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Impaired endothelial function and insulin action in first-degree relatives of patients with type 2 diabetes mellitus

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Abstract

First-degree relatives (FDR) of patients with type 2 diabetes mellitus are at increased risk of developing type 2 diabetes mellitus. We studied if endothelial dysfunction of the resistance vessels is present and may coexist with metabolic insulin resistance in FDR. Male FDR (n = 13; 26 ± 1 years; body mass index, 25 ± 1 kg m^2 [mean ± SEM]) and matched control subjects (CON) (n = 22; 25 ± 1 years; body mass index, 24 ± 1 kg m^2) were studied by hyperinsulinemic (40 mU $min^{-1}\cdot m^{-2}$) isoglycemic clamp combined with brachial arterial and deep venous catheterization of the forearm. Forearm blood flow (FBF) was measured by venous occlusion plethysmography upon stimulation with systemic hyperinsulinemia (291 ± 11 pmol/L, pooled data from both groups) and upon intraarterial infusion of adenosine (ADN) and acetylcholine (ACH) ± hyperinsulinemia. Forearm blood flow response to ADN and ACH was less in FDR vs CON ($P < .05$); systemic hyperinsulinemia added to the FBF effect of ADN in CON ($P < .05$) but not in FDR. In addition, FDR demonstrated impaired FBF to hyperinsulinemia (2.1 ± 0.2 vs 4.0 ± 0.6 mL 100 mL^{-1} min^{-1}) in FDR and CON, respectively ($P < .05$). Both M-value (5.0 ± 0.7 vs 7.0 ± 0.5 mg min^{-1} kg^{-1}) and forearm glucose clearance (0.6 ± 0.1 vs 1.4 ± 0.4 mL 100 $mL^{-1}\cdot min^{-1}$) were diminished in FDR compared with CON (all $P < .05$). FDR demonstrated endothelial dysfunction of the resistance vessels in addition to impaired insulin-stimulated increase in bulk flow. Moreover, FDR demonstrated whole-body insulin resistance as well as decreased basal and insulin-stimulated forearm glucose uptake. It remains to be established whether FDR also demonstrate impaired insulin-stimulated microvascular function.

1. Introduction

First-degree relatives (FDR) of patients with type 2 diabetes mellitus are at increased risk of developing type 2 diabetes mellitus. This is due to a mixture of genetic inheritance and adoption of the family lifestyle. In addition to the insulin resistance found in young FDR [1-3], studies have also demonstrated endothelial dysfunction [4-9]. In studies of endothelial function in FDR, the noninvasive flow-mediated vasodilatation (FMD) technique with detection of the brachial artery diameter by ultrasound technique has predominately been used [4-9]. This technique primarily evaluates the conduit vessels, whereas venous occlusion plethysmography combined with intraarterial infusion of endothelial-dependent vasodilators such as acetylcholine (ACH) or adenosine (ADN) evaluates the resistance vessels [10]. The latter method is considered the criterion standard for assessing the endothelial function of the resistance vessels [11]. Both methods provide information about the endothelium and can supplement but not replace each other [12]. However, there is a lack of studies that specifically address the endothelial function of the resistance vessels in FDR.

The effects of insulin on metabolism and vascular tissue are closely linked [13]. Insulin promotes vasodilatation and capillary recruitment, thereby increasing the nutritive flow in various tissues and particularly in skeletal muscle [14-16]. Insulin stimulates bulk flow as an endothelium-dependent vasodilator via nitric oxide production [17,18]; but the much smaller and protracted vasodilator effect of submaximal doses of insulin compared with the effect of an nitric oxide–dependent vasodilator, for example, ACH, indicates that other mechanisms may be present. Furthermore, the fact that insulin potentiates the vasodilator effect of ACH in healthy subjects

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[19], but not in non–insulin-treated patients with type 2 diabetes mellitus [20], shows that insulin resistance is present also at the vascular level in type 2 diabetes mellitus. Interestingly, the potentiating defect is reversible with regular insulin treatment [20], which also improves endothelium-dependent vasodilatation [21,22]. As FDR have been shown to be insulin resistant in terms of whole-body insulin-mediated glucose uptake and as endothelial function may also be impaired (at the conduit vessels) [4-8], it seems plausible that the vascular insulin sensitivity in FDR is decreased as well; but this has to our knowledge not previously been investigated.

