Nutrition, Metabolism & Cardiovascular Diseases (xxxx) xxx, xxx

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

# Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique

Eleonora Fusco [a], Margherita Pesce [b], Vanessa Bianchi [a], Emioli Randazzo [a], Silvia Del Ry [c], Diego Peroni [a], Marco Rossi [b], Giovanni Federico [a,*]

[a] Unit of Pediatric Endocrinology and Diabetes, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy
[b] Department of Clinical and Experimental Medicine, Pisa University, Pisa, Italy
[c] Laboratory of Biochemistry and Molecular Biology, Institute of Clinical Physiology, CNR, Pisa, Italy

*Received 9 March 2019; received in revised form 29 July 2019; accepted 6 September 2019*
*Handling Editor: F. Galletti*
*Available online* ■ ■ ■

**KEYWORDS**
Obesity;
Skin;
Microcirculation;
Peripheral artery tonometry;
Laser-Doppler flowmetry

**Abstract** *Background and aims:* Childhood obesity promotes adverse changes in cardiovascular structure and function. This study evaluated whether alterations in skin microcirculation were already present in obese adolescents in a pre-clinical phase of cardiovascular disease.
*Methods and results:* After an overnight fasting 22 obese adolescents and 24 normal-weight controls of similar age and gender distribution underwent clinical and blood examination and assessment of microvascular function by using two non-invasive techniques such as Peripheral Artery Tonometry (PAT) and Laser-Doppler Flowmetry (LDF).

As compared to normal weight subjects, obese children had higher blood pressure, were significantly more hyper-insulinemic and insulin resistant, showing significantly higher plasma total cholesterol, LDL cholesterol, triglycerides and alanine aminotransferase (ALT).

LDF showed lower pre- and post-occlusion forearm skin perfusion (perfusion units/second (PU/sec); median [IQR]) in obese than in normal weight subjects (pre-occlusion: 1633.8 [1023.5] vs. 2281.1 [1344.2]; $p = 0.015$. Post-occlusion: 4811.3 [4068.9] vs. 7072.8 [7298.8]; $p = 0.021$), while PAT revealed similar values of reactive hyperemia index (RHI).

In entire population, fat mass % (FM%) was an independent determinant of both pre-and post-occlusion skin perfusion. Finally, being obese was associated with a higher risk to have a reduction of both pre- and post-occlusion skin perfusion (OR = 5,82 and 9,27, respectively).
*Conclusion:* LDF showed very early, pre-clinical, vascular involvement in obese adolescents, characterized by impaired skin microcirculation, possibly reflecting a more diffuse microvascular dysfunction to other body tissues. Whether changing life style and improving weight may reverse such pre-clinical alterations remains to be established.


*Acronyms:* ALT, Alanine Aminotransferase; AUC, Area under the curve; BMI, Body mass index; BP, Blood pressure; CRP, C-reactive protein; FM %, Fat mass %; HC, Hip Circumference; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; IMT, Carotid intima-media thickness; LDF, Laser-Doppler Flowmetry; PAT, Peripheral Artery Tonometry; PU/sec, Perfusion units/second; RHI, Reactive hyperemia index; WC, Waist circumference; WC/H, WC/Height ratio.

* Corresponding author. U.O. Pediatria, Unit of Pediatric Endocrinology and Diabetes, Azienda Ospedaliero-Universitaria Pisana, Via Roma 67, 56126 Pisa, Italy.
*E-mail address:* giovanni.federico@med.unipi.it (G. Federico).

https://doi.org/10.1016/j.numecd.2019.09.007
0939-4753/

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

## Introduction

Apart from a possible recent plateau in the prevalence of obesity in high-income countries [1], its prevalence has dramatically increased in the past 30 years becoming a public health problem [2]. Obesity in childhood increases the possibility of being obese as adult representing a risk for adulthood cardiovascular morbidity and mortality [3,4]. Adult obesity is associated with a number of cardiovascular functional and structural abnormalities, recognized as established biomarkers of cardiovascular risk, including large vessel abnormality such as increased carotid intima-media thickness (IMT) [3] and heart dysfunction [5]. Some of these alterations were described also in obese children and adolescents [6–8] with conflicting results [9–12]. Recently, we reported that obese children and adolescents demonstrated structural and functional alterations of heart and large vessels reflecting both physiologic adaptations to increase in body size and adverse effect of metabolically active intra-abdominal adiposity [13]. Some previous data suggested that these changes were fully reversible with weight loss: subjects who had been obese in youth, but were non-obese as adults, had carotid IMT values comparable to subjects who had been consistently non-obese from childhood to adulthood, whereas those who had remained obese from childhood to adulthood had increased carotid IMT [14–16].

