2016•2017

FACULTY OF MEDICINE AND LIFE SCIENCES

Master of Biomedical Sciences

Master's thesis

Heart rate responses during exercise in obese adolescents: relation with cardiovascular health

Supervisor :
Prof. Dr. Guy MASSA
Prof. dr. Dominique HANSEN

Marjolein Beyens

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences

Transnational University Limburg is a unique collaboration of two universities in two countries: the University of Hasselt and Maastricht University.

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List of abbreviations

A mitral	trans-mitral peak late diastolic velocity
AF	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BAT	brown adipose tissue
BF	breathing frequency
BIA	bioelectrical impedance analysis
BMI	body Mass Index
BP	blood pressure
Ca2+	calcium
CIMT	carotid intima media thickness
CO	cardiac output
CPET	cardiopulmonary testing
CRI	chronotropic index
CRP	c-reactive protein
DT	deceleration time
E mitral	trans-mitral peak early diastolic velocity
E'	early diastolic mitral annular velocity
ECG	electrocardiogram
EF	ejection fraction
FFA	free fatty acids
GGT	gamma-glutamyl transpeptidase
Hb	haemoglobin
HbA1c	glycosylated haemoglobin
HC	hip circumference
HDL	high-density lipoprotein
HOMA-IR	homeostatic model assessment for insulin resistance
HR	heart rate
HRR	heart rate recovery
HRreserve	heart rate reserve
Ht	haematocrit
IL-6	interleukin-6
IOTF	International Obesity Task Force
K^+	potassium
LA	left atrium
LDL	low-density lipoprotein
LV	left ventricle
MetS	metabolic syndrome
MRF	metabolic risk factor
NASH	non-alcoholic steatohepatitis
NG	lean group
OG	obese group
OITF	International Obesity Task Force
OUES	oxygen uptake efficiency slope
PAQ-A	physical activity questionnaire for adolescents
Pdia	diastolic blood pressure
$PETCO_2$	end-tidal CO_2 tension
$PETO_2$	end-tidal O_2 tension
Psys	systolic blood pressure
QTc	corrected QT
RAS	renal renin-angiotensin system
RER	respiratory exchange ratio
RIA	radioimmunoassay
rpm	revolutions per minute
SFC	skinfold calliper
T2DM	type 2 diabetes mellitus
T4	thyroxine
TAG	triacylglycerol
TNF-α	tumour necrosis factor-α
TSH	thyroid stimulating hormone
TTE	transthoracic echocardiography
VCO_2	carbon dioxide output
Vd/Vt	physiological dead-space/tidal volume ratio
VE	expired volume
VE/VCO_2	ventilatory equivalents for CO_2
VE/VO_2	ventilatory equivalents for O_2
VO_2	oxygen uptake
VO_2/HR	oxygen pulse
Vt	tidal volume
VT1	first ventilatory threshold
VT2	second ventilatory threshold
WAT	white adipose tissue
WC	waist circumference
WHO	World Health Organisation
WHR	waist-hip ratio
WIV-ISP	Belgian scientific institute of public health

Abstract

Introduction: Childhood obesity is a growing epidemic associated with important cardiovascular, gastrointestinal, respiratory and orthopaedic comorbidities. In adults with obesity, a changed peak heart rate (HRpeak) during exercise is independently related to an elevated risk for adverse cardiovascular events. However, the prognostic relevance of this parameter in adolescents with obesity remains to be explored.

Objectives: The goal of this project was to study the association between HR responses and (i) cardiac function, (ii) cardiovascular autonomic function, (iii) atherosclerosis and (iv) cardiac electrophysiology to assess the importance of HR responses during exercise as a prognostic tool for cardiovascular disease in obese adolescents.

Methods: Twenty-four obese (OG BMI: 32.1 ± 4.3 kg/m^2, age: 13.4 ± 1.1 years) and 24 lean (LG BMI: 19.6 ± 2.5 kg/m^2, age: 13.9 ± 1.5 years) adolescents performed a maximal cardiopulmonary exercise test (CPET), with assessment of cardiovascular and respiratory outcomes. Subject groups were age and gender matched, Tanner stage and physical activity levels were assessed. Transthoracic echocardiography (TTE) was performed, as well as measurement of carotid intima media thickness (CIMT). Fasting blood samples were collected to evaluate glycaemic control, liver function and cardiovascular risk factors. Multivariate regression models were used to explore relationships between CPET outcomes and secondary measurements.

Results: HRpeak was not different between two groups (p=0.97). However, heart rate reserve (HR reserve) (p=<0.01) and heart rate recovery (HRR) at 30 (p=0.03), 60 (p=0.02) and 120 seconds (p=<0.01) after exercise were decreased in the OG. Other CPET outcomes were not significantly altered except for maximal power output (p=0.01). HRreserve and HRR were associated with metabolic risk factors seen in obesity and factors indicative of cardiovascular abnormalities.

Conclusion: Significant alterations in HRreserve and HRR suggest cardiovascular autonomic function is distorted in obese adolescents. Although the data indicates that HRpeak is not changed in obese adolescents, HRreserve and HRR might be of prognostic value for cardiovascular disease risk in obese adolescents. Further research should be implemented to discover the associations between cardiac autonomic function and adverse cardiac events and the aetiology behind the development of distorted autonomic function in obese adolescents.

1. Introduction

1.1. Obesity: prevalence and health effects

The World Health Organisation (WHO) defines overweight and obesity as an abnormal or excessive fat accumulation that may have adverse health effects. Defining obesity is essential in observing prevalence rates as well as monitoring and intervening with the process of weight gain (1). Body mass index (BMI [kg/m^2]) is the ratio of weight to height that is commonly used to classify weight status. BMI is calculated by dividing one's weight in kilograms by the square of their height in meters.

On a population level, BMI provides a useful, rapid measure of overweight and obesity. However, when considering individuals, it should be used with caution as the same BMI between individuals might not correspond to the same degree of fatness or adiposity. In adults, overweight is defined as a BMI greater than or equal to 25 kg/m^2 and obesity as a BMI greater than or equal to 30 kg/m^2 by the WHO (2). When defining childhood overweight and obesity, age, as well as gender, needs to be considered additionally. The International Obesity Task Force (IOTF) cut-offs have been widely used to assess prevalence rates for childhood overweight and obesity and are defined by values of BMI at age of 18 years (3).

According to a report by the Belgian scientific institute of public health (WIV-ISP), the prevalence rates of obesity have risen to 7% in children from 2 to 17 years old in 2013 in Belgium. The prevalence of overweight children is even higher, one in five children are too heavy considering their age and height. Moreover, both these numbers have risen since 1997 (4). Worldwide, an estimated 170 million children (aged less than 18 years old) are now overweight (2012) (5). Although significant efforts are being made to prevent and reduce the prevalence of childhood obesity, obesity continues to become even more prevalent (5, 6)

Childhood obesity is caused by diverse genetic and non-genetic factors. The "thrifty gene" hypothesis partially explains the global obesity epidemic by natural selection favouring a thrifty energy metabolism in times of limited food supply and high energy expenditure (7). Current low-activity lifestyles and an overabundance of high-energy foods, both characteristics of western countries, combined with the genetic predisposition of the thrifty genes, could lead to obesity. Even disregarding this theory, low levels of physical activity and easier access to high energy foods, have been implicated as important factors in the development of paediatric obesity (8, 9).

The residual energy that is left over when caloric intake is higher than caloric expenditure leads to energy stored in the form of triacylglycerol (TAG) in adipose tissue (see Figure 1). The excessive storage that occurs in obesity eventually incites an excess release of free fatty acids (FFA), due to excessive sympathetic nervous system stimulation and resulting increase in lipolysis. Excess FFA in the bloodstream lead to lipotoxicity, as lipids create oxidative stress in cells (10). The fat cells or adipocytes that form the adipose tissue do not only store TAGs, but also form an important endocrine tissue, releasing a wide range of so-called adipokines. Adipokines range from proteo-hormones such as leptin and adiponectin, that help regulate fat mass and appetite (11, 12), to pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) (13). Particularly white adipose tissue (WAT) in visceral fat depots, rather than brown adipose tissue (BAT), contribute to the creation of a low-grade inflammatory state by excretion of these cytokines (14). Adipocytes also activate fat-associated macrophages which in turn also excite inflammation (15).

The low-grade inflammation alongside the created lipotoxicity form an intricate network that makes obese patients at greater risk of developing cardiovascular diseases such as high blood pressure and atherosclerosis, endocrine disorders such as insulin resistance and type 2 diabetes mellitus (T2DM) and gastrointestinal problems e.g. gallstones and non-alcoholic fatty liver disease or non-alcoholic steatohepatitis (NASH) (7, 16, 17). Hypertension in obesity is mostly caused by adipokines that lead to excessive secretion of hormones of the renal renin-angiotensin system (RAS), which enhance endothelial vasomotor tone (18). Atherosclerosis is provoked by increased inflammation and further exacerbated by insulin resistance (19). The insulin resistance, in turn, is caused by inflammatory processes that decrease insulin sensitivity and the effect of lipotoxicity in the islets of the pancreas, which decrease secretion of insulin by the β-cells (20). T2DM emerges when insulin resistance worsens and when dyslipidaemia arises from the increased FFAs and TAGs in circulation (21). Gallstones become more prevalent in subjects with obesity due to enhanced lipolysis and in turn saturation of bile, promoting the formation of gallstones (22). NASH arises when inflammatory adipokines and lipotoxicity work together in the liver to cause the fatty and inflamed state (19).

