Diminished Insulin-Mediated Forearm Blood Flow and Muscle Glucose Uptake in Young Men with Low Birth Weight

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Key Words

Acetylcholine • Adenosine • Endothelial function • Insulin • Insulin sensitivity

Abstract

Background: Low birth weight (LBW) is associated with increased risk of type 2 diabetes and cardiovascular disease. We studied endothelial function and insulin sensitivity in young men with LBW ($n = 22$) and controls ($n = 22$). **Methods:** Insulin sensitivity and endothelial function was studied with venous occlusion plethysmography and intra-arterial infusions of adenosine and acetylcholine, before and during a hyperinsulinemic isoglycemic clamp. **Results:** Forearm blood flow response to systemic hyperinsulinemia was diminished in LBW compared to controls ($p < 0.05$). Fractional arteriovenous glucose extraction was similar, and consequently insulin-stimulated forearm glucose clearance was diminished in LBW compared with controls (0.8 ± 0.09 vs. $1.4 \pm 0.36 \text{ ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$, respectively, $p < 0.05$). Forearm blood flow response to adenosine and acetylcholine with or without insulin stimulation did not differ between groups. Whole-body glucose uptake was lower in LBW than controls (8.7 ± 0.5 and $9.1 \pm 0.6 \text{ mg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ lean body mass); however, this was not significant. **Conclusions:** Forearm blood flow response to insulin is impaired in LBW,

whereas the response to adenosine and acetylcholine is preserved. The impaired insulin-mediated increase in bulk flow in LBW may be due to an impairment of insulin-mediated capillary recruitment independent of – or preceding – whole-body insulin resistance in LBW subjects.

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Introduction

Lifestyle factors and genetic predisposition play a significant role in the lifetime risk of developing insulin resistance and cardiovascular disease. Furthermore, according to the theory of fetal origin of diseases [1], humans born with a low birth weight (LBW) for gestational age have an increased risk of developing these conditions. In LBW, discrete abnormalities in glucose and fat metabolism have been reported [2–5] and, moreover, there is also an association with LBW and increased incidence of hypertension and cardiovascular disease in later adult life [6, 7].

It is controversial if LBW is also associated with endothelial dysfunction [8–12]. In studies [8, 10, 11] that have reported endothelial dysfunction in LBW, endothelial function has been estimated by measurements of flow-mediated brachial artery dilation (examining the conduit

vessels). However, in studies [9, 12] where intra-arterial infusion of endothelium-dependent vasodilators [e.g. acetylcholine (ACH)] was used to examine the resistance vessels, no difference between LBW and controls (CON) was found.

Adenosine (ADN) is a partly endothelial-dependent vasodilator [13, 14]; however, peripheral vasoactivity in response to ADN has not, to our knowledge, been measured in healthy LBW and appropriate control subjects before. Reduced coronary artery blood flow response to ADN is associated with insulin resistance [15], and hyperinsulinemia potentiates the vasodilatation response of ADN in the coronary arteries of healthy subjects [16].

Insulin stimulates bulk flow and capillary recruitment via endothelial dependent pathways [17, 18], and an intact vascular insulin response is obligatory for an optimal metabolic insulin response [17, 19, 20]. The vascular insulin response in LBW to systemic insulin infusion has, to our knowledge, not been examined before. Therefore, the aim of this study was to characterize the blood flow responses to insulin, ACH and ADN, and to measure insulin-mediated glucose uptake rates in whole body and in the forearm (primarily representing skeletal muscle) in subjects with LBW during steady-state conditions. We performed a hyperinsulinemic, isoglycemic clamp in combination with a-v catheterization of the forearm, allowing pharmacological stimulation of forearm blood flow before and during systemic hyperinsulinemia. In accordance with observations in obese subjects [21, 22], decreased vascular insulin sensitivity could be present in LBW, which in turn could contribute to metabolic insulin resistance.

