation of BMI and improved body composition compared to placebo. Therefore, metformin may be considered a safe additional therapy in combination with lifestyle intervention.

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Contributors statement

MP van der Aa collected the data, carried out the data analyses, drafted the initial manuscript, and approved the final manuscript as submitted. MAJ Elst collected the data, critically reviewed the manuscript, and approved the final manuscript as submitted. EMW van de Garde supervised the data analysis, critically reviewed the manuscript, and approved the final manuscript as submitted. EGAH van Mil conceptualized and designed the study, critically reviewed the manuscript, and approved the final manuscript as submitted. MMJ van der Vorst and CAJ Knibbe conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

Conflict of interest

The authors declare no conflict of interest.

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SUPPLEMENTARY MATERIALS TO CHAPTER 6B

Supplemental table 1. Comparison of baseline data of participants lost to follow up and participants who completed the first part of the study.

	Follow up complete (n=43)	Lost to follow up (n=18)	P-value
Age	13.4 (11.7-15.2)	12.38 (11.4-15.3)	0.61
Gender			n (%)
- Boys	14 (82.4)	3 (17.6)	0.21
- Girls	29 (65.9)	15 (34.1)	
BMI	30.0 (28.3-35.0)	32.4 (28.9-35.2)	0.45
BMI-SDS	3.25 (2.86-3.65)	3.44 (2.99-3.63)	0.49
HbA1c	33 (31-34)	31 (30-35)	0.17
HOMA-IR	4.08 (2.40-5.88)	4.10 (3.23-5.77)	0.55

Supplemental table 2. Baseline characteristics of all randomized participants Reported values are median (Interquartile range) or numbers (%).

	Metformin (n= 31)	Placebo (n=30)
Clinical measurements		
Age (yr)	13.6 (11.8-15.2)	12.9 (11.4-15.2)
Gender, n (%)		
- Boys	7 (22.6)	10 (33.3)
- Girls	24 (77.4)	20 (66.7)
Height (cm)	162.0 (156.0-168.0)	162.6 (159.4-166.3)
Height-SDS	-0.01 (-0.56-0.64)	0.34 (-0.27-1.17)
Weight (kg)	80.2 (72.4-92.7)	87.5 (74.2-98.7)
BMI (kg/m2)	30.3 (28.2-34.5)	32.0 (28.9-36.3)
BMI-SDS	3.20 (2.72-3.55)	3.44 (3.10-4.02)
Hip circumference (cm)	102.5 (94.5-108.0)	103.4 (96.0-113.3)
Waist circumference (cm)	99.0 (94.0-107.8)	103.0 (99.5-114.0)
Waist-to hip ratio	1.00 (0.95-1.06)	1.04 (0.93-1.10)
Systolic blood pressure (mmHg)	118 (115-124)	121 (116-127)
Diastolic blood pressure (mmHg)	68 (61-72)	67 (59-76)
Tanner stage, n (%)		
- Prepubertal (TS 1)	5 (16.1)	5 (16.7)
- Pubertal (TS 2-4)	23 (74.2)	19 (65.5)
- Postpubertal (TS 5)	3 (9.7)	5 (16.7)
Family-history, first and/or second degree, n (%)		
Obesity	28 (90.3)	24 (80.0)
Diabetes mellitus	21 (67.7)	15 (50.0)
Hypercholesterolemia	20 (64.5)	14 (46.7)
Hypertension	22 (71.0)	20 (66.7)
Cardiovascular disease	19 (61.3)	19 (63.3)
Highest level of education, n (%)		
Participant		
- Lowest	7 (22.6)	11 (36.7)
- Low	20 (64.5)	10 (33.3)
- Middle	4 (12.9)	9 (30.0)
- High	0 (0)	0 (0)
Father		
- Lowest	3 (9.7)	4 (13.3)
- Low	11 (35.5)	9 (30.0)
- Middle	10 (32.3)	11 (36.7)
- High	4 (12.9)	4 (13.3)
- Unknown	3 (9.7)	2 (6.7)
Mother		
- Lowest	1 (3.2)	5 (16.7)
- Low	14 (45.2)	12 (40.0)
- Middle	9 (29.0)	10 (33.3)
- High	6 (19.4)	1 (3.3)
- Unknown	1 (3.2)	2 (6.7)

Supplemental table 2. Baseline characteristics of all randomized participants Reported values are median (Interquartile range) or numbers (%). (continued)

	Metformin (n= 31)	Placebo (n=30)
Biochemical measurements		
Glucose 0' (mmol/l)	4.8 (4.6-5.0)	4.8 (4.5-4.9)
Glucose 120' (mmol/l)	5.8 (6.1-7.0)	5.9 (5.1-6.9)
Insulin 0' (mU/l)	20.0 (13.0-27.0)	18.0 (12.0-26.0)
Insulin 120' (mU/l)	103.0 (67.0-146.0)	75.5 (52.5-131.0)
HOMA-IR	4.09 (2.60-6.27)	4.04 (2.52-5.59)
HbA1c (mmol/mol)	33 (30-34)	32 (30-34)
Cholesterol (mmol/l)	4.7 (3.9-5.2)	4.5 (4.1-5.0)
HDL (mmol/l)	1.14 (1.02-1.27)	1.14 (1.01-1.42)
LDL (mmol/l)	2.9 (2.3-3.1)	2.4 (2.1-3.2)
TG (mmol/l)	1.4 (1.0-1.7)	1.5 (1.0-1.8)
Total cholesterol/HDL-ratio		
ALT (U/l)	20 (16-28)	21 (15-27)
Kreatinin (µmol/l)	50 (48-55)	52 (47-59)
Vitamin B12 (pmol/l)	365 (267-429)	337 (253-427)
Bio-impedance		*n=29*
Body fat (%)	39.3 (36.9-44.0)	41.5 (36.8-46.1)
Fat mass (kg)	32.1 (27.1-39.4)	36.1 (28.1-46.8)
Fat free mass (kg)	47.7 (43.5-52.5)	51.0 (44.3-54.7)
Quality of Life by IWQOL-Kids	*n=30*	*n=24*
Section 1, Physical comfort	25.5 (21.8-28.3)	24.5 (19.0-27.0)
Section 2, Body esteem	32.5 (26.0-38.3)	30.0 (25.3-36.8)
Section 3, Social life	26.5 (23.0-29.0)	26.0 (24.3-27.8)
Section 4, Family relations	30.0 (29.0-30.0)	30.0 (28.0-30.0)
Physical fitness	*n=22*	*n=16*
Shuttle walk test, distance in m	1500 (1118-1500)	1500 (1065-1500)
9 meter sprinttest (sec)	2.50 (2.40-2.69)	2.69 (2.03-2.91)
10x5 m sprinttest (sec)	21.52 (19.30-23.94)	20.28 (19.20-23.00)
Situps in 30 seconds (n)	21 (18-30)	20 (17-24)
Time to stand up from supine position	2.25 (1.85-2.86)	2.40 (1.80-2.72)

Reported values are median (Interquartile range) or numbers (%).
Abbreviations: SDS – standard deviation score; BMI – body mass index; HOMA-IR - homeostasis model assessment for insulin resistance; HDL – high density lipoprotein; LDL – low density lipoprotein; ALT – alanine aminotransferase; IWQOL – impact of weight on quality of life.

Chapter 7

Eighteen-month treatment with metformin in obese adolescents

Results on change in BMI in an outpatient clinic compared to results obtained in a clinical trial

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Submitted

Abstract

Background

In a recent randomized controlled trial (RCT) in obese adolescents, 18 months treatment with metformin versus placebo was reported to lead to stabilisation of the BMI. This study aimed to compare the effect of metformin on BMI in obese adolescents in daily practice versus results obtained in an RCT.

Methods

Obese adolescents treated off-label with metformin in daily practice in an outpatient clinic with a follow up of ≥18 months were identified. Anthropometric and biochemical data were collected at baseline and at 18 months. Patients treated with metformin for 18 months in an RCT were used for comparison. BMI was compared between the two groups.

Results

Nineteen patients (median age 14.3 (interquartile range 11.7-15.7) years, BMI 31.3 (28.8-33.8) kg/m^2) treated in daily practice were compared to 23 patients receiving metformin in the RCT (age 13.6 (12.6-15.3) years, BMI 29.8 (28.1-34.5) kg/m^2). BMI change after 18 months was -0.36 (-2.10-1.58) vs +0.22 (-2.87-1.27) kg/m^2 for the two groups, respectively. In the multivariable model, BMI change was not statistically significantly different between the two groups (p=0.61).

Conclusion

Treatment with metformin in obese adolescents in daily practice results in a in BMI change comparable to the change observed in the RCT.

Clinical Trial Registration

ClinicalTrials.gov number: NCT01487993

Introduction

Childhood obesity is rising, as well as attention for obesity treatments. Cornerstone in the treatment of obesity is lifestyle intervention, that have proven to lead to a decrease in body mass index (BMI) after 6-12 months [1]. Longer-term effects have been described to be marginal additive [2]. To potentially improve the effects of life-style interventions, additional pharmacological interventions have been suggested and studied [3-5].

Metformin is one of these pharmacological agents used in adolescents with obesity. Metformin is registered for the treatment of type 2 diabetes in children aged 10 years and older. It is frequently used off-label for the treatment of children with obesity. In a systematic review and meta-analysis, a reduction in BMI of -1.38 (95%CI -1.93- -0.82) kg/m^2 was reported for metformin after 6 months of treatment [6]. However, the effect after >12 months of treatment was not significantly different compared to placebo [6]. As such, it seems that the maximum effect of metformin is achieved after 6-9 months of treatment, since in studies of 48 weeks and 18 months smaller effects for change in BMI were reported [7, 8]. In our RCT of 18 months, BMI decreased during the first 9 months of metformin treatment, comparable to the results in the study of 48 weeks. After 18 months, the BMI returned back to baseline in the metformin group with an increase compared to baseline in the placebo group.

Generally, it is believed that patients who participate in a trial are more likely to change their behaviour, because they are frequently monitored. This phenomenon is also known as the Hawthorne effect [9, 10]. The Hawthorne effect is considered as one of the explanations for improvements in health in clinical trials. As a result, the effects observed in clinical trials might be larger than the results that can be obtained in daily clinical practice. Such a difference in effect can also be referred to as the efficacy-effectiveness gap. The aim of the present study is to compare the effects of metformin treatment in addition to lifestyle intervention on change in BMI between obese adolescents treated with metformin in daily clinical care and obese adolescents treated with metformin as participants to a corresponding randomized placebo controlled trial (RCT). In addition, the effects on glucose metabolism between the two groups are compared.

Methods

Patients

In this study, two groups of patients were compared, i.e. the daily clinical practice group and the RCT group. The daily clinical practice group consisted of patients who

were treated off-label with metformin in the pediatric obesity outpatient clinic of the St Antonius Hospital Nieuwegein/Utrecht between January 1 2007 and July 31 2015. The data of these patients were collected retrospectively. The patients in the daily clinical practice group were included if they were aged 10-16 years at start of metformin therapy, were obese (defined as BMI-SDS > 2.3) and had a follow up time of at least 18 months. According to the intention to treat principle, patients should have started metformin, but treatment with metformin for the complete 18 month follow up was not required. Patients were excluded if they had type 2 diabetes mellitus. As standard of care, to all patients in the obesity out-patient clinic a multidisciplinary lifestyle intervention programme is offered. Ethical approval of the study protocol (Protocol number Z-11.27) was obtained from the local Medical Ethical Committee of the St. Antonius Hospital. As only routinely collected information was used and analysed anonymously, the need for written informed consent of the children and their parents was waived.

The other group, i.e. the RCT group, consisted of the patients of the metformin arm of a RCT on metformin versus placebo in obese children [8, 11]. The inclusion criteria for the patients in the RCT were age 10-16 years, obesity (defined as BMI-SDS > 2.3), insulin resistance (defined as HOMA-IR ≥ 3.4) and being of Caucasian origin. The Medical Ethical Committee of the St. Antonius hospital, Nieuwegein/Utrecht, the Netherlands, approved the RCT study protocol and written informed consent was obtained from participants (if applicable) and parents. The RCT was registered in the Clinical Trials register (ClinicalTrials.gov number NCT01487993).

Data collection

For the daily clinical practice group, the outpatient pediatrician identified the patients in the outpatient clinic after which a double-check was performed by identifying all patients who visited the pediatric obesity outpatient clinic between 2006 and 2014 using the 'Diagnose behandel combinatie (Diagnosis treatment combination) code (*DBC*-code) 'adiposity'. The medical files of these obese children were screened for treatment with metformin. No additional patients were identified (Figure 1).

From the included patients, data were extracted from the electronic patient files. Baseline (t=0) was defined as date of start of metformin therapy. Collected baseline data were age, sex, height (cm), weight (kg), fasted plasma glucose (FPG) in mmol/l, fasted plasma insulin (FPI) in mU/l, and HbA1c in mmol/mol. BMI was calculated from height and weight (BMI = weight (kg)/ (height (m))^2. BMI-SDS was calculated by the "TNO Groeicalculator voor professionals" (https://groeiweb.pgdata.nl/calculator.asp), which is a web application developed by the Dutch Organisation for Applied Scientific Research (TNO) calculating age and sex adjusted height and BMI standard deviation scores. Impaired fasted glucose was defined as FPG ≥ 5.6 mmol/l; hyperinsulinemia as FPI > 15 μU/ml. For insulin resistance, the Homeostasis Model Assessment for Insulin

Resistance (HOMA-IR) was calculated: (FPG(mmol/l) * FPI (mu/l)) / 22.5 [12); insulin resistance was defined as HOMA-IR ≥ 3.4.

