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Vascular Function in Children with Low Birthweight and Its Relationship with Early Markers of Cardiovascular Risk

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Key Words
Low birthweight · Children · Endothelial function · Cardiovascular risk factors

Abstract
Low birthweight (LBW) increases the risk of developing cardiovascular diseases (CVD). Few studies have established its impact at early ages. ***Aims:*** To study endothelial function (EF) and arterial stiffness (AS) and their relationship to early markers of CVD risk in children with LBW. ***Methods:*** In children with LBW (4–6 years; n = 53), anthropometric, haemodynamic and laboratory parameters, including HOMA-IR, hs-CRP, adiponectin and leptin, were determined. EF and AS were evaluated by digital pulse plethysmography. Data were compared with a control group (n = 33). ***Results:*** In both groups, anthropometric parameters remained within normal limits. Insulin and HOMA-IR had normal values, but they were significantly augmented in LBW children. LBW children showed higher leptin and hs-CRP levels than the control group. The LBW group had decreased EF (37.5 ± 5.6%) compared with the control group (75.0 ± 11.9%; $p < 0.01$), however without differences in AS. In LBW children, EF was negatively correlated with waist circumference, leptin, hs-CRP and with a cumulative score of risk factors. ***Conclusions:*** LBW children display altered EF that is related to early changes in CVD risk factors. The differences found in the metabolic parameters might indicate a pro-inflammatory state. This hypothesis is also supported by the laboratory findings and the correlation between EF and the number of CVD risk factors, suggesting that very early lifestyle interventions may be needed.

Introduction

Cardiovascular diseases (CVD) represent the main cause of mortality and morbidity in the industrialised world. The hypothesis by Barker et al. [1] postulates the existence of a direct relationship between low birthweight (LBW) and CVD in adulthood. It is known that alterations in endothelial function (EF) are implicated in the early physiopathology of CVD [2]. In this sense, the antecedent of LBW has been associated with impaired EF [3]. However, the signalling mechanisms underlying the physiopathology of vascular damage in LBW and their influence on adult health have not been elucidated. A compensatory increase in body weight, with an increase in body mass index (BMI) and fat mass observed in LBW children, could be involved in the development of CVD [4]. In addition, increased CVD risk may be associated

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with central or abdominal obesity, which is manifested clinically as an increased waist circumference. In prepuberal and puberal children, waist circumference is a good predictor of metabolic disease and CVD in adulthood [5]. On the other hand, it has been recognised that reduced insulin sensitivity, a characteristic of LBW-related conditions, may be present as early as the first year of life [6, 7]. However, despite the increasing amount of data showing that hyperinsulinaemia and insulin resistance during childhood may be risk factors for CVD [8], the impact of waist circumference on CVD risk in infants has not been studied extensively.

It has been proposed that the impact of LBW on CVD risk could appear early in life. In this sense, it has been demonstrated that young adults with LBW have increased CVD risk that is associated with altered EF [9]. Moreover, alterations in the EF from 9 years of age in children with LBW have been described [10, 11].

In individuals with vascular damage, not only alterations in EF but also alterations in vascular compliance, such as arterial stiffness (AS), have been demonstrated. In hypertensive patients, alterations in AS are associated with an increased incidence of cardiovascular events [12]. Therefore, it has been proposed that EF and AS could be used as independent prognostic indicators for CVD [13]. Recently, several non-invasive techniques have been developed to assess vascular function in humans. EF can be evaluated by a digital plethysmographic method involving flow-mediated dilatation (FMD) after an ischaemic stimulus (hyperaemic response) [14, 15]. In previous work from our laboratory, we studied EF in asymptomatic adults with Chagas disease [16] as well as in obese adolescents [17]. Our laboratory has also used plethysmographic study of digital volume waveform to evaluate the AS [17].

On the other hand, it is well known that an inflammatory process is involved in the physiopathology of CVD. One marker of inflammation is the high-sensitivity C-reactive protein (hs-CRP). It has been shown that hs-CRP levels are a good predictor of CVD, and hs-CRP cut-offs for low, moderate and high CVD risk have been established [18].

Other factors that may be associated with CVD are the increased leptin and decreased adiponectin levels. Increased leptin levels may be associated with the inflammatory process and possibly also with the increased morbidity associated with obesity [19]. Adiponectin, on the other hand, could reduce the production and activity of inflammatory cytokines [20]. Insulin resistance is also an important risk factor for obesity, hypertension and CVD. Therefore, measurements of the levels of leptin, insulin and adiponectin are also often included in attempts to predict CVD risk [21].

