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

2016

ABBREVIATIONS

ABPM	ambulatory BP monitoring
BF	body fat
BMI	body mass index
BP	blood pressure
CAN	cardiac autonomic neuropathy
CRF	cardiorespiratory fitness
CV	cardiovascular
CVD	cardiovascular diseases
DBHR	heart rate variation during deep breathing
DBP	diastolic blood pressure
ED	exercise duration
HELENA	Healthy Lifestyle in Europe by Nutrition in Adolescence cross-sectional study
HDL-c	high density lipoprotein cholesterol
HOMA	homeostasis model assessment
HR	heart rate
HR_0	resting heart rate
HRpeak	peak heart rate
LAT	lactic acidosis threshold
LAT-BW	lactic acidosis threshold normalised for body weight
MS	metabolic syndrome
OGTT	oral glucose tolerance test
OT	fall in systolic blood pressure on standing
PA	physical activity
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/L	standing-lying heart rate ratio
SH	rise in diastolic blood pressure during sustained handgrip
SNS	sympathetic nervous system
TC	total cholesterol
TC/HDL-c ratio	total cholesterol / high density lipoprotein cholesterol ratio
VO_2max	maximal oxygen consumption
VO_2peak	peak oxygen consumption
VO_2rest	resting oxygen consumption
VO_2peak-BW	peak oxygen consumption normalised for body weight
VO_2rest-BW	resting oxygen consumption normalised for body weight

I. INTRODUCTION

The prevalence of childhood overweight and obesity has increased at a high speed during the past three decades while appears to be stabilising at different levels in different countries; it remains high and a significant public health issue.

Obesity-associated hypertension, hyperinsulinaemia, impaired glucose tolerance and dyslipidaemia are considered to be separate and independent risk factors for cardiovascular (CV) and cerebrovascular diseases in adulthood.

The clustering of these risk factors, called metabolic syndrome (MS), has also been shown in both children and adults. Our working group has demonstrated the frequency of MS, which was 8.9% in obese children.

Poor physical activity (PA) and cardiorespiratory fitness (CRF) during these periods of life seems to be associated with later CV risk factors. PA and CRF in obese children are generally decreased. Measurements of CRF are preferable in relation to those of PA, due to their greater objectivity and lower propensity to errors. Physical exercise requires the interaction of physiologic mechanisms that enable the CV and respiratory systems to support the increased energy demands of contracting muscles. Both systems are consequently stressed during exercise. Cardiopulmonary exercise testing can be used provide an objective assessment of exercise capacity and impairment. In obese individual needs greater than normal CV and ventilatory responses in order to perform any physical work. 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.

Long duration of obesity in adulthood can be associated with an increased incidence of ischemic heart disease, malignant ventricular arhythmias 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. Alterations in the autonomic nervous system can cause disturbances in the function of CV system- 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, which result in resting tachycardia, abnormal myocardial blood flow regulation and impaired cardiac function. Obesity and metabolic syndrome are characterized by sympathetic nervous system (SNS)

predominance in the basal state and reduced SNS responsiveness after various sympathetic stimuli. Hyperglycaemia plays a key role in the development and progression of CAN. Autonomic dysfunction impairs exercise tolerance, increases resting heart rate and blood pressure and alters cardiac output responses to exercise. 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. Cardiovascular reflex testing may help us identify children at high risk of unexplained sudden death and hypertension without preventive efforts in adulthood.

It is well known that CAN is associated with abnormal diurnal blood pressure (BP) variables at adulthood. Ambulatory blood pressure monitoring (ABPM) provides the opportunity to study BP patterns during the day and night. 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. O'Brien *et al.* were the first to draw attention to the negative prognostic value of the absence of nocturnal BP fall in hypertensive patients. These so-called non-dippers may have more pronounced 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). Abnormal 24-hour ABPM profiles have been observed in diabetic and hypertensive patients, both adults and children. However, no studies have been performed on obese children.

Resting heart rate (RHR) reflects sympathetic nerve activity and it is an accessible clinical measurement. A significant association between RHR and cardiovascular mortality has been reported in some epidemiologic studies. Based on epidemiologic data and inferences from clinical trials the results showed that high RHR is undesirable in terms of CV disease. However, the importance of RHR as a prognostic factor and potential therapeutic outcome has not been formally explored in childhood.

