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Cardiac function in adolescents with obesity: cardiometabolic risk factors and impact on physical fitness

Cardiac diastolic function in obese adolescents

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Abstract

Objective

To gain greater insights in the aetiology and clinical consequences of altered cardiac function in obese adolescents. We aimed to examine cardiac structure and function in obese adolescents, and to examine associations between altered cardiac function/structure and cardiometabolic disease risk factors or cardiopulmonary exercise capacity.

Methods

In 29 obese (BMI 31.6 ± 4.2 kg/m², age 13.4 ± 1.1 years) and 29 lean (BMI 19.5 ± 2.4 kg/m², age 14.0 ± 1.5 years) adolescents fasted blood samples were collected to study haematology, biochemistry, liver function, glycaemic control, lipid profile and hormones, followed by a transthoracic echocardiography to assess cardiac structure/function, and a cardiopulmonary exercise test (CPET) to assess cardiopulmonary exercise parameters. Regression analyses were applied to examine relations between altered echocardiographic parameters and blood parameters or CPET parameters in the entire group.

Results

In obese adolescents left ventricular septum thickness, left atrial diameter, mitral A wave velocity, E/e' ratio were significantly elevated ($p < 0.05$), as opposed to lean controls, while mitral e' wave velocity was significantly lowered ($p < 0.01$). Elevated homeostatic model assessment of insulin resistance and blood insulin, c-reactive protein and uric acid concentrations (all significantly elevated in obese adolescents) were independent risk factors for an altered cardiac diastolic function ($p < 0.01$). An altered cardiac diastolic function was not related to exercise tolerance but to a delayed heart rate recovery ($p < 0.01$). ‘

59 *Conclusion*

60 In obese adolescents an altered cardiac diastolic function was independently related to
61 hyperinsulinemia and whole-body insulin resistance, and only revealed by a delayed heart rate
62 recovery (HRR) during CPET. This indicates that both hyperinsulinemia, whole-body insulin
63 resistance and delayed HRR could be regarded as clinically relevant outcome parameters.

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INTRODUCTION

Obesity among adolescents has increased considerably over the past decades and is becoming one of the most serious epidemic preventable health concerns worldwide^{1, 2}. Obesity is characterized by excessive lipid accumulation in adipose tissue and ectopic fat accumulation, thereby contributing adiposopathy³. This process leads to systemic inflammation, oxidative stress, altered blood adipokine concentrations, endocrine abnormalities and insulin resistance³. As a result, obesity has been recognized as an altered health state leading to hypertension, type 2 diabetes mellitus, dyslipidemia, nonalcoholic fatty liver disease and atherosclerosis^{4, 5}. These pathophysiological processes are already present in obese adolescents, thereby increasing the risk for cardiovascular comorbidities and premature death during adulthood^{5, 6}. Hence, early detection of cardiovascular abnormalities is important since control of these abnormalities is more effective in early stages of this disease and/or from a young age. Indeed, it has been shown that obesity is associated with the first early signs of adverse cardiac remodeling and cardiac dysfunction in adolescents, leading to a clinical syndrome known as the obesity cardiomyopathy^{5, 7-10}. Left ventricular hypertrophy and an increased left ventricular mass is commonly observed, being an independent predictor of heart failure and sudden death in adulthood¹¹. Next to structural changes, obese adolescents also demonstrate a diminished cardiac systolic and diastolic function^{12, 13}.

However, the exact contribution of cardiometabolic risk factors to the development of these structural and functional cardiac abnormalities remains speculative^{14, 15}. Several studies have shown that components of the metabolic syndrome, in particular hypertension and insulin resistance, are involved in the development of adverse cardiac remodeling and cardiac dysfunction^{10, 16, 17}. It may thus be hypothesized that normalisation of blood pressure and/or insulin sensitivity may be important to offer cardioprotection, although the exploration of cardiac dysfunction to a wider range of subject characteristics (such as biochemistry,

hematology, endocrinology and glycemic control) remains to be examined. Such studies may reveal novel risk factors and thus may improve prevention strategies. In this regard, detailed exploration of relations between cardiometabolic risk factors and cardiac function/structure are relevant as this may lead to novel cardioprotective treatments.

Moreover, the influence of alterations in cardiac structure and function on cardiopulmonary exercise capacity of obese adolescents remains to be studied. During cardiopulmonary exercise testing (CPET), a disturbed cardiac chronotropic response and a reduced peak workload are often observed¹⁸⁻²¹. However, the contribution of early structural and functional myocardial alterations to such exercise dysfunctions has not been fully examined in obese adolescents. In one recent study it was observed for the first time that a lowered exercise capacity was independently related to an impaired myocardial contractility¹³. Such studies are however important as they provide greater insights in the clinical repercussions (such as physical functioning) of a worse cardiac function/structure in obese adolescents.

The present study aims to gain greater insights in the aetiology of cardiac dysfunction in obese adolescents, and in the clinical impact of such dysfunctions. Therefore, the primary aim of this study is to examine cardiac structure and function by comparing echocardiographic parameters between lean and obese adolescents. Secondary aims of the study are to examine associations between altered echocardiographic parameters and cardiometabolic disease risk factors (risk factor analysis), and to examine associations between altered echocardiographic parameters and CPET parameters in lean and obese adolescents (clinical impact analysis). It was hypothesized that 1. specific echocardiographic abnormalities are present in obese adolescents, 2. specific cardiometabolic disease risk factors relate to such echocardiographic parameters and 3. these specific alterations in cardiac function/structure relate to specific abnormalities in cardiopulmonary function and exercise capacity in obese adolescents.