In the light of these considerations, we hypothesised that vascular function and early markers of CVD risk could be altered in children with a history of LBW. The objective of the present work was to test this hypothesis by studying vascular function (EF and AS) and its relationship to early markers of CVD risk in children with LBW.

Materials and Methods

Male and female children 4–6 years of age were recruited from the Instituto de Maternidad Nuestra Señora de las Mercedes (Tucumán, Argentina). The exclusion criteria were as follows: history of diabetes; CVD or other pathologies; regular use of medications; clinical report of infections at the moment of inclusion; congenital infections; chromosomal disorders; malformations, and neonatal asphyxia. The mothers of the children were all healthy non-smokers who did not use medications or special diets and who did not suffer from hypertension, diabetes or glucose intolerance and had not experienced multiple pregnancies.

Children with antecedent LBW (n = 53; 23 boys) were compared with a group of controls with similar characteristics without LBW (n = 33; 18 boys). A standard deviation score (SDS) for birthweight was calculated for the average birthweight of each group (see the section Statistical Analysis). According to institutional guidelines, an informed consent form was signed by the parents of all participants for both the assessment of vascular function and the obtaining of blood samples.

Anthropometric parameters (weight, height, BMI, and waist circumference) were measured and the percentiles for age and sex were calculated according to the guidelines of the Argentinean Paediatric Society [22]. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also measured and the percentiles of these values were calculated for height percentile, age, and sex according to the guidelines of the Argentinean Society of Hypertension [23].

Laboratory tests including fasting glucose (Abbott kit; Germany), lipid profile (Abbott kit; Germany), insulinaemia (Abbott kit; Germany), HOMA-IR, quantitative insulin sensitivity check index (QUICKI) and blood count were performed. Plasma levels of hs-CRP, adiponectin (radioimmunoassay LINCO Research) and leptin (Leptin IRMA Test DSL-23100) were also determined. To discard confounders in the biochemical parameters, we correlated the values with BMI SDS (see the section Statistical Analysis).

Assessment of EF

EF was evaluated by a non-invasive plethysmographic technique previously used in our laboratory; this technique captures the changes in the amplitude of the digital volume waveform after a hyperaemic response [16, 17]. To record and measure the amplitude of the digital pulse wave, the plethysmograph was connected to an electrocardiograph using an adapter designed by our laboratory. The electrocardiograph was calibrated (1 mm = 0.1 mV) to obtain a standard chart recording at a velocity of 25 mm/s for the

posterior analysis. The hyperaemic response was obtained by FMD. For this purpose, SBP and DBP were determined with an aneroid sphygmomanometer, under the recommended conditions for its correct determination in children [23]. After this, a photoelectric transducer was placed on the child's index finger. Prior to starting the recording phase, the child was asked to hold his breath for 10 s (pre-occlusion phase). To obtain complete arterial occlusion, the sphygmomanometer cuff was then insufflated to a pressure of 50 mm Hg above SBP for 5 min (occlusion phase). Subsequently, the sphygmomanometer cuff was deflated; after 2 min, another 10 s of apnoea was required to obtain the post-occlusion recording (post-occlusion phase). The register obtained from each child was scanned to measure the amplitude of the pulse wave: height of the valley/peak using ImageJ 1.43u software (Bethesda, Md., USA). In each child, 10 waves from each phase were averaged to compare pre-occlusion versus post-occlusion (delta: Δ).

To evaluate endothelium-independent vasodilatation, 1% nitroglycerine cream (NTG), an exogenous NO donor, was applied topically to the contralateral index finger of a group of children, and the changes in the record were measured.

Assessment of AS

The AS index was evaluated using the plethysmography system. The procedures were similar to those used for the evaluation of EF. After the child's blood pressure was measured, a graphic record of 10 pulse waves was obtained in a simultaneous record of the electrocardiogram. This was done to check the corresponding diastolic and systolic phases of the pulse wave and to rule out the waves produced by ectopic beats. The register obtained from each child was scanned to determinate the stiffness index which was calculated by the formula: $a(100) / b$ where a = the amplitude of the maximal systolic peak in millimetres and b = the amplitude of the maximal diastolic peak in millimetres. In each subject, 10 pulse waves were averaged. In 1 control child, AS could not be calculated because the pulse wave record did not present a dicrotic notch but rather a sine wave.