Subjects and Methods

Subjects

We recruited 44 healthy, white, male subjects born at term [gestational week 40 ± 0.2 and 40 ± 0.1 weeks in LBW and CON, respectively ($p > 0.05$)] via the Danish Birth Registry. Twenty-two were born with LBW (<10 percentile: birth weight $2,530 \pm 50$ g) and 22 were CON subjects, born with normal birth weight (50–75 percentile: birth weight $3,797 \pm 49$ g; $p < 0.001$, LBW vs. CON). The 2 groups were matched groupwise according to age, BMI and physical fitness (table 1). Body composition was determined by DEXA scanning (Lunar Prodigy Advance; GE Healthcare, USA). $\text{VO}_{2\text{max}}$ was measured on a bicycle ergometer with a stepwise incremental test using the leveling off criterion (Oxycon Pro; Jaeger Instruments, Höchberg, Germany).

None of the subjects had a family history of type 2 diabetes. Six subjects had 1 first-degree relative with hypertension. Two subjects were smokers (15 cigarettes per day, 1 subject in each group).

Table 1. Basal characteristics

	CON (n = 22)	LBW (n = 22)
Age, years	25 ± 0.2	26 ± 0.5
Height, m	1.85 ± 0.01	$1.79 \pm 0.01^*$
Weight, kg	83 ± 2	$75 \pm 3^*$
BMI, kg/m^2	24 ± 1	23 ± 1
Body fat, %	18.6 ± 1.7	19.7 ± 1.4
Waist-to-hip ratio	0.86 ± 0.005	0.87 ± 0.06
$\text{VO}_{2\text{max}}$, ml/min/kg	43.5 ± 1.3	42.6 ± 2.7
Systolic blood pressure, mm Hg	128 ± 2	126 ± 4
Diastolic blood pressure, mm Hg	69 ± 2	69 ± 3
Glucose, mmol/l	5.3 ± 0.06	5.4 ± 0.07
Insulin, pM	35 ± 3	38 ± 5
C-peptide, pM	513 ± 25	531 ± 42
HbA1C, %	5.1 ± 0.1	5.1 ± 0.1
Total cholesterol, mmol/l	3.9 ± 0.2	4.1 ± 0.2
HDL, mmol/l	1.3 ± 0.1	1.3 ± 0.1
LDL, mmol/l	2.2 ± 0.1	2.3 ± 0.2
Triglyceride, mmol/l	0.9 ± 0.1	1.1 ± 0.2
Homocysteine, $\mu\text{mol/l}$	9.8 ± 0.5	11.1 ± 0.2
hsCRP, nmol/l	25.1 ± 6.6	27.5 ± 8.4
ICAM, ng/ml	227 ± 8	217 ± 11
VCAM, ng/ml	610 ± 19	593 ± 38
von Willebrand factor, Kiu/l	0.90 ± 0.07	0.86 ± 0.05

All values are means $\pm$ SEM. All blood samples were obtained after an overnight fast. Glucose is arterial blood.

* $p < 0.05$, LBW vs. CON.

The study was approved by the regional ethics committee [No. (01) 262546] and all procedures were performed in accordance with the guidelines of the Declaration of Helsinki. Informed written consent was obtained from all subjects before participation.

Experimental Protocol

All subjects were provided with a standardized isocaloric nutritionally recommended diet (55 E% of carbohydrates, 15 E% of protein, and 30 E% of fat) 4 days prior to the study. After 10 h overnight fasting, the subjects reported to the laboratory. Electrocardiogram and heart rate were monitored by precordial electrodes. Arterial catheters (Becton Dickinson, UK) were inserted in the brachial arteries of both arms for blood sampling (control arm), blood pressure monitoring (control arm) and for infusion of vasodilators (intervention arm). Venous catheters (18 G Venflon; Medex Medicine, UK) were inserted in the medial antecubital veins of both arms. In the intervention arm they were inserted in a retrograde direction (for blood sampling) and in the control arm they were antegrade (for infusion of insulin and glucose). In 3 CON and 1 LBW subjects, 1 ($n = 3$) or both ($n = 1$) arterial lines could not be inserted, and arterialized blood samples from a heated hand vein were used as replacement. In 5 CON subjects and 1 LBW subject, it was not possible to insert the retrograde venous catheter.

