

**PHYSICAL FITNESS AND AUTONOMIC DYSFUNCTIONS IN
CHILDHOOD OBESITY**

PhD thesis

Author: Katalin Török, M.D.

**Department of Paediatrics, Medical Faculty,
University of Pécs**

Head of the Doctoral School: Gábor L. Kovács MD, PhD, Dsc

Programme leader: Dénes Molnár MD, PhD, Dsc

Tutor: Dénes Molnár MD, PhD, Dsc

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ABBREVIATIONS

A	age
ABPM	ambulatory BP monitoring
ANOVA	analysis of variance
AT	anaerobic threshold
AUC	area under the curve
BF	body fat
BMI	body mass index
BP	blood pressure
BW	body weight
CAN	cardiac autonomic neuropathy
CI 95%	confidence interval 95%
CRF	cardiorespiratory fitness
CV	cardiovascular
CVD	cardiovascular diseases
DBHR	heart rate variation during deep breathing
DBP	diastolic blood pressure
DBP₀	diastolic blood pressure at rest,
DBP_{peak}	diastolic blood pressure at peak level
ED	exercise duration
HELENA-CSS	Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study
HDL-c	high density lipoprotein cholesterol
HOMA	homeostasis model assessment
HOMA-IR	homeostasis model assessment index
HR	heart rate
HR₀	resting heart rate
HR_{peak}	peak heart rate
IGTT	impaired glucose tolerance test
IMCL	intramyocellular fat
LAT	lactic acidosis threshold
LAT-BW	lactic acidosis threshold normalised for body weight
LBM	lean body mass
LR –	negative likelihood ratios
LR +	positive likelihood ratios
MS	metabolic syndrome
MVPA	moderate to vigorous physical activity
OGTT	oral glucose tolerance test
OT	fall in systolic blood pressure on standing
PA	physical activity
PWC	physical working capacity
PWC-130	physical working capacity at 130 beats/min
PWC-130-BW	physical working capacity at heart rate 130 normalised for body weight
PWC-150	physical working capacity at 150 beats/min
PWC-150-BW	physical working capacity at heart rate 150 normalised for body weight
PWC-170	physical working capacity at 170 beats/min

PWC-170-BW	physical working capacity at heart rate 170 normalised for body weight
RHR	resting heart rate
ROC	receiver operating characteristics
S	final speed
S/L	standing-lying heart rate ratio
SBP	systolic blood pressure,
SBP₀	systolic blood pressure at rest
SBP_{peak}	systolic blood pressure at peak level,
SH	rise in diastolic blood pressure during sustained handgrip
SNS	sympathetic nervous system
T2DM	type 2 diabetes mellitus
TC	total cholesterol
TC/HDL-c ratio	total cholesterol / high density lipoprotein cholesterol ratio
VCO₂	carbon dioxide production
VO₂	oxygen consumption
VO₂max	maximal oxygen consumption
VO₂peak	peak oxygen consumption
VO₂peak-BW	peak oxygen consumption normalised for body weight
VO₂rest-BW	resting oxygen consumption normalised for body weight
W/H	waist per hip ratio

CONTENT

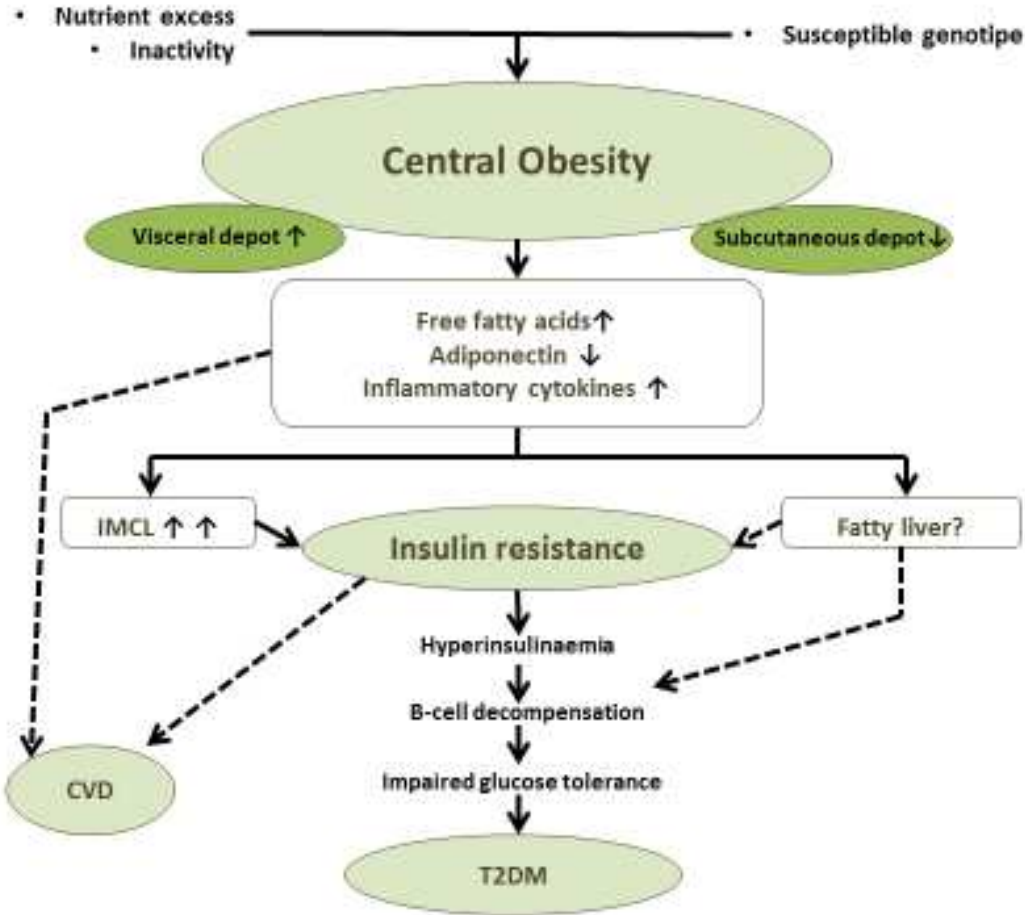
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I. INTRODUCTION

The prevalence of childhood overweight and obesity has increased at a high speed during the past three decades in most industrialised countries, and in several low income countries, especially in urban areas. Prevalence doubled or trebled between the early 1970s and late 1990s in Australia, Brazil, Canada, Chile, Finland, France, Germany, Greece, Japan, the UK and the USA. In the United States, the prevalence of obesity in children aged 6-11 years and 12-19 years increased from 4% and 6% in 1971-1974 to 15% in both age groups in 1999-2000 [1,2]. In Hungary, the prevalence of overweight and obesity among school children increased from 11.8% in the 1980s to 16.3% in the 1990s, then to 25.7% in 2005 [4,5]. The 2013 data showed in Hungary 30.3% of boys and 24.9% of girls were overweight or obese and 7.9% of boys and 6.1% of girls were obese in ages 2-19 years [3]. The increasing prevalence seems to have slowed or plateaued in many countries. The 2011 review data from nine countries representing numerous regions around the globe including Western Europe, North America, Asia and Oceania showing a slowing trend and in some countries a decline in the prevalence of childhood obesity and overweight between 1995 and 2008 [6]. While the prevalence of childhood overweight and obesity appears to be stabilising at different levels in different countries, it remains high and a significant public health issue. Obesity is associated with significant health problems in the pediatric age group and is an important early risk factor for adult morbidity and mortality, including cardiovascular diseases (mainly heart disease and stroke), diabetes, musculoskeletal disorders, especially osteoarthritis and certain types of cancer (endometrial, breast and colon). At least 2.6 million people on the world each year die as a result of being overweight or obese.

Many of the metabolic and cardiovascular complications of obesity are already present in childhood and are closely related to the presence of insulin resistance/hyperinsulinaemia, the most common abnormality seen in obesity [7,8]. The obesity-related morbidities that emerge early in childhood are alterations in insulin resistance and fatty infiltration of the liver (Figure 1); although an accelerated atherogenic process is present, the clinical cardiovascular lesions appear later [9]. Childhood obesity can adversely affect almost every organ system and often has serious consequences in addition to those mentioned, including hypertension, dyslipidaemia, insulin resistance or diabetes, fatty liver disease, and psychosocial complications. Obesity-associated hypertension, hyperinsulinaemia, impaired glucose tolerance and dyslipidaemia are considered to be separate and independent risk factors for cardiovascular and cerebrovascular diseases in adulthood [8,10,11]. Recently a growing level of interest has focused on new risk factors for cardiovascular disease, such as insulin, leptin, adiponectin, homocysteine and urinary albumin excretion. A recent study carried out among American youth demonstrated that half of them presented at least one cardiometabolic risk factor [12].

Figure 1 Mechanisms of obesity related morbidities



IMCL: intramyocellular fat, **CVD:** cardiovascular diseases, **T2DM:** type 2 diabetes mellitus

The aggregation of cardiovascular risk factors (hyperinsulinaemia, impaired glucose tolerance, dyslipidaemia and hypertension) has been well demonstrated in obese individuals [13,14,15]. The clustering of these risk factors, called metabolic syndrome (MS), has also been shown in both children and adults [8]. Our working group has investigated the occurrence of MS and its components in childhood obesity [16]. We demonstrated that only 14.4% of obese children were free from any risk factors. The frequency of MS was 8.9% in obese children. We could not prove any gender difference in the prevalence of MS in obese children. Obese children compared to non-obese children had a 19.35 times higher risk of developing at least

one cardiometabolic risk factor (odds ratio 19.35, CI 11.59 ± 32.3) and a 6.29 times higher risk of having more than one risk factor (odds ratio 6.29, CI 2.96 ± 13.4). The duration of obesity was significantly longer in subjects with three or four risk factors than in those with fewer than three risk factors. This finding supports the hypothesis that the development of MS is a long-term process. The cardiovascular risk factors tend to track into adulthood when they are left untreated. Concerning the definition of MS in children and adolescents, no consensus has been reached yet [17]. From a methodological perspective, the use of a clustered cardiometabolic risk score derived from systolic blood pressure, HOMA-IR (homeostasis model assessment index), triglycerides, TC/HDL-c ratio, VO₂max and the sum of four skinfolds) is also recommended in the studies examine the effect of main metabolic cardiovascular diseases risk factors [18,19].

It is well known that sedentary lifestyle, obesity, decreased physical fitness and cardiovascular risk factors are interrelated [20,21]. Cross-sectional studies have documented the relationship between physical activity (PA), physical fitness and health, and a number of cardiovascular risk factors already during childhood and adolescence [22,23]. Longitudinal studies have shown that the degree of physical fitness during childhood and adolescence may determine physical fitness as an adult. In addition, poor physical fitness during these periods of life seems to be associated with later cardiovascular (CV) risk factors such as hyperlipidaemia, hypertension and obesity [24,25]. Martinez-Gómez *et al.* found that adolescents with a high level of sedentary behaviour (using accelerometry) had less favourable systolic blood pressure, triglyceride and glucose levels and a higher cardiovascular risk score [26]. Recent observations suggest that preventive efforts focused on maintaining physical fitness and activity through puberty may have favourable health

benefits in later years. Physical activity is a behaviour, because of its complex nature, that is difficult to assess under free-living conditions. No single method is available to quantify all dimensions of the activity (intensity, frequency, duration). These can be measured using subjective methods, such as questionnaires, or objective methods, such as motion sensors or heart-rate monitors. Physical fitness encompasses all the physical qualities of a person. The state of physical fitness can be considered an integrated part of all the functions and structures involved in the performance of physical exertion. Health-related physical fitness involves cardiorespiratory fitness (CRF), muscle strength, speed and agility, coordination, flexibility and body composition. These can be assessed by the cardiopulmonary exercise test, jump test and shuttle run test.