To determine the impact of percentile of weight for gestational age, we divided the LBW group into two subgroups: adequate for gestational age (LBW/AGA; ≥10th percentile according to the guidelines of the Argentinean Paediatric Society) and small for gestational age (LBW/SGA; <10th percentile according to the same guidelines).

To evaluate the possible relationship between the number of CVD risk factors present with EF and AS in each child, a semiquantitative score (grade 0–7) was calculated for each participant based on the presence of altered values of the CVD risk factors: obesity [24]; triglycerides [24]; HDL cholesterol [24], blood pressure [24] glucose [24], hs-CRP [18] and HOMA-IR [25]. The scale was scored as follows: grade 0 = absence of risk factors; grade 1 = presence of 1 of the 7 risk factors; grade 2 = presence of 2 of the 7 risk factors, and similarly through grade 7 = presence of all 7 CVD risk factors.

Statistical Analysis

The data were expressed as the mean ± standard error. A statistical probability of less than 5% ($p < 0.05$) was considered significant. Graph-Pad Prism 4.0 and the statistical software package Statistica 5.0 were used to calculate statistical parameters. Student's t test or the Pearson correlation coefficient was used when necessary.

Table 1. Clinical characteristics of children studied

	Control (n = 33)	LBW (n = 53)
Age, years	5.2±0.1	5.1±0.1
Body weight, kg	19.5±0.6	20.4±0.6
Percentile	61.0±4.8	51.3±7.3
BMI	16.6±0.3	16.7±0.4
Percentile	70.3±4.9	64.7±4.4
Height, cm	110.4±0.1	107.7±0.1
Percentile	55.3±4.9	52.7±5.8
Waist circumference, cm	56.7±1.5	54.9±0.9
Percentile	60.9±6.8	54.2±11.1
Blood pressure		
Systolic, mm Hg	89.3±1.6	94.3±1.1**
Percentile	52.4±3.5	60.8±2.7*
Diastolic, mm Hg	61.5±1.5	63.0±1.3
Percentile	70.5±3.7	76.2±2.5
Heart rate, beats/min	92.0±1.6	95.6±1.8

* $p < 0.05$; ** $p < 0.01$; unpaired t test.

Biochemical parameters values were correlated with BMI SDS. For this purpose, BMI SDS was calculated by adjusting it to age and sex using local normative data [22]. A similar adjustment was performed for EF and AS. In a similar way, height SDS was calculated.

Birthweight SDS was calculated for the average birthweight of each group. For this purpose, each child's birthweight was adjusted to the SD of his/her own percentile for specific gestational age and sex according to the guidelines of the Argentinean Paediatric Society [22].

Results

In the LBW group, birthweight was 2,296 ± 31 g (n = 53) and in the control group birthweight was 3,341 ± 76 g (n = 33; $p < 0.001$). Both LBW and control children were born at term. The average gestational age was 38.1 ± 0.2 weeks (range 37–41 weeks) in LBW children and 38.5 ± 0.2 weeks (range 37–40 weeks) in controls (p = n.s.). The percentiles of birthweight were 12.2 ± 1.5 for LBW children versus 72.3 ± 4.1 for controls ($p < 0.001$). It is necessary to note that the differences between the LBW group and controls were maintained when we adjusted birthweight for SDS (–0.9 ± 0.1 for LBW vs. 0.7 ± 0.1 for controls; $p < 0.001$). There was no overlap in birthweight between the 2 groups. In addition, we calculated the difference between the real and the expected 50th percentile of birthweight for each particular gestational age, expressed as a percentage of the expected percentile. The

Table 2. Laboratory test in children studied

	Control (n = 33)	LBW (n = 53)
Fasting glucose, mg/dl	54±1	57±8
Insulin, mU/ml	4.2±0.3	6.0±0.5**
HOMA-IR	0.8±0.1	1.1±0.1*
QUICKI	0.35±0.02	0.27±0.00***
Cholesterol, mg/dl		
Total	103±2	110±2
HDL	32.3±3.1	32.8±0.8
LDL	62.6±2.1	64.9±1.9
Triglycerides, mg/dl	55.9±3.6	62.0±3.4
Leptin, ng/ml	0.9±0.1	1.6±0.2*
Adiponectin, υg/ml	6.8±0.2	7.8±0.4
hs-CRP, mg/l	0.5±0.1	1.5±0.4*

Data adjusted for BMI SDS; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; unpaired t test.

