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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 ($p<0.01$). '

Conclusion

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

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 adiposopathy[3]. This process leads to systemic inflammation, oxidative stress, altered blood adipokine concentrations, endocrine abnormalities and insulin resistance[3]. 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 adulthood[11]. 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 observed[18-21]. 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 contractility[13]. 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 (>95th 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 (weight/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 = systolic BP + (2 x 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 Adolescents[27].

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) / 405[28].

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*-score= (individual value – sample mean) / 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 encouragement[31]. 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 determined[32].

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 method[35]. 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 ± 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 adolescents[5]. 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

(based on observed W_{peak} in obese vs. lean adolescents), it was calculated that a sample size of 26 obese individuals and 26 healthy controls had to be included in the present study[37]. Taking into account a drop-out rate of 10%, the number of participants to include in this study was at least 29 lean and 29 obese adolescents, resulting in a final sample size of 58 subjects.

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±10bpm; p=0.006), 1.0 (-23±11 vs. -30±14bpm; p=0.036) and 2.0 (-35±12 vs. -45±12bpm; 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 β=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 β=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 β=0.378; p=0.004) and blood uric acid concentration (SC β=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 β=-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 β=0.483; p<0.001) and a lower cardiac output (SC β=-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 β=-0.473; p<0.001 and SC β=-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 β=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 hypertrophy[40-43] through the activation of insulin-like growth factor-1 receptors[44]. 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 ventricle[46-48]. 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 fibroblasts[49-52]. Furthermore, obesity may also be associated with altered cardiac diastolic function via the effect of inflammation[53]. 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 matrix[54]. 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 $\dot{V}O_{2peak}$ and a lower myocardial contractility in obese adolescents[13]. However, this study was limited by the small sample size and the correction of $\dot{V}O_{2peak}$ for body weight (ml/kg/min), thus increasing the risk for an artificial relation[13]. Indeed, when $\dot{V}O_{2peak}$ 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 $HRR_{0.5min}$, whereas the E/A ratio was positively related to HRR_{2min}. 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 HRR_{1min} in the same order of magnitude (obese: -22±10bpm; lean: -29±10bpm) as measured in the present study[57]. 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 responsiveness[59]. It has been proposed that early HRR, reflected by $HRR_{0.5min}$ and HRR_{1min}, could be considered as markers of cardiac parasympathetic reactivity[60]. 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/m^2 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 is 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.

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

AUTHOR CONTRIBUTIONS

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

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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 ± SD. Abbreviations: HR: Heart rate. *P<0.05.