The aim of this study was therefore to evaluate the endothelial function of the resistance vessels with plethysmography and intraarterial infusions of endothelial-dependent vasodilators and to evaluate the metabolic and vascular insulin sensitivity in a population of healthy FDR compared with matched controls. We hypothesized that in FDR the endothelial function of the resistance vessels would follow the impaired function of the conduit vessels. Secondly, we hypothesized that both the vascular and metabolic response to submaximal insulin would be impaired in FDR. Finally, we hypothesized that, if the vascular response to insulin was similar in FDR and controls, the response to combined insulin and (other) endothelial-dependent vasodilators would unmask a possible vascular insulin resistance in FDR.

2. Research design and methods

2.1. Subjects

Thirty-five young white men were recruited to the study. The control group (CON) of 22 subjects was recruited via the Danish Birth Registry (born at term and birth weight within the 50%-75% percentile) and had no relatives with type 2 diabetes mellitus. The FDR group was recruited via their parents who attended Steno Diabetes Center, Gentofte, Denmark (10 subjects) and via advertisement (3 subjects). All were born at term with birth weight average around the 50% percentile and with no records of gestational diabetes of the mother. The inclusion criterion was at least 1 parent with type 2 diabetes mellitus and 1 second-degree relative with type 2 diabetes mellitus. Seven subjects had more than 1 second-degree relative with type 2 diabetes mellitus. The 2 groups were similar according to sex, age, body mass index (BMI), and physical fitness. Body composition was measured with dual-energy x-ray absorptiometry scanning (Lunar Prodigy Advance, GE Health Care, Chalfort St. Giles, United Kingdom).

Maximal oxygen consumption was measured on a bicycle ergometer with a stepwise incremental test using the leveling off criterion (Jaeger Instruments, Höchberg, Germany).

Two FDR subjects were smokers with an average of 10 cigarettes per day, 1 CON subject smoked 15 cigarettes per day, and 2 CON were light smokers (3-4 cigarettes per day).

The study was approved by the regional ethical committee (reference 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 the subjects before participation.

2.2. Experimental protocol

All subjects were provided a standardized isocaloric nutritionally recommended diet (ie, 55 E% carbohydrates, 15 E% of protein, 30 E% fat) 4 days before the study.

After 10 hours of overnight fast, the subjects reported to the laboratory at 8.00 AM. Electrocardiogram and heart rate were monitored by precordial electrodes. Arterial catheters (Becton-Dickinson, Oxford, United Kingdom) were inserted in the brachial arteries of both arms for blood sampling, mean arterial pressure (MAP) and blood pressure monitoring (control arm), and infusion of vasodilators (intervention arm). Venous catheters (Medex Medicine, Rossendale, United Kingdom) were inserted in the medial antecubital veins of both arms. In the intervention arm, the venous catheter was inserted in the retrograde direction (for blood sampling); and in the control arm, the venous catheter was inserted in the antegrade direction (for infusion of insulin and glucose). In 2 CON subjects and 1 FDR subject, it was not possible to insert more than one arterial line; and arterialized blood samples from a heated hand vein were used in replacement. In 5 CON subjects and 3 FDR subjects, it was not possible to insert the retrograde venous catheter.

2.3. Blood flow and arteriovenous differences—measurements and calculations

We measured forearm blood flow (FBF) simultaneously in both arms by venous occlusion mercury-in-Silastic strain-gauge plethysmography (Hokanson EC6 and E20, DE Hokanson, Bellevue, WA). During the entire experiment, the subject was placed in the supine position with both arms rested on custom-made triangular cushions, ensuring the passive return of blood in the arm to the heart. During measurements, a rapid cuff inflator on the upper arm was set at 40 mm Hg to occlude venous outflow from the forearm. Forearm blood flow was measured at cycles of 7 seconds every 15 seconds and calculated as an average of 4 to 5 consecutive readings. Around the wrists, small cuffs were positioned and inflated to suprasystolic pressures during plethysmography recordings and blood sampling to exclude the circulation of the hand and the contribution of the arteriovenous (a-v) shunts, respectively. All the plethysmography readings were analyzed by 1 single investigator (MS).