In a study by Martin-Matillas *et al.* the association between adolescents' PA levels and their relatives' PA participation and encouragement were examined [27]. The results showed that relatives' PA encouragement was more strongly associated with adolescents' PA levels than relatives' PA participation. Their findings indicate that younger adolescents are more physically active than their older counterparts, and PA shows a decreasing trend with age.

Ruitz *et al.* observed negative associations between CRF and insulin resistance, blood pressure and low-grade inflammatory proteins in young people with relatively high levels of total and central body fat [28]. The study by Jiménez-Pavón *et al.* found a negative association between PA and serum leptin concentration [29].

A randomised, controlled trial investigated the effects of a PA program on systemic blood pressure and early markers of atherosclerosis in 44 prepubertal obese children, without dietetic or psychological intervention [30]. At baseline, 24-hour blood pressure, arterial stiffness, body mass index, body fat and insulin resistance

indices were higher, while VO_2 max was lower in obese compared with lean subjects. The three-month PA program (three 60-minute sessions per week) resulted in significant reductions in systemic blood pressure, BMI (body mass index) z-score and total and abdominal adiposity, and increases in fat-free mass and CRF. However, a recent worldwide survey in adolescents revealed that only 12-42% of 13-year olds and 8-37% of 15-year olds met the recommended 60 min moderate- to-vigorous intensity daily PA [31]. Consequently, the promotion of PA has become an international public health concern.

Physical performance of obese children is generally decreased, particularly in activities requiring lifting of the body [32]. Considerable controversy exists as to whether this decreased exercise capacity is due to increased weight *per se*, to a lack of PA or to the metabolic consequences of fatness [20,33].

Physical exercise requires the interaction of physiologic mechanisms that enable the cardiovascular and respiratory systems to support the increased energy demands of contracting muscles. Both systems are consequently stressed during exercise; their ability to respond adequately to this stress is a measure of their physiologic competence. Cardiopulmonary exercise testing can be used provide an objective assessment of exercise capacity and impairment. The oxygen consumption (VO_2) at which this anaerobic supplementation of the aerobic energy exchange begins has been termed the anaerobic threshold (AT). The AT is defined as the level of exercise VO_2 above which aerobic energy production is supplemented by anaerobic mechanisms and is reflected in an increase in the lactate and lactate/pyruvate ratio in muscle or arterial blood. Above the AT the net increase in lactic acid production results in an acceleration of the rate of increase in carbon dioxide production (VCO_2) relative to VO_2 . Maximal VO_2 should be contrasted with the maximum or peak VO_2 ,

which is simply the highest VO_2 achieved for a given, presumed maximal exercise effort.

The body clearly has an upper limit for oxygen utilisation at a particular state of fitness. This is determined by the maximal cardiac output. In obese subjects more energy is needed to move heavy legs in cycling exercise or to move the large body mass while ambulating in addition to that needed to perform external work. As adipose tissue is added to the body, commensurate growth of the heart and blood vessels to meet this abnormally elevated metabolic requirement of muscular activity does not occur. An obese individual needs greater than normal cardiovascular and ventilatory responses in order to perform any physical work. Constraints are imposed on the maximal performance of the cardiovascular and ventilatory systems by obesity, especially in extremely obese subjects. Because of the large mass, resting cardiac output per kilogram of lean body weight is already high. During exercise, further increase in cardiac output is limited. The added mass on the chest wall and the constraining pressure from the abdomen effectively “chest strap” the patient and cause the resting end-expiratory lung volume to be reduced. In addition, pulmonary vascular resistance can be increased, primarily as a result of pulmonary insufficiency, but also possibly from mechanical kinking of blood vessels at low lung volume. The maximum VO_2 and AT are low when related to body weight, but they are usually normal when related to height and lean body mass. Observational studies have shown that childhood adiposity is associated with an unfavourable metabolic profile, which continues into adulthood. Because of the strong inverse correlation between cardiorespiratory fitness and fatness, it is possible that deleterious consequences ascribed to adiposity may be partially due to the influence of low CRF. The positive influence of PA on both fitness and fatness has also been

recently shown [34]. Higher levels of CRF and PA are associated with a reduced incidence of metabolic-related diseases in adults [35].

Studies examining associations between PA, CRF and metabolic risk factors are limited and generally confined to questionnaire-based assessments of PA, which often lack the necessary accuracy, especially in children. Recently it has been shown that there might be an independent, inverse relationship between objectively measured PA and metabolic risk factors after adjustment for CRF in children.

The study by Rizzo *et al.* suggests that total PA does not have an independent effect on metabolic risk factors in children and adolescents and that CRF is more strongly correlated to metabolic risk than total PA [36]. Body fat seems to play a pivotal role in the association of CRF with metabolic profile. Higher levels of CRF and PA are associated with a reduced incidence of metabolic-related diseases in adults. It is known that aerobic exercise results in physiological adaptations of skeletal muscle cells. Some of the physiological adaptations that might furnish protective mechanisms in relation to metabolic syndrome factors include an increase in capillary supply to skeletal muscles, increase in the activities of enzymes in the mitochondrial electron transport chain, and a concomitant increase in mitochondrial volume and density. Additionally, an increased substrate use with a decrease of carbohydrate oxidation, as well as improved insulin sensitivity, may play a part in the protective adaptations triggered by PA on metabolic risk factors.

There is increasing evidence that physically active subjects have a better cardio-metabolic profile than less active ones [18].

Long duration of obesity in adulthood can be associated with an increased incidence of ischemic heart disease, malignant ventricular arrhythmias and sudden cardiac death. Apart from the components of metabolic syndrome, autonomic

nervous system dysfunction may also be involved in the development of cardiovascular complications [37]. Alterations in the autonomic nervous system can cause disturbances in the function of cardiovascular, gastrointestinal and genitourinary systems and regulation of energy metabolism as well [38]. Among these consequences the most unfavourable is the involvement of the cardiovascular system - the cardiac autonomic neuropathy (CAN). CAN results from damage to the autonomic nerve fibres that innervate the heart and blood vessels, increased baroreceptor intima-media thickness, reduced vascular distensibility and endothelial dysfunction. CAN causes alterations in heart-rate and modulation of vascular dynamics, which result in resting tachycardia, abnormal myocardial blood flow regulation and impaired cardiac function [39,40]. Obesity and metabolic syndrome are characterized by sympathetic nervous system (SNS) predominance in the basal state and reduced SNS responsiveness after various sympathetic stimuli [41,42]. SNS activity is associated with both energy balance and metabolic syndrome. Reciprocal associations exist between SNS activity and food intake. Sympathetic activation via the hypothalamic regulatory feedback reduces food intake and inhibits leptin production and secretion [41]. Leptin furthermore inhibits ectopic fat accumulation, prevents β -cell dysfunction and protects the β -cell from cytokine- and fatty acid-induced apoptosis [43]. Activation of the SNS also has additional effects on energy homeostasis through incremental changes in resting metabolic rate and thermogenesis [44,45]. Hyperglycaemia plays a key role in the activation of the metabolic and/or redox state of the cell, which impairs nerve vascular perfusion, contributing to the development and progression of CAN [46,47]. Initially, CAN is characterized by increased sympathetic activity with increased resting heart rate [48]. The initial augmentation in cardiac sympathetic activity with subsequent

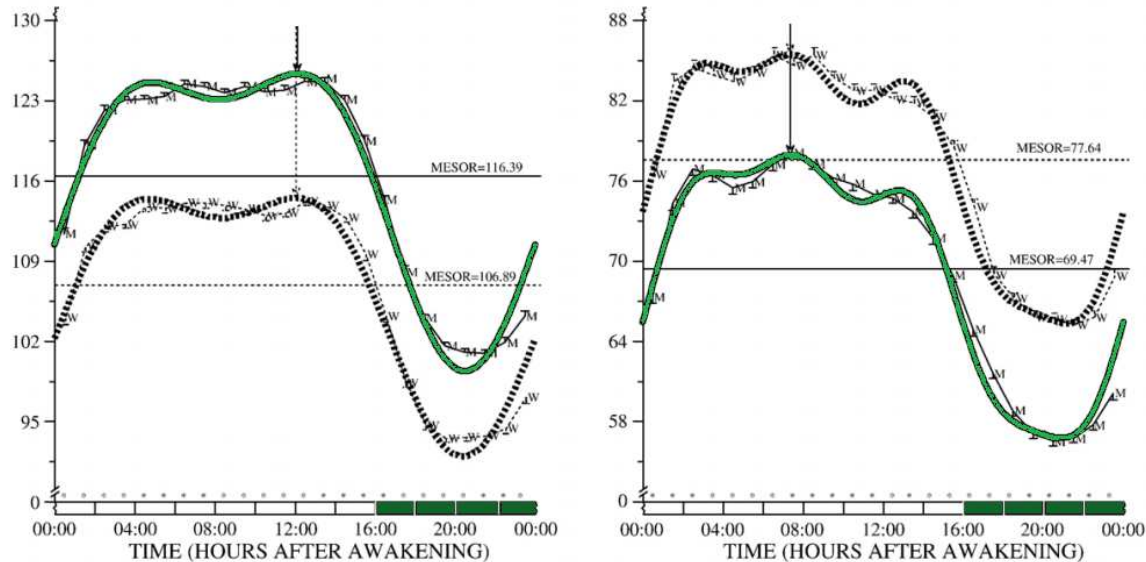
abnormal norepinephrine signalling and metabolism increases mitochondrial oxidative stress and calcium dependent apoptosis and may therefore contribute to myocardial injury, thereby explaining the increased risk of cardiac events and sudden death [49,50]. Autonomic dysfunction impairs exercise tolerance, increases resting heart rate and blood pressure and alters cardiac output responses to exercise [50]. Exercise leads to significant improvements in cardiac autonomic function in obesity and diabetes and can decrease the associated comorbidities that can be maintained over time [51].

It is well known that cardiovascular autonomic neuropathy is associated with abnormal diurnal blood pressure (BP) variables at adulthood [52,53]. Ambulatory blood pressure monitoring provides the opportunity to study blood pressure patterns during the day and night. An endogenous basis for the 24-hour variation, a relationship between the circadian clock and the BP rhythm, is suggested by laboratory rodent studies showing that lesioning of the suprachiasmatic nucleus (the master circadian clock located in the hypothalamus of the brain) abolishes the circadian rhythms of BP and heart rate (HR) without affecting the sleep-wake and motor activity 24-hour cycles [54-56]. The sleep-wake cycle results from the alternation of mutually inhibitory interactions of arousing or activating systems on the one hand, and of hypnogenic or deactivating systems on the other hand. Cyclic variation in autonomic nervous system activity plays an important role not only in the mechanism of the sleep-wake rhythm, but also in mediating the influence of sleep and wakefulness on cardiovascular function and BP, in general [57]. Serotonin, arginine vasopressin, vasoactive intestinal peptide, somatotropin, insulin and steroid hormones and metabolites are involved in the induction of sleep, whereas corticotropin-releasing hormone, ACTH, thyrotropin-releasing hormone, endogenous

opioids and prostaglandin E₂ are involved in activation and arousal. The cyclic 24-hour pattern in these chemical mediators is bound to be reflected in the phasic oscillations in cardiovascular function (Figure 2) [58]. Other variables are also involved in BP regulation. For example, previous research has demonstrated predictable circadian variability in plasma renin activity, angiotensin-converting enzyme, angiotensin II, aldosterone, atrial natriuretic peptide, and catecholamines [59,60]. In human beings, plasma renin activity peaks during the last hours of night-time sleep and is at its nadir in the early afternoon [61]. This variation seems to be related to the rest-activity rhythm rather than the environmental light and dark cycle. The circadian pattern in renin activity influences other factors integrating the renin-angiotensin-aldosterone system, including the circulating levels of angiotensin I and angiotensin II [62]. Plasma aldosterone also presents a circadian pattern with high hormone concentrations at the beginning of daily activity and reduced ones at the beginning of nocturnal rest. The high amplitude circadian rhythm in atrial natriuretic peptide also plays a role in 24-hour BP regulation. Circadian rhythms in autonomic nervous system function are well known; sympathetic tone is dominant during the diurnal activity span, while vagal tone is dominant during most of the night-time sleep span [63]. This day-night oscillation of the autonomic nervous system is strongly linked to the sleep-wake circadian rhythm and plays a dominant role in the observed 24-hour BP variation in both normotension and uncomplicated essential hypertension. In persons with normal BP and uncomplicated essential hypertension, BP declines to lowest levels during night-time sleep, rises abruptly with morning awakening and attains near peak values during the first hours of diurnal activity (Figure 2). In so-called normal dippers, the sleep-time BP mean is lower by 10-20%

compared to daytime mean. In addition, the typical circadian pattern of BP exhibits two daytime peaks.

