2016•2017
FACULTEIT GENEESKUNDE EN LEVENSWETENSCHAPPEN
master in de biomedische wetenschappen

Masterproef

Cardiac chronotropic responses during exercise and relations to cardiometabolic health in obese adolescents

Promotor :
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Scriptie ingediend tot het behalen van de graad van master in de biomedische wetenschappen

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universiteit hasselt | Maastricht University

Abstract

Background Adults with obesity may display disturbed cardiac chronotropic responses during cardiopulmonary exercise testing (CPET), which relates to worse cardiometabolic health and increased cardiovascular risk. Whether specific cardiac chronotropic responses are present in obese adolescents, and relate to cardiometabolic and cardiovascular risk, remains uncertain.

Aim To investigate whether cardiac chronotropic responses during CPET may be a sensitive and specific independent indicator for cardiometabolic and cardiovascular risk factors. We hypothesized that aberrant cardiac responses are present during CPET, and these relate to worse cardiometabolic health in obese adolescents.

Methods Twenty-four obese (BMI 32.1 ± 4.3 kg/m^2, age 13.4 ± 1.1 years) and 24 lean (BMI 19.6 ± 2.5 kg/m^2, age 13.9 ± 1.5 years) adolescents performed a CPET with comparison of cardiopulmonary exercise parameters and ECG variables. Transthoracic echocardiography (TTE) and carotid ultrasound measurements were performed to assess cardiac function and carotid artery intima thickness. Haematology, chemistry, liver function, glycaemic control, lipid profile and endocrine hormones were studied in fasting blood samples. Regression analyses were performed to examine relations between aberrant cardiac responses and subject characteristics, blood parameters, ECG variables and TTE variables.

Results Peak cycling power output ($p = 0.011$) and heart rate reserve ($p = 0.002$) were lower in obese adolescents. Compared with lean controls, obese adolescents demonstrated a significantly decreased heart rate recovery HRR, 0.5 ($p = 0.012$), 1 ($p = 0.021$) and 2 ($p = 0.007$) minutes after cessation of exercise. In addition, HRR is independently associated with a worse metabolic risk profile, cardiometabolic health, cardiovascular risk factors and electrophysiological parameters.

Conclusion Obese adolescents demonstrate impaired heart rate recovery (HRR) after CPET, whereby reduced post exercise HRR in obese adolescents suggests impaired autonomic function. HRR is independently associated with worse cardiometabolic health.

Preface

It is June 2017 and I realize I am really going to graduate now. Over the past eight months, I had the pleasure of participating in the study of “chronotropic responses in obese adolescents during exercise”. This project was performed at the Jessa hospital at the department of Pediatric Endocrinology in Hasselt, Belgium. It was a great experience with a lot of educational and enjoyable moments. This thesis is the result of the committed hands and hearts of many people.

Foremost among those is my promoter prof. dr. Dominique Hansen, who gave me the opportunity to perform this study within his research group. Thank you very much for your time, guidance, suggestions, knowledge, enthusiasm and educational moments! In addition, big thanks to my second promoter prof. dr. Massa for the guidance and knowledge.

I also want to thank dr. Torab Al Hatawe for his time to perform all echocardiographic analysis. Furthermore, I want to thank the entire nursing staff for all the support they provide during my senior internship. Thanks to the staff of the heart rehabilitation center for all the support and funny moments!

And last but not least, I would thank Marjolein Beyens for the great and enjoyable cooperation during this project! And thanks to my fellow student Hajar Boujemaa for her support and many funny moments!

Once again, thanks to everyone who helped me to finish this project successfully!

List of abbreviations

AD-SVF	Adipose-derived stromal vascular fraction
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BF	Breathing frequency
BIA	Bio-electrical impedance
BMI	Body mass index
BP	Blood pressure
CIMT	Carotid intima thickness
CPET	Cardiopulmonary exercise testing
CPET	Cardiopulmonary exercise testing
CRP	C-reactive protein
CVD	Cardiovascular disease
2-DE	Two-dimensional
ECG	Electrocardiography
GGT	Gamma-glutamyl transpeptidase
HbA1c	Glycated haemoglobin
HDL-C	High density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment of insulin resistance
HR	Heart rate
HRR	Heart rate recovery
IL	Interleukin
IMT	Intima-media thickness
IOTF	International Obesity Task Force
LDL	Low-density lipoprotein
LVM	Left ventricular mass
MAP	Mean arterial pressure
MetS	Metabolic syndrome
M-mode	Motion mode
MSC	Mesenchymal stem cells
OUES	Oxygen uptake efficiency slope
PAQ	Dutch physical activity questionnaire
$PETCO_2$	End-tidal carbon dioxide pressure
$PETO_2$	End-tidal oxygen pressure
RER	Respiratory gas exchange ratio
SD	Standard deviation
T2DM	Type 2 diabetes mellitus
T4	Free thyroxine
TG	Triglycerides
TNF-α	Tumor necrosis factor alpha
TSH	Thyroid-stimulating hormone
TTE	Transthoracic echocardiography
VCO2	Respiratory carbon dioxide production
Vd/Vt	Tidal volume/dead space volume
VE	Expiratory volume
VE/VCO_2	Equivalents for carbon dioxide production
VE/VO_2	Equivalents for oxygen uptake
VLDL-C	very low-density lipoprotein cholesterol
VO2	Respiratory oxygen uptake
VO_2/HR	Oxygen pulse
Vt	Tidal volume
VT1	First ventilatory threshold
VT2	Second ventilatory threshold
W	Cycling power output
WHR	Waist-to-hip ratio

Contents

1. INTRODUCTION

During the 20th century, environmental changes such as consumption of junk food and sedentary behavior became a new way of living. This led to a mismatch between adaptive biological characteristics and the modern environmental conditions [1]. Obesity among adolescents has increased considerably over the past decades and is becoming one of the most serious epidemic preventable health concerns worldwide [2, 3]. Recent data indicate the prevalence of overweight children and adolescents has increased remarkably up to 24% in 2013, of which approximately 6% are obese, in developed countries [2]. In Belgium prevalence rates of obesity in adolescents of 3.5% have been registered [4]. If this global health problem is not tackled effectively, predictive models suggest an annual increase of 1.3 million overweight and obese children in the European Union [3]. Although the exact causes and origin have not yet been identified, obesity is generally accepted to be a complex multifactorial disease arising from a combination of sedentary lifestyle, dietary changes, metabolism, culture, socioeconomic status and genetic predisposition [5].

Obesity is characterized by excessive lipid accumulation in adipose tissue and ectopic fat storage in other organs. In addition, fat storage in adipose tissue contributes to anatomical and functional abnormalities of adipocytes and adipose tissue, resulting in a pathophysiological process termed adiposopathy [6]. This process is exposed by systemic inflammation, oxidative stress, altered adipokine levels, endocrine abnormalities and insulin resistance [6]. As a result, obesity has been described as a phenotype of numerous significant adverse effects, including hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia, metabolic syndrome, nonalcoholic fatty liver disease, and early development of atherosclerosis [7, 8].

Although it was thought that these adverse effects only occur in adulthood, it has become clear that obesity related pathophysiological processes are now being seen in obese adolescents. In addition, obesity in adolescents increases the risk for cardiovascular comorbidities and premature death, independent of obesity during adulthood [8, 9]. Therefore, early detection of cardiovascular abnormalities is essential since control of these abnormalities is more effective in early stages of disease.

1.1. Adipose tissue and organ crosstalk

White adipose tissue is a complex, essential, and highly metabolic organ, whose primary function relates to the storage of triglycerides (TG) during energy consumption [10]. In addition to adipocytes as main cellular component, this heterogeneous tissue contains several cell types, including pre-adipocytes, mesenchymal stem cells (MSC), fibroblasts, endothelial cells, vascular smooth muscle cells, mast cells, granulocytes, lymphocytes, macrophages and extracellular

matrix, collectively termed the adipose-derived stromal vascular fraction (AD-SVF) [11]. Although adipose tissue has long been considered as a nonfunctional passive depot for lipid storage, research has revealed that adipose tissue has to be regarded as an active endocrine organ that secretes a unique profile of adipokines, a wide range of regulatory mediators that exhibit hormone characteristics [10, 12, 13]. Adipokines comprise immunomodulatory factors such as cytokines and chemokines, vasoactive and coagulation factors, regulators of glucose, energy and lipoprotein metabolism, leptin and adiponectin [14]. As a result, adipose tissue plays a pivotal role in interorgan crosstalk achieved through endocrine, paracrine and autocrine signaling [13]. This allows white adipose tissue to regulate both adipocyte metabolism and distant organs and tissues, including skeletal muscle, the brain, the liver, the pancreas and the cardiovascular system [13].

White adipose tissue contains adipocytes, consisting of small lipid droplets, with normal metabolic functions in lean individuals. In addition, resident macrophages in the AD-SVF display an anti-inflammatory alternatively activated M2 phenotype, which secrete anti-inflammatory cytokines such as interleukin (IL) 10 [15]. However, impaired lipid turnover in adipocytes is an essential process in the development of obesity and its complications due to both increased lipid storage and decreased lipid removal [15]. As a result, a reduced net mobilization of fatty acids leads to adipocyte hypertrophy and adipose tissue dysfunction, reflected by an altered adipokine secretion profile [12]. Furthermore, increased macrophage infiltration and a switch to classically activated M1 macrophages, producing pro-inflammatory cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α), contribute to low-grade chronic inflammation [12, 15]. Next to macrophage recruitment and the phenotypic switch from M2 to M1 macrophages, through cytokine and chemokine secretion, other hypothetical processes have been demonstrated to initiate an inflammatory response in adipose tissue [15]. First, adipocyte hypertrophy leads to local hypoxia and adipocyte apoptosis due to insufficient vasculature and oxygen supply [16]. In addition, as a result of inadequate nutrient supply, necrotic adipocytes are surrounded by aggregated macrophages to constitute crown-like structures and subsequent release of their cellular content [15]. Next to adipose tissue dysfunction, the increasing adipose tissue mass also leads to excessive free fatty acid release, which in turn results in ectopic storage of lipids within liver, skeletal muscle, and the pancreatic insulin-secreting beta cells [13] (Figure 1).