More recently, on the basis of the demonstrated role of the microvascular abnormalities in cardiovascular risk and outcomes in obese subjects, other studies assessed microvascular function in obese/overweight children and adolescents, showing conflicting results. The majority of these studies used Peripheral Artery Tonometry (PAT), a non-invasive technique using photoplethysmography to measure changes in pulse wave amplitude in the fingers, reflecting small vessel digital arterial function [17]. The technique is validated for the study of microvascular endothelial function [18,19]. To our knowledge, only one study [20] employed Laser-Doppler Flowmetry (LDF), a non-invasive method used to assess cutaneous blood flow based in the reflection of a beam of laser light. When the laser light hits moving blood cells, wavelength alterations occur correlated to number/velocity of red blood cells [21–23]. LFD has been largely used to assess microcirculatory function in adults with obesity [24–26], arterial hypertension [27], and in smokers [28], assuming that skin microcirculation mirrors microvascular function in other body tissues, including heart [29]. One study [25] reported a weak correlation between PAT and LDF, possibly reflecting the different entities of microvascular function.

The aim of our study was to assess microvascular reactivity in response to ischemia in obese adolescents by using LDF, in addition to PAT, to better verify whether obesity affects microvascular function in a very early, preclinical stage.

## Methods

### *Study population*

Twenty-two obese children, referred as outpatients to the Unit of Pediatric Endocrinology and Diabetes, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy, and 24 age- and sex-matched normal weight subjects, were enrolled in the study (Table 1).

Normal weight adolescents were healthy subjects, who repeated blood examination after a short duration intervening disease (i.e. upper respiratory and urinary tract infections, acute abdominal pain not requiring surgery and not due to inflammatory bowel disease). At the time of discharge from the hospital, F. E. and B. V. described to the adolescents needing to repeat a blood sampling, as well as to their parents, the study protocol, offering them to participate to it. Only subjects not assuming drugs from at least two weeks before blood sampling and showing white cells count and C-reactive protein into the appropriate reference ranges were enrolled. None of the enrolled subject was a smoker or

**Table 1** Characteristics of study population.

| | Normal weight | Obese | p |
|---|---|---|---|
| N | 24 | 22 | |
| Female:Male | 11:13 | 13:9 | n.s. |
| Age (years) | 15.20 ± 1.56 | 14.11 ± 2.53 | n.s. |
| Height (cm) | 166.65 ± 8.24 | 162.87 ± 12.13 | n.s. |
| Height z-score | 0.28 ± 1.05 | 0.70 ± 1.52 | n.s. |
| Weight (kg) | 54.11 ± 7.46 | 87.20 ± 21.23 | 0.0001 |
| BMI (kg/m$^2$) | 19.43 ± 1.77 | 32.49 ± 5.50 | 0.0001 |
| BMI z-score | −0.15 ± 0.75 | 2.94 ± 0.70 | 0.0001 |
| Fat mass (%) | 14.6 [10.4] | 37.4 [12.8] | 0.0001 |
| Skeletal muscle mass (kg) | 40 [11.5] | 49 [18.9] | n.s. |
| WC (cm) | 66 [7] | 93 [19] | 0.0001 |
| WC/H | 0.40 [0.025] | 0.56 [0.085] | 0.0001 |
| HC (cm) | 88 [6] | 107.5 [16] | 0.0001 |
| Fasting Glucose (mmol/L) | 4.55 [0.38] | 4.49 [0.44] | n.s. |
| Fasting insulin (μU/mL) | 14.2 [3.82] | 18.2 [12.1] | 0.033 |
| HOMA-IR index | 2.8 [0.47] | 3.8 [2.21] | 0.037 |
| HbA1c (mmol/mol) | 33.3 [4.37] | 33.3 [3.27] | n.s. |
| Total-cholesterol (mmol/L) | 3.6 ± 0.3 | 4.2 ± 0.7 | 0.001 |
| LDL-cholesterol (mmol/L) | 1.86 [0.28] | 2.8 [0.67] | 0.0004 |
| HDL-cholesterol (mmol/L) | 1.26 [0.20] | 1.06 [0.36] | 0.044 |
| Triglycerides (mmol/L) | 0.62 [0.14] | 1.07 [0.49] | 0.0001 |
| ALT (U/L) | 17 [9] | 26 [13] | 0.0022 |
| Systolic BP (mmHg) | 111.7 ± 14.3 | 115 ± 10.5 | n.s. |
| Systolic BP (percentile) | 40.9 [37.9] | 57.1 [44.3] | 0.0275 |
| Diastolic BP (mmHg) | 55 ± 5.8 | 63 ± 10 | 0.0025 |
| Diastolic BP (percentile) | 20 [18.5] | 42.9 [43.8] | 0.0059 |
| Heart rate (bpm) | 67.6 ± 10.3 | 72.3 ± 9.7 | n.s. |

Data are expressed as mean ± SD or median [IQR] for skewed variables.

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

drinker. Body Mass Index (BMI) was calculated in all by using the formula weight (kg)/height (m)$^2$ [30]. We used the same National reference data [30] to calculate BMI z-score and Height z-score. Obesity was assessed according to the definition of the international Task Force on Obesity in childhood and using population reference data specific for age and sex for BMI [31].