MATERIALS AND METHODS

Subjects

Obese adolescents were recruited from the pediatric clinic of the Jessa hospital (Hasselt, Belgium) and lean adolescents were recruited by means of publication of advertisements in the Jessa hospital and Hasselt University. Participants were between 11 and 17 years of age and free from any known chronic cardiovascular, renal, pulmonary or orthopaedic disease. The International Obesity Task Force criteria and body fat percentage ($>95^{\text{th}}$ percentile) were used to categorize the participants into a lean and obese group^{22, 23}. Twenty-nine obese adolescents and 30 lean adolescents were included in this study. All participants and their parents/legal guardians received oral and written information about the aim and protocol of the study and gave their written informed consent prior to participation. The study protocol was approved by the medical ethical committee of the Jessa hospital and Hasselt University (Hasselt, Belgium) and was performed according to the Declaration of Helsinki. The present study is registered at clinicaltrials.gov as NCT03516721.

Study design

The study was carried out according to an observational, cross-sectional design and performed at the Jessa hospital (Hasselt, Belgium). From midnight prior to examination, all subject refrained from consuming food, with the exception of water *ad libitum* to prevent changes on biochemical analysis and exercise physiology. First, in fasted state (at least ten hours after the last meal) anthropometry and body composition were assessed, followed by examination of blood pressure, Tanner stage and physical activity level. Next, a venipuncture was executed. After a standardized meal, containing 296 kcal, composed of 3g of fat, 56g of carbohydrate and 9g of protein, echocardiography and CPET were performed.

Anthropometry and body composition

Body height was measured to the nearest 0.1 cm using a wall-mounted Harpenden stadiometer (ICD 250 DW, De Grood Metaaltechniek, Nijmegen, the Netherlands), with participants barefoot. Body weight (in underwear) was determined using a digital-balanced weighting scale to the nearest 0.1 kg (Seca 770, Hamburg, Germany). BMI was calculated from weight and height measurements ($\text{weight}/\text{height}^2$). Standard deviation scores (SDS) were calculated as described by Cole *et al.*²². Waist and hip circumferences were measured to the nearest 0.1 cm using a flexible metric measuring tape with participants barefoot (in underwear) in standing position. Waist circumference was measured at the midpoint between the lower rib margin and the top of the iliac crest. Hip circumference was measured at the widest circumference of the hip at the level of the greater trochanter. Waist-to-hip ratio was calculated by dividing waist circumference (cm) by hip circumference (cm). Body composition was evaluated using skinfold measurements. The thickness of the triceps and subscapular skinfolds were measured in triplicate at the left side of the body to the nearest 0.1 mm using an Harpenden skinfold caliper (Baty, West Sussex, UK), at the following sites: triceps, halfway between the acromion process and the olecranon process; biceps, at the same level as the triceps skinfold, directly above the center of the cubital fossa; subscapular, about 2 cm below the tip of the scapula, at an angle of 45° to the lateral side of the body; and suprailiac, about 2 cm above the iliac crest, in the axillary line. The mean value of the triplicate measurements was used in the analysis. Skinfold measurements were performed by the same observer. The percentage of body fat was calculated using the equation reported by Slaughter *et al.*²⁴.

Blood pressure, pubertal development stage and physical activity evaluation

Blood pressure (BP) was measured in supine position using an electronic sphygmomanometer (Omron®, Omron Healthcare, IL, USA) after a resting period of five minutes. Mean arterial pressure (MAP) was calculated as $MAP = \text{systolic BP} + (2 \times \text{diastolic BP}) / 3$. Pubertal status was assessed using Tanner's scale, which defines physical features of development based on external primary and secondary sex characteristics, according to observation by a pediatrician and the adolescents' own opinion based on a figure^{25, 26}. The level of physical activity was determined using the validated Dutch Physical Activity Questionnaires for Adolescents²⁷.

Biochemical analysis

Venous blood samples were taken for the measurement of blood parameters. Plasma glucose, iron, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), uric acid, calcium concentrations, lipid profile (blood total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations), c-reactive protein (CRP), thyroid-stimulating hormone (TSH), free thyroxine (FT4), cortisol and serum insulin concentrations were automatically assessed on Roche Cobas 8000 (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Blood glycated haemoglobin concentration (HbA1c) was measured using ion exchange chromatography (Menarini HA-8180 HbA1c auto-analyser, Menarini Diagnostics, Diegem, Belgium). Serum leptin concentration was measured using radioimmunoassay (RIA; LINCO Research Inc., Saint Louis, MI, USA). Blood haemoglobin, haematocrit, and leukocytes were automatically assessed using high-volume haematology analyser Siemens Advia 2120 (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Whole-body insulin resistance/sensitivity was estimated using the

homeostatic model assessment of insulin resistance (HOMA-IR) = fasting glucose level (mg/dl) x fasting insulin level (μU/ml) / 405²⁸.

Metabolic risk profile

The metabolic risk score was determined with the aid of the following variables: waist circumference, MAP, triglycerides/HDL-C ratio and fasting insulin concentration, as described by Martinez-Vizcaino *et al.*²⁹. The standardized value of each variable was calculated as follows: $z\text{-score} = (\text{individual value} - \text{sample mean}) / \text{standard deviation (SD)}$.