II. AIMS OF THE STUDY

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

2. 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. 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. 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 assessing the CV risk factors in our cohort of 180 obese children, 22 boys with multiple CV risk factors (MS group) and 17 boys being free of any CV risk factor (Obese group) were included into the study. Healthy boys with normal weight matched for age served as controls (Control group; n=29). MS was defined as the simultaneous occurrence of obesity, hyperinsulinaemia, hypertension and both or at least one of the impaired glucose tolerance and dyslipidemia.

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 (m^2). 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. 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.

Examinations and laboratory measurements

BP 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. If the average of the three BP values was above the 95^{th} percentile for age and sex, 24-hour ABPM was performed. Children with mean ABPM values exceeding the 95^{th} percentile value for height and sex were considered hypertensive.

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 μU/mL (mean + 2 SD of 100 non-obese Hungarian children) and/ or postload peak serum insulin during OGTT >150 μU/mL; impaired glucose tolerance – fasting blood glucose ≥5.6 mmol/L or 2-hour blood glucose during OGTT ≥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); hypercholesterolaemia – total fasting cholesterol concentration >5.2 mmol/L. 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.*

Plasma glucose was measured by glucose oxidase method. Serum cholesterol, triglyceride and HDL-cholesterol levels were determined by enzymatic method using Boehringer kits. 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 CV 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. ECG was continuously monitored throughout the test. BP was measured each minute by auscultation. Respiratory variables were measured by means of a Jaeger EOS-Sprint exercise metabolic measurement system. The O_2 and the CO_2 concentrations were determined from the mixed expired air and the volume of the expired air was measured using a pneumotachograph. Exercise duration (ED), resting heart rate (HR_0), peak heart rate (HRpeak), 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.

RESULTS

Obese children with or without MS, demonstrated a significantly shorter ED than the controls. HR_0 and HR_{peak} comparisons are demonstrated in Figure 1/1. PWC-170 and, when PWC-170 was normalised for body weight comparisons are demonstrated in Figure 1/2.

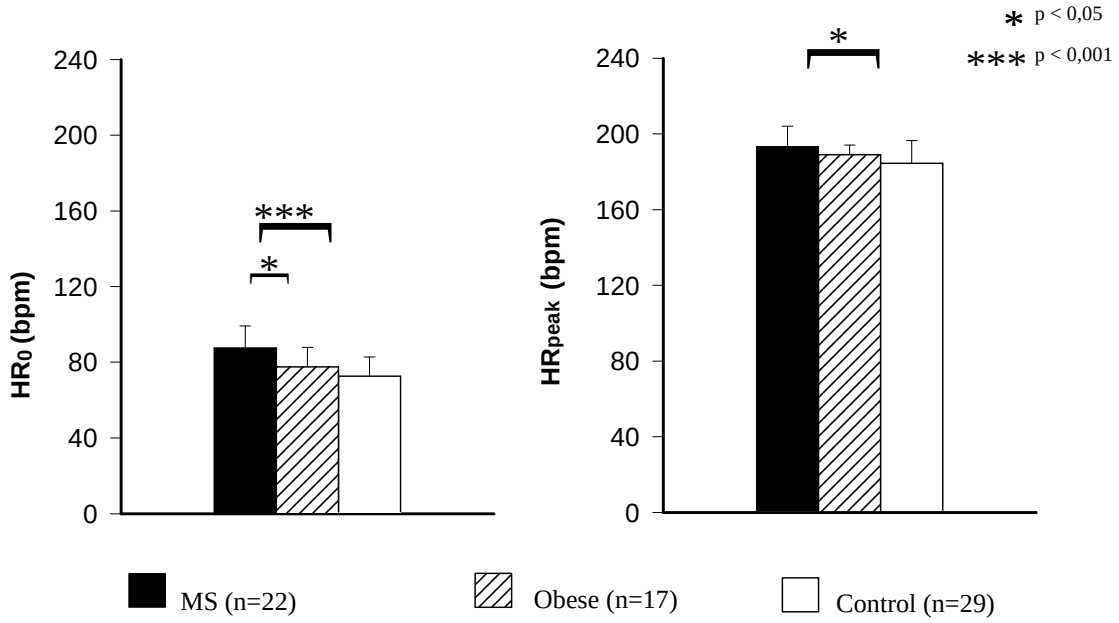
The VO_2peak and the LAT were also significantly lower in the obese groups when normalised for the body weight (Table 1/1).