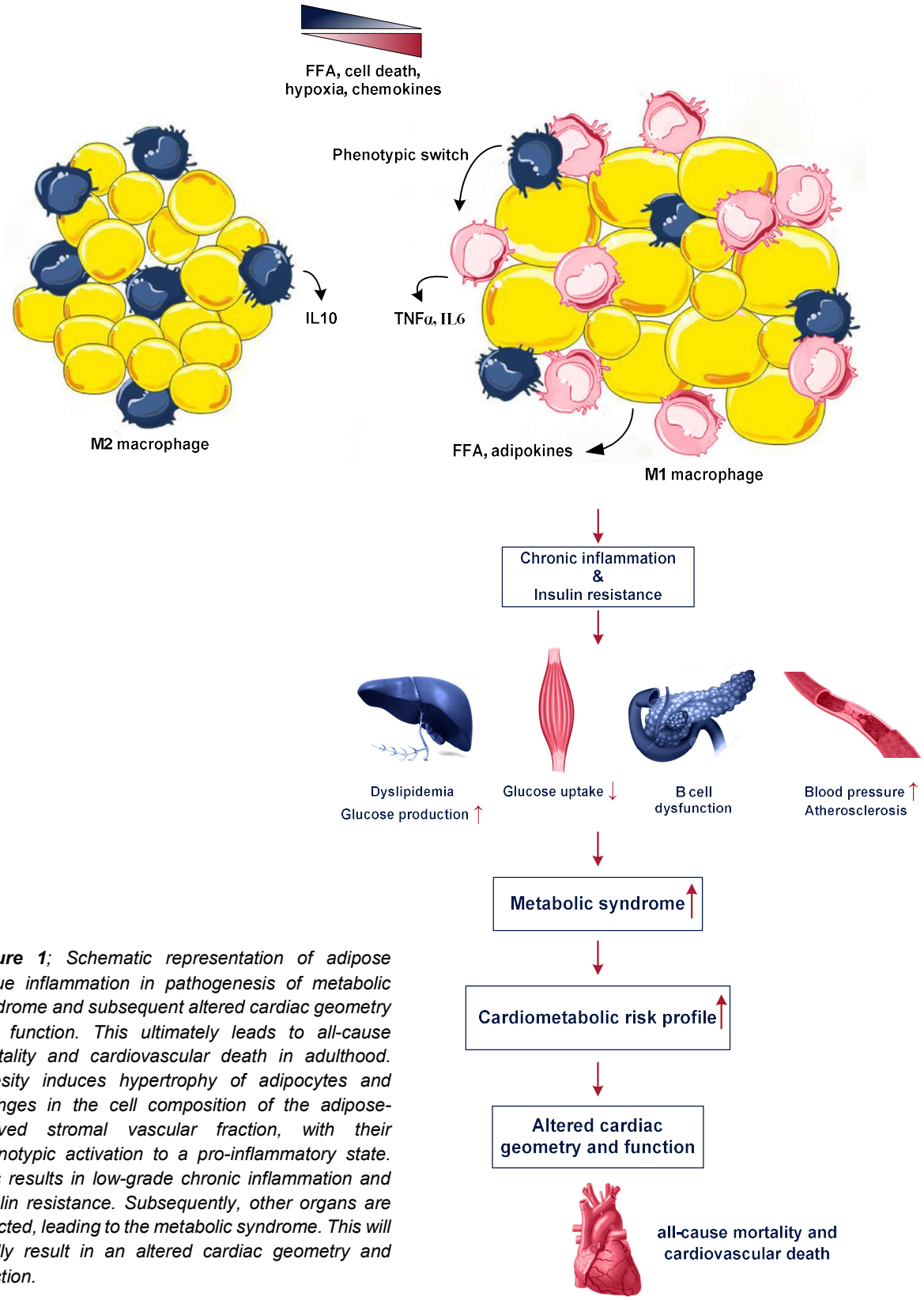

***Figure 1**; Schematic representation of adipose tissue inflammation in pathogenesis of metabolic syndrome and subsequent altered cardiac geometry and function. This ultimately leads to all-cause mortality and cardiovascular death in adulthood. Obesity induces hypertrophy of adipocytes and changes in the cell composition of the adipose-derived stromal vascular fraction, with their phenotypic activation to a pro-inflammatory state. This results in low-grade chronic inflammation and insulin resistance. Subsequently, other organs are affected, leading to the metabolic syndrome. This will finally result in an altered cardiac geometry and function.*

1.2. Obesity and the metabolic syndrome

Adolescent obesity can adversely affect nearly every organ system, due to ectopic lipid accumulation and a proinflammatory state, resulting in serious consequences [12, 15]. A number of these consequences are a cluster of cardiovascular risk factors, including insulin resistance, abdominal obesity, hypertension and atherogenic dyslipidemia, collectively referred to the metabolic syndrome (MetS) [5, 17]. Insulin resistance has been proposed as a major underlying mechanism responsible for the MetS [18]. Ectopic lipid accumulation in skeletal muscles and the liver contributes to local insulin resistance, resulting in metabolic complications [18]. In addition, due to insulin resistance pancreatic beta cells will increase insulin secretion, leading to a state of hyperinsulinemia in order to maintain euglycemia. However, at a certain moment, beta cells are not able to maintain this state and impaired fasting glucose appears. Petersen *et al*. demonstrated that de novo lipogenesis and hepatic triglyceride synthesis were both increased in the insulin resistant subjects and is possibly *attributed* to hyperinsulinemia [19]. This mechanism promotes increased expression levels of the hepatic sterol regulatory element binding protein-1c [20, 21]. In this way, it has been shown that energy distribution is markedly different between insulin sensitive and insulin resistant subjects. Insulin resistance in skeletal muscle promotes dyslipidemia by stimulating the conversion of energy intake into hepatic de novo lipogenesis [19]. This conversion leads to higher plasma very low-density lipoprotein cholesterol (VLDL-C) levels, whereby VLDL-C triglycerides can be exchanged for high-density lipoprotein cholesterol (HDL-C) cholesterol. Triglyceride-rich HDL-C particles then undergo hydrolysis of its triglyceride, resulting in apolipoprotein A-I protein dissociation, clearance and reduced HDL-C concentrations [21]. Furthermore, insulin resistance and/or hyperinsulinemia raises blood pressure (BP) by a number of mechanisms, including the antinatriuretic effect of insulin, impaired endothelium dependent vasodilatation and increased sympathetic activity of the nervous system [22]. In addition, obesity is also characterized by decreased circulating adiponectin levels, and accumulating evidence indicates that adiponectin possesses antihypertensive effects through an endothelial-dependent mechanism [23]. Apart from the degree of obesity, it is suggested that body fat distribution contributes to a cardiometabolic risk profile. Multiple studies have been shown that visceral adipose tissue in particular is critical for the development of cardiometabolic abnormalities, since visceral adipose tissue displays different phenotypic, physiological and functional characteristics compared to subcutaneous adipose tissue [24]. Although it is known that the MetS is highly prevalent in obese adults, it is far more common among adolescents than previously reported [25].

1.3. Obesity and cardiovascular health

The MetS has a significant effect on cardiac health in obese adolescents and epidemiological studies have shown that the MetS in adolescents is a predictor of cardiovascular disease (CVD) in adulthood [5, 26, 27]. It is well known that CVD risk factors have effects on cardiovascular function and structure, including intima-media thickness (IMT), left ventricular mass and endothelial dysfunction [26, 28]. It has been shown that early stages of the atherogenic process are detectable in obese adolescents [26]. Furthermore, the Bogalusa Heart Study has demonstrated that adolescent obesity causes an increase in the extent of lesions in the coronary arteries, which may reflect the influence of clustering of risk factors in the MetS [29]. Several studies have shown that IMT of peripheral arterial vessels, a surrogate marker of atherosclerosis, is increased at an early stage among obese adolescents, whereby risk factor related increase in IMT is associated with CV events in adults [30, 31]. Left ventricular hypertrophy, established as a strong independent predictor of heart failure and sudden death in adults, has also been related to obesity in adolescents [32]. It has been shown that left ventricular mass (LVM) was independently associated with fat mass and lean body mass [32].

Next to these structural changes, obese adolescents also demonstrate diminished myocardial function [28, 33]. Previous studies have indicated a reduced systolic and diastolic function, which was related to excess adiposity [28]. A significantly lower left ventricular ejection fraction was found in obese adolescents compared to their lean counterparts. In addition, reduced myocardial contractility and relaxation was strongly associated with total body weight [33].

1.4. Obesity and cardiopulmonary exercise testing (CPET)

In adolescents with obesity cardiopulmonary exercise testing (CPET) has become an important clinical examination providing valuable information with regard to the integrative exercise responses, including the pulmonary, cardiovascular and muscular systems [34-37]. The cardiovascular and pulmonary system respond to a progressive-intensity exercise stimulus by enhancing oxygen delivery to the exercising muscles to meet the aerobic energy demands. In addition, metabolic byproducts such as carbon dioxide, produced due to increased metabolism and lactic acid buffering, should be removed. The ability to perform physical exercise depends on the capacity of the cardiovascular and pulmonary system to supply oxygen to the working muscles and to remove carbon dioxide from the bloodstream. Exercise capacity during CPET is assessed using measurements of respiratory oxygen uptake (VO_2), carbon dioxide production (VCO_2) and ventilatory parameters [38]. In addition, electrocardiographic (ECG) monitoring is used to determine specific abnormal electrophysiological morphologies and cardiac responses during

CPET [38]. Interestingly, if pathologies are present leading to exercise intolerance, CPET identifies which physiologic system is responsible and, therefore, CPET provides valuable diagnostic and prognostic information.

During CPET, mechanical constraints in ventilation, an elevated risk for hypoxia and chronotropic incompetence or compromised cardiac function (e.g. lowered heart rate (HR) recovery, chronotropic index and stroke volume) are often observed in obese adults [39, 40]. In addition, several studies regarding exercise capacity and cardiopulmonary responses to maximal endurance exercise testing have been performed in obese adolescents [37]. Despite these previous investigations in obese adolescents it remains controversial whether cardiopulmonary disturbances can be observed consistently during CPET. However, a number of studies have reported a suboptimal response to exercise, in particular a reduced peak heart rate (HR_{peak}) and peak cycling power output (W_{peak}) [37, 41, 42]. Adult obesity modifies cardiac behavior, including resting HR and chronotropic incompetence, which has a marked effect on exercise capacity [40]. Therefore, chronotropic variables are the most important factors that affect exercise performance. It has been shown that both peak and resting HR account for over forty percent of variability of exercise capacity [40]. Interestingly, resting HR and HR response to exercise, including a blunted HR increase, low chronotropic index and HR recovery, are important predictors of all-cause mortality and cardiovascular death, at least in adults [43, 44]. These changes in HR during and recovery from CPET are mediated by the balance between sympathetic and vagal activity of the autonomic nervous system [45]. Adverse cardiovascular outcomes associated with the MetS may be mediated by autonomic dysfunction, whereby obesity is characterized by sympathetic predominance and a decrease in vagal activity in the basal state, where reduced sympathetic responsiveness has been observed during exercise [40, 44]. Therefore, these multiple exercise risk markers could provide valuable clinical information regarding cardiometabolic health. Nonetheless HR behavior during CPET has not been described in obese adolescents.