Clinical and auxological examination, blood sampling and instrumental procedures were performed in each subject in the morning, after an overnight fasting. In particular, PAT and LDF were carried out in the same morning in the same subject. FM % and skeletal muscle mass (kg) were measured using the Tanita BC-418 Segmental Body Composition Analyzer (Tanita Corporation, Tokyo, Japan). Blood pressure (BP) was measured by trained investigators according to a standardized protocol [32]. Briefly, BP was taken on the left arm with the subject sitting, using an aneroid sphygmomanometer; the cuff had bladder long enough to encircle at least one-half of the upper arm without overlapping and width that covered at least two-thirds of the upper arm. The average of three BP measurements was used for analysis and for calculating systolic and diastolic BP percentiles by comparing values found in our subjects with BP reference data for children and adolescents according to sex, age and height percentiles [33].

Blood samples were collected in all the subjects by venipuncture. Blood samples to evaluate blood glucose and lipids were collected in lithium-heparin containing vials, while those for insulin assay were gathered in EDTA containing vials. After collection, samples were quickly separated by centrifugation for 15 min at 4 °C, and plasma was stored frozen at −80 °C, in 1-mL aliquots, in polypropylene tubes until assay that was performed within one month. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was calculated according the formula: fasting plasma insulin in μU/mL x fasting plasma glucose in mmol/L/22.5 [34,35].

The protocol of the study followed the principles of the Declaration of Helsinki and was approved by the institutional Ethics Committee. Parents and subjects involved in the study gave their informed consent to participate, as appropriate.

### *Biochemical parameter assays*

Blood glucose, total cholesterol, HDL and LDL cholesterol fractions and triglycerides were measured by a Cobas Integra 400 analyzer (Roche, Italy) using appropriate commercial kits (Cobas Integra 400 Glucose HK; enzymatic reference method with hexokinase, Cobas Integra 400 Cholesterol; enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine, Cobas Integra 400 HDL-Cholesterol and LDL-Cholesterol plus 2nd generation; homogeneous enzymatic colorimetric assays, Cobas Integra 400 Triglycerides; enzymatic, colorimetric method with glycerol phosphate oxidase and 4-aminophenazone).

HbA1c was assessed by HPLC (G7 HPLC Analyzer, Tosoh Bioscience Inc., South San Francisco, USA; reference range 23−43 mmol/mol). Circulating insulin levels were measured by a commercial immunoassay kit (Access® Ultrasensitive Insulin, Beckman Coulter Inc., Fullerton, CA, USA), with a sensitivity of 0.03 μU/mL and a precision of <10% CV.

### *Peripheral arterial tonometry (PAT)*

Reactive hyperemia index (RHI), a measure for endothelial function, was assessed by the EndoPAT 2000 device (Itamar Medical, Israel) and calculated on PAT signal changes using a computerized automated algorithm (software version 3.1.2) provided with the device. This technique involves finger probes to assess arterial pulse wave amplitudes at the fingertips.

Measurements were performed as previously described [36]. Briefly, subjects, after an overnight fasting, were in supine position for a minimum of 20 min before testing, in a quiet, temperature-controlled (21−24 °C) room with dimmed lights. The Endo-PAT pneumatic probes were placed on the index finger of both hands to register arterial pulse wave amplitudes, i.e. the difference between the highest and lowest point of a pulse wave. The subjects were asked to remain as still as possible and silent during the entire measurement period. Each recording consisted of 5 min of baseline measurement, 5 min of occlusion measurement, and 5 min post-occlusion measurement (hyperemic period). Occlusion of the brachial artery was performed on the nondominant upper arm with a manometer cuff inflated to supra-systolic pressure (at least 40 mmHg above the systolic BP). The software calculated the RHI, that is the ratio of the mean pulse wave amplitude between 90 and 150 s after deflation divided by a pre-occlusion period value during 210 s before occlusion, divided by the same ratio for the control arm to correct for changes in systemic vascular tone [37].

### *Laser-Doppler Flowmetry (LDF)*

Skin blood perfusion was assessed by means of LDF as previously described [28]. Briefly, skin blood flux was recorded at the level of right forearm by means of a LDF apparatus (Periflux PF4, Perimed, Järfälla, Sweden), equipped with a not heated probe (PF408). This apparatus allowed skin blood flux to be detected in a cutaneous tissue volume of about 1 mm and measured in perfusion units (PU) (1 PU = 10 mV). The LDF probe was fixed to the medial surface of right forearm, the precise measurement site being selected so as to avoid proximity to any of the larger blood vessels, hairs and blemishes. The laser characteristics were: 780 nm wavelength, 10 Hz−19 KHz bandwidth, 0.1 s time constant, 32 Hz sampling frequency. Probe calibration was performed, before each test session, by a specific device (Perimed, Järfälla, Sweden) containing colloidal latex particles whose Brownian motion provides

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

the standard values. The LDF signal was recorded continuously by an interfaced computer (Compaq, Hewlett Packard, Netherlands) equipped with a dedicated software (Perisoft, Perimed, Järfälla, Sweden).

Basal skin blood perfusion was recorded for 10 min under basal condition at the level of right forearm. Then, right forearm ischemia was induced by inflating a pneumatic cuff (which was positioned on the right arm prior to the test) to 30 mm Hg above the systolic BP of the subject, for 5 min, to compress the brachial artery. After this time, the pneumatic cuff was instantaneously deflated and forearm skin blood perfusion was then recorded for 10 min.