Time points for blood sampling, flow measurements, and the infusion regimen of vasodilators are shown in Figs. 1 and 2. Adenosine was infused intraarterially in three 5-minute consecutive intervals at 50, 150, and 500 μg min^{-1}. After 5 minutes of infusion at each step, measurements of FBF started as described above. When blood flow had returned to baseline values (≈20 minutes), intraarterial infusion of ACH in three 5-minute consecutive intervals at 15, 30, and 60 μg min^{-1} began. At t = 210 minutes (Figs. 1 and 2), a

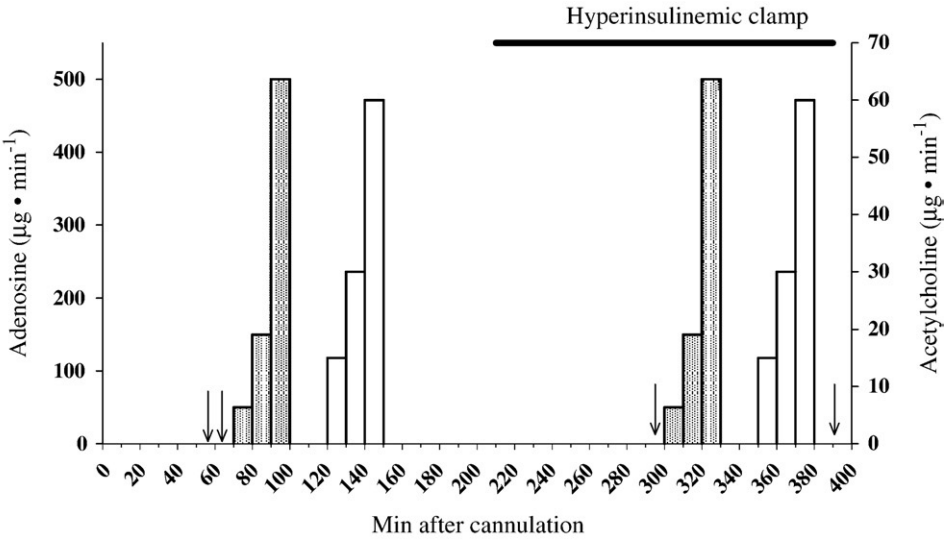

Fig. 1. Protocol for the experiment. The x-axis is time after insertion of arterial and venous catheters. Hatched columns are intraarterial infusions of ADN (left y-axis), and white columns are intraarterial infusions of ACH (right y-axis). At the end of each infusion step, FBF was measured by venous occlusion plethysmography. Arrows (↓) indicate time points for additional arteriovenous blood sampling. From $t = 210$ minutes, a 3-hour hyperinsulinemic (40 mU min^{-1} m^{-2}) isoglycemic clamp was added.

hyperinsulinemic isoglycemic clamp was initiated; and the intraarterial infusions of ADN and ACH were repeated 90 minutes into the clamp. Glucose infusion rates were averaged for 10-minute periods. Whole-body insulin-mediated glucose uptake rates were calculated as the mean of steady-state glucose infusion rates during the last 30 minutes of the clamp (from clamp time 150 to 180 minutes) (M-value). Baseline FBF and glucose a-v differences were measured twice at $t = 60$ minutes, just before infusion of ADN. Clamp FBF and a-v differences were measured at $t = 300$ minutes (clamp time 90 minutes) and at $t = 390$ minutes (clamp time 180 minutes). There was no significant difference between flow and fractional forearm glucose extraction measured at these 2 time points, and average values were used. Fractional glucose extraction across the intervention arm (forearm) was calculated as the a–v difference divided by the arterial glucose concentration and expressed in percentage. Glucose clearance (intervention arm) was calculated as the a–v difference divided by the arterial glucose concentration and multiplied by the blood flow (expressed as milliliters per 100 milliliters per·minute). Vascular conductance was calculated as FBF divided by MAP and expressed in milliliters per 100 milliliters per·minute·per millimeter of mercury.