Table 1/1 Original LAT and VO_2peak values, and those normalised for body weight (LAT-BW, VO_2peak-BW) (mean ± SD)

	MS (n=22)	Obese (n=17)	Control (n=29)
LAT (L/min)	1.53 ± 0.42	1.53 ± 0.48	1.61 ± 0.28
LAT-BW (L/min)	1.33 ± 0.37 ∗	1.50 ± 0.37 @	1.78 ± 0.37
VO_2peak (L/min)	2.70 ± 0.60	2.51 ± 0.74	2.47 ± 0.39
VO_2peak-BW (L/min)	2.19 ± 0.42 ∗	2.43 ± 0.45 @	2.91 ± 0.43

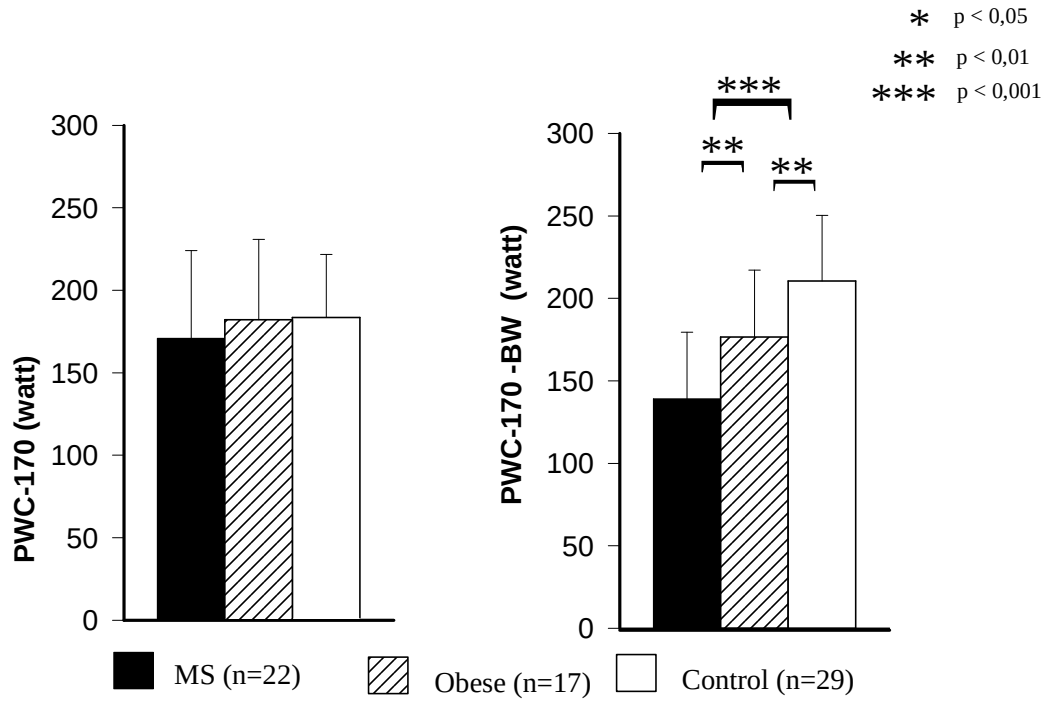
LAT: lactic acidosis threshold; LAT-BW: lactic acidosis threshold normalised for body weight; VO_2peak: peak oxygen consumption; VO_2peak-BW: peak oxygen consumption normalised for body weight; MS: Metabolic syndrome; ∗ $p<0.05$ MS vs Control; @ $p<0.05$ Obese vs Control

Figure 1/1 Resting and peak heart rate



$HR_{0:}$ Resting heart rate, HRpeak: peak heart rate, MS: Metabolic syndrome

Figure 1/2 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

CONCLUSIONS

Our results demonstrated that children with MS had significantly lower physical performance as measured by ED and body weight corrected PWC-170, VO_2peak 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. 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 ± SD)

	Boys (n=23)			Girls (n=24)		
Age (years)	12.7	±	2.5	14.1	±	2.4
Body weight (kg)	82.9	±	17.5	80.4	±	15.3
BMI (kg/m^2)	31.1	±	4.4	30.5	±	3.9
BF (kg)	31.0	±	7.6	34.7	±	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. 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). RHR was 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 were performed by the same person. Reference ranges were used from literature (data obtained in 130 healthy children aged 6 to 18 years). RHR, S/L and SH test results were age dependent.