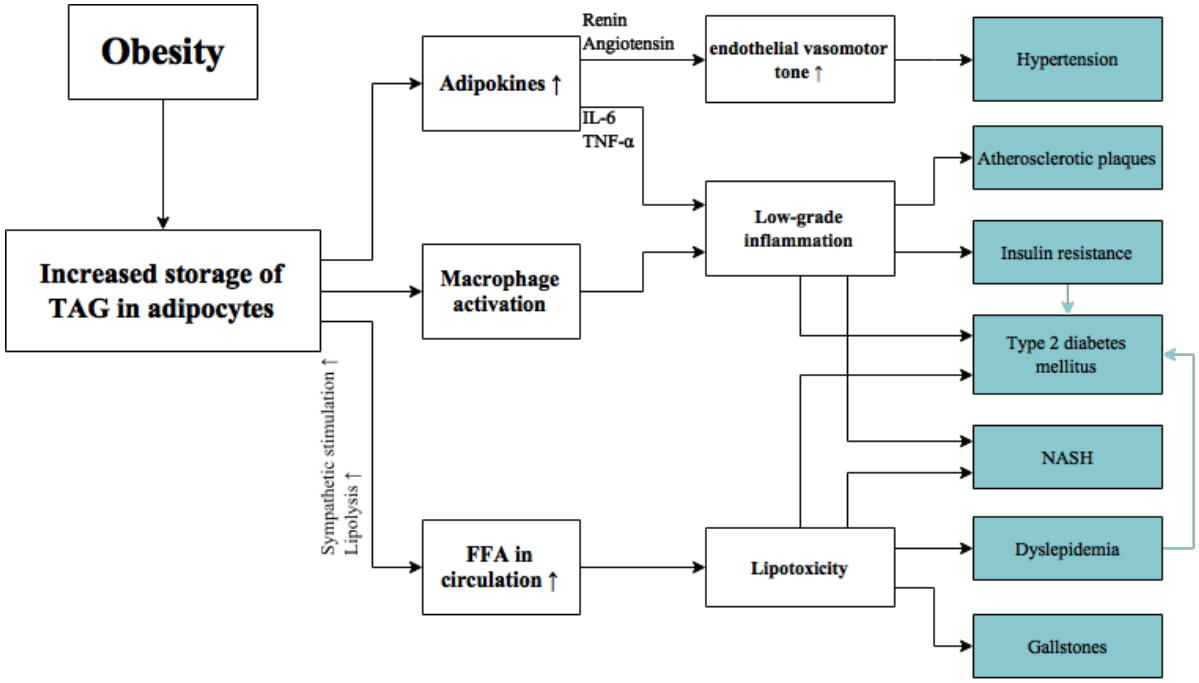

Figure 1: Pathology of obesity.
Abbreviations: TAG, triacylglyceride; FFA, free fatty acids; NASH, non-alcoholic steatohepatitis (10).

Before these conditions fully establish themselves, a cluster of risk factors identified as the metabolic syndrome (MetS) is seen in patients. The MetS risk factors include hypertension, hypertriglyceridemia, low HDL cholesterol and hyperinsulinemia, and are early, though reversible, stages of the comorbidities mentioned above (23).

Beyond these issues, obesity can cause problems of respiratory and orthopaedic origins. Comorbidities involving the respiratory system include obesity hypoventilation syndrome or Pickwick syndrome, resulting from the accumulation of adipose tissue, confining the upper respiratory tracts and adversely affecting breathing (16). The low-grade inflammatory state can exacerbate this condition by affecting bronchioles (24). The increased pressure on the joints due to weight can cause flatfoot and combined with inflammatory effects of adipokines can cause osteoarthritis. Obese adolescents can also experience psychosocial effects because of the social stigma associated with obesity, quality of life can, therefore, be reduced when compared to lean peers (16). Furthermore, not only does obesity contribute to illness in adolescence, it leads to increased morbidity and mortality in adulthood, even if the weight is normalised in adulthood (25). Lastly, obesity significantly increases the risk of certain cancers, such as breast cancer and kidney cancer (26).

Next to the disease and psychosocial burden on the patients, society carries a substantial medical and financial burden. Should the obese status persist through adulthood, costs are estimated to be much higher. A German study estimates a cost up to € 207 000 per obese adult (27). The total cost of overweight and obesity in European countries is 0.7% to 8% of the total healthcare expenditure, in which obesity is the main contributor (4).

1.2. Obesity and exercise

Other negative effects of excess body mass in adolescents are suboptimal responses to exercise. Often found is a reduced peak oxygen uptake per kg of lean tissue (28). Some studies reported a reduced peak cycling power output (29, 30) or a reduction in peak heart rate (HRpeak)(29, 31) in obese adolescents compared to their lean counterparts. Other studies mention changes in heart rate reserve (HRreserve) and heart rate recovery (HRR) after exercise (32, 33). In obese adults, HRpeak is independently related to an elevated glycosylated haemoglobin (HbA1c) concentration, which is used as a biomarker in diabetes diagnosis (34). Moreover, HRpeak is correlated with the risk of major adverse cardiovascular events and premature death and lower exercise tolerance (34, 35). Despite the established association between HRpeak and prognosis in obese adults, the validity of HRpeak as a prognostic marker in obese adolescents has not yet been explored. Assessing HRpeak using cardiopulmonary testing (CPET) would then provide a non-invasive, inexpensive and safe early predictor for cardiovascular incidents later in life (36). CPET allows the researcher to study the responses of both the cardiovascular and ventilatory systems to exercise stress. Gas exchange measurements of oxygen (O_2) and carbon dioxide (CO_2) are accompanied by an electrocardiogram (ECG), heart rate monitoring and blood pressure measurements. Gas exchange at the airways is interconnected with cardiac output and pulmonary blood flow as well as extraction of O_2 and feedback of CO_2 by the muscles, which is visualised in Figure 2. Because of the multifaceted outcomes CPET provides, a large range of dysfunctions can be discovered, even if they are only present when stress, i.e. exercise is put upon the body (37).

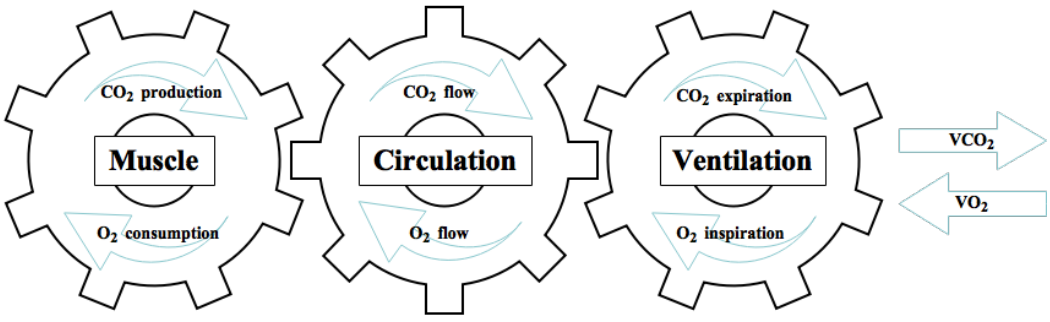

Figure 2: Coupling between muscle metabolism, blood circulation and ventilation, the foundation of cardiopulmonary testing(37).

In line with findings in obese adults, exercise testing and particularly HRpeak and heart rate responses may indicate several asymptomatic abnormalities in obese adolescents (38). For example, blood catecholamine (39) and potassium (40) concentrations during exercise are lowered in some obese adolescents compared to leans. Furthermore, obesity in adolescents can be associated with structural abnormalities of the heart or ventricular stiffness (41) and impaired blood pressure regulation and cardiovascular autonomic dysfunction (42). Some studies have also found atherosclerosis (43) in obese adolescents or cardiac electrophysiological abnormalities (44).

1.3. Hypothesis and objectives

The goal of this investigation was to examine whether HR responses during exercise testing may be an early indicator for cardiovascular abnormalities in obese adolescents. We hypothesised that HR responses during exercise testing are a sensitive and specific marker for cardiac dysfunction, cardiovascular autonomic abnormalities, atherosclerosis and cardiac electrophysiology abnormalities in obese adolescents.

The first aim of the proposed project was to compare CPET outcomes, HR responses in particular, between two groups, an obese group and a lean group. Secondly, the association between HR responses and (i) cardiac function, (ii) cardiovascular autonomic function, (iii) blood potassium concentrations at rest and at peak exercise, (iv) atherosclerosis, and (v) cardiac electrophysiology will be studied. In this regard, the diagnostic value of HR responses during maximal exercise testing will be clarified in obese adolescents, and the physiology behind the development of heart disease in obese adolescents can be explored.

2. Materials and methods

2.1. Subjects and study design

Forty-eight adolescents between the ages of 12 and 16 were recruited between November 2016 and May 2017 into the current cohort case-control clinical study. Subjects were split up into two groups of 24 subjects: the obese group (OG) and lean group (NG). The subjects were classified as obese or of normal weight based on BMI (kg/m^2) according to the age- and gender-specific IOTF centile curves. Overweight and obesity were classified using BMI cut-off points matching an adult BMI of over 25 and 30 kg/m^2 respectively. Normal weight was classified with BMI cut-off points consistent with adult BMI of between 18,5 and 25 kg/m^2.

Obese participants were recruited from the childhood obesity clinic at Jessa Hospital vzw (Hasselt, Belgium) where they were referred to for overweight/obesity or indications of underlying pathologies, (for instance insulin resistance). Patients were invited to take part in the study if they were not using any medications with effects on exercise performance at the time and if they were free from clinically diagnosed T2DM or cardiovascular or respiratory diseases. The lean group was recruited in several manners: recruitment posters with a small description and contact information were circulated in different places, a classified advertisement was placed in a local newspaper and word-of-mouth also provided a few participants. Interested subjects were invited to participate in the study if they were not currently using any performance-altering medication and if they were free from chronic cardiovascular, respiratory or metabolic diseases.

Ethics approval was acquired from the medical ethical committees of both Jessa Hospital vzw and Hasselt University. Written informed consent was obtained from every participant and their parent or legal guardian after careful explanation of the study aims and methods. The study was conducted according to the principles outlined in the Helsinki Declaration as stated by the World Medical Association in 2013.