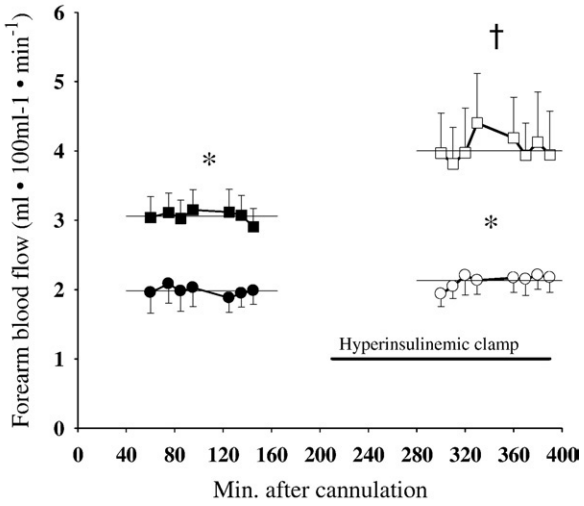

Fig. 2. Forearm blood flow in the nonintervention (control) arm in 12 FDR (circles) and in 19 CON (squares). Measurements were done by venous occlusion plethysmography and were performed at baseline (closed symbols) and during systemic hyperinsulinemia (open symbols). The hyperglycemic isoglycemic clamp was performed from $t = 210$ to $t = 390$ minutes (thick horizontal line). Thin horizontal lines indicate the mean value of individual averaged blood flow measurements. The Δ value (baseline vs hyperinsulinemia) was significantly ($P = .016$) greater in CON (1.1 ± 0.5) compared with FDR (0.1 ± 0.1). *Significant difference between FDR and CON ($P < .05$). †Significant increase with hyperinsulinemia in CON ($P < .05$). Values are mean ± SEM.

2.4. Infusates and infusion regimens

ADN and ACH solutions were prepared from stock solutions (5 mg mL^{-1} [Item Development, Stocksund, Sweden] and 10 mg mL^{-1} [Michol-E, Novartis Pharma, Basle, Switzerland], respectively) and isotonic sodium chloride. For the hyperinsulinemic isoglycemic clamp, insulin (Actrapid 100 U/mL, Novo Nordisk, Copenhagen, Denmark) was administered as a 2-mL bolus followed by a constant intravenous infusion (40 mU $min^{-1}·m^{-2}$). Arterial blood was sampled every 5 to 10 minutes, and glucose (20%) infusion rates were adjusted accordingly to measured plasma glucose concentrations. Potassium level was measured during the clamp; and if needed, oral potassium (750 mg Kaleorid, LEO Pharma, Ballerup, Denmark) was given.

2.5. 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 by enzyme-linked immunosorbent assay technique (ELISA) (DAKO ELISA kit 6219 and 6218, Dublin, OH, respectively). Glycosylated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (Tosoh Biosciences, Montgomeryville, PA). Total cholesterol and high-density lipoprotein (HDL) cholesterol were analyzed with an enzymatic colorimetric test (Roche Diagnostic, Mannheim, Germany). Low-density lipoprotein (LDL) cholesterol was calculated from the Friedewald formula [23]. Plasma triglyceride concentration was determined with Triglyceride GPO-PAP (Roche Diagnostic). High-sensitivity C-reactive protein (hsCRP) was measured on automatic analyzer (Hitachi 912, Boehringer, Mannheim, Germany). Homocysteine was measured with reagent Axis-Shield Enzymatic Homocysteine FHER100 (Thermo Konelab 30i, Clinical Diagnostics, Vantaa, Finland). Adhesion molecules (soluble intercellular adhesion molecule–1 [sICAM-1] and soluble vascular cell adhesion molecule–1 [sVCAM-1]) were analyzed with ELISA Immunoassay (R&D Systems, Minneapolis, MN). Von Willebrand factor (vWF) was measured by ELISA technique at Rigshospitalet, Copenhagen, Denmark. E-selectin was analyzed with ELISA technique (RayBiotech, Norcross, GA).

2.6. Statistics

Statistical analysis was done using Sigma Stat (St. Louis, MO) version 3.1. Student *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 (Kolmogorov-Smirnov test). Furthermore, 2-way analysis of variance for repeated measures was used when analyzing continuous variables (blood flow responses to vasodilators). In case of a significant interaction between the 2 variables (group and time), an all-pairwise multiple comparison procedure (Holm-Sidak method) was used to locate differences at specific time points. Spearman rank order test was used to determine the correlation between forearm and whole-body glucose clearance rates. A P value less than .05 was considered significant in 2-tailed testing. All data are reported as mean ± SEM.