Echocardiography

All subjects underwent a standardized transthoracic echocardiographic examination using a commercial ultrasound system (Vivid 7, GE Health Medical, Milwaukee, Wisconsin, USA) and a phased array matrix transducer (GE M4S, 1.5 - 3.6 MHz, Vivid 7 ultrasound system, GE Health Medical, Milwaukee, Wisconsin, USA). Two-dimensional and motion mode echocardiographic parameters were obtained with subjects lying in supine or left lateral semirecumbent position and standard parasternal and apical views were used, as described by Anderson *et al.*³⁰. The cross-sectional area of the aortic valve was calculated from the diameter of the left ventricular outflow tract (LVOT) using the parasternal long axis view. The velocity time integral (VTI) was measured at the LVOT site from the apical five chamber view with pulsed-wave Doppler echocardiography. Cardiac output was calculated from the estimated stroke volume using the VTI of flow through the LVOT, the cross-sectional area of the aortic valve and the heart rate recorded during the echocardiography measurement. Diastolic function was assessed using transmittal inflow patterns, left ventricular ejection fraction, mitral annulus velocity and left atrial (LA) diameter. Transmittal inflow patterns were obtained using pulsed-wave Doppler echocardiography. Peak early (E) and late (A)

diastolic velocities, the E/A ratio and the deceleration time of early filling velocity were determined using apical 4 chamber views. Ejection fraction was measured using apical 4 chamber views and determined using the biplane modified Simpsons method. Mitral annulus early diastolic velocity (e') and late diastolic velocity were determined using 4 chamber views at septal and lateral mitral annulus and the E/e' ratio was assessed. Left ventricular (LV) septal wall thickness, LV diameter and LA diameter were measured using parasternal long axis views. All transthoracic echocardiographic and Doppler assessments and analyses were performed by the same cardiologist and stored digitally until analysis using EchoPAC software (GE Health Medical, Milwaukee, Wisconsin, USA).

Cardiopulmonary exercise testing (CPET)

CPET was performed up to volitional exhaustion using an electronically braked cycle ergometer (eBike, GE Medical systems, Milwaukee, Wisconsin, USA), controlled by the Cardiosoft electrocardiography software (Cardiosoft 6.6, GE Medical systems, Freiburg, Germany). At the beginning of each test day, a gas and volume calibration was performed according to manufacturer's instructions. During the test, environmental temperature was kept stable at 19-21°C. The exercise test (ramp protocol) included a one-minute pre-exercise resting period, a one-minute unloaded warm-up cycling phase, an incremental exercise cycling period with an initial workload of 40W and an increasing workload of 20W per minute. During warm-up cycling and incremental exercise a cycling frequency of 60 to 70 revolutions per minute (rpm) had to be maintained. The test was ended when the subject failed to maintain a pedal frequency of at least 60 rpm. All subjects were verbally encouraged during exercise testing to achieve maximal effort, based on a respiratory gas exchange ratio (RER) ≥ 1.05 and subjective opinion of an experienced tester who confirmed whether a maximal exercise test was executed, based on subjective features as described by Bongers *et*

$al.$, including dyspnea, sweating, facial flushing, clear unwillingness to continue and a sustained drop in the participant's pedaling frequency from 60 rpm despite verbal encouragement³¹. After cessation of exercise, workload was set at 45W at which subjects cycled during two minutes for active recovery with a cycling frequency of 50 rpm. At the end of the test the Borg rating scale was determined³².

With the aid of continuous pulmonary gas exchange analysis (Jaeger MasterScreen CPX Metabolic Cart, CareFusion Germany GmbH, Hoechberg, Germany) oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$) minute ventilation ($\dot{V}E$), equivalents for oxygen uptake ($\dot{V}E/\dot{V}O_2$) and carbon dioxide production ($\dot{V}E/\dot{V}CO_2$), tidal volume ($\dot{V}t$), breathing frequency (BF) and the respiratory gas exchange ratio (RER) were collected breath-by-breath and averaged every ten seconds. Using a 12-lead electrocardiography device (KISS™ Multilead, GE Medical systems, Freiburg, Germany) heart rate (HR) was monitored and averaged every ten seconds. From this parameter oxygen pulse ($\dot{V}O_2/HR$) was calculated. Heart rate recovery (HRR) was defined as the reduction in heart rate from peak exercise level (HR_{peak}) to the rate 30 seconds, one minute and two minutes after cessation of exercise testing and designated as $HRR_{0.5min}$, HRR_{1min} and HRR_{2min} ^{33, 34}. The oxygen uptake efficiency slope was calculated using all exercise data by a linear least square regression of $\dot{V}O_2$ on the logarithmic of $\dot{V}E$ ³¹. First ventilatory threshold (VT1) was determined using the V-slope method³⁵. Secondly, ventilatory threshold (VT2) was determined, using the $\dot{V}E$ vs. $\dot{V}CO_2$ plot, on the point where $\dot{V}E$ increases out of proportion to $\dot{V}CO_2$ ³⁶. Exercise tolerance was assessed by the peak workload (W_{peak}).

Statistical analysis

Statistical analysis was performed by IBM SPSS® version 24.0 (IBM SPSS Statistics for Windows, Chicago, IL, USA). Data were expressed as mean $\pm$ SD. Shapiro-Wilk test was used to test normality of the data ($p < 0.05$). Comparisons between groups were tested using the chi-square test for categorical variables. Differences between continuous variables were assessed using independent sample T-tests for normally distributed data and Mann-Whitney U-tests for abnormally distributed data. A two-way repeated measures ANOVA was used to assess whether there were differences in HRR after cessation of exercise testing between obese and lean adolescents: an interaction effect was evaluated, where group (obese vs. lean adolescents) was a between-subjects factor, and time (HRR_{0.5min}, HRR_{1min} and HRR_{2min}) was a within-subjects factor. A post-hoc analysis (Bonferroni post-hoc comparison test) was performed when the between-subjects factor was statistically significant. Multivariate linear regression analysis was applied to examine relations between altered echocardiographic variables and blood parameters or CPET parameters. In these regression analyses, variables with an abnormal distribution were log-transformed, and corrections for age, sex and Tanner stage were made. Variables with a beta-coefficient < 0.1 were left out of consideration. Relations between altered echocardiographic parameters and the metabolic risk score were examined by partial correlations and adjusted for age, sex and Tanner stage. A p -value < 0.05 (2-tailed) was considered statistically significant.

