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Insulin secretion response during oral glucose tolerance test is related to low cardiorespiratory fitness in obese adolescents

Abstract

Background: The obesity paradox refers to a category of subjects who may be less prone to develop co-morbidities, such as type 2 diabetes. Cardiorespiratory fitness (CRF) has been identified as one of the key factors. We aimed at exploring the difference in insulin metabolism between fit and unfit obese adolescents.

Methods: We recruited 22 obese adolescents and assessed CRF during an incremental treadmill test. According to a cut-off at 80% of predicted maximal oxygen consumption (VO_2max), subjects were separated into low or normal CRF. Body composition was determined by densitometry. Serum levels of insulin were measured sequentially during an oral glucose tolerance test and insulin secretion responses were calculated.

Results: Compared to adolescents with normal CRF, the ones with low CRF had higher insulin resistance indices (p=0.023) and insulin secretion response (p=0.010), independently of the body mass index z-score.

Conclusions: Interventions in obese adolescents should focus on the maintenance or improvement of CRF to at least 80% of predicted VO_2max. Indeed, this cut-off was significantly related to insulin secretion responses, independently of the adiposity level. A CRF above the proposed cut-off may prevent the development of insulin resistance.

Keywords: adolescent; cardio-respiratory fitness; insulin sensibility; obesity paradox.

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Introduction

Childhood obesity leads to the development of many complications, such as type 2 diabetes (T2DM), resulting in an increased morbidity and premature mortality in adulthood (1). The prevalence of abnormal glucose metabolism or T2DM is increasing in youth, especially for subjects at risk (2). Furthermore, insulin resistance is frequent in overweight adolescents, long before the occurrence of glucose dysfunction, and is related to the severity of obesity (3, 4). This condition plays a crucial role, as it is also implicated in the development of other co-morbidities such as dyslipidemia, hypertension, non-alcoholic fatty liver disease and the metabolic syndrome (5, 6).

The obesity paradoxes have been widely discussed in the adult population (7). One of those paradoxes refers to a subgroup of obese subjects who may be protected against obesity-related complications. The role of cardiorespiratory fitness (CRF) as a protecting factor has been proposed (8–10), with the demonstration that metabolically healthy obese subjects were fitter than unhealthy ones (8). This paradox has been poorly investigated in obese adolescents.

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This study aimed at looking at the difference in insulin levels, measured sequentially during an oral glucose tolerance test (OGTT), between fit and unfit obese adolescents.

Materials and methods

Study design and subjects

This study is embedded in a prospective cross-sectional protocol which aimed at measuring cardiovascular risk factors in obese adolescents. The main study population was composed of 25 obese adolescents (55% of females), aged 10–16 years (mean: 14.0±0.9), living in an urban area. They were recruited at the Pediatric Obesity Clinic of the University Hospitals of Geneva.

Inclusion criteria for the main study were: 1) onset of puberty, 2) no diagnosis of hypertension, 3) no antihypertensive or anti-diabetic treatment, 4) no use of medication affecting glucose and lipid metabolisms, 5) no history of familial hypertension or dyslipidemia and 6) absence of diabetes or other chronic diseases. Based on these criteria, we had to exclude one subject after the initial laboratory testing because of previously unknown familial hypercholesterolemia.

For the purpose of this project, only subjects from the main study who performed a cardiorespiratory fitness test were analyzed (22/24).

Once informed, a written consent was obtained from both parents and adolescents. The Mother and Child Ethics Committee of the University Hospitals of Geneva approved the study.

Measures

Anthropometrics and body composition We assessed body weight (kg) in light clothes (panties and tee-shirt) and height (cm) without shoes. Body mass index (BMI) was calculated as weight/height squared (kg/m^2) and z-scores were derived using the World Health Organization references (11). Mean BMI and BMI z-scores were 30.5±5.4 (kg/cm^2) and 2.6±0.7, respectively. Pubertal development (Tanner's stages) was determined by clinical examination.