The software calculated both basal and post-ischemic skin perfusion (in PU per second) as area under the pre-occlusion LDF curve (pre-occlusion "area under curve": AUC), registered during 5 min before pneumatic cuff inflation, and as post-occlusion area under post-occlusion LDF curve (post-occlusion AUC), registered during 5 min after pneumatic cuff deflation, respectively. Maximal post-occlusion skin perfusion was expressed (in PU) as the maximal skin blood flux value (peak-flux) after cuff deflation.

### *Statistical analysis*

Data are expressed as mean ± SD, unless otherwise specified. After data had been tested to be normally distributed, they were analyzed by Student's T test for unpaired samples to detect differences among groups. Skewed variables were expressed as median (IQR) and log converted before statistical analysis performed with parametric tests.

In planning the study protocol, we determined that, at $\alpha = 0.05$, a sample of 24 normal weight subjects and 22 obese adolescents had a power (1-β) >90% in detecting a minimal significant clinical effect expressed by non-centrality parameter $\phi = 1$.

We used contingency analysis to evaluate categorical variables to explore the possible influence of being obese on pre- and post-occlusion AUC LDF results. We categorize pre- and post-occlusion AUC results found in obese as reduced or increased according to their median values observed in normal weight subjects. The association is expressed as odds ratio (OR), considering that a OR = 1 indicates no influence. Relations between variables were assessed by bivariate and multivariate linear regression analysis. A p-value < 0.05 was considered significant. Statistical analysis was performed by JMP software, version 12 (SAS Institute Inc, Cary, NC, USA).

## Results

Table 1 summarizes some results we observed in the studied population. As expected, obese adolescents had a significant higher BMI and BMI z-score, FM %, waist circumference (WC), WC/Height ratio (WC/H) and Hip Circumference (HC) than normal weight subjects.

Fasting glucose and HbA1c blood levels were similar in obese and in normal weight subjects in the presence of higher fasting insulin and HOMA values in obese indicating that they had increased insulin resistance.

In comparison with normal weight subjects, obese adolescents showed significantly higher levels of total-cholesterol, LDL-cholesterol and triglycerides, but lower HDL-cholesterol. In particular, values of LDL-cholesterol and triglycerides were borderline high indicating a degree of dyslipidemia [38].

Blood ALT levels were significantly higher in obese than in normal weight subjects suggesting the presence of NAFLD [39].

As regards blood pressure, when it was reported in mmHg obese adolescents had higher diastolic BP, while the systolic one was similar in comparison with normal weight subjects. When BP was reported as percentiles, after normalization for age, sex and height, also systolic BP values were significantly higher in obese than in normal weight subjects. Heart rate showed a modest, not significant, increase in obese in comparison with normal weight subjects.

Table 2 shows results obtained by LDF and PAT analyses. At LDF obese adolescents had significantly lower pre- and post-occlusion AUC, while peak-flux was similar between the two groups. The reduced AUC values indicated the presence of an altered microcirculation in obese subjects. As regards PAT, RHI values were similar between obese and normal weight adolescents.

As shown in Table 3, pre- and post-occlusion AUC, considered as dependent variables in a bivariate correlation analysis, correlated inversely and significantly with BMI z-score, WC/H, FM % and triglycerides.

Including pre- and post-occlusion AUC as dependent variables and BMI z-score, WC/H, FM % and triglycerides as covariates in a multiple regression model, we observed that FM % was an independent determinant of both pre- and post-occlusion AUC (Table 4).

Contingency analysis showed that an obese subject had a higher risk in reduction of both pre-occlusion AUC with OR = 5,82 and post-occlusion AUC with OR = 9,27. In other words, being obese increases by > 5 and > 9 folds, over normal weight subjects, the risk of having a reduced pre- and post-occlusion AUC.

## Discussion

This study showed some degree of preclinical skin microvascular alterations, recognized by LDF, in a group of

**Table 2** Assessment of vascular function.

| | Normal weight | Obese | p |
|---|---|---|---|
| Pre-occlusion AUC (PU/sec.) | 2281.1 [1344.2] | 1633.8 [1023.5] | 0.015 |
| Post-occlusion AUC (PU/sec.) | 7072.8 [7298.8] | 4811.3 [4068.9] | 0.021 |
| Peak-flux (PU) | 86.06 ± 33.53 | 73.93 ± 26.84 | n.s. |
| RHI | 1.86 ± 0.51 | 1.80 ± 0.62 | n.s. |

Data are expressed as mean ± SD or median [IQR] for skewed variables.

