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ORIGINAL ARTICLE

Association between nocturnal blood pressure dipping and insulin resistance in children affected by NAFLD

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Abstract The aim of this study was to analyse the relationship between insulin–glucose metabolism, nocturnal blood pressure dipping and nonalcoholic fatty liver disease (NAFLD) in obese adolescents without diabetes. One hundred one consecutive children, with biopsy-proven NAFLD, were included in this study. Blood samples were drawn for the analyses of liver function tests, insulin–glucose metabolism and lipid profile appraisal. An ambulatory blood pressure measurement (ABPM) was performed. Seventy-six children (75.3 %) were systolic nondippers, and 23 of them were diastolic nondippers (30.3 %). No differences were found in the anthropometric parameters between the two groups. When compared to the systolic dippers, the systolic nondippers had higher medians of mean nocturnal blood pressure, glucose at 0, 60 and 120 min in the oral glucose tolerance test (OGTT), OGTT insulin at all time points and insulin-resistance values. No correlation of histopathological features with dipping/nondipping statuses was found. *Conclusions*: We found an association between a nocturnal blood pressure fall and measures of insulin levels, independent of obesity, or daytime blood pressure levels, among the obese patients with NAFLD. Although no association between nondipping profiles and NAFLD was observed in our study, further studies

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with a longer term follow-up are needed, to better elucidate the complex link between these particular entities.

Keywords Dipping · Hypertension · NAFLD · Children · Obesity

Abbreviations

ALT	Alanine transferases
AST	Aspartate transferases
ABPM	Ambulatory blood pressure measurement
BP	Blood pressure
GGT	Gamma-glutamyl-transpeptidase
HSC	Hepatic stellate cells
HOMA	Homeostatic model assessment
IQR	Interquartile range
ISI	OGTT-derived insulin sensitivity index
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
NAFLD	Nonalcoholic fatty liver disease
OGTT	Oral glucose tolerance test
RAAS	Renin–angiotensin–aldosterone system
NASH	Steatohepatitis
SNS	Sympathetic nervous system
TG	Total triglycerides

Introduction

The worldwide obesity epidemic is changing the face of paediatric hypertension. A growing body of evidence shows an alarmingly high prevalence of overweight and obesity among children and adolescents, not only in the USA, but also in other developed countries, and to some extent, in the developing world as well. The most recent National Health and Nutrition Examination Study (NHANES) data shows that nearly 18 % of children and adolescents meet the criteria for obesity and 32 % meet the criteria for overweight (currently defined as body mass index [BMI] ≥95th and ≥85th percentiles for age and gender, respectively) [25].

Following several decades of trending downwards, the prevalence of elevated blood pressure (BP), among US children and adolescents, has been increasing since the late 1980s. The increment in childhood obesity, which preceded this upward trend by about 10 years, is likely to be the major contributor to these changes. It is estimated that the prevalence of paediatric hypertension ranges between 3 and 11 % in different countries, and it is three times more frequent in obese than in normal-weight children [8, 27, 32]. The prevalence of hypertension increases progressively with an increasing body mass index, and some studies have detected hypertension in over 30–40 % of obese children [18, 31].

Ambulatory blood pressure measurement (ABPM) can more precisely characterise changes in BP patterns and detect abnormalities of the circadian variation of BP over a day and night period [30]. In adolescents, night-time (sleep) systolic BP normally drops by 10 % from daytime (awake) BP, which is the phenomenon called nocturnal dipping [30]. Obese adolescents are at a higher risk of reduced nocturnal dipping [7], and it is reported that nondipping BP profiles may be associated with an increased risk of BP-related complications [34]. Recent evidence suggests that there is an association between reduced nocturnal dipping and impaired glucose metabolism, and this association is even more frequent in obese adolescents [17, 7]. However, there have only been a few studies analysing the association between insulin–glucose metabolism and nocturnal dipping in obese and nondiabetic adolescents [34].