RESULTS

Our results are demonstrated in Table 2/2, 2/3 and 2/4.

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)	19	40.4
S/L	17	36.2
DBHR (beats/min)	15	31.9
SH (mmHg)	8	17
RHR (beats/min)	6	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 ± SEM)

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

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 hypertensions 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 rhytm of blood pressure pattern in obese children

PATIENTS AND METHODS

Patients and sampling

73 obese children (51 males, 22 females; age [mean ± SD]: 14.2 ± 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. 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.

Blood pressure measurements

BP was measured during the morning in the outpatient clinic on three separate occasions by a physician using a mercury sphygmomanometer. The average of the three measurements was used to give the office systolic and diastolic BPs. An office BP consistently greater than or equal to the 95th percentile of the BP distribution in a normal reference population was considered as hypertensive. Multiple BP measurements were then carried out by ABPM monitoring 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. The ABPM days were always performed over a working day (Monday to Friday). For evaluation, the ABPM Report Management System program was used. 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

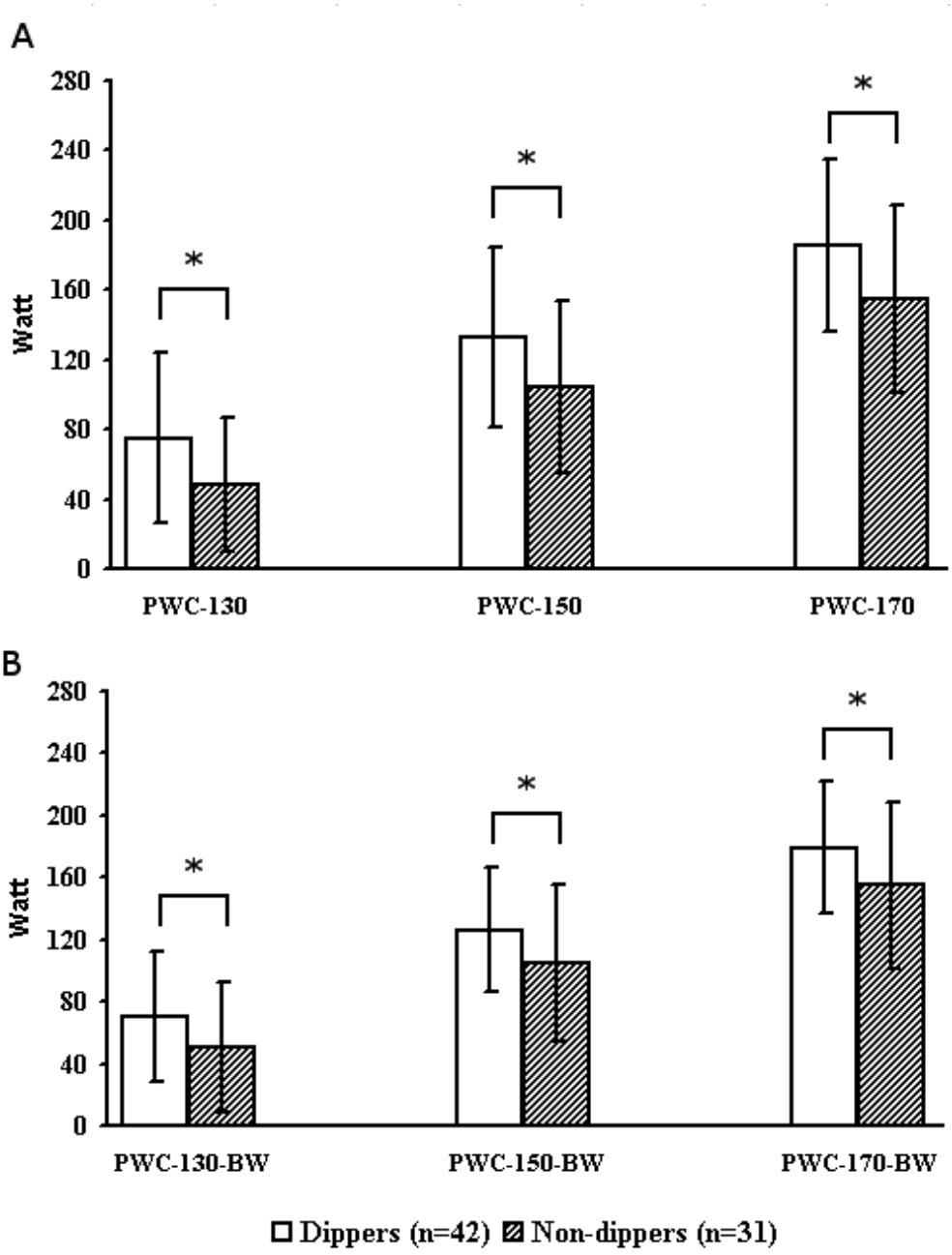
according to Soergel *et al.* Children with mean 24-hour systolic or diastolic ABPM values exceeding the 95th percentile for height and sex was considered hypertensive. Masked hypertension was defined as increased 24-h ABPM value in the presence of normal office blood pressure. Nocturnal BP fall was calculated by subtracting nighttime values from daytime values of BP 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 BP at night.