Figure 2 Circadian pattern of SBP (left) and HR (right) in young clinically healthy normotensive men and women sampled by ABPM for 48 consecutive hours



Hermida et al. *Advanced Drug Delivery Reviews* 59 (2007) 904–922

M: men, W: women, SBP: Systolic blood pressure, HR: Heart rate.

* $p < 0.05$ difference in hourly mean between groups

(thin lines) hourly means and standard errors, (continuous line) data collected from men, (dashed line) data collected from women, (thick lines) nonsinusoidal-shaped curves represented around means and standard errors correspond to the best-fitted waveform model determined by population multiple-component analysis for each gender. Arrows descending from upper horizontal axis point to the circadian orthophase (rhythm crest time). The lower horizontal axis represents circadian time of sampling (in hours after awakening from nocturnal sleep). Average duration of sleep across all individuals is represented by the dark bar on the lower horizontal axis.

Using the ratio calculated as $100 \times [(\text{mean diurnal BP} - \text{mean nocturnal BP}) / \text{mean diurnal BP}]$, patients have been classified as dippers or non-dippers (diurnal/nocturnal ratio $< 10\%$). More recently, this classification has been extended by dividing the patients into four possible groups: extreme-dippers (diurnal/nocturnal ratio $\geq 20\%$), dippers (diurnal/nocturnal ratio $\geq 10\%$), non-dippers (diurnal/nocturnal ratio $< 10\%$) and inverse-dippers or risers (ratio $< 0\%$, indicating nocturnal BP above the diurnal mean). Alteration of the circadian rhythm of the neurohumoral factors that

affect the autonomic nervous system and cardiovascular system, secondary to various pathological conditions, results in persistent change of the 24-hour BP pattern [64]. The absence of a nocturnal blood pressure decrease is also emerging as an index for future target-organ damage, particularly to the heart (left ventricular hypertrophy, congestive heart failure and myocardial infarction), brain (stroke) and kidney (albuminuria and progression to end-stage renal failure). The association of left ventricular hypertrophy and vagal deactivation may lead to prolongation of the corrected QT interval, potentially facilitating ventricular arrhythmias in non-dipper hypertensive patients. In healthy subjects blood pressure values are substantially lower at night than during daytime activities [65,66]. O'Brien *et al.* were the first to draw attention to the negative prognostic value of the absence of nocturnal blood pressure fall in hypertensive patients [67]. These so-called non-dippers may have more pronounced target-organ damage than their dipping counterparts and may be exposed to a higher incidence of cardiovascular complications.

Studies using ambulatory BP monitoring (ABPM) have shown that disturbances of the BP profile during sleep may also be harmful in normotensive subjects [68].

Putz and colleagues have found elevated blood pressure values and a higher frequency of non-dippers among people with impaired glucose tolerance in the absence of cardiovascular autonomic neuropathy even after adjustment for BMI, suggesting that dysregulated glycaemic control by itself has a detrimental effect on blood pressure regulation [69].

H. Izzedine *et al.* have found by spectral analysis of heart rate variability, concomitant to ambulatory blood pressure monitorings, that the extent of loss in the day/night rhythm of BP is associated with a proportional nocturnal sympathetic predominance in diabetic patients [70,71]. To confirm this relation Van Ittersum *et al.*

observed that a lower fall of diastolic blood pressure (DBP) and of heart rate during sleep was associated with autonomic dysfunction in normoalbuminuric type 1 diabetic patients, suggesting incipient damage of the parasympathetic nervous system [72].

Resting heart rate (RHR) reflects sympathetic nerve activity [73,74] and it is an accessible clinical measurement. A significant association between RHR and cardiovascular mortality has been reported in some epidemiologic studies [73,75-77]. Based on epidemiologic data and inferences from clinical trials the results showed that high RHR is undesirable in terms of cardiovascular disease. However, the importance of RHR as a prognostic factor and potential therapeutic outcome has not been formally explored and therefore, despite suggestive evidence, is not generally accepted [78].

II. AIMS OF THE STUDY

1. This is of concern for the future population health, as cardio-metabolic risk factors in children and adolescents predict coronary heart disease and mortality in adulthood [79]. There is increasing evidence that physically active subjects have a better cardio-metabolic profile than less active ones [18]. However, less is known about the impact of sedentary behaviours on different cardio-metabolic risk factors. Measurements of physical fitness are preferable in relation to those of physical activity, due to their greater objectivity and lower propensity to errors. Furthermore, physical fitness is better correlated with cardiovascular diseases in adults [80]. Physical fitness in obese children is generally decreased. **Our aims were to compare the cardiorespiratory response to exercise of control children and of obese children with and without MS.**

2. Clinical features of autonomic neuropathy in childhood are not often seen (the prevalence in diabetic children is 1-2%); however, these are a known feature of in diabetic, hypertonic and obese adults [37,81,106-110]. Searching for early signs of autonomic nervous system dysfunction is an important tool to characterise clinically important subgroups of overweight children. Cardiovascular reflex testing may help us identify children at high risk of unexplained sudden death and hypertension without preventive efforts in adulthood. Our hypothesis that subclinical signs of cardiovascular autonomic neuropathy were detectable in obese children and adolescents due to altered glucose metabolism, insulin resistance and hyperinsulinaemia. **Our aims were to investigate the function of the autonomic**

nervous system in obese children with different cardiovascular risk factors to evaluate the occurrence of subclinical autonomic disturbances.

3. The lack of nocturnal decline in blood pressure can be detected in several pathological conditions. Abnormal 24-hour blood pressure profiles have been observed in diabetic and hypertensive patients, both adults and children [68,82-85]. However, no studies have been performed on obese children. **The purpose of our examination was to evaluate the circadian rhythm of blood pressure pattern in obese children and to investigate whether the lack of normal diurnal rhythm of blood pressure was associated with cardiovascular risk factors.**

4. Identifying good predictors for cardio-metabolic risk factors is essential to assist in the development of actions designed to improve cardiometabolic health of young people. **The *Healthy Lifestyle in Europe by Nutrition in Adolescence* cross-sectional study (HELENA-CSS) brought the opportunity to test the hypothesis that RHR is a good predictor for cardio-metabolic risk factors in paediatric populations. Thus, the objective of this study was to analyse the predictive power and accuracy of RHR as a screening measure for individual and clustered cardiovascular diseases risk in adolescents.**

III. CLINICAL INVESTIGATIONS

1. Investigation of the cardiorespiratory response to exercise of obese children with and without MS compare to the control children

PATIENT AND METHODS

Patients and sampling

180 obese children (103 males, 77 females), referred to the Obesity Clinic of the Department of Paediatrics, University of Pecs were included into the study after the exclusion of endocrinological disorders, or obesity syndromes. After assessing the cardiovascular risk factors in our cohort of 180 obese children, 22 boys with multiple cardiovascular risk factors (MS group) and 17 boys being free of any cardiovascular risk factor (Obese group) were included into the study. Healthy boys with normal weight matched for age served as controls (Control group; n=29). The anthropometric parameters of these groups are shown in Table 1/1. MS was defined as the simultaneous occurrence of obesity, hyperinsulinaemia, hypertension and both or at least one of the impaired glucose tolerance and dyslipidemia. The definition we used was adapted from the adult MS definitions with cut-off described below [16].

Methods

Anthropometric measurements

Weight and height were measured by standard beam scale and Holtain stadiometer, respectively. Relative BMI was calculated as the ratio of weight (kg) and height (m²). Body composition was estimated according to the method of Parizkova and Roth from the sum of five skinfolds (biceps, triceps, subscapular, suprailiac and calf) as

measured by Holtain caliper [86]. We considered children as obese if their body weight exceeded the expected weight for height by more than 20 % and body fat content (BF) was higher than 25 % in males and 30 % in females [87].

Examinations and laboratory measurements

Blood pressure was measured in each subject at least 3 times on 3 separate days by the same observer using mercury-gravity manometer with proper cuff size, according to the method recommended by the Second Task Force on Blood Pressure Control in Children [88]. If the average of the three blood pressure values was above the 95th percentile for age and sex, 24-hour ABPM was performed. Children with mean ABPM values exceeding the 95th percentile value for height and sex were considered hypertensive [89].

Fasting blood samples were collected, and a 2-hour oral glucose tolerance test (OGTT) was performed with administration of the standard 1.75 g/kg (maximum 75 g) glucose. Definitions used for the obesity-related metabolic conditions were as follows: hyperinsulinaemia - fasting serum insulin > 20 μ U/mL (mean + 2 SD of 100 non-obese Hungarian children) and/ or postload peak serum insulin during OGTT >150 μ U/mL [90]; impaired glucose tolerance – fasting blood glucose \geq 5.6 mmol/L or 2-hour blood glucose during OGTT \geq 7.8 mmol/L (American Diabetes Association criteria); dyslipidaemia- high fasting triglyceride (>1.1 mmol/L [<10 years]; >1.5 mmol/L [>10 years]) or low fasting HDL-cholesterol (<0.9 mmol/L) concentration (criteria of the Hungarian Lipid Consensus Conference [93]); hypercholesterolaemia – total fasting cholesterol concentration >5.2 mmol/L [91]. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) index (fasting insulin x fasting glucose / 22.5), as described by Matthews et al. [92].

Plasma glucose was measured by glucose oxidase method [93]. Serum cholesterol, triglyceride and HDL-cholesterol levels were determined by enzymatic method using Boehringer kits [94-96]. Plasma immunoreactive insulin levels were measured with commercially available radioimmunoassay kits from the Institute of Isotopes of the Hungarian Academy of Sciences.

To evaluate the association between the physical fitness level and multiple cardiovascular risk factors, a multistage test - involving an incremental treadmill test - was performed.

Exercise testing procedure

After arrival in the laboratory, the subjects rested for 30 minutes. The exercise test was performed on a treadmill (Jaeger EOS-Sprint), according to a multistage protocol. The protocol involved 3 minutes of lying on the belt, 3 minutes sitting on a chair and standing 3 minutes on the treadmill. After these initial phases the belt speed and the inclination were increased every 30 seconds, such that the estimated work rate increased in a linear fashion until the predicted maximum load (Watt/kg) was reached. We used Jones' prediction in determining the predicted maximum exercise capacity [97], using the age, sex, weight and height. At least one bipolar chest ECG lead was continuously monitored throughout the test, and the beat to beat R-R intervals were registered. Blood pressure was measured each minute by auscultation. Respiratory variables were measured by means of a Jaeger EOS-Sprint exercise metabolic measurement system. The metabolic system consists of a highly linear pneumotach including pressure transducer, amplifier and digital integrator with temperature compensation; a highly accurate gas analyser for O₂, CO₂; an

automatic calibration system; and the barometric pressure transducer and temperature sensor. The O₂ and the CO₂ concentrations were determined from the mixed expired air and the volume of the expired air was measured using a pneumotachograph. The subjects breathed through a tight-fit face mask and a non-rebreathing respiratory valve into the pneumotachograph and a mixing bag. The air from the mixing bag was continuously sampled by the gas analyzers that were previously calibrated with known gas mixtures. The pneumotachograph was calibrated with a 2 liter syringe prior to each test.