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

**Table 3** Bivariate correlation between microcirculation vs. clinical and laboratory data.

| Response variable | Regressors | R | T | p |
|---|---|---|---|---|
| **Pre-occlusion AUC** | Age (years) | 0.06 | 0.39 | 0.69 |
| | BMI z-score | 0.41 | −2.88 | 0.0063 |
| | WC/H | 0.44 | −3.14 | 0.0032 |
| | Height z-score | 0.14 | 0.95 | 0.34 |
| | Fat mass (%) | 0.56 | −4.30 | 0.0001 |
| | Skeletal muscle mass (kg) | 0.13 | 0.85 | 0.40 |
| | Fasting insulin (μU/mL) | 0.04 | −0.3 | 0.76 |
| | HOMA-IR | 2.41 | −0.05 | 0.96 |
| | Total-cholesterol (mmol/L) | 0.23 | −1.50 | 0.14 |
| | LDL-cholesterol (mmol/L) | 0.27 | −1.84 | 0.073 |
| | Triglycerides (mmol/L) | 0.32 | −2.20 | 0.033 |
| **Post-occlusion AUC** | Age (years) | 0.07 | 0.05 | 0.96 |
| | BMI z-score | 0.40 | −2.78 | 0.0083 |
| | WC/H | 0.43 | −3.01 | 0.0045 |
| | Height z-score | 0.24 | 1.62 | 0.11 |
| | Fat mass (%) | 0.55 | −4.24 | 0.001 |
| | Skeletal muscle mass (kg) | 0.1 | 0.76 | 0.44 |
| | Fasting insulin (μU/mL) | 0.02 | −0.27 | 0.78 |
| | HOMA-IR | 0.04 | −0.28 | 0.77 |
| | Total-cholesterol (mmol/L) | 0.25 | −1.68 | 0.10 |
| | LDL-cholesterol (mmol/L) | 0.28 | −1.91 | 0.06 |
| | Triglycerides (mmol/L) | 0.34 | −2.30 | 0.0267 |

**Table 4** Multivariate linear regression for Pre- and Post-occlusion AUC.

| | B (SD) | T | p |
|---|---|---|---|
| **Pre-occlusion AUC** | | | |
| Intercept | 8.48 (1.40) | 6.05 | <0.0001 |
| BMI z-score | 0.11 (0.14) | 0.77 | 0.445 |
| WC/H | −0.53 (1.32) | −0.40 | 0.690 |
| Triglycerides (mmol/L) | −0.07 (0.24) | −0.28 | 0.778 |
| Fat mass (%) | −0.51 (0.19) | −2.68 | 0.011 |
| **Post-occlusion AUC** | | | |
| Intercept | 5.92 (1.97) | 5.03 | <0.0001 |
| BMI z-score | 0.157 (0.20) | 0.77 | 0.444 |
| WC/H | −0.62 (1.87) | −0.33 | 0.741 |
| Triglycerides (mmol/L) | −0.17 (0.34) | −0.51 | 0.614 |
| Fat mass (%) | −0.70 (0.26) | −2.63 | 0.0123 |

obese adolescents. In particular, compared with normal weight subjects, obese adolescents exhibited a defect in forearm skin perfusion, as shown by a significant reduction in pre- and post-occlusion AUC, respectively, while peak-flux did not differ between obese and normal weight subjects.

Our finding of reduced post-occlusion skin perfusion in obese adolescents, even in the presence of a preserved skin post-occlusion peak-flux, is consistent with the occurrence of a microvascular dysfunction. It should be underlined that, while post-occlusion peak-flux reflects an instantaneous, endothelial-dependent [27,40] response during post-ischemic re-perfusion, post-occlusion AUC mirrors the whole microcirculatory post-occlusion re-perfusion response, that is largely dependent on the myogenic reactivity of microvascular wall [41] and on the accumulation in the ischemic skin of vasodilator mediators [42].

Since it is known that insulin induces recruitment of capillaries, vasodilatation, and influences vasomotion [43], the normoglicemic hyperinsulinism, due to increased insulin resistance, showed by our obese subjects needs some considerations. Under normal circumstances, in fact, perivascular adipose tissue, that is increased in obesity, functions in a paracrine fashion, directly reducing smooth muscle tone and enhancing insulin-dependent vasodilatation [25,44].

Change of this tissue function in healthy obese women has been shown to lead to a decreased insulin-dependent microvascular recruitment and vasodilator capacity that, in normal circumstances, represent a significant mediator in the relationship between obesity and metabolic insulin sensitivity [45].

In addition, it was demonstrated that lowering BMI by gastric bypass surgery, both in patients with and without type 2 diabetes mellitus, improved the reduced hyperemic response to postocclusive reactive hyperemia found in obese in comparison with healthy control subjects [24]. Therefore, it might be that changes in the perivascular adipose tissue function and insulin resistancy contributed to impair insulin-dependent vasodilatation in the skin of our obese adolescents.

Thus, our findings suggest that a very early microvascular impairment, in the absence of a detectable degree of endothelial dysfunction, as suggested by the preserved RHI values, was already present in our obese subjects.

The ratio of maximal to baseline LDF would be a better measure of vascular reactivity than absolute values of skin perfusion. However, the lower pre-occlusion skin perfusion we observed in our obese in comparison with normal weight subjects made this ratio less suitable than the absolute LDF values to detect a possible impairment of vascular reactivity.

The difference between our own data and those from the previous LDF study in obese adolescents [20], reporting a higher skin post-occlusion peak-flux in obese than in normal weight control subjects, is only apparent. The authors of that study, in fact, attributed their results to an impaired vasoconstriction following cuff-induced ischemia, due to endothelial dysfunction that, as above reported, was not revealed by PAT in our obese adolescents. Thus, if correct in our assumption then the preserved post-occlusion peak-flux we observed in our obese adolescents may simply reflect a different stage, possibly earlier in our series, in vascular system involvement. The same may be true for RHI results, found reduced by some authors [46], including us [47], or normal by others [10].