Nonalcoholic fatty liver disease (NAFLD) today represents the most common liver disease in children and adolescents in industrialised countries. It is a clinic-pathological entity that ranges from simple steatosis to an advanced form of steatohepatitis (NASH), with inflammation and fibrosis, which can progress to cirrhosis and an end-stage liver disease [3]. The pathophysiological mechanisms leading to NAFLD/NASH are still not completely understood, but it is now considered to be a multipathogenetic disorder, and insulin resistance has been indicated as a key event in the pathogenesis [24, 1].

Therefore, on the basis of this data, we hypothesise that there is an association between insulin–glucose metabolism and nocturnal BP dipping, among obese and nondiabetic adolescents with fatty liver disease. The aim of this study was to analyse the relationship between insulin–glucose metabolism, nocturnal dipping, and liver disease, in obese adolescents without diabetes.

Methods

Patients

We prospectively included in the present study children and adolescents with biopsy-proven NAFLD, as observed in the Hepato-Metabolic Department of Bambino Gesù Children's Hospital, Rome, Italy, from January 2011 to July 2013. All of the patients were tested for secondary causes of steatosis, including alcohol abuse, total parenteral nutrition and the use of drugs known to precipitate steatosis (e.g. valproate, amiodarone or prednisone) or those drugs that affect body weight and blood pressure. Hepatitis A, B, C, cytomegalovirus and Epstein–Barr virus infections were ruled out by appropriate tests. Autoimmune liver disease, metabolic liver disease, Wilson's disease, celiac disease and alpha-1-antitrypsin deficiency were ruled out using standard clinical,

laboratory and histological criteria. The study design conformed to the ethical guidelines of the Declaration of Helsinki (1975), and it was approved by the Ethics Committee of Bambino Gesù Children's Hospital and Research Institute.

Anthropometric measures

Body weight was measured to the nearest 0.5 kg on a standard physician's beam scale with the child only wearing underwear and no shoes. Height was measured to the nearest 0.5 cm by using a wall-mounted stadiometer, without shoes, heels together, with the child's heels, buttocks, shoulders and head against the vertical wall, with the line of sight aligned horizontally. BMI was calculated by dividing weight (kilograms) by height squared (metres squared). A BMI *Z* score (SDS) was calculated according to national BMI reference tables [4]. The waist circumference (WC) measurement was taken midway between the tenth rib and the iliac crest and was recorded to the nearest millimetre. A nonelastic flexible tape measure was employed with the subject in a standing position [21].

Laboratory assessment

Alanine and aspartate transferases (ALT and AST), gamma-glutamyl-transpeptidase (GGT), total triglycerides (TG) and both low-density (LDL) and high-density lipoprotein (HDL) cholesterol were evaluated using standard laboratory methods. Plasma insulin was measured using a radioimmunoassay (Myria Technogenetics, Milan, Italy). All participants underwent a standard oral glucose tolerance test (OGTT) performed with 1.75 g of glucose per kilogram of body weight (up to 75 g), and glucose and insulin were measured at 0, 30, 60, 90 and 120 min. The degree of insulin sensitivity/resistance was determined via the homeostatic model assessment (HOMA) and by the OGTT-derived insulin sensitivity index (ISI) [20]. Both the HOMA and the OGTT-derived ISI have a significant correlation with the gold standard euglycemic hyperinsulinemic glucose clamp technique [35]. A HOMA value >2, or an ISI value <6, was considered an indication of insulin resistance.