Exercise duration (ED), resting heart rate (HR₀), peak heart rate (HR_{peak}), physical working capacity at 170 beat/min (PWC-170), peak oxygen consumption (VO_{2peak}) and the lactic acidosis threshold (LAT) were determined. LAT was determined by the V-slope method [98]

Statistical analysis

Statistical analysis was conducted using SPSS version 11.5. Distribution was tested for each variable by Kolmogorov-Smirnov test. Means, standard deviations were calculated with standard methods. The level of significance was taken as $p < 0.05$.

Statistical significance of the means were analysed with analysis of variance (ANOVA), and the statistical significance was tested by Scheffe post hoc test. Variables were normalised for body weight, using body weight as covariant.

RESULTS

Boys with MS had a significantly higher body weight (BW), lean body mass (LBM) and body fat (BF) compared to obese and control groups. Obese boys also had significantly higher body weight, LBM and BF (Table 1/1). Since there were

significant differences in BW, LBM and BF between the three groups, variables of the physical fitness were normalised for BW.

Serum insulin was significantly higher in MS group as compared to obese and control groups. Serum total cholesterol, triglyceride and blood pressure values were significantly higher in MS group, as compared to the control group. In the obese group only the systolic blood pressure was significantly higher than in the control group (Table 1/2).

Obese children with or without MS, demonstrated a significantly shorter ED than the controls. In the MS group markedly shorter ED was observed as compared to the Obese group (Figure 1/1).

HR_0 was significantly higher in obese groups than in controls. This difference was more pronounced in the MS group. However, there was no difference in the HR_{peak} between MS and obese groups. HR_{peak} on the other hand, was significantly higher in obese children with MS as compared to controls. The peak heart rate response of obese children with no MS (Obese group) did not differ from that of controls (Figure 1/2).

Absolute values of PWC-170 were not different in the 3 groups, however, when PWC-170 was normalised for body weight, it was significantly lower in obese as compared to controls and further decreased in obese children with MS (Figure 1/3).

The VO_{2peak} and the LAT were also significantly lower in the obese groups when normalised for the body weight (Table 1/3).

Table 1/1 Anthropometric data of patients (mean \pm SD)

	MS (n=22)	Obese (n=17)	Control (n=29)
Age (years)	14.16 \pm 1.88	14.15 \pm 2.58	15.25 \pm 1.03
Body weight (kg)	97.29 \pm 15.3 * #	82.57 \pm 15.68 @	64.27 \pm 8.50
BMI (kg/m ²)	34.05 \pm 3.37 * #	29.31 \pm 3.80 @	20.52 \pm 2.59
W/H	0.89 \pm 0.05 *	0.85 \pm 0.05 @	0.79 \pm 0.04
LBM (kg)	70.24 \pm 11.7 * #	61.26 \pm 11.08 @	52.97 \pm 6.00
BF (kg)	27.04 \pm 4.25 * #	21.31 \pm 4.95 @	11.29 \pm 2.92

* p<0.05 MS vs Control # p<0.05 MS vs Obese @ p<0.05 Obese vs Control

MS: Metabolic syndrome, BMI: body mass index, W/H: waist per hip ratio, LBM: lean body mass, BF: body fat

Table 1/2 Cardiovascular risk factor values in patients (mean \pm SD)

	MS (n=22)	Obese (n=17)	Control (n=29)
Insulin (μ U/ml)	26.22 \pm 16.07*#	11.93 \pm 5.69	13.20 \pm 6.20
Cholesterol (mmol/l)	4.51 \pm 0.99*	4.01 \pm 0.62	3.62 \pm 0.63
Triglyceride (mmol/l)	1.62 \pm 0.89*	1.39 \pm 0.54	1.02 \pm 0.49
Systolic blood pressure (mmHg)	143.63 \pm 18.65*	135.88 \pm 20.48@	123.79 \pm 9.32
Diastolic blood pressure (mmHg)	82.95 \pm 9.71*	78.82 \pm 8.39	73.62 \pm 7.42

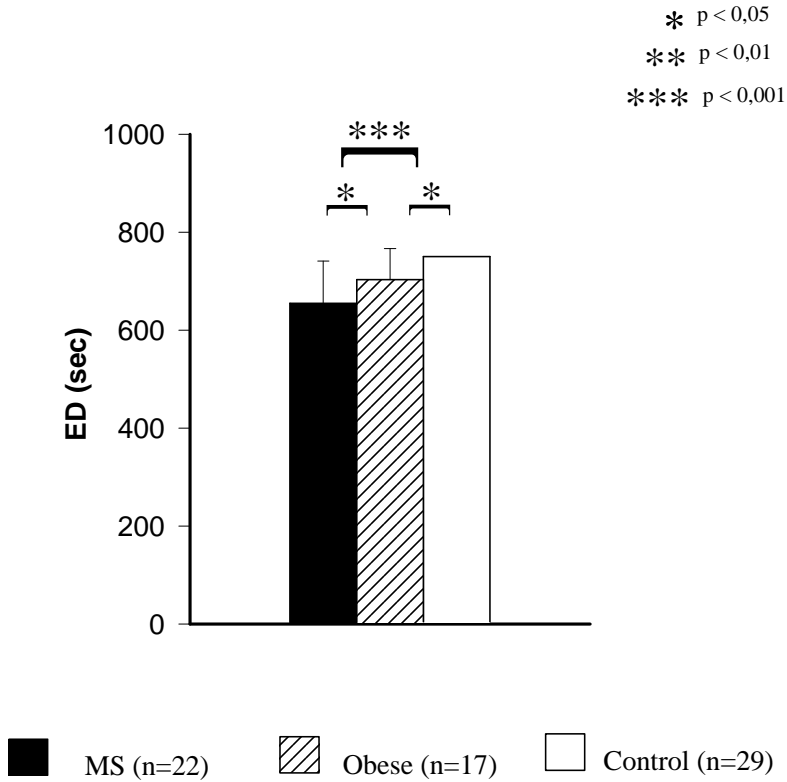
* p<0.05 MS vs Control # p<0.05 MS vs Obese @ p<0.05 Obese vs Control, MS: Metabolic syndrome

Table 1/3 Original LAT and VO₂peak values, and those normalised for body weight (LAT-BW, VO₂peak-BW) (mean \pm SD)

	MS (n=22)	Obese (n=17)	Control (n=29)
LAT (L/min)	1.53 \pm 0.42	1.53 \pm 0.48	1.61 \pm 0.28
LAT-BW (L/min)	1.33 \pm 0.37 *	1.50 \pm 0.37 @	1.78 \pm 0.37
VO₂peak (L/min)	2.70 \pm 0.60	2.51 \pm 0.74	2.47 \pm 0.39
VO₂peak-BW (L/min)	2.19 \pm 0.42 *	2.43 \pm 0.45 @	2.91 \pm 0.43

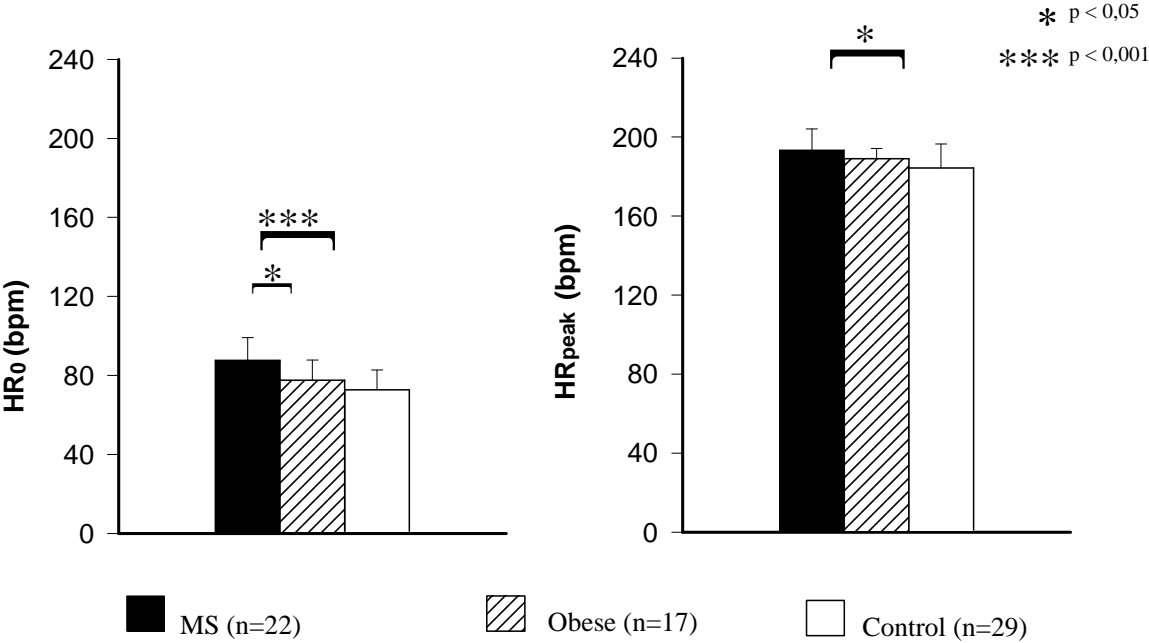
LAT: lactic acidosis threshold; LAT-BW: lactic acidosis threshold normalised for body weight; VO₂peak: peak oxygen consumption; VO₂peak-BW: peak oxygen consumption normalised for body weight; MS: Metabolic syndrome; * p<0.05 MS vs Control; @ p<0.05 Obese vs Control

Figure 1/1 Exercise duration



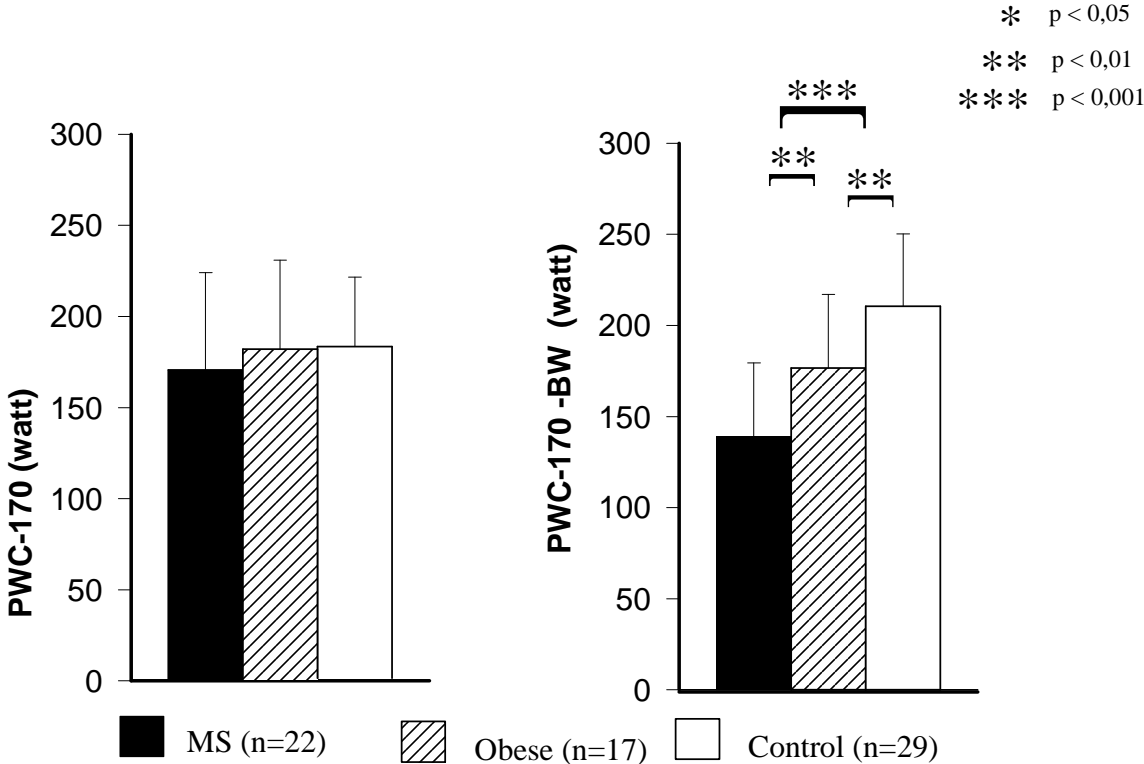
ED: exercise duration, MS: metabolic syndrome

Figure 1/2 Resting and peak heart rate



HR₀: Resting heart rate, HR_{peak}: peak heart rate, MS: Metabolic syndrome

Figure 1/3 Physical working capacity at heart rate 170, and physical working capacity at heart rate 170 normalised for body weight



PWC-170: Physical working capacity at heart rate 170 in absolute values, PWC-170-BW: Physical working capacity at heart rate 170 normalised for body weight, MS: Metabolic syndrome

DISCUSSION

The relationship between physical performance and obesity, on the one hand, and physical performance and atherosclerotic risk factors, on the other hand, have been studied by several authors, with conflicting results.