3. Results

The anthropometric characteristics and biochemical data are outlined in Table 1. Fat tissue was located relatively more in the trunk area in FDR compared with CON (trunk fat–body fat ratio: 0.58 ± 0.01 and 0.48 ± 0.01, respectively [$P <$ .0001]) and less in the legs (leg fat–body fat ratio: 0.29 ± 0.01 and 0.37 ± 0.01, respectively [$P <$ .0001]). However BMI and waist circumference did not reveal any significant difference between groups. Arm fat percentage and the arm fat–body fat ratio were also similar between groups ($P >$.05). Fasting plasma insulin concentrations, hsCRP, homocysteine, and LDL cholesterol were significantly higher in FDR compared with CON.

Table 1
Subject characteristics

	CON	FDR	
	n = 22 (men)	n = 13 (men)	
Age (y)	25.1 ± 0.2	26.4 ± 1.2	NS
Height (m)	1.85 ± 0.01	1.84 ± 0.02	NS
Weight (kg)	83.3 ± 3.3	83.1 ± 2.1	NS
BMI	24.3 ± 0.5	24.9 ± 0.9	NS
VO_2 max (mL min^{-1} kg^{-1})	43.5 ± 1.5	39.1 ± 1.9	NS
Body fat (%)	18.6 ± 1.7	25.0 ± 2.3	$P <$ .05
Fat-free mass (kg)	63.5 ± 1.0	58.6 ± 1.5	$P <$ .05
Waist (cm)	85.5 ± 1.8	89.7 ± 2.9	NS
Waist-hip ratio	0.86 ± 0.01	0.88 ± 0.02	NS
Systolic blood pressure (mm Hg)	128 ± 2	126 ± 3.1	NS
Diastolic blood pressure (mm Hg)	68 ± 2	71 ± 3	NS
Fasting arterial glucose ($mmol \cdot L^{-1}$)	5.3 ± 0.1	5.5 ± 0.1	NS
HbA_{1c} (%)	5.1 ± 0.1	5.1 ± 0.1	NS
Insulin ($pmol \cdot L^{-1}$)	35 ± 3	47 ± 5	$P <$ .05
C-peptide ($pmol \cdot L^{-1}$)	513 ± 25	663 ± 51	$P <$ .01
Total cholesterol ($mmol \cdot L^{-1}$)	3.9 ± 0.2	4.5 ± 0.3	$P =$ .0597
LDL ($mmol \cdot L^{-1}$)	2.2 ± 0.1	2.8 ± 0.3	$P <$ .05
Triglyceride ($mmol \cdot L^{-1}$)	0.9 ± 0.1	1.1 ± 0.1	NS
HDL ($mmol \cdot L^{-1}$)	1.3 ± 0.1	1.2 ± 0.1	NS
Homocysteine ($\mu mol \cdot L^{-1}$)	9.8 ± 0.5	12.3 ± 0.6	$P <$ .005
hsCRP ($nmol \cdot L^{-1}$)	25 ± 7	99 ± 18	$P <$ .001
sICAM-1 ($ng \cdot mL^{-1}$)	227 ± 8	228 ± 15	NS
sVCAM-1 ($ng \cdot mL^{-1}$)	611 ± 19	695 ± 65	NS
vWF ($kIU \cdot L^{-1}$)	0.90 ± 0.07	0.94 ± 0.07	NS
E-selectin ($pg \cdot mL^{-1}$)	191 ± 33	307 ± 57	$P =$ .076

VO_2 max indicates maximal oxygen consumption; NS, not significant.

3.1. Vasodilatation responses

Average MAP at baseline (FDR: 78 ± 2 mm Hg, CON: 86 ± 1 mm Hg) compared with average MAP during hyperinsulinemia (FDR: 78 ± 2 mm Hg, CON: 85 ± 1 mm Hg) was not different in either group; however, MAP in the FDR was significantly lower than the MAP in CON both at baseline and during hyperinsulinemia.