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 (93.4±17 vs 83.1±17.5, $p<0.05$), but there was no significant difference between the two groups in the degree of obesity (BMI, body fat). 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.

There was no significant difference in office BPs and average daytime systolic and diastolic BP values between the two groups, while average nighttime values were significantly elevated in non-dippers. Incidence of CV 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 (93.4±17 vs 45.2±83.9, $p<0.001$) on the basis of ABPM, the prevalence of hypertension was similar in the two groups when office BP measurements were considered. The prevalence of masked hypertension was also significantly higher in the non-dippers (19.0±32.3, $p<0.001$). 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). Resting and peak oxygen consumption was also higher in dippers than in non-dippers (Figure 3/2), however, the differences disappered, 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.

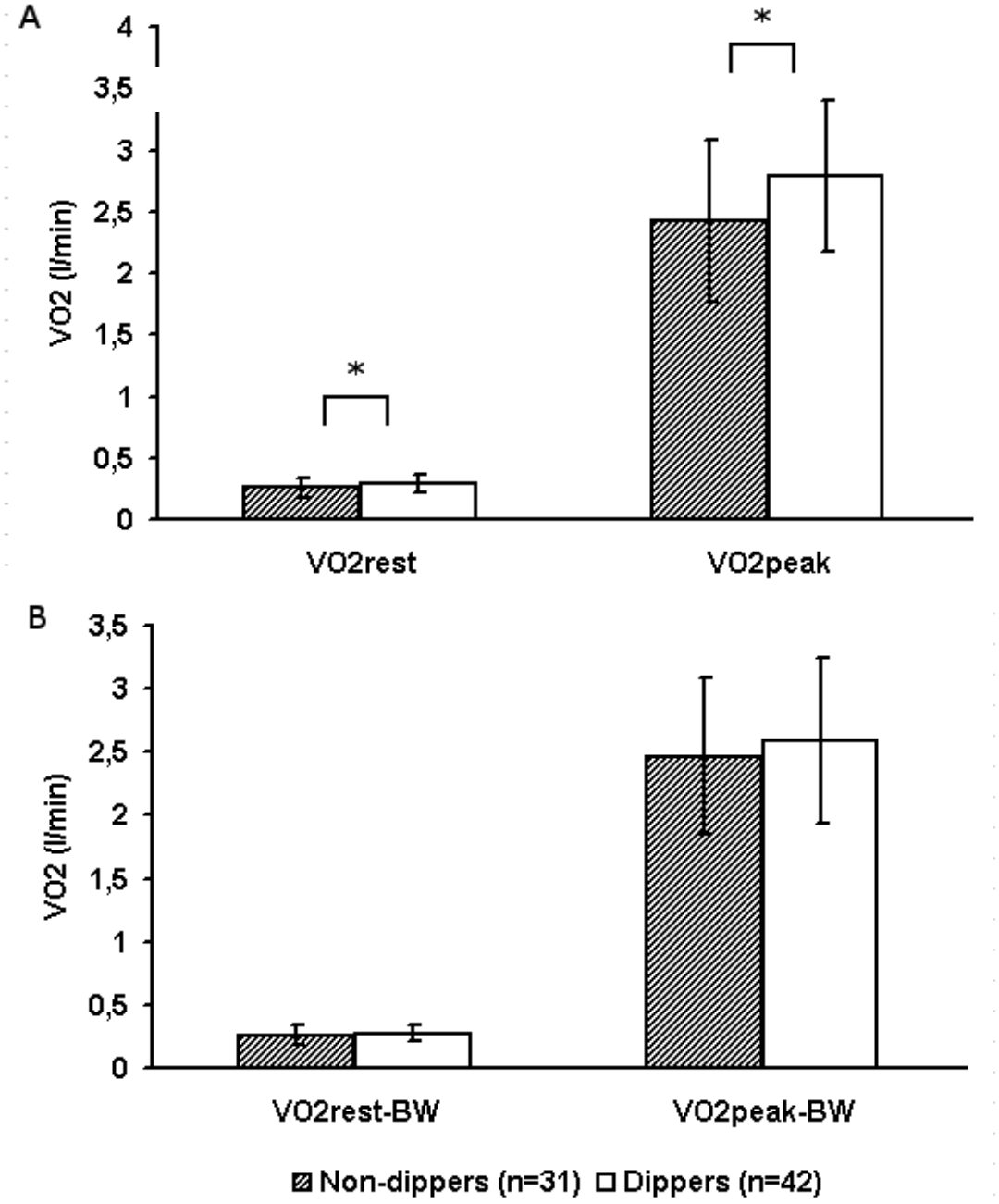
Figure 3/1 Physical working capacity at heart rate 130, -150, -170 in absolute values (A), and normalised for body weight (B) in dippers and non-dippers (mean ± SD)