Table 3. Laboratory test in the LBW/SGA and the LBW/AGA subgroups

	LBW/SGA (n = 26)	LBW/AGA (n = 27)	Significance, p
Fasting glucose, mg/dl	79.1±1.4	75.5±1.6	n.s.
Insulin, mU/ml	7.5±0.6	8.9±1.2	n.s.
HOMA-IR	1.4±0.1	1.7±0.2	n.s.
QUICKI	0.4±0.02	0.4±0.1	n.s.
Cholesterol, mg/dl			
Total	151.1±4.7	150.0±3.4	n.s.
HDL	45.6±1.8	44.4±1.6	n.s.
LDL	88.4±4.5	89.4±2.9	n.s.
Triglycerides, mg/dl	88.4±6.6	81.7±6.7	n.s.
Leptin, ng/ml	2.2±0.3	2.2±0.4	n.s.
Adiponectin, μg/ml	10.8±0.7	10.8±0.8	n.s.
hs-CRP, mg/l	2.2±0.7	1.9±0.7	n.s.

deviations from the 50th percentile were –76.0 ± 0.9% in LBW subjects and 44.6 ± 8.3% in controls ($p < 0.001$).

The clinical characteristics of the children who participated in the study are shown in table 1. In both groups, anthropometric and haemodynamic parameters were within normal limits. The LBW group showed no significant differences with respect to the control group in either mean or percentile values for body weight, BMI, height, waist circumference, DBP and heart rate. In relation to SBP, although this parameter remained within normal limits, the LBW group showed significant increases in both mean and percentile values (table 1). Similarly, pulse pressure was increased in the LBW group (control group 28 ± 1 mm Hg vs. LBW group 31 ± 1 mm Hg; $p < 0.05$). BMI SDS was similar in both groups (control group 0.66 ± 0.14; n = 33 vs. LBW group 0.73 ± 0.18; n = 53; p = n.s.). Height SDS was also similar in both groups (control group 0.19 ± 0.1; n = 33 vs. LBW group 0.12 ± 0.1; n = 53; p = n.s.). A positive correlation between SBP and DBP was observed only in the LBW group (Pearson r: 0.7065; 95% CI: 0.5371–0.8211; $p < 0.001$).

No difference was found in any variable shown in table 1 when statistical analysis was performed after dividing the LBW group into LBW/AGA and LBW/SGA subgroups (data not shown).

Table 2 presents the results of the laboratory testing of the children. The LBW group showed no significant differences from the control group in fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides. With respect to insulin, although HOMA-IR and QUICKI fell within the normal limits in both groups, the LBW group showed a significant difference with respect to the control group (table 2). In the LBW group, leptin levels and hs-CRP showed increased values compared to those of the control group (table 2). The adiponectin values were similar in the two groups. It is necessary to note that, in all cases, the differences between the LBW and the control groups were maintained when we adjusted these variables for BMI SDS (table 2).

Within the LBW group, no significant differences in the laboratory test results of LBW/AGA and LBW/SGA were found (table 3).

In the LBW group, but not in the control group, positive correlations of leptin levels and hs-CRP, BMI, waist circumference, total cholesterol, LDL cholesterol and triglycerides were found (table 4). These correlations were not shown for adiponectin.

In the LBW group, but not in the control group, the hs-CRP level was negatively correlated with HDL cholesterol and triglycerides were positively correlated with weight, BMI, waist circumference, total cholesterol, LDL cholesterol and insulin (table 4). On the other hand, a negative correlation between triglycerides and HDL cholesterol was found (table 4). In the LBW group, HOMA-IR was positively correlated with waist circumference, weight and BMI (table 4). Similarly, in the LBW group, triglyceride levels were correlated with HOMA-IR and SBP (fig. 1). In the control group, none of the above-mentioned correlations were found.

Figure 2a, b shows typical digital pulse/wave plethysmography records obtained from children in the control and LBW groups. An endothelial-dependent assay ob-

