

Original article

# Chronotropic incompetence is more frequent in obese adolescents and relates to systemic inflammation and exercise intolerance

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## Abstract

**Background:** Adults with obesity may display disturbed cardiac chronotropic responses during cardiopulmonary exercise testing, which relates to poor cardiometabolic health and an increased risk for adverse cardiovascular events. It is unknown whether cardiac chronotropic incompetence (CI) during maximal exercise is already present in obese adolescents and, if so, how that relates to cardiometabolic health.

**Methods:** Sixty-nine obese adolescents (body mass index standard deviation score =  $2.23 \pm 0.32$ , age =  $14.1 \pm 1.2$  years; mean  $\pm$  SD) and 29 lean adolescents (body mass index standard deviation score =  $-0.16 \pm 0.84$ , age =  $14.0 \pm 1.5$  years) performed a maximal cardiopulmonary exercise testing from which indicators for peak performance were determined. The resting heart rate and peak heart rate were used to calculate the maximal chronotropic response index. Biochemistry (lipid profile, glycemic control, inflammation, and leptin) was studied in fasted blood samples and during an oral glucose tolerance test within obese adolescents. Regression analyses were applied to examine associations between the presence of CI and blood or exercise capacity parameters, respectively, within obese adolescents.

**Results:** CI was prevalent in 32 out of 69 obese adolescents (46%) and 3 out of 29 lean adolescents (10%). C-reactive protein was significantly higher in obese adolescents with CI compared to obese adolescents without CI ( $p = 0.012$ ). Furthermore, peak oxygen uptake and peak cycling power output were significantly reduced ( $p < 0.05$ ) in obese adolescents with CI vs. obese adolescents without CI. The chronotropic index was independently related to blood total cholesterol (standardized coefficient  $\beta = -0.332$ ;  $p = 0.012$ ) and C-reactive protein concentration (standardized coefficient  $\beta = -0.269$ ;  $p = 0.039$ ).

**Conclusion:** CI is more common in the current cohort of obese adolescents, and is related to systemic inflammation and exercise intolerance.

**Keywords:** Adolescents; Cardiometabolic health; Exercise tolerance; Heart rate; Obesity

## 1. Introduction

The prevalence of obesity has increased considerably among adolescents over the past several decades to become one of the most significant health concerns worldwide.<sup>1</sup> Recent data indicate that, globally, the number of children and adolescents with obesity increased to 62 million by 2016.<sup>1</sup> Obese adolescents face major health and socio-economic impacts,<sup>2,3</sup> which include being at

greater risk for developing endothelial dysfunction, hypertension, insulin resistance, nonalcoholic fatty liver disease, respiratory and orthopedic disorders, or for suffering from psychosocial difficulties, even into adulthood.<sup>4–6</sup> In addition to the medical complications related to obesity in adolescents, suboptimal physiological responses to exercise have also been observed;<sup>7</sup> in particular, a reduced peak cycling power output<sup>7–9</sup> and peak oxygen uptake/kg lean tissue mass.<sup>7</sup>

In adults with obesity (those with or without type 2 diabetes mellitus), chronotropic incompetence (CI) is often observed.<sup>10–12</sup> CI is defined as the inability of the heart rate (HR) to increase in

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accordance with the metabolic demand, leading to a lowered peak HR ( $HR_{peak}$ ) ( $<80\%$  of maximal predicted  $HR_{peak}$ ).<sup>13,14</sup> It has been shown that both peak and resting HR account for over forty percent of the variability explaining exercise capacity, and that this kind of modification in cardiac behavior leads to reductions in exercise capacity.<sup>12</sup>

In adult individuals who are obese, CI is independently related to an elevated risk for major adverse cardiovascular events (e.g., acute myocardial infarction or heart failure) and premature death.<sup>15,16</sup> There are several mechanisms that could potentially account for the association of obesity and CI, including a blunted catecholamine response, an abnormal baroreflex function, or a decreased catecholamine sensitivity and beta-adrenergic receptor density, all of which ultimately lead to autonomic dysfunction.<sup>17,18</sup> This supports the concept that abnormalities in autonomic balance may precede manifestations of cardiovascular disease and may contribute to the early identification of persons at high risk for sudden death.<sup>16</sup>

Despite the well-established association between CI and prognosis of all-cause mortality in adults with obesity,<sup>15,16</sup> the prevalence of CI in adolescents with obesity has not been investigated, nor has its association with cardiometabolic health or exercise tolerance. These are important questions to answer, however, as they may affect the methodology for prescribing/monitoring exercise intensity based on HR and lead us to a deeper understanding of how adolescent obesity may affect cardiometabolic health and exercise performance.