Fig. 1. Protocol for the experiment. The x-axis is time after insertion of arterial and venous catheters. Shaded columns are intra-arterial infusions of ADN (left y-axis) and white columns are intra-arterial infusions of ACH (right y-axis). The arrows indicate forearm blood flow measurement by venous occlusion plethysmography and arteriovenous blood was sampled.

We measured forearm blood flow (FBF) simultaneously in both arms by venous occlusion mercury-in-Silastic strain-gauge plethysmography (Hokanson EC6 and E20; Marcom, Vemmeløv, Denmark). Blood flow was measured at cycles of 7 s and calculated as an average of 5–7 consecutive readings. Around the wrists small cuffs were positioned and inflated to suprasystolic pressures during plethysmography recordings and blood sampling, in order to exclude the circulation of the hand and the contribution of the a-v shunts, respectively. All measurements and analyses were carried out by a single investigator (M.S.).

Time points for the infusion regimen of vasodilators are shown in figure 1. ADN was infused intra-arterially in three 5-min intervals at each rate (see below). At the end of each interval, FBF were measured and arteriovenous blood sampled. When blood flow had returned to baseline values, the intra-arterial infusions commenced with infusions of ACH. At $t = 210$ min, the clamp was started. At $t = 300$ min (90 min after start of the insulin infusion), the intra-arterial infusion regimen with ADN and ACH infusions was repeated.

Calculations

Glucose infusion rates were averaged for 10-min periods (fig. 2) and whole-body insulin-mediated glucose uptake rates were calculated as the mean of steady-state glucose infusion rates during the last 30 min of the clamp ($t = 150$ – 180 min clamp time; M-value). Baseline FBF and a-v differences were measured at $t = 60$ min, just before the start of the infusions. Clamp FBF and a-v differences were measured at $t = 300$ min (clamp time 90 min) and at $t = 390$ min (clamp time 180 min). There was no significant difference between flow and forearm fractional glucose extraction at the 2 latter time points, thus an average of these time points was used.

Fig. 2. Glucose infusion rates averaged for periods of 10 min in 22 subjects born with LBW (circles) and 22 CON subjects (squares) during a 3-hour hyperinsulinemic, isoglycemic clamp. No significant difference between the glucose infusion rates in the 2 groups was observed. Values are means $\pm$ SEM.

Fractional glucose extraction across the intervention arm (forearm) was calculated as the a-v difference divided by the arterial glucose concentration and expressed in percent. Glucose clearance (intervention arm) was calculated as glucose extraction multiplied by the FBF (expressed as $\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$) and glucose uptake was calculated as glucose a-v difference multiplied by the FBF (expressed as $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$).

Forearm vascular conductance was calculated as FBF divided by mean arterial pressure (MAP) and expressed as $\text{ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1}$.

Respiratory coefficient across the forearm was calculated as carbon dioxide production over the forearm (v-a) divided by oxygen consumption (a-v).

Infusates and Infusion Regimens

ADN and ACH solutions were prepared from stock solutions ($5 \text{ mg} \cdot \text{ml}^{-1}$; Item Development AB, Stocksund, Sweden, and Michol-E $10 \text{ mg} \cdot \text{ml}^{-1}$; Novartis Pharma AG, Basel, Switzerland, respectively) and isotonic sodium chloride. Intra-arterial infusion rates were 50, 150 and $500 \mu\text{g} \cdot \text{min}^{-1}$ (ADN) and 15, 30 and $60 \mu\text{g} \cdot \text{min}^{-1}$ (ACH).