Since subjects did not regularly visit the obesity out-patient clinic, windows were created to define times of visit. The windows were: t=6 months (day 180 (range 120-240)), t=12 months (day 360 (range 300-420)) and t=18 months (day 540 (range 450-630). Data extracted from follow up visits were date of visit, height (cm), weight (kg), fasted plasma glucose (FPG) in mmol/l, fasted plasma insulin (FPI) in mU/l, and HbA1c in mmol/mol.

Patients from the RCT group visited the outpatient clinical three monthly. During these visits height (cm) and weight (kg) was measured and with these data BMI and BMI-SDS were calculated. Vena punctures were performed every visit to measure FPG and FPI (every 3 months) and HbA1c (every 6 months). A detailed description of this group is described elsewhere [11].

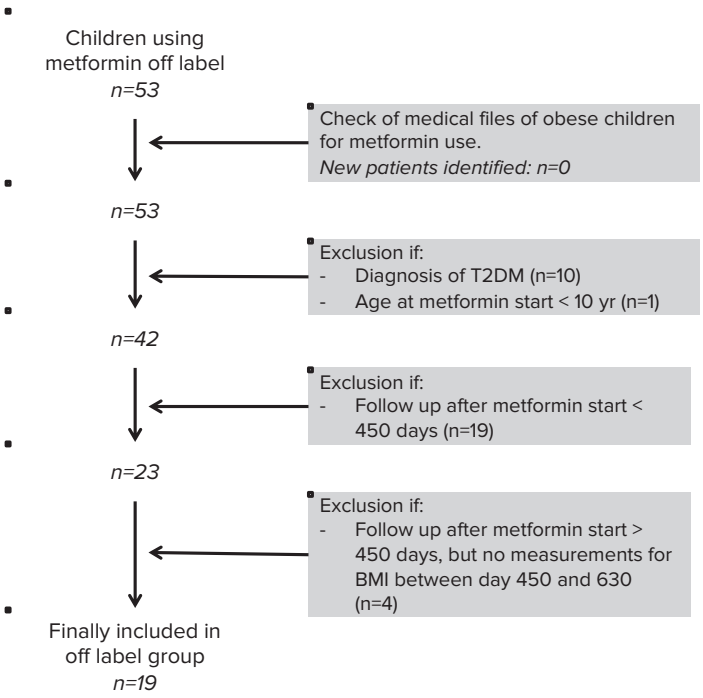


Figure 1. Flowchart of included patients in the daily clinical practice group

Statistical analysis

Since most parameters were not normally distributed in the RCT group, all data were reported as median (interquartile range). Baseline characteristics of continuous data were compared using the Mann-Whitney U test. For dichotomous data the chi squared

test was used. The change in BMI (ΔBMI) and BMI-SDS (ΔBMI-SDS) between baseline and t=18 months was compared between the two groups (off label treatment versus RCT-treatment) using the Mann Whitney U test. Subsequently, a multivariable linear regression analysis was conducted to assess the effect of trial participation (yes/no) adjusted for potential confounding factors. The latter model was constructed in triple, with ΔBMI, ΔBMI-SDS and ΔFPG as outcomes of interest. All variables that differed at baseline between the two groups ($p<0.10$) were considered as potential confounders, and added stepwise to the model. Variables were retained in the final regression model if the coefficient of trial participation changed >10%. In case of missing data regarding the outcome of interest, the case was excluded from that analysis. All analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Patients

In the daily clinical practice group, 19 patients were identified who were treated with metformin for 18 months (Figure 1). For the RCT group, the data of 23 patients were eligible for analysis. In table 1 baseline characteristics of both groups are displayed. In the daily clinical practice group, more boys (57.9%) than girls (42.1%) were included, whereas the participants in the RCT were more girls (73.9%) than boys (26.1%). The participants in the daily clinical practice group were of multi-ethnic origin, with 11/19 (58%) being Caucasian. Other ethnicities were Asian (n=2), African (n=2), North-African (n=2), and Hindustani (n=2). In the RCT all participants were of Caucasian origin as a result of the inclusion criteria. Both groups were equal in age, height, weight and BMI at baseline. Morbid obesity (defined as BMI-SDS ≥3.0) was more prevalent in the daily clinical practice group, i.e. 15/19 (78.9%) were morbidly obese, versus 13/23 (56.5%) in the RCT group, which was not significant (p=0.13). Baseline differences were observed for FPG, FPI and HOMA-IR, with significantly higher prevalences of impaired fasted glucose (FPG ≥ 5.6 mmol/l), hyperinsulinemia (FPI > 15 μU/ml) and insulin resistance (HOMA-IR ≥ 3.4) in the daily clinical practice group (Table 1).

Table 1. Baseline characteristics of patients treated with off label metformin and treated with metformin in a randomized clinical trial

	Daily clinical practice group (n=19)	RCT group (n=23)	P-value chi^2	P-value Mann-Whitney
Gender				
- Boys	- 11 (57.9)	- 6 (26.1)	**0.037**	
- Girls	- 8 (42.1)	- 17 (73.9)		
Age (years)	14.3 (11.7-15.7)	13.6 (12.6-15.3)		0.99
Etnicity				
- Caucasian	- 11 (57.9)	- 23 (100)	NA	
- Other	- 8 (42.1)	- 0 (0)		
Height (cm)	168.3 (161.5-177.2)	162.9 (159.9-168.0)		0.08
Weight (kg)	92.5 (75.2-104.0)	82.2 (75.4-92.7)		0.16
BMI (kg/m^2)	31.3 (28.8-33.8)	29.8 (28.1-34.5)		0.66
BMI-SDS	3.23 (3.05-3.64)	3.10 (2.72-3.52)		0.37
BMI-SDS ≥ 3.0	15 (78.9)	13 (56.5)	0.13	
Tanner stage:				
- Prepubertal (TS1)	- 3 (15.8)	- 3 (13.0)	0.42	
- Pubertal (TS2-4)	- 4 (26.4)	- 17 (74.0)		
- Postpubertal (TS5)	- 1 (5.6)	- 3 (13.0)		
- Unknown	- 10 (52.6)	- 0 (0)		
FPG (mmol/l)	5.0 (4.8-5.6)	4.8 (4.7-5.0)		**0.015**
FPG ≥ 5.6 mmol/l	5 (26.3)	0 (0)	NA	
FPI (µU/ml)	31.0 (22.0-41.9)	18.0 (11.0-27.0)		**0.005**
FPI > 15 µU/ml	17 (89.5)	14 (60.9)	**0.036**	
HOMA-IR	7.74 (4.48-8.96)	4.00 (2.30-6.36)		**0.003**
HOMA-IR ≥ 3.4	17 (89.5)	10 (43.5)	**0.019**	
HbA1c (mmol/mol)	36 (32-39)$^{\#}$	33 (31-34)		0.052

Data are presented as median (interquartile range) or n (%). $^{\#}$ n=14

Abbreviations: RCT – randomized clinical trial; BMI (-SDS) – body mass index (standard deviation score); FPG – fasted plasma glucose; FPI – fasted plasma insulin; HOMA-IR – homeostasis model assessment for insulin resistance

Change in BMI over 18 months

In table 2, the results after 18 months of treatment are presented. Median ΔBMI over 18 months in the daily clinical practice group was -0.36 (-2.10-1.58) kg/m^2, versus +0.22 (-2.87-1.27) kg/m^2 in the RCT group, which is not a statistically significant difference (p=0.69) (Figure 2). The corresponding changes in BMI-SDS were -0.15 (-0.54- -0.05) and -0.12 (-0.50-0.08) for the off label and RCT group, respectively (p=0.99) (Figure 2). In the multivariable linear regression analyses, study participation was not associated

with ΔBMI nor ΔBMI-SDS. Variables that influenced the coefficient for study participation with more than 10% were gender in the ΔBMI model, and gender, height and insulin resistance for BMI-SDS, with final regression coefficients of –0.40 (-1.93 – 1.13) (p=0.61) and -0.02 (-0.31 – 0.28) (p=0.90), respectively.

Change in glucose metabolism over 18 months

At baseline, the daily clinical practice group had significant higher levels of FPG (table 1) while impaired fasted glucose was present in 4/19 patients, vs. 0/23 patients in the off label versus the RCT group. Univariate analysis of the ΔFPG showed a significant difference between both groups, with an increase of +0.2 (0.0-0.3) mmol/l in the daily clinical practice group and a decrease of -0.2 (-0.5-0.0) mmol/l in the RCT group (p=0.001) (Figure 3). This remained significant in a multivariate analysis model containing IR at baseline (p<0.001). For ΔFPI, ΔHOMA-IR and ΔHbA1c no significant difference between the groups was observed (Table 2).

Table 2. Results after 18 months of treatment

	Daily clinical practice group (n=19)			RCT group (n=23)			Delta
	t=0	t=18	Δ t=18- t=0	t=0	t=18	Δ t=18- t=0	p-value
BMI (kg/m^2)	31.0 (27.9-32.8)	30.5 (26.0-32.4)	-0.36 (-2.10-1.58)	29.8 (28.1-34.5)	29.9 (26.3-33.6)	0.22 (-2.87-1.27)	0.686
BMI-SDS	3.23 (3.05-3.64)	3.00 (2.43- 3.37)	-0.15 (-0.54- 0.05)	3.10 (2.72-3.52)	2.90 (2.34-3.39)	-0.12 (-0.50-0.08)	0.990
FPG (mmol/l)	5.0 (4.8-5.6)	5.4 (5.0-5.7)[a]	0.2 (0.0-0.3)[a]	4.8 (4.7-5.0)	4.6 (4.4-4.8)	-0.2 (-0.5-0.0)	**0.001**
FPI (µU/ml)	31.0 (21.0-41.9)	19.6 (11.0-34.0)[a]	-5.0 (-20.5-6.3)[a]	18.0 (11.0-27.0)	15.0 (10.0-20.0)	-3.0 (-13.0-6.0)	0.661
HOMA-IR	7.16 (4.31-8.96)	4.29 (2.52-9.43)[a]	-1.03 (-4.48-1.88)[a]	4.00 (2.30-6.36)	3.00 (2.00-4.29)	-1.00 (-3.17-1.47)	0.802
HbA1c	34 (32-39)[a]	34 (33-36)[a]	-1.0 (-3.5-3.5)[b]	33 (31-34)	34 (31-34)[c]	1.0 (-1.0-2.3)[c]	0.480

Data are presented as median (interquartile range). [a] n=14;[b] n=6; [c] n=22.
Abbreviations: BMI (-SDS) – body mass index (standard deviation score); FPG – fasted plasma glucose; FPI – fasted plasma insulin; HOMA-IR – homeostasis model assessment for insulin resistance.