During one day hospitalisation, all participants were subjected to following exams in a chronological order.

1. Anthropometry, assessment of physical activity and maturation stage
2. Fasting blood sampling
3. Echocardiography and carotid intima thickness measurement
    - Standardized meal

4. Maximal cardiopulmonary exercise testing with blood lactate and potassium assessment at rest and peak exercise

2.2. Anthropometry, assessment of physical activity and maturation stage

Height was measured to the nearest 0.1 cm using a Harpenden wall-mounted stadiometer (De Grood metaaltechniek, Nijmegen, The Netherlands). Body weight was determined in fasting condition and in underwear to the nearest 0.1 kg on a digital scale (Seca, California, USA). BMI was then calculated from the ratio of weight (in kg) to squared height (m^2). BMI was defined using the age- and gender-specific IOTF centile curves (3). Waist and hip circumference (WC; HC) were determined using measurement tape. WC was measured across the umbilicus, HC was determined at the height of the iliac crest. Concurrently waist-hip ratio was calculated (WHR, waist circumference (cm)/ hip circumference (cm)). Fat percentage was assessed in two manners. Firstly, by using bioelectrical impedance analysis (BIA) with the Bodystat 1500 MDD (EuroMedix POC, Leuven, Belgium) (45) and secondly by means of a Harpenden skinfold calliper (SFC) (Baty International, West Sussex, UK). Skinfold thickness at the medial triceps area and subscapular area were then used to calculate body fat percentage according to a protocol by Slaughter et al., 1988 (46, 47). Blood pressure was evaluated using an automatic blood pressure monitor from Omron (Hoofddorp, The Netherlands). Physical maturity was assessed using the Tanner scale, which defines physical measurements of development based on external primary and secondary sex characteristics(48, 49). It was assessed in accordance with the observation by a paediatrician and the adolescents' own opinion using a graphic depiction. The level of physical activity was determined using the validated Dutch physical activity questionnaire for adolescents (PAQ-A)(50). The result is an activity score between 1 and 5, a score of 1 indicates low physical activity, whereas a score of 5 indicates high physical activity. The score is based on physical activity over the past week during school time, over lunch, right after school, in the evening and during the weekend.

2.3. Fasting blood samples

Next, fasting blood samples were collected for the analysis of: plasma glucose, insulin, iron, ferritin, thyroid-stimulating hormone (TSH), free T4, cortisol, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, calcium (Ca^{2+}), potassium (K^{+}), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and c-reactive protein (CRP) concentrations (Cobas ®8000 modular analyser (F. Hoffmann-La Roche Ltd., Basel, Switzerland)). Blood glycated haemoglobin concentration ($HbA1_C$) was measured using ion exchange chromatography (Menarini HA-8180 HbA1C auto-analyser, Menarini Diagnostics, Diegem, Belgium). Blood leptin concentration was

measured using radioimmunoassay (RIA) (LINCO Research Inc., Saint Louis, MI, USA). Blood haemoglobin, haematocrit, and white blood cells were automatically evaluated using Siemens Advia 2120 (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Information on which collection tubes were used can be found in Appendix 1. From fasting glucose and insulin concentration , homeostatic model assessment for insulin resistance (HOMA-IR) was calculated by: fasting glucose (mg/dl) * fasting insulin concentration (µU/ml) / 405 (51). From blood parameter data, together with anthropometric data, a single-factor model, metabolic risk factor (MRF), underlying the MetS was created(52). MRF was calculated as the sum of standardized WC (cm), standardized insulin concentration (pmol/l), standardized triglyceride/HDL ratio and standardized mean arterial pressure (MAP), divided by four. Standard score or z-scores were calculated by subtracting the population mean from the value and dividing this number by the standard deviation (53).

2.4. Echocardiography and carotid intima thickness measurement

Ultrasound procedures were performed using the Vivid 7 Ultrasound Machine by GE Healthcare (Milwaukee, USA). Transthoracic echocardiography (TTE) with tissue Doppler was executed by an experienced cardiologist with the patient in a reclined position, using the GE M4S Matrix Array sector transducer (GE Healthcare, Milwaukee, USA). Left-ventricular (LV) systolic and diastolic function was assessed using following parameters: ejection fraction (EF), LV septum width (mm), LV diameter (mm), left-atrial (LA) diameter (mm), trans-mitral peak early diastolic velocity (E mitral [cm/sec]), trans-mitral peak late diastolic velocity (A mitral [cm/sec]), mitral E/A ratio, deceleration time (DT [sec]), E/E'- ratio (E'=Early diastolic mitral annular velocity) and cardiac output (CO [l/min])(54). Ejection fraction was estimated using Simpson's rule algorithm (55) on the apical two and four chamber views. LV septum width, LV diameter and LA diameter were assessed on the parasternal long axis (PLAX). E mitral, A mitral, DT and CO were evaluated in the apical four chamber view. An algorithm was used to evaluate left ventricular diastolic function by assessment of left atrial pressure (LAP), visualised in Appendix 2 (56).

Following echocardiography, carotid intima media thickness (CIMT) was measured by ultrasound of the lateral cervical area using the GE 9L-D linear transducer with the patient in supine position. CIMT is measured in end-diastolic position on the far wall of the left and right common carotid artery just inferior to the bifurcation (57). All ultrasound images were analysed EchoPAC Clinical Workstation Software (GE Healthcare, Milwaukee, USA).

2.5. Maximal cardiopulmonary testing

After consumption of a standardized meal (382 kcal, 9.75 g fat, 62.5 g carbohydrates, 10.6 g protein), a maximal CPET was executed on an electronically braked cycle and corresponding CardioSoft software from GE Healthcare (Milwaukee, USA). After one minute of sitting on the bike and one minute of unloaded cycling, the exercise test was initiated with pedalling at a starting load of 40 W, thereafter the resistance increased continuously with 20 W every minute. A cycling frequency of 60 to 70 revolutions per minute (rpm) had to be maintained. By continuous pulmonary gas exchange analysis, oxygen uptake (VO_2), carbon dioxide output (VCO_2) and other respiratory parameters (expired volume (VE), ventilatory equivalents for O_2 (VE/VO_2), ventilatory equivalents for CO_2 (VE/VCO_2), tidal volume (Vt), physiological dead-space-tidal volume ratio (Vd/Vt), breathing frequency (BF), respiratory exchange ratio (RER(VO_2/ VCO_2), end-tidal O_2 tension ($PETO_2$), end-tidal CO_2 tension ($PETCO_2$), oxygen pulse (VO_2/HR)) were collected breath-by-breath and averaged every 10 seconds. Heart rate (HR) was monitored and averaged every 10 seconds and more specific cardiac electrophysiological parameters were continuously monitored using a 12-lead ECG device (KISS™ multilead, GE Healthcare, Milwaukee, USA). The test was ended when the patient failed to maintain a pedal frequency of at least 60 rpm or indicated that they were exhausted. The 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, based on subjective features (fatigue, laboured and fast breathing, leg muscle pain). Perceived exertion of the respiratory system and muscles was evaluated using the Borg scale, to assess the intensity level the patient experienced. After stopping the test, 2 minutes of recovery was tracked while subjects continued to cycle unloaded at 50 rpm. Parameters were assessed at rest, defined by the lowest HR during the one minute of rest on the bike, and at peak exercise. Peak exercise was defined by the highest VO_2 value the subject could achieve before starting recovery. In recovery, heart rate recovery (HRR (△HRpeak-HR)) was assessed at 30, 60, 90 and 120 seconds (where data was available at this time point). Heart rate reserve (HRreserve) was calculated by extracting HR at rest (HRrest) from HR at peak (HRpeak). Chronotropic index(CRI) was calculated using the following formula: (HR at peak exercise − resting HR)/(predicted HRpeak − resting HR)(58). Predicted HRpeak was estimated using the formula proposed by Tanaka et al. (2001) (208 - 0.7 x age)(59, 60).

The oxygen uptake efficiency slope (OUES) was calculated using all exercise data (from start up to recovery) by a linear least square regression of VO_2 on the logarithmic of VE (61). The first ventilatory threshold (VT1) was estimated from the slope of HR against VO_2, at the point that HR starts to increase disproportionally compared to VO_2. The second ventilatory threshold (VT2) was determined on the VE/VCO_2 slope, at the point where VE starts to increase more than VCO_2 does.

Venous blood was drawn at two times points, at rest, just before the CPET, and at peak exercise. In these samples, potassium and lactate concentrations were determined. Venous blood was collected in lithium heparin tubes (Vacutainer®, BD, New Jersey, USA) and potassium concentration was established using Cobas ®8000 modular analyser (F. Hoffmann-La Roche Ltd., Basel, Switzerland). Lactate concentrations in venous blood were determined using Cobas Accutrend®Plus from F. Hoffmann-La Roche Ltd. (Basel, Switzerland).

The electrocardiogram was analysed using 50 second ECG-traces at rest and at peak exercise, and averaging the measurements. PR-interval, RR-interval, p-top width, QRS-complex width, Sokolow-index and QT-interval were measured using CardioSoft software from GE Healthcare (Milwaukee, USA) (see Appendix 3). Corrected QT-interval (QTc) was then calculated using Bazett's Formula (QT Interval / √(RR-interval)).

2.6. Statistics

Continuous data was checked for normality within both groups. Afterwards, continuous parameters were compared between OG and LG using either a Mann-Whitney U test (in case of non-parametric data) or an independent samples t-test (normally distributed data). Chi-Square analysis was used to compare discrete data (Tanner stage, sex). P-values of ≤ 0.05 were considered significant (2-tailed). Correlations were made using Spearman's rank correlation coefficient for non-parametric data. Multivariate linear regression was used to determine the relationship between CPET parameters that were significantly different between groups and other significantly altered parameters, grouped as metabolic parameters, cardiovascular parameters and electrocardiography parameters. All statistical analyses were performed using IBM® SPSS® Statistics for Macintosh, Version 24.0 (IBM Corp, Armonk, NY).