As regards the lower pre-occlusion skin perfusion, we observed in our obese in comparison with normal weight controls, we hypothesized that it might reflect a raised

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

basal vasoconstriction in skin microcirculation due to an increased sympathetic tone, as reported in obese adults [48].

A study on Italian obese adolescents reported an altered profile of cardiovascular autonomic regulation with higher values of systolic BP, higher Low Frequency variability power of systolic BP (an index of vasomotor sympathetic regulation) and smaller spontaneous baroreflex gain in obese than in normal weight subjects, in the presence of similar values of heart rate and heart rate variability [49].

The higher BP percentiles we observed in our obese adolescents in comparison with normal weight individuals, in addition to suggest that they had a certain degree of vascular involvement, support, at least in part, our hypothesis of a dysregulated sympathetic tone in these subjects even in the presence of only marginally, non-significantly, increased heart rate.

It is interesting to note that BMI z-score, WC/H ratio, triglycerides and FM % were inversely related with both pre- and post-occlusion skin perfusion. Analyzing these variables in a multivariate regression model, only FM % was an independent determinant of pre- and post-occlusion AUC. In addition, having an excess in FM % increased the risk of developing an alteration in microcirculation that was more than 6 and 9 folds over normal weight adolescents for reduced pre- and post-occlusion skin perfusion, respectively. Taking together, these findings are consistent with a negative, early, impact of obesity on microvascular function already present during adolescence.

In conclusion, our study shows that LDF may be a useful technique to recognize alterations in skin microcirculation in obese adolescents in a very early, pre-clinical stage of vascular involvement. This finding is important if one considers that skin microcirculation mirrors microcirculation in other body tissues, including heart tissue. It implies that alterations we found in the skin in obese adolescents may reflect a more diffuse microvascular dysfunction, which may be a further factor favoring cardio-metabolic impairment associated to obesity. If these data will be confirmed in other studies, a further step in this field of investigation could be to assess whether changing life style and improving weight may reverse such pre-clinical, functional, alterations contributing to preserve cardiovascular health of obese adolescents during adulthood.

## Conflicts of interest

The authors have nothing to disclose.

## Acknowledgments

The study was partially supported by grants of Tuscany Region (Regione Toscana Research Call “Innovation in Medicine 2009”, Project code B55E09000560002).

## References

[1] Gregg EW, Shaw JE. Global health effects of overweight and obesity. N Engl J Med 2017;377(1):80–1. https://doi.org/10.1056/NEJMe1706095.

[2] GBD 2015 Obesity Collaborators. Health effects of overweight and obesity in 195 countries over 25 years. N Engl J Med 2017;377:13–27. https://doi.org/10.1056/NEJMe170609510.1056/NEJMoa1614362.

[3] Raitakari OT, Juonala M, Viikari JS. Obesity in childhood and vascular changes in adulthood: insights into the Cardiovascular Risk in Young Finns Study. Int J Obes 2005;29(Suppl 2):S101–4. https://doi.org/10.1038/sj.ijo.0803085.

[4] Bastien M, Poirier P, Lemieux I, Després JP. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis 2014;56(4):369–81. https://doi.org/10.1016/j.pcad.2013.10.016.

[5] Alpert MA, Omran J, Mehra A, Ardhanari S. Impact of obesity and weight loss on cardiac performance and morphology in adults. Prog Cardiovasc Dis 2014;56(4):391–400. https://doi.org/10.1016/j.pcad.2013.09.003.

[6] Kelly AS, Barlow SE, Rao G, Inge TH, Hayman LL, Steinberger J, et al. Severe obesity in children and adolescents: identification, associated health risks, and treatment approaches: a scientific statement from the American Heart Association. Circulation 2013;128(15):1689–712. https://doi.org/10.1161/CIR.0b013e3182a5cfb3.

[7] Mangner N, Scheuermann K, Winzer E, Wagner I, Hoellriegel R, Sandri M, et al. Childhood obesity: impact on cardiac geometry and function. JACC Cardiovasc Imaging 2014;7(12):1198–205. https://doi.org/10.1016/j.jcmg.2014.08.006.

[8] Zachariah JP, Ingul CB, Marx GR. Linking pediatric obesity to subclinical alterations in cardiac structure and function. JACC Cardiovasc Imaging 2014;7(12):1206–8. https://doi.org/10.1016/j.jcmg.2014.09.006.

[9] Cote AT, Phillips AA, Harris KC, Sandor GG, Panagiotopoulos C, Devlin AM. Obesity and arterial stiffness in children: systematic review and meta-analysis. Arterioscler Thromb Vasc Biol 2015;35(4):1038–44. https://doi.org/10.1161/ATVBAHA.114.305062.

[10] Tryggestad JB, Thompson DM, Copeland KC, Short KR. Obese children have higher arterial elasticity without a difference in endothelial function: the role of body composition. Obesity (Silver Spring, MD) 2012;20(1):165–71. https://doi.org/10.1038/oby.2011.309.