Fat mass (FM, kg), fat-free mass (FFM, kg), percentage of total body fat (%) and percentage of abdominal fat (%) were measured using dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy™, Lunar Corp., Madison, WI, USA). Manual analysis, with the "regions of interest" feature, was performed on total body scans to assess abdominal fat. The upper border was defined as the distal margin of the lower ribs, and the lower border was defined as just superior to the supra-iliac crest. The lateral margins were placed outside the body, so that all abdominal but no arm tissue was included (12).

Glucose and insulin metabolisms All subjects underwent an OGTT in the morning (8:00 AM) after a 12-h overnight fast. Timed blood samples (at –15, 0, 30, 60, 90 and 120 min) were collected for the measurement of plasma glucose and insulin concentrations (13), which were measured using standard automated techniques (SYNCHRON LX20®) and radioimmunoassay (Access® ultrasensitive insulin, Beckman Coulter Ireland Inc.), respectively.

Insulin resistance was assessed using the homeostasis model [HOMA-IR=fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5]. An HOMA-IR>3 was considered abnormal (14). Insulin sensitivity index (ISI) was calculated according to the method described by Matsuda and DeFronzo (13), and the beta-cell function was evaluated by the insulin secretion responses during the OGTT. The total area under the curve was calculated by the trapezoid integration procedure ($AUC_{insulin}$) (15). Glucose response during the OGTT was also evaluated. Impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or T2DM was defined according to the American Diabetes Association.

Cardiorespiratory fitness CRF was determined as peak oxygen consumption (peakVO_2) assessed by direct gas analysis (Vmax Spectra™, Vyasis Healthcare, GE, USA) during a multi-stage treadmill test (Marquette 2000™, GE, USA). After a graded warm-up (2 min at 4 and 2 min at 5 km/h), the subject either walked fast or ran on the treadmill at a constant speed, which varied by age and physical capacity (6–9 km/h). The grade of the treadmill was increased by 2.5% every 2 min until the subject was exhausted. Maximal heart rate (beat/min), respiratory quotient (RQ) and VO_2 were recorded at the end of the test. The VO_2 was considered maximal if clinical signs of exhaustion and at least one of the following criteria were present: heart rate>95% of predicted maximal heart rate for age (i.e., 220–age), RQ>1.05, or oxygen plateau with a <2 mL/kg/min increase in VO_2 with increasing work rate. CRF was considered low if the peakVO_2 was <80% of predicted maximal VO_2 (16, 17).

Statistical analysis

Statistical analyses were performed using the SPSS software 18.0 (Chicago, IL, USA). The initial number of subjects needed was calculated for the main cardiovascular outcome. However, a power calculation analysis with an alpha error of 5% showed that, with our sample size, statistical power was of 97.5% for $AUC_{insulin}$, 73.9% for HOMA-IR and 53.3% for ISI. Data are presented as mean and standard deviation (SD). Statistical differences between groups or gender were analyzed using an independent Student's t-test and χ^2. We used analysis of co-variance (ANCOVA) to adjust the groups' comparison for the percentage of abdominal fat, one-way ANOVA with the Bonferroni post-hoc test to compare some variables among pubertal stages and a quadratic regression analysis to evaluate the relationship between insulin sensitivity and secretion. We had no missing data. Differences were considered significant if $p<0.05$.

Results

Patients' characteristics

The complete physical characteristics of the subjects have been reported previously (18). Only data on fasting glucose and insulin concentrations are duplicated in the present study. Characteristics of the 22 subjects who performed the cardiorespiratory test are presented according to their VO_2max in Table 1.

Table 1 Characteristics of obese subjects.