3. Results

3.1. Participant characteristics

Characteristics of the participants are shown in Table 1. To summarize, the study population consisted of 48 adolescent males and females divided into the OG (n=24) and the LG (n=24). Groups were age matched (age OG: 13.4±1.1 years, age LG: 13.9±1.5 years, p=0.32) and male and female subjects were proportioned equally (p=0.77). Physical maturity levels were uniformly distributed among groups (p=0.52). Body length was not different between two groups (OG: 166.4±8.7 cm, LG: 167.3±9.1 cm, p=0.51). In line with the study design, mean weight and BMI were respectively 61% and 64% higher in the OG (weight OG: 89.3±16.3 kg, weight LG: 55.3±11.4 kg, p=<0.01; BMI OG: 32.1±4.3 kg/m^2, BMI LG: 19.6±2.5 kg/m^2, p=<0.01). WC and HC, as well as WHR were higher in the oG (WC OG: 104.4±13.4 cm, WC LG 67.8±6.5 cm, p=<0.0; HC OG 105.0±8.6 cm, HC LG 78.9±8.9 cm, p=<0.01; WHR OG: 0.99±0.07, WHR LG 0.87±0.11, p=<0.01). BIA showed fat percentage to be 161% higher in OG, SFC calculations indicates a 181% higher fat percentage in OG. (BIA Fat% OG: 36.7±6.9, BIA Fat% LG 14.0±7.9, p=<0.01; SFC Fat% OG: 48.9±11.1, SFC Fat% LG 17.4±5.9, p= <0.01). Physical activity level was higher in the LG (PAQ-A score OG: 2.1±0.6, PAQ-A score LG 2.6±0.5, p=0.02).

Table 1: Patient characteristics

	Obese subjects (n=24)	Lean subjects (n=24)	
	Mean ± SD	Mean ± SD	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	
Weight (kg)	89.3 ± 16.3	55.3 ± 11.4	**<0.01**
Length (cm)	166.4 ± 8.7	167.3 ± 9.1	0.51
BMI (kg/m2)	32.1 ± 4.3	19.6 ± 2.5	**<0.01**
WC (cm)	104.4 ± 13.4	67.8 ± 6.5	**<0.01**
HC (cm)	105.0 ± 8.6	78.9 ± 8.9	**<0.01**
WHR	0.99 ± 0.07	0.87 ± 0.11	**<0.01**
% Fat mass		±	**<0.01**
BIA	36.7 ± 6.9	14.0 ± 7.9	**<0.01**
SFC	48.9 ± 11.1	17.4 ± 5.9	**<0.01**
Tanner stage			0.52
I (n)	2	2	
II (n)	0	1	
III (n)	6	4	
IV (n)	2	6	
V (n)	11	11	
PAQ-A score	2.1 ± 0.6	2.6 ± 0.5	**0.02**

Abbreviations: BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist hip ratio; BIA, bioelectrical impedance analysis; SFC, skinfold calliper

3.2. Cardiopulmonary testing parameters

Table 2 lists the parameters of the CPET from OG and LG, compared between groups. Blood lactate concentration at rest was different between the two groups (OG:1.3±0.7, LG:1.7±0.7, p=0.02). Systolic and diastolic blood pressure were higher in the obese patients (Psys OG: 124.9±12.1 mmHg, Psys LG: 113.7±10.3 mmHg, p=<0.01; Pdias OG: 77.2±9.8 mmHg, Pdias OG 69.1±7.5 mmHg, p=<0.01).

At peak exercise only one parameter was significantly different between groups: cycling power output. With a mean of 158.8 W (SD: 36.2W), peak cycling power output in the OG was 20% lower than in the LG (190.3±44,5, p=0.01).

Table 2: Cardiopulmonary testing parameters

	Obese subjects (n=24)			Lean subjects (n=24)			
	Mean	±	SD	Mean	±	SD	p-value
Rest							
VO_2 (ml/min)	351.9	±	83.7	315.4	±	111.9	0.05
VCO_2 (ml/min)	315.9	±	80.5	264.8	±	96.2	0.05
VE (l/min)	10.6	±	2.6	9.1	±	2.9	0.07
VE/VO_2	0.031	±	0.007	0.030	±	0.006	0.53
VE/VCO_2	0.034	±	0.004	0.035	±	0.004	0.36
Vt (l)	0.66	±	0.27	0.65	±	0.23	0.09
Vd/Vt	14.7	±	4.8	15.8	±	6.2	0.52
BF (breaths/min)	17.4	±	4.4	15.7	±	5.0	0.22
RER	0.90	±	0.12	0.84	±	0.11	0.09
$PETO_2$ (mmHg)	112.0	±	6.1	111.1	±	6.8	0.65
$PETCO_2$ (mmHg)	34.8	±	3.1	33.7	±	3.4	0.25
VO_2HR (ml/beat)	3.9	±	1.0	4.4	±	1.7	0.30
Psys (mmHg)	124.9	±	12.1	113.7	±	10.3	**<0.01**
Pdias (mmHg)	77.2	±	9.8	69.1	±	7.5	**<0.01**
Borg Legs	9.9	±	1.8	8.6	±	2.0	**0.02**
Borg Lungs	9.3	±	2.5	8.3	±	1.4	0.22
Lactate Rest (mmol/l)	1.3	±	0.7	1.7	±	0.7	**0.02**
K Rest (mmol/l)	3.8	±	0.2	4.0	±	0.3	0.05
Peak exercise							
VO_2 (ml/min)	2059.1	±	454.7	2251.4	±	553.5	0.21
VCO_2 (ml/min)	2525.0	±	595.0	2782.0	±	714.1	0.14
VE (l/min)	75.6	±	19.8	84.5	±	22.3	0.15
VE/VO_2	0.037	±	0.005	0.038	±	0.006	0.59
VE/VCO_2	0.030	±	0.003	0.031	±	0.004	0.41
Vt (l)	1.9	±	0.5	1.9	±	0.5	0.83
Vd/Vt	18.8	±	2.8	18.4	±	2.8	0.61
BF (breaths/min)	41.2	±	8.1	45.3	±	9.2	0.11
RER	1.23	±	0.07	1.23	±	0.07	0.69
$PETO_2$ (mmHg)	114.1	±	18.4	119.3	±	5.1	0.17
$PETCO_2$ (mmHg)	40.9	±	18.6	35.8	±	4.3	0.07
VO_2HR (ml/beat)	11.1	±	2.2	12.1	±	2.9	0.16
Borg Legs	15.8	±	3.3	16.5	±	2.0	0.64
Borg Lungs	16.3	±	2.2	15.6	±	1.9	0.55
Cycling power output (W)	158.8	±	36.2	190.3	±	44.5	**0.01**
Lactate Peak (mmol/l)	4.9	±	2.5	4.5	±	1.4	0.82
K Peak (mmol/l)	4.2	±	0.6	4.4	±	0.5	0.43

Abbreviations: VO_2, oxygen uptake; VCO_2, carbon dioxide output; VE, expired volume; VE/VO_2, ventilatory equivalents for O_2; VE/VCO_2, ventilatory equivalents for CO_2; Vt, tidal volume; Vd/Vt, Physiological dead-space-tidal volume ratio; BF, breathing frequency; RER, respiratory exchange ratio; $PETO_2$, end-tidal O_2 tension; $PETCO_2$, end-tidal CO_2 tension; VO_2/HR, oxygen pulse; Psys, systolic blood pressure; Pdia, diastolic blood pressure; K, potassium;

The VT1 was reached at a higher percentage of VO_2peak in obese subjects (OG: 52.9±6.1%, LG:47.7±6.8%, p=0.01). VT1 however was not different between groups (OG:1072.5±185.2 ml/min; LG: 1072.5±296. p=1.00). The VT2 and the percentage of the VO_2peak at which it was attained were not different between groups (Table 3).

Table 3: Ventilatory efficiency parameters during CPET

	Obese subjects (n=24) Mean ± SD	Lean subjects (n=24) Mean ± SD	p-value
OUES	2195.8 ± 501.2	2229.4 ± 501.5	0.82
VT1(ml/min)	1072.5 ± 185.2	1072.5 ± 296.2	1.00
VT2(ml/min)	1565.4 ± 379.4	1802.9 ± 502.0	0.12
VT1 (% of VO_2 peak)	52.9 ± 6.1	47.7 ± 6.8	**0.01**
VT2 (% of VO_2 peak)	76.4 ± 9.9	80.0 ± 9.1	0.19

Abbreviations: OUES, oxygen uptake efficiency slope (VE/VCO_2 slope); VT1, first ventilatory threshold; VT2, second ventilatory threshold

Resting HR was elevated in OG with 79.9 bpm compared to 64.7 bpm in LG (SD OG: 13.3, SD OG: 10.7, p=<0.01). Peak HR was not lowered in the OG (OG: 185.7±11.5bpm; LG: 185.8±9.6 bpm, p-value 0.97). Chronotropic responses between groups were not different, as indicated by CRI which was similar in both groups (OG: 0.86±0.09; LG: 0.90±0.07; p=0.74). HRreserve however was higher in lean subjects compared to their obese counterparts (OG: 106.4±17.8 bpm, LG:126.5±25.6, p=<0.01). During recovery from peak exercise, heart rate decline in the OG was delayed compared to the LG, as demonstrated by HRR at 30, 60 and 120 sec which are all lowered in obese subjects (HRR30s OG: 9.8±7.2, HRR30s LG: 16.5±10.1, p=0.01; HRR60s OG: 22.5±10.8, HRR60s LG: 31.4±14, p=0.02; HRR120s OG: 35.4±11.8, HRR120s LG: 46.1±11.5, p=0.01)(Table 4). This is visualised in Figure 3.