For the hyperinsulinemic isoglycemic clamp, a 50-ml insulin solution was prepared from insulin (Actrapid $100 \text{ U} \cdot \text{ml}^{-1}$; Novo Nordisk, Copenhagen, Denmark), saline, and 2.5 ml of the subject's own plasma and administered as a 2-ml bolus followed by a constant intravenous infusion [$40 \text{ mU} \cdot \text{min}^{-1} \cdot \text{m}^2$ ($^{-1}$)]. Arterial blood was sampled every 5–10 min and glucose (20%) infusion rates were adjusted accordingly. Potassium level was measured throughout the clamp and oral supplements (750 mg Kaleorid; LEO Pharma A/S, Ballerup, Denmark) were given if necessary. The coefficient of variation for the clamps performed in this study was 4.1%.

Biochemical Analyses

Plasma concentrations of glucose, potassium and blood gases were analyzed on an automatic analyzer (ABL 735; Radiometer, Copenhagen, Denmark). Concentrations of insulin and C-peptide in plasma were measured with commercially available kits (Dako ELISA, UK). Glycosylated haemoglobin (HbA1C) was measured by high-performance liquid chromatography on a Bio-Rad Variant™ (Bio-Rad Laboratories, Hercules, Calif., USA). Total cholesterol and HDL-cholesterol was analyzed with an enzymatic colorimetric test (Roche Diagnostic, Mannheim, Germany). LDL cholesterol was calculated from the Friedewald formula [23] and VLDL cholesterol was calculated as triglycerides divided by 2.2. Plasma triglyceride concentration was determined with Triglyceride GPO-PAP (Roche Diagnostic). HsCRP was measured with a 912 Automatic Analyzer (Hitachi, Boehringer, Mannheim, Germany). Homocysteine was measured with reagent Axis-Shield Enzymatic Homocysteine FHER100 on Thermo Konelab 30i, Software version 6.5.h. ICAM-1 and VCAM-1 were analyzed by commercially available kits (ELISA Immunoassay; R&D Systems, Minneapolis, Minn., USA). Von Willebrand factor (vWF) was analyzed by ELISA at the department of biochemistry at Rigshospitalet, Copenhagen, Denmark.

Statistics

Statistical analysis was performed using Sigma Stat version 3.1. Student's *t* test or a nonparametric Mann-Whitney test was used when analyzing data on single measurements, depending on whether data were normally distributed or not. ANOVA for repeated measures was used when analyzing continuous variables. A *p* value of <0.05 was considered significant in 2-tailed testing. All data are reported as mean $\pm$ SEM. If there were missing data points in the ANOVA analysis a general linear approach (the adjusted sums of squares) was used. Only complete data sets are reported.

Several parameters may be used in power calculations; however, the main objectives were the vascular responses to vasodilators. Power was calculated to above 80% to detect a 15% difference between the groups in blood flow response to insulin at the level of 5% significance.

Results

Subject characteristics are outlined in table 1. Generally LBW were proportionally smaller than CON (e.g. lower height and weight), but with the same relation between lean body and fat mass. All other parameters including lipid profile were similar.

Response to Insulin

With insulin infusion, plasma insulin concentration increased to 294 ± 10 and 309 ± 16 pmol·l⁻¹ in CON and LBW, respectively. The M-value was not different between CON and LBW (7.0 ± 0.5 and 6.8 ± 0.5 mg·min⁻¹·kg body weight⁻¹; fig. 2). When expressed relative to lean body mass, the M-value also did not differ be-

Fig. 3. Forearm blood flow in the control arm of 22 subjects born with LBW (circles) and 22 CON (squares) subjects. Measurements were done by venous occlusion plethysmography. Measurements were done in the basal period (closed symbols) and during insulin infusion (open symbols). In both groups forearm blood flow increased significantly in response to insulin. * *p* < 0.05. For difference in forearm blood flow under insulin infusion between the 2 groups: *p* = 0.110. Values are means $\pm$ SEM.

tween groups (9.1 ± 0.6 and 8.7 ± 0.5 mg·min⁻¹·kg lean body mass⁻¹ in CON and LBW, respectively). The experimental design allowed for simultaneous evaluation of the FBF response to systemic hyperinsulinemia in both arms (intervention arm and control arm). In general, the FBF response was comparable in the 2 arms but different between the groups. In CON and LBW subjects, FBF increased significantly in both arms in response to insulin. (fig. 3, 4). However, the insulin-stimulated FBF was lower in LBW compared to CON subjects (*p* < 0.05 and *p* = 0.110 in intervention and control arm, respectively).