Liver histology

The clinical indication for a biopsy was either to assess the presence of NASH and the degree of fibrosis and/or to rule out other potential liver diseases [33]. A liver biopsy was performed in all children, after an overnight fast, using an automatic core biopsy 18-gauge needle (Biopince, Amedic, Sweden), under general anaesthesia and ultrasound guidance [26]. A Sonoline Omnia ultrasound machine (Siemens, Munich, Germany), equipped with a 5-MHz probe (5.0 C 50, Siemens), and a biopsy adaptor were employed. The length of liver specimen was recorded, and only samples with a length ≥15 mm and including at least 5–6 complete portal tracts were considered adequate for the purpose of the study. Biopsies were routinely processed (i.e. formalin-fixed and paraffin-embedded), and sections of the liver tissue were stained with haematoxylin-eosin, Van Gieson's stain, periodic acid-Schiff diastase stain and Prussian blue stain. Biopsies were evaluated by a single hepatopathologist who was purposely blind to the clinical and laboratory data. Steatosis, inflammation, hepatocyte ballooning and fibrosis were scored using the NAFLD Clinical Research Network (CRN) criteria [13]. Briefly, steatosis was graded on a 4-point scale: grade 0=steatosis involving <5 % of hepatocytes; grade 1=steatosis involving up to 33 % of hepatocytes; grade 2=steatosis involving 33–66 % of hepatocytes and grade 3=steatosis involving >66 % of hepatocytes. Lobular inflammation, too, was graded on a 4-point scale: grade 0=no foci; grade 1=<2 foci per 200× field; grade 2=2–4 foci per 200× field and grade 3 = >4 foci per 200× field. Hepatocyte ballooning was graded from 0 to 2: 0=none; 1=few balloon cells and 2=many/prominent balloon cells. The stage of fibrosis was quantified using a 5-point scale: stage 0=no fibrosis; stage 1=perisinusoidal or periportal; 1a=mild, zone 3, perisinusoidal; 1b=moderate, zone 3, perisinusoidal; 1c=portal/periportal; stage 2=perisinusoidal and portal/periportal; stage 3=bridging and stage 4=cirrhosis.

Features of steatosis, lobular inflammation and hepatocyte ballooning were combined, to obtain the NAFLD activity score (NAS). As recently recommended by the NASH Clinical Research Network [13], a microscopic diagnosis based on an overall injury pattern (steatosis, hepatocyte ballooning, inflammation), as well as the presence of additional lesions (e.g. zonality of lesions, portal inflammation and fibrosis), was assigned to each case. Accordingly, biopsies were subdivided into not-NASH and definite NASH subcategories.

Blood pressure measurement

All subjects underwent a physical examination, with the measurement of blood pressure at rest, using a calibrated Tycos aneroid sphygmomanometer. Systolic and diastolic blood pressures were measured in the right arm in the seated position. A correct measurement of blood pressure required the use of a cuff that was appropriate to the size of the child's upper right arm, as indicated in the criteria of the Task Force for Blood Pressure Control in Children [23]. The blood pressure that was used for analysis was the mean of three separate measurements taken at 3–5-min intervals. Blood pressure was recorded twice on each examination, and the average of each of the systolic and diastolic blood pressure measurements was used to determine the blood pressure level. To define a subject as hypertensive, blood pressure tables, as adjusted for gender, age and height, were used [23]. A child was defined as

normotensive if their blood pressure was below the 90th percentile. If the child's blood pressure, either systolic or diastolic, was at, or above, the 95th percentile, the child was considered hypertensive after three examinations, during which the blood pressure was still at the 95th percentile. A child was clearly hypertensive if their blood pressure was over the 95th percentile [23]. According to the available evidence, weight reduction, obtained by behavioural modification (regular physical activity and dietary changes), associated to low sodium diet represents the first-line therapeutic approach to BP alterations [23]. Only if left ventricular hypertrophy is associated, pharmacological options were considered.

Ambulatory blood pressure monitoring

All patients underwent a 24-h ambulatory blood pressure monitoring (Spacelab 90207, Spacelab Inc, Redmond, WA). An appropriate cuff size was chosen with the same criteria as was used at rest. The subject was instructed to relax the arm during a blood pressure measurement. Measurements that produced a pulse pressure of less than 20 mmHg, or a heart rate of less than 40 beats per minute, were regarded as errors and excluded automatically by the recorder. If any of the parameters could not be measured correctly, the measurement was repeated within 3 min. The device was set to obtain readings every 15 min from 7:00 a.m. to 11:00 p.m. and every 30 min from 11:00 p.m. to 7:00 a.m. Awake and sleep periods were established by a daily-activities diary as kept by each patient. Ambulatory blood pressure readings were only accepted when at least 75 % of the measurements were successful. The mean of 24-h daytime and night-time systolic and diastolic blood pressures, the 24-h mean of arterial pressure and the 24-h mean pulse pressure, together with the presence of physiological nocturnal dipping, were determined. To define hypertension, reference values were obtained and used from the table of oscillometric mean ABPM values for healthy children, as adjusted for gender and height [30]. The percentage of night-time reduction in BP ('dipping') was calculated as (daytime BP−night-time BP)*100/daytime BP. Nondipping was defined as a nocturnal BP reduction of <10 %.