1.5. Aim and hypothesis

Although several studies have been shown the established association between cardiometabolic disease risk and disturbances during CPET in adulthood, data are lacking with regard to the cardiac chronotropic responses in relation to cardiometabolic health and cardiovascular risk in obese adolescents. Therefore, the primary aim of the present study is to investigate whether cardiac chronotropic responses during CPET may be a sensitive and specific independent predictor for various cardiovascular abnormalities, such as altered blood catecholamine and/or potassium concentrations during maximal exercise testing, structural myocardial abnormalities,

diastolic dysfunction or atherosclerosis, which have all been detected in obese adolescents. This includes the analysis of anthropometric parameters, exercise capacity, blood parameters, glucose tolerance, cardiac function, carotid intima thickness, cardiac electrophysiology, cardiac autonomic function, and changes in blood parameters during exercise in obese and lean adolescents.

We hypothesize that worse cardiac chronotropic responses are independently related to cardiovascular comorbidities and, therefore, can be used as an indicator for these cardiovascular comorbidities in obese adolescents during CPET.

2. MATERIAL AND METHODS

2.1. Subjects

Obese adolescents were recruited from the paediatric clinic of Jessa hospital (Hasselt, Belgium) and lean adolescents were recruited by means of publication of advertisements in Jessa hospital and Hasselt University. Participants were between 11 and 17 years of age and free from any chronic cardiovascular, renal, pulmonary or orthopaedic disease, and free form medication which modifies the heart rate. The International Obesity Task Force (IOTF) criteria were used to categorize the participants into a lean and obese group [46]. Twenty-four obese adolescents and twenty-four 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 Jessa hospital and Hasselt University (Belgium) and was executed according to the Declaration of Helsinki (revised version, October 2013, Fortaleza, Brazil).

2.2. Study design

The study was carried out according to an observational case-control, cross-sectional, design and executed in Jessa hospital. From midnight prior to a one-day hospitalization, all subjects refrained from consuming food, with the exception of water at libitum, to prevent short term effects on outcome parameters. First, anthropometric measurements, Tanner stage, venipuncture and an oral glucose tolerance test were accomplished in a fasted state (Figure 2). After a standardized meal, containing 296 kcal, composed of 3 g of fat (5%), 56 g of carbohydrate (82%) and 9 g of protein (13%), echocardiography and a CPET were performed.

2.3. Measurements

2.3.1. 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. Body mass index (BMI) was calculated directly from weight and height measurements (weight/height²). BMI classes were defined according to age- and gender-specific IOTF centile curves passing through BMI 30 kg/m^2 as ‘class I obesity’, through BMI 35 kg/m^2 as ‘class II obesity’, and through BMI 40 kg/m^2 as ‘class III obesity’ [46, 47].

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 (WHR) was calculated by dividing waist circumference (cm) by hip circumference (cm). Body composition was evaluated using skinfold measurements and bio-electrical impedance (BIA) analysis. 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 centre 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*. (1988) [48, 49]. Tetrapolar BIA measurement was performed using the Bodystat 1500 MDD® monitoring unit (EuroMedix, Leuven, Belgium) with subjects in supine position [50]. Electrodes were placed on the dorsal surface of the wrist and hand, just behind the middle finger, and the right ankle and food, just behind the middle toe, according to manufacturer's instructions. The percentage of body fat, lean tissue mass and body water content was calculated using the formula of Houtkooper [51].

2.3.2. Blood pressure, pubertal development stage and physical activity evaluation

Blood pressure 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 paediatrician and the adolescents' own opinion based on a figure [52, 53]. The level of physical activity is determined using the validated Dutch physical activity questionnaire for adolescents (PAQ-A) [54]. A PAQ score of 1 indicates low physical activity, whereas a score of 5 indicates high physical activity.

2.3.3. Biochemical analysis

After catheter placement, venous blood samples were taken for the measurement of biochemical, hormonal and haematological 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), and c-reactive protein (CRP) concentration, blood ferritin, thyroid-stimulating hormone (TSH), free thyroxine (T4), cortisol and serum insulin concentration were automatically assessed on Roche Cobas 8000 (Roche Diagnostics International Ltd, Rotkreuz, Switerzerland). Blood glycated haemoglobin concentration (HbA1c) was measured using ion exchange chromatography (Menarini HA-8180 HbA1c auto-analyser, Menarini Diagnostics, Belgium). Blood 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). 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 [55].

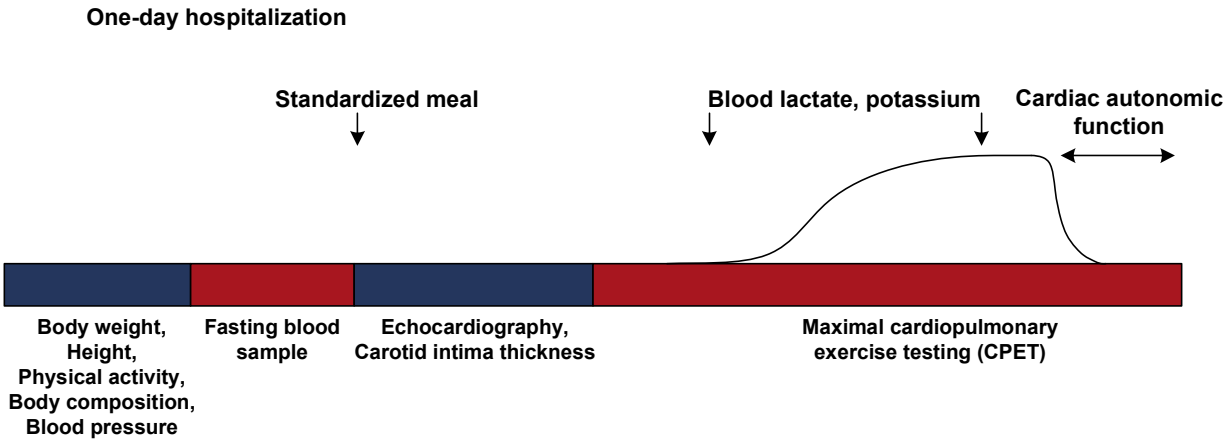

***Figure 2**; Schematic representation of different measurements at various time points during a one-day hospitalization. All subjects underwent several measurements in exact chronology. First, body weight, height, blood pressure, body composition and physical activity were determined, followed by blood sample collection, echocardiography and carotid intima thickness measurements. Blood lactate, epinephrine and potassium concentrations will be measured at rest and during peak exercise. During recovery period of cardiopulmonary exercise testing cardiac autonomic function was assessed.*

2.3.4. Metabolic risk profile

The metabolic risk score, a continuous score reflecting a composite metabolic risk factor profile, was determined with the aid of the following variables: waist circumference, MAP, triglycerides/HDL-C ratio and fasting insulin, as described by Martinez-Vizcaino *et al*. [56]. The standardized value of each variable was calculated as follows: *z*-score = (value – mean) / standard deviation (SD). The *z*-scores of the individual risk factors were summed to create the metabolic risk score where a lower metabolic risk score indicated a better overall CVD risk factor profile.

2.3.5. Cardiopulmonary exercise testing (CPET)

CPET was performed up to volitional exhaustion using an electronically braked cycle ergometer (eBike, GE Medical systems, Milwaukee, USA), controlled by the Cardiosoft electrocardiography software (Cardiosoft 6.6, GE Medical systems, Freiburg, Germany). Prior to every exercise test, gas and volume calibration was executed according to manufacturer's instructions. During the test, environmental temperature was kept stable at 19-21°C. The exercise test protocol included a one-minute pre-exercise resting period, a one-minute warm-up cycling with no additional resistance applied to the pedals (unloaded exercise), an incremental exercise cycling with an initial workload of 40 W and an increasing workload of 20 W per minute until exhaustion or other symptoms limited the test. 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 patient failed to maintain a pedal frequency of at least 60 rpm. All subjects were verbally encouraged during exercise testing to achieve a maximal exercise test, 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 (fatigue, dyspnea, leg muscle pain) [57]. The adult criterion of VO_2 reaching a plateau with a further increase in work is often not observed in children [58], and due to the potential presence of chronotropic incompetence in obese adolescents [37], HR_{peak} was not used as a criterion for maximal exercise effort. 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.

With the aid of continuous pulmonary gas exchange analysis (Jaeger MasterScreen CPX Metabolic Cart, CareFusion Germany GmbH, Hoechberg, Germany) oxygen uptake (VO_2), carbon dioxide output (VCO_2) expiratory volume (VE), equivalents for oxygen uptake (VE/VO_2) and carbon dioxide production (VE/VCO_2), tidal volume (Vt), tidal volume/dead space volume (Vd/Vt) ratio, breathing frequency (BF), respiratory gas exchange ratio (RER), end-tidal oxygen ($PETO_2$) and carbon dioxide ($PETCO_2$) pressure were collected breath-by-breath and averaged every ten

seconds. Using a 12-lead ECG device (KISS™ Multilead, GE Medical systems, Freiburg, Germany) heart rate (HR) was monitored and averaged every ten seconds. From this parameter oxygen pulse (VO_2/HR) was calculated. The oxygen uptake efficiency slope (OUES) was calculated using all exercise data by a linear least square regression of VO_2 on the logarithmic of VE [59]. First ventilatory threshold (VT1) was determined using the V-slope method and was expressed in ml/min and in percentage of peak VO_2 [60]. Second ventilatory threshold (VT2) was determined, using the VE vs. VCO_2 plot, on the point where VE increases out of proportion to VCO_2 and expressed in ml/min and in percentage of peak VO_2 [61].

From continuous 12-lead ECG monitoring, following parameters were calculated: PR-interval, P-wave duration, QRS-width, QT-interval, QTc-interval, which was calculated as QTc = QT (ms) / √RR (sec), Sokolov-index, QT_{peak} interval), Sokolov-index, QT_{peak} interval. In addition, maximal cycling power output (W_{max}) was reported. A selection of these parameters reflects ventilatory function (VE, VE/VO_2, VE/VCO_2, Vt, Vd/Vt, $PETO_2$, $PETCO_2$, RR, SaO_2%) or cardiac function (HR, VO_2/HR, PR-interval, P-wave duration, QRS-interval, QT-interval, QTc-interval. Age predicted maximal heart rate was determined using the formula 208 – (0.7 x age) [62]. Prior to and at the end of the test the Borg rating scale, a visual analogue measure to assess perceived exertion, was determined and the reason to end the test was recorded [63]. In addition, blood lactate and potassium concentration were automatically assessed on Roche Cobas 8000 (Roche Diagnostics International Ltd, Rotkreuz, Switzerzerland) at rest and during peak exercise. Autonomic function, reflected by heart rate recovery (HRR), was defined as the reduction in heart rate from peak exercise level (HR_{peak}) to the rate 0.5, one and two minutes after cessation of exercise testing and designated as HRR0.5, HRR1 and HRR2 [64].