[11] Dangardt F, Osika W, Volkmann R, Gan LM, Friberg P. Obese children show increased intimal wall thickness and decreased pulse wave velocity. Clin Physiol Funct Imaging 2008;28(5):287–93. https://doi.org/10.1111/j.1475-097X.2008.00806.x.

[12] Van Putte-Katier N, Rooman RP, Haas L, Verhulst SL, Desager KN, Ramet J, et al. Early cardiac abnormalities in obese children: importance of obesity per se versus associated cardiovascular risk factors. Pediatr Res 2008;64(2):205–9. https://doi.org/10.1203/PDR.0b013e318176182b.

[13] Kozakova M, Morizzo C, Bianchi V, Marchetti S, Federico G, Palombo C. Hemodynamic overload and intra-abdominal adiposity in obese children: relationships with cardiovascular structure and function. Nutr Metab Cardiovasc Dis 2016;26(1):60–6. https://doi.org/10.1016/j.numecd.2015.10.002.

[14] Juonala M, Raitakari M, Viikari JS, Raitakari OT. Obesity in youth is not an independent predictor of carotid IMT in adulthood. The Cardiovascular Risk in Young Finns Study. Atherosclerosis 2006;185(2):388–93. https://doi.org/10.1016/j.atherosclerosis.2005.06.016.

[15] Freedman DS, Dietz WH, Tang R, Mensah GA, Bond MG, Urbina EM, et al. The relation of obesity throughout life to carotid intima-media thickness in adulthood: the Bogalusa Heart Study. Int J Obes Relat Metab Disord 2004;28(1):159–66. https://doi.org/10.1038/sj.ijo.0802515.

[16] McCrindle BW. Cardiovascular consequences of childhood obesity. Can J Cardiol 2015;31(2):124–30. https://doi.org/10.1016/j.cjca.2014.08.017.

[17] Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. Am Heart J 2003 Jul;146(1):168–74. https://doi.org/10.1016/S0002-8703(03)00094-2.