Obesity may be associated with a decrement in exercise performance particularly at maximal work levels. Davies *at al.* [33] found that during maximal exercise there was a marked decrement in exercise performance in obese females as compared with controls. During maximal performance the absolute $VO_2\text{max}$ was the same in obese and non-obese subjects but for a given body weight, lean body mass $VO_2\text{max}$ was significantly reduced. During light exercise when oxygen intake for a given work output was standardized for body weight it was shown that obese patients exercised within the normal range of aerobic energy expenditure. Zanconato *at al.* [99] performed maximal exercise testing on 23 obese children aged 9 to 14 years, who had lower endurance time and $VO_2\text{max/kg}$ values than the controls, but their absolute $VO_2\text{max}$ values were not significantly different from the controls.

There are data indicating that hyperinsulinaemia, which is ubiquitously associated with obesity, might have a direct or indirect effect on the cardiovascular system, and consequently, on exercise performance. Hyperinsulinaemic obese children had significantly lower physical working capacity than the non-hyperinsulinaemic ones, in spite of their similar anthropometric characteristics and lipid profiles [100].

The majority of obese children, especially those with MS, had resting tachycardia, which can be explained by elevated sympathetic nervous system activity in response to hyperinsulinaemia. In our earlier investigations we could detect increased norepinephrine levels in obese children with hypertension and

hyperinsulinaemia [101]. While in some cases a decreased activity of the sympathetic nervous system is emphasized in the aetiology of obesity, some data suggest that overfeeding and hyperinsulinaemia stimulates the sympathetic nervous system [102-104]. In addition, it has been demonstrated that an increase in brain insulin reduces neuropeptide Y and its gene expression in the arcuate nucleus which results in the stimulation of the sympathetic nervous system [105,106].

Fripp et al. [20] demonstrated a correlation between physical fitness and risk factors for atherosclerosis in the male adolescent population. They also found that higher levels of fitness were associated with better risk profiles (decreased body mass index, lower systolic and diastolic blood pressure and triglyceride levels, and higher high-density lipoprotein levels). The multiple linear regression analysis demonstrated that body mass index accounted for much of the variation in fitness parameters.

Our results demonstrated clearly that children with MS had significantly lower physical performance as measured by ED and body weight corrected PWC-170, VO_2 peak and AT values than obese children without metabolic disturbances. The question, whether the metabolic alterations or the decreased physical activity are responsible for the poor physical performance in children with MS, cannot be answered at present and further investigations are warranted.

2. Investigation of the occurrence of subclinical autonomic disturbances in obese children

PATIENTS AND METHODS

47 obese children (23 boys, 24 girls), referred to the Obesity Clinic of the Department of Paediatrics, University of Pécs, participated in the study after the exclusion of any endocrinological disorders or obesity syndromes. None of the subjects had any disorders nor had been treated with any drugs during the study or during the preceding two weeks. A normal resting electrocardiogram was a condition for inclusion in the study.

The anthropometric parameters of these children are shown in Table 2/1.

Table 2/1 Anthropometric data of patients (mean \pm SD)

	Boys (n=23)			Girls (n=24)		
Age (years)	12.7	\pm	2.5	14.1	\pm	2.4
Body weight (kg)	82.9	\pm	17.5	80.4	\pm	15.3
BMI (kg/m ²)	31.1	\pm	4.4	30.5	\pm	3.9
BF (kg)	31.0	\pm	7.6	34.7	\pm	8.4

BMI (body mass index), BF (body fat)

Anthropometric measurements

We used the same methods as in investigation 1.

Cardiovascular reflex tests

Autonomic function was investigated by bedside cardiovascular tests [111,112]. The cardiovascular tests employed included: mean resting heart rate for a period of 1 minute (RHR); heart rate variation during deep breathing (DBHR); standing-lying heart rate ratio (S/L); fall in systolic blood pressure on standing (OT); and rise in

diastolic blood pressure during sustained handgrip (SH). Heart rates were determined with a routine electrocardiographic device (Medicor ER31-A) by RR intervals; for blood pressure measurements a digital blood pressure device (Omron HEM-400 C) was used. After arrival at the laboratory and following a one-hour rest, tests for autonomic function were performed by the same person. Reference ranges for each cardiovascular test from literature were generated from data obtained in 130 healthy children aged 6 to 18 years [111]. Resting heart rate, standing-lying heart rate ratio and handgrip test results were age dependent.

Statistical analysis

Statistical analysis was conducted using SPSS version 11.5. Distribution was tested for each variable by Kolmogorov-Smirnov test. Means, standard deviations were calculated with standard methods. The level of significance was taken as $p < 0.05$.

Statistical differences were assessed using the unpaired Students t-test, chi-square analysis.

RESULTS

Using reference ranges [111], autonomic tests were negative only 6 (12.8%) obese children (Table 3/2). 22 (46.8%) had only one abnormal test and 19 (40.4%) had two or more abnormal tests. In obese children a significantly decreased deep breathing heart rate variation, a greater fall in systolic blood pressure to standing and a lower response during the handgrip test were observed (Table 2/4). RHR and heart rate response to standing from a lying position (S/L) did not differ between obese and control groups. The most frequent abnormal autonomic test result was the blood pressure response from lying to standing (OT) (Table 2/3).

Table 2/2 Abnormal autonomic test results

Abnormal autonomic test results	Obese children (n=47)	
	n	%
3	5	10.6
2	14	29.8
1	22	46.8
0	6	12.8

Table 2/3 The frequency of abnormal test results

Abnormal test results	%
OT (mmHg)	40.4
S/L	36.2
DBHR (beats/min)	31.9
SH (mmHg)	17
RHR (beats/min)	12.8

OT: fall in systolic blood pressure on standing; S/L: heart rate response to standing from a lying position (standing lying ratio); DBHR: heart rate variation during deep breathing; SH: rise in diastolic blood pressure during sustained handgrip; RHR: mean resting heart rate for a period of 1 minute.

Table 2/4 Cardiovascular autonomic test results (mean \pm SEM)

	Obese children (n=47)			Control (n=130)		
RHR (beats/min)	81.7	\pm	1.9	84.2	\pm	2.2
DBHR (beats/min)	24.9*	\pm	1.3	32.8	\pm	0.6
S/L	1.1	\pm	0.1	1.3	\pm	0.1
OT (mmHg)	-9.3*	\pm	1.2	-2.5	\pm	1.5
SH (mmHg)	10.7*	\pm	1.4	12.8	\pm	1.3

* $p < 0.05$ Obese vs Control

OT: fall in systolic blood pressure on standing; S/L: heart rate response to standing from a lying position (standing lying ratio); DBHR: heart rate variation during deep breathing; SH: rise in diastolic blood pressure during sustained handgrip; RHR: mean resting heart rate for a period of 1 minute.

CONCLUSION

In obesity as a consequence of dysfunction of the lesion of the parasympathetic nervous system the heart rate increases, physiological respiratory arrhythmia decreases and the ratio of standing to lying heart rate decreases. Dysfunction of the sympathetic nervous system is associated with an exaggerated orthostatic fall in systolic blood pressure and with the blunting of the rise in diastolic blood pressure during sustained handgrip. In this study we found decreased deep breathing heart rate variation, a higher fall in systolic blood pressure on standing and a lower response to the handgrip test in obese children as early signs of autonomic dysfunction. An abnormal cardiovascular test result was found in 5% of the healthy reference population [111,112]. Lower sympathetic and parasympathetic activity was found by Peterson *et al.* (1988) with an inverse correlation of sympathetic and parasympathetic activity with increasing body fat [113]. Rossi *et al.* (1989) also reported a lower parasympathetic function but no alteration in sympathetic functions [114]. In obese women higher sympathetic and parasympathetic activities were found by Gao *et al.* (1996) [115]. Hofman *et al.* (2000) found a decrease in sympathetic and parasympathetic functions [116]. Karason *et al.* (2000) suggested that obese patients have increased sympathetic and decreased parasympathetic activity, improving after weight loss [117].

In conclusion, cardiovascular autonomic dysfunction is not a rare phenomenon in obese children and adolescents. Search for early signs of autonomic nervous system dysfunction should be detect clinically important subgroups of overweight children, those who might have high risk at adulthood for unexplained sudden death, those in whom hypertension develops and are necessary to involve these children in the preventive exercise programs. Further studies are necessary to investigate how long

term exercise programmes influence the cardiac autonomic functions of obese young patients.

3. The examination of the circadian rhythm of blood pressure pattern in obese children

PATIENTS AND METHODS

Patients and sampling

73 obese children (51 males, 22 females; age [mean \pm SD]: 14.2 \pm 2.3 years; age range in males [minimum - maximum]: 7.1 - 18.4 years, in females: 8.8 - 18.2 years) referred to the Outpatient Clinic for Obesity of the Department of Paediatrics, University of Pécs, were included into the study after the exclusion of endocrinological disorders, or obesity syndromes. Anthropometric data and laboratory parameters were obtained. Following these measurements 24-hour ABPM and treadmill exercise tests were performed. Concerning the methods of anthropometric- and laboratory measurements and exercise testing procedures we refer to investigation 1. In this study we used Cole's age- and gender-specific BMI values [118]. Children with BMI values exceeding the international value for BMI corresponding to 30 kg/m² at age 18 were considered obese.

Blood pressure measurements

Blood pressure was measured during the morning in the outpatient clinic on three separate occasions by a physician using a mercury sphygmomanometer (first and fifth phases of Korotkoff sounds taken as the systolic blood pressure and diastolic blood pressure, respectively). The average of the three measurements was used to give the office systolic and diastolic blood pressures. An office blood pressure consistently greater than or equal to the 95th percentile of the blood pressure

distribution in a normal reference population was considered as hypertensive [119]. Multiple blood pressure measurements were then carried out by ambulatory blood pressure monitoring (ABPM) with a non-invasive recorder (Meditech, Hungary) using the oscillometric method. The proper cuff size was selected (10 cm x 13 cm or 13 cm x 24 cm) according to the circumference of the nondominant arm. All individuals were asked not to change their usual daily activities, to keep still at the times of measurement, to note the occurrence of unusual events or poor sleep in a diary. The ABPM days were always performed over a working day (Monday to Friday). For evaluation, the ABPM Report Management System program was used, which calculates mean blood pressure and heart rate during day, night, and over the whole 24-h period separately. Systolic, diastolic blood pressure and heart rate values were monitored with the sampling time set 20-minutes during daytime, and 30-minutes during sleep. The duration of these periods were adjusted to the individual timetable of the child. Each ABPM dataset was first automatically scanned to remove artefactual readings. At least 64 successful recordings (84%) were required for a valuable evaluation of ABPM, otherwise the patients were not included in the study. The recording was then analysed to obtain 24-hour, day-time and night-time average systolic, diastolic blood pressure and heart rate. Upper limits of normal values (95th percentile) were used according to Soergel *et al.* [89]. Children with mean 24-hour systolic or diastolic ABPM values exceeding the 95th percentile for height and sex were considered hypertensive. Masked hypertension was defined as increased 24-h ABPM value in the presence of normal office blood pressure. Nocturnal blood pressure fall was calculated by subtracting nighttime values from daytime values of blood pressure and expressed as a percentage of the daytime level. Children were divided into two groups based on the presence (dippers) or absence (non-dippers) of

a normal (more than 10 %) reduction in both the systolic and diastolic blood pressure at night [120].