2.3.6. Echocardiography

All subjects underwent a standardized transthoracic echocardiographic (TTE) examination using a commercial ultrasound system (Vivid 7, GE Health Medical, Milwaukee, Wisconsin) and a phased array matrix transducer (GE M4S, 1.5 - 3.6 MHz, Vivid 7 ultrasound system, GE Health Medical, Milwaukee, Wisconsin). Two-dimensional (2-DE) and motion mode (M-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 before [65]. Diastolic dysfunction was assessed using transmittal inflow patterns, left ventricular ejection fraction, mitral annulus velocity, left atrium 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 four chamber views at septal and lateral mitral annulus and the E/e' ratio was assessed. Left ventricular (LV) septal wall thickness, LV diameter and left atrium diameter were measured parasternal long axis views. All TTE and Doppler assessments and analyses were performed by the same cardiologist and stored digitally until analysis using EchoPAC software (GE Health Medical, Milwaukee, Wisconsin).

2.3.7. Carotid intima thickness (CIMT)

2-DE bilateral examination of the common carotid arteries were obtained using a 3 - 8 MHz linear-array transducer (GE 9LD, Vivid 7 ultrasound system, GE Health Medical, Milwaukee, Wisconsin). Imaging was performed with subjects in supine position, and the head was externally rotated 45° and retroflexed by approximately 20°. Carotid intima thickness (CIMT) was assessed from both the right and left common carotid arteries just proximal to the carotid bulb [66].

2.3. Statistical analysis

Statistical analysis was performed by IBM SPSS® version 24.0 (IBM SPSS Statistics for Windows, Chicago, IL, USA). Data were expressed as means ± 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 an independent samples *t* test for normally distributed data and Mann-Whitney U test for abnormally distributed data, with Holm–Bonferroni corrections for multiple comparisons. Automatic forward stepwise multivariate regression analysis (with information criterion) was used to examine relations between aberrant CPET parameters (significantly different between lean and obese adolescents) and subject characteristics, blood parameters, ECG variables and echocardiographic variables at rest and at peak effort. Next, multivariate linear regression analysis was applied to examine relations between aberrant cardiac chronotropic responses and subject characteristics, CPET parameters, blood parameters, ECG variables and echocardiographic variables with significant predictive power for aberrant exercise responses out of the automatic forward stepwise multivariate regression analysis ($p < 0.05$). Regression analyses were made for three different categories: cardiometabolic health, reflected by subject characteristics and blood parameters, cardiovascular risk factors, reflected by CPET parameters and echocardiographic parameters, and electrophysiological morphologies. In this regression analyses, variables with an abnormal distribution were log transformed. All independent variables were corrected for age,

gender, Tanner stage and body height. Variables with a beta coefficient lower than 0.1 were left out of consideration. Pearson univariate correlations were performed to examine associations between normal distributed independent aberrant CPET parameters and variables out of automatic stepwise multivariate regressions analysis. Spearman univariate correlations were used for abnormally distributed data. *P*-values < 0.05 (2-tailed) was considered statistically significant.

3. RESULTS

3.1. Subject characteristics

A total of 48 participants (24 obese and 24 lean adolescents) were eligible and completed the study. The proportion of male and female subjects was comparable ($p = 0.77$) between obese (male: n = 13, female: n = 11) and lean (male: n = 14, female: n = 10) adolescents (Table 1). No statistical differences were found in age (13.4 ± 1.1 vs. 13.9 ± 1.5 years; $p = 0.32$), body height (166.4 ± 8.7 vs. 167.3 ± 9.1 cm, respectively; $p = 0.51$) and Tanner stage ($p = 0.52$) between obese and lean adolescents. As expected, body weight (89.3 ± 16.3 vs. 55.3 ± 11.4 kg; $p < 0.001$), BMI (32.1 ± 4.3 vs. 19.6 ± 2.5 kg/m^2; $p < 0.001$), waist circumference (104.4 ± 13.4 vs 67.8 ± 6.5 cm; $p < 0.001$), hip circumference (105.0 ± 8.6 vs. 78.9 ± 8.9 cm; $p < 0.001$) and waist- to-hip ratio (0.99 ± 0.07 vs. 0.87 ± 0.11, respectively; $p < 0.001$) were elevated in the obese group, compared to their lean counterparts. With regard to body composition, body fat (36.7 ± 6.9 vs. 14.0 ± 7.9 %; $p < 0.001$), lean body mass (63.3 ± 6.9 vs. 86.0 ± 7.9 %; $p < 0.001$) and body water content (48.9 ± 5.7 vs. 64.0 ± 6.9 %; $p < 0.001$), measured using BIA, were different between groups. Percentage of body fat (48.9 ± 11.1 vs. 17.4 ± 5.9 %; $p < 0.01$), measured using skinfold measurements, and the sum of skinfolds (131.5 ± 32.2 vs. 36.6 ± 13.8 mm, respectively; $p < 0.01$) were higher in obese subjects compared to lean adolescents. In obese subjects, systolic BP (124.9 ± 12.1 vs. 113.7 ± 10.3 mmHg; $p = 0.001$), diastolic BP (77.2 ± 9.8 vs. 69.1 ± 7.5 mmHg; $p = 0.003$) and MAP (93.1 ± 8.5 vs. 83.9 ± 7.7 mmHg, respectively; $p < 0.001$) were all elevated, compared to lean subjects. Physical activity level was higher ($p = 0.013$) in lean adolescents (2.56 ± 0.52) compared to obese adolescents (2.14 ± 0.58).

3.2. Blood parameters

Blood haemoglobin concentration (13.4 ± 0.9 vs. 14.1 ± 1.1 g/dl; $p = 0.015$) and haematocrit (38.7 ± 2.3 vs. 39.4 ± 7.9 %; $p = 0.013$) were higher in obese adolescents, compared to lean adolescents. With regard to cardiovascular risk factors, plasma CRP (4.8 ± 7.5 vs. 0.6 ± 2.0 mg/dl; $p < 0.001$), LDL-C (93.5 ± 26.5 vs. 76.0 ± 22.2 mg/dl; $p = 0.017$) and triglyceride (105.0 ± 63.7 vs. 68.5 ± 32.1 mg/dl; $p = 0.031$) concentrations were increased, whereas plasma HDL-C (46.2 ± 11.4 vs. 62.3 ± 11.7 mg/dl) was lower in obese adolescents (Table 2). Plasma total cholesterol concentration did not vary significantly ($p = 0.442$) between groups. In comparison with lean adolescents, obese adolescents showed an increased fasting insulin concentration (25.4 ± 16.9 vs. 10.5 ± 5.5 µU/l; $p < 0.001$) and HOMA-IR (5.6 ± 3.9 vs. 2.2 ± 1.3; $p < 0.01$). In obese adolescents, blood ALT (29.8 ± 16.8 vs. 16.6 ± 7.3 U/l; $p < 0.01$) and GGT (19.4 ± 8.1 vs. 13.2 ±

4.2 U/l; p = 0.003) concentrations were higher, in contrast to lean adolescents. Plasma leptin concentration was increased (p < 0.001) in obese subjects (48.5 ± 23.0 vs. 8.1 ± 6.2 µg/ l; p < 0.001), compared to their lean counterparts.

***Table 1**; Subject characteristics of obese and lean individuals.*

General features	Obese subjects (n=24)	Lean subjects (n=24)	P-value
Age (years)	13.4 ± 1.1	13.9 ± 1.5	0.32
Sex			0.77
Male (n)	13	14	
Female (n)	11	10	
Body weight (kg)	89.3 ± 16.3	55.3 ± 11.4	**<0.001**
Body height (cm)	166.4 ± 8.7	167.3 ± 9.1	0.51
Body mass index (kg/m²)	32.1 ± 4.3	19.6 ± 2.5	**<0.001**
Waist (cm)	104.4 ± 13.4	67.8 ± 6.5	**<0.001**
Hip (cm)	105.0 ± 8.6	78.9 ± 8.9	**<0.001**
Waist-to-hip ratio	0.99 ± 0.07	0.87 ± 0.11	**<0.001**
Body fat (%)	36.7 ± 6.9	14.0 ± 7.9	**<0.001**
Lean body mass (%)	63.3 ± 6.9	86.0 ± 7.9	**<0.001**
Body water (%)	48.2 ± 5.7	64.0 ± 6.9	**<0.001**
Sum skinfold (mm)	131.5 ± 32.2	36.6 ± 13.8	**<0.001**
Body fat (%)	48.9 ± 11.1	17.4 ± 5.9	**<0.001**
PAQ-A score	2.14 ± 0.58	2.56 ± 0.52	**0.013**
Systolic BP (mmHg)	124.9 ± 12.1	113.7 ± 10.3	**0.001**
Diastolic BP (mmHg)	77.2 ± 9.8	69.1 ± 7.5	**0.003**
Mean arterial pressure (mmHg)	93.1 ± 8.5	83.9 ± 7.7	**<0.001**
Development stage			
Tanner stage 1 (n)	2	2	
Tanner stage 2 (n)	0	1	
Tanner stage 3 (n)	6	4	
Tanner stage 4 (n)	2	6	
Tanner stage 5 (n)	11	11	0.52

Data are expressed as means ± SD. Abbreviations: PAQ-A: Dutch physical activity questionnaire for adolescents,
BP: Blood pressure. Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.
P-values <0.05 (2-tailed) was considered statistically significant.