Characteristics	Normal CRF (n=11)	Low CRF (n=11)
Gender female, %	45	64
Age, years	14.1±0.6	13.9±1.2
Pubertal stages (II/III/IV/V)	1/6/1/3	3/4/2/2
Anthropometrics		
Weight, kg	76.4±12.0	87.2±18.9
Weight z-score	1.7±0.5	2.2±0.6[a]
Height, cm	164.0±7.2	162.5±5.4
Height z-score	0.2±1.0	0.4±1.0
BMI, kg/cm^2	28.3±2.8	32.8±6.5[a]
BMI z-score	2.2±0.4	2.9±0.8[a]
FFM, kg	43.6±7.3	43.0±8.4
FM, kg	29.8±7.8	42.0±11.1[a]
Percentage total body fat, %	40.5±6.9	49.1±4.6[a]
Percentage abdominal fat, %	45.3±8.1	55.5±4.0[a]
Glucose and insulin metabolisms		
Fasting glucose, mmol/L	4.4±0.2	4.6±0.4
Fasting insulin, mUl/L	8.8±4.6	12.7±6.0
HOMA-IR	2.1±0.9	3.6±1.5[a]
2-h glucose, mmol/L	5.6±0.7	6.0±0.8
2-h insulin, mUl/L	47.0±37.6	88.4±43.3[a]
Maximal insulin level, mUl/L	75.4±35.8	169.6±76.5[a]
$\{AUC\}_{insulin}$	312±123.3	584.2±58.9[a]
ISI	6.4±3.1	3.6±3.2
Cardiorespiratory fitness		
peakVO_2, mL/kg/min	42.7±5.3	31.7±4.0[a]
% of predicted peakVO_2	88.6±5.0	68.8±10.7[a]
Absolute peakVO_2, L/min	3.22±0.59	2.73±0.46[a]
peakVO_2 corrected per FFM, mL/kgFFM/min	73.9±4.1	64.0±6.5[a]
peakVO_2 corrected per FM, mL/kgFM/min	113.0±9.4	67.3±12.8[a]
Maximal heart rate, beat/min	187.2±8.2	181.5±7.6
% of predicted max heart rate	97.3±4.9	94.6±4.3
Maximal RQ	1.02±0.07	1.1±0.12

Results are expressed as mean and SD. [a]p<0.05 between normal and low CRF. CRF, cardiorespiratory fitness; BMI, body mass index; FFM, fat-free mass; FM, fat mass; HOMA-IR, homeostasis model assessment; AUC, area under the curve; ISI, insulin sensitivity index; peakVO_2, peak oxygen consumption during exercise; RQ, respiratory quotient.

Glucose and insulin metabolisms

The HOMA-IR was normal in 77% (17/22) of subjects and none of them presented IFG and/or IGT upon OGTT. Insulin levels measured at different time points during the OGTT for all subjects are presented in Figure 1A, and the relationship between insulin secretion (AUC) and sensitivity (ISI) is presented in Figure 2. There was no difference in insulin or glucose concentrations between gender or among pubertal stages (p>0.05 for all).

Cardiorespiratory fitness

All subjects reached exhaustion during the treadmill test, 64% (14/22) of them attained the 95% of predicted maximal heart rate and 55% (12/22) had an RQ above 1.05. In total, 17 subjects fulfilled the criteria for the maximal test. As all subjects reached exhaustion, we decided to compare peakVO_2 rather than VO_2max in order to analyze all of them. There was no difference in pubertal stages or gender for peakVO_2 or maximal heart rate, but an RQ above 1.05 was more frequently achieved in girls (girls: 9/12, boys: 3/10; p=0.035).

Half (11/22) of all subjects were considered to have low CRF with a peakVO_2 under the 80% of predicted maximal value. There was no difference in the number of subjects fulfilling the criteria for the maximal test between the two groups (8/11 in normal CRF; 9/11 in low CRF; p=0.611). The characteristics' comparison between the two different cardiorespiratory statuses is presented in Table 1.