Therefore, this study aims to (1) examine the prevalence of CI in adolescents with obesity and (2) understand whether CI is related to cardiometabolic health and exercise tolerance in obese adolescents. Based on literature from studies involving obese adults,<sup>10,12</sup> we hypothesize that CI will be more prevalent in obese vs. lean adolescents, and that CI relates to a worse cardiometabolic health and exercise performance.

## 2. Methods

The study was carried out according to an observational, cross-sectional design and was performed at Jessa Hospital (Hasselt, Belgium), as described previously.<sup>19</sup> From midnight prior to a 1-day hospitalization, all subjects refrained from consuming food (with the exception of water *ad libitum*) to prevent short-term metabolic effects on outcome parameters. After registration of general characteristics, anthropometry, body composition, Tanner stage, and blood pressure were measured. In addition, venous blood samples were collected (in a fasted state), and an oral glucose tolerance test (OGTT) was performed to examine participants' cardiometabolic health. One hour prior to cardiopulmonary exercise testing (CPET), a standardized meal (total energy = 296 kcal; composed of 3 g fats, 56 g carbohydrates, and 9 g proteins) was consumed.

### 2.1. Subjects

Adolescents with obesity were recruited from the pediatric clinic of Jessa Hospital. Data from lean adolescents were taken from a previous study performed by our research group and used only to determine the CI prevalence.<sup>19</sup> Participants were

between 11 and 17 years of age and free from any known chronic cardiovascular, renal, pulmonary or orthopedic disease. The International Obesity Task Force criteria and body fat percentage ( $>95$ th percentile) were used to categorize the participants into a lean group and an obese group.<sup>20,21</sup> Sixty-nine obese adolescents (body mass index (BMI) standard deviation score (SDS) =  $2.23 \pm 0.32$ , age =  $14.1 \pm 1.2$  years; mean  $\pm$  SD) and 29 lean adolescents (BMI SDS =  $-0.16 \pm 0.84$ , age =  $14.0 \pm 1.5$  years) were included into the 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 (2013). The present study is registered at clinicaltrials.gov as NCT04185753.

### 2.2. Auxological parameters

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 was determined to the nearest 0.1 kg using a digital-balanced weighing scale (Seca 770; Seca GmbH, Hamburg, Germany) with participants in underwear. The BMI was calculated from weight and height measurements ( $\text{weight}/\text{height}^2$ ). The body height and BMI SDS were calculated using the LMS method, as described by Cole et al.:<sup>20,22</sup>  $\text{body height-SDS} = (\text{body height}/M) L - 1)/(L \times S)$  and  $\text{BMI-SDS} = (\text{BMI}/M) L - 1)/(L \times S)$ , where M is the median BMI by age, S the coefficient of variation of BMI, and L expresses the skewness of the BMI distribution in terms of the Box-Cox power needed to transform the data to near normality.<sup>22</sup> Waist and hip circumferences were measured in triplicate to the nearest 0.1 cm using the flexible metric measuring tape Seca 201 (Seca GmbH), with participants in standing position, barefoot and in underwear. 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 by tetrapolar bioimpedance measurement using the Bodystat 1500 MDD<sup>®</sup> monitoring unit (EuroMedix, Leuven, Belgium) with subjects in supine position.<sup>23</sup> Electrodes were placed on the dorsal surface of the wrist and hand (just behind the middle finger) and the ankle and foot (just behind the middle toe) according to manufacturer's instructions. Body fat percentage was calculated using the formula of Houtkooper.<sup>24</sup>

### 2.3. Blood pressure, pubertal development stage, and physical activity evaluation

Blood pressure was measured in supine position using an electronic sphygmomanometer (Omron, Omron Healthcare, Lake Forest, IL, USA) after a resting period of 5 min. Pubertal status was assessed using Tanner's scale, which defines physical features of development according to external primary and

secondary sex characteristics; the assessment relied on observations by a pediatrician as well as on the adolescents' own opinions based on a figure.<sup>25,26</sup> Level of physical activity was determined using the validated Dutch Physical Activity Questionnaires for Adolescents (PAQ-A).<sup>27</sup>

#### 2.4. Biochemical analysis

Venous blood samples were taken from participants for the measurement of blood parameters. Plasma glucose, lipid profile (blood total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein cholesterol, and triglyceride concentrations), C-reactive protein (CRP), and serum insulin concentrations were automatically assessed on Roche Cobas 8000 (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Blood glycated hemoglobin concentration was measured using ion exchange chromatography (Menarini HA-8180 hemoglobin concentration auto-analyzer, Menarini Diagnostics, Diegem, Belgium). Serum leptin concentrations were measured using radioimmunoassay (RIA; LINCO Research Inc., St. Louis, MO, USA). All coefficients of variation for these assays were less than 15%.