***Table 2**; Biochemical, haematological and hormonal parameters in obese and lean adolescents.*

	Obese (n = 24)	Lean (n = 24)	p-value
Haematology			
Haemoglobin (g/dl)	13.4 ± 0.9	14.1 ± 1.1	**0.015**
Haematocrit (%)	38.7 ± 2.3	39.4 ± 7.9	**0.013**
Leukocyte count (x10^9/l)	7.0 ± 1.4	8.7 ± 8.4	0.148
Chemistry			
Iron (µg/dl)	81.8 ± 34.8	108.8 ± 39.4	0.017
Ferritin (µg/l)	58.5 ± 31.8	48.6 ± 27.4	0.257
Calcium (mmol/l)	2.31 ± 0.35	2.42 ± 0.07	0.200
Uric acid (mg/dl)	5.6 ± 0.9	5.2 ± 1.1	0.145
Cardiovascular risk factors			
C-reactive protein (mg/l)	4.8 ± 7.5	0.6 ± 2.0	**<0.001**
Total cholesterol (mg/dl)	158.4 ± 33.5	152.0 ± 22.2	0.442
Low-density lipoprotein cholesterol	93.5 ± 26.5	76.0 ± 22.2	**0.017**
High-density lipoprotein cholesterol	46.2 ± 11.4	62.3 ± 11.7	**<0.001**
Triglycerides (mg/dl)	105.0 ± 63.7	68.5 ± 32.1	**0.031**
Glycemic control			
Fasting glucose (mg/dl)	88.9 ± 6.1	86.1 ± 6.6	0.135
Fasting insulin (µU/l)	25.4 ± 16.9	10.5 ± 5.5	**<0.001**
Glycated haemoglobin (%)	5.3 ± 0.3	5.2 ± 0.2	0.137
HOMA-IR	5.6 ± 3.9	2.2 ± 1.3	**<0.001**
Liver function			
Aspartate aminotransferase (U/l)	23.7 ± 7.3	24.4 ± 8.7	0.898
Alanine aminotransferase (U/l)	29.8 ± 16.8	16.6 ± 7.3	**<0.001**
Gamma-glutamyl transpeptidase (U/l)	19.4 ± 8.1	13.2 ± 4.2	**0.003**
Alkaline phosphatase (U/l)	188.0 ± 91.6	216.5 ± 121.5	0.558
Endocrinology			
Thyroid stimulating hormone (mU/l)	2.7 ± 1.0	2.2 ± 0.8	0.069
Free thyroxine (pmol/l)	14.1 ± 3.1	14.2 ± 1.8	0.558
Cortisol (µg/dl)	9.8 ± 6.2	7.8 ± 3.7	0.344
Leptin (µg/l)	48.5 ± 23.0	8.1 ± 6.2	**<0.001**

Data are expressed as means ± SD. Abbreviations: HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.
Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.
P-values <0.05 (2-tailed) was considered statistically significant

3.3. Metabolic risk profile

In comparison with lean adolescents, obese adolescents had a higher (0.63 ± 013 vs. -0.47 ± 0.28, respectively; $p < 0.001$) metabolic risk profile (Figure 3).

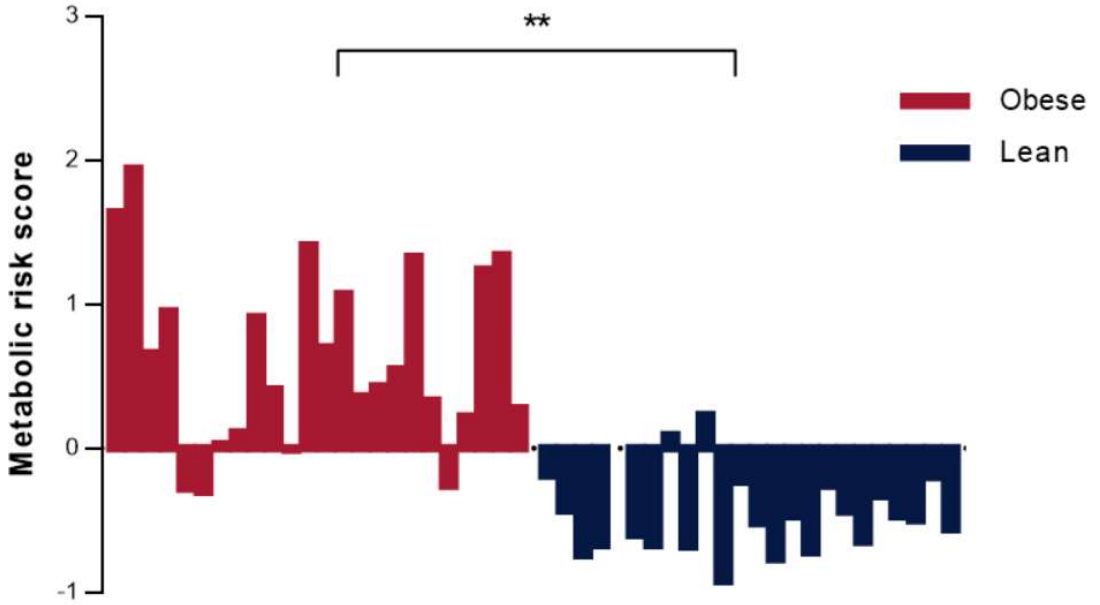

***Figure 3**; Metabolic risk score, reflected by a cluster of metabolic risk factors including,* waist circumference, MAP, triglycerides/HDL-C ratio and fasting insulin, of obese (n=24) and lean (n=24) adolescents. Comparisons between groups were performed using the independent-samples t-test. ** $p < 0.01$ lean vs. obese adolescents.

3.4. Exercise tolerance and cardiopulmonary function

A reduced W_{peak} (158.8 ± 36.2 vs. 190.3 ± 44.5 W; $p = 0.011$) and gross exercise efficiency (0.208 ± 0.019 vs. 0.223 ± 0.023 %; $p = 0.002$) was found in obese adolescents, compared to lean adolescents. VT1 as percentage of VO_{2peak} was different between obese (52.9 ± 6.1 %) and lean adolescents (47.7 ± 6.8 %; $p = 0.006$). At rest, a significant difference in blood potassium (3.80 ± 0.21 vs. 3.97 ± 0.33 mmol/l; $p = 0.045$, respectively) and lactate (1.3 ± 0.7 vs. 1.7 ± 0.7 mmol/l; $p = 0.023$, respectively) concentration was found between obese and lean adolescents (Table 3). No statistically significant differences were found between other CPET parameters ($p > 0.05$).

***Table 2**; Exercise capacity of obese and lean adolescents.*

Peak parameters	Obese (n = 24)	Lean (n = 24)	p-value
Oxygen uptake (ml/min)	2059.1 ± 454.7	2251.4 ± 553.5	0.208
Carbon dioxide output (ml/min)	2525.0 ± 595.0	2782.0 ± 714.1	0.138
Minute ventilation (l/min)	75.6 ± 19.8	84.5 ± 22.3	0.149
Oxygen uptake efficiency slope (ml/Logl)	2195.8 ± 501.2	2229.3 ± 501.5	0.818
ventilatory equivalent O_2	0.037 ± 0.005	0.038 ± 0.006	0.592
ventilatory equivalent CO_2	0.030 ± 0.003	0.031 ± 0.004	0.406
Tidal volume (l)	1.87 ± 0.49	1.90 ± 0.48	0.829
Breathing frequency (breaths/min)	41 ± 8	45 ± 9	0.109
Respiratory exchange ratio	1.23 ± 0.07	1.23 ± 0.07	0.690
End-tidal tensions of oxygen (mmHg)	114.1 ± 18.4	119.3 ± 5.1	0.167
End-tidal tensions of carbon dioxide (mmHg)	40.9 ± 18.6	35.8 ± 4.3	0.073
Oxygen pulse (ml O_2 / heart beat)	11.1 ± 2.2	12.1 ± 2.9	0.161
Borg dyspnea	15 ± 2	16 ± 2	0.553
Borg legs	16 ± 3	17 ± 2	0.636
Cycling power output (W)	158.8 ± 36.2	190.3 ± 44.5	**0.011**
Ventilatory threshold 1 (ml/min)	1072.5 ± 185.2	1072.5 ± 296.2	1.000
Ventilatory threshold 2 (ml/min)	1565.4 ± 379.4	1802.9 ± 502.0	0.117
Ventilatory threshold 1 (% peak VO_2)	52.9 ± 6.1	47.7 ± 6.8	**0.006**
Ventilatory threshold 2 (% peak VO_2)	76.4 ± 9.9	80.0 ± 9.1	0.194
Gross efficiency (%)	0.208 ± 0.019	0.223 ± 0.023	**0.002**

Data are expressed as means ± SD. Abbreviations: W: Watt, bpm: beats per minute.

Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test. P-values <0.05 (2-tailed) was considered statistically significant.

***Table 3**; Blood potassium and lactate concentrations at rest and at peak exercise in obese and lean adolescents.*

	Obese (n = 24)	Lean (n = 24)	p-value
Rest			
Potassium (mmol/l)	3.80 ± 0.21	3.97 ± 0.33	**0.045**
Lactate (mmol/l)	1.3 ± 0.7	1.7 ± 0.7	**0.023**
Peak exercise			
Potassium (mmol/l)	4.20 ± 0.65	4.37 ± 0.52	0.432
Lactate (mmol/l)	4.9 ± 2.5	4.5 ± 1.4	0.820

Data are expressed as means ± SD.

Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.

P-values < 0.05 (2-tailed) was considered statistically significant.

3.4.1. Chronotropic response

A significant higher resting HR (79.9 ± 13.3 vs. 64.6 ± 10.4 bpm; p < 0.001) but lower HR reserve (106.4 ± 17.8 vs. 123.9 ± 21.8 bpm, respectively; p = 0.002) was found in obese adolescents in comparison with lean adolescents (Table 4). No statistically significant difference between groups was found in peak HR and maxCRI (p < 0.05). Short term HR recovery, reflected by HRR0.5 (9.9 ± 7.2 vs. 16.5 ± 10.1 bpm; p = 0.012) and HRR1 (22.5 ± 10.8 vs. 31.4 ± 14.0 bpm; p = 0.021), was reduced in obese adolescents, compared to lean adolescents (Figure 4). Compared to lean adolescents, a smaller two-minute HRR was found in obese adolescents (35.4 ± 11.8 vs. 46.1 ± 11.4 bpm; p = 0.007, respectively).