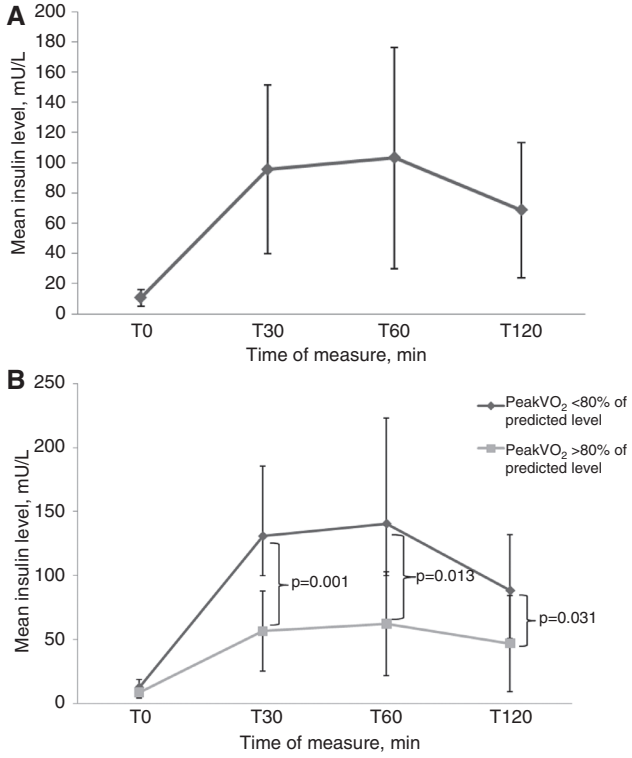

Figure 1 Evolution of insulin concentrations according to time of measurement after glucose overload. (A) In all subjects: bars refer for standard deviations; T=time of sampling. (B) According to cardiorespiratory fitness levels: bars refer for standard deviations; T=time of sampling.

Absolute and FM- or FFM-corrected peakVO_2 were statistically different between the two CRF groups (absolute: p=0.040; FM- and FFM-corrected: p<0.001). There were no differences among gender or pubertal stages for normal or low CRF. Compared to subjects with normal CRF, adolescents with low CRF had an ISI divided by almost 2, with a difference approaching the significance (p=0.062). Insulin resistance indices and insulin levels during the OGTT, except at the fasting state, were also significantly higher in this group (Figure 1B).

As subjects with low CRF had also a higher BMI z-score, we performed an ANCOVA with adjustment for the BMI z-score, in order to compare HOMA-IR, ISI and $AUC_{insulin}$ between the two CRF groups. We found that they stayed significantly different between the two CRF groups (HOMA-IR: F=20.0, p<0.001; $AUC_{insulin}$: F=9.5, p=0.001; ISI: F=4.5, p=0.026). We performed the same analyses adjusting for body composition, gender or pubertal stage (all variables) and found that HOMA-IR and $AUC_{insulin}$ remained significantly different. However, results for insulin sensitivity were less clear with nonsignificant results when adjusting for gender or pubertal stage (all variables).

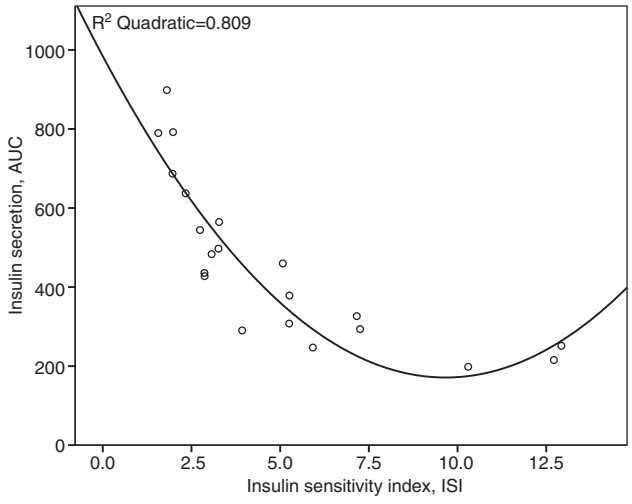

Figure 2 Relationship between insulin secretion and sensitivity. The plain line represents the quadratic regression line.