Statistical analysis

Statistical analysis was conducted using SPSS version 11.5. Distribution was tested for each variable by Kolmogorov-Smirnov test. Means, standard deviations were calculated with standard methods. The level of significance was taken as $p < 0.05$.

All comparison of mean values for the dippers and non-dippers were tested by Student's t test for independent samples. The Univariate Analysis of Variance was conducted to test differences between groups, when physical working capacity (PWC) and oxygen consumption values were normalised for body weight. PWC and oxygen consumption were used as dependent variables, and weight as a covariant. The frequency of cardiovascular risk factors were tested using chi-square analysis.

RESULTS

42 % of obese children (41 % of boys, 45 % of girls) were non-dipper. The age of the two groups did not differ significantly. The dippers were significantly heavier than non-dippers ($p < 0.05$), but there was no significant difference between the two groups in the degree of obesity (BMI, body fat %) (Table 3/1). No differences could be detected between dippers and non-dippers either in the fasting serum levels of total cholesterol, triglyceride, HDL-cholesterol, or in serum glucose and insulin levels at fasting and at 120 minutes of oral glucose tolerance test and in the HOMA-IR (Table 3/2).

There was no significant difference in office blood pressures and average daytime systolic and diastolic blood pressure values between the two groups, while average nighttime values were significantly elevated in non-dippers ($p < 0.001$) (Table 3/3).

Incidence of cardiovascular risk factors (hyperinsulinaemia, impaired glucose tolerance, dyslipidaemia) was similar in the two groups. While the prevalence of hypertension was significantly higher in the non-dippers than among dippers ($p<0.001$) on the basis of ABPM, the prevalence of hypertension was similar in the two groups when office blood pressure measurements were considered (Table 3/2). The prevalence of masked hypertension was also significantly higher in the non-dippers ($p<0.001$) (Table 3/2). Comparing the results of the spiroergometric stress test, exercise performances measured at heart rate 130, -150, -170 (PWC-130, -150, -170) were significantly lower in the non-dipper group ($p<0.05$) (Figure 3/1). In other words, dippers achieved the same level of work load at a lower heart rate, while the non-dipper group produced a relatively tachycardic heart rate response to exercise. Resting and peak oxygen consumption was also higher in dippers than in non-dippers (Figure 3/2), however, the differences disappeared, when oxygen consumption values were corrected for body weight (Figure 3/2). Neither the duration of exercise nor resting and peak values of heart rate, resting and peak blood pressure values or anaerobic threshold, were different in the two groups (Table 3/4).

Table 3/1 Anthropometric data of patients (mean \pm SD)

	Dippers (n=42)	Non-dippers (n=31)
Male/Female	30/12	21/10
Age (years)	14.9 \pm 2.1	13.4 \pm 2.3
Height (cm)	169.7 \pm 11.7	164.1 \pm 13.7
Weight (kg)	93.4* \pm 17.0	83.1 \pm 17.5
BMI (kg/m ²)	32.3 \pm 4.0	30.6 \pm 4.2
BF (%)	38.9 \pm 5.0	38.6 \pm 4.1

* $p<0.05$ Dippers vs Non-dippers
 BMI (body mass index), BF (body fat)

Table 3/2 Values and frequency of cardiovascular risk factors in dippers and non-dippers

	Dippers (n=42)		Non-dippers (n=31)	
	mean ± SD	%	mean ± SD	%
Insulin₀ (mU/ml)	23.2 ± 14.9		20.9 ± 11.1	
Insulin₁₂₀ (mU/ml)	169.7 ± 128.4		124.6 ± 81.0	
Blood glucose₀ (mmol/l)	4.3 ± 0.6		4.3 ± 0.8	
Blood glucose₁₂₀ (mmol/l)	6.1 ± 1.1		6.0 ± 1.3	
Cholesterol (mmol/l)	4.3 ± 0.8		4.2 ± 0.9	
Triglyceride (mmol/l)	1.5 ± 0.7		1.4 ± 0.8	
HDL-cholesterol (mmol/l)	1.3 ± 0.3		1.3 ± 0.3	
HOMA-IR	4.7 ± 3.5		3.9 ± 2.2	
Hyperinsulinaemia		55.0		51.6
IGTT		30.0		16.1
Dyslipidaemia		46.6		32.3
Hypertension				
ABPM		45.2		83.9**
Office blood pressure		42.4		45.2
Masked hypertension		19.0		32.3**

**p <0.001 Dippers vs Non-dippers
IGTT (impaired glucose tolerance test)

Table 3/3 Results of 24-hour blood pressure monitoring and office blood pressures (mean \pm SD)

	Dippers (n=42)	Non-dippers (n=31)
SBP daytime (mmHg)	132.4 \pm 9.5	128.8 \pm 10.6
DBP daytime (mmHg)	77.0 \pm 7.5	75.4 \pm 9.0
SBP nighttime (mmHg)	111.8** \pm 7.7	122.5 \pm 11.0
DBP nighttime (mmHg)	58.9** \pm 5.6	70.3 \pm 9.4
SBP ϕ (mmHg)	120.3 \pm 11.3	122.3 \pm 11.2
DBP ϕ (mmHg)	75.5 \pm 9.2	70.8 \pm 10.3

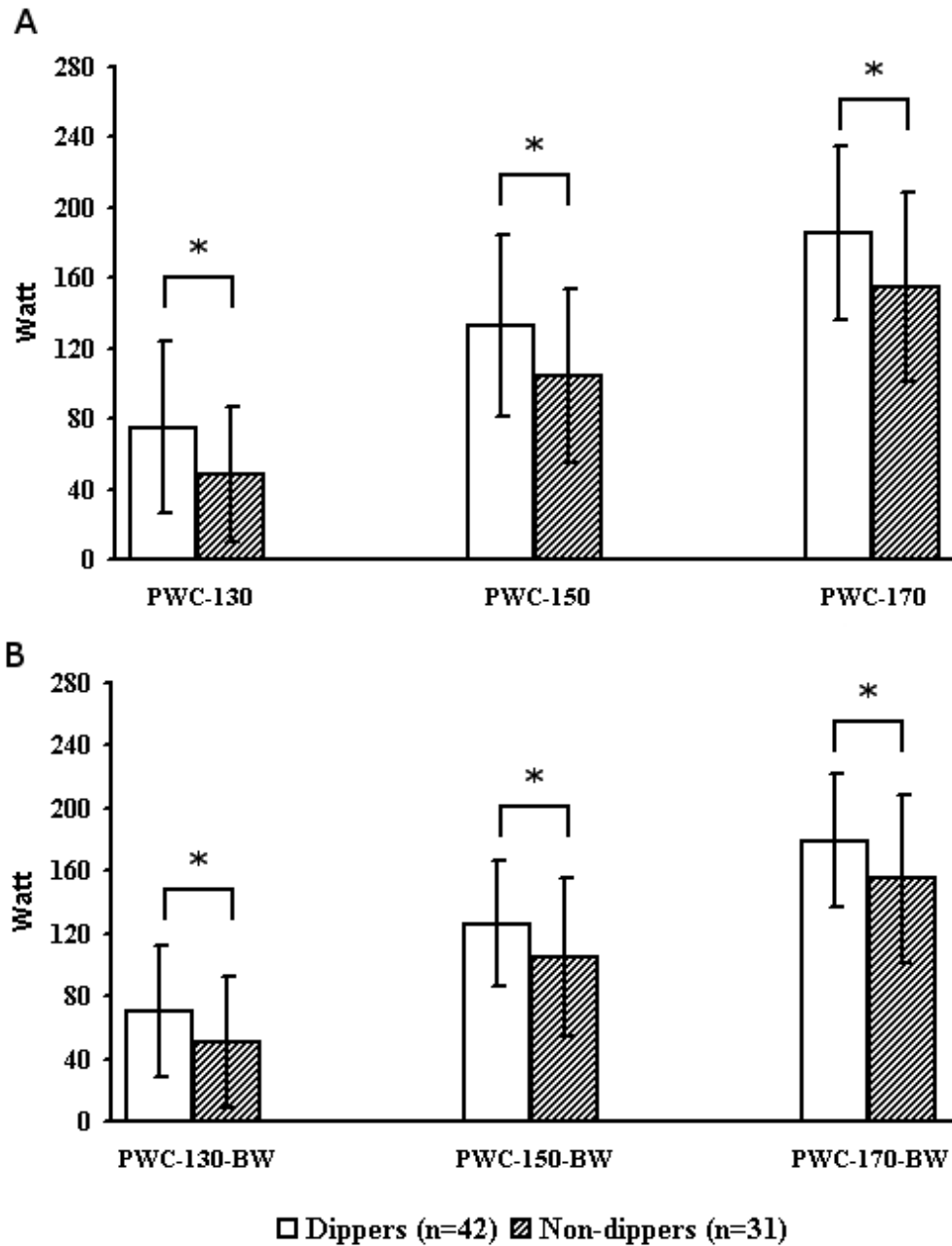
**p <0.001 Dippers vs Non-dippers
 SBP: systolic blood pressure, DBP: diastolic blood pressure
 ϕ office blood pressures

Table 3/4 Cardiorespiratory function parameters in dippers and non-dippers (mean \pm SD)

	Dippers (n=42)	Non-dippers (n=31)
ED (sec)	683.0 \pm 80.6	692.0 \pm 64.2
HR₀ (bpm)	86.7 \pm 9.0	91.2 \pm 11.7
HR_{peak} (bpm)	190.2 \pm 11.5	194.8 \pm 8.8
SBP₀ (mmHg)	141.4 \pm 15.9	143.7 \pm 15.7
SBP_{peak} (mmHg)	206.1 \pm 17.4	203.8 \pm 17.7
DBP₀ (mmHg)	89.8 \pm 9.2	87.9 \pm 10.4
DBP_{peak} (mmHg)	67.5 \pm 10.3	66.7 \pm 9.0
AT (L/min)	1.7 \pm 0.4	1.6 \pm 0.5