Table 4. Correlation of laboratory variables in LBW children

	Pearson r	95% CI	p
Leptin with hs-CRP	0.85587	0.7333 to 0.9244	<0.001
Leptin with BMI	0.5260	0.2336 to 0.7311	<0.01
Leptin with waist circumference	0.6316	0.3731 to 0.7991	<0.001
Leptin with total cholesterol	0.4004	0.07740 to 0.6473	<0.05
Leptin with LDL cholesterol	0.4380	0.1225 to 0.6730	<0.01
Leptin with triglycerides	0.43417	0.1271 to 0.6756	<0.01
hs-CRP with HDL cholesterol	–0.2943	–0.5251 to –0.02319	<0.05
Triglycerides with weight	0.2719	0.001638 to 0.5051	<0.05
Triglycerides with BMI	0.3248	0.05970 to 0.5471	<0.05
Triglycerides with waist circumference	0.2989	0.02819 to 0.5287	<0.05
Triglycerides with total cholesterol	0.4533	0.2084 to 0.6446	<0.01
Triglycerides with LDL cholesterol	0.3133	0.04688 to 0.5381	<0.05
Triglycerides with insulin	0.3823	0.1248 to 0.5915	<0.01
Triglycerides with HDL cholesterol	–0.3382	–0.5576 to –0.07470	<0.05
HOMA-IR with waist circumference	0.5335	0.3049 to 0.7039	<0.001
HOMA-IR with weight	0.4299	0.1805 to 0.6274	<0.01
HOMA-IR with BMI	0.4148	0.1666 to 0.6186	<0.001

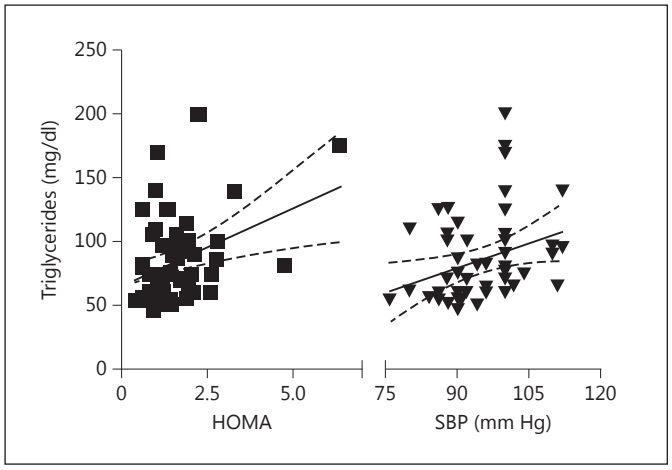


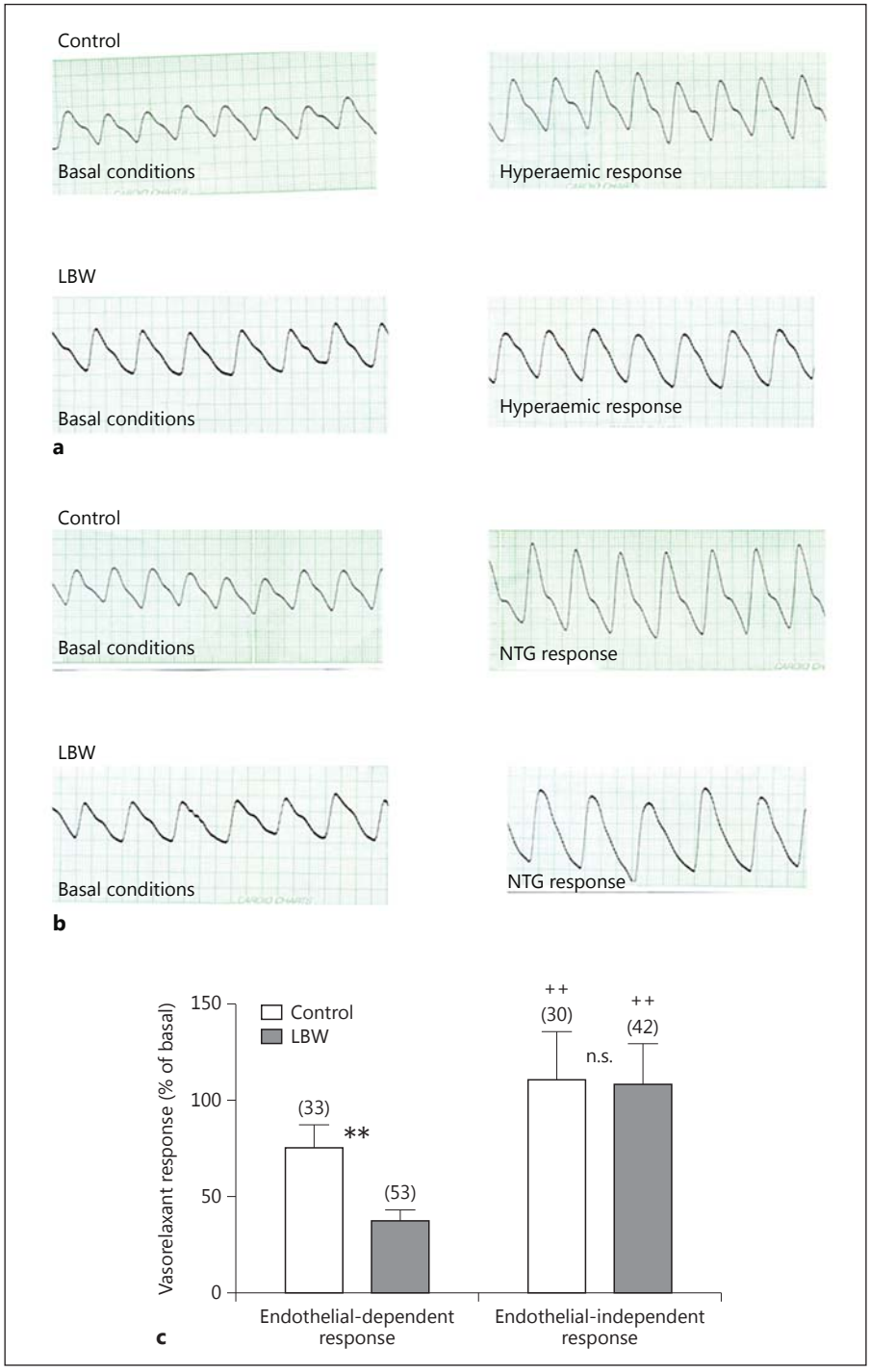
Fig. 1. Correlation between triglycerides with HOMA-IR (Pearson r: 0.3611; 95% CI: 0.1005–0.5753; $p < 0.01$; n = 53) and triglycerides with SBP (Pearson r: 0.3095; 95% CI: 0.04268–0.5351; $p < 0.05$; n = 53) in the LBW group.