Fractional glucose extraction across the forearm increased by a factor 3–4 in both groups with insulin infusion (*p* < 0.05 for both groups), but remained similar between the groups (fig. 4). Consequently, forearm glucose clearance rates were similar at baseline but higher (*p* < 0.05) in CON during insulin infusion (fig. 4).

In both CON and LBW we found an increase in forearm respiratory coefficient from baseline to insulin stimulation (CON from 0.67 ± 0.09 to 0.78 ± 0.04 , *p* < 0.05; LBW from 0.61 ± 0.05 to 0.75 ± 0.04 , *p* < 0.05). The increase from baseline to insulin stimulation was not different between the groups.

Fig. 4. **a** FBF in the intervention arm in 22 subjects born with LBW and 22 CON subjects. **b** Arteriovenous glucose extraction fraction, calculated as $[(a-v)/a] \times 100$, in 20 LBW and 15 CON. **c** Forearm glucose clearance rates (FBF $\times$ glucose extraction) in 20 LBW and 15 CON. Baseline values (black bars) were obtained

before infusion of vasodilators or insulin (see fig. 1). During insulin infusion, measurements were done at $t = 300$ min (90 min into the clamp) and at $t = 390$ (180 min into the clamp) (white bars). ^a $p < 0.05$ vs. baseline. ^b $p < 0.05$, LBW vs. CON. Values are means $\pm$ SEM.

Fig. 5. ADN-induced increase in FBF in 22 subjects born with LBW (circles) and 21 CON subjects (squares). The infusion protocol was repeated during insulin infusion (open symbols and dashed lines). Insulin added significantly ($p < 0.05$) to the effect of ADN stimulation of FBF in both groups. Values are means $\pm$ SEM.

Fig. 6. ACH-induced increase in FBF in 22 subjects born with LBW (circles) and 21 CON subjects (squares). The infusion protocol was repeated during insulin infusion (open symbols and dashed lines). Insulin added significantly ($p < 0.05$) to the effect of ACH stimulation of FBF in LBW but not in CON ($p = 0.18$). Values are means $\pm$ SEM.

Vasodilator Response and Inflammatory Markers

Average MAP at baseline (CON 86 ± 1 mm Hg, LBW 86 ± 2 mm Hg) compared with average MAP during hyperinsulinemia (CON 85 ± 2 mm Hg, LBW 87 ± 2 mm Hg) was not different in either group nor was there any difference in MAP between the groups.

In response to intra-arterial infusion of ADN, FBF increased dose-dependently ($p < 0.001$) and similarly ($p >$

0.05) in both groups (fig. 5). With the addition of insulin, the FBF response to ADN significantly increased compared with ADN infusion alone (average increase CON $28 \pm 7\%$, LBW $27 \pm 1\%$; fig. 5).

In response to intra-arterial infusion of ACH, FBF increased dose-dependently ($p < 0.001$) and similarly ($p > 0.05$) in both groups (fig. 6). With the addition of insulin, FBF response to ACH increased on average by $29 \pm 9\%$

Fig. 7. Forearm glucose uptake vs. forearm blood flow in CON (a) and LBW (b) during ADN (circles) and ACH infusions (squares) with (open symbols) and without (closed symbols) hyperinsulinemia. Closed and open triangular symbols refer to

baseline data with and without concomitant hyperinsulinemia, respectively [corresponding to time points 295 min and 55–65 min after cannulation, respectively (see fig. 1)]. Values are means $\pm$ SEM.

($p < 0.05$) in LBW, but in CON the increase of $16 \pm 0.8\%$ did not attain statistical significance ($p = 0.18$; fig. 6).

To examine if there were any changes in forearm glucose uptake due to increased FBF, forearm glucose uptake was calculated at each infusion step with and without hyperinsulinemia (fig. 7a, b).