*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 ± SD)



*p <0.05 Dippers vs Non-dippers
VO_2rest-BW: resting oxygen consumption normalised for body weight, VO_2peak: maximal oxygen consumption normalised for body weight

CONCLUSION

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. The lack of the normal circadian BP rhythm is considered an early sign of autonomic neuropathy, which could be a first sign of modified vascular reactivity. Whether the lack of the nocturnal fall of BP 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. 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 *Healthy Lifestyle in Europe by Nutrition in Adolescence (*HELENA) cross-sectional study aimed to describe the lifestyle and nutritional status of European adolescents. Data collection took place between October 2006 and December 2007 in 10 europen cityes. The general inclusion criteria for HELENA were age range of 12.5-17.5 years.

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).

Resting Heart Rate (RHR)

The RHR and BP 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. 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. The definition of obesity (including overweight) was based on international BMI cutoffs proposed by *Cole et al*.

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. Finally, the VO_2max in ml/kg/min was estimated by the Leger equation (boys and girls: VO_2max = 31.025 + (3.238 x S x 3.248 x A) + (0.1536 x S x A); A the age, S the final speed S = 8 + 0.5 last stage compleed.

Cardiovascular risk factors

Blood samples were obtained for a third of the HELENA-study participants. Blood samples were taken in the morning after an overnight fast. Blood was collected, immediately placed on ice and centrifuged and transported 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). Insulin levels were measured using an Immulite 200 analyser (DPC Bierman GmbH). The HOMA calculation was used as a measurement of insulin resistance.

A clustered cardiovascular risk index was created from the following variables: systolic blood pressure, HOMA index, triglycerides, TC/HDL-c ratio, VO_2max and the sum of four skinfolds. The standardized value of each variable was calculated as follows: (value-mean)/SD, separately for boys and girls and by 1-yr age groups. For variables characterized by a lower metabolic risk with increasing values (VO_2max), 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 ≥1SD.

RESULTS

The proportion of boys had significantly performing physical activity the recommended amount of physical activity (≥60min/day) was higher than girls (27.7(23.3-32.1), 60.7(55.4-66.1, $p<0.05$). 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 (78.9(77.8-80.0), 80.6(79.3-81.8, $p<0.05$).

The accuracy of prediction of RHR for the six factors individual CVD risk factors and for the cluster of CVD separately by sex seen was analysed using a more accurate analysis (ROC curve). For all CVD risk factors, the RHR have a high sensitivity, low specificity and accuracy (area under of curve), regardless of sex. RHR is not a good predictor of CVD risk factors in this population, regardless of sex, age and level of physical activity.