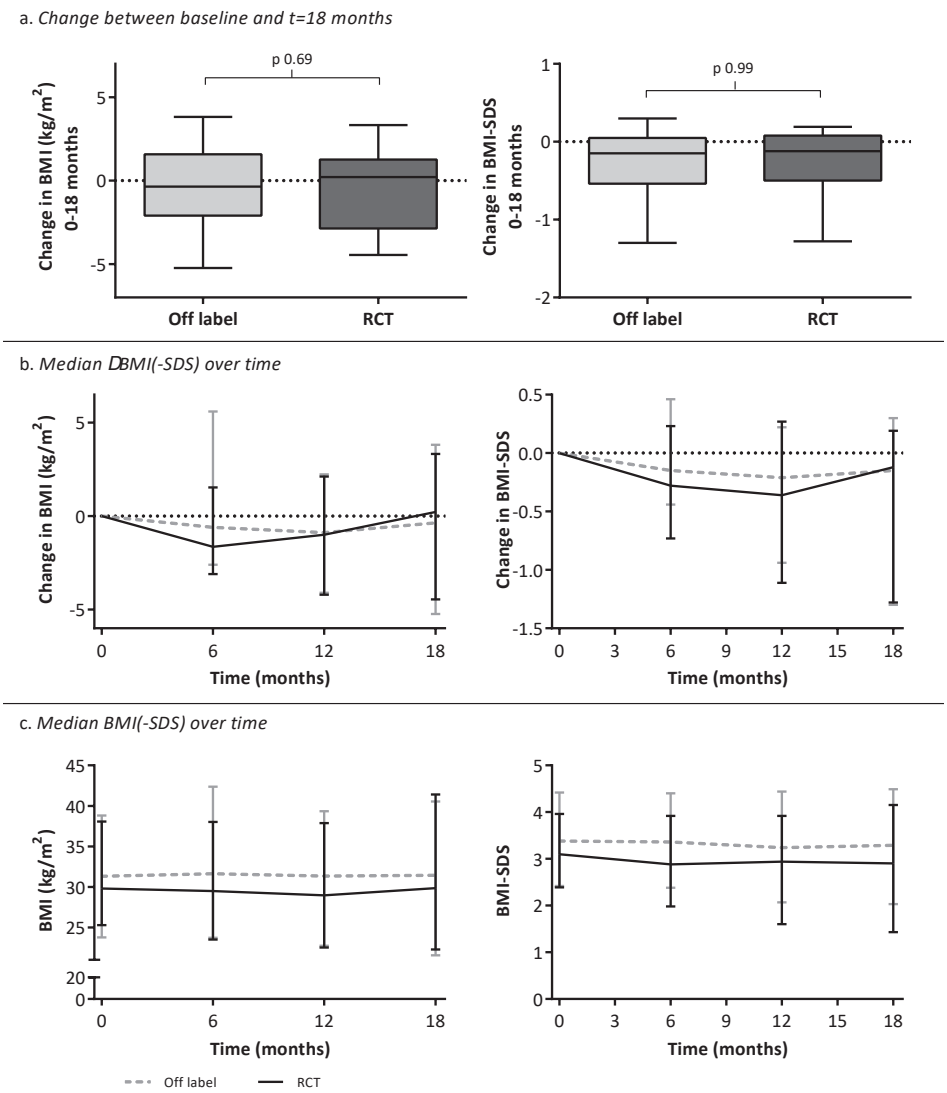
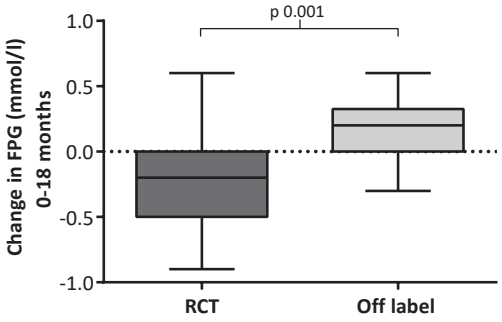
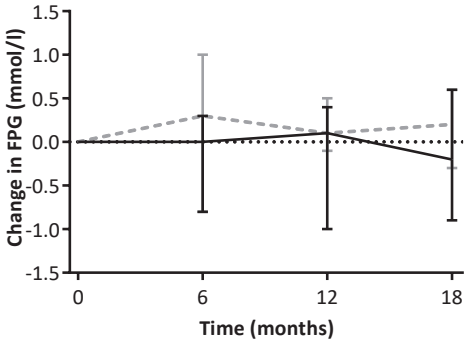


Figure 2. Change in BMI (left column) and BMI-SDS (right column) over 18 months of treatment
a. Change between baseline and t=18 months; b. Median ΔBMI(-SDS) over time; c. Median BMI(-SDS) over time. Graphs b and c represent median (min-max).

a. *Change between baseline and t=18 months*



b. *Median change in FPG over time*



c. *Median FPG over time*

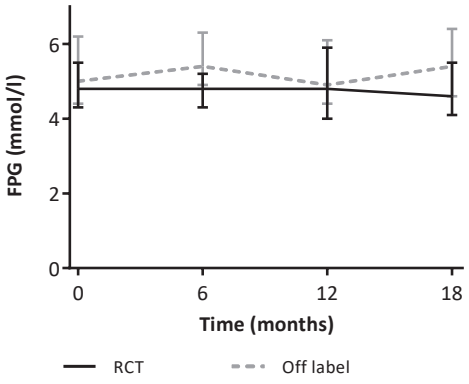


Figure 3. Change in fasted plasma glucose

a. Change between baseline and t=18 months; b. Median ΔFPG over time; c. Median FPG over time Graphs b and c represent median (min-max).

Discussion

In this observational study, we compared treatment results of metformin in obese adolescents treated in daily clinical practice in an outpatient pediatric obesity clinic with results of a RCT in obese adolescents. We observed that metformin treatment in obese adolescents in daily clinical practice was associated with change in BMI similar to the change during metformin treatment in obese adolescents in a RCT.

The RCT was the first study reporting on the effects of metformin versus placebo during 18-month treatment, showing a ΔBMI of +0.22 (-2.87-1.27) kg/m^2 in the metformin group. This small increase in ΔBMI at 18 months was initially preceded by a substantial decrease in ΔBMI in the metformin arm at 9 months, while in the placebo arm of this RCT ΔBMI from baseline to 18 months was found to increase significantly compared to the metformin arm (i.e. ΔBMI +1.17 (-0.26-2.37) kg/m^2, (p=0.015)) [8]. In the current study, the course of BMI and BMI-SDS over time in the daily clinical practice group, also showed an initial decrease in BMI and BMI-SDS in accordance with the RCT group. After 6-12 months, the median BMI and BMI-SDS started to increase again (Figure 2 and 3), which could be an indication that the effect of metformin fades out after a certain period of treatment. This is in line with the findings in the meta-analysis of McDonagh, where the effect after >6 months was -0.79 (95%CI -1.63 – 0.06) kg/m^2 compared to 6 months of treatment -1.38 (95%CI -1.93- -0.82) kg/m^2 [6]. Although the effect of metformin might fade out over time, it remains unclear whether prolonged use (>18 months) of metformin is not effective any more (i.e. children treated with metformin return to their previous BMI-percentile), or whether it will result in persisting lower BMI-values compared to placebo. For the 18-month treatment, our study shows that the change in BMI upon metformin in daily clinical practice was similar to results as obtained in a RCT after treatment of 18 months [8].

In contrast to the ΔBMI, the ΔFPG was different between both groups after 18 months, with an increase in FPG in the daily clinical practice group. Next to ΔFPG, baseline FPG, FPI and HOMA-IR were significantly higher in this group compared to the RCT group. Especially the difference in HOMA-IR is remarkable, since inclusion in the RCT required a HOMA-IR ≥ 3.4, while no criteria for HOMA-IR were used for the daily clinical practice group. Selection bias for treatment with metformin in daily clinical practice is a possible explanation. The clinician might tend to reserve off label treatment with metformin for children with increased levels of FPG, FPI or HOMA-IR. Another explanation could be the difference in ethnicity between both groups. The participants of the RCT were all Caucasian, whereas the daily clinical practice group was multi-ethnic. Since some ethnicities are at higher risk for T2DM than others, for example, African-Americans, Asians and South-Indians [13], this might result in higher prevalence rates of T2DM precursors in these groups. In our daily clinical practice population 5 children had impaired fasted glucose at baseline, of which 2 were of

Hindustan origin and 2 of North-African (Moroccan) origin. Regarding the influence of ethnicity on the effect of metformin, a study by Williams et al found a better glycemic response in African-Americans compared to European Americans [14]. Nagi et al. found no difference in effect of metformin in Caucasian and Asian subgroups [15]. Based on these studies the influence of ethnicity on the glycemic response to metformin can neither be confirmed nor ruled out. Therefore, the difference in ΔFPG in our study remains not well explained, and since in the RCT group all participants are Caucasian further analysis of our data was not possible.

A limitation of our study is the retrospective data collection in the daily clinical practice group. For FPG, FPI and HOMA-IR this resulted in 26% and for HbA1c 68% missing data after 18 months of treatment, despite of the use of time windows for the visits after 6, 12 and 18 months, ultimately reducing our patient number from 19 to 14 patients for the analysis of FPG, FPI and HOMA-IR, and 6 patients for HbA1c. As a benefit of the retrospective data collection in the outpatient clinic, patients were not aware of being studied.

Another limitation is the incomplete information on lifestyle intervention of the daily clinical practice group. In daily clinical practice a lifestyle intervention programme is offered as standard care to all patients, but is not clear whether all patients attended these programmes and whether all programmes were comparable. Some patients in daily clinical practice received dietary advice by a dietician, whereas others received limited dietary advice by the paediatrician. To all participants of the RCT a lifestyle programme consisting of dietary advice and physical training twice weekly was offered.

Conclusions

In this study BMI remained stable over 18 months in adolescents in daily practice, which is comparable to the results obtained under the strict circumstances of a RCT. It is reassuring that metformin added to lifestyle interventions in daily practice is associated with a similar change in BMI as observed during metformin use in experimental conditions.

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Section 4

Conclusions and perspectives

Chapter 8

Conclusions and perspectives on diagnosis and treatment of obese children with insulin resistance

Summary and conclusions

Introduction and background

Last decades, the prevalence of childhood obesity is rising, with rates up to 17.4% in the U.S. [1]. In the Netherlands, the prevalence of childhood obesity varied in 2010 from 1.8% in native boys to 8.4% in boys of Turkish descent [2]. For children standard deviation scores (SDS), z-scores, or percentiles are used to the define overweight and obesity [3-5]. Cut-off values used in the Netherlands are BMI-SDS > 1.1 (BMI > p85) for overweight and BMI-SDS > 2.3 (BMI > p95) for obesity [6].

Childhood obesity is a strong predictor for obesity in adulthood. Odds ratios for obese children to become obese adults varied from OR 1.3 for obese children aged 1-2 years to an OR of 22.3 for obese children aged 10-14 years [7, 8]. Besides psychological consequences, childhood obesity has multiple somatic consequences, affecting almost all organ tracts. Cardiovascular and metabolic consequences are common, including hypertension, dyslipidemia, endothelial dysfunction, insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [9-11]. IR is described as an early sign in the development of metabolic and cardiovascular consequences in obesity [12-14]. Although insulin resistance is related to obesity, not all obese children are insulin resistant, and not all insulin resistant children are obese [15]. However, insulin resistance levels do increase with the level of overweight [16, 17].

Prevalence, diagnosis and follow up of children with insulin resistance

In view of the increasing incidence of obesity in children, insight into the epidemiology of the pre-diabetic state IR seems important. In **Chapter 2**, a systematic review was presented to give an overview of all population-based studies reporting on the prevalence and incidence rates of IR in childhood [18]. Eighteen population-based studies were identified, describing prevalence rates varying between 3.1 and 44 %. This variation could be explained partly by different definitions for IR. The results show that overweight and obese children had higher prevalence rates than normal weight children. In seven out of thirteen studies reporting sex-specific results, girls seemed to be more affected than boys. Since different definitions were used in most studies, comparison of prevalence rates between studies was impeded. It was concluded that consensus on the definition for IR in children is needed to allow for comparisons between different studies.

This variation in definitions for IR was further investigated in **Chapter 3**. Published definitions (methods and cut-off values) to define IR in pediatric populations were applied to a population of patients with obesity from a pediatric outpatient clinic. In 103 identified articles, 146 IR definitions were reported based on 14 different methods. Definitions based on fasted blood samples were used 137 times, whereas oral/intra-

venous glucose tolerance test derived methods were used 9 times. The homeostasis model for the assessment of insulin resistance (HOMA-IR) and fasted plasma insulin (FPI) were the most frequently used fasted methods (83 and 37 times, respectively). A wide range in cut-off values to define IR was observed, resulting in prevalence rates in the predefined obese pediatric population between 5.5% (FPI > 30 mU/l) and 72.3% (Insulin sensitivity index$_{Matsuda}$ ≤ 7.2). The findings of this study underlined the need for a uniform definition for IR.

Currently, the recommended screening to identify children at risk for diabetes and its precursors impaired glucose tolerance (IGT) and IR is fasted plasma glucose (FPG). In **Chapter 4,** the value of FPI to calculate the HOMA-IR in addition to screening with FPG to detect children with IR, impaired glucose tolerance or T2DM, was evaluated [19]. For this, routinely collected data of an oral glucose tolerance test (OGTT) of 311 obese children (10.8±3.2 years) were evaluated. Screening according to the guidelines, using FPG with a cut-off ≥ 5.6 mmol/l was compared to screening with FPG ≥ 5.6 mmol/l combined with HOMA-IR (cut-off value ≥ 3.4). Diabetes and IGT were defined according to the American Diabetes Association (ADA) criteria [20]. Cases of IR, IGT or T2DM identified on the basis of screening with FPG ≥ 5.6 mmol/l, compared to screening with FPG ≥ 5.6 mmol/l or HOMA-IR ≥ 3.4, were respectively four (80%) vs five (100%) for T2DM, 7 (28%) vs 16 (64%) for IGT and 0 (0%) vs 93 (100%) for IR. In conclusion, screening with FPG and FPI to calculate HOMA-IR has equal burden compared to screening with FPG alone, and identifies all patients with diabetes, and more patients with precursors of diabetes.

To date, the recommended screening interval for children at risk for T2DM, i.e. for example children with overweight or obesity and IR, is 3 years. In **Chapter 5,** a follow up study was performed in children at risk for T2DM, to evaluate weight, insulin sensitivity, and progression to T2DM approximately 3 years after being diagnosed with overweight/obesity and IR (measured by HOMA-IR) [21]. Out of 86 invited children, 44 (mean age 15.4 ± 3.6 years) participated. Medical history, physical examination, and laboratory workup were performed. While the mean BMI-SDS significantly increased from 2.9 to 3.4, the mean HOMA-IR significantly decreased from 5.5 to 4.6 (baseline vs follow up visit). Change in HOMA-IR was due to a decrease in mean FPI (24.1 vs 21.1, p=0.073). Although the increase in BMI-SDS in these children is worrisome, none of the children at risk for T2DM developed T2DM during the screening interval of three years proposed by the American Diabetes Association.