As a consequence of the higher FBF during insulin stimulation but unaltered MAP, the averaged forearm vascular conductance was higher during insulin stimulation compared with baseline (CON 0.12 ± 0.01 and 0.17 ± 0.02 ml · 100 ml⁻¹ · min⁻¹ · mm Hg⁻¹ baseline and insulin stimulation, respectively, $p < 0.05$; LBW 0.11 ± 0.01 and 0.15 ± 0.02 ml · 100 ml⁻¹ · min⁻¹ · mm Hg⁻¹ baseline and insulin stimulation, respectively, $p < 0.05$) with no differences between groups ($p > 0.05$). HsCRP, vWF, homocysteine and the markers of endothelial function sICAM-1 and sVCAM-1 were similar between the groups (table 1).

Discussion

The major finding of the present study is that LBW subjects had diminished vasodilator response to systemic hyperinsulinemia, while the response to intra-arterial infusion of endothelial-dependent vasodilators did not differ between LBW and CON. Fractional forearm glucose extraction was not different between the groups, and consequently insulin-mediated forearm glucose clear-

ance was lower in LBW compared with CON, while insulin-stimulated whole-body glucose uptake was normal.

To our knowledge, it has not previously been shown that FBF response to systemic hyperinsulinemia is impaired in young healthy LBW subjects. In contrast to the present study, FBF response to insulin has, in fact, previously been shown to be similar in LBW and CON subjects [9]. However, in the previous study local intra-arterial insulin infusion for 20 minutes was used and this could probably explain why the difference in flow responses was not found [9]. Insulin has a slow vasodilatation onset effect on the bulk flow. In the present study, steady-state blood flow measurements were carried out at 90 and 180 min of systemic hyperinsulinemia, at a time when steady-state glucose homeostasis prevailed. Furthermore, lack of blood flow response to local hyperinsulinemia, as opposed to systemic hyperinsulinemia, has previously been noted [24]. Our finding of increased respiratory coefficient across the forearm with insulin stimulation verifies that metabolic rate did increase, but to the same extent in the 2 groups. We cannot exclude the possibility that other known (e.g. insulin-mediated release of ADN and/or ATP from platelets [25]) or unknown vasoactive substances released from LBW and CON in a differential manner due to different glucose utilization rates may explain the differences in blood flow. But, altogether, the data does not support the notion that differences in metabolic rate from the forearm muscles controlled the insulin-stimulated FBF.

The set of data allows for an evaluation of forearm glucose uptake rates when the FBF is otherwise increased (i.e. by intra-arterial infusion of ADN or ACH; fig. 7). Whether or not hyperinsulinemia is present, increasing FBF to values above approximately $10 \text{ ml} \cdot 100 \text{ ml}^{-1} \cdot \text{min}^{-1}$ does not result in a further increase in glucose uptake rates. Thus, increased glucose delivery to the forearm muscles above a certain threshold is under these conditions counteracted by a decrease in extraction of glucose from the blood. The role of blood flow in the regulation of muscle glucose uptake has been detailed elsewhere [26].

We did not measure insulin-mediated capillary recruitment in the present study, but others have found a positive association between impaired post-ischemic capillary recruitment in the skin and LBW in children [27] and in adults [28]. Additionally, in 3 months the cutaneous maximal hyperemic response of old LBW infants was found to be impaired, whereas the cutaneous endothelial-dependent vasodilatation was not affected [29]. In neither of these aforementioned studies was macrovessel endothelial function addressed, wherefore a possible association between the conditions of the macrovessels and microvessels in LBW remains unknown. However, our data of impaired forearm insulin-mediated blood flow and normal forearm glucose extraction leading to decreased forearm glucose clearance could be the consequence of capillary recruitment impairment.

A similar whole-body insulin-stimulated glucose uptake (M-value) in LBW and CON subjects has been demonstrated before in subjects who were comparable to the subjects used in the present study [9]. We cannot completely discharge an effect of differences between LBW and CON subjects in hepatic glucose production, which we did not measure. However, in a previous study, it was shown that LBW had a greater suppression of hepatic glucose production compared with CON subjects [30]. If this finding is extrapolated to the present study, our conclusion that insulin-mediated whole-body glucose uptake rates are not decreased in LBW subjects may be somewhat weakened.