#### 2.5. Insulin sensitivity and beta cell function

A standard 5-point OGTT was performed for assessment of whole-body/tissue-specific insulin sensitivity and beta cell function. Subjects ingested a solution (200 mL) containing 75 g dextrose, and venous blood samples were obtained at 0 min, 30 min, 60 min, 90 min, and 120 min for assessment of glucose and insulin concentration. Blood samples were automatically assessed on Roche Cobas 8000 (Roche Diagnostics International Ltd.). From glucose and insulin concentrations, homeostatic model assessment for insulin resistance was calculated by: fasting glucose (mg/dL)  $\times$  fasting insulin (mU/L)/405.<sup>28</sup> Tissue-specific insulin resistance was calculated using the hepatic insulin resistance index and the muscle insulin sensitivity index. The hepatic insulin resistance index was calculated as the product of the area under curve (AUC) for glucose and insulin during the first 30 min of the OGTT (glucose<sub>0-30</sub>(AUC in mg/dL h)  $\times$  insulin<sub>0-30</sub>(AUC in mU/L h)), and the muscle insulin sensitivity index was calculated as the rate of decay of glucose concentration during the OGTT divided by the mean insulin concentration during the OGTT (in mg/dL/min/mU/L). The rate of decay was calculated as the slope of the least square fit to the decline in glucose concentration from peak to nadir, as described by Vogelzangs et al.<sup>29</sup> In addition, whole-body insulin sensitivity index was calculated by: 10000/ $\sqrt{(\text{fasting glucose (mg/dL)} \times \text{fasting insulin (mU/L)}) \times (\text{mean glucose during OGTT (mg/dL)} \times \text{mean insulin during OGTT (mU/L)})}$ .<sup>28</sup> The quantitative insulin sensitivity check index (QUICKI) was calculated as: 1/log(fasting insulin (mU/L) + log(fasting glucose (mg/dl))).<sup>28</sup> Beta cell function was estimated by calculation of the insulinogenic index, that is, the ratio of increment of insulin (mU/L) and glucose (mg/dL) in the first 30 min of the OGTT.<sup>30</sup> The AUC for glucose and insulin for the 2-h period was calculated using the trapezoidal rule.<sup>31</sup>

#### 2.6. CPET

CPET was performed up to volitional exhaustion using an electronically braked cycle ergometer (eBike, GE Medical systems, Milwaukee, WI, 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 between 19°C and 21°C. The exercise test (ramp protocol) included a 1-min pre-exercise resting period, a 1-min unloaded warm-up cycling phase, as well as an incremental exercise cycling period with an initial workload of 40 W and an increasing workload of 20 W per minute. During warm-up cycling and incremental exercise, a cycling frequency of 60–70 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, measured by a respiratory gas exchange ratio of  $\geq 1.05$  and the subjective opinion of an experienced tester who confirmed whether or not a maximal exercise test was executed. The tester's expert opinion was based on subjective features described by Bongers et al.,<sup>32</sup> including dyspnea, sweating, facial flushing, a clear unwillingness to continue, and a sustained drop in the participant's pedaling frequency from 60 rpm despite verbal encouragement. After cessation of exercise, workload was set at 45 W; subjects cycled for 2 min during active recovery with a cycling frequency of 50 rpm.

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, breathing frequency, and the respiratory gas exchange ratio were determined breath by breath, and data were averaged every 10 s. Using a 12-lead electrocardiography device (KIS Multilead, GE Medical systems, Freiburg, Germany) HR was monitored and averaged every 10 s. From this parameter, oxygen pulse ( $\dot{V}O_2/HR$ ) was calculated. Age-predicted maximal HR was calculated using the equation  $HR = 220 - \text{age in years}$ . Maximal chronotropic response index ( $CRI_{\max}$ ) was calculated using the following equation:  $(\text{actual } HR_{\text{peak}} - HR_{\text{rest}})/(\text{predicted } HR_{\text{peak}} - HR_{\text{rest}})$ .<sup>13</sup> CI was defined as the inability to achieve a  $CRI_{\max} \geq 0.80$ .<sup>13</sup> The  $\dot{V}O_2$  efficiency slope was calculated by a linear least square regression of  $\dot{V}O_2$  on the logarithmic of  $\dot{V}E$  using all exercise data.<sup>32</sup> The first ventilatory threshold was determined using the V-slope method.<sup>33</sup> The second ventilatory threshold 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  $\dot{V}CO_2$ .<sup>34</sup> Exercise tolerance was assessed by the peak workload.