Treatment of obese children with insulin resistance

In the second part of this thesis, the effect of long-term treatment with metformin in obese children with IR was presented. With the rising prevalence of childhood obesity, and thereby of IR, the risk of complications in childhood rises as well. To prevent

these complications, lifestyle intervention is the cornerstone in treatment. However, long-term efficacy of lifestyle intervention is questionable [22]. As adolescents with obesity and IR may be refractory to lifestyle intervention therapy alone [23], additional off-label metformin therapy is often applied [24, 25]. Metformin has been shown to be moderately effective to reduce BMI in adolescents with obesity and hyperinsulinemia [26-32]. However, data on long-term efficacy and safety are lacking. In **Chapter 6a,** the study protocol of the Metformin study was presented [33]. The primary objective of the Metformin study was to determine the effect of adding metformin treatment to lifestyle-intervention in reducing BMI in adolescents with obesity and IR. The Metformin study is a multi-centre prospective study, that consists of two parts of each 18 months: a double-blind randomized placebo-controlled trial (part 1) and an open-label follow up study (part 2). During part 1 the participants were given Metformin 1000 mg or placebo twice daily and were offered a lifestyle intervention program over 18 months. During part 2, no structured lifestyle intervention program was offered. All participants who still met the criteria for the use of metformin were free to choose whether they would use metformin in part 2. Primary endpoints were change in BMI and IR measured by the HOMA-IR. Secondary endpoints were safety and tolerability of metformin. Other endpoints were body fat percentage and HbA1c. In **Chapter 6b,** the results of part 1 of the Metformin study were presented [34]. Forty-two participants completed the 18 month-study (66% girls, median age 13 (12-15) years, BMI 30.0 (28.3-35.0) kg/m^2 and HOMA-IR 4.08 (2.40-5.88)). Median ΔBMI at 18 months was +0.2 (-2.9-1.3) kg/m^2 (metformin) versus +1.2 (-0.3-2.4) kg/m^2 (placebo) (p=0.015). No significant difference was observed for HOMA-IR. No serious adverse events were reported. Median change in fat percentage was -3.1 (-4.8-0.3) vs -0.8 (-3.2-1.6)% (p=0.150), in fat mass -0.2 (-5.2-2.1) vs +2.0 (1.2-6.4) kg (p=0.007), in fat free mass +2.0 (-0.1-4.0) vs +4.5 (1.3-11.6) kg (p=0.047), and in ΔHbA1c +1.0 (-1.0-2.3) vs +3.0 (0.0-5.0) mmol/mol (p=0.020) (metformin vs placebo). To conclude, long-term treatment with metformin in adolescents with obesity and IR results in stabilisation of BMI and improved body composition compared to placebo. Its seems therefore that metformin may be useful as additional therapy next to lifestyle intervention in adolescents with obesity and IR.

Because treatment effects reported in clinical trials may differ from the effects in daily clinical practice, the aim of **Chapter 7** is to compare the effects of metformin (in addition to a lifestyle intervention program) on change in BMI between adolescents with obesity treated with metformin in daily clinical practice and adolescents who participated in the RCT (Chapter 6). For this study, all adolescents with obesity treated off-label with metformin in our pediatric obesity outpatient clinic, with clinical follow up of at least 18 months from start of treatment were identified. Anthropometric data (age, height, weight, body mass index) and laboratory parameters (FPG, FPI and HbA1c) were collected at baseline and at t=18 months. Change in BMI after 18 months was

compared between the two groups. Nineteen patients (median age 14.3 (interquartile range 11.7-15.7) years, BMI 31.3 (28.8-33.8) kg/m^2, BMI-SDS 3.23 (3.05-3.64)) in the daily clinical practice group were compared to 23 patients receiving metformin during the RCT (age 13.6 (12.6-15.3) years, BMI 29.8 (28.1-34.5) kg/m^2, BMI-SDS 3.10 (2.72-3.52). Change in BMI after 18 months was -0.36 (-2.10-1.58) vs +0.22 (-2.87-1.27) kg/m^2 for the two groups, respectively. In the multivariable model, the changes in BMI were not statistically significantly different (p=0.61). In these populations, treatment with metformin in adolescents with obesity in daily clinical practice is associated with a change in BMI similar to the change observed during metformin treatment in obese adolescents in a RCT. This finding further supports considering metformin as an add-on therapy next to lifestyle intervention.

Perspectives

The importance of a uniform definition for IR and how to get to a uniform definition

In this thesis, the lack of a uniform definition for IR in children and adolescents has become clear. As a result of this lack of uniform definition for IR, the incidence and prevalence of IR in pediatric populations remains unclear [18]. Differences in prevalence rates between populations can in part be explained by the use of different definitions. With a uniform definition for IR, it will be possible to compare prevalence and incidence rates between populations and trends over time. In clinical practice, a clear definition and cut-off value will help clinicians to identify children at risk for T2DM and other cardiometabolic complications. For the follow up of children with IR, the factors resulting in a physiological increase or decrease of insulin concentration, such as age and pubertal stage, have to be taken into account in the definition and cut-off value for use in the follow up in clinical practice.

Although IR is an important risk factor for T2DM and cardiometabolic complications [11, 14]., other risk factors should not be ignored. In most patients a combination of risk factors results in the development of T2DM or other complications. These risk factors are combined in the metabolic syndrome, also called insulin resistance syndrome or syndrome X. For the metabolic syndrome however, there is no consensus on the best definition for the use in pediatric populations either [35, 36]. At least six definitions for the metabolic syndrome in pediatric patients have been reported [36-41]. These definitions all include criteria for overweight, blood pressure and blood lipids, with various cut-off values. The criterion on blood glucose and/or insulin varies: four definitions include impaired fasted glucose (with different cut-off points in each definition) [37, 38, 40, 41]; the other two definitions include impaired fasted glucose (with different cut-off

points in each definition), hyperinsulinemia or increased HOMA-IR as criterion [36, 39]. A uniform definition for IR could be applied in these definitions combining the most important risk factors for cardiometabolic complications.

Towards a uniform definition for IR in children

A uniform definition for IR in children should meet certain criteria to be of use in daily clinical practice. First, it should be accurate. The gold standard is the euglycemic-hyperinsulinemic clamp study [42]. However, this clamp study is not suitable for daily clinical practice because of the invasive, time consuming character and high burden for the patients. Many surrogate measures have been developed and compared to the euglycemic-hyperinsulinemic clamp study [43-45]. The correlations of measures based on the OGTT are comparable to the measures based on fasted samples [43, 46, 47]. However, the surrogate measures based on fasted samples have lower burden than OGTT-based measures, which is preferable for use in daily clinical practice. The most frequently studied fasted measures in pediatric populations, i.e. HOMA-IR, QUICKI and FPI, have moderate to strong correlations with IR assessed with the euglycemic-hyperinsulinemic clamp, respectively 0.51-0.81, 0.43-0.91 and 0.48-0.92 [46-51]. Therefore, this criterion does not distinguish in which method would be the best to use. To minimize the burden for the patients as much as possible, preferably fingertip capillary blood testing should be used. However, the accordance between insulin measured from capillary blood and blood from an antecubital venous puncture was poor (coefficient of variation 36.0%) [52].

A second criterion is the reproducibility of the test. Data available from adult studies showed a coefficient of variation (CV) for HOMA-IR of 11.8% (7.8-11.9), for QUICKI 1.8% (1.1 – 2.9) and for FPI 13.4% (8.8 – 21.9) [53]. The low CV reported for the QUICKI was however debated by Antuna et al. because this measure is composed of log transformed values of FPG and FPI [54]. When the CV of log transformed HOMA-IR values are compared to the CV of the QUICKI, similar, low CV's were found for both measures. Since all of these formulas are based on the same measurements of glucose and insulin, the CV is not discriminating between HOMA-IR and QUICKI either.

In summary, the three surrogate measures for IR based on fasted samples are comparable to each other, with equal burden for the patients.

Factors influencing the insulin concentration, such as age, pubertal stage [55-57], ethnicity [58, 59], and gender [60] have to be considered when defining cut-off values for IR in children. The available (population based) studies present percentiles for FPI and HOMA-IR levels for gender and age [36, 61-66]; and percentiles for HOMA-IR and QUICKI by Tanner stage and by Tanner stage and gender [67]. None of these studies differentiated between ethnic groups, and most studies included participants of only

one ethnicity. In a study by Chiu et al, it was found that ethnicity was an independent factor influencing the insulin sensitivity indices [58].

To produce age, pubertal stage, ethnicity and gender specific reference values for FPI, HOMA-IR and QUICKI the data of the performed studies could be used, complemented with data from additional new studies. The available large populations based studies are predominantly performed in Caucasian children. These studies provide data for HOMA-IR and FPI in European, mainly Caucasian children aged 3-10.9 years (n=7074 children) [36, 66]; for FPI in European children aged 7-20 years (n=1976) [63]; and for FPI and HOMA-IR in Caucasian children 9-16 years (n=2244) [65]. Two large population based studies in children from different ethnicities have been performed: one study in Mexican-American children aged 6-18 years (n=3701) providing values for HOMA-IR [61]; and a study by Yi et al. performed in Asian children aged 10-20 years (n=2716), providing data for FPI and HOMA-IR [64]. Additional data for Tanner stage and other ethnicities are required, as well as values for Asian children under the age of 10. Based on the values for FPI, HOMA-IR and QUICKI from these large studies, cut-off values could be defined. As cut-off values, the 95th percentile or a SD-score of 2 for gender, age or pubertal stage and ethnicity could be used.

Since there seems to be no advantage for the use of FPI above HOMA-IR or QUICKI, the clinician could use the measure he or she prefers, in combination with age, gender, pubertal stage and ethnicity specific cut-off values. For the comparison of prevalence rates and incidence rates, the use of one measurement is preferred. In our studies we used the HOMA-IR, since this was to our opinion the most frequently used, well-known measure in pediatrics. Furthermore, the calculation for HOMA-IR is easier than QUICKI to perform.

Preventive interventions in children with obesity at risk for cardiometabolic complications

Screening and follow up of obese children at risk for T2DM was discussed in chapter 4 and 5. The current ADA recommendations apply to children with overweight or obesity and additional risk factors for T2DM [20]. No specific recommendations are available for children with overweight or obesity without additional risk factors [20, 68]. On the basis of screening for risk factors and complications, children with obesity are classified with mild, moderate, high or very high risk of complications. The Dutch guideline 'Diagnosis and treatment of obesity in adults and children' differentiates in their treatment recommendations based on this 'weight-related health risk' ('Gewichtsgerelateerd Gezondheidsrisico') [68]. Children at higher risk, should receive a more intensive lifestyle intervention program.

There have been many studies assessing the effect of lifestyle intervention programs in overweight and obese children [22, 69-73]. Only a few studies compared the effects

of these programs between children with different degrees of overweight or obesity. A study by Rijks et al. showed that the effects of a lifestyle intervention program, with a follow up of 24 months, were similar in overweight, obese and morbidly obese children with respect to change in BMI z-score. After 12 months, cardiovascular risk factors such as blood pressure, cholesterol, FPG and HbA1c improved equally in all groups [74]. In contrast to this, Knop et al. described more effects in extremely obese children (<10 years) compared to obese children. For adolescents (≥ 10 years), the obese group had a better result of lifestyle intervention than extremely obese group [75]. In large prospective studies, the risk of T2DM and cardiovascular risk factors in adulthood was similar for both normal weight adults who were overweight or obese during childhood, and adults who had normal weight during childhood [76].

In view of this, the question is, whether we should focus on obese children with additional risk factors only, or consider overweight and obese children without risk factors as well, with respect to screening, follow up and preventive lifestyle interventions. In our study on screening obese children for T2DM, described in chapter 4, we excluded children with overweight [19]. In chapter 5, where the follow up of children at risk for T2DM was described, overweight and obese children were included [21]. Children with overweight had lower levels of HOMA-IR, compared to children with obesity (HOMA-IR 3.3 vs 4.8, respectively). However, the mean HOMA-IR of 3.3 was only slightly below the threshold of 3.4 used as cut-off value. Since the consequences of childhood overweight and obesity seem reversible provided normal weight is achieved in adulthood [76], lifestyle intervention should in our opinion be offered to all overweight and obese children. Children with overweight have risk factors for complications as described in different studies [77-80]. An economic evaluation of interventions for childhood obesity showed that for both overweight and obese children, lifestyle interventions are potentially cost effective on the long-term [81]. To improve the (long-term) effects of lifestyle intervention, use of e-health, web-based interventions and the use of smartphones should be further investigated. The currently available studies on the use of these technologies showed improved compliance and response, and lower dropout rates [82-84]. As mentioned in the general introduction, parental motivation is important as well. A combination of parent-only interventions and web-based or smartphone support could be an interesting field for future research to improve the effect of lifestyle interventions.

To conclude, both children with overweight and obesity have risk factors for cardiometabolic complications. Current guidelines apply to children with obesity only. Since the long-term risk of complications for both overweight and obese children is reversible provided they become normal weight adults, screening and preventive treatment of overweight children should be considered. Consequently, lifestyle interventions should be offered to both overweight and obese children.