Baseline FBF was significantly lower in FDR compared with CON measured in the control arm ($P =$ .007, Fig. 2) and in the intervention arm ($P =$ .025, Fig. 3). In the FDR, a forearm vasodilatation response to hyperinsulinemia was not observed in the control arm (the arm without the vasodilator infusions; baseline: 2.0 ± 0.2 mL 100 mL^{-1} min^{-1} vs insulin-stimulated: 2.1 ± 0.2 mL 100 $mL^{-1} \cdot min^{-1}$ [Fig. 2]), whereas a slight increase with insulin was seen in the intervention arm (baseline: 2.0 ± 0.2 vs insulin-stimulated: 2.7 ± 0.2 mL 100 $mL^{-1} \cdot min^{-1}$, $P =$ .05 [Fig. 3]). In contrast, in CON subjects, FBF increased significantly from baseline to hyperinsulinemia in both the control arm (baseline: 3.1 ± 0.3 vs insulin-

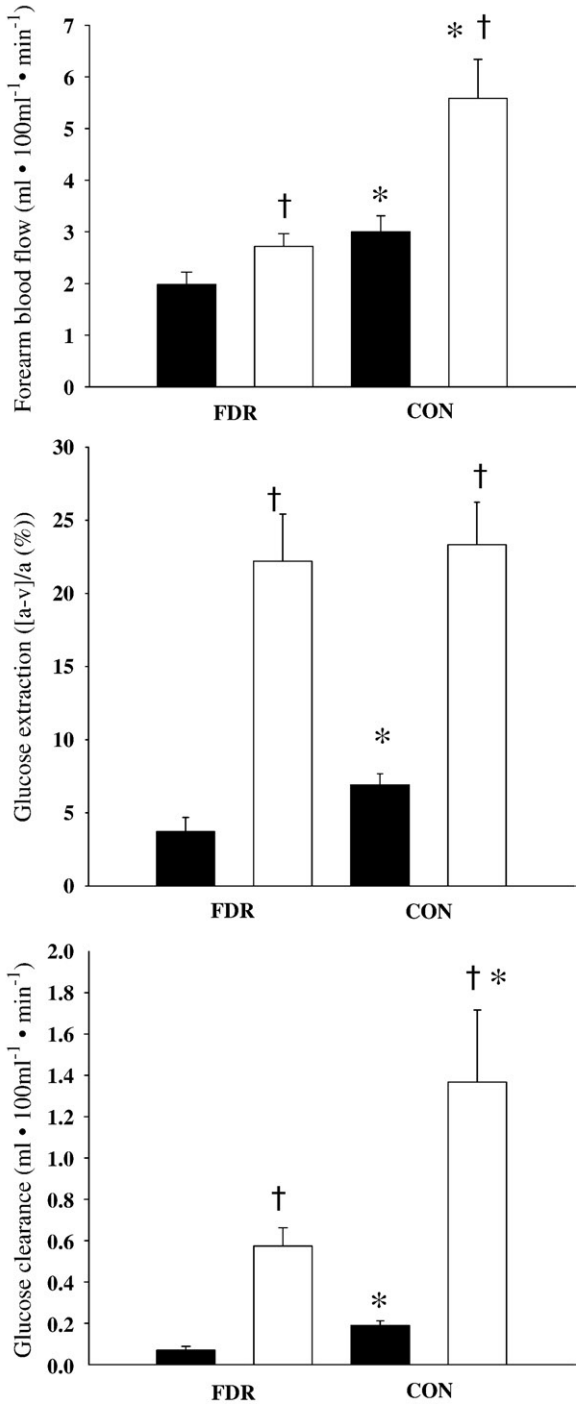

Fig. 3. First-degree relatives to patients with type 2 diabetes mellitus (n = 10) and CON (n = 15) had an isoglycemic hyperinsulinemic clamp performed in combination with forearm arteriovenous catheterization and blood flow measurements by plethysmography. Black bars are baseline values, and white bars are values obtained during hyperinsulinemia. A, Forearm blood flow. B, Arteriovenous glucose extraction ([a-v]/a × 100%). C, Forearm glucose clearance rates (FBF × glucose extraction). Baseline values were obtained before infusion of vasodilators or insulin (Fig. 1). Data from the hyperinsulinemic state are average of measurements obtained after 90 and 180 minutes of hyperinsulinemia because these were not significantly different from each other. *Significant difference between FDR and CON ($P < .05$). †Significant difference between baseline and hyperinsulinemia within each group ($P < .05$). Values are mean ± SEM.