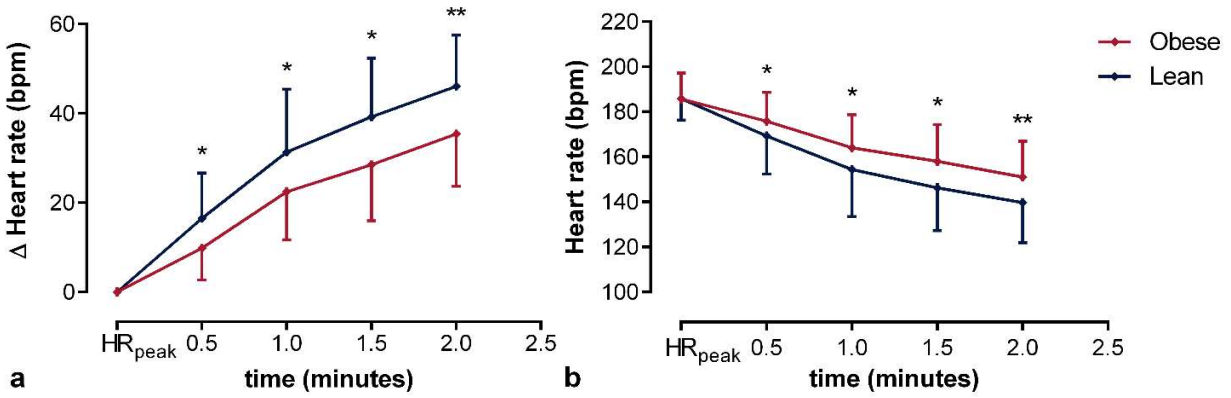

***Figure 4**; Heart rate response (a) and delta heart rate (b) 0.5, 1 and 2 minutes in recovery period of both obese and lean adolescents. Differences between groups were tested using the Mann-Whitney U test with Holm-Bonferroni corrections. * p < 0.05; ** p < 0.01 lean vs. obese. Abbreviations: HR: Heart rate.*

***Table 4**; Heart rate behavior during CPET in obese and lean adolescents.*

	Obese (n = 24)	Lean (n = 24)	p-value
Resting heart rate (bpm)	79.9 ± 13.3	64.6 ± 10.4	**< 0.001**
Peak heart rate (bpm)	185.7 ± 11.5	185.8 ± 9.60	0.968
Heart rate reserve (bpm)	106.4 ± 17.8	123.9 ± 21.8	**0.002**
MaxCRI	0.89 ± 0.09	0.90 ± 0.07	0.858

Abbreviations: MaxCRI: maximal chronotropic response index
Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.
P-values < 0.05 (2-tailed) was considered statistically significant.

3.5. Electrophysiological parameters

At rest, an increased PR interval was found in obese adolescents (136 ± 24 ms), compared to lean adolescents (149 ± 16 ms; p = 0.027). In addition, a lower QT interval was found in obese vs. lean adolescents (319 ± 53 vs. 344 ± 50 ms, respectively; P = 0.047), whereas no differences were found when corrected for heart rate (p > 0.05). In contrast, a reduced Sokolov index was demonstrated in obese adolescents (3.1 ± 1.7 vs. 3.7 ± 1.2 mV; p = 0.003). During peak exercise, Sokolov index (2.6 ± 1.0 vs. 3.7 ± 1.1 mV; p = 0.001) and PR interval (95 ± 18 vs. 105 ± 14 ms, respectively; p = 0.034) were lower in obese adolescents, compared to lean adolescents (Table 5).

***Table 5**; ECG variables at rest and during maximal exercise testing in obese and lean adolescents.*

	Obese subjects (n=24)	**Lean subjects (n=24)**	**p-value**
Rest			
PR interval (ms)	136 ± 24	149 ± 16	**0.027**
P wave duration (ms)	86 ± 14	89 ± 13	0.556
QRS width (ms)	87 ± 11	83 ± 11	0.116
QT interval (ms)	319 ± 53	344 ± 50	**0.047**
QTc interval (ms)	399 ± 60	391 ± 21	0.149
Sokolov index (mV)	3.1 ± 1.7	3.7 ± 1.2	**0.003**
Peak exercise			
PR interval (ms)	95 ± 18	105 ± 14	**0.034**
P wave duration (ms)	71 ± 14	71 ± 13	0.950
QRS width (ms)	79 ± 10	76 ± 10	0.244
QT interval (ms)	222 ± 36	223 ± 13	0.373
QTc interval (ms)	392 ± 61	391 ± 21	0.143
Sokolov index (mV)	2.6 ± 1.0	3.7 ± 1.1	**0.001**

Data are expressed as means ± SD. Abbreviations: QTc: corrected QT interval.
Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.
P-values <0.05 (2-tailed) was considered statistically significant.

3.4. Echocardiographic parameters

An increased CIMT was found in lean adolescents (0.54 ± 0.06 vs. 0.51 ± 0.06 mm; p = 0.017), compared to obese adolescents (Table 6). With regard to left ventricular morphology and function, no statistical differences were found between obese and lean adolescents, with the exception of a significant increased LV septum thickness (8.0 ± 0.82 vs. 7.1 ± 1.0 mm; p = 0.003) in obese adolescents. Left atrial diameter was increased in obese adolescents (34.1 ± 4.50 vs. 29.0 ± 3.90 mm, respectively; p < 0.001), compared to lean adolescents. No differences in diastolic function were found between obese and lean adolescents (p > 0.05).

***Table 6**; Morphological and functional echocardiographic parameters of obese and lean adolescents.*

	Obese (n = 24)	Lean (n = 24)	p-value
Carotid intima thickness (mm)	0.51 ± 0.06	0.54 ± 0.06	**0.017**
Left ventricle			
LV septum thickness (mm)	8.0 ± 0.82	7.1 ± 1.0	**0.003**
LV diameter (mm)	45.4 ± 4.00	46.2 ± 3.50	0.500
Cardiac output (l/min)	5.2 ± 0.8	4.9 ± 1.1	0.257
LV ejection fraction (%)	61.0 ± 6.10	60.7 ± 6.10	0.991
Left atrium			
LA diameter (mm)	34.1 ± 4.50	29.0 ± 3.90	**< 0.001**
LV diastolic function			
Mitral E wave velocity (cm/s)	83.6 ± 16.5	82.3 ± 13.9	0.601
Mitral A wave velocity (cm/s)	52.5 ± 13.1	45.9 ± 8.0	0.058
E/A ratio	1.7 ± 0.4	1.8 ± 0.5	0.156
Mitral deceleration time (ms)	154.8 ± 22.30	145.8 ± 26.1	0.223
Mitral e' wave velocity	9.3 ± 1.8	10.3 ± 1.8	0.071
E/e' ratio	9.2 ± 1.9	8.2 ± 1.6	0.055

Data are expressed as means ± SD. Abbreviations: LV: Left ventricular, LA: Left atrium.
Comparisons between two groups were performed using the independent-samples t-test or Mann-whitney U test.
P-values <0.05 (2-tailed) was considered statistically significant

3.6. Relations between worse cardiac chronotropic responses during exercise and cardiometabolic health in obese adolescents

Multivariate linear regression analysis showed that 33 percent of the variance ($p < 0.001$) of a smaller HRR1 was explained by a worse metabolic risk profile (SC $\beta = -0.330$; $p = 0.017$). Univariate analysis showed a significant negative correlation between HRR0.5 and the metabolic risk profile of both obese and lean adolescents ($r = -0.387$; $p = 0.007$). In addition, 34 percent of the variance ($p < 0.001$) of a lower HRR1 (SC $\beta = -0.381$; $p = 0.006$) and HRR2 (SC $\beta = -0.440$; $p = 0.004$) was explained by a worse metabolic risk profile (Table 7). A negative correlation was found between 1-min ($r = -0.413$; $p = 0.005$) and 2-min ($r = -0.492$; $p = 0.001$) HRR and worse metabolic risk profile (Figure 4). HR reserve was inversely associated ($p < 00.1$) with the metabolic risk profile (SC $\beta = -0.382$; $p = 0.015$).

***Table 7**; Multivariate regression explaining heart rate recovery and heart rate reserve.*

	R^2	R^2 adjusted	beta	SC (B)	p-value
HRR 0.5 minute	0.333	0.299			
Gender			-0.336	-0.424	**0.003**
Metabolic risk			-0.397	-0.330	**0.017**
HRR 1 minute	0.337	0.303			
Gender			-0.189	-0.410	**0.003**
Metabolic risk			-0.257	-0.381	**0.006**
HRR 2 minutes	0.193	0.173			
Metabolic risk			-0.446	-0.440	**0.004**
HR reserve	0.235	0.195			
Body length			0.004	0.437	**0.006**
Metabolic risk			-0.080	-0.382	**0.015**

Abbreviations: HR: heart rate. HRR: Heart rate recovery.
The independent variable metabolic risk factor was log transformed.
Regression model was corrected for age, gender, Tanner stage and body length.

Higher HRR0.5 was associated ($p < 0.001$) with a lower leptin concentration (SC $\beta = -0.454$; $p < 0.001$) and higher alkaline phosphatase concentration (SC $\beta = 0.406$; $p = 0.001$). With regard to cardiovascular risk, HHR0.5 was inversely associated ($p < 0.001$) with the mitral A wave velocity (SC $\beta = -0.558$; $p < 0.001$). In addition, 28 percent of the variance ($p = 0.001$) of a higher HRR0.5 was explained by sex (SC $\beta = -0.274$; $p = 0.013$) and a higher PR_{peak} interval (SC $\beta = 0.337$; $p = 0.017$).

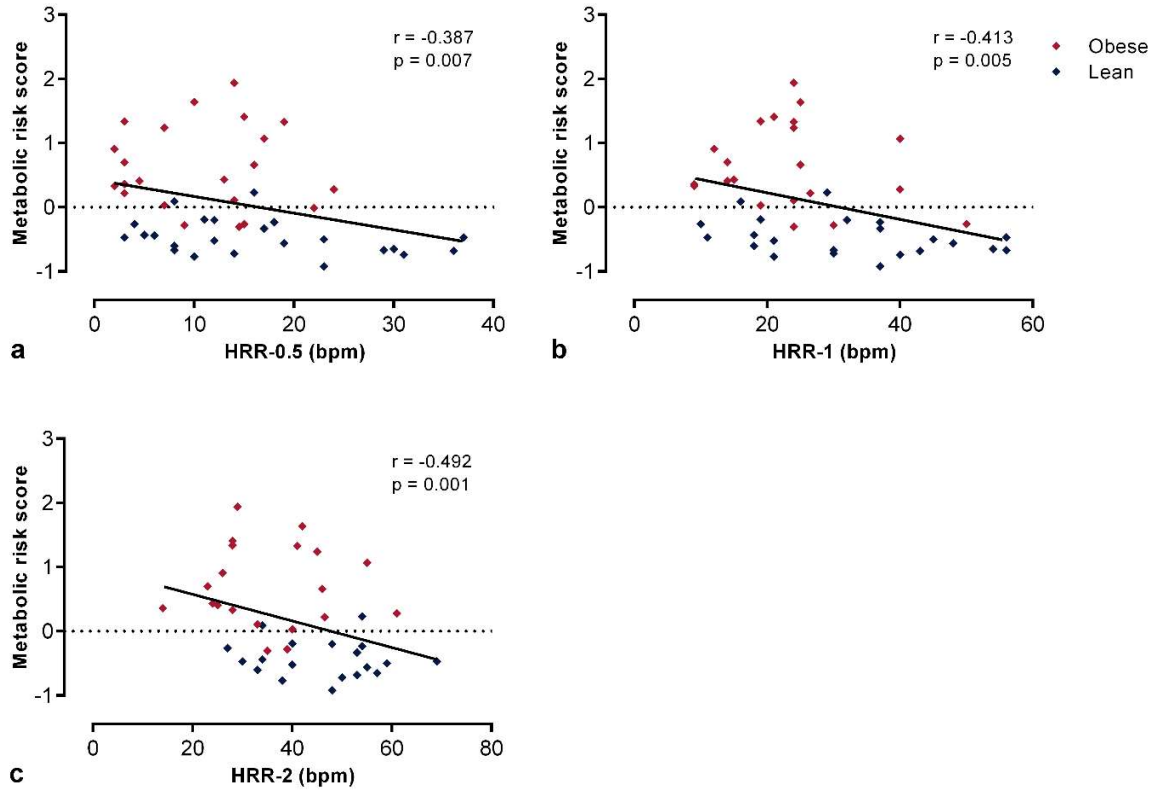

***Figure 5**; Analysis of univariate correlations between the metabolic risk profile and heart rate recovery, (a) half a minute (n = 47), (b) one minute (n = 45) and (c) two minutes (n = 43) after cessation of exercise testing of obese and lean adolescents. Abbreviations: ΔHR: delta heart rate.*