The use of metformin in addition to lifestyle intervention in children with obesity

In the chapters 6 and 7 of this thesis, the effects of metformin in the treatment of children with obesity were described. It was found that metformin over 18 months resulted in a stabilization of BMI, whereas the participants receiving placebo continued gaining weight. Moreover, children with obesity treated in daily practice with metformin had similar results regarding the stabilization of BMI. The effects of metformin on change in BMI has been studied in many short-term trials, which have been analyzed in two meta-analyses, reporting a reduction in mean BMI of -1.42 ((95%CI -2.02 - -0.83) kg/m^2 (based on 5 studies) [26] and -1.38 (95%CI -1.93- -0.82) kg/m^2 (based on 8 studies) [85]. Long-term data are limited to a study of 48 weeks (mean ΔBMI -0.9(±0.5) kg/m^2 (metformin) versus +0.2(±0.5) kg/m^2 (placebo), p=0.03) [86], and our RCT of 18 months (median ΔBMI +0.2 (-2.9-1.3) kg/m^2 (metformin) versus +1.2 (-0.3-2.4) kg/m^2 (placebo) p=0.015) [34]. Regarding side-effects, especially gastro-intestinal side-effects are common, with up to 74% of the participants reporting nausea and 61% reporting diarrhoea in our study. Vitamin B12 deficiency occurred in 13%. In most cases, the gastro-intestinal side-effects are self-limiting; in 6% of cases, side effects resulted in treatment cessation [34]. Based on this evidence, globally there are three scenarios for the future use of metformin in the treatment of children of obesity. These scenarios will be discussed here, and the arguments are listed in table 1.

Table 1. Scenarios for future use of metformin in children with obesity

	Pro	Contra
Scenario 1:		
No use of metformin in obese children	- No risk of side effects, vitamin B12 deficiency or ketoacidosis - No exposure to a therapy of which the mechanism is partially unknown - No overtreatment / unnecessary use of medication	- No opportunity to benefit from effects of metformin on BMI [27-32, 88-90)
Scenario 2:		
Metformin to be used in all obese children if 1 year lifestyle intervention fails	- Equal treatment for obese children who do not benefit from lifestyle intervention alone - Potential benefit against limited burden of side effects	- Evidence for effectivity of metformin mainly in children with obesity and IR or other risk factors [27-32, 88-90) - Side effects and vitamin B12 deficiency, ketoacidosis
Scenario 3:		
Metformin in a select population of children with obesity and IR, hyperinsulinemia or other risk factors	- Available evidence applicable on this population - Effect on BMI (short-term and long-term) and IR (short-term)	- Side effects and vitamin B12 deficiency, ketoacidosis - Mechanism unknown - Effects of prolonged use (>18 months) unknown

In the first scenario, metformin is not used for the treatment of children with obesity. As a benefit of this scenario there is no risk of adverse events, such as gastro-intestinal complaints, vitamin B12 deficiency and (the scarcely occurring complication) keto-acidosis. Furthermore, children and adolescents are not exposed to a therapy with possible side-effects. It is suggested that metformin results in weight loss because of multilevel influence on the neuropeptides regulating appetite, and thereby reducing food intake [87]. However, weight loss during metformin therapy might also be due to side-effects, since patients with nausea and/or diarrhea have reduced caloric intake because of these side-effects. As such, it is debatable whether the use of metformin is justified given the relatively small reduction in BMI that is obtained.

On the other hand, in view of the broad spectrum of complications due to childhood obesity every small improvement in BMI could be helpful in reducing the risk of complications. Compared to invasive surgical options the complications of metformin are mild and mostly self-limiting and the burden of treatment is relatively low.

As a second scenario, metformin therapy can be initiated in all obese children, for instance in case lifestyle intervention during 1 year does not result in an improvement in BMI. Metformin would be offered to all children with obesity, with or without additional risk factors. It is unknown how motivation for lifestyle intervention is influenced by the availability of pharmacological interventions. In this scenario, all children with obesity have equal chances to benefit from the effect of metformin, against the burden of possible mild side-effects of metformin. Since most studies on the efficacy of metformin to achieve weight loss included patients with obesity and insulin resistance or other additional risk factors, the evidence use of metformin in children with obesity without any other risk factor is scarce [27-32, 88-90]. In the only study that did not require insulin resistance or other additional risk factors, mean baseline levels for HOMA-IR were 3.8±2.8 in the metformin group and 5.0±3.5 in the placebo group [86]. Based on these values the majority of the participants in this study can be thought to suffer from insulin resistance, and the results of this study cannot be interpreted as if the participants have obesity without any additional risk factor.

In the third scenario, metformin is prescribed to a select population of children with obesity and IR, hyperinsulinemia or other risk factors. These risk factors could be ethnicity, a family history of T2DM, or a family history of premature cardiovascular events. As described in the second scenario, most studies regarding the effect of metformin in obesity included children with obesity and IR or hyperinsulinemia. The before mentioned evidence on the (long term) use of metformin with respect to change in BMI is applicable to this population. For this group, the additional risk factors for the development of T2DM or other complications could justify the risk of gastro-intestinal side effects, vitamin B12 deficiency or ketoacidosis. Moreover, besides the effect on BMI, short-term studies on the use of metformin show an improvement in insulin resistance

as well [27, 28, 32, 42, 88]. This finding was however not confirmed in the 48-weeks study by Wilson et al. nor in our 18 months study [34, 86].

The question is whether metformin should also be given to children under the age of 10 years with obesity and IR, hyperinsulinemia or other risk factors. For these younger children the evidence is limited. Three studies included children < 10 years [27, 29, 32], but in 2 of them the mean age was comparable to studies including children aged 10 years and above. Only the study by Yanovski et al. included relatively young participants (6-12 years) with a mean age of 10.1 (±1.6) years for the metformin group and 10.4 (±1.4) years for the placebo group. Changes in BMI over 6 months were -0.78 (95%CI -1.54 - -0.01)kg/m^2 vs +0.32 (-0.54 – 1.18) and in BMI-SDS (-0.11 (-0.16 - -0.05) vs -0.04 (-0.1 – 0.02) for the metformin and placebo groups, respectively [32]. These results are comparable to other studies with 6 months follow up in older children. No studies with longer treatment duration in children under the age of ten years are available. Future research could focus on these younger children with obesity and IR, hyperinsulinemia or other risk factors.

Although metformin has beneficial effects there are some disadvantages, for example the above mentioned side-effects. Furthermore, data on the effects of prolonged use of metformin (>18 months) are not yet available, and it is therefore not clear whether treatment with metformin should stop after 18 months or be continued. In the second part of our RCT (chapter 6), follow up data of the participants are collected [33]. In the first 18-month part of our study, the effect seemed to fade out during the treatment (Figure 1). The results of the follow up study are therefore important to provide data on the effect of prolonged treatment with metformin (up to 36 months).

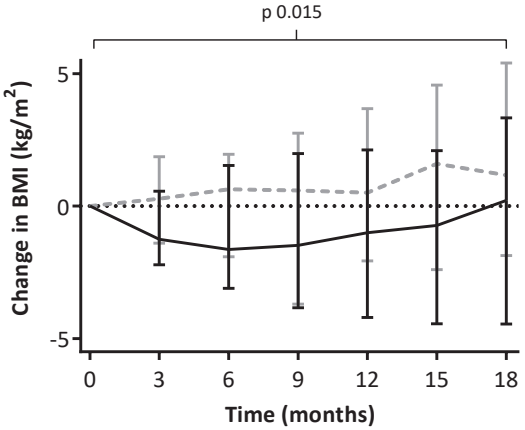

Figure 1. Effect of metformin on ΔBMI over 18 months

In conclusion, whether metformin should be applied in childhood obesity is open for discussion. Since metformin is safe with acceptable burden for the patients, we think it should be considered in pediatric patients with obesity and IR, hyperinsulinemia or other risk factors. It remains debatable whether metformin should be started when lifestyle intervention alone has failed or as a first step combined with lifestyle intervention. Another group of interest are children under the age of 10 years.

In this thesis, we aimed to study the epidemiology of IR, the screening and follow up of obese children at risk for T2DM, and the effect of metformin treatment in children with obesity and IR.

Prevalence rates for IR reported by population-based studies vary from 3.1 up to 44%. A comparison between these studies was not possible, since all studies used different definitions for IR. This difference in definitions was visualized by calculation of the prevalence rate of IR in an outpatient population of obese children using the reported definitions for IR. Depending on the definition, prevalence rates varied between 5.5 and 72.3% in this population. Therefore, a uniform definition for IR is essential to compare prevalence rates in populations. Moreover, since many factors influence the insulin concentration, specific cut-off levels for IR for age, pubertal stage, ethnicity and gender should be defined.

For screening on T2DM in obese children, a comparison was made between calculation of IR based on FPG and FPI with the use of HOMA-IR and on FPG alone. Screening with FPG and HOMA-IR was found to identify more children with IR and IGT, and with T2DM, compared to screening with FPG alone. In addition, as the recommended screening interval for children at risk of T2DM is 3 years, we performed a follow up study of children with obesity and IR. Even though during follow up their BMI-SDS increased, none of the children developed T2DM, leading to the conclusion that for now a screening interval of 3 years can be considered adequate.

Finally, long-term treatment of children with obesity and IR with metformin in addition to lifestyle intervention was studied in a RCT. In children treated with metformin for 18 months, BMI stabilized, whereas BMI increased in children receiving placebo. Based on these results, treatment with metformin in addition to lifestyle intervention in children with obesity and IR could be considered. This is underlined by comparing the results of metformin treatment on BMI in the RCT to the results in adolescent with obesity treated with metformin in daily clinical practice. These results were comparable to each other. In order to further optimize the effects of a combined treatment of metformin with life style intervention, and to obtain data on the optimal treatment duration, long term follow up of these children is needed. For long-term follow up, the results of the second part of our RCT have to be awaited.

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Chapter 9

Nederlandse samenvatting/
Dutch summary

Samenvatting en conclusies

Introductie en achtergrond

In de afgelopen decennia is de prevalentie van obesitas bij kinderen toegenomen, met prevalenties tot 17.4% in de V.S. [1]. In Nederland varieerde de prevalentie van obesitas bij kinderen in 2010 van 1.8% bij jongens van Nederlandse afkomst tot 8.4% bij jongens van Turkse afkomst [2]. Om overgewicht en obesitas bij kinderen vast te stellen wordt gebruik gemaakt van standaarddeviatie scores (SDS), z-scores of percentielen [3-5]. De afkapwaarden die in Nederland worden gebruikt zijn BMI-SDS>1.1 voor overgewicht (BMI>p85) en BMI-SDS>2.3 voor obesitas (BMI>p95) [6].

Obesitas op de kinderleeftijd is een sterke voorspeller voor obesitas als volwassene. Odd's ratio's (OR) voor kinderen met obesitas om als volwassenen obees te zijn lopen uiteen van 1.3 voor kinderen van 1-2 jaar oud met obesitas tot 22.3 voor kinderen met obesitas van 10-14 jaar oud [7,8]. Behalve psychologische gevolgen, zijn er ook verscheidene somatische gevolgen van obesitas bij kinderen, die zich in praktisch alle orgaansystemen kunnen uiten. Veelvoorkomend zijn cardiovasculaire en metabole gevolgen zoals hypertensie, dyslipidemie, endotheel dysfunctie, insuline resistentie (IR) en type 2 diabetes mellitus (T2DM) [9-11]. IR is beschreven als een vroeg stadium in het ontstaan van de cardiovasculaire en metabole gevolgen van obesitas [12-14]. Hoewel IR gerelateerd is aan obesitas, zijn niet alle kinderen met obesitas insuline resistent, en niet alle insuline resistente kinderen hebben obesitas [15]. Echter, de mate van IR loopt wel op met de mate van overgewicht [16,17].

Prevalentie, diagnose en follow-up van kinderen met insuline resistentie

Met het oog op de toenemende incidentie van obesitas bij kinderen, is het belangrijk om inzicht te krijgen in de epidemiologie van IR. In **Hoofdstuk 2** is een systematische review van de literatuur beschreven, waarin een overzicht wordt gegeven van alle population-based studies die prevalentie en/of incidentie cijfers van IR op de kinderleeftijd rapporteren [18]. Achttien studies werden gevonden, en de gerapporteerde prevalenties liepen uiteen van 3.1% tot 44%. Deze variatie kon gedeeltelijk worden verklaard door het gebruik van verschillende definities van IR in de afzonderlijke studies. Uit de gegevens werd duidelijk dat bij kinderen met overgewicht en obesitas de prevalentie van IR hoger is dan bij kinderen met een normaal gewicht. Dertien studies presenteerden prevalentiewaarden voor jongens en meisjes, in 7 studies hiervan lijken meisjes vaker IR te hebben dan jongens. Doordat verschillende definities voor IR werden gebruikt in de meeste studies, konden de prevalenties in de studies niet onderling worden vergeleken. Op basis hiervan werd geconcludeerd dat overeenstemming over de definitie voor IR bij kinderen noodzakelijk is om verschillende studies te kunnen vergelijken.