tained under hyperaemic conditions is shown in figure 2a: the left record corresponds to the pre-occlusion phase (basal conditions) and the right record corresponds to the post-occlusion phase (hyperaemic response). The endothelial-independent response, assayed by topical NTG, is shown in figure 2b: there the left records correspond to basal conditions and the right records correspond to the NTG response.

The averages of these responses are shown in figure 2c. The endothelial-dependent response was significantly higher in the controls than in the LBW group (fig. 2c, first group of bars), whereas the endothelial-independent response was similar in the two groups (fig. 2c, second group of bars). The difference in EF was maintained when it was adjusted for BMI SDS (control group 49 ± 8%; n = 33 vs. LBW group 27 ± 4%; n = 53; $p < 0.001$). We did not find differences between the LBW/SGA and LBW/AGA subgroups in EF (LBW/SGA subgroup 42 ± 9%; n = 26 vs. LBW/AGA subgroup 33 ± 7%; n = 27; p = n.s.). It is necessary to note that the differences in EF between the LBW group and the control group (fig. 2c, first group of bars) were maintained when we compared the control group with the LBW/SGA subgroup ($p < 0.02$) and the LBW/AGA subgroup ($p < 0.02$).

In the LBW group, but not in the control group, EF was negatively correlated with hs-CRP (fig. 3a), waist circumference (Pearson r: –0.306; $p < 0.05$) and leptin level (Pearson r: –0.333; $p < 0.05$).

The LBW group and control group showed no significant differences in AS (control group 64.0 ± 2.2%; n = 32 vs. LBW group 65.0 ± 1.9; n = 53; p = n.s.). This result was unchanged when AS was adjusted for BMI SDS (control group 47 ± 7%; n = 32 vs. LBW group 47 ± 5%; n = 53; p = n.s.). Likewise, we did not find differences between the LBW/SGA and LBW/AGA subgroups in AS (LBW/SGA subgroup 62 ± 3; n = 26 vs. LBW/AGA subgroup 67 ± 3; n = 27; p = n.s.). Furthermore, no differences were obtained when we compared the control group with the LBW/SGA subgroup (p = n.s.) and the LBW/AGA subgroup (p = n.s.).



Color version available online

Fig. 2. Vasorelaxant response to hyperaemic manoeuvres and NTG. **a** A typical tracing from the endothelial-dependent response assay using hyperaemia. **b** The endothelial-independent response assays obtained using NTG in a control child and in an LBW child. **c** Average of these responses. ** $p < 0.01$ control vs. LBW; n.s. = not significant control vs. LBW; $^{++}$ $p < 0.01$ hyperaemic vs. NTG response ANOVA and Newman Keuls post-test. The data are expressed as the mean ± standard error. The numbers of children tested are shown in parentheses.