The sample size calculation was performed using GPower v. 3.1 (Düsseldorf, Germany). Ingul *et al.* have shown an increased e' (effect size: 1.70) and E/e' ratio (effect size: 1.55) in obese adolescents⁵. Based on a statistical power > 0.8 and a two-sided alpha of 0.05 it was calculated that a sample size of 8 obese individuals and 8 healthy controls had to be included in the present study. In addition, a secondary outcome parameter with regard to exercise tolerance was included. Using the same values as stated above and an effect size of 0.93

287 (based on observed W_{peak} in obese vs. lean adolescents), it was calculated that a sample size
288 of 26 obese individuals and 26 healthy controls had to be included in the present study³⁷.
289 Taking into account a drop-out rate of 10%, the number of participants to include in this study
290 was at least 29 lean and 29 obese adolescents, resulting in a final sample size of 58 subjects.
291
292

RESULTS

Subject characteristics

A total of 58 participants (29 obese and 29 lean adolescents) were eligible and completed the study. Due to previously undetected anaemia, data from one lean adolescent were excluded (Suppl. 1). Sex, age, body height, body height-SDS and Tanner stage were comparable between groups ($p>0.05$, Table 1). Body weight, BMI, BMI-SDS, waist circumference, hip circumference and waist-to-hip ratio were higher ($p<0.001$) in obese subjects. Percentage of body fat and the sum of skinfolds were higher ($p<0.001$) in obese adolescents. In obese subjects, systolic BP, diastolic BP and MAP were all higher ($p<0.01$) compared to lean subjects, whereas the physical activity level was lower ($p=0.023$) in obese adolescents.

Blood parameters

Blood haemoglobin, haematocrit, iron and HDL cholesterol concentration were lower ($p<0.05$) in obese adolescents (Table 2). Blood leukocytes, uric acid, CRP, LDL cholesterol, triglycerides, triglyceride-to-HDL cholesterol ratio, glucose, insulin, ALT, GGT and leptin concentrations were higher ($p<0.05$) in obese adolescents. HOMA-IR was elevated ($p<0.001$) in obese adolescents compared to lean adolescents.

Metabolic risk

An increased metabolic risk score (0.61 ± 0.68 vs. -0.55 ± 0.31 ; $p<0.001$) was found in obese adolescents compared to lean adolescents, respectively.

Left ventricular structure and function

An increased LV septum thickness ($p=0.003$) and LA diameter ($p<0.001$) were found in obese adolescents (Table 3). A higher mitral A wave velocity ($p=0.028$) and E/e' ratio ($p=0.005$)

were found, whereas a lower mitral e' wave velocity ($p=0.009$) was demonstrated in obese adolescents.

Exercise tolerance, cardiopulmonary function and heart rate recovery

A reduced W_{peak} ($p=0.010$) was found in obese adolescents compared to lean adolescents (Table 4). A time ($p<0.001$) and group ($p=0.009$) effect was found for HRR (Figure 1). Post-hoc analysis showed a delayed heart rate recovery at 0.5 (-10 ± 7 vs. -16 ± 10 bpm; $p=0.006$), 1.0 (-23 ± 11 vs. -30 ± 14 bpm; $p=0.036$) and 2.0 (-35 ± 12 vs. -45 ± 12 bpm; $p=0.005$) minutes after cessation of exercise in obese adolescents, respectively. None of the other CPET parameters differed between groups ($p>0.05$).

Relation between metabolic risk score and aberrant echocardiographic parameters

A positive partial correlation (corrected for age, sex and Tanner stage) was found between the metabolic risk score and LV septum thickness ($r=0.380$; $p=0.007$), LA diameter ($r=0.337$; $p=0.018$) and E/e' ratio ($r=0.367$; $p=0.01$), whereas a negative partial correlation was found between the metabolic risk score and mitral e' wave velocity ($r=-0.316$; $p=0.03$) in the entire group.

Relations between altered echocardiographic parameters and cardiometabolic health

With regard to cardiac morphology, a higher LV septum thickness was independently (model $r^2=0.136$; $p=0.005$) related to a higher blood insulin concentration (standardized coefficient of beta (SC β)= 0.368 ; $p=0.006$; Table 5) and a higher LA diameter was independently (model $r^2=0.233$; $p<0.001$) related to a higher HOMA-IR (SC $\beta=0.482$; $p<0.001$). Multivariate regression analyses of altered echocardiographic parameters reflecting diastolic function showed that a higher mitral A wave velocity was independently (model $r^2=0.128$; $p=0.008$) related to a higher blood CRP concentration (SC $\beta=0.358$; $p=0.006$) and a higher E/e' ratio was independently (model $r^2=0.327$; $p<0.001$) related to a higher HOMA-IR (SC $\beta=0.378$; $p=0.004$) and blood uric acid concentration (SC $\beta=0.321$; $p=0.013$). Furthermore, a higher mitral e' wave velocity was independently (model $r^2=0.199$; $p=0.001$) related to a lower blood insulin concentration (SC $\beta=-0.446$; $p=0.001$).