Dit verschil in definities voor IR werd verder onderzocht in **Hoofdstuk 3**. Alle gepubliceerde definities (methodes en afkapwaarden) voor IR in kinderpopulaties werden toegepast op een populatie van patiënten met obesitas uit een kinderpolikliniek. Er werden 103 artikelen gevonden, waarin 146 definities voor IR gebaseerd op 14 methoden werden gerapporteerd. In 137 gevallen was de definitie gebaseerd op een methode met nuchter afgenomen bloedmonsters, tegen 9 gevallen gebaseerd op een methode waarbij waarden uit een orale of intraveneuze glucosetolerantie test werden gebruikt. De 'homeostasis model for the assessment of insulin resistance' (HOMA-IR) en nuchter plasma insuline (FPI) waren de meest gebruikte nuchtere methoden (respectievelijk 83 en 37 keer). Een grote spreiding in de gebruikte afkapwaarden werd gevonden, welke resulteerde in prevalentiewaarden in een vooraf vastgestelde populatie van obese kinderen van 5.5% (FPI > 30 mU/l) tot 72.3% (Insulin sensitivity $index_{Matsuda} \leq 7.2$). Deze bevindingen benadrukten het belang van een eenduidige definitie voor IR.

Op dit moment is nuchter plasma glucose (FPG) de aanbevolen screening om kinderen die risico lopen op diabetes en voorlopers hiervan, gestoorde glucosetolerantie (IGT) en IR, op te sporen. In **Hoofdstuk 4** werd de toegevoegde waarde van het bepalen van FPI om de HOMA-IR te berekenen, naast FPG als screening voor IR, IGT en T2DM, onderzocht [19]. Hiervoor werd gebruik gemaakt van routinematig verzamelde gegevens van een orale glucosetolerantie test (OGTT) van 311 kinderen (10.8±3.2 jaar) van een kinderpolikliniek voor obesitas. Screening volgens de richtlijn, met FPG ≥ 5.6 mmol/l, werd vergeleken met een gecombineerde screening van FPG ≥ 5.6 mmol/l en HOMA-IR (afkapwaarde ≥ 3.4). Diabetes en IGT werden gedefinieerd aan de hand van de American Diabetes Association (ADA) criteria [20]. Het aantal opgespoorde gevallen van IR, IGT en T2DM op basis van screening met FPG, vergeleken met screening met FPG en HOMA-IR, was respectievelijk 4 (80%) versus 5 (100%) voor T2DM, 7 (28%) versus 16 (64%) voor IGT, en 0 (0%) versus 93 (100%) voor IR. Hieruit werd geconcludeerd dat door screening met FPG en FPI om de HOMA-IR te berekenen, alle patiënten met T2DM en meer patiënten met voorlopers van diabetes werden opgespoord, terwijl de belasting voor de patiënt gelijk blijft.

In de huidige richtlijnen is het aanbevolen screeningsinterval drie jaar voor kinderen die risico lopen op T2DM, bijvoorbeeld kinderen met overgewicht of obesitas en IR. In **Hoofdstuk 5** werd een follow-up studie verricht bij kinderen met een verhoogd risico op T2DM. Hierin werden gewicht, insuline gevoeligheid en progressie naar T2DM geëvalueerd circa drie jaar nadat overgewicht/obesitas en IR (gemeten met HOMA-IR) was vastgesteld bij deze kinderen [21]. Zesentachtig kinderen werden uitgenodigd, 44 (gemiddelde leeftijd 15.4 ± 3.6 jaar) kinderen namen deel. Anamnese, lichamelijk onderzoek en laboratoriumonderzoek werden verricht. Ondanks dat de gemiddelde BMI-SDS steeg van 2.9 naar 3.4, daalde de gemiddelde HOMA-IR van 5.5 naar 4.6

(baseline versus follow-up meting). De verandering in HOMA-IR werd veroorzaakt door een afname in de gemiddelde FPI (24.1 vs 21.1, p=0.073). Hoewel de stijging in BMI-SDS bij deze groep kinderen die risico loopt op T2DM zorgelijk is, ontwikkelde niemand tijdens dit door de ADA aanbevolen screeningsinterval van 3 jaar T2DM.

Behandeling van kinderen met obesitas en insuline resistentie

In het tweede deel van dit proefschrift, werd het effect van lange termijn behandeling met metformine bij obese kinderen met IR beschreven. Met de stijgende prevalentie van obesitas bij kinderen, en daarmee ook van IR, stijgt ook het risico op complicaties van obesitas op kinderleeftijd. Om deze complicaties te voorkomen, is interventie noodzakelijk. Leefstijlinterventie is de hoeksteen van de behandeling. Op de lange termijn is de effectiviteit van leefstijlinterventie echter minder gunstig [22]. Bij adolescenten met obesitas en IR is het effect van enkel leefstijlinterventie vaak onvoldoende [23], waardoor off-label therapie met metformine aan de behandeling wordt toegevoegd [24, 25]. In meerdere onderzoeken is gebleken dat metformine effectief is in het verlagen van de BMI bij adolescenten met obesitas en een hyperinsulinemie [26-32]. Echter, gegevens over de effectiviteit op de lange termijn ontbreken. In **Hoofdstuk 6a** is het studieprotocol van de Metformin studie beschreven [33]. Het primaire doel van de Metformin studie is het onderzoeken van het effect van toevoeging van metformine aan leefstijlinterventie op het verlagen van de BMI bij obese adolescenten met IR. De Metformin studie is een prospectieve, multicentrum studie, die uit 2 delen van 18 maanden bestaat: een dubbelblinde, gerandomiseerde, placebogecontroleerde studie (deel 1), en een open-label follow-up studie (deel 2). Tijdens deel 1 van de studie kregen de deelnemers tweemaal daags metformine 1000 mg of placebo, en een leefstijlinterventie programma gedurende 18 maanden. Tijdens deel 2 werd geen gestructureerd leefstijlinterventie programma aangeboden. Deelnemers die nog aan de criteria voor gebruik van metformine voldeden, mochten kiezen of zij in deel 2 metformine wilden gebruiken. De primaire eindpunten waren verandering in BMI en in IR (gemeten met de HOMA-IR). Secundaire eindpunten waren de veiligheid en verdraagbaarheid van metformine. Overige eindpunten waren percentage lichaamsvet en HbA1c.

In **Hoofdstuk 6b** werden de resultaten van deel 1 van de Metformin studie gepresenteerd [34]. Tweeënveertig deelnemers hebben dit 18 maanden durende deel van de studie volledig afgerond (66% meisjes, mediane leeftijd 13 (12-15) jaar, BMI 30.0 (28.3-35.0) kg/m^2 en HOMA-IR 4.08 (2.40-5.88)). De mediane ΔBMI na 18 maanden was +0.2 (-2.9-1.3) kg/m^2 (metformin) versus +1.2 (-0.3-2.4) kg/m^2 (placebo) (p=0.015). Er werd geen significant verschil gevonden voor HOMA-IR. Er werden geen ernstige ongewenste voorvallen (bijwerkingen) gemeld. De mediane verandering in vet percentage was -3.1 (-4.8-0.3) vs -0.8 (-3.2-1.6)% (p=0.150), in vet massa -0.2 (-5.2-2.1) vs +2.0

(1.2-6.4)kg (p=0.007), in vetvrije massa +2.0 (-0.1-4.0) vs +4.5 (1.3-11.6)kg (p=0.047), en in ΔHbA1c +1.0 (-1.0-2.3) vs +3.0 (0.0-5.0)mmol/mol (p=0.020) (metformin vs placebo). Concluderend resulteert lange termijn behandeling met metformine bij adolescenten met obesitas en IR in een stabilisatie van de BMI, en een verbeterde lichaamssamenstelling vergeleken met behandeling met placebo. Metformine zou bruikbaar kunnen zijn als therapie toegevoegd aan leefstijlinterventie bij adolescenten met obesitas en IR.

Resultaten van behandeling in trials kunnen afwijken van de resultaten die in de dagelijkse praktijk worden bereikt. Daarom was het doel van **Hoofdstuk 7** om het effect van metformine (toegevoegd aan behandeling met leefstijlinterventie) op verandering in BMI bij adolescenten met obesitas te vergelijken tussen adolescenten met obesitas behandeld in de dagelijkse praktijk en adolescenten die deelnamen aan een RCT (Hoofdstuk 6). In deze studie werden alle adolescenten geïncludeerd met obesitas die behandeld zijn met metformine en minimaal 18 maanden poliklinisch vervolgd op onze obesitas kinderpolikliniek. Antropometrische gegevens (leeftijd, geslacht, lengte, gewicht, BMI) en laboratoriumgegevens (FPG, FPI en HbA1c) werden verzameld bij start van de behandeling en na 18 maanden. De verandering in BMI werd vergeleken tussen de 2 groepen. Negentien patiënten (mediane leeftijd 14.3 (interkwartielbereik 11.7-15.7) jaar, BMI 31.3 (28.8-33.8) kg/m^2, BMI-SDS 3.23 (3.05-3.64)) in de groep uit de dagelijkse praktijk werden vergeleken met 23 patiënten die metformine kregen tijdens de RCT (leeftijd 13.6 (12.6-15.3) jaar, BMI 29.8 (28.1-34.5) kg/m^2, BMI-SDS 3.10 (2.72-3.52). De verandering in BMI was respectievelijk -0.36 (-2.10-1.58) vs +0.22 (-2.87-1.27) kg/m^2 voor de 2 groepen. In het multivariabele model, was dit verschil in BMI niet statistisch significant verschillend (p=0.61). Daarop werd geconcludeerd dat behandeling met metformine bij adolescenten met obesitas in de dagelijkse praktijk een vergelijkbare verandering in BMI oplevert als de verandering die werd gemeten bij adolescenten die deelnamen aan een RCT. Deze bevinding ondersteunt de overweging om metformine toe te voegen aan behandeling met leefstijlinterventie.

Perspectieven

Het belang van en de weg naar een uniforme definitie voor IR

In dit proefschrift is het ontbreken van een uniforme definitie voor IR bij kinderen en adolescenten duidelijk geworden. Door dit gebrek aan een uniforme definitie voor IR, blijven de incidentie en prevalentie van IR in pediatrische populaties ook onduidelijk [18]. De verschillen in prevalentie tussen populaties kunnen deels verklaard worden door het gebruik van verschillende definities. Met een uniforme definitie voor IR, is het mogelijk om de prevalentie en incidentie tussen populaties, en de trends in de

tijd te vergelijken. In de dagelijkse klinische praktijk zal een duidelijke definitie en afkapwaarde artsen helpen bij het identificeren van kinderen die risico lopen op T2DM en andere cardio-metabole complicaties. De factoren die leiden tot een fysiologische toename of afname van insuline concentratie, zoals leeftijd en puberteitsstadium moeten worden meegenomen in de definitie en afkapwaarde bij gebruik in de follow-up van kinderen met IR.

Hoewel IR een belangrijke risicofactor is voor T2DM en cardio-metabole complicaties [11, 14], moeten andere risicofactoren niet worden vergeten. Bij de meeste patiënten resulteert een combinatie van risicofactoren in het ontstaan van T2DM of andere complicaties. Deze risicofactoren zijn samengevoegd in het metabool syndroom, ook wel insulineresistentie syndroom of syndroom X genoemd. Voor het metabool syndroom is er echter, net als voor IR, geen consensus welke definitie het beste is voor het gebruik bij kinderen [35, 36]. Ten minste zes definities voor het metabool syndroom bij kinderen zijn vermeld in de literatuur [36-41]. Deze definities bevatten allen criteria voor overgewicht, bloeddruk en lipiden, met verschillende afkapwaarden. Het criterium voor bloedglucose en/of insuline varieert: vier definities bevatten verminderde nuchtere glucose (met verschillende afkapwaarden per definitie) [37, 38, 40, 41]; de andere twee definities bevatten verminderde nuchtere glucose (met verschillende afkapwaarden per definitie), hyperinsulinemie of verhoogde HOMA-IR als criterium [36, 39]. Een uniforme definitie voor IR zou kunnen worden toegepast binnen deze definities die de belangrijkste risicofactoren voor cardio-metabole complicaties combineren.

Naar een uniforme definitie voor IR bij kinderen

Een uniforme definitie voor IR bij kinderen moet voldoen aan bepaalde criteria voor het gebruik in de dagelijkse klinische praktijk. Ten eerste moet deze nauwkeurig zijn. De gouden standaard is de euglycemische-hyperinsulinemische clamp studie [42]. Echter, deze clamp-studie is niet geschikt voor de dagelijkse praktijk vanwege het invasieve en tijdrovende karakter en de hoge belasting voor de patiënt. Vele surrogaat methodes zijn ontwikkeld en vergeleken met de euglycemische-hyperinsulinemische clamp studie [43-45]. De correlaties met de gouden standaard van de methodes gebaseerd op een OGTT en de methodes op basis van nuchtere bloedmonsters zijn vergelijkbaar [43, 46, 47]. Echter, de methodes op basis van nuchtere bloedmonsters hebben een lagere belasting voor de patiënt dan de methodes op basis van de OGTT. Deze lagere belasting heeft de voorkeur bij gebruik in de dagelijkse klinische praktijk. De bij kinderen meest bestudeerde methodes gebaseerd op nuchtere bloedmonsters zijn de HOMA-IR, QUICKI en FPI; deze hebben matige tot sterke correlaties met IR gemeten met de gouden standaard, respectievelijk 0.51-0.81, 0.43-0.91 en 0.48-0,92 [46-51]. Hierdoor is de nauwkeurigheid niet onderscheidend tussen de verschillende

methodes gebaseerd op nuchtere bloedmonsters. Om de belasting voor de patiënt zoveel mogelijk te beperken, zou bloed verkregen uit een vingerprik kunnen worden gebruikt. Echter, de overeenstemming tussen insuline gemeten in capillair bloed (vingerprik) en bloed uit een venapunctie was slecht (variatiecoëfficiënt 36.0%) [52].