Statistical analysis

Descriptive statistics were reported as being of median and interquartile range (IQR). The IQR was calculated as the difference between the 25th and 75th percentile.

The most continuous variables were not normally distributed, and all were reported as being of the 25th, 50th and 75th percentiles. Between-group comparisons of medians were performed using quantile regression [14]. Categorical variables were reported as numbers and frequencies. Univariable logistic regression was used to evaluate the association between dipping and logeISI (continuous), z-BMI (continuous) and liver steatosis (categorical: 1=mild, 2=moderate, 3=severe) [11]. Multivariable logistic regression was used to estimate the joint contribution of loge-ISI, z-BMI and liver steatosis to dipping [11]. Fractional polynomials were used to test whether the univariable and the multivariable relationships of dipping with continuous predictors were linear. They were found to be so in all cases [28]. Statistical significance was set to a p value <0.05, and all statistical tests were two-tailed.

Results

We included in the study 101 patients with biopsy-proven NAFLD (54 males, median age 11 years, range 8–14 years). All patients were overweight, or obese, according to age-adjusted BMI. No subject was diabetic, and all were basically normotensive.

As for blood pressure, 76 children (75.3 %) were systolic nondippers and 23 of them were also diastolic nondippers (30.3 %).

As reported in Table 1, which summarises the characteristics of the studied patients, no differences were found in age, weight, height, BMI and WC, between the dipper and nondipper groups.

When compared to the systolic dippers, the systolic nondippers had higher medians of mean nocturnal SBP, OGTT glucose at 0, 60 and 120 min, OGTT insulin at all time points and HOMA and lower values of ISI.

The histopathological features of the children are given in Table 2; 24 % of the children had mild steatosis, 56 % of the children had moderate steatosis, and 20 % of the children had severe steatosis. No correlation of histopathological features with dipping/nondipping statuses was found.

Figure 1 shows the probability of systolic dipping as a function of $\log_e$ISI, BMI (SDS) and liver fat. Such a probability was calculated from a univariable logistic regression model using systolic dipping as the outcome. As reported in Fig. 1, there was a strong inverse association with $\log_e$ISI ($p<0.001$), but no association with BMI ($p=0.32$) and liver fat ($p=0.24$). At the point of multivariable logistic regression, only the contribution of $\log_e$ISI to systolic dipping was significant; the inclusion of BMI and liver fat was associated with only a modest change in the logit of logeISI (−3.6, 95 %CI −5.1 to −2.0 for univariable vs. −4.1, 95 %CI −5.8 to −2.3 for the multivariable model).

Discussion

During the past decade, there has been an increasing interest in childhood and adolescent blood pressure as a determinant for