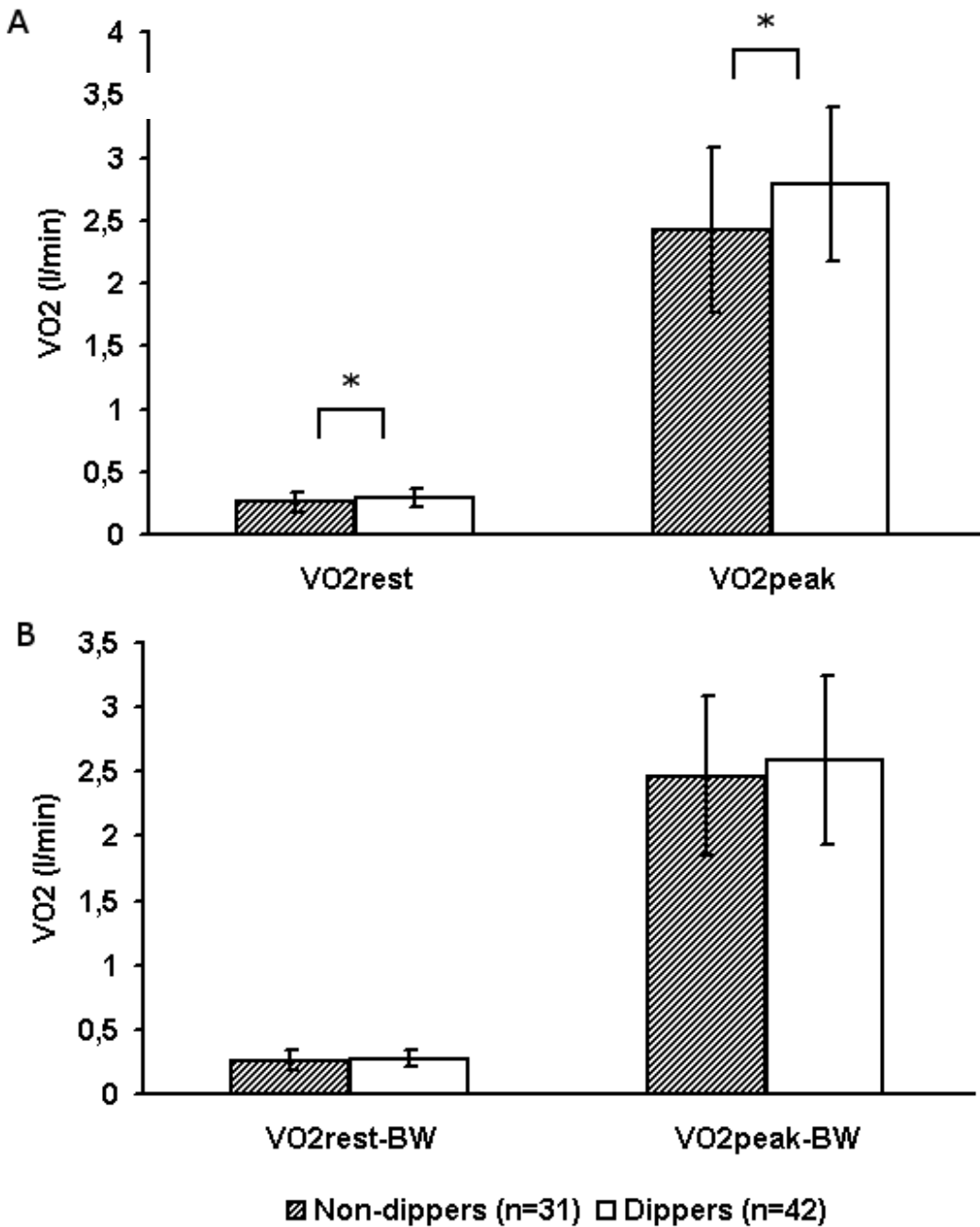
ED: exercise duration, HR₀ :resting heart rate , HR_{peak}: peak heart rate, SBP₀ : systolic blood pressure at rest - average of lying, sitting and standing values, SBP_{peak}: systolic blood pressure at peak level, DBP₀ :diastolic blood pressure at rest- average of lying, sitting and standing values, DBP_{peak}: diastolic blood pressure at peak level, AT: anaerobic threshold

Figure 3/1 Physical working capacity at heart rate 130, -150, -170 in absolute values (A), and normalised for body weight (B) in dippers and non-dippers (mean \pm SD)



*p < 0.05 Dippers vs Non-dippers
 PWC-130, PWC-150, PWC-170: Physical working capacity at heart rate 130, -150, -170 in absolute values, PWC-130-BW, PWC-150-BW, PWC-170-BW: Physical working capacity at heart rate 130, -150, -170 normalised for body weight

Figure 3/2 Resting and peak oxygen consumption in absolute values (A), and normalised for body weight (B) in dippers and non-dippers (mean \pm SD)



*p < 0.05 Dippers vs Non-dippers
 VO₂rest-BW: resting oxygen consumption normalised for body weight, VO₂peak: maximal oxygen consumption normalised for body weight

DISCUSSION

Among adults, non-dippers have been reported to be at greater risk for increased left ventricular mass index and higher cardiovascular disease mortality rates [121]. Few data are available on the importance of non-dipping in children. Lurbe *et al.* have recently reported that nocturnal hypertension predicts the development of microalbuminuria, an early renal impairment in diabetic adolescents [122].

According to the present findings the lack of normal nocturnal fall in blood pressure was a frequent phenomenon (42%) among obese children. The frequency of non-dipping was similar in the two genders. The prevalence of hypertension on the basis of ABPM and masked hypertension was also significantly higher in non-dipper obese children. They had lower physical working capacity, due to a more tachycardic heart rate response to exercise, as compared to dippers. The higher incidence of hypertension on the basis of ABPM in the non-dipper group is explained by their significantly higher night-time blood pressure values [123]. The results of the present study indicate that masked hypertension is not uncommon condition in non-dipper obese children. In the study of Lurbe *et al.* the prevalence of masked hypertension was 4.3% in overweight, 10.4% in moderately obese and 4.2% in severely obese children [124]. Since Lurbe *et al.* studied a general population, therefore the results can not be compared to the present study investigating obese children referred to the obesity centrum. In adults, masked hypertension is associated with increased left ventricular mass and a worse cardiovascular prognosis [125]. The frequency of other cardiovascular risk factors was similar in the two groups. Increased fasting insulin levels were reported by Della Mea *et al.* [126] in non-dipper adults, but we could not prove this in obese children.

The pathomechanism of non-dipping in obese children and the link between non-dipping phenomenon and decreased exercise performance cannot be explained on the basis of the present results. In adults this phenomenon has been attributed, at least partially, to the increase in sympathetic or decrease in parasympathetic activity [127]. Weight reduction was shown to increase significantly the blood pressure fall during the night [128]. The lack of the normal circadian blood pressure rhythm is considered an early sign of autonomic neuropathy, which could be a first sign of modified vascular reactivity [129]. In diabetic patients with neuropathy, the circadian blood pressure rhythm disappears [77] and their physical performance is decreased [131]. Whether the lack of the nocturnal fall of blood pressure is associated with autonomic neuropathy and the latter is linked to low physical performance in obese children needs further investigation.

The clinical and prognostic value of a non-dipping nocturnal blood pressure profile still remains the source of controversial debate. The limited reproducibility of nocturnal variations could explain conflicting conclusions. This is presumably related to the fact that the quality and depth of sleep as well as the mental and physical activity during daytime can markedly vary from one recording session to another. In normotensive adults there is a good reproducibility of ABPM after intervals as long as 1 year [132]. Good reproducibility of ABPM was also found in healthy normotensive children after several months [133]. The study of Rucki *et al.* indicates that a single ABPM measurement is not sufficient for definitive classification of young individuals into hypertensives or normotensives [134]. According to Rucki *et al.* 25.6% of hypertensive children showed shifting between the dipping and non-dipping pattern [134]. To avoid some of these potential methodological problems, in the present study we standardized recording techniques and tried to achieve a good compliance.

4. Analysing the predictive power and accuracy of RHR as screening measure for individual and clustered cardiovascular risk in adolescents.

METHODS

Study population

The HELENA-CSS aimed to describe the lifestyle and nutritional status of European adolescents. Data collection took place between October 2006 and December 2007 in the following cities: Athens and Heraklion in Greece, Dortmund in Germany, Ghent in Belgium, Lille in France, Pecs in Hungary, Rome in Italy, Stockholm in Sweden, Vienna in Austria, and Zaragoza in Spain. Further information about the study design has been published elsewhere [135,136]. Participants were recruited at schools. To ensure that the heterogeneity of social background of the population would be represented, schools were randomly selected after stratification by school zone or district. The general inclusion criteria for HELENA were age range of 12.5-17.5 years [135].

From a sample of 3528 adolescents who met the HELENA general inclusion criteria, one third of the school classes were randomly selected in each centre for blood collection, resulting in a total of 1089 adolescents. For the purposes of the present study, adolescents with valid data for sedentary behaviour, accelerometry, cardiorespiratory fitness, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), insulin, glucose, systolic blood pressure and triceps, biceps, subscapular and supra-iliac skinfolds were finally included in the analysis (n=769). The study sample did not differ in sex distribution, mean age, mean BMI and mean values of CRF from the full HELENA sample (all $p>0.05$).

Resting Heart Rate (RHR)

The RHR were measured in all centers using the same type of oscillometric monitor device OMRON[®] M6 (HEM 70001) which has been approved by the British Hypertension Society, all devices were calibrated previously [137]. Measurements were taken twice (10 min apart) and the lowest value was retained.

Physical examination

Waist circumference, height, weight and four skinfold thicknesses (on the left side from biceps, triceps, subscapular, supra-iliac) were measured following a standardized protocol [118]. The definition of obesity (including overweight) was based on international BMI cutoffs proposed by Cole et al. [118] from several different countries. Systolic and diastolic blood pressure measurements by the arm blood pressure oscillometric monitor device OMRON[®] M6 (HEM 70001) which has been approved by the British Hypertension Society [137]. Measurements were taken twice (10 min apart) and the lowest value was retained.

Cardiorespiratory fitness

Participants ran between two lines 20 m apart, keeping the pace with audio signals. The initial speed was 8.5 km/h, and each minute speed was increased by 0.5 km/h. Participants had to run in a straight line and to pivot on the lines. The test finished when subjects stopped due to fatigue or when they failed to reach the end line concurrent with the signals on two consecutive occasions. The last completed stage or half-stage was recorded. Finally, the VO₂max in ml/kg/min was estimated by the Leger equation (boys and girls: $VO_2\text{max} = 31.025 + (3.238 \times S \times 3.248 \times A) +$

$(0.1536 \times S \times A)$; A the age, S the final speed $S = 8 + 0.5$ last stage completed) [138], [139]. Physical fitness levels were described in detail elsewhere [140].

Cardiovascular risk factors

Blood samples were obtained for a third of the HELENA-CSS participants. Blood samples were taken in the morning after an overnight fast. Blood was collected, immediately placed on ice and centrifuged. Immediately after centrifugation, the samples were stored and transported at 4-7°C to the central laboratory in Bonn (Germany) and stored there at -80°C until assayed. Triglycerides, TC, HDL-c and glucose were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were measured using an Immulite 200 analyser (DPC Bierman GmbH, Bad Nauheim, Germany). The homeostasis model assessment (HOMA) calculation was used as a measurement of insulin resistance (fasting glucose x fasting insulin/ 22.5) [92].

A clustered cardiovascular risk index was created from the following variables: systolic blood pressure, HOMA index, triglycerides, TC/HDL-c ratio, VO_2 max and the sum of four skinfolds. The standardized value of each variable was calculated as follows: $(\text{value} - \text{mean}) / \text{SD}$, separately for boys and girls and by 1-yr age groups. For variables characterized by a lower metabolic risk with increasing values (VO_2 max), Z scores were multiplied by -1. To create the metabolic risk score, all the Z-scores were summed, where the lowest values are indicative of a better cardio-metabolic risk profile. Finally, all those subjects at or above age and gender specific cut-offs, subjects were classified as having metabolic risk when they accumulated $\geq 1\text{SD}$ [18,19].

Statistical analysis

The descriptive analyses were presented as means (quantitative variables) and percentages (qualitative variables) and confidence intervals 95% (95% CI).

All cardiovascular risk factors variables were entered as fixed factors. Education of the mother, MVPA, waist circumference and months from menarche for girls were entered as covariates. Receiver operating characteristics (ROC) curve analysis was applied to calculate the relationship between clustered and individual cardiovascular risk factors (were used binary outcome) and RHR. ROC curve provides the whole spectrum of specificity/sensitivity values for all the possible cut-offs. The area under the curve (AUC) is determined from plotting sensitivity versus 1 – specificity of a test as the threshold varies over its entire range. Taking into account the suggested cut-off points, the test can be non-informative/test equal to chance less accurate ($0.5 < \text{AUC} < 0.7$); moderately accurate ($0.7 > \text{AUC} \leq 0.9$); highly accurate ($0.9 > \text{AUC} < 1.0$); and perfect discriminatory tests ($\text{AUC} = 1.0$) [141]. In addition, ROC curve indexes of each cut-off point were calculated through the determination of positive and negative predictive values, overall misclassification rate, positive and negative likelihood ratios, and Youden Index [142].