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007

[18] Bruyndonckx L, Radtke T, Eser P, Vrints CJ, Ramet J, Wilhelm M, et al. Methodological considerations and practical recommendations for the application of peripheral arterial tonometry in children and adolescents. Int J Cardiol 2013;168(4):3183–90. https://doi.org/10.1016/j.ijcard.2013.07.236.
[19] Rubinshtein R, Kuvin JT, Soffler M, Lennon RJ, Lavi S, Nelson RE, et al. Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. Eur Heart J 2010;31(9):1142–8. https://doi.org/10.1093/eurheartj/ehq010.
[20] Schlager O, Willfort-Ehringer A, Hammer A, Steiner S, Fritsch M, Giurgea A, et al. Microvascular function is impaired in children with morbid obesity. Vasc Med 2011;16(2):97–102. https://doi.org/10.1177/1358863X11400780.
[21] Nilsson GE, Tenland T, Oberg PA. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE (Inst Electr Electron Eng) Trans Biomed Eng 1980;27(10):597–604. https://doi.org/10.1109/TBME.1980.326582.
[22] Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol Sci 2006 Sep;27(9):503–8. Epub 2006 Jul 31, https://doi.org/10.1016/j.tips.2006.07.008.
[23] Treu CM, Lupi O, Bottino DA, Bouskela E. Sidestream dark field imaging: the evolution of real-time visualization of cutaneous microcirculation and its potential application in dermatology. Arch Dermatol Res 2011 Mar;303(2):69–78. https://doi.org/10.1007/s00403-010-1087-7.
[24] Rossi M, Nannipieri M, Anselmino M, Pesce M, Muscelli E, Santoro G, et al. Skin vasodilator function and vasomotion in patients with morbid obesity: effects of gastric bypass surgery. Obes Surg 2011;21(1):87–94. https://doi.org/10.1007/s11695-010-0286-9.
[25] Van der Heijden DJ, Van Leeuwen MAH, Janssens GN, Lenzen MJ, Van de Ven PM, Eringa EC, et al. Body mass index is associated with microvascular endothelial dysfunction in patients with treated metabolic risk factors and suspected coronary artery disease. J Am Heart Assoc 2017 Sep 14;6(9):e006082. https://doi.org/10.1161/JAHA.117.006082.
[26] Mastantuono T, Di Maro M, Chiurazzi M, Battiloro L, Starita N, Nasti G, et al. Microvascular blood flow improvement in hyperglycemic obese adult patients by hypocaloric diet. Transl Med UniSa 2016 Nov 1;15:1–7.
[27] Rossi M, Bradbury A, Magagna A, Pesce M, Taddei S, Stefanovska A. Investigation of skin vasoreactivity and blood flow oscillations in hypertensive patients: effect of short-term antihypertensive treatment. J Hypertens 2011;29(8):1569–76. https://doi.org/10.1097/HJH.0b013e328348b653.
[28] Rossi M, Pistelli F, Pesce M, Aquilini F, Franzoni F, Santoro G, et al. Impact of long-term exposure to cigarette smoking on skin microvascular function. Microvasc Res 2014;93:46–51. https://doi.org/10.1016/j.mvr.2014.03.001.
[29] Holowatz LA, Thompson-Torgerson CS, Kenney WL. The human cutaneous circulation as a model of generalized microvascular function. J Appl Physiol (1985) 2008;105(1):370–2. https://doi.org/10.1152/japplphysiol.00858.2007.
[30] Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. Br Med J Clin Res 2000;320(7244):1240–3. https://doi.org/10.1136/bmj.320.7244.1240.
[31] Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. Stat Med 1992;11(10):1305–19. https://doi.org/10.1002/sim.4780111005.
[32] National high blood pressure education program working group on high blood pressure in children and adolescents, the fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics 2004;114:555–76. 114(2 Suppl 4th Report), https://doi.org/10.1161/01.HYP.0000143545.54637.af.
[33] Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics 2017 Sep;140(3):e20171904. https://doi.org/10.1542/-peds.2017-1904.
[34] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412–9. https://doi.org/10.1007/BF00280883.
[35] D'Annunzio G, Vanelli M, Pistorio A, Minuto N, Bergamino L, Lafusco D, et al. Insulin resistance and secretion indexes in healthy Italian children and adolescents: a multicentre study. Acta Biomed 2009;80:21–8.
[36] Bruyndonckx L, Hoymans VY, Van Craenenbroeck AH, Vissers DK, Vrints CJ, Ramet J, et al. Assessment of endothelial dysfunction in childhood obesity and clinical use. Oxidative Med Cell Longevity 2013;2013(1):174782. https://doi.org/10.1155/2013/174782.
[37] Moerland M, Kales AJ, Schrier L, van Dongen MG, Bradnock D, Burggraaf J, et al. Evaluation of the EndoPAT as a tool to assess endothelial function. Int J Vasc Med 2011;2012:904141. https://doi.org/10.1155/2012/904141.
[38] Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents; national heart, lung, and blood Institute. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: summary report. Pediatrics 2011 Dec;128(Suppl 5):S213–56. https://doi.org/10.1542/peds.2009-2107C.
[39] Schwimmer JB, Dunn W, Norman GJ, Pardee PE, Middleton MS, Kerkar N, et al. SAFETY study: alanine aminotransferase cutoff values are set too high for reliable detection of pediatric chronic liver disease. Gastroenterology 2010 Apr;138(4):1357–64. https://doi.org/10.1053/j.gastro.2009.12.052. 1364.e1-2.
[40] Cracowski JL, Roustit M. Current methods to assess human cutaneous blood flow: an updated focus on laser-based-techniques. Microcirculation 2016 Jul;23(5):337–44. https://doi.org/10.1111/micc.12257.
[41] Lorenzo S, Minson CT. Human cutaneous reactive hyperaemia: role of BKCa channels and sensory nerves. J Physiol 2007 Nov 15;585(Pt 1):295–303. https://doi.org/10.1113/jphysiol.2007.143867.
[42] Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. Microcirculation 2012 Jan;19(1):47–64. https://doi.org/10.1111/j.1549-8719.2011.00129.x.
[43] Serné EH, IJzerman RG, Gans RO, Nijveldt R, De Vries G, Evertz R, et al. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. Diabetes 2002 May;51(5):1515–22. https://doi.org/10.2337/diabetes.51.5.1515.
[44] Greenstein AS, Khavandi K, Withers SB, Sonoyama K, Clancy O, Jeziorska M, et al. Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. Circulation 2009 Mar 31;119(12):1661–70. https://doi.org/10.1161/CIRCULATIONAHA.108.821181.
[45] Meijer RI, Serné EH, Korkmaz HI, van der Peet DL, de Boer MP, Niessen HW, et al. Insulin-induced changes in skeletal muscle microvascular perfusion are dependent upon perivascular adipose tissue in women. Diabetologia 2015 Aug;58(8):1907–15. https://doi.org/10.1007/s00125-015-3606-8.
[46] Mahmud FH, Hill DJ, Cuerden MS, Clarson CL. Impaired vascular function in obese adolescents with insulin resistance. J Pediatr 2009;155(5):678–82. https://doi.org/10.1016/j.jpeds.2009.04.060.
[47] Del Ry S, Cabiati M, Bianchi V, Caponi L, Maltinti M, Caselli C, et al. C-type natriuretic peptide is closely associated to obesity in Caucasian adolescents. Clin Chim Acta 2016;460(1):172–7. https://doi.org/10.1016/j.cca.2016.06.045.
[48] Marinos A, Gamboa A, Celedonio JE, Preheim BA, Okamoto LE, Ramirez CE, et al. Hypertension in obese black women is not caused by increased sympathetic vascular tone. J Am Heart Assoc 2017;6(11):e006971. https://doi.org/10.1161/JAHA.117.006971.
[49] Lucini D, de Giacomi G, Tosi F, Malacarne M, Respizzi S, Pagani M. Altered cardiovascular autonomic regulation in overweight children engaged in regular physical activity. Heart 2013 Mar;99(6):376–81. https://doi.org/10.1136/heartjnl-2012-302616.

Please cite this article as: Fusco E et al., Preclinical vascular alterations in obese adolescents detected by Laser-Doppler Flowmetry technique, Nutrition, Metabolism & Cardiovascular Diseases, https://doi.org/10.1016/j.numecd.2019.09.007