#### 2.7. Statistical analysis

Statistical analysis was performed by SPSS Version 24.0 (IBM Corp., Armonk, NY, USA). Data were expressed as means  $\pm$  SD. A Shapiro–Wilk test was used to test normality of the data ( $p < 0.05$ ). Comparisons between groups were

tested using the  $\chi^2$  test for categorical variables. Differences between continuous variables of subject characteristics were assessed using a one-way analysis of variance with a Bonferroni *post hoc* comparison test for normally distributed data and the Kruskal–Wallis test (Dunn's *post hoc* comparison test) for non-normally distributed data. Differences between continuous variables (e.g., blood and CPET) were assessed using independent samples *t* tests for normally distributed data and Mann-Whitney *U* tests for abnormally distributed data within obese adolescents. A two-way repeated measures analysis of variance was used to assess whether there were differences in HR behavior during exercise testing between obese adolescents with CI and those without: an interaction effect was evaluated where group (obese vs. obese with CI) was a between-subjects factor, and time (percentage of  $\dot{V}O_{2\text{peak}}$ ) 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 the  $\text{CRI}_{\text{max}}$  and variables of cardiometabolic health, corrected for age, sex, and Tanner stage. Variables with a beta-coefficient  $<0.1$  were left out of consideration. A *p* value of  $<0.05$  (2-tailed) was considered statistically significant.

The sample size was based on a previous study by Marinus et al.<sup>35</sup> that showed obese adolescents had a significantly decreased  $\text{HR}_{\text{peak}}$  ( $192 \pm 8$  bpm vs.  $183 \pm 11$  bpm).<sup>35</sup> Based on the difference in  $\text{HR}_{\text{peak}}$  (effect size  $d=0.96$ ), a statistical power of  $>0.8$ , and a 2-sided  $\alpha$  of 0.05, it was calculated that a sample size of 19 obese individuals with CI and 19 obese adolescents without CI must be included in the present study. In addition, a secondary outcome parameter with regard to systemic inflammation was included. Huang et al.<sup>36</sup> found that hsCRP concentrations were significantly different between adults with and without CI. Using the same values as stated above and an effect size of 0.39, it was calculated that a sample size of 16 obese individuals with and 16 without CI must be included in the present study. Taking into account a drop-out rate of 10%, the number of participants needed to be raised to at least 21 obese adolescents with CI and 21 obese adolescents without CI, which resulted in a final sample size of 42 subjects.

### 3. Results

#### 3.1. Subject characteristics

A total of 98 participants (69 obese and 29 lean adolescents) were eligible and completed the study. Due to previously undetected anemia, data from 1 lean adolescent was excluded. By design, body weight ( $p < 0.001$ ), BMI ( $p < 0.001$ ) and BMI-SDS ( $p < 0.001$ ), waist circumference ( $p < 0.001$ ), hip circumference ( $p < 0.001$ ), waist-to-hip ratio ( $p < 0.001$ ), percentage of body fat ( $p < 0.001$ ), and systolic blood pressure (SBP) ( $p < 0.001$ ) were elevated in obese vs. lean adolescents (Table 1). Compared with their lean counterparts, physical activity levels (PAQ-A score) were lower in obese adolescents with CI ( $p=0.020$ ) but similar in those without. Age, sex, body height, body height-SDS, diastolic blood pressure

(DBP), and Tanner stage were comparable between groups ( $p > 0.05$ ) (Table 1).

#### 3.2. Prevalence of CI in lean vs. obese adolescents

CI was present in 32 out of 69 (46%) obese adolescents and 3 out of 29 (10%) lean adolescents. These CI prevalence rates were significantly different between lean and obese adolescents ( $p < 0.01$ ). No differences in clinical features were found between obese adolescents with and without CI, except for SBP ( $124 \pm 12$  mmHg vs.  $137 \pm 26$  mmHg;  $p=0.032$ ).

#### 3.3. CI and its relation to cardiometabolic health within obese adolescents

##### 3.3.1. Blood parameters

Only CRP was significantly higher ( $p=0.012$ ) in obese adolescents with CI ( $4.9 \pm 3.2$  mg/L) than in obese adolescents without CI ( $2.2 \pm 2.2$  mg/L). With respect to glycemic control and lipid profile, no differences were found between obese adolescents with or without CI ( $p > 0.05$ ) (Table 2).