Table 4: Heart rate responses

	Obese subjects (n=24) Mean ± SD	Lean subjects (n=24) Mean ± SD	p-value
HRrest (bpm)	79.9 ± 13.3	64.7 ± 10.7	**<0.01**
HRpeak (bpm)	185.7 ± 11.5	185.8 ± 9.6	0.97
CRI	0.86 ± 0.09	0.90 ± 0.07	0.74
HRreserve	106.4 ± 17.8	126.5 ± 25.6	**<0.01**
HRR			
30 s	9.8 ± 7.2	16.5 ± 10.1	**0.01**
60 s	22.5 ± 10.8	31.4 ± 14	**0.02**
120 s	35.4 ± 11.8	46.1 ± 11.5	**0.01**

Abbreviations: HR, heart rate; HRreserve, heart rate reserve (△HRpeak-HRrest); HRR, heart rate recovery (△HRpeak - HR at 30, 60, 120s)

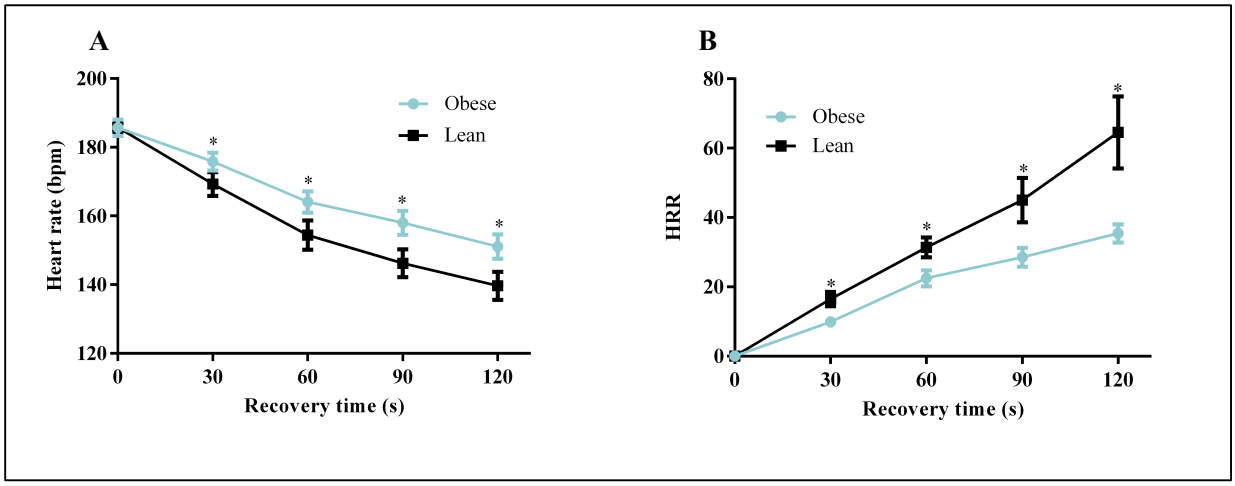

Figure 3: Heart rate changes during recovery from peak exercise in obese and lean subjects. (A) The decline in heart rate over time, starting at HRpeak. (B) Course of HRR. * p-value<0.05 between groups

Abbreviations: HRR, heart rate recovery

3.3. Blood parameters

Markers of glycaemic control are listed in Table 5. Fasting blood glucose was not altered between groups (OG: 88.9±6.1 mg/dl, LG: 86.1±6.6, -p=0.14), blood insulin concentration was higher in the OG (OG: 176.7±117.3 pmol/l, LG: 73.1±38.4 pmol/l, p=<0.01). HOMA-IR, also had significant different values between groups (OG: 5.6±3.9, LG: 2.2±1.3, p=<0.01). Mean blood leptin concentration in the obese subjects was higher than in lean subjects (OG: 25.4±16.9 ng/ml, LG 10.5±5.5 ng/ml, p=<0.01).

Liver function was disturbed in obese subjects, as indicated by elevated blood ALT and GGT concentrations (ALT OG: 29.8±16.8 IU/L, ALT LG: 16.6±7.3 IU/L, p=<0.01; GGT OG: 19.4±8.1 IU/L, GGT LG: 13.2±4.2 IU/L, p=<0.01).

Blood HDL concentrations were reduced and blood LDL and triglyceride concentrations were increased (HDL OG: 46.2±11.4 mg/dl, LG: 62.3±11.7 mg/dl, p=<0.01; LDL OG :93.5±26.5 mg/dl, LG 76.0±22.2 mg/dl, p=0.02; triglycerides OG: 105.0±63.7 mg/dl, LG 68.5±32.1 mg/dl, p=0.03). In obese subjects blood CRP concentration was 4.8 (±7.5) mg/dl and in lean subjects 0.6 (±2.0) mg/dl and thus lower (p=<0.01) (Table 5).

The remaining biochemical and haematological blood values tested did not show many dissimilarities among the two groups. Blood iron concentrations in obese subjects were reduced in the OG (OG: 81.8±34.8 µg/dl, LG: 108.8±39.4 µg/dl, p=0.02). Haemoglobin and haematocrit concentrations were

both lowered in obese subjects (Hb OG: 13.4±0.9 g/dl, LG: 14.1±1.1 g/dl, p=0.02; Ht OG: 38.7±2.3%, 39.4±7.9%, p=0.01) (Table 5).

Table 5: Blood parameters

	Obese subjects (n=24)	Lean subjects (n=24)	
	Mean ± SD	Mean ± SD	p-value
Glycemic control			
Glucose (mg/dl)	88.9 ± 6.1	86.1 ± 6.6	0.14
Insulin (pmol/l)	176.7 ± 117.3	73.1 ± 38.4	**<0.01**
HbA1C (mg/dl)	5.3 ± 0.3	5.2 ± 0.2	0.13
HOMA-IR	5.6 ± 3.9	2.2 ± 1.3	**<0.01**
Leptin (ng/ml)	25.4 ± 16.9	10.5 ± 5.5	**<0.01**
Liver function markers			
AST (IU/L)	23.7 ± 7.3	24.4 ± 8.7	0.90
ALT (IU/L)	29.8 ± 16.8	16.6 ± 7.3	**<0.01**
GGT (IU/L)	19.4 ± 8.1	13.2 ± 4.2	**<0.01**
ALP (IU/L)	188.0 ± 91.6	200.1 ± 120.3	0.91
Uric acid (µmol/L)	7.4 ± 8.9	5.1 ± 1.1	0.45
Atherosclerosis risk factors			
Total cholesterol (mg/dl)	158.4 ± 33.5	152.0 ± 22.2	0.74
HDL (mg/dl)	46.2 ± 11.4	62.3 ± 11.7	**<0.01**
LDL (mg/dl)	93.5 ± 26.5	76.0 ± 22.2	**0.02**
Triglycerides (mg/dl)	105.0 ± 63.7	68.5 ± 32.1	**0.03**
CRP (mg/dl)	4.8 ± 7.5	0.6 ± 2.0	**<0.01**
Other bloodparameters			
Iron (µg/dl)	81.8 ± 34.8	108.8 ± 39.4	**0.02**
Calcium (mmol/l)	2.3 ± 0.3	2.4 ± 0.1	0.20
ferritin (ng/ml)	58.5 ± 31.8	48.6 ± 27.4	0.26
TSH (IU/ml)	2.7 ± 1.0	2.2 ± 0.8	0.07
free T4 (pmol/L)	14.1 ± 3.1	14.2 ± 1.8	0.56
cortisol (ng/ml)	9.8 ± 6.2	7.8 ± 3.7	0.34
Hb (g/dl)	13.4 ± 0.9	14.1 ± 1.1	**0.02**
Ht (%)	38.7 ± 2.3	39.4 ± 7.9	**0.01**
Leukocytes (cells/µl)	7.0 ± 1.4	8.7 ± 8.4	0.15

Abbreviations: HbA1C, glycosylated haemoglobin; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, c-reactive protein; TSH, thyroid stimulating hormone; T4, thyroxine; Hb, Haemoglobin; Ht, Haematocrit

3.4. Metabolic risk factor

Obese subjects had a significantly higher MRF, with a mean of 0.63(±0.63), compared to a mean MRF in the lean subjects of -0,47 (±0.28) (p=<0.01).

3.5. Electrocardiogram parameters

At rest, RR-intervals of the obese subjects were smaller than those of the lean subjects (OG: 641.7±319.0, LG: 802.8±172.8, p=<0.01) (Table 6). Both at rest and at peak exercise the PR-interval was longer in the LG, both times by around 10% (Rest PR-interval OG: 135.9±398.8 ms, LG: 149.3±16.3 ms, p=0.03, Peak PR-interval OG: 94.5±18.3, LG: 105.0±14.4, p=0.03). Sokolow-index was also significantly different at both time points. At rest, it was 3.1(±1.7) mV in the OG and 4.0 (±1.2) mV in the LG (p=<0.01). At peak exercise the same relationship is seen, again Sokolow-index in the OG was smaller than in de LG (OG: 2.6±1.0mV, LG: 3.7±1.1, p=<0.01).