A negative correlation between EF and AS was found only in the LBW group (fig. 3a). It is necessary to note that this correlation was maintained when we divided the LBW into the LBW/SGA subgroup (Pearson r: –0.4942; 95% CI: –0.7361 to –0.1405; n = 26; $p < 0.01$) and the LBW/AGA subgroup (Pearson r: –0.5162; 95% CI: –0.7531 to –0.1610; n = 27; $p < 0.01$).

With respect to the presence of CVD risk factors described for metabolic syndrome in children [24], we found that 4 of the children in the LBW group (7.5%)

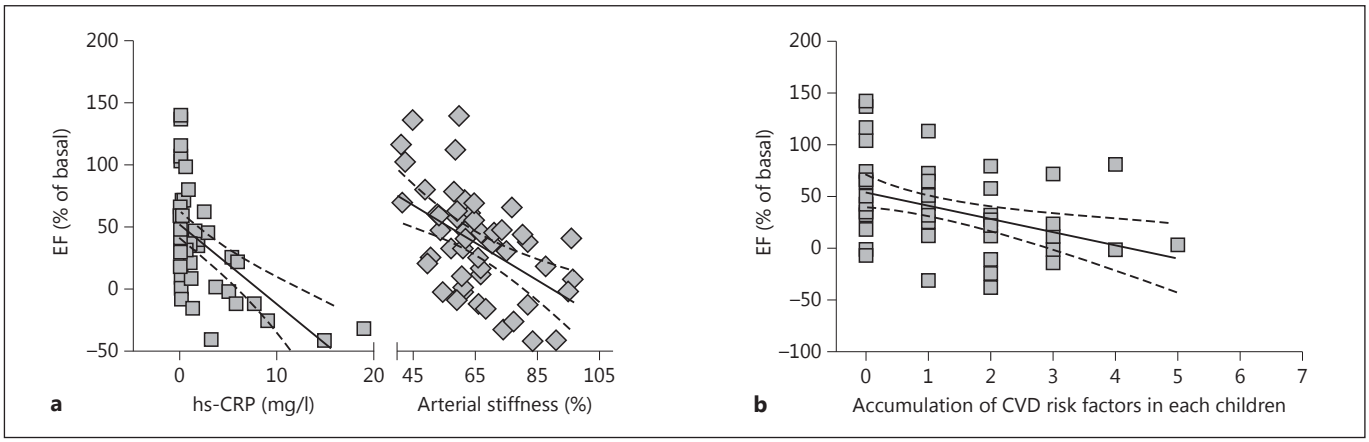


Fig. 3. a Correlation between EF and hs-CRP (Pearson r: –0.5664; 95% CI: –0.7269 to –0.3470; $p < 0.0001$; n = 53) and correlation between EF and AS (Pearson r: –0.5102; 95% CI: –0.6859 to –0.2782; $p < 0.001$; n = 53) in the LBW group. **b** Correlation between EF and accumulation of CVD risk factors (score 0–7) present in each LBW child (Pearson: –0.3905; 95% CI: –0.5978 to –0.1343; $p < 0.01$; n = 53).

Table 5. Presence of CVD risk factors described for metabolic syndrome in children

	Obesity n (%)	Blood pressure, n (%)	Glucose n (%)	HDL cholesterol n (%)	Triglycerides, n (%)	Metabolic syndrome (3 or more), n (%)
LBW group (n = 53)	8 (15.0)	18 (33.9)	0 (0)	13 (24.5)	10 (18.9)	4 (7.5)
Control group (n = 33)	0 (0)	5 (15.1)	0 (0)	3 (9.0)	2 (6.0)	0 (0)
Significance, p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Significance: difference between percentages.

presented metabolic syndrome, whereas none (0%) of the children in the control group did (table 5).

Regarding the relationship between the number of CVD risk factors and vascular function, we found a negative correlation between EF and the number of CVD risk factors in the LBW children (fig. 3b). No correlation between these parameters was found in the control group (Pearson r: 0.1075; 95% CI: –0.2449 to 0.4349; p = n.s.; n = 33) and no correlation between the number of CVD risk factors and AS was found in either the LBW or the control group (data not shown).