##### 3.3.2. Exercise tolerance and cardiopulmonary function

A reduction in the  $\dot{V}O_{2\text{peak}}$  (mL/min:  $p=0.005$ ; mL/min/kg:  $p < 0.001$ ),  $\dot{V}CO_{2\text{peak}}$  ( $p < 0.001$ ),  $\dot{V}E_{\text{peak}}$  ( $p < 0.001$ ),  $Vt_{\text{peak}}$  ( $p=0.029$ ),  $\text{BF}_{\text{peak}}$  ( $p=0.022$ ), oxygen uptake efficiency slope ( $p=0.035$ ),  $\text{RER}_{\text{peak}}$  ( $p=0.001$ ), peak workload ( $p < 0.001$ ), and  $\text{CRI}_{\text{max}}$  ( $p < 0.001$ ) was observed in obese adolescents with CI vs. obese adolescents without CI (Table 3). Although the resting HR was comparable between both obese groups, time ( $p < 0.001$ ), group ( $p < 0.001$ ), and time  $\times$  group interaction ( $p < 0.001$ ) effects were found for the HR response during CPET (Fig. 1). The HR increase becomes significantly less steep at 50% of  $\dot{V}O_{2\text{peak}}$  in obese adolescents with CI, in comparison to obese adolescents without CI (50%  $\dot{V}O_{2\text{peak}}$ :  $p=0.023$ ; 60%–100%  $\dot{V}O_{2\text{peak}}$ :  $p < 0.001$ ).

##### 3.3.3. Relation between $\text{CRI}_{\text{max}}$ and cardiometabolic health in obese adolescents

The  $\text{CRI}_{\text{max}}$  was independently related to blood total cholesterol (standardized coefficient  $\beta = -0.332$ ;  $p=0.012$ ) and CRP concentration (standardized coefficient  $\beta = -0.269$ ;  $p=0.039$ ), which together explained about 21% of the variance in  $\text{CRI}_{\text{max}}$  (model  $r^2 = 0.201$ ;  $p=0.004$ ; adjusted for age, sex, and Tanner stage).

### 4. Discussion

In the present study, CI was found to be highly prevalent in obese adolescents and related to poor cardiometabolic health as well as exercise intolerance.

CI was prevalent in 32 out of 69 obese adolescents (46% of cohort) and in 3 out of 29 lean adolescents (10% of cohort). These CI prevalence rates were significantly different between lean and obese adolescents ( $p < 0.01$ ). Moreover, CI starts to appear at a workload that corresponds to about 50% of the  $\dot{V}O_{2\text{peak}}$ , after which a less steep slope in HR response is present up to peak exercise. These data thus confirm observations

Table 1  
Subject characteristics of obese and lean individuals (*n* or mean  $\pm$  SD).

General features	Lean ( <i>n</i> = 29)	Obese ( <i>n</i> = 37)	Obese CI ( <i>n</i> = 32)	<i>p</i>
<b>Age (year)</b>	14.0 $\pm$ 1.50	14.1 $\pm$ 1.30	14.1 $\pm$ 1.10	0.382
<b>Sex (<i>n</i>)</b>				0.489
Male	16	21	15	
Female	13	16	17	
<b>Body weight (kg)</b>	54.7 $\pm$ 10.8* <sup>#</sup>	91.9 $\pm$ 18.6	96.4 $\pm$ 21.0	<0.001
<b>Body height (cm)</b>	166.8 $\pm$ 8.9	166.9 $\pm$ 9.5	166.6 $\pm$ 7.4	0.977
<b>Body height-SDS</b>	0.68 $\pm$ 1.00	0.72 $\pm$ 1.17	0.69 $\pm$ 1.05	0.979
<b>BMI (kg/m<sup>2</sup>)</b>	19.5 $\pm$ 2.4* <sup>#</sup>	32.7 $\pm$ 4.4	34.6 $\pm$ 5.9	<0.001
<b>BMI-SDS</b>	-0.16 $\pm$ 0.84* <sup>#</sup>	2.20 $\pm$ 0.31	2.27 $\pm$ 0.33	<0.001
<b>Waist circumference (cm)</b>	67.4 $\pm$ 6.2* <sup>#</sup>	101.5 $\pm$ 12.2	101.4 $\pm$ 12.0	<0.001
<b>Hip circumference (cm)</b>	78.7 $\pm$ 8.3* <sup>#</sup>	106.5 $\pm$ 8.8	107.0 $\pm$ 10.3	<0.001
<b>Waist-to-hip ratio</b>	0.86 $\pm$ 0.11* <sup>#</sup>	0.95 $\pm$ 0.07	0.95 $\pm$ 0.08	<0.001
<b>Body fat (%)</b>	14.1 $\pm$ 7.6* <sup>#</sup>	36.0 $\pm$ 5.2	39.2 $\pm$ 6.8	<0.001
<b>PAQ-A score</b>	2.51 $\pm$ 0.50 <sup>#</sup>	2.24 $\pm$ 0.66	2.04 $\pm$ 0.57	0.020
<b>SBP (mmHg)</b>	114 $\pm$ 10*	137 $\pm$ 26 <sup>#</sup>	124 $\pm$ 12	<0.001
<b>DBP (mmHg)</b>	70 $\pm$ 7	75 $\pm$ 21	73 $\pm$ 11	0.393
<b>CI (<i>n</i>)</b>	29/3	0/37	32/32	<0.001
<b>Development stage (<i>n</i>)</b>				0.759
Tanner Stage 1	2	3	1	
Tanner Stage 2	2	1	2	
Tanner Stage 3	4	6	6	
Tanner Stage 4	7	3	6	
Tanner Stage 5	14	17	17	