The statistical software package Stata version 12.0 (Stata Corp., college Station, TX, USA) was used for all statistical calculations.

RESULTS

The proportion of boys had significantly performing physical activity the recommended amount of physical activity (≥ 60 min/d) was higher than girls. Among CVD risk factors, males showed higher significant levels for SBP and TC/HDL, while

girls had higher plasma concentrations of TC, HDL-c and triglycerides. Boys had also higher RHR than their female peers (**Table 4/1**).

The accuracy of prediction of RHR for the six factors individual CVD risk factors and for the cluster of CVD separately by sex seen in **Table 4/2**. For all CVD risk factors, the RHR have a high sensitivity, low specificity and accuracy (area under of curve), regardless of sex.

Table 4/1 Characteristics of the study population.

Variables	Girls (n= 393) mean or % (95%CI)	Boys (n= 376) mean or % (95%CI)
Age (years)	14.8 (14.7 - 14.9)	14.8 (14.7 - 14.9)
Tanner Stage (%)		
1 and 2 (pre-pubertal)	7.3 (5.0 – 9.6)	7.1 (4.7 – 9.5)
3 and 4 (pubertale)	65.6 (61.6 – 70.1)	64.4 (59.9 – 68.9)
5 (post-pubertal)	26.8 (23.9 – 28.9)	28.5 (24.3 – 32.8)
Education of mother		
Lower education	8.4 (6.0 - 10.7)	8.9 (6.3 - 11.4)
Lower secondary education	30.3 (26.4 - 34.2)	27.6 (23.6 - 31.7)
Higher secondary education	30.7 (26.8 - 34.6)	29.6 (25.4 - 33.8)
University degree	30.6 (26.8 - 34.6)	33.9 (25.4 - 33.8)
MVPA		
< 60 min/d	72.3 (67.9 - 76.7)	39.3 (33.9 - 44.6)
≥ 60 min/d	27.7 (23.3 - 32.1)	60.7 (55.4 - 66.1)
Sedentary behavior by questionnaire		
> 4 h/d	20.4 (18.3 - 22.5)	38.8 (36.2 - 41.5)
2 - 4 h/d	36.3 (33.8 - 38.8)	39.1 (36.4 - 41.7)
< 2 h/d	43.3 (40.7 - 45.8)	22.1 (19.9 - 24.4)
Months from menarche	24.0 (22.4 - 25.6)	
Height (cm)	162.3 (161.8 - 162.9)	169.3 (168.5 - 170.1)
Weight (kg)	56.7 (55.9 - 57.6)	61.0 (59.9 - 62.2)
BMI (kg/m²)	21.5 (21.2 - 21.8)	21.1 (20.8 - 21.5)
Obesity (%) by Cole	3.0 (1.3 - 4.6)	5.5 (3.1 - 7.8)
Waist circumference (cm)	70.6 (70.0 - 71.3)	74.4 (73.6 - 75.2)
VO₂max (ml/kg/min)	84.8 (84.4 - 85.2)	83.4 (83.0 - 83.8)
Tryglicerides (mg/dl)	73.3 (70.3 - 76.4)	64.4 (61.6 - 67.2)
HDLc (mg/dl)	60.0 (56.1 - 62.9)	53.0 (52.1 - 53.9)
Total cholesterol (mg/dl)	166.9 (164.6 - 169.2)	153.8 (151.6 - 156.1)
TC/HDL-c	2.99 (2.93 - 3.04)	3.02 (2.96 - 3.09)
Systolic Blood Pressure (mmHg)	116.2 (115.3 - 117.1)	124.4 (123.1 - 125.8)
HOMA index	2.38 (2.20 - 2.56)	2.28 (2.12 - 2.43)
∑ Four skinfolds	53.6 (51.5 - 55.8)	52.2 (49.9 - 54.4)
Resting heart rate (bpm)	78.9 (77.8 - 80.00)	80.6 (79.3 - 81.8)
Metabolic risk (%)	15.3 (11.8 - 18.9)	15.6 (11.9 - 19.4)

95% CI: confidence interval of 95%; BMI: body mass index; MVPA: Moderate to vigorous physical activity; HDLc= High-density lipoprotein cholesterol; TC= Total cholesterol. Significance difference ($p < 0.05$) between girls and boys are in bold

Table 4/2 Accuracy of resting heart rate in screening of individual and clustered cardio-metabolic risk factors in adolescents

Cardiovascular risk factors	Sensitivity (%)	Specificity (%)	Area Under the Curve	CI 95%		LR +	LR –	Youden Index
				Lower	Upper			
Clustered								
metabolic risk								
Male	80.7	17.2	49.0	43.4	54.5	0.95	1.12	27.1
Female	90.3	18.1	54.2	50.0	58.4	1.10	0.53	29.2
TC/HDL-c								
Male	77.0	18.0	47.5	42.3	52.7	0.94	1.27	26.8
Female	84.8	19.6	52.3	47.9	56.6	1.06	0.77	29.1
VO2max								
Male	85.5	18.5	52.0	47.3	56.7	1.05	0.78	30.4
Female	89.5	19.1	54.3	50.5	58.2	1.11	0.55	33.3
∑ Four skinfolds								
Male	81.4	18.8	50.1	45.8	54.5	1.00	0.99	30.9
Female	83.9	19.7	51.8	47.6	56.0	1.04	0.82	30.6
HOMA index								
Male	89.1	19.7	54.4	49.9	58.9	1.11	0.55	27.4
Female	87.8	19.8	53.8	48.8	58.7	1.09	0.62	25.8
Systolic Blood Pressure								
Male	61.4	15.6	38.5	32.5	44.5	0.73	2.48	22.0
Female	72.9	18.1	45.5	39.5	51.5	0.89	1.50	24.0
Tryglicerides								
Male	80.3	18.6	49.4	44.4	54.5	0.99	1.06	27.4
Female	87.7	20.0	53.9	49.4	58.2	1.10	0.62	28.0

CI 95%: confidence interval 95%, LR +: Positive likelihood ratios, LR –: Negative likelihood ratios, HDLc: High-density lipoprotein cholesterol, TC: total cholesterol.

DISCUSSION

This study analyzed the predictive power and accuracy of RHR as a screening measure for individual and clustered CVD risk factors in a large sample of European adolescents. The main finding was that RHR is not a good predictor of CVD risk factors in this population, regardless of sex, age and level of physical activity. Our

hypothesis is biologically plausible, since the onset of these factors in adolescence is strongly associated with increased risk of CVD in adulthood [141,147,151].

Girls had lower RHR than boys (statistically significant), this difference can be partially explained by two reasons: 1) the girls have a higher VO₂max and this increased aerobic capacity decreases RHR; 2) boys have a higher accumulation abdominal fat (measured by waist circumference) than girls, and visceral fat has been associated with higher sympathetic activity [143,144,145]. This activation is a key mechanism underlying the effect of intra-abdominal fat accumulation on the development of hypertension [146].

Although RHR has been recently showed to be a good predictor for CVD in adults [73], our findings do not confirm these results in adolescents. These differences may explained by the fact that the analyses carried out in adults considered as risk values into percentiles of the RHR [73,75-77] which is intrinsically associated with the distribution of the variable within the sample. In our study, we analyzed the predictive value using a more accurate analysis (ROC curve) that the distribution in percentiles. Another important point is that the onset of CVD takes several years [148], and here only we compare with risk factors for diseases.

Fernandes *et al.* [149] found that higher RHR is associated with higher levels of SBP regardless of nutritional status in children, however the authors also used percentiles to classify the RHR. There is evidence that obese adolescents have higher levels of SBP [149], which might also be translated into having higher RHR. However, accurate measurement of RHR is difficult, and the biological parameter has no advantage over the use of other CVD risk factors, since RHR has been shown to have an accuracy of less than 55% for all the factors. Another important point which may explain the absence of good prediction is the fact that adolescents

are in the process of biological maturation and maturation-related hormones influence the sympathetic activation [150] can be reflects vagal nerve activity that controls the RHR [73,74].

The principal message from our study is that RHR not being a good predictor of cardio-metabolic health during the adolescence.

The strengths of this study are that samples were collected in different countries using the same methodology, appropriate statistical analysis controlling for potential confounding factors were performed as well as the analysis of the efficiency of RHR such a predictor for different individual CVD risk factors and clustered CVD. On the other hand, diverse geographic origin of the sample and multilevel analysis are some of the main strengths of our investigation.

In this study there are some limitations such as its cross-sectional design; consequently, causality cannot be established. Moreover, it has not been possible to adjust the analysis for other factors potentially associated with BP, eg. genetic, intrauterine development and inflammatory indicators.

CONCLUSIONS

In conclusion, the RHR is a poor predictor of individual and clustered CVD risk factors. Furthermore, the estimates based on RHR are not accurate. According to our findings, the use of RHR as an indicator of cardiovascular risk in adolescents may result in a biased screening of cardiovascular health in both sexes.

IV. SUMMARY OF NEW OBSERVATIONS

1. Investigation

Hyperinsulinaemic obese children had significantly lower physical working capacity than the non-hyperinsulinaemic ones, in spite of their similar anthropometric characteristics and lipid profiles. Children with MS had significantly lower physical performance as measured by ED and body weight corrected PWC-170, VO_2 peak and AT values than obese children without metabolic disturbances.

2. Investigation

Cardiovascular autonomic dysfunctions are not rare among obese children and adolescents. Search for early signs of autonomic nervous system dysfunction should be detect clinically important subgroups of overweight children, those who might have high risk at adulthood for unexplained sudden death, those in whom hypertension develops and in whom obesity is not an important health hazard.

3. Investigation

The lack of normal nocturnal fall in blood pressure was a frequent phenomenon (42%) among obese children. The frequency of non-dipping was similar in the two genders. Most of the non-dipper obese children are hypertensive on the basis of ABPM, and their physical performance is decreased. The clinical consequences of non-dipping in obese children are presently unknown due to the absence of long-term follow-up studies (cardiac hypertrophy, subclinical atherosclerosis, renal dysfunction). The results of the present study indicate that

masked hypertension is a common condition in non-dipper obese children (32.2%).

4. Investigation

RHR is a poor predictor of individual CVD risk factors and of clustered CVD and the estimates based on RHR are not accurate. The use on RHR as an indicator of CVD risk in adolescents may produce a biased screening of cardiovascular health in both sexes.

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VII. TITLES OF PUBLICATIONS

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- 14.12th November 2014, Welsh Paediatric Cardiovascular Network Telemedicine Facilitated Academic Teaching Program, Cardiff. K Török: Coarctation of the aorta
- 15.21th January 2015, Welsh Paediatric Cardiovascular Network Telemedicine Facilitated Academic Teaching Program, Cardiff. K Török: Assessment of ventricular septum defects
- 16.6th March 2015, CALM meeting- meeting of Welsh adult cardiologists, research cardiologists and paediatric cardiologists, Cardiff. K Török, A Pateman, V Ofoe: Syncope in a 6 year old
- 17.18th March 2015, Welsh Paediatric Cardiovascular Network Telemedicine Facilitated Academic Teaching Program, Cardiff. K Török: Anderson Tawil syndrome
- 18.25th March 2015, Welsh Paediatric Cardiovascular Network Telemedicine Facilitated Academic Teaching Program, Cardiff. K Török: Echocardiographic assessment of mitral valve diseases
- 19.12th June 2015, CALM meeting- meeting of Welsh adult cardiologists, research cardiologists and paediatric cardiologists, Cardiff. K Török: "Pierced to the very heart"
- 20.17th June 2015, Welsh Paediatric Cardiovascular Network Telemedicine Facilitated Academic Teaching Program, Cardiff. K Török: Echocardiographic assessment of aortic valve diseases