Relations between aberrant CPET parameters and echocardiographic parameters

A higher W_{peak} was independently (model $r^2=0.590$; $p<0.001$) related to a higher LV diameter (SC $\beta=0.483$; $p<0.001$) and a lower cardiac output (SC $\beta=-0.266$; $p=0.010$). A higher $HRR_{0.5min}$ (model $r^2=0.404$; $p<0.001$) and HRR_{1min} (model $r^2=0.369$; $p<0.001$) were independently related to a lower mitral A wave velocity (SC $\beta=-0.473$; $p<0.001$ and SC $\beta=-0.535$; $p<0.001$, respectively). A higher HRR_{2min} was independently (model $r^2=0.201$; $p<0.001$) related to a higher E/A ratio (SC $\beta=0.449$; $p=0.001$).

DISCUSSION

In this study, we observed that obese adolescents present early signs of altered cardiac diastolic function which were independently related to insulin resistance and hyperinsulinemia, and only being revealed by a delayed HRR during CPET. This is the first study which relates altered cardiac diastolic function to cardiometabolic health and altered cardiopulmonary function during exercise in obese adolescents.

Relations between altered echocardiographic parameters and cardiometabolic health

In obese adolescents the LA diameter, mitral A wave velocity, and E/e' ratio were significantly increased, whereas the mitral e' wave velocity was significantly decreased, compared to lean controls. All these parameters reflect left ventricular diastolic function, thereby suggesting that obesity, even at young age, is related to altered cardiac diastolic function. In addition, an increased LV septum thickness was found in obese adolescents, which provides further evidence that obese adolescents already exhibit early signs of LV hypertrophy, next to altered diastolic function^{5, 7, 10, 38, 39}. In the present study, significant relationships were observed between elevated metabolic risk scores and altered echocardiographic outcomes, including elevated LV septum thickness, LA diameter, E/e' ratio or lowered mitral e' wave velocity. As the metabolic risk score is a composite score, it was further examined which of those different composites, or other health indicators, were related to cardiac function and structure. Interestingly, an increased LV septum thickness was independently associated with higher serum insulin concentrations. Several studies have shown that insulin resistance and the accompanying compensatory hyperinsulinemia could be involved in the development of LV hypertrophy⁴⁰⁻⁴³ through the activation of insulin-like growth factor-1 receptors⁴⁴. These receptors enhance anabolic effects on the myocardium and directly promote LV hypertrophy^{42, 44, 45}. The presence of LV hypertrophy would be expected

to predispose LV diastolic dysfunction by an impaired relaxation and subsequent reduced compliance of the left ventricle⁴⁶⁻⁴⁸. Indeed, in this study a significantly diminished diastolic function (as evidenced by a higher mitral A wave velocity and E/e' ratio, and lower mitral e' wave velocity) in obese adolescents was noticed. Altered cardiac diastolic function was independently related to a higher HOMA-IR and serum insulin concentration. As a result, insulin resistance and hyperinsulinemia may play, at least in part, an important role in the development of impaired cardiac diastolic dysfunction. Although the exact mechanisms explaining the role of insulin resistance and hyperinsulinemia in the development of cardiac diastolic dysfunction have not been fully elucidated, potential mechanisms may include endothelial dysfunction, oxidative stress, interstitial fibrosis and increased collagen production from fibroblasts⁴⁹⁻⁵². Furthermore, obesity may also be associated with altered cardiac diastolic function via the effect of inflammation⁵³. This is consistent with results from the present study as a greater mitral A wave velocity was independently related to elevated blood CRP concentrations. Indeed, it has been demonstrated that inflammation may reduce myocardial function by remodeling of the extracellular matrix⁵⁴. Interestingly, we also found a relationship between elevated blood uric acid concentrations and a higher E/e' ratio. Elevated serum uric acid levels are frequently observed in obese individuals, and accumulating evidence indicates that hyperuricemia plays a key role in the development and progression of cardiac diastolic dysfunction by promoting cardiomyocyte hypertrophy, inflammation and oxidative stress, which in turn leads to myocardial fibrosis and associated cardiac diastolic dysfunction^{55, 56}.

Therefore, data from the present study support the idea that hyperinsulinemia might be related to the development of LV hypertrophy in obese adolescents. In addition, elevated HOMA-IR, blood insulin, CRP and uric acid concentrations could be related to alterations in cardiac diastolic function in obese adolescents. It thus follows that it should be explored whether

decreasing blood insulin concentrations and/or the inflammatory state, with the aid of lifestyle changes or pharmacological interventions, may exert cardioprotective effects in obese adolescents.

Relations between aberrant CPET parameters and echocardiographic parameters

Although a reduced exercise capacity was noticed in obese adolescents (as evidenced by a reduced W_{peak}), this was not related to a difference in cardiac diastolic function. In fact, none of the peak exercise parameters were related to cardiac function or structure in the entire group. These data are in contrast with a previous study reporting a relation between a lower $\dot{V}O_{2\text{peak}}$ and a lower myocardial contractility in obese adolescents¹³. However, this study was limited by the small sample size and the correction of $\dot{V}O_{2\text{peak}}$ for body weight (ml/kg/min), thus increasing the risk for an artificial relation¹³. Indeed, when $\dot{V}O_{2\text{peak}}$ was corrected for lean tissue mass, these relations disappeared. It thus remains questionable whether cardiac dysfunction would actually affect exercise capacity in obese adolescents. The only exercise parameter that seemed to be related to cardiac function in the present study was HRR, where a delayed HRR was independently associated with altered cardiac diastolic function. The mitral A wave velocity was inversely associated with $\text{HRR}_{0.5\text{min}}$, whereas the E/A ratio was positively related to $\text{HRR}_{2\text{min}}$. These data thus indicate that a delayed HRR is an early marker for the potential presence of altered cardiac diastolic function in obese adolescents and could be regarded as a clinically relevant outcome parameter during CPET. Our findings are consistent with previous research in obese adults and adolescents, which reported a markedly impaired HRR^{57, 58}. Gondoni *et al.* showed that obese adolescents had a slowed $\text{HRR}_{1\text{min}}$ in the same order of magnitude (obese: $-22 \pm 10\text{bpm}$; lean: $-29 \pm 10\text{bpm}$) as measured in the present study⁵⁷. However, considering the rather low predictive power of the regression models, caution is warranted in clinical use. Although the dynamics of HRR are attributed to