Notes: Comparisons between groups were tested using the  $\chi^2$  test for categorical variables. For continuous variables, comparisons between groups were tested using a one-way analysis of variance (Bonferroni *post hoc* comparison test) for normally distributed data and the Kruskal-Wallis test (Dunn's *post hoc* comparison test) for non-normally distributed data. The *p* values reported represent the overall analysis of variance.

\* *p* < 0.05, compared with obese group; <sup>#</sup> *p* < 0.05, compared with obese CI group.

Abbreviations: BMI = body mass index; BMI-SDS = body mass index standard deviation score; CI = chronotropic incompetence; DBP = Diastolic blood pressure; PAQ-A = Dutch Physical Activity Questionnaire for Adolescents; SBP = systolic blood pressure; SDS = standard deviation score.

in adults with obesity and/or type 2 diabetes mellitus.<sup>12,14</sup> This finding is clinically relevant because it shows that in nearly half of the obese adolescents, basing intensities for physical activity and exercise on predicted maximal HR is invalid. In fact, such an approach will lead to an overestimation of the target HR during exercise and physical activity, which should be avoided in order to maintain therapy adherence.<sup>37</sup> Based on current findings (Fig. 1), such an overestimation could already be occurring during low-to-moderate-intensity activities.

However, the etiology of CI in obese adolescents remains to be explored in greater detail. In adults with obesity, it has been shown that CI is caused, at least in part, by a suppressed catecholamine synthesis and release during exercise,<sup>11</sup> probably due to the exaggerated cortisol response seen in obese individuals<sup>38,39</sup> or the exaggerated secretion of (non-)cholinergic and neuropeptides from adipocytes.<sup>40</sup> Indeed, previous studies show that the synthesis and release of epinephrine is significantly suppressed in obese adolescents during exercise,<sup>41,42</sup> which leads to less stimulation of the atrial sinus node and thus impedes proper HR responses during exercise. Remediation for such an aberrant response in catecholamine release during exercise is currently not available and it is questionable whether this anomaly could be attenuated by exercise training.<sup>43</sup> Together with the suppressed catecholamine release, the lower potassium levels observed during exercise in obese individuals<sup>11</sup> may also contribute to the manifestation of

CI in obese adolescents. However, potassium concentrations were not measured in the present study.

Furthermore, the presence of diastolic dysfunction in obese adolescents has already been shown.<sup>19,44,45</sup> Such diastolic dysfunction induces delayed myocardial relaxation, impaired left ventricular filling, and increased myocardial stiffness, all which could result in an inability to increase stroke volume adequately to the degree of effort during exercise, as seen in other populations.<sup>14</sup> For these reasons, diastolic dysfunction may be involved in the chronotropic incompetent response seen in this study.

In the present study it was observed that CI was independently related to blood CRP and total cholesterol concentrations (model  $r^2 = 0.201$ , *p* < 0.05). This is in accordance with the previous research of Huang et al.<sup>36</sup> wherein higher concentrations of CRP, as well as N-terminal pro-brain natriuretic peptide, were found in non-obese subjects with CI. The findings of the present study therefore support the hypothesis that obese adolescents with CI may present a pronounced systemic inflammatory status. Together with this higher level of systemic inflammation, cholesterol may play an important role in the development of endothelial damage/dysfunction.<sup>46</sup> Steinberg et al.<sup>46</sup> showed that even an increase in cholesterol within the (high-) normal range, can contribute to endothelial damage in healthy subjects. This endothelial damage could lead to increased arterial stiffness and, eventually, attenuated HR responses during

Table 2  
Cardiometabolic risk factors and parameters regarding glycemic control in obese adolescents with and without chronotropic incompetence (mean  $\pm$  SD).