Table 6: ECG-parameters

	Obese subjects (n=24) Mean ± SD	Lean subjects (n=24) Mean ± SD	p-value
Rest			
RR-interval (ms)	641.7 ± 319.0	802.8 ± 172.8	**<0.01**
PR-interval (ms)	135.9 ± 398.8	149.3 ± 16.3	**0.03**
P-top-width (ms)	86.0 ± 13.7	88.8 ± 13.1	0.47
QRS-width (ms)	87.4 ± 10.5	82.5 ± 10.7	0.12
Sokolow index (mV)	3.1 ± 1.7	4.0 ± 1.2	**<0.01**
QT-interval (ms)	319.0 ± 53.0	343.6 ± 50.3	0.05
QTc (ms)	398.8 ± 60.3	389.7 ± 67.1	0.15
Peak Exercise			
RR-interval (ms)	321.3 ± 20.8	324.4 ± 16.6	0.57
PR-interval (ms)	94.5 ± 18.3	105.0 ± 14.4	**0.03**
P-top-width (ms)	71.0 ± 13.6	70.7 ± 13.3	0.95
QRS-width (ms)	79.0 ± 9.6	75.7 ± 10.0	0.24
Sokolow index (mV)	2.6 ± 1.0	3.7 ± 1.1	**<0.01**
QT-interval (ms)	222.2 ± 35.5	222.8 ± 13.2	0.37
QTc (ms)	392.2 ± 61.1	391.0 ± 21.2	0.14

QTc : QT interval corrected for heart rate

3.6. Ultrasound parameters

CIMT in lean subjects was on average 0.04 mm thicker than CIMT in obese subjects (OG:.0.50±0.05 mm, LG: 0.54±0.06 mm, p=0.02).

LV septum was widened in obese subjects with an average of 0.9 mm compared to lean subjects (OG: 8.0±0.8 mm, LG: 7.1±1.0 mm, p=<0.01) (Table 7). The diameter of the LA was also broader in the OG (OG:34.1±4.5 mm, LG: 29.0±3.9 mm, p=<0.01). Although no significant differences were seen in other parameter, trends are seen in velocity of the mitral A wave and the E/E'-ratio. Both are higher in the obese subjects. An algorithm was used to the rise in LAP, however in both groups no subjects classified as LAP↑.

Table 7: Ultrasound parameters

	Obese subjects (n=24)			Lean subjects (n=24)			
	Mean	±	SD	Mean	±	SD	p-value
CIMT (mm)	0.50	±	0.05	0.54	±	0.06	**0.02**
TTE parameters							
EF (%)	60.9	±	6.0	60.7	±	6.1	0.89
LV septum width (mm)	8.0	±	0.8	7.1	±	1.0	**<0.01**
LV diameter (mm)	45.4	±	4.0	46.2	±	3.5	0.50
LA diameter (mm)	34.1	±	4.5	29.0	±	3.9	**<0.01**
E mitral (cm/sec)	83.6	±	16.5	82.3	±	13.9	0.60
A mitral (cm/sec)	52.5	±	13.1	45.9	±	8.0	0.06
mitral E/A ratio	1.7	±	0.4	1.8	±	0.5	0.16
DT (ms)	154.8	±	22.3	145.8	±	26.2	0.22
E/E'	9.2	±	1.9	8.2	±	1.6	0.06
CO (l/min)	5.2	±	0.8	4.9	±	1.1	0.26
LAP ↑ (n)	0			0			1

CIMT, carotid intima media thickness; EF, Ejection fraction; LV, left ventricle; LA, left atrium; E mitral, transmitral peak early diastolic velocity; A mitral, transmitral peak late diastolic velocity; DT, deceleration time; E', Early diastolic mitral annular velocity; CO, Cardiac output; LAP, left atrial pressure

3.7. Association between metabolic risk factor and heart rate responses

Since metabolic risk factor and HRreserve, HRR30s, HRR60s and HRR120s proved changed in obese the OG, the association between these factors was further investigated. The associations were first evaluated using linear regression and concomitant correlation (Figure 4). All three HRR parameters were significantly correlated with metabolic risk (MRF). HRreserve was negatively correlated with a correlation coefficient (r) of -0.297 (p=0.05), but the correlation was not significant. The correlations between HRR and MRF differ between time points, although all relationships are significantly negative. At 30 seconds, HRR is correlated with an r of -0.393 (p=0,006). At 60 seconds the relation becomes even stronger, and the correlation coefficient is -0.414 (p=0.004). After another minute of recovery, the relationship with MRF returns to the same value as at 30 seconds (r: -0.392, p=0,014).

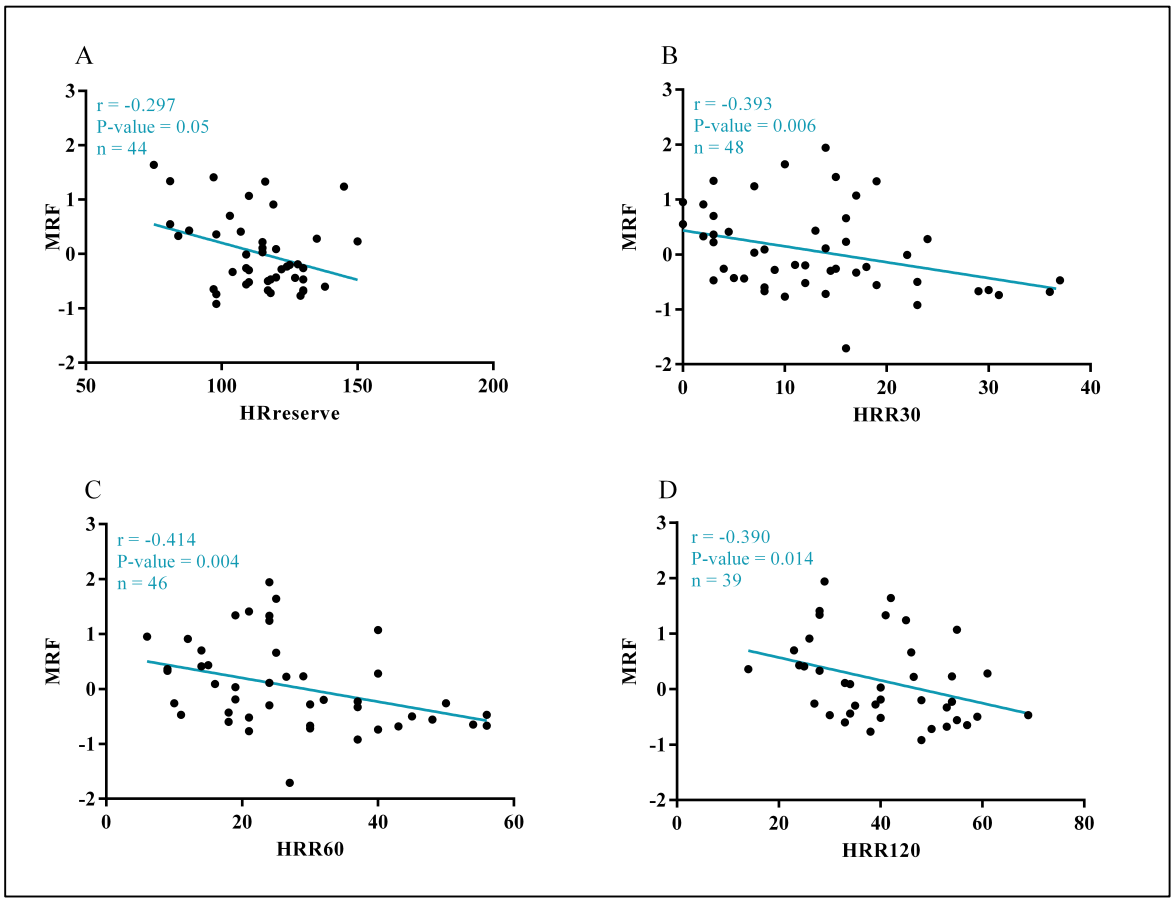

Figure 4: Correlations between MRF and (A) HRreserve, (B), HRR30s, (C) HRR60s, (D) HRR120s.

Abbreviations: MRF, metabolic risk factor; HRreserve, heart rate reserve (△HRpeak-HRrest); HRR, heart rate recovery (△HRpeak - HR at 30, 60, 120s)

Next, an adjusted linear model was used to associate MRF with each of the four autonomic heart function parameters. For each parameter, the best fitted model was chosen (according to adjusted R^2), initially adjusting the models for age, length, tanner stage and sex (Table 8). Even after adjusting, MRF stays a significant negative predictor for HRreserve, HRR30sec, HRR60sec, and HRR120sec. The model for MRF and HRreserve had a coefficient of determination (adjusted R^2) of 0.253 and the standardized coefficient β (SC β) for MRF in this model was -0.408 (p=0.009). MRF had an SC β of -0.33(p=0.017) in the model for HRR30 with an adjusted R^2 of 0.295. The corrected model between HRR60 gives MRF an SC β of -0.381(p=0.006), the goodness of fit in this model is represented by adjusted R^2 of 0.301. The last model, for MRF and HRR120, has an adjusted R^2 of 0.139 and gives MRF an SC β of -0.466.

3.8. Multivariate models for heart rate responses

Multivariate models were created for autonomic heart function parameters HRreserve, HRR30s, HRR60s and HRR120s and (i) metabolic parameters, (ii) cardiovascular parameters and (iii) ECG parameters (Table 8). For all predictors in the models, correlations were evaluated, results can be found in Appendix 4.

3.8.1. Metabolic parameter models for heart rate responses

HRreserve was negatively associated with WHR (adjusted R^2: 0.243, SC β: -0.298, p=<0.01). HRR30sec was significantly associated with blood leptin and ALP concentrations (adjusted R^2: 0.482). The relationships were different in direction, as blood leptin had a negative association with an SC β of -0.454(p=<0.01) and blood ALP was positively associated (SC β: 0.406, p=<0.01). The model compiled for HRR60s contained blood ALP, triglycerides, iron and Hb concentration (adjusted R^2: 0.540). Triglyceride concentration had a negative relationship (SC β: -0.394, p=<0.01), the rest of the predictors were positively related to HRR60s (SC β ALP: 0.405, p=<0.01; SC β iron: 0.229, p=0.05; SC β Hb: 0.297, p=0.01). HRR at 120s is predicted with an adjusted R^2 of 0.515 by blood CRP, triglyceride and ferritin concentrations. CRP had an SC β of -0.303 (p=0.013), triglyceride concentration had an SC β of -0.275 (p=0.01, and lastly SC β for ferritin is 0.5472 (p=<0.01).