Discussion

The association between CRF and insulin metabolism has been poorly studied in obese children and adolescents, in contrast to the adult population. In our study, we aimed to explore the difference in insulin metabolism between fit and unfit obese adolescents. We therefore investigated sequential measurements of insulin levels after glucose overload, and we demonstrated that unfit obese subjects had higher insulin resistance indices and insulin secretion response compared to fit subjects. This difference was independent of the adiposity level.

It is well known that cardiovascular diseases risks are different between fit and unfit adult subjects (8, 19), with better insulin sensitivity in those with high CRF (20–22). Indeed, subjects with insulin resistance or T2DM have generally reduced CRF concomitantly to a decreased capacity to transport and oxidize glucose, as well as a decrement in cellular glycogen synthase activity (23). We therefore divided obese adolescents into normal and low CRF (peakVO_2<80% of predicted VO_2max) (16, 17). Using this cut-off, the mean peakVO_2 by gender in each group (data not shown) corresponded to the normative data proposed by the European Group of Pediatric Work Physiology, i.e., a peakVO_2 of ≥35 mL/kg/min for girls and ≥40 mL/kg/min for boys (24). In addition, 50% of our subjects had low CRF. This difference in CRF was not due to body composition, as absolute and corrected peakVO_2 values for FM or FFM remained significantly different between

the two groups. This highlights the fact that regardless of the difference in lean or fat mass, the low CRF group has lower muscle oxidative capacity compared to the normal CRF group.

The main finding of this study is that low CRF in obese adolescents was associated with significantly higher insulin indices: higher HOMA-IR, as well as higher 2-h insulin and insulin secretion response during the OGTT. These differences were independent of the BMI z-score or body composition. Furthermore, the ISI was almost twofold lower, suggesting an increased risk to develop diabetes. Insulin sensitivity is known to depend of the amount of oxidative and insulin-sensitive type 1 muscular fibers, muscular lipid content, as well as fat and/or lipid droplet size into the muscle cells (25). In our study, lean tissue did not differ between adolescents with normal or low CRF, which may explain the trend in ISI between groups. In fact, this difference was significant when adjusting for adiposity.

The identification of subjects with normal or low CRF is essential, as it may predict the development of the metabolic syndrome (26), as well as obesity-related complications later in life (8). Indeed, Ruiz et al. demonstrated that healthy children with low CRF (using similar cut-offs) had higher cluster of metabolic risk factors (19). Forty to forty-four percent of healthy children did not reach the cut-off, which mirrors our findings. Ortega et al. suggested that CRF plays a key role in mortality and morbidity prognosis, as they demonstrated that metabolically healthy but obese phenotype adults had higher fitness and lower obesity-related complications (8). Furthermore, children with low CRF may beneficiate of exercise training program, as they are more prone to improve CRF and reduce cardiovascular and metabolic risk factors (27).

Strengths and limitations of the study

The strengths of this study are the sequential measurements of insulin and glucose concentrations performed during the OGTT, as well as the objective measures of CRF. Furthermore, no obese adolescents showed abnormal glucose metabolism making the comparison between groups more reliable.

This study suffers from the following limitations. First, the sample size is limited and may adversely affect the power of our analysis and limit the reliability of the subgroup analysis based on gender and pubertal status. Second, the cross-sectional nature of the study reduces our ability to make inferences about temporal link between CRF and insulin metabolism changes. Third, the age group studied coincides with pubertal development, which greatly influences insulin sensitivity.

Our study demonstrated that low CRF in obese adolescents was strongly related to increased insulin resistance and secretion. We suggest that interventions in youth should focus on the maintenance or improvement of the CRF level above the 80% of predicted maximal VO_2, as it is significantly related to better beta-cell function. A CRF above recognized cut-offs might prevent the development of insulin resistance. Finally, further studies are needed to determine if normal CRF during childhood and adolescences may delay or reduce the development of obesity-related complications later in life.

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Conflicts of interest statement: The authors have no conflicts of interest to declare.

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