stimulated: 4.0 ± 0.6 mL 100 mL^{-1}·min^{-1}, $P = .027$ [Fig. 2]) and in the intervention arm (baseline: 3.0 ± 0.3 vs insulin-stimulated: 5.6 ± 0.8 mL 100 mL^{-1}·min^{-1}, $P = .001$ [Fig. 3]). Hence, the increase from baseline to hyperinsulinemia (Δ value) was greater in CON compared with FDR (control arm: 1.1 ± 0.5 vs 0.1 ± 0.1 [$P = .016$], intervention arm: 2.6 ± 0.3 vs 0.7 ± 0.3 mL 100 mL^{-1}·min^{-1} [$P = .001$], respectively). In FDR, the vascular conductance was similar at baseline compared with insulin stimulation (0.09 ± 0.01 and 0.11 ± 0.02 mL 100 mL^{-1}·min^{-1} mm Hg^{-1}, respectively [$P > .05$]), whereas in CON, the higher FBF during insulin stimulation but unaltered MAP resulted in a higher vascular conductance 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 [$P < .05$]).

In response to intraarterial infusion of ADN or ACH, FBF always increased dose dependently ($P < .001$); but FBF was significantly lower in FDR compared with CON during ADN (FDR: 4.7 ± 0.6, 6.7 ± 1.1, and 9.7 ± 2.3 mL 100 mL^{-1}·min^{-1} at 50, 150, and 500 μg min^{-1}, respectively) (CON: 6.9 ± 0.6, 11.3 ± 1.3, and 19.0 ± 2.6 mL 100 mL^{-1}·min^{-1} at 50, 150, and 500 μg min^{-1}, respectively) ($P < .01$) (Fig. 4) as well as during ACH stimulation (FDR: 5.6 ± 0.7, 7.3 ± 1.2, and 10.8 ± 1.9 mL 100 mL^{-1}·min^{-1} at 15, 30, and 60 μg min^{-1}, respectively) (CON: 9.2 ± 1.2, 13.6 ± 1.8, and 17.5 ± 2.6 mL 100 mL^{-1}·min^{-1} at 15, 30, and 60 μg ·min^{-1}, respectively) ($P < .05$) (Fig. 5). With addition of insulin (ie, repetition of the ADN infusion protocol during the clamp), the FBF response to ADN was less in FDR compared with CON. (FDR: 6.0 ± 0.6, 8.7 ± 0.8, and 13.1 ± 2.8 mL 100 mL^{-1}·min^{-1} at 50, 150, and 500 μg min^{-1}, respectively) (CON: 10.6 ± 1.4, 17.9 ± 2.5, and 22.0 ±

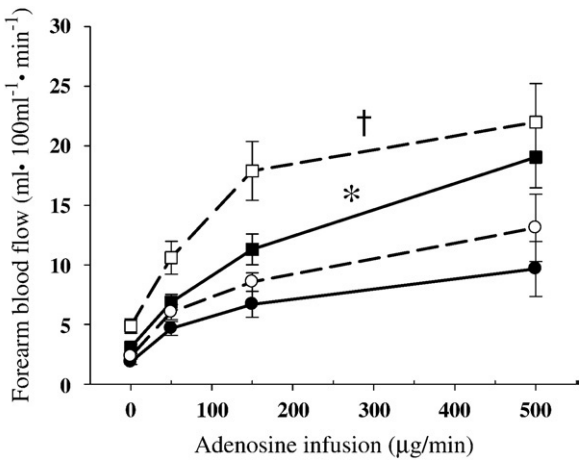

Fig. 4. Adenosine-induced increase in FBF in 12 FDR (circles) and in 21 CON (squares). The infusion protocol was repeated during a hyperinsulinemic isoglycemic clamp (open symbols). The effect of ADN on FBF was higher in CON compared with FDR (main effect: $P < .01$, interaction: $P = .004$). Insulin added significantly to the effect of ADN in CON (main effect: $P < .001$, interaction: $P = .051$) but not in FDR. *Significant difference ($P < .01$) between FDR and CON. †Significant effect ($P < .001$) of insulin. Values are mean ± SEM.