different physiological mechanisms, autonomic imbalance could possibly influence HRR to a certain extent. Several studies have been shown that HRR could be used as an indicator of autonomic nervous system mediated responses, in particular parasympathetic reactivation. In this case a reduction in HRR may be indicative of decreased autonomic nervous responsiveness⁵⁹. It has been proposed that early HRR, reflected by HRR_{0.5min} and HRR_{1min}, could be considered as markers of cardiac parasympathetic reactivity⁶⁰. In addition, gradual withdrawal of sympathetic activity is becoming more important later in recovery (HRR_{2min})⁶⁰. However, future studies are necessary to elucidate the use of HRR as a marker of autonomic function during cardiopulmonary exercise testing in obese adolescents.

Limitations

Three obese adolescents had a BMI between 28-30kg/m² which can possibly lead to skewed data. However, we included these adolescents as 'obese' since they had a body fat percentage of >95th percentile. Since cardiac function and body fat percentage are highly correlated, we can assume these adolescents will represent the obese population. Furthermore, we did not directly measure autonomic function (i.e. heart rate variability). Although it has been shown that HRR can be used as a marker for cardiac autonomic function, prospective studies are needed to explore whether autonomic dysfunction during exercise is present in obese adolescents.

CONCLUSION

In obese adolescents, altered cardiac diastolic function is independently related to insulin resistance or hyperinsulinemia, and is only revealed by a delayed HRR during CPET. Therefore, the cardioprotective effect of interventions leading to lower serum insulin concentrations or elevations in insulin sensitivity should be explored in obese adolescents.

458 Additionally, it could be hypothesized that HRR during CPET may be used as clinically
459 relevant marker for alterations in cardiac diastolic function.

461 **AUTHOR CONTRIBUTIONS**

462 P.D., G.M. and D.H. conceived and designed the study design. WMA.F., M.B. and G.M.
463 included the participants. WMA.F. and M.B. performed the measurements. T.A.H performed
464 the echocardiographic measurements and analyzed the echocardiographic data. WMA.F.,
465 M.B., D.H. and G.M. analyzed the data. WMA.F. and M.B. performed the statistical analysis.
466 WMA.F., D.H. and G.M. wrote the manuscript. T.A.H., I.F., P.D., K.V. and B.O.E. critically
467 reviewed the manuscript. All authors gave their final approval of the manuscript to be
468 submitted.

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CONFLICT OF INTEREST

No conflict of interest was declared.

Supplementary information is available at International Journal of Obesity's website.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet* 2014; **384**(9945): 766-781.
2. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Int. J. Pediatr. Obes.* 2006; **1**(1): 11-25.
3. McCrindle BW. Cardiovascular consequences of childhood obesity. *Can. J. Cardiol.* 2015; **31**(2): 124-30.
4. Herouvi D, Karanasios E, Karayianni C, Karavanaki K. Cardiovascular disease in childhood: the role of obesity. *Eur. J. Pediatr.* 2013; **172**(6): 721-32.
5. Ingul CB, Tjonna AE, Stolen TO, Stoylen A, Wisloff U. Impaired cardiac function among obese adolescents: effect of aerobic interval training. *Arch. Pediatr. Adolesc. Med.* 2010; **164**(9): 852-9.
6. Bjorge T, Engeland A, Tverdal A, Smith GD. Body mass index in adolescence in relation to cause-specific mortality: a follow-up of 230,000 Norwegian adolescents. *Am J Epidemiol* 2008; **168**(1): 30-7.
7. Chinali M, de Simone G, Roman MJ, Lee ET, Best LG, Howard BV *et al.* Impact of obesity on cardiac geometry and function in a population of adolescents: the Strong Heart Study. *J. Am. Coll. Cardiol.* 2006; **47**(11): 2267-73.
8. Dhuper S, Abdullah RA, Weichbrod L, Mahdi E, Cohen HW. Association of obesity and hypertension with left ventricular geometry and function in children and adolescents. *Obesity* 2011; **19**(1): 128-33.
9. Dusan P, Tamara I, Goran V, Gordana ML, Amira PA. Left ventricular mass and diastolic function in obese children and adolescents. *Pediatr. Nephrol.* 2015; **30**(4): 645-52.
10. Porcar-Almela M, Codoner-Franch P, Tuzon M, Navarro-Solera M, Carrasco-Luna J, Ferrando J. Left ventricular diastolic function and cardiometabolic factors in obese normotensive children. *Nutr Metab Cardiovasc Dis* 2015; **25**(1): 108-15.
11. Brady TM. The Role of Obesity in the Development of Left Ventricular Hypertrophy Among Children and Adolescents. *Curr. Hypertens. Rep.* 2016; **18**(1): 3.
12. Rowland TW. Effect of obesity on cardiac function in children and adolescents: a review. *J. Sports Sci. Med.* 2007; **6**(3): 319-26.
13. Dias KA, Spence AL, Sarma S, Oxborough D, Timilsina AS, Davies PSW *et al.* Left ventricular morphology and function in adolescents: Relations to fitness and fatness. *Int. J. Cardiol.* 2017; **240**: 313-319.

14. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW *et al.* Obesity and the metabolic syndrome in children and adolescents. *N. Engl. J. Med.* 2004; **350**(23): 2362-74.
15. Van Putte-Katier N, Rooman RP, Haas L, Verhulst SL, Desager KN, Ramet J *et al.* Early cardiac abnormalities in obese children: importance of obesity per se versus associated cardiovascular risk factors. *Pediatr. Res.* 2008; **64**(2): 205-9.
16. de las Fuentes L, Brown AL, Mathews SJ, Waggoner AD, Soto PF, Gropler RJ *et al.* Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass. *Eur. Heart J.* 2007; **28**(5): 553-9.
17. Shah AS, Dolan LM, Khoury PR, Gao Z, Kimball TR, Urbina EM. Severe Obesity in Adolescents and Young Adults Is Associated With Subclinical Cardiac and Vascular Changes. *J. Clin. Endocrinol. Metab.* 2015; **100**(7): 2751-7.
18. Drinkard B, Roberts MD, Ranzenhofer LM, Han JC, Yanoff LB, Merke DP *et al.* Oxygen-uptake efficiency slope as a determinant of fitness in overweight adolescents. *Med. Sci. Sports Exerc.* 2007; **39**(10): 1811-6.
19. Hansen D, Marinus N, Remans M, Courtois I, Cools F, Calsius J *et al.* Exercise tolerance in obese vs. lean adolescents: a systematic review and meta-analysis. *Obes. Rev.* 2014; **15**(11): 894-904.
20. Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, Ducimetiere P. Heart-rate profile during exercise as a predictor of sudden death. *N. Engl. J. Med.* 2005; **352**(19): 1951-8.
21. Norman AC, Drinkard B, McDuffie JR, Ghorbani S, Yanoff LB, Yanovski JA. Influence of excess adiposity on exercise fitness and performance in overweight children and adolescents. *Pediatrics* 2005; **115**(6): e690-6.
22. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012; **7**(4): 284-94.
23. McCarthy HD, Cole TJ, Fry T, Jebb SA, Prentice AM. Body fat reference curves for children. *Int. J. Obes.* 2006; **30**(4): 598-602.
24. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD *et al.* Skinfold equations for estimation of body fatness in children and youth. *Hum Biol* 1988; **60**(5): 709-23.
25. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch. Dis. Child.* 1969; **44**(235): 291-303.
26. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch. Dis. Child.* 1970; **45**(239): 13-23.
27. Bervoets L, Van Noten C, Van Roosbroeck S, Hansen D, Van Hoorenbeeck K, Verheyen E *et al.* Reliability and Validity of the Dutch Physical Activity

- Questionnaires for Children (PAQ-C) and Adolescents (PAQ-A). *Archives of public health = Archives belges de sante publique* 2014; **72**(1): 47.
28. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**(7): 412-419.
29. Martinez-Vizcaino V, Martinez MS, Aguilar FS, Martinez SS, Gutierrez RF, Lopez MS *et al.* Validity of a single-factor model underlying the metabolic syndrome in children: a confirmatory factor analysis. *Diabetes Care* 2010; **33**(6): 1370-2.
30. Andersen OS, Smiseth OA, Dokainish H, Abudiab MM, Schutt RC, Kumar A *et al.* Estimating Left Ventricular Filling Pressure by Echocardiography. *J. Am. Coll. Cardiol.* 2017; **69**(15): 1937-1948.
31. Bongers BC, Hulzebos EH, Helbing WA, Ten Harkel A, van Brussel M, Takken T. Response profiles of oxygen uptake efficiency during exercise in healthy children. *European journal of preventive cardiology* 2016; **23**(8): 865-73.
32. Lamb KL, Eston RG, Corns D. Reliability of ratings of perceived exertion during progressive treadmill exercise. *Br. J. Sports Med.* 1999; **33**(5): 336-9.
33. Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H *et al.* Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 1994; **24**(6): 1529-35.
34. Okutucu S, Karakulak UN, Aytemir K, Oto A. Heart rate recovery: a practical clinical indicator of abnormal cardiac autonomic function. *Expert Rev. Cardiovasc. Ther.* 2011; **9**(11): 1417-30.
35. Wasserman K, Stringer WW, Casaburi R, Koike A, Cooper CB. Determination of the anaerobic threshold by gas exchange: biochemical considerations, methodology and physiological effects. *Z Kardiol* 1994; **83 Suppl 3**: 1-12.
36. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986; **60**(6): 2020-7.
37. Marinus N, Bervoets L, Massa G, Verboven K, Stevens A, Takken T *et al.* Altered gas-exchange at peak exercise in obese adolescents: implications for verification of effort during cardiopulmonary exercise testing. *J Sports Med Phys Fitness* 2017; **57**(12): 1687-1694.
38. Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B *et al.* Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. *J. Am. Coll. Cardiol.* 2004; **43**(8): 1399-404.
39. de Simone G, Izzo R, De Luca N, Gerds E. Left ventricular geometry in obesity: Is it what we expect? *Nutr Metab Cardiovasc Dis* 2013; **23**(10): 905-12.