Een tweede criterium waarnaar moet worden gekeken is de reproduceerbaarheid van de test. Gegevens beschikbaar uit volwassen studies toonden een variatiecoëfficiënt (VC) voor HOMA-IR van 11.8% (7.8-11.9), voor QUICKI van 1.8% (1.1-2.9) en voor FPI van 13.4% (8.8-21.9) [53]. De lage VC gerapporteerd voor de QUICKI werd echter door Antuna et al. ter discussie gesteld vanwege het gebruik van log-getransformeerde waarden van FPG en FPI in de formule voor QUICKI [54]. Wanneer de VC van log-getransformeerde HOMA-IR waarden worden vergeleken met de VC van de QUICKI, hebben beide methodes vergelijkbare lage waardes. Omdat alle methodes zijn gebaseerd op dezelfde metingen van glucose en insuline, maakt de VC niet het onderscheid tussen HOMA-IR, QUICKI en FPI.

Samenvattend zijn deze drie alternatieve methodes voor het bepalen van IR gebaseerd op nuchtere bloedmonsters vergelijkbaar met elkaar, met een gelijke belasting voor de patiënten.

Bij het bepalen van afkapwaarden voor IR bij kinderen, moeten factoren die van invloed zijn op de insuline concentratie, zoals leeftijd, puberteitsstadium [55-57], etniciteit [58, 59] en geslacht [60] worden meegenomen. Er zijn percentielen voor FPI en HOMA-IR voor geslacht en leeftijd [36, 61-66] beschikbaar uit (population-based) studies; en percentielen voor HOMA-IR en QUICKI voor Tanner stadium en voor Tanner stadium per geslacht [67]. Geen van deze studies maakte onderscheid tussen etnische groepen; de meeste studies includeerden deelnemers van één etniciteit. In een studie van Chiu et al. bleek echter dat etniciteit een onafhankelijke factor is die de insulinegevoeligheid indices beïnvloedt [58].

Om specifieke afkapwaarden voor leeftijd, puberteitsstadium, etniciteit en geslacht te genereren voor FPI, HOMA-IR en QUICKI, kunnen de gegevens van de beschikbare studies worden gebruikt, aangevuld met gegevens van nieuwe studies. De beschikbare grote population-based studies werden voornamelijk uitgevoerd bij Kaukasische kinderen. Deze beschikbare studies bevatten gegevens voor FPI en HOMA-IR bij Europese (voornamelijk Kaukasische) kinderen van 3-10.9 jaar (n=7074 kinderen) [36, 66], voor FPI bij Europese kinderen in de leeftijd 7-20 jaar (n=1976) [63], en voor FPI en HOMA-IR bij Kaukasische kinderen 9-16 jaar (n = 2244) [65]. Er zijn twee population-based studies bij kinderen van andere etnische groepen uitgevoerd: één onderzoek bij Mexicaans-Amerikaanse kinderen in de leeftijd 6-18 jaar (n = 3701) met waarden voor HOMA-IR [61]; en een studie van Yi et al. uitgevoerd in Aziatische kinderen in de leeftijd 10-20 jaar (n = 2716), met gegevens voor de FPI en HOMA-IR [64]. Aanvullende gegevens zijn nog nodig van Tanner stadium bij andere dan de Kaukasische etniciteit,

en voor Aziatische kinderen jonger dan 10. Op basis van de waarden voor FPI, HOMA-IR en QUICKI uit de beschikbare en nieuwe studies zou een afkapwaarde kunnen worden gedefinieerd. Als afkapwaarden, kunnen de 95e percentiel of een SD-score van 2 voor geslacht, leeftijd of puberteitsstadium, en etniciteit worden gebruikt.

Aangezien er geen duidelijk voordeel lijkt voor het gebruik van FPI, HOMA-IR of QUICKI, kan de behandelend arts zelf de methode kiezen die zijn of haar voorkeur heeft, waarbij de voor leeftijd, geslacht, puberteitsstadium en etniciteit specifieke afkapwaarde wordt toegepast. Voor het vergelijken van prevalentie en incidentie-waarden, is het gebruik van één methode aan te bevelen. In onze studies hebben we gebruik gemaakt van de HOMA-IR, omdat deze het meest gebruikt en bekend is in kindergeneeskundige studies. Bovendien is de berekening van de HOMA-IR eenvoudiger dan de die van de QUICKI.

Preventieve interventies bij kinderen met obesitas en een verhoogd risico op cardiometabole complicaties

Screening en follow-up van obese kinderen met een verhoogd risico op T2DM werd besproken in hoofdstuk 4 en 5. De huidige ADA-aanbevelingen gelden voor kinderen met overgewicht of obesitas en andere risicofactoren voor T2DM [20]. Er zijn geen specifieke aanbevelingen beschikbaar voor kinderen met overgewicht of obesitas zonder bijkomende risicofactoren [20, 68]. Kinderen met obesitas worden ingedeeld in categorieën met licht, matig, sterk of extreem verhoogd risico op complicaties, op basis van screening op risicofactoren en complicaties. De Nederlandse richtlijn 'Diagnostiek en behandeling van obesitas bij volwassenen en kinderen' differentieert op basis van dit 'Gewichtsgerelateerd Gezondheidsrisico' tussen verschillende aanbevolen behandelingen [68]. Kinderen met een hoger risico, moet een intensiever leefstijlinterventie programma krijgen.

Er zijn veel studies naar het effect van leefstijl interventieprogramma's bij kinderen met overgewicht en obesitas geweest [22, 69-73]. Slechts een paar van deze studies vergeleken de effecten van deze programma's tussen kinderen met een verschillende mate van overgewicht of obesitas. In studie van Rijks et al. werd aangetoond dat in een leefstijlinterventie programma met een follow-up van 24 maanden, de verandering in BMI z-score vergelijkbaar was voor kinderen met overgewicht, obesitas en morbide obesitas. Na 12 maanden werd een vergelijkbare verbetering gevonden in cardiovasculaire risicofactoren zoals bloeddruk, cholesterol, FPG en HbA1c in alle groepen [74]. In tegenstelling hierop, beschreven Knop et al. dat meer effect van leefstijlinterventie werd gevonden bij morbide obese kinderen (<10 jaar) in vergelijking met obese kinderen. Voor adolescenten (≥ 10 jaar), had de obese groep een beter resultaat dan de morbide obese groep [75].

Het risico op T2DM en cardiovasculaire risicofactoren bij volwassen met normaal gewicht die als kind overgewicht of obesitas hadden, was in grote prospectieve stu-

dies gelijk aan het risico bij volwassenen met een normaal gewicht die een normaal gewicht hadden als kind [76]. Met het oog hierop, is het de vraag of we screening, follow-up en preventieve leefstijlinterventies alleen moeten richten op obese kinderen met bijkomende risicofactoren, of ook op kinderen met overgewicht of obesitas zonder risicofactoren. In onze studie over het screenen van obese kinderen op T2DM, beschreven in hoofdstuk 4, hebben we kinderen met overgewicht uitgesloten [19]. In hoofdstuk 5, waar de follow-up van de kinderen met een risico op T2DM werd beschreven, werden zowel kinderen met overgewicht als obese kinderen opgenomen [21]. Kinderen met overgewicht hadden een lagere HOMA-IR, vergeleken met kinderen met obesitas (HOMA-IR 3.3 versus 4.8, respectievelijk). Echter, de gemiddelde HOMA-IR van 3.3 was slechts iets onder onze afkapwaarde van 3.4. Omdat de gevolgen van overgewicht en obesitas op kinderleeftijd omkeerbaar lijken als een normaal gewicht is bereikt op volwassen leeftijd [76], moet naar onze mening leefstijlinterventie worden aangeboden aan alle kinderen met overgewicht en obesitas. Ook bij kinderen met overgewicht komen risicofactoren voor complicaties voor, zoals beschreven in verschillende studies [77-80]. Uit een economische evaluatie van interventies voor overgewicht bij kinderen is gebleken dat voor zowel kinderen met overgewicht als met obesitas, leefstijlinterventies kosteneffectief kunnen zijn op de lange termijn [81]. Om de (lange termijn) effecten van leefstijlinterventie te verbeteren, moet het gebruik van e-health, web-based interventies en het gebruik van smartphones verder worden onderzocht. De momenteel beschikbare studies over het gebruik van deze technologieën toonde betere therapietrouw en een lagere uitval [82-84]. Zoals is beschreven in de algemene inleiding, is motivatie van ouders eveneens belangrijk. Een combinatie van interventies gericht op ouders en web-based of smartphone ondersteuning is een interessante focus voor toekomstig onderzoek om het effect van leefstijlinterventies verbeteren.

Samenvattend hebben zowel kinderen met overgewicht als kinderen met obesitas risicofactoren voor cardio-metabole complicaties. De huidige richtlijnen zijn enkel gericht op kinderen met obesitas. Omdat het lange termijn risico op complicatie voor zowel kinderen met overgewicht als obesitas omkeerbaar is als zij als volwassenen een normaal gewicht hebben, zou screening en preventieve behandeling ook voor kinderen met overgewicht moeten worden overwogen. Leefstijlinterventie zou dus zowel aan kinderen met obesitas als aan kinderen met overgewicht moeten worden aangeboden.

Het gebruik van metformine naast leefstijlinterventie bij kinderen met obesitas

In hoofdstuk 6 en 7 van dit proefschrift werden de effecten van metformine in de behandeling van kinderen met obesitas beschreven. Er werd gevonden dat behandeling met metformine gedurende 18 maanden resulteerde in een stabilisatie van de BMI, terwijl deelnemers die placebo kregen bleven toenemen in gewicht. Daarnaast

werd gevonden dat kinderen met obesitas die in de dagelijkse praktijk 18 maanden werden behandeld met metformine, vergelijkbare resultaten bereikten met betrekking tot stabilisatie van de BMI. Het effect op korte termijn van behandeling met metformine is in meerdere korte termijn studies onderzocht. Deze resultaten zijn samengevoegd in 2 meta-analyses, waarin een afname van de gemiddelde BMI van -1.42 ((95%CI -2.02 - -0.83) kg/m^2 (gebaseerd op 5 studies) [26] en -1.38 (95%CI -1.93- -0.82) kg/m^2 (gebaseerd op 8 studies) werd gerapporteerd [85]. Lange termijn gegevens zijn beperkt tot een studie van 48 weken (gemiddelde ΔBMI -0.9 (± 0.5) kg/m2 (metformine) versus 0.2 (± 0.5) kg/m^2 (placebo), p=0.03) [86], en onze RCT van 18 maanden (mediane ΔBMI +0.2 (-2.9-1.3) kg/m^2 (metformine) versus 1.2 (-0.3-2.4) kg/m^2 (placebo) p=0.015) [34]. Vooral gastro-intestinale bijwerkingen komen vaak voor, met tot 74% van de deelnemers die misselijkheid en 61% die diarree rapporteerden in onze studie. Vitamine B12 deficiëntie trad op bij 13%. In de meeste gevallen, gingen de gastro-intestinale bijwerkingen vanzelf over; in 6% van de gevallen resulteerde bijwerkingen in staken van de behandeling [34]. Op basis van deze gegevens, zijn er grofweg drie scenario's voor het toekomstige gebruik van metformine bij de behandeling van kinderen van obesitas. Deze scenario's worden hier besproken en de argumenten zijn opgesomd in tabel 1.

Table 1. Scenario's voor toekomstig toepassing van metformine bij kinderen met obesitas

	Pro	Contra
Scenario 1: Geen gebruik van metformine bij kinderen met obesitas	- Geen risico op bijwerkingen, vitamine B12 deficiëntie of ketoacidose - Geen blootstelling aan therapie waarvan het werkingsmechanisme gedeeltelijk onopgehelderd is - Geen overbehandeling/onnodig gebruik van medicatie	- Geen kans om te profiteren van effect van metformine op BMI [27-32, 88-90]
Scenario 2: Metformine bij alle kinderen met obesitas als levensstijlinterventie gedurende 1 jaar heeft gefaald	- Gelijke behandeling voor alle obese kinderen die niet geen effect hebben van alleen leefstijlinterventie - Mogelijk voordeel van therapie tegen lage belasting t.g.v. bijwerkingen	- Bewijs voor effectiviteit van metformine voornamelijk bij kinderen met obesitas en IR of andere risicofactoren [27-32, 88-90] - Bijwerkingen, vitamine B12 deficiëntie en ketoacidose
Scenario 3: Metformine bij een geselecteerde populatie van kinderen met obesitas en IR, hyperinsulinemie of andere risicofactoren.	- Beschikbare bewijs van toepassing op deze populatie - Effect op BMI (korte- en lange termijn) en IR (korte termijn)	- Bijwerkingen, vitamine B12 deficiëntie en ketoacidose - Mechanisme (deels) onopgehelderd - Effecten van langduriger gebruik (>18 maanden) onbekend

In het eerste scenario, wordt metformine niet toegepast in de behandeling van kinderen met obesitas. Een voordeel van dit scenario is dat er geen kans is op ongewenste effecten, zoals maag-darmklachten, vitamine B12 deficiëntie en (zeldzaam voorkomende) ketoacidose. Kinderen worden dus niet blootgesteld aan een therapie met mogelijke bijwerkingen. Er wordt verondersteld dat gebruik van metformine leidt tot gewichtsverlies door invloed op meerdere punten bij in de regulatie van de eetlust door neuropeptiden, en daardoor de voedselinname vermindert [87]. Echter, gewichtsverlies tijdens het gebruik van metformine zou ook kunnen worden veroorzaakt door bijwerkingen, met name de maag-darmklachten, omdat patiënten die misselijk zijn of diarree hebben daardoor mogelijk ook een lagere calorie-inname hebben. Hierdoor is het de vraag of het gebruik van metformine is gerechtvaardigd gezien de relatief kleine vermindering van BMI die wordt behaald.