	Obese (n = 37)	Obese CI (n = 32)	<i>p</i>
<b>Cardiovascular risk factors</b>			
C-reactive protein (mg/L)	2.2 $\pm$ 2.2	4.9 $\pm$ 3.2	0.012
Total cholesterol (mg/dL)	152 $\pm$ 33	158 $\pm$ 27	0.388
LDL cholesterol (mg/dL)	85 $\pm$ 24	93 $\pm$ 25	0.282
HDL cholesterol (mg/dL)	44 $\pm$ 11	42 $\pm$ 10	0.497
Triglycerides (mg/dL)	112 $\pm$ 67	123 $\pm$ 75	0.550
Triglyceride-to-HDL cholesterol ratio	2.8 $\pm$ 2.0	3.3 $\pm$ 2.6	0.571
Leptin ( $\mu$ g/L)	46.9 $\pm$ 22.0	8.7 $\pm$ 6.3	0.703
<b>Glycemic control</b>			
Fasting glucose (mg/dL)	91 $\pm$ 6	92 $\pm$ 6	0.636
Fasting insulin (mU/L)	24 $\pm$ 13	28 $\pm$ 5	0.096
Glycated hemoglobin (%)	5.4 $\pm$ 0.3	5.4 $\pm$ 0.2	0.313
HOMA-IR	5.4 $\pm$ 3.2	6.1 $\pm$ 3.7	0.116
MISI (mg/dL/min/mU/L)	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.710
HIRI ((mg/dL) $\times$ h $\times$ (mU/L) $\times$ h)	51.2 $\pm$ 18.9	58.1 $\pm$ 21.0	0.179
WBISI	2.38 $\pm$ 1.22	1.95 $\pm$ 1.17	0.140
QUICKI	0.31 $\pm$ 0.03	0.30 $\pm$ 0.03	0.347
IGI (mU/L/mg/dL)	3.46 $\pm$ 1.93	4.96 $\pm$ 3.81	0.191
AUC glucose ((mg/dL) $\times$ h)	512 $\pm$ 86	484 $\pm$ 77	0.195
AUC insulin ((mU/L) $\times$ h)	702 $\pm$ 670	680 $\pm$ 438	0.883

Note: Comparisons between the 2 groups were performed using the independent-samples *t* test or Mann-Whitney *U* test.

Abbreviations: AUC = area under curve; HDL = high-density lipoprotein; HIRI = hepatic insulin resistance index; HOMA-IR = homeostatic model assessment of insulin resistance; IGI = insulinogenic index; LDL = low-density lipoprotein; MISI = muscle insulin sensitivity index; QUICKI = quantitative insulin sensitivity check index; WBISI = whole-body insulin sensitivity index.

exercise.<sup>47,48</sup> Furthermore, as demonstrated by Lambert et al.,<sup>49</sup> an increase in total cholesterol is accompanied by an increase in sympathetic activation, thereby inducing

sympathovagal dysbalans, which may lead to the development of CI in young females. Because of the frequent activation of sympathetic nerves, down-regulation of the  $\beta$ -adrenergic receptors may occur, leading to post-synaptic desensitization and thus to a disturbed sympathetic driven HR regulation during exercise.<sup>50</sup> However, the exact role of hypercholesterolemia and systemic inflammation in the development or progression of CI in obese adolescents remains to be investigated in detail. Nevertheless, CI clearly relates to a poor cardiometabolic health profile and should be addressed in obese adolescents in order to prevent future cardiovascular events.

CI was also shown to be associated with significant reductions in exercise tolerance, as indicated by a reduction in peak oxygen uptake and peak cycling power output in obese adolescents with CI (*p* < 0.05). These results confirm previous findings from studies in adult populations.<sup>51</sup> It could thus be hypothesized that exercise limitations in obese adolescents may be due to central factors, as they are in adults, and not simply due to skeletal muscle dysfunction.

This study may have been prone to some limitations. First, catecholamine and potassium levels were not measured but should be included in further research. In addition, prospective studies need to confirm measurements regarding autonomic function as well as one of the hypotheses that autonomic imbalance is a key determinant of HR behavior during exercise.