- 634 40. Azevedo A, Bettencourt P, Almeida PB, Santos AC, Abreu-Lima C, Hense HW *et al.*
635 Increasing number of components of the metabolic syndrome and cardiac structural
636 and functional abnormalities--cross-sectional study of the general population. *BMC*
637 *Cardiovasc. Disord.* 2007; **7**: 17.
638
- 639 41. Atabek ME, Akyuz E, Selver Eklioglu B, Cimen D. The relationship between
640 metabolic syndrome and left ventricular mass index in obese children. *J. Clin. Res.*
641 *Pediatr. Endocrinol.* 2011; **3**(3): 132-8.
642
- 643 42. Sung JK, Kim JY. Obesity and preclinical changes of cardiac geometry and function.
644 *Korean circulation journal* 2010; **40**(2): 55-61.
645
- 646 43. Urbina EM, Gidding SS, Bao W, Elkasabany A, Berenson GS. Association of fasting
647 blood sugar level, insulin level, and obesity with left ventricular mass in healthy
648 children and adolescents: The Bogalusa Heart Study. *Am. Heart J.* 1999; **138**(1 Pt 1):
649 122-7.
650
- 651 44. Pires KM, Buffolo M, Schaaf C, David Symons J, Cox J, Abel ED *et al.* Activation of
652 IGF-1 receptors and Akt signaling by systemic hyperinsulinemia contributes to cardiac
653 hypertrophy but does not regulate cardiac autophagy in obese diabetic mice. *J. Mol.*
654 *Cell. Cardiol.* 2017; **113**: 39-50.
655
- 656 45. Sasson Z, Rasooly Y, Bhesania T, Rasooly I. Insulin resistance is an important
657 determinant of left ventricular mass in the obese. *Circulation* 1993; **88**(4 Pt 1): 1431-
658 6.
659
- 660 46. Chakko S, Mayor M, Allison MD, Kessler KM, Materson BJ, Myerburg RJ.
661 Abnormal left ventricular diastolic filling in eccentric left ventricular hypertrophy of
662 obesity. *Am. J. Cardiol.* 1991; **68**(1): 95-8.
663
- 664 47. Fischer M, Baessler A, Hense HW, Hengstenberg C, Muscholl M, Holmer S *et al.*
665 Prevalence of left ventricular diastolic dysfunction in the community. Results from a
666 Doppler echocardiographic-based survey of a population sample. *Eur. Heart J.* 2003;
667 **24**(4): 320-8.
668
- 669 48. Alpert MA, Omran J, Bostick BP. Effects of Obesity on Cardiovascular
670 Hemodynamics, Cardiac Morphology, and Ventricular Function. *Current obesity*
671 *reports* 2016; **5**(4): 424-434.
672
- 673 49. Mizushige K, Yao L, Noma T, Kiyomoto H, Yu Y, Hosomi N *et al.* Alteration in left
674 ventricular diastolic filling and accumulation of myocardial collagen at insulin-
675 resistant prediabetic stage of a type II diabetic rat model. *Circulation* 2000; **101**(8):
676 899-907.
677
- 678 50. Goldstein RH, Poliks CF, Pilch PF, Smith BD, Fine A. Stimulation of collagen
679 formation by insulin and insulin-like growth factor I in cultures of human lung
680 fibroblasts. *Endocrinology* 1989; **124**(2): 964-70.
681
- 682 51. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B *et al.* Evidence for an
683 independent and cumulative effect of postprandial hypertriglyceridemia and

- hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 2002; **106**(10): 1211-8.
52. Aroor AR, Mandavia CH, Sowers JR. Insulin resistance and heart failure: molecular mechanisms. *Heart Fail. Clin.* 2012; **8**(4): 609-17.
 53. Wu CK, Yang CY, Lin JW, Hsieh HJ, Chiu FC, Chen JJ *et al.* The relationship among central obesity, systemic inflammation, and left ventricular diastolic dysfunction as determined by structural equation modeling. *Obesity* 2012; **20**(4): 730-737.
 54. Prabhu SD. Cytokine-induced modulation of cardiac function. *Circ. Res.* 2004; **95**(12): 1140-53.
 55. Iliesiu A, Campeanu A, Dusceac D. Serum uric acid and cardiovascular disease. *Maedica* 2010; **5**(3): 186.
 56. Jia G, Habibi J, Bostick BP, Ma L, DeMarco VG, Aroor AR *et al.* Uric acid promotes left ventricular diastolic dysfunction in mice fed a Western diet. *Hypertension* 2015; **65**(3): 531-9.
 57. Gondoni LA, Titon AM, Nibbio F, Augello G, Caetani G, Liuzzi A. Heart rate behavior during an exercise stress test in obese patients. *Nutr Metab Cardiovasc Dis* 2009; **19**(3): 170-6.
 58. Laguna M, Aznar S, Lara MT, Lucia A, Ruiz JR. Heart rate recovery is associated with obesity traits and related cardiometabolic risk factors in children and adolescents. *Nutr Metab Cardiovasc Dis* 2013; **23**(10): 995-1001.
 59. Kizilbash MA, Carnethon MR, Chan C, Jacobs DR, Sidney S, Liu K. The temporal relationship between heart rate recovery immediately after exercise and the metabolic syndrome: the CARDIA study. *Eur. Heart J.* 2006; **27**(13): 1592-6.
 60. Freeman JV, Dewey FE, Hadley DM, Myers J, Froelicher VF. Autonomic nervous system interaction with the cardiovascular system during exercise. *Prog. Cardiovasc. Dis.* 2006; **48**(5): 342-62.

Figure legend

Figure 1

Heart rate response from HR_{peak}, 0.5, 1 and 2 minutes in recovery period of both obese (n=29) and lean (n=29) adolescents. Differences in heart rate recovery after cessation of exercise testing between obese and lean adolescents were tested using a two-way repeated measures ANOVA. A Bonferroni post-hoc comparison test was performed when the between-subjects factor was statistically significant. Data are expressed as mean $\pm$ SD. Abbreviations: HR: Heart rate. *P<0.05.