Higher HRR1 was associated ($p < 0.001$) with a higher alkaline phosphatase (SC β = 0.405; p = 0.001), haemoglobin (SC β = 0.297; p = 0.010) and iron se (SC β = 0.229; p = 0.046) concentration, and a lower triglyceride concentration (SC β = -0.394; p = 0.001), Table 8. With regard to cardiovascular risk, HHR1 was inversely associated (p = 0.002) with the mitral A wave velocity (SC β = -0.421; p = 0.009) and positively related to oxygen pulse at peak exercise (SC β = 0.369; p = 0.020). In addition, 44 percent of the variance ($p < 0.001$) of a higher HRR1 was explained by increased PR interval at peak exercise (SC β = 0.369; p = 0.008) and PR interval at rest (SC β = 0.278; p = 0.043). With regard to long-term HRR, 55 percent of the variance in HRR2 ($p < 0.001$) was explained by a higher ferritin concentration (SC β = 0.472; $p < 0.001$) and a lower triglyceride (SC β = -0.334; p = 0.006) and C-reactive protein concentration (SC β = -0.303; p = 0.013). An increased E/A ratio (SC β = 0.314; p = 0.043) explained 10 percent of the variance in HRR2 (p = 0.043). In addition, HRR2 was associated ($r^2 = 0.24$; p = 0.001) with a higher PR interval at peak exercise (SC β = 0.490; p = 0.001). HR reserve was inversely associated (r^2 =

0.281; p = 0.002) with waist-to-hip ratio (SC β = -0.298; p = 0.037). With regard to cardiovascular risk, 16 percent of the variance (p = 0.008) of a higher HRR2 was explained by a higher carbon dioxide output (SC β = 0.404; p = 0.043). All aberrant parameters significantly correlated with the independent variables from the regression analysis (p < 0.05), with the exception of blood haemoglobin concentration and oxygen pulse (APPENDIX A, Table 9).

***Table 8**; Multivariate regression explaining heart rate recovery and heart rate reserve.*

	R^2	R^2 adjusted	beta	SC (B)	p-value
HRR 0.5 minute					
Cardiometabolic health	0.506	0.482			
Leptin			-0.361	-0.454	**<0.001**
Alkaline phosphatase			0.725	0.406	**0.001**
Cardiovascular risk	0.311	0.295			
Mitral A wave velocity			-2.415	-0.558	**<0.001**
Electrophysiological	0.281	0.246			
PR_{peak}			0.009	0.337	**0.017**
Sex			-0.274	-0.350	**0.013**
HRR 1 minute					
Cardiometabolic health	0.585	0.540			
Alkaline phosphatase			0.432	0.405	**0.001**
Triglycerides			-0.396	-0.394	**0.001**
Haemoglobin			0.064	0.297	**0.010**
Iron			0.001	0.229	**0.046**
Cardiovascular risk	0.358	0.314			
Mitral A wave velocity			-1.121	-0.421	**0.009**
Oxygen $pulse_{peak}$			0.920	0.369	**0.020**
Electrophysiological	0.436	0.390			
PR_{peak}			0.005	0.369	**0.008**
PR_{rest}			0.003	0.278	**0.043**
HRR 2 minute					
Cardiometabolic health	0.552	0.515			
Ferritin			0.552	0.472	**<0.001**
Triglycerides			-0.526	-0.334	**0.006**
C-reactive protein			-0.241	-0.303	**0.013**
Cardiovascular risk	0.099	0.076			
E/A ratio			1.050	0.314	**0.043**
Electrophysiological	0.240	0.221			
PR_{rest}			0.008	0.490	**0.001**
HR reserve					
Cardiometabolic health	0.281	0.243			
Body height			0.004	0.001	**0.004**
Waist-to-hip ratio			-0.219	-0.298	**0.037**
Cardiovascular risk	0.163	0.142			
VCO_{2peak}			0.300	0.404	**0.043**

Abbreviations: HR: heart rate. HRR: Heart rate recovery. VCO_{2peak}: Carbon dioxide output at peak exercise.
All variables with an abnormal distribution were log transformed.
Regression model was corrected for age, gender, Tanner stage and body length.

4. DISCUSSION AND CONCLUSION

In the present study, cardiopulmonary responses, in particular chronotropic responses, during CPET were compared between obese and lean adolescents. In addition, we investigated associations between aberrant cardiac chronotropic responses and cardiometabolic health indicators, cardiovascular risk factors and electrophysiological morphologies. The main findings of the present study demonstrate a diminished HRR and HR reserve in obese adolescents, as opposed to their lean counterparts. In addition, an inverse association of HRR and HR reserve with cardiometabolic health indicators, cardiovascular risk factors and electrophysiological parameters was observed.

An inadequate decline in HR after cessation of exercise was observed in obese adolescents, compared to lean adolescents. Autonomic nervous system mediated responses, in particular parasympathetic reactivation, are a major determinant of HRR, whereby a reduction in HRR may be indicative of decreased autonomic nervous responsiveness [67]. It has been proposed that short-term HRR, reflected by heart rate recovery 0.5 minute after cessation of exercise (HRR0.5) and heart rate recovery one minute after cessation of exercise (HRR1), could be considered as markers of cardiac parasympathetic reactivity, with gradual withdrawal of sympathetic activity becoming more important later in recovery (long-term HRR, HRR2) [68]. In our study, obese adolescents showed a smaller HR decline in both short-term and long-term HRR. These findings are consistent with previous research in obese adults and adolescents, which reported a marked impairment of HRR, one, three and five minutes after cessation of exercise [40, 69]. In addition, slower HRR following exercise has been associated with obesity and the MetS and several of its components in children and adolescents [70, 71]. Our data showed a significantly increased metabolic risk profile in obese adolescents and confirm the hypothesis that a worse metabolic risk profile was independently associated with a decreased short-term and long-term HRR. Although the association between dysregulation of the autonomic nervous system and MetS has been widely studied, the exact cause and origin is not fully understood. Some studies have proposed that the sympathetic nervous system plays a key role in the development of the MetS [72-74]. In contrast, according to Kizilbash *et al*., decreased HRR did not precede the development of the MetS, but appeared after MetS components were present in adults [67]. Furthermore, Laguna *et al*. demonstrated that MetS components, including waist circumference, sum of five skinfolds, systolic BP, diastolic BP, HDL-C and HOMA-IR, were associated with short-term and long-term HRR in obese children [69]. Our findings confirm that the association between metabolic risk factors and HRR seems to exist in obese adolescents. In addition, cardiometabolic risk factors,

including a reduced blood HDL-C concentration and higher blood LDL-C, fasting insulin, CRP, triglyceride and leptin concentration and HOMA-IR, were all significantly different in obese adolescents, compared to lean adolescents. All these biochemical parameters are used to calculate different metabolic risk scores and are, therefore, also possibly involved in autonomic dysfunction. Although the exact causes of autonomic dysfunction are not fully clear it is suggested that hyperleptinemia, insulin resistance and compensatory hyperinsulinemia are associated with autonomic imbalance, possibly due to impaired baroreflex sensitivity and nerve damage [75].
Multiple linear regression analysis also showed that short-term HRR was associated with sex. A previous study regarding the relation between HRR and sex was reported by Nilsson *et al*., who found that among 75-year-old subjects the MetS components were more strongly correlated to HRR in females compared to males [76]. These results has consistently been reported by Lin *et al*., who found a relatively higher correlation between HRR and metabolic risks in female adolescents [70].

An inverse association of HRR with cardiometabolic health indicators was found. Kaufman *et al*. noted that autonomic dysfunction in obese children was associated with blood leptin and C-reactive protein concentrations, primarily mediated by adipose tissue [77, 78]. In addition, Shishehbor *et al*. suggested that there was a strong association between abnormal HRR2 and blood triglyceride-to-HDL-C ratio [78]. These results were also found in our study, where HRR0.5 was related to blood leptin concentrations, HRR 1 to blood triglyceride concentration and HRR2 was related to blood triglyceride and C-reactive protein concentration. All these biochemical variables are involved in insulin resistance and therefore may possibly contribute to impaired short-term and long-term HRR. Furthermore, HRR1 was significantly associated with blood haemoglobin levels. A study of Lutfi *et al*. has been demonstrated that haemoglobin concentrations are related to heart rate variability, a biomarker for autonomic function. They showed that high blood haemoglobin concentrations were associated with increased parasympathetic and sympathetic activity, and their findings were comparable with other reports [79-83]. Gehi *et al*. revealed that increased odds ratios of having low heart rate variability was related to each 1g/dl decrease in blood haemoglobin levels [84]. Therefore, lower blood haemoglobin concentrations may be responsible, at least in part, for reduced heart rate variabilities. In addition, blood haemoglobin was significantly lower in obese adolescents and confirms the relationship between autonomic dysfunction and lower blood haemoglobin concentration. Iron concentration was also a predictor of HRR1, where ferritin concentration was related to HRR2. These parameters may explain, in part, the association between blood

haemoglobin concentration and autonomic dysfunction. In a previous study, alterations in autonomic function were found in patients with iron deficiency anemia [83]. In these patients, autonomic dysfunction was explained due to low oxygen tension in tissues.
Although blood alkaline phosphatase concentration was not statistically different between both groups, a positive association was found between blood alkaline phosphatase concentration and short-term HRR. These findings are inconsistent with a study performed by Kumar *et al*., where no significant association was found between indices of autonomic dysfunction and alkaline phosphatase in young adults [85]. This is the first study suggesting a relation between alkaline phosphatase and short-term HRR in adolescents. Since blood alkaline phosphatase concentration is a sensitive and reliable indicator of osteoblast activity, decreased blood alkaline phosphatase concentrations suggest lower osteoblast activity [86]. Obesity possibly affects bone metabolism through several mechanisms. First, adipocytes and osteoblasts are derived from a common MSC, whereby obesity causes increased adipocyte differentiation and reduced osteoblast differentiation and subsequent bone formation [87]. In addition, increased blood leptin concentrations in obese adolescents may affect bone formation and in turn blood alkaline phosphatase concentration [87]. Although this hypothesis may be applicable to obese adolescents, the relation between alkaline phosphatase and HRR in lean adolescents remains unknown. Therefore, further studies are necessary to explain the link between blood alkaline phosphatase concentrations and HRR.