3.8.2. Cardiovascular parameter models for heart rate responses

HRreserve was associated with VCO_2 at peak exercise (SC β: 0.404, p=<0.01), adjusted R^2 for this model is 0.142. The model assembled for HRR30s contained only mitral A-wave velocity as a predictor and had an adjusted R^2 of 0.295 (SC β A mitral -0.558, p=0.01). HRR at 60sec was positively correlated with peak VO_2/HR (SC β: 0.369, p=0.02) and negatively associated with mitral A-wave velocity (SC β: -0.421, p=0.01). This model had a goodness of fit indicated with an adjusted R^2 of 0.314. The model for HRR120s predictive with an adjusted R^2 of 0.076 compiling only Mitral E/A ratio (SC β: 0.314, p=0.04).

3.8.3. Electrophysiological parameter models for heart rate responses

Again, no significant predictors could be found for HRreserve and consequently a model was not generated. HRR at 30sec was correlated with peak PR-interval (adjusted R^2: 0.246) (SC β: 0.337, p=0.02). HRR60s was significantly associated with PR-interval both at rest and at peak exercise (adjusted R^2: 0.390). SC β for resting PR-interval is 0.278 (p=0.04) and for peak PR-interval it is 0.369 (p=0.01). The model composed for HRR120s contained only resting PR-interval (SC β: 0.490, p=<0.01) and the model was predictive with an adjusted R^2 of 0.221.

Table 8: Multivariate adjusted models for heart rate responses

	HRreserve			HRR 30			HRR60			HRR120		
	Adj. R^2	SC β	p-value	Adj. R^2	SC β	p-value	Adj. R^2	SC β	p-value	Adj. R^2	SC β	p-value
Metabolic Risk model	0.253[a]			0.299[a]			0.301[a]			0.139[b]		
Metabolic Risk		-0.408	**0.01**		-0.330	**0.02**		-0.358	**0.01**		-0.466	**<0.01**
Metabolic parameters model	0.243[c]			0.482			0.540			0.515		
WHR		-0.298	**0.037**									
Leptin (ng/ml)					-0.454	**<0.01**						
ALP (IU/L)					0.406	**<0.01**		0.405	**<0.01**			
Triglycerides (mg/dl)								-0.394	**<0.01**		-0.334	**<0.01**
CRP (mg/dl)											-0.303	**0.013**
Iron (µg/dl)								0.229	**0.05**			
ferritin (ng/ml)											0.472	**<0.01**
Hb (g/dl)								0.297	**0.01**			
Cardiovascular parameters model	0.142			0.295			0.314			0.076		
Peak VCO_2 (ml/min)		0.404	**0.008**									
Peak VO_2/HR								0.369	**0.02**			
A mitral (cm/sec)					-0.558	**0.01**		-0.421	**0.01**			
Mitral E/A ratio											0.314	**0.04**
ECG parameters model				0.246[a]			0.390[a]			0.221		
Rest PR-interval (ms)								0.278	**0.04**		0.490	**<0.01**
Peak PR-interval (ms)					0.337	**0.02**		0.369	**<0.01**			

Abbreviations: Adj., adjusted; HRreserve, heart rate reserve (△HRpeak-HRrest); HRR, heart rate recovery (△HRpeak - HR); SCβ, standardized coefficient β; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; CRP, c-reactive protein; Hb, Haemoglobin; VCO2, carbon dioxide output; VO2/HR, oxygen pulse; E mitral, transmitral peak early diastolic velocity; A mitral, transmitral peak late diastolic velocity

[a], adjusted for sex

[b], adjusted for age, length, tanner stage and sex

[c], adjusted for body length

4. Discussion

The aim of this clinical study was to examine whether HR responses during cardiopulmonary exercise testing may be an early indicator for cardiovascular anomalies in obese adolescents. The main findings of our study are that heart rate reserve as well as heart rate recovery are significantly altered in obese subjects. Moreover, significant associations are found between these parameters and parameters indicative of cardiovascular function.

4.1. Markers for cardiovascular abnormalities

Comparing the OG and LG, many important different changes are visible, which are associated with cardiovascular outcomes. First, evidence of the MetS is seen in the obese population. This cluster of four risk factors, leading to increased risk of cardiovascular disease, can be observed the OG (62). Psys and Pdia are elevated (hypertension), triglyceride concentration in the blood is augmented (hypertriglyceridemia), the concentration of blood HDL cholesterol is lower and blood insulin concentration is higher (hyperinsulinemia). MRF confirms this finding: obese subjects have an overall much higher score. This factor is based on triglyceride/HDL ratio, insulin concentration, MAP (a measure blood pressure) and additionally, WC (52). WC had been indicated as a marker of cardiovascular health, and WHR, another parameter that was substantially heightened in obese subjects, is often used in cardiometabolic risk assessment in children (63, 64).

In the results from the ECG analyses, differences in resting RR-interval are seen, however, RR-interval in this case is only a proxy for HR, it represents the time between two heart depolarisations and consequent heart beats (65). HRrest is significantly higher in the obese groups and thus follows that the RR-interval is shorter. PR-interval again is shortened in the OG, though, this time both at rest and at peak exercise. At rest, the shorter PR-interval could be associated with the faster HR, but since the HRpeak is the same in both groups, the PR-interval difference at peak exercise cannot be explained in this way. What is more, other studies have found opposite results, that PR-interval is longer in obese adolescents (66) and that in adults it prolonged PR interval has been associated with adverse cardiovascular outcomes (67).

Sokolow-index was significantly higher both at rest and at peak exercise in the LG. A high Sokolow-index can indicate left ventricular hypertrophy (68). Sokolow-index, however, is based upon the signal transduction of the electrical activity of the heart. In obese individuals, the signal must travel through a

larger amount of tissue, and thus some of the signal is lost (69). In this way, the higher Sokolow-index in the LG can be rationalised and is does not imply any effects on cardiovascular health.

CIMT in lean subjects is significantly higher in lean subjects, this is contradictory to other findings (70, 71). A possible clarification for this finding is the relationship between androgen concentrations and CIMT, in which higher androgen concentrations are associated with thicker carotid intima media (71, 72). However, androgens were not tested for in our study population, so this remains speculation. Nonetheless, lower concentrations of testosterone have been reported in obese adolescents (73).

In echocardiography, a larger left ventricular septum width is seen in obese subjects. LV septum width can be a predictor for left ventricular hypertrophy, and this has been shown to be present in obese adolescent, possibly due to the effects of high blood pressure (74). Left atrial diameter, an important risk factor for atrial fibrillation in adults (75), is also enlarged in the OG. No other parameters were significantly different between the two groups, but a trend is seen in E/e' ratio, which is higher in obese subjects and is a measure of left ventricular diastolic function. A higher ratio, in this case, can predict a worse outcome, it indicates reduced passive filling capacity of the left ventricle during diastole (76).

4.2. Cardiopulmonary testing outcomes

Our data suggests that peak cycling power output is significantly lowered in obese subjects. Nadeau (2009) and Mendelson (2012) confirm these findings (29, 30). HRpeak, however, was not different between groups in our population, which is contradictory to some other findings (29, 31), but again, other studies find the same (30). Peak cycling power output is dependent on the interconnection between intrinsic characteristics of the muscle tissue (amount of tissue, efficiency of energy metabolism,…), the cardiovascular function (efficient distribution of oxygen) and ability of the respiratory system to extract oxygen from the air and elimination of carbon dioxide from the bloodstream (37). Since VO_2 peak is the same in both groups, metabolic demand must be greater in the OG, for the same workload. A few mechanisms can explain this phenomenon. Obese subjects experience a higher demand on muscles due to the increased weight (77) and ventilatory work requires extra metabolic power to compensate for the pressure that is put on the chest by the increase in weight (78). Moreover, obese adults show an increased proportion of type IIb- muscle fibers. This type of fibers, which are more glycolytic, is less efficient in metabolism and is less resistant to fatigue (79).

During recovery from exercise, HRR is significantly slowed in obese subjects. Also, HRreserve is significantly lowered in the OG. This difference in HR reserve, which is defined as the peak HR minus the resting HR, is the effect of the higher HRrest in the obese patients since the HRpeak is not different

between groups. Other studies have also seen this occurrence of a lower resting HR in obese adolescents (80, 81). A possible clarification for this higher resting HR is dysregulation of the autonomic nervous system (ANS). The sympathetic nervous system (SNS) has been shown to be over- active, while parasympathetic nervous system (PNS) activity is reduced in obese adolescents (33, 82). However, other studies have found an opposite effect and found an under-activation of the SNS as well as the PNS (83, 84). Our findings confirm the first outcomes since a higher resting HR is the symptom of over-activation of the SNS and possible reduced PNS activity. The finding that HRR recovery is slowed in the obese subjects confirms this and was established by other studies that find the same (32, 85). HRR was calculated as the difference between HRpeak and the HR 30, 60 and 120 seconds after the secession of the maximal exercise test. HRR can be used as a measure of ANS dysfunction. The short term HRR, at 30 and 60 seconds at could be considered the consequence of cardiac parasympathetic activity, while HRR at 120 seconds is related to withdrawal of sympathetic activity and to the clearance of adrenal influences (86). Furthermore, slowed HRR is associated with higher mortality risk (87, 88).

The correlations between MRF and these autonomic heart function parameters are often seen in literature. Many studies speak of dysfunctional autonomic function in the presence of metabolic syndrome in obese adolescents, of which MRF is a predictor (89, 90).

In the models with other metabolic parameters for HRR, an association is seen between blood leptin concentration and HRR30. Evidence suggests leptin stimulates sympathetic activity (91), and as leptin resistance and an increase in blood leptin are often seen in obese patients (92), it could be one of the initiating factors of dysfunction of the ANS.