CONCLUSIONS

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_2peak 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 hypertensions develops and in whom obesity is not an important health hazard.

3. Investigation

The lack of normal nocturnal fall in BP 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-diping 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.

VI. ACKNOWLEDGMENTS

I would like to express my thanks to Professor Dénes Molnár, who supported me throughout all my experimental and clinical work. I thank him for his never-ending encouragement in my studies and during the writing of my thesis.

I am grateful to Professor Gyula Soltész, who supported me at the beginning of my experimental and clinical work.

I would like to express my gratitude to Professor Zoltán Szelényi, who supported my research work in the Department of Pathophysiology. I am also grateful to János Pórszász, who helped to plan the exercise test protocol and gave a lot of practical advice and invaluable input.

I am grateful to Professor István Wittmann, who gave the opportunity to do the autonomic neuropathy tests in the laboratory of the 2nd Department of Internal Medicine and Nephrology Centrum.

Special thanks to Magdolna Szűcs, Magdolna Baranyai(†) and Judit Girán from the Department of Pathophysiology, to Ágnes Tarján and to the nurses and doctors of Endocrine-diabetes ward from the Department of Paediatrics, who helped in my experimental work.

Thank to Sára Jeges for statistical advice.

I am grateful to Dr Dirk Wilson Consultant Paediatric Cardiologist at Cardiff University Children's Hospital for reviewing my thesis.

I express my thanks to my collegues, friends and family for their help, encouragement and support during my studies and work.

VII. TITLES OF PUBLICATIONS RELATED TO THE THESIS

Articles related to the thesis

1. Gy. Csábi, K. Török, S. Jeges, D. Molnár: Presence of metabolic cardiovascular syndrome in obese children. **Eur J Pediatr** 159: 91-94, 2000 (**IF: 1.318; SCI: 209**)
2. Török K., Csák B., Molnár D.: Csökkent fizikai teljesítőképesség multimetabolicus cardiovascularis szindrómában. **Obesitologia Hungarica** 1 (1): 13-16, 2000
3. Török K.: Multimetabolikus szindróma és fizikai teljesítőképesség. (Az Apáthy Alapítvány és a Pediáter 1999-ben kiírt pályázatán I. díjas pályamunka) **Pediáter** 9 (2): 81-89, 2000
4. K. Török, Z. Szelényi, J. Pórszász, D. Molnár: Low physical performance in obese adolescent boys with metabolic syndrome. **Int J Obesity** 25: 966-970, 2001 (**IF: 2.196; SCI: 39**)
5. Török K., Pálfi A., Molnár D., Szelényi Z.: A vérnyomás cirkadián változása elhízott gyemekeknél. **Magyar Orvos** 16:(4) pp.41-45, 2008
6. K. Török, A. Pálfi, Z. Szelényi, D. Molnár: Circadian variability of blood pressure in obese children. **Nutrition, Metabolism & Cardiovascular Diseases** 18: 429-435, 2008 **(IF:3.565; SCI: 12)**
7. A. C. Ferreira de Moraes, A. J. F. Cassenote, C. Leclercq, J. Dallongeville, Y. Manios, K. Török, M. González-Gross, K. Widhalm, A. Kafatos, H. Barbosa de Carvalho, L. A Moreno: Resting heart rate is not a good predictor of a clustered cardiovascular risk score in adolescents. The HELENA Study. **Plos One** ; 10:(5) Paper e0127530. 11 p., 2015 **(IF:3.534)**

Published abstracts related to the thesis

1. K. Török, D. Molnár: Physical fitness in obese adolescents with multimetabolic syndrome. (abs) **Int J Obesity** 23 (Suppl.5): S118, 1999
2. K. Török, I. Wittmann, D. Molnár: Cardiovascular autonomic dysfunction in obese children. (abs) **Int J Obesity** 25 (Suppl.2): S79, 2001
3. K. Török, I. Wittmann, D. Molnár: Cardiovascular autonomic dysfunction in obese children. (abs.) **Acta Physiologica Hungarica** 89: 191, 2002
4. K. Török, I. Wittmann, D. Molnár: Cardiovascular autonomic dysfunction in obese children. (abs.) **J Pediatric Endocrinology & Metabolism** 15 (Suppl 4):1090, 2002
5. K. Török, Z. Szelényi, D. Molnár: Circadian variability of blood pressure and physical fitness in obese children. (abs) **Slovenska pediatrija** 11: 44, 2004