Table 1 Anthropometric and metabolic measurements of the children

	Nondippers (n=76)			Dippers (n=25)		
	P_{25}	P_{50}	P_{75}	P_{25}	P_{50}	P_{75}
Age (years)	10	11	14	9	11	13
Weight (kg)	52	64	79	59	66	85
Height (m)	1.46	1.53	1.63	1.48	1.54	1.71
Body mass index (kg/m^2)	23.9	27.3	30.3	26.0	28.0	31.0
Body mass index (SDS)	1.35	1.94	2.30	1.75	2.05	2.44
WC (cm)	86	90	97	86	89	96
Mean daily SBP (mmHg)	106	112	116	107	111	116
Mean daily DBP (mmHg)	60	63	65	61	65	67
Mean diurnal SBP (mmHg)	107	114	119	112	115	121
Mean diurnal DBP (mmHg)	63	66	69	65	69	72
Mean nocturnal SBP (mmHg)	101	106	111	98	101*	108
Mean nocturnal DBP (mmHg)	53	57	60	53	55	60
Heart rate (BPM)	72	79	86	75	80	87
OGTT glucose 0 min (mg/dl)	77	82	90	73	77**	80
OGTT glucose 30 min (mg/dl)	122	133	149	107	122	137
OGTT glucose 60 min (mg/dl)	135	145	169	116	122*	143
OGTT glucose 90 min (mg/dl)	120	130	148	102	116	134
OGTT glucose 120 min (mg/dl)	104	120	133	93	105*	129
OGTT insulin 0 min (mg/dl)	9	14	19	5	6**	9
OGTT insulin 30 min (mg/dl)	78	118	162	47	70**	94
OGTT insulin 60 min (mg/dl)	96	121	136	47	58**	90
OGTT insulin 90 min (mg/dl)	64	112	149	41	53**	72
OGTT insulin 120 min (mg/dl)	71	103	149	47	65**	83
HOMA	1.73	2.85	4.27	0.86	1.03**	1.76
ISI	1.95	2.70	3.91	4.52	6.06**	8.15

*p<0.05 and **p<0.001 vs. dippers at quantile regression of P50

P_X Xth percentile

cardiovascular (CVD) risk. Systolic BP, diastolic BP, pulse pressure (PP) (i.e. the difference between SBP and DBP) and dipping all seem to play crucial roles in CVD [2, 22]. Interestingly, in fact, previous studies have demonstrated that obese adolescents are at a higher risk of reduced nocturnal dipping, and individuals with a nondipping pattern may be at an increased risk of BP-related complications, when compared with those with a normal dipping pattern [7, 34]. Previously, Westerstahl and Marcus reported an association between dipping and the measurement of insulin levels. This association was independent of obesity, or daytime BP levels among obese nondiabetic adolescents; this association was also describing a high prevalence, being approximately 50 % of nondipping, among obese adolescents [7]. According to this data, in the present study, we also found an association between a nocturnal BP fall and measurements of insulin levels (ISI), independent of obesity or daytime BP levels, among the obese nondiabetic patients with NAFLD. When compared to the systolic dippers, in fact, the systolic nondippers had higher medians of OGTT glucose and insulin at all time points and HOMA and lower ISI values. Noteworthy, we found a higher percentage of nondipper patients (75 %) in respect to previous studies. This data is probably due to the characteristics of the population evaluated in the present study. Our patients, in fact, are all obese and affected by NAFLD. Previous data demonstrated a positive correlation between altered BP status and NAFLD [16]. Therefore, it is possible that our results may be due to the particular phenotype of the population, as characterised by patients with fatty liver and exhibiting a high risk of a nondipping BP profile.

Although the mechanism underlying the association between impaired nocturnal dipping and insulin–glucose metabolism is not fully elucidated, many studies have suggested that hyperinsulinemia and insulin resistance may increase sympathetic nerve activity [31, 10, 12, 5, 19, 9], leading to an increased peripheral vascular resistance and BP elevation, through the renin–angiotensin–aldosterone system (RAAS) [15, 36]. Although the activity of a sympathetic nervous

Table 2 Liver histopathology of children affected by NAFLD

	N	Percent
Steatosis		
1	24	23.8
2	57	56.4
3	20	19.8
Inflammation		
0	7	6.9
1	67	66.3
2	27	26.7
Portal inflammation		
1	55	54
2	46	45
Fibrosis		
0	34	33.7
1	58	57.4
2	7	6.9
3	2	2.0
Ballooning		
0	59	58.4
1	25	24.8
2	17	16.8
NAS		
1	2	2.0
2	20	19.8
3	25	24.8
4	19	18.8
5	23	22.8
6	8	7.9
7	4	4.0

system was not evaluated in our study, the increasing trend for heart rate, along with the percentile of BMI observed in our series, seems to indirectly support the hypothesis for sympathetic nervous system hyperactivity in this kind of patient.