Aan de andere kant, gezien het brede spectrum van complicaties van obesitas op de kinderleeftijd, kan elke kleine verbetering in BMI bijdragen aan het verminderen van het risico op complicaties. Vergeleken met invasieve chirurgische ingrepen, zijn de complicaties van metformine mild, en gaan meestal vanzelf over, waardoor de belasting van de behandeling relatief laag is.

In het tweede scenario, kan metformine worden gestart bij alle kinderen met obesitas als levensstijlinterventie gedurende 1 jaar niet heeft geleid tot een verbetering van de BMI. Metformine zou dan worden aangeboden aan alle kinderen met obesitas, met of zonder bijkomende risicofactoren. In dit scenario hebben alle kinderen met obesitas gelijke kansen om te profiteren van het effect van metformine, tegen de kans op de mogelijke milde bijwerkingen van metformine. Overigens, is het onbekend hoe motivatie voor levensstijlinterventie wordt beïnvloed door de inzetten van farmacologische interventies. Aangezien de meeste studies over de werkzaamheid van metformine op gewichtsverlies bij patiënten met obesitas en IR of andere bijkomende risicofactoren zijn uitgevoerd [27-32, 88-90], is het bewijs voor gebruik van metformine bij kinderen met obesitas zonder andere risicofactor schaars. In de enige studie waarin geen IR of andere bijkomende risicofactoren was vereist, was de gemiddelde uitgangswaarden voor HOMA-IR 3.8 ± 2.8 in de metformine groep en 5.0 ± 3.5 in de placebogroep [86]. Op basis van deze waarden kan worden verondersteld dat de meerderheid van de deelnemers in deze studie IR zal hebben gehad en kunnen de resultaten van dit onderzoek niet worden geïnterpreteerd alsof deelnemers obesitas zonder extra risicofactor hadden.

In het derde scenario wordt metformine voorgeschreven aan een selecte populatie van kinderen met obesitas en IR, hyperinsulinemie of andere risicofactoren. Deze risicofactoren kunnen etniciteit, familiair voorkomen van T2DM, of familiair voorkomen van cardiovasculaire aandoeningen op vroege leeftijd zijn. Zoals beschreven in het tweede scenario zijn de meeste studies naar het effect van metformine bij obesitas

bij kinderen met obesitas en IR of hyperinsulinemie uitgevoerd. De eerder vermelde gegevens over (lange termijn) gebruik van metformine met betrekking tot de veranderingen in BMI zijn van toepassing op deze populatie. Voor deze risicogroep zou het hogere risico op het ontwikkelen van T2DM of andere complicaties de kans op gastro-intestinale bijwerkingen, vitamine B12 deficiëntie of ketoacidose bij het gebruik van metformine rechtvaardigen. Bovendien toonden een aantal korte termijn studies naast het effect op BMI ook een verbetering van de insulineresistentie [27, 28, 32, 42, 88]. Deze bevinding werd noch bevestigd in het onderzoek van 48 weken van Wilson et al., noch in onze studie van 18 maanden [34, 86].

De vraag is of metformine ook moet worden gegeven aan kinderen jonger dan 10 jaar met overgewicht en IR, hyperinsulinemie of andere risicofactoren. Voor deze jongere kinderen is het bewijs beperkt. In drie studies werden kinderen <10 jaar opgenomen [27, 29, 32], maar in 2 hiervan was de gemiddelde leeftijd vergelijkbaar met studies die enkel kinderen van 10 jaar en ouder opnamen. Alleen het onderzoek van Yanovski et al. bestond uit relatief jonge deelnemers (6-12 jaar) met een gemiddelde leeftijd van 10.1 (±1.6) jaar voor de metformine groep en 10.4 (±1.4) jaar voor de placebogroep. Veranderingen in de BMI na 6 maanden waren -0.78 (95%BI -1.54 - -0.01) kg/m^2 versus 0.32 (-0.54 - 1.18) en in de BMI-SDS (-0.11 (-0.16 - -0.05) vs -0.04 (-0.1 - 0.02) voor respectievelijk de metformine en de placebogroep [32]. Deze resultaten zijn vergelijkbaar met studies van 6 maanden bij oudere kinderen. Er zijn geen studies met een langere behandelduur bij kinderen jonger dan tien jaar beschikbaar. Toekomstig onderzoek zou zich kunnen richten op deze jongere kinderen met obesitas en IR, hyperinsulinemie of andere risicofactoren.

Hoewel metformine voordelen heeft, zijn er ook een aantal nadelen, bijvoorbeeld de bovengenoemde bijwerkingen. Bovendien zijn de gegevens over de effecten van langdurig gebruik van metformine (>18 maanden) nog niet beschikbaar, en het is daarom nog niet duidelijk of behandeling met metformine moet stoppen na 18 maanden of moet worden voortgezet. In het tweede deel van onze RCT (hoofdstuk 6), worden follow-up gegevens van de deelnemers verzameld [33]. In de eerste 18 maanden van de studie, lijkt het effect te verminderen gedurende de behandeling (figuur 1). De resultaten van de follow-up studie zijn daarom van belang om inzicht te krijgen in het effect van langdurige behandeling met metformine (maximaal 36 maanden).

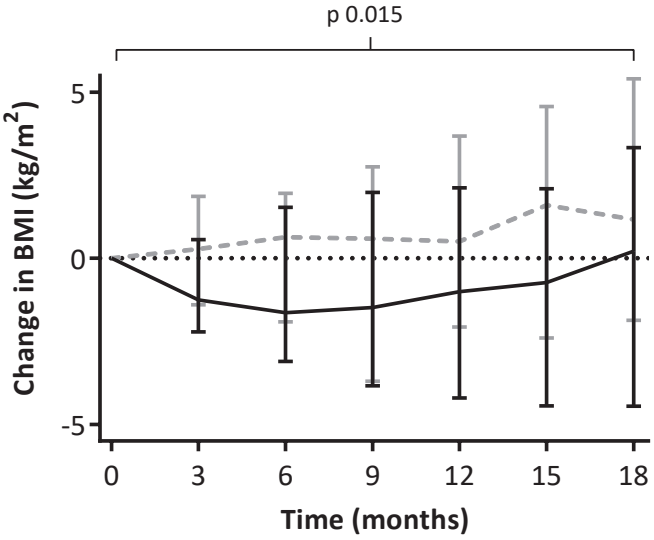

Figure 1. Effect van metformine op ΔBMI gedurende 18 maanden

Al met al, blijft het gebruik van metformin bij kinderen met obesitas ter discussie staan. Aangezien het gebruik van metformin veilig is, met een acceptabele belasting voor de patiënten, denken wij dat gebruik van metformine moet worden overwogen bij kinderen met obesitas en IR, hyperinsulinemie of andere risicofactoren. De vraag blijft wanneer metformine zou moeten worden gestart: nadat leefstijlinterventie heeft gefaald, of als eerste stap in de behandeling in combinatie met leefstijlinterventie. Daarnaast is ook de groep kinderen jonger dan 10 jaar nog een interessant gebied voor verder onderzoek.

Samenvattend hebben we ons in dit proefschrift gericht op het bestuderen van de epidemiologie van IR, de screening en follow-up van kinderen met obesitas en verhoogd risico op T2DM, en het effect van behandeling met metformine bij kinderen met obesitas en IR.

Gerapporteerde prevalentiewaarden voor IR in population-based studies variëren van 3.1 tot 44%. Een vergelijking tussen de verschillende populaties was niet mogelijk, aangezien de studies verschillende definities voor IR hanteren. Dit verschil in definities werd zichtbaar gemaakt door berekening van de prevalentie van IR op basis van de gerapporteerde definities voor IR in één poliklinische populatie van obese kinderen. Afhankelijk van de definitie, varieerde de prevalentie van 5.5 tot 72.3% in deze populatie. Dit benadrukt dat een uniforme definitie voor IR essentieel is om de prevalentie te vergelijken tussen populaties. Aangezien veel factoren de insuline concentratie beïnvloeden, moeten er specifieke afkapwaarden voor IR voor leeftijd, puberteitsstadium, etniciteit en geslacht moeten worden vastgesteld.

Voor het screenen op T2DM bij obese kinderen, werd een vergelijking gemaakt tussen screening met FPG en HOMA-IR (berekend uit FPG en FPI) en screening met enkel FPG. Bij screening met een combinatie van FPG en HOMA-IR werden meer kinderen met IR, IGT en T2DM opgespoord dan bij screening met enkel FPG. Aanvullend werd in een follow-up studie bij kinderen met overgewicht of obesitas en IR, het aanbevolen screeningsinterval voor T2DM van 3 jaar geëvalueerd. Hoewel de BMI-SDS van deze groep toenam, ontwikkelde geen enkel kind T2DM, waarop werd geconcludeerd dat 3 jaar een veilig screeningsinterval is.

Tot slot werd het effect van lange termijn behandeling met metformine in aanvulling op leefstijlinterventie bij kinderen met obesitas en IR onderzocht in een RCT. Bij de kinderen die metformine kregen gedurende 18 maanden, stabiliseerde de BMI, terwijl de BMI steeg bij kinderen die placebo kregen. Op basis hiervan, kan metformine als aanvulling op leefstijlinterventie bij kinderen met obesitas en IR worden overwogen. Dit werd nogmaals benadrukt de resultaten uit de RCT te vergelijken met resultaten bij adolescenten met obesitas die in de dagelijkse praktijk werden behandeld met metformine. Deze resultaten waren vergelijkbaar met elkaar. Om een optimaal effect te behalen uit de gecombineerde behandeling, en om te bepalen wat de optimale behandelduur is, is lange termijn follow-up van deze kinderen noodzakelijk. Voor gegevens over de lange termijn follow up, zullen de resultaten van het tweede deel van onze RCT moeten worden afgewacht.

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Appendices

Curriculum Vitae

Marloes van der Aa was born on November 12, 1985 in Twello, The Netherlands. In 2003 she graduated from the 'Gymnasium Apeldoorn' and started to study medicine at the Utrecht University. She graduated from medical school in December 2009. From January-December 2010 Marloes worked as a resident at the department of internal medicine of "Ziekenhuis Gelderse Vallei" in Ede. In 2011 she started her PhD project described in this thesis at the pediatric department of the St. Antonius Hospital in Nieuwegein under supervision of dr. M.M.J. van der Vorst, Prof. dr. A. de Boer and Prof. dr. C.A.J. Knibbe. Marloes commenced the postgraduate general practitioner (GP) training in the UMC Utrecht Julius Center in September 2013. In November 2016 she graduates as GP.

List of publications

Publications related to this thesis

van der Aa MP, Fazeli Farsani S, Kromwijk LA, de Boer A, Knibbe CA, van der Vorst MM. How to screen obese children at risk for type 2 diabetes mellitus? Clinical Pediatrics 2014 Apr;53(4):337-42

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Van der Aa MP, Elst, MAJ, van de Garde EMW, van Mil EGAH, Knibbe CAJ, van der Vorst MMJ. Long-term treatment with metformin in obese, insulin-resistant adolescents: results of a randomized, double-blinded placebo-controlled trial. Nutrition & Diabetes 2016 Aug 29;6(8):e228

Other publications

Fazeli Farsani S, van der Aa MP, van der Vorst MM, Knibbe CA, de Boer A. Global trends in the incidence and prevalence of type 2 diabetes in children and adolescents: a systematic review and evaluation of methodological approaches. Diabetologia. 2013 Jul;56(7):1471-88

Elst MAJ, van der Aa MP, van Mil EGAH, van der Vorst MMJ. Screening op type 2-diabetes mellitus: de heilige graal? Tijdschrift voor Kindergeneeskunde. 2015 March; 83(1): 27-35

Nawoord

Mijn proefschrift is af! Dat was niet gelukt zonder de hulp en steun van de medewerkers van afdelingen Kindergeneeskunde, Klinische Farmacie en Fysiotherapie van het St. Antonius Ziekenhuis, en de afdeling Kindergeneeskunde van het Jeroen Bosch Ziekenhuis. Uiteraard mag ik de patiënten die deelnamen aan de studies niet vergeten te vermelden, zonder hen waren de beschreven onderzoeken niet mogelijk geweest.

De volgende mensen in het bijzonder hebben bijgedragen aan het tot stand komen van dit proefschrift:

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Zoals hierboven blijkt, lever je een grote prestatie nooit alleen. Niet alleen de mensen die daadwerkelijk een bijdrage aan de studies hebben geleverd waren belangrijk, maar ook het stevige thuisfront.

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