

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

Peer-reviewed author version

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VERBOVEN, Kenneth; DENDALE, Paul; OP 'T EIJNDE, Bert; MASSA, Guy &
HANSEN, Dominique (2018) Cardiac function in adolescents with obesity:
cardiometabolic risk factors and impact on physical fitness. In: International journal of
obesity, 43 (7), p. 1400-1410.

DOI: 10.1038/s41366-018-0292-x

Handle: <http://hdl.handle.net/1942/28121>

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

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4 Cardiac diastolic function in obese adolescents

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19 Keywords: exercise tolerance, obesity, adolescents, diastolic function, cardiometabolic risk,
20 physical fitness, heart rate recovery

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22 Conflicts of interest: none declared.

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33

34 **Abstract**

35 *Objective*

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

40

41 *Methods*

42 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
43 14.0 ± 1.5 years) adolescents fasted blood samples were collected to study haematology,
44 biochemistry, liver function, glycaemic control, lipid profile and hormones, followed by a
45 transthoracic echocardiography to assess cardiac structure/function, and a cardiopulmonary
46 exercise test (CPET) to assess cardiopulmonary exercise parameters. Regression analyses
47 were applied to examine relations between altered echocardiographic parameters and blood
48 parameters or CPET parameters in the entire group.

49

50 *Results*

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

58

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.

64

65

66

67 INTRODUCTION

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

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

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

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

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

116

117 **MATERIALS AND METHODS**

118 **Subjects**

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

131

132 **Study design**

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

141 **Anthropometry and body composition**

142 Body height was measured to the nearest 0.1 cm using a wall-mounted Harpenden
143 stadiometer (ICD 250 DW, De Grood Metaaltechniek, Nijmegen, the Netherlands), with
144 participants barefoot. Body weight (in underwear) was determined using a digital-balanced
145 weighting scale to the nearest 0.1 kg (Seca 770, Hamburg, Germany). BMI was calculated
146 from weight and height measurements (weight/height²). Standard deviation scores (SDS) were
147 calculated as described by Cole *et al.*²². Waist and hip circumferences were measured to the
148 nearest 0.1 cm using a flexible metric measuring tape with participants barefoot (in
149 underwear) in standing position. Waist circumference was measured at the midpoint between
150 the lower rib margin and the top of the iliac crest. Hip circumference was measured at the
151 widest circumference of the hip at the level of the greater trochanter. Waist-to-hip ratio was
152 calculated by dividing waist circumference (cm) by hip circumference (cm). Body
153 composition was evaluated using skinfold measurements. The thickness of the triceps and
154 subscapular skinfolds were measured in triplicate at the left side of the body to the nearest 0.1
155 mm using an Harpenden skinfold caliper (Baty, West Sussex, UK), at the following sites:
156 triceps, halfway between the acromion process and the olecranon process; biceps, at the same
157 level as the triceps skinfold, directly above the center of the cubital fossa; subscapular, about
158 2 cm below the tip of the scapula, at an angle of 45° to the lateral side of the body; and
159 suprailiac, about 2 cm above the iliac crest, in the axillary line. The mean value of the
160 triplicate measurements was used in the analysis. Skinfold measurements were performed by
161 the same observer. The percentage of body fat was calculated using the equation reported by
162 Slaughter *et al.*²⁴.

163

164 **Blood pressure, pubertal development stage and physical activity evaluation**

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

172

173 **Biochemical analysis**

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

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

190

191 **Metabolic risk profile**

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

196

197 **Echocardiography**

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

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

222

223 **Cardiopulmonary exercise testing (CPET)**

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

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

243 With the aid of continuous pulmonary gas exchange analysis (Jaeger MasterScreen CPX
244 Metabolic Cart, CareFusion Germany GmbH, Hoechberg, Germany) oxygen uptake ($\dot{V}O_2$),
245 carbon dioxide output ($\dot{V}CO_2$) minute ventilation ($\dot{V}E$), equivalents for oxygen uptake ($\dot{V}E/$
246 $\dot{V}O_2$) and carbon dioxide production ($\dot{V}E/ \dot{V}CO_2$), tidal volume ($\dot{V}t$), breathing frequency
247 (BF) and the respiratory gas exchange ratio (RER) were collected breath-by-breath and
248 averaged every ten seconds. Using a 12-lead electrocardiography device (KISS™ Multilead,
249 GE Medical systems, Freiburg, Germany) heart rate (HR) was monitored and averaged every
250 ten seconds. From this parameter oxygen pulse ($\dot{V}O_2/HR$) was calculated. Heart rate recovery
251 (HRR) was defined as the reduction in heart rate from peak exercise level (HR_{peak}) to the rate
252 30 seconds, one minute and two minutes after cessation of exercise testing and designated as
253 $HRR_{0.5min}$, HRR_{1min} and HRR_{2min} ^{33, 34}. The oxygen uptake efficiency slope was calculated
254 using all exercise data by a linear least square regression of $\dot{V}O_2$ on the logarithmic of $\dot{V}E$ ³¹.
255 First ventilatory threshold (VT1) was determined using the V-slope method³⁵. Secondly,
256 ventilatory threshold (VT2) was determined, using the $\dot{V}E$ vs. $\dot{V}CO_2$ plot, on the point where
257 $\dot{V}E$ increases out of proportion to $\dot{V}CO_2$ ³⁶. Exercise tolerance was assessed by the peak
258 workload (W_{peak}).