In conclusion, the present study shows that CI is more common in the current cohort of obese adolescents, and relates to systemic inflammation and exercise intolerance.

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Table 3  
Cardiopulmonary function during cardiopulmonary exercise testing in obese adolescents with and without chronotropic incompetence (mean  $\pm$  SD).

	Obese normal (n = 37)	Obese CI (n = 32)	<i>p</i>
$\dot{V}O_{2peak}$ (mL/min)	2303 $\pm$ 459	1999 $\pm$ 414	0.005
$\dot{V}O_{2peak}$ per weight (mL/min/kg)	25.5 $\pm$ 5.5	21.0 $\pm$ 3.6	<0.001
$\dot{V}O_{2peak}$ (mL/min)	2750 $\pm$ 517	2268 $\pm$ 458	<0.001
Peak minute ventilation (L/min)	80 $\pm$ 17	64 $\pm$ 15	<0.001
Peak tidal volume (L)	2.00 $\pm$ 0.51	1.75 $\pm$ 0.38	0.029
Peak breathing frequency (breaths/min)	41 $\pm$ 7	37 $\pm$ 7	0.022
Oxygen uptake efficiency slope	2492 $\pm$ 529	2232 $\pm$ 465	0.035
Peak ventilatory equivalent $\dot{V}O_2$	33.6 $\pm$ 6.8	31.0 $\pm$ 5.0	0.078
Peak ventilatory equivalent $\dot{V}CO_2$	28.6 $\pm$ 3.4	27.8 $\pm$ 2.8	0.264
Peak respiratory exchange ratio	1.20 $\pm$ 0.08	1.14 $\pm$ 0.07	0.001
Peak oxygen pulse (mL/heart beat)	12.1 $\pm$ 2.5	11.6 $\pm$ 2.5	0.305
Peak workload (W)	172 $\pm$ 31	137 $\pm$ 27	<0.001
Ventilatory threshold 1 (mL/min)	1184 $\pm$ 231	1151 $\pm$ 231	0.552
Ventilatory threshold 2 (mL/min)	1693 $\pm$ 347	1577 $\pm$ 330	0.178
Resting heart rate (bpm)	97 $\pm$ 15	101 $\pm$ 15	0.287
Peak heart rate (bpm)	191 $\pm$ 6	173 $\pm$ 11	<0.001
Maximal chronotropic response index	0.87 $\pm$ 0.06	0.69 $\pm$ 0.09	<0.001

Note: Comparisons between the 2 groups were performed using the independent-samples *t* test or Mann-Whitney *U* test.

Abbreviations: bpm = beats per minute;  $\dot{V}O_{2peak}$  = peak oxygen uptake;  $\dot{V}CO_{2peak}$  = peak carbon dioxide output; W = watt.

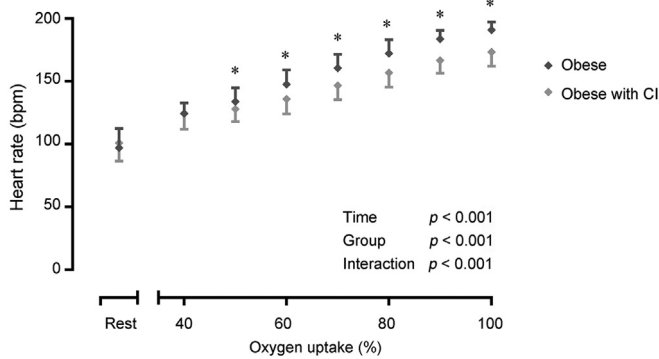


Fig. 1. Heart rate response at rest and during exercise testing in obese individuals with ( $n=32$ ) and without ( $n=37$ ) CI. Differences in heart rate response were tested using a two-way repeated measures ANOVA. A Bonferroni *post hoc* comparison test was performed when the between-subjects factor was statistically significant. Data are expressed as mean  $\pm$  SD. \*  $p < 0.05$ . ANOVA = analysis of variance; bpm = beats per minute; CI = chronotropic incompetence.

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#### Authors' contributions

WMAF, CK, NM, KV, and LvR contributed to the acquisition, analysis, or interpretation of data for the work and drafted the manuscript; BOE and PD contributed to the conception or design of the work and critically revised the manuscript; RZ contributed to the acquisition, analysis, or interpretation of data for the work and critically revised the manuscript; GM and DH contributed to the conception or design of the work, contributed to the acquisition, analysis, or interpretation of data for the work, and critically revised the manuscript. All authors have read and approved the final version of the manuscript, and agreed with the order of presentation of the authors.

#### Competing interests

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