Oral presentations related to the thesis

1. 1997. szeptember, Szombathely, A Magyar Diabetes Társaság Gyermekdiabetes Szekcióülése: Török K, Soltész Gy, Molnár D: Fizikai teljesítőképesség, cardiovascularis adaptatio diabeteses gyermekekben
2. 1988. május 27., Szeged, MGYT Országos Nagygyűlése
   Török K, Dr. Molnár D, Orbán K: Testsúlycsökkenés hatása a cardiorespiratoricus adaptatiora gyermekkorban
3. 30-31 October, 1998 Budapest. 2nd Conference of the International Group for Prevention of Atherosclerosis in Childhood.
   D. Molnar, T. Decsi, Gy. Csábi, K. Török, I. Burus. Multimetabolic sydrome and other risk factors of atherosclerosis in childhood
4. 9th European Congress on Obesity, 3-6 June 1999, Milano, Italy
   K. Török, D. Molnár: Physical fitness in obese adolescents with multimetabolic syndrome (poster)

5. 8. Tagung mitteleuropaischer Lander “Padiatrische Forschung”, 18. Juni 1999, Wien
   K. Török, D. Molnár: Physical fitness in obese adolescents with multimetabolic syndrome
6. 1999. szeptember 19-20., Siófok, A Magyar Elhízásellenes Alapítvány XII. Konferenciája:
   Török K, Molnár D: Anyagcsere gondozás klinikánkon - törekvéseink a gondozás hatékonyságának fokozására
7. 2000. szeptember 7-9., Szolnok, A Magyar Táplálkozástudományi Társaság XXV. Vándorgyűlése:
   Török K, Erhardt É, Csábi Gy, Molnár D: "Életmód tábor" - fogyókúrás gyermektáborok tapasztalatai
8. 2000. szeptember 14-17., Dobogókő, A Magyar Elhízástudományi Társaság I. kongresszusa és a Magyar Elhízásellenes Alapítvány XV. országos konferenciája:
   Török K, Angsterné Tarján Á, Rugási E, Erhardt É, Molnár D: Nyári táboroztatás és sportolás szerepe a gyermekkori elhízás kezelésében
9. 10-11 November, 2000 Pécs, European Childhood Obesity Group 10th Workshop
   K Török, Dénes Molnár: Cardiovascular autonomic dysfunction in obese children – preliminary results
10. Mandulavirágzási Tudományos Napok, Pécs, 2001. március 5-8.:
    Török K: Kardiovaszkuláris autonóm neuropathia gyermekkorban
11. 2001. június 15-16., Pécs, A Magyar Gyermekorvosok Társasága Nagygyűlése: Török K, Garábi B, Wittmann I, Molnár D: Cardiovascularis autonóm neuropathia gyermekkori elhízásban (poszter)
12. 2002. május 30-június 2., Debrecen. A Magyar Diabetes Társaság XVI. Kongresszusa
    Török K, Wittmann I, Molnár D: Cardiovascularis autonom neuropathia gyermekkori elhízásban
13. 2002. június 13-15., Tatabánya A Magyar Gyermekorvosok Társasága Nagygyűlése: Dr. Török K., Pálfi A., Dr. Molnár D.: A vérnyomás circadián változása és a fizikai terhelhetőség elhízott gyermekekben
14. 2002. szeptember 13-14., Budapest, A Magyar Elhízástudomány 10. Jubileumi Kongresszusa:
    Török K., Wittmann I., Molnár D.: Kardiovaszkuláris autonom neuropátia kövér gyermekekben
15. 2003. június 2-4., Pécs, A Magyar Élettani Társaság LXVII. Vándorgyűlése: Rugási E., Török K., Molnár D., Ángyán L.: A rendszeres gyógyúszás hatása a túlsúlyos gyermekek testösszetételére és fizikai terhelhetőségére (poszter)
16. Paediatric Research of Central European Countries, June 4, 2004, Bled, Slovenia: K Török, Z Szelényi, D Molnár: Circadian variability of blood pressure and physical fitness in obese children (poster)