259

260

261

262 **Statistical analysis**

263 Statistical analysis was performed by IBM SPSS® version 24.0 (IBM SPSS Statistics for
264 Windows, Chicago, IL, USA). Data were expressed as mean \pm SD. Shapiro-Wilk test was
265 used to test normality of the data ($p < 0.05$). Comparisons between groups were tested using
266 the chi-square test for categorical variables. Differences between continuous variables were
267 assessed using independent sample T-tests for normally distributed data and Mann-Whitney
268 U-tests for abnormally distributed data. A two-way repeated measures ANOVA was used to
269 assess whether there were differences in HRR after cessation of exercise testing between
270 obese and lean adolescents: an interaction effect was evaluated, where group (obese vs. lean
271 adolescents) was a between-subjects factor, and time (HRR_{0.5min}, HRR_{1min} and HRR_{2min}) was a
272 within-subjects factor. A post-hoc analysis (Bonferroni post-hoc comparison test) was
273 performed when the between-subjects factor was statistically significant. Multivariate linear
274 regression analysis was applied to examine relations between altered echocardiographic
275 variables and blood parameters or CPET parameters. In these regression analyses, variables
276 with an abnormal distribution were log-transformed, and corrections for age, sex and Tanner
277 stage were made. Variables with a beta-coefficient < 0.1 were left out of consideration.
278 Relations between altered echocardiographic parameters and the metabolic risk score were
279 examined by partial correlations and adjusted for age, sex and Tanner stage. A p-value < 0.05
280 (2-tailed) was considered statistically significant.

281 The sample size calculation was performed using GPower v. 3.1 (Düsseldorf, Germany).
282 Ingul *et al.* have shown an increased e' (effect size: 1.70) and E/e' ratio (effect size: 1.55) in
283 obese adolescents⁵. Based on a statistical power > 0.8 and a two-sided alpha of 0.05 it was
284 calculated that a sample size of 8 obese individuals and 8 healthy controls had to be included
285 in the present study. In addition, a secondary outcome parameter with regard to exercise
286 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

293 **RESULTS**

294 **Subject characteristics**

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

303

304 **Blood parameters**

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

310

311 **Metabolic risk**

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

314

315 **Left ventricular structure and function**

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

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

320

321 **Exercise tolerance, cardiopulmonary function and heart rate recovery**

322 A reduced W_{peak} (p=0.010) was found in obese adolescents compared to lean adolescents
323 (Table 4). A time (p<0.001) and group (p=0.009) effect was found for HRR (Figure 1). Post-
324 hoc analysis showed a delayed heart rate recovery at 0.5 (-10±7 vs. -16±10bpm; p=0.006), 1.0
325 (-23±11 vs. -30±14bpm; p=0.036) and 2.0 (-35±12 vs. -45±12bpm; p=0.005) minutes after
326 cessation of exercise in obese adolescents, respectively. None of the other CPET parameters
327 differed between groups (p>0.05).