Cardiovascular risk factors, in particular parameters reflecting diastolic function, were independently associated with impaired autonomic function. Diastolic function of the LV is assessed by the ratio of peak velocity flow in early diastole (Mitral E wave velocity) to peak velocity flow in late diastole, which is caused by atrial contraction (Mitral A wave velocity). Mitral A wave velocity was inversely associated with HRR0.5 and HRR1, where the E/A ratio was positively related to HRR2. A study performed in elderly men demonstrated a positive correlation between E/A ratio and HRR1 after exercise [88]. These data may indicate that LV relaxation progressively decreases with impaired HRR, resulting in an increase of left atrial pressure and left ventricular diastolic filling pressure, eventually leading to symptoms of heart failure. Skaluba *et al*. stated that even mild increases in LV filling pressure were associated with autonomic dysfunction [89]. In addition, Poirier *et al*. demonstrated a relation between left ventricular diastolic dysfunction and cardiac autonomic neuropathy in men with uncomplicated type II diabetes [90]. This may explain the significantly increased left atrial diameter in obese adolescents since left atrial enlargement is a robust predictor of diastolic burden [91]. This is supported by a previous study which also found left arterial enlargement in obese adolescents [92]. A possible cause of diastolic dysfunction is LV

hypertrophy due to higher peripheral resistance in obese adolescents [89]. Our results confirm this hypothesis since LV septum thickness was significantly increased in obese adolescents, compared to lean adolescents. Furthermore, it is proven that LV hypertrophy as well as left atrial enlargement are associated with adverse cardiac events and worse prognosis [91].
A lower oxygen pulse at peak exercise was associated with reduced HRR1. Oxygen pulse is a robust estimator of cardiac stroke volume during exercise using the Fick equation [93]. Since an impaired LV diastolic function has an adverse effect on preload and stroke volume, there could be a possible link between the association of diastolic impairment, decreased stroke volume at peak exercise and autonomic imbalance. This indicates a blunted stroke volume response to exercise in subjects with parasympathetic autonomic dysfunction. A previous study in female adolescents with type II diabetes showed that stroke volume was lower during submaximal exercise [94]. In addition, a possible relation was suggested between cardiac autonomic neuropathy and LV diastolic dysfunction [94].
A significant higher CIMT was found in lean adolescents, compared to obese adolescents. These results are in contrast to other studies, which showed a higher CMIT in obese adolescents [95, 96]. A possible reason for this finding is not known and more studies are necessary to clarify these results.

A smaller PR interval at rest was inversely associated with HRR1 and HRR2. In addition, PR interval at rest was significantly lower in obese adolescents, which could be explained by the inverse relationship of PR interval and HR [97]. This relationship was also seen in our results and is corroborated with previous findings, which indicate the existence of a positive correlation between BMI and resting HR in obese individuals [98]. An increased resting HR may be related, at least in part, to autonomic imbalance present in obese individuals. These results are consistent with previous studies showing a relationship between a higher resting HR and sympathetic predominance in obese young people and adolescents [99, 100]. HRR0.5 and HRR1 were also associated with PR interval at peak exercise. However, these findings should be taken with caution since interindividual PR intervals were measured in different leads. Therefore, further investigation is necessary to explain this relationship.
Our study showed decreased HR reserve values in obese adolescents, and may be attributable to an increased resting HR. A positive association was found between HR reserve and waist-to-hip ratio, an index of abdominal adiposity [101]. Since BMI is positively correlated to abdominal adiposity, it is suggested that increased waist-to-hip ratio is related to higher resting heart rate, and subsequent HR reserve. A lower carbon dioxide output at peak exercise was also related to

a lower HR reserve. A decreased carbon dioxide output at peak exercise may be indicative for exercise intolerance. A significantly decreased W_{peak} was found in obese adolescents. In a meta-analysis of our group W_{peak} was reduced in obese adolescents in two out of three available studies [37]. However, this difference did not reach meta-analytical statistical significance. A possible reason for the reduced W_{peak} is due to the higher oxygen costs to move the heavy limbs during cycling exercise. Furthermore, obesity in adolescents has a profound effect on respiratory mechanics due to increased adipose tissue mass on the thoracic cave and increased pressure in the abdomen, leading to an increase in ventilatory work. In addition, increased abdominal pressure may restrict diaphragmatic descent during inspiration, reducing inspiratory capacity [102]. This is consistent with our results which showed no differences in peak oxygen uptake, despite a significant decreased W_{peak} in obese adolescents. In addition, a significant reduction of gross efficiency was shown in obese adolescents, which indicates a diminished mechanical efficiency. Therefore, obese adolescents require increased oxygen uptake to do a given amount of external work. It has been well established this increased O_2 cost of work during incremental exercise occurs once the cycling power output exceeds the lactate threshold, where this decreased mechanical efficiency is considered as a sign of decreased efficiency of muscle contraction, subsequently leading to exercise intolerance [103]. Zoladz *et al.* have been shown that during CPET efficiency is reduced by 20% above the lactate threshold and this decreased efficiency is related to a diminished exercise tolerance [104]. In our study, a significant lower VT1 was explored in obese adolescents, with the assumption that VT1 occurs at very similar levels of intensity to the lactate threshold [105], thereby suggesting mechanical efficiency is reduced at an earlier stage. It Is proposed that an impairment of skeletal muscle oxidative metabolism negatively affects this reduced exercise tolerance [106]. However, the exact relationship between muscle fatigue and decreased efficiency during exercise above the lactate threshold remains elusive and further studies are warranted.

Our findings suggest a relation between worse cardiometabolic health and cardiovascular risk in obese adolescents. This would indicate that autonomic dysfunction, reflected by HRR, may provide important clinical information regarding cardiometabolic health and impaired LV diastolic function. In this way, HRR can be used as a valuable prognostic marker with regard to early detection of risk factors for cardiometabolic disease in obese adolescents. Early detection is important since adolescence has been proposed as a sensitive period for the development of obesity and cardiovascular diseases, also in adulthood [107]. To date, clinicians and physiologists do not taking into account CPET parameters in their evaluation, or have even ignored it. However,

exercise testing provides valuable clinical information and is recommended due to the low cost and easy applicability. With regard to obese adolescent, individuals that experience abnormal cardiopulmonary functions during CPET should deserve greater attention during clinical follow-up and might be eligible for other treatment options.

In conclusion, this study demonstrates that obese adolescents have impaired heart rate recovery (HRR) after CPET, whereby reduced post exercise HRR in obese adolescents suggests impaired autonomic function. In addition, HRR is independently associated with worse cardiometabolic health.

4.4. Limitations and future perspectives

In this study, we were restricted to only one measure determining autonomic function. Therefore, it would be useful to confirm these findings using other markers that might estimate autonomic function. In addition, physical activity was measured using the PAQ-A questionnaire. Although this is a reliable and valid tool for measuring physical activity, the use of accelerometers may be a better option. Furthermore, percentage fat was measured using BIA and skinfold thickness. These measurements are not accurately enough to make a correct estimation of adiposity. Therefore, it is recommended in future research to use DEXA scan or MRI. Preceding echocardiography and CPET, a standard oral glucose intolerance test was performed in only the obese subjects, where they had to ingest 75g of glucose, and may affect exercise performance and CPET parameters.
It is recommended to focus on future research on longitudinal studies in obese adolescents to investigate whether obese adolescents will be confronted with cardiometabolic diseases, and to look of HRR becomes better after weight reduction. In addition, more controlled studies with a larger sample size are required to confirm our findings and to elucidate the mechanisms involved in this complex interplay between HRR, obesity and the metabolic syndrome. In addition, in future research sex has taken into account since it seems there are difference in autonomic function between males and females.

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APPENDIX A

***Table 9**; Correlation analysis of aberrant chronotropic responses and independent variables form regression analysis.*

	r	p-value
HRR 0.5 minute		
Cardiometabolic health		
Leptin	-0.462	**0.001**
Alkaline phosphatase	0.578	**<0.001**
Cardiovascular risk		
Mitral A wave velocity	-0.398	**0.007**
Electrophysiological		
PR_{peak}	0.387	**0.007**
HRR 1 minute		
Cardiometabolic health		
Alkaline phosphatase	0.508	**<0.001**
Triglycerides	-0.480	**0.001**
Haemoglobin	0.222	0.138
Iron		
Cardiovascular risk		
Mitral A wave velocity		
Oxygen $pulse_{peak}$	0.255	0.087
Electrophysiological		
PR_{peak}	0.562	**<0.001**
PR_{rest}	0.489	**0.001**
HRR 2 minute		
Cardiometabolic health		
Ferritin		
Triglycerides	-0.411	**0.009**
C-reactive protein	-0.468	**0.003**
Cardiovascular risk		
E/A ratio	0.617	**<0.001**
Electrophysiological		
PR_{rest}	0.489	**0.002**
HR reserve		
Cardiometabolic health		
Waist-to-hip ratio	-0.369	**0.014**
Cardiovascular risk		
VCO_{2peak}	0.142	**0.008**

Abbreviations: HR: heart rate. HRR: Heart rate recovery. VCO_{2peak}: Carbon dioxide output at peak exercise.
Pearson correlation was used for all variables with a normal distribution, and Spearman was used for abnormal distributed data.

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Cardiac chronotropic responses during exercise and relations to cardiometabolic health in obese adolescents

Richting: **master in de biomedische wetenschappen-klinische moleculaire wetenschappen**
Jaar: **2017**

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Datum: **8/06/2017**