An association with both HRR30 and HRR60 is seen with blood ALP concentration. The higher the concentration, the better the outcome in the form of higher HRR. ALP is mostly formed in the liver and bone tissue and higher concentrations of serum ALP have been associated with MetS (93). However, multiple forms of ALP are found in the body, evidence suggests that intestinal alkaline phosphatase deficiency is linked to development of MetS and T2DM, and that higher concentration s are protective (94). This could explain the link with HRR, although further evaluation is needed.

Triglyceride concentration in the blood is negatively associated with HRR60 and HRR120, this can be explained trough the mechanisms of MetS, since hypertriglyceridemia is a risk factor included in the MetS (23). Blood CRP, a marker for inflammation, is negatively correlated with HRR120, evidence suggest an association between inflammatory pathways and the ANS (95). Lastly in the metabolic parameters models, positive associations were found between blood iron and Hb concentrations and

HRR60 and ferritin and HRR120. Although no evidence exist linking these mechanisms, blood iron, Hb and ferritin are all involved in the transport of oxygen in the blood, and deficiencies can implicate anemia (96). Moreover, anemia has been associated with ASN dysfunction (97). Linking these two mechanisms can explain the associations between HRR and blood iron, Hb and ferritin concentrations.

The current study suggests HRreserve is associated positively with peak VCO_2. A high peak VCO_2 suggest a better overall exercise performance (98), possibly this explains the association with better HRreserve outcomes. Peak VO_2/HR is also positively associated with HRR 60. A higher oxygen pulse, again, is a sign of improved exercise performance since more oxygen per heart beat is pumped, possibly due to higher stroke volumes (99). Stroke volume during exercise is regulated by the SNS (100), it is thus likely that normal function of the SNS is related to better HRR outcomes.

HRR30 and HRR60 are negatively associated with mitral A-wave velocity. Decreased velocity of the A-wave can point to diastolic dysfunction. Although SNS over-activation has been linked to systolic dysfunction(101), its role in diastolic dysfunction remains to be investigated(102). Mitral E/A ratio is positively associated with HRR120. In normal diastolic function, E-wave velocity is higher than the A-wave velocity, and a higher ratio is thus better. However, a much higher E/A ratio can indicate diastolic dysfunction (103). Conversely, because all our patients were below this boundary of E/A function, this association is positive.

The lasts models include ECG-parameters. They indicated PR-interval at rest and at peak exercise are associated with HRR. It is likely that PR-interval is related more to HR than any abnormalities in electrophysiological function, and that the association is more related to HR. Resting HR confirms this theory, a longer PR-interval would indicate a lower HR and that is a positive predictor for HRR. However, because HRpeak is not different between groups, the correlation with peak PR-interval is not clear. Evidence suggests that, normally, peak PR-intervals decrease with exercise due to withdrawal of parasympathetic tone(104). Since HRR dysregulation implies that PNS activity is reduced, this cannot elucidate the association.

5. Conclusion

Significant alterations in HRreserve and HRR suggest cardiovascular autonomic function is distorted in obese adolescents. HRreserve and HRR are associated with metabolic risk factors seen in obesity and factors indicative of cardiovascular abnormalities. Although the data indicates that HR at peak exercise is not changed in obese adolescents, HRreserve and HRR might be of prognostic value for cardiovascular disease risk in obese adolescents. Further research should be implemented to discover the associations between cardiac autonomic function and adverse cardiac events and the aetiology behind the development of distorted autonomic function in obese adolescents.

6. Limitations

Our study has a few limitations. Although the target sample size was achieved in this study and sufficient power was generated, a larger sample size could provide more sturdy associations between outcomes. Fat percentage was assessed using BIA and SCF, but more valid methods of assessing body composition are available, for example dual-energy X-ray absorptiometry (DXA). An important implication for performance in the CPET could be amount of physical activity. The PAQ-A that was used in this study provides an idea about physical activity, but questionnaires are always subject to bias form the participant. Systematic data-collection using accelerometers could provide more objective and correct information on the physical activity levels.

7. Future perspectives

Heart rate recovery and heart rate reserve in adolescents deserve more attention. Possible longer recovery time with cardiopulmonary evaluation could provide more information still about the heart rate recovery. Echocardiography during exercise testing would also provide more insight into cardiac function during exercise. Heart rate variability (HRV) is also often used to evaluate cardiac autonomic function, using spectrum analysis on the RR-intervals of the ECG could provide an insight into the HRV during exercise.

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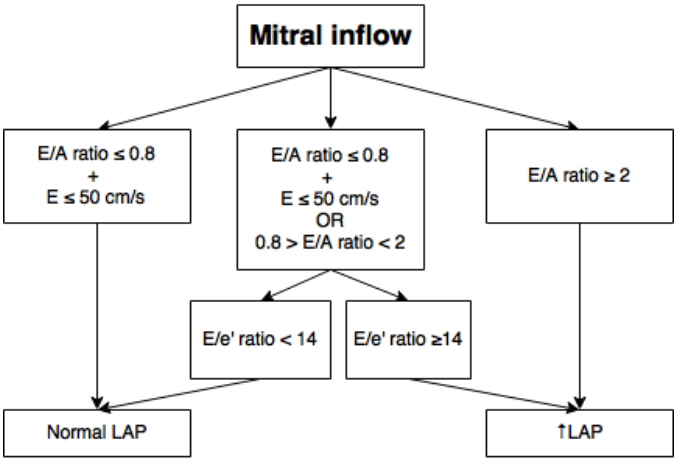
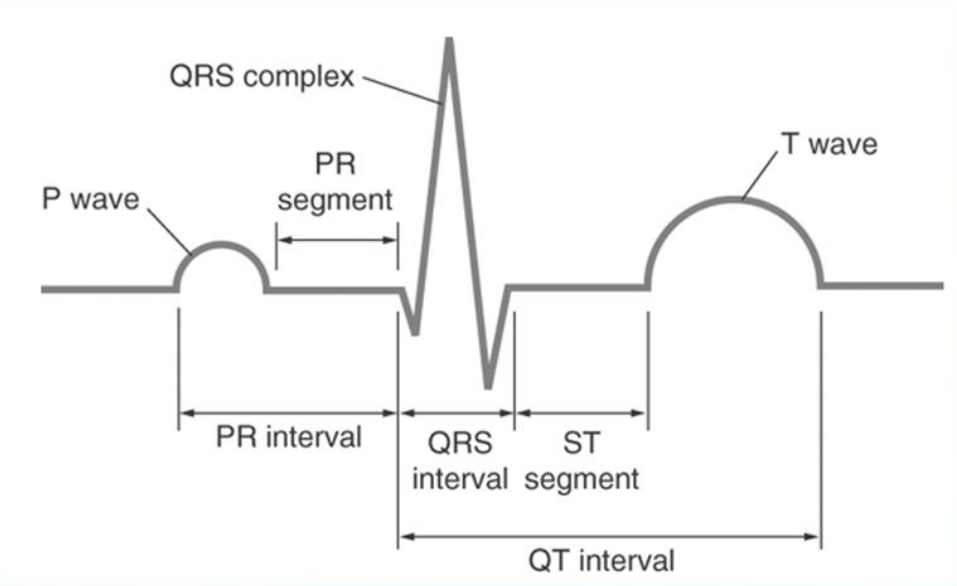
9. Appendices

Testing variable	Additive	Product	Brand
Glucose CRP	Fluoride- Oxalaat	Vacutainer ® Blood collection tube	BD (New Jersey, USA)
Iron AST GGT ALT Uric Acid K Ca Cholesterol HDL LDL Triglycerides	Lithium heparine		
ALP Ferritin TSH Free T4 Cortisol Insulin Hb1ac	Clot activator		
Leptin Hemoglobine Hematocrit White blood cells	K2EDTA		

Appendix 1: Blood collection tubes used in fasting blood sampling



Appendix 2: Algorithm to evaluate left atrial pressure (LAP) (56)



Appendix 3: Intervals of the ECG (65)

	HRreserve		HRR 30		HRR60		HRR120	
	r	p-value	r	p-value	r	p-value	r	p-value
Metabolic Risk model								
Metabolic Risk	-0.297	0.05	-0.393	0.0006	-0.414	0.004	-0.39	0.014
Metabolic parameters model								
WHR	-0.369	0.014						
Leptin (ng/ml)			-0.462	0.001				
ALP (IU/L)			0.578	<0.001	0.508	<0.001		
Triglycerides (mg/dl)					-0.48	0.001	-0.411	0.009
CRP (mg/dl)							-0.468	0.003
Iron (μg/dl)					0.373	0.012		
ferritin (ng/ml)							0.033	0.844
Hb (g/dl)					0.222	0.138		
Cardiovascular parameters model	0.142							
Peak VCO_2 (ml/min)		0.008						
Peak VO_2/HR					0.255	0.087		
A mitral (cm/sec)			-0.398	0.007	-0.36	0.018		
Mitral E/A ratio							0.617	<0.001
ECG parameters model								
Rest PR-interval (ms)					0.489	0.001	0.489	0.002
Peak PR-interval (ms)			0.387	0.007	0.562	0		

Abbreviations: r, Spearman's rho correlation coefficient; HRreserve, heart rate reserve (△HRpeak-HRrest); HRR, heart rate recovery (△HRpeak - HR); AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; CRP, c-reactive protein; Hb, Haemoglobin; VCO2, carbon dioxide output; VO2/HR, oxygen pulse; E mitral, transmitral peak early diastolic velocity; A mitral, transmitral peak late diastolic velocity

Appendix 4: Correlations between predictors of the heart rate response models

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Heart rate responses during exercise in obese adolescents: relation with cardiovascular health

Richting: **Master of Biomedical Sciences-Environmental Health Sciences**
Jaar: **2017**

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Beyens, Marjolein

Datum: **8/06/2017**