Considering the association between a nondipping profile and insulin resistance, it may be useful to investigate, in further studies, the nocturnal glico-insulinemic pattern associated with ABPM, in order to better explain the mechanism of interaction concerning 'nocturnal hypertension/insulin resistance'.

In view of the vast amount of studies demonstrating a central role played by insulin resistance in the pathogenesis of NAFLD, we have investigated the possible correlation between the type and the severity of histological lesions of NAFLD and BP status. To the best of our knowledge, this is the first study that has analysed the possible association between dipping, insulin metabolism and biopsy-proven NAFLD. In the other available studies, in fact, NAFLD was diagnosed only on the basis of ultrasonographic parameters (fatty liver) [6, 16], without any histopathological evaluation. The possible correlation between histological features of NAFLD, mainly fibrosis and dipping, was expected; this was established on the basis of recent studies demonstrating that catecholamines induce in vitro with a profibrotic phenotypic change of the hepatic stellate cells (HSC) [29]. Moreover, it has been reported that HSC adrenoceptors are upregulated in human livers with NAFLD cirrhosis. These findings could suggest that sympathetic nervous system activation might be involved in the progression to NASH and fibrogenesis induction and suggest a potential role for adrenoceptor antagonists as an anti-fibrotic. Further studies are needed to better elucidate the underlying pathogenetic mechanisms and to define the possible use of these agents.

However, in our study, no correlations between histopathological NAFLD features and dipping/nondipping statuses were found. The absence of this correlation in our study might be due to the young age of the enrolled patients (median age 11 years) and to the initial stage of hepatic and cardiovascular disease (in the present study only 9/101 patients had an F2 or F3 fibrosis index). In conclusion, the present study confirms the previous observation of an association between a nocturnal BP fall and insulin metabolism. Although no association between a nondipping profile and NAFLD damage was observed in our studies, a longer term observation period,

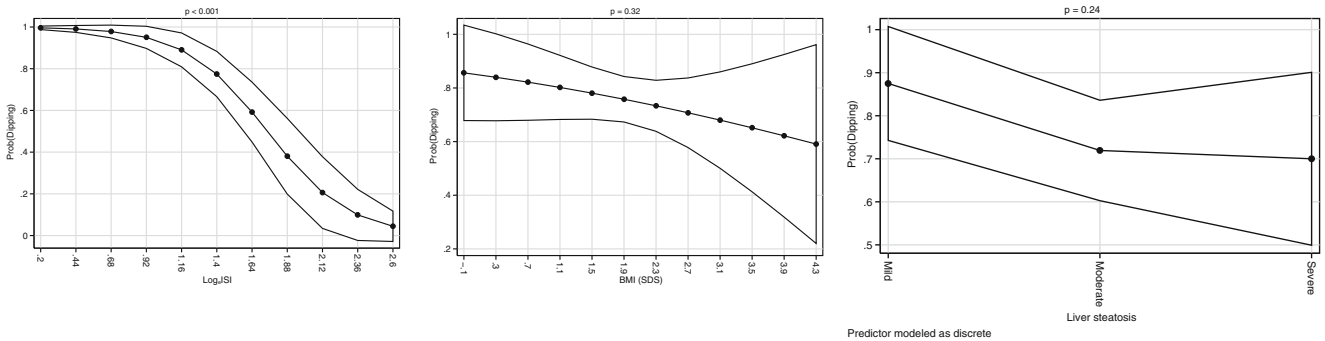

Fig. 1 Probability of systolic dipping as a function of log ISIe, BMI (SDS) and liver fat. Values are point estimates and 95 % confidence intervals and are obtained from univariable logistic regression

together with an examination of an older population, is needed, to better elucidate the complex link between these entities.

Furthermore, according to both of our previous and current findings, we suggest that screening with ABPM, OGTT and working up to NAFLD in childhood obesity should be included in a general paediatric practice, for instance, in a specialty clinic, for both an early detection and the efficient management of obesity-related comorbidities.

Conflict of interest No author has any conflict of interest, financial or otherwise related to the content of the paper.

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