3.2 mL 100 mL^{-1}·min^{-1} at 50, 150, and 500 μg min^{-1}, respectively) ($P < .05$). Thus, in FDR, ADN and insulin combined did not increase FBF further, whereas in CON subjects, the combination (ADN + insulin) elicited an FBF significantly higher ($P < .001$) compared with ADN stimulation alone (Fig. 4). With ACH stimulation, no additional effect of hyperinsulinemia FBF was observed in either group (Fig. 5).

3.2. Whole-body and forearm glucose uptake

Glucose infusion rates during the hyperinsulinemic isoglycemic clamp averaged for periods of 10 minutes were lower in FDR compared with CON (Fig. 6), resulting in a significantly lower M-value (5.0 ± 0.7 vs 7.0 ± 0.5 mg min^{-1} kg^{-1}, respectively [$P < .05$]). Plasma insulin concentrations during the clamp were not different between groups (283 ± 28 [FDR] and 294 ± 10 [CON] pmol L^{-1} [$P > .05$]). Although FDR had a significantly lower lean body mass (LBM) and higher whole-body fat mass, M-value relative to LBM was still lower in FDR compared with CON (7.0 ± 0.8 and 9.1 ± 0.6 mg min^{-1} kg^{-1} LBM [$P < .05$]).

Baseline fractional glucose extraction across the forearm was significantly lower in FDR compared with CON (Fig. 3), whereas the insulin-stimulated fractional glucose extraction was not different between the 2 groups (Fig. 3). Forearm glucose clearance was lower in FDR compared with CON at baseline (0.07 ± 0.02 and 0.19 ± 0.02 mL 100 mL^{-1}·min^{-1}, respectively [$P = .0009$]) and increased with hyperinsulinemia (to 0.57 ± 0.09 and 1.37 ± 0.35 mL 100 mL^{-1}·min^{-1}, respectively), with the difference between FDR and CON still significant($P = .028$) (Fig. 4). Forearm respiratory coefficient (RQ) did not change significantly from baseline

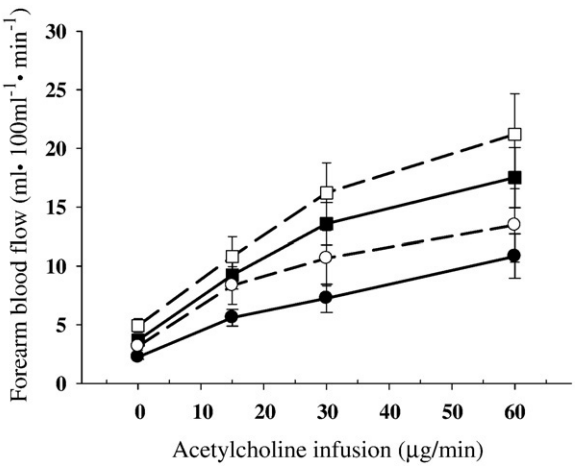

Fig. 5. Acetylcholine-induced increase in FBF in 12 FDR (circles) and in 21 CON (squares). The infusion protocol was repeated during a hyperinsulinemic isoglycemic clamp (open symbols). The effect of ACH on FBF was higher in CON compared with FDR (main effect: $P = .021$, interaction: $P = .120$). There was no additional effect of insulin on ACH-stimulated FBF in either group. *Significant difference ($P = .021$) between FDR and CON. Values are mean ± SEM.

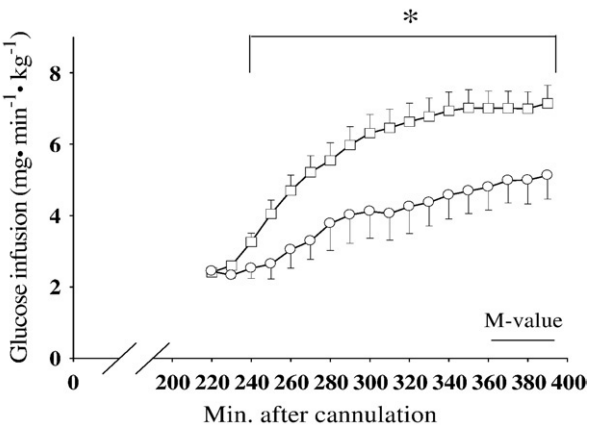

Fig. 6. Glucose infusion rates averaged for periods of 10 minutes in 13 FDR (circles) and 22 CON (squares) subjects