Discussion

The results of this study showed that, although children with LBW presented anthropometric, haemodynamic and laboratory parameters within normal limits, they also displayed premature endothelial dysfunction. The impaired capacity for endothelium-dependent vasodilatation present in these children was correlated with increased levels of hs-CRP and leptin. In the present work, these pro-inflammatory markers appear to be more involved in early endothelial dysfunction than in arterial stiffness, because AS was not increased in LBW children and no correlation of hs-CRP or leptin levels with AS was found.

Although it is known that LBW is a risk factor for the development of CVD in adulthood [26–28], its impact on vascular function in childhood is unclear. In relation to EF, a decrease in FMD has been shown in prepuberal children with LBW [10, 11]. This is in agreement with our findings that alterations in EF can occur early in life (at 4–6 years of age) and with the findings of Martin et al. [29], who showed that an unfavourable epigenetic environment, induced by inadequate levels of micronutrients,

could produce not only LBW but also endothelial dysfunction in newborns. On the other hand, these findings are in disagreement with the report of Hovi et al. [30], who did not find impaired FMD in young adults with histories of very LBW. A possible explanation for the discrepancies in these findings is that the study by Hovi et al. included children with very LBW who were born preterm. It has been reported that preterm birth attenuates the association between LBW and endothelial dysfunction [31] and that LBW attributable to prematurity does not increase the risk of CVD in 15-year-old subjects [32]. This is supported by the fact that we excluded preterm children in our study.

We found no differences in AS in the LBW and control groups, even after adjusting for BMI SDS in order to discard confounders. This finding is in disagreement with the findings of Leeson et al. [11] and Hovi et al. [30] who showed increased carotid intima-media thickness measured by ultrasonography in prepuberal children and young adults with a history of LBW. On the other hand, in agreement with our findings, Chan et al. [33] did not find alterations in AS, measured by the augmentation index, in prepuberal children with LBW.

To the best of our knowledge, the present study is the first to demonstrate a lack of alteration in AS in LBW children at very young ages. A hypothesis that would explain the lack of alteration in AS is that, at this age, reversible physical changes in the vessel wall predominate, and it is these changes that, over the long term, cause arterial remodelling. However, due to the limitations of the present study, including the lack of a prospective design and the relatively small number of children investigated, further testing of this hypothesis in additional studies will be necessary to elucidate the significance of early changes in vascular stiffness in LBW children.

In relation to CVD risk factors, there is no clear evidence for their presence in children with LBW. As mentioned above, we found elevated hs-CRP values in the LBW group. In adulthood, elevated hs-CRP is found in the presence of endothelial dysfunction even without elevation of other markers of inflammation [34]. hs-CRP is not only considered a marker of CVD risk [35], but its elevation also increases cardiovascular damage in several pathologies [36]. The hs-CRP values measured in our LBW children have been correlated, in the adult population, with the presence of moderate cardiovascular risk [18]. On the other hand, the fact that only in the LBW group were the hs-CRP values negatively correlated with HDL cholesterol and positively correlated with leptin and the finding that leptin was positively correlated with BMI, waist circumference, total cholesterol, LDL cholesterol and triglycerides provide evidence that CVD risk factors are not only present in children with LBW but that they are also associated with each one.

In relation to the impact of LBW on the pathological conditions associated with CVD, it has been demonstrated that LBW increases the risk of diabetes in adulthood [37] and is associated with altered sensitivity to insulin in prepuberal children with very LBW [38]. The significant increase in HOMA-IR in LBW and its correlation with anthropometric variables (BMI, weight and waist circumference) observed in our LBW population could indicate that risk factors related to alterations in glycaemic homeostasis and arterial blood pressure act together to increase cardiovascular risk in this population. Support for this idea is found in the higher glucose levels and SBP levels observed in LBW children. Thus, in addition to the classic cardiovascular risk factors, we found changes in other parameters such as hs-CRP and HOMA-IR, which in the light of recent research [39, 40] would be expected to have a great impact on CVD risk.

In conclusion, LBW children display altered EF that is related to early changes in CVD risk factors. Although anthropometric, laboratory and haemodynamic parameters remained within normal limits in these children, the significant differences found for leptin and hs-PCR in LBW children and controls may indicate the presence of a pro-inflammatory state. On the other hand, the significant differences found in the metabolic parameters might indicate that failure to make lifestyle interventions at this age could result in these children developing CVD. This hypothesis is also supported by the laboratory findings and the correlation between EF and the number of CVD risk factors, suggesting that very early lifestyle interventions may be needed.

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