The apparent discrepancy of a lower forearm glucose clearance in LBW in the face of a normal whole-body insulin-stimulated glucose uptake has several possible explanations. The differences cannot be attributed to differences in forearm composition. The relationship between arm fat mass and arm lean mass did not differ between groups (0.18 ± 0.02 vs. 0.19 ± 0.02 in LBW and CON, respectively, $p > 0.05$). The forearm constitutes only a small part of the whole-body skeletal muscle mass

and therefore a decreased glucose clearance of the forearm could be compensated for by a slightly higher glucose uptake rate in the rest of the skeletal muscles (or adipose tissue). Additionally, the insulin sensitivity in the forearm skeletal muscle may not be fully representative of the entire skeletal muscle in the body [31]. However, the possibility remains that insulin-stimulated glucose uptake is reduced in other skeletal muscle groups to the same extent as in the forearm muscle, and we therefore cannot exclude that increased glucose uptake in nonmuscle tissue, including liver and adipose tissue, may counterbalance reduced muscle glucose uptake in LBW subjects.

In accordance with the present study, a divergent response of insulin on vessel vasodilatation and on whole-body glucose uptake rates was previously found in a study in patients with mild hypertension [32] in which the coronary vasoreactivity to insulin was blunted, but whole-body glucose uptake rates were normal. Thus, it seems that vascular insulin resistance precedes, or can exist without, metabolic insulin resistance. The finding of an impaired insulin vasoreactivity in patients with mild hypertension [32] is intriguing because it is well documented [33, 34] that LBW later in life is associated with an increased risk of cardiovascular disease including hypertension.

We found no difference in the vasodilator responses to ACH and ADN between CON and LBW. The ACH data are in accordance with other published data on the forearm [9, 12], but the response to ADN is a novel finding. The facilitating effect of insulin on the endothelium-dependent vasodilator responses as demonstrated in other studies [9, 35] was also found in LBW. This may at a first glance seem contradictory due to the impaired effect in LBW of insulin alone (fig. 4); however, it should be noted that during ADN and ACH stimulation, FBF rates were considerably higher than with insulin alone. Thus the 2 conditions are not comparable. ADN's effect in the forearm vasculature is partly nitric oxide-dependent [13, 14] and the short half-life ($<10 \text{ s}$) makes it suitable for repeated tests. By using intra-arterial infusion, general sympathetic activation is avoided which otherwise could counteract the vasodilator effect via activation of vasoconstrictor α -receptors in the vascular smooth muscle in the arterioles.

In agreement with the similar resistance vessel vascular response in LBW and CON, biomarkers of inflammation and endothelial function (hsCRP, ICAM-1, VCAM-1, homocysteine and vWF) were similar in LBW and CON. Consequently, it can be concluded that the impaired vascular insulin response observed in LBW may

exist without endothelial damage/dysfunction and low-grade inflammation. To our knowledge VCAM and ICAM have not been measured in LBW and a matched control group before. Increased homocysteine [36] and vWF [12] concentrations in plasma have been reported previously in 69 boys and girls who were between 8 and 13 years old and had LBW (homocysteine), and in 12 adults with LBW (vWF). The latter finding was not accompanied by an impaired endothelial function [12]. The discrepancy between the present study and the previous studies may be explained by differences in age and study group size.

In summary, we found diminished insulin-mediated FBF and glucose clearance rates in subjects with LBW compared with a well-matched group of CON. The vasodilator responses to ADN and ACH and biomarkers of inflammation were not different between the groups. The impaired insulin-mediated increase in bulk flow in

LBW may be due to an impairment of insulin-mediated capillary recruitment in these subjects. The decreased insulin-mediated forearm glucose clearance did not translate into significantly decreased whole-body insulin-mediated glucose uptake in LBW, albeit the M-value was slightly lower in LBW compared with CON.

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