328

329 **Relation between metabolic risk score and aberrant echocardiographic parameters**

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

335

336 **Relations between altered echocardiographic parameters and cardiometabolic health**

337 **With regard to cardiac morphology,** a higher LV septum thickness was independently (model
338 $r^2=0.136$; $p=0.005$) related to a higher blood insulin concentration (standardized coefficient of
339 beta (SC β)= 0.368 ; $p=0.006$; Table 5) **and** a higher LA diameter was independently (model
340 $r^2=0.233$; $p<0.001$) related to a higher HOMA-IR (SC $\beta=0.482$; $p<0.001$). **Multivariate**
341 **regression analyses of altered echocardiographic parameters reflecting diastolic function**
342 **showed that** a higher mitral A wave velocity was independently (model $r^2=0.128$; $p=0.008$)
343 related to a higher blood CRP concentration (SC $\beta=0.358$; $p=0.006$) **and** a higher E/e' ratio
344 was independently (model $r^2=0.327$; $p<0.001$) related to a higher HOMA-IR (SC $\beta=0.378$;
345 $p=0.004$) and blood uric acid concentration (SC $\beta=0.321$; $p=0.013$). **Furthermore,** a higher
346 mitral e' wave velocity was independently (model $r^2=0.199$; $p=0.001$) related to a lower blood
347 insulin concentration (SC $\beta=-0.446$; $p=0.001$).

348

349 **Relations between aberrant CPET parameters and echocardiographic parameters**

350 A higher W_{peak} was independently (model $r^2=0.590$; $p<0.001$) related to a higher LV diameter
351 (SC $\beta=0.483$; $p<0.001$) and a lower cardiac output (SC $\beta=-0.266$; $p=0.010$). A higher
352 $HRR_{0.5min}$ (model $r^2=0.404$; $p<0.001$) and HRR_{1min} (model $r^2=0.369$; $p<0.001$) were
353 independently related to a lower mitral A wave velocity (SC $\beta=-0.473$; $p<0.001$ and SC $\beta=-$
354 0.535 ; $p<0.001$, respectively). A higher HRR_{2min} was independently (model $r^2=0.201$;
355 $p<0.001$) related to a higher E/A ratio (SC $\beta=0.449$; $p=0.001$).

356

357

358 **DISCUSSION**

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

364

365 **Relations between altered echocardiographic parameters and cardiometabolic health**

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

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

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

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

411

412 **Relations between aberrant CPET parameters and echocardiographic parameters**

413 Although a reduced exercise capacity was noticed in obese adolescents (as evidenced by a
414 reduced W_{peak}), this was not related to a difference in cardiac diastolic function. In fact, none
415 of the peak exercise parameters were related to cardiac function or structure in the entire
416 group. These data are in contrast with a previous study reporting a relation between a lower
417 $\dot{V}O_{2\text{peak}}$ and a lower myocardial contractility in obese adolescents¹³. However, this study was
418 limited by the small sample size and the correction of $\dot{V}O_{2\text{peak}}$ for body weight (ml/kg/min),
419 thus increasing the risk for an artificial relation¹³. Indeed, when $\dot{V}O_{2\text{peak}}$ was corrected for lean
420 tissue mass, these relations disappeared. It thus remains questionable whether cardiac
421 dysfunction would actually affect exercise capacity in obese adolescents. The only exercise
422 parameter that seemed to be related to cardiac function in the present study was HRR, where a
423 delayed HRR was independently associated with altered cardiac diastolic function. **The** mitral
424 A wave velocity was inversely associated with $\text{HRR}_{0.5\text{min}}$, whereas the E/A ratio was
425 positively related to $\text{HRR}_{2\text{min}}$. These data thus indicate that a delayed HRR is an early marker
426 for the potential presence of altered cardiac diastolic function in obese adolescents and could
427 be regarded as a clinically relevant outcome parameter during CPET. **Our findings are**
428 **consistent with previous research in obese adults and adolescents, which reported a markedly**
429 **impaired HRR^{57, 58}. Gondoni *et al.* showed that obese adolescents had a slowed $\text{HRR}_{1\text{min}}$ in**
430 **the same order of magnitude (obese: -22 ± 10 bpm; lean: -29 ± 10 bpm) as measured in the**
431 **present study⁵⁷. However, considering the rather low predictive power of the regression**
432 **models, caution is warranted in clinical use. Although the dynamics of HRR are attributed to**

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

442

443 **Limitations**

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

452

453 **CONCLUSION**

454 In obese adolescents, altered cardiac diastolic function is independently related to insulin
455 resistance or hyperinsulinemia, and is only revealed by a delayed HRR during CPET.
456 Therefore, the cardioprotective effect of interventions leading to lower serum insulin
457 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.

460

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.

469

470

471 **ACKNOWLEDGEMENTS**

472 We would like to thank all adolescents for their participation and guidance of their parents
473 during this study. Furthermore we thank the clinicians from the department of pediatrics at the
474 Jessa hospital for all the support in this study.

475

476 **CONFLICT OF INTEREST**

477 No conflict of interest was declared.

478

479 **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 \pm SD. Abbreviations: HR: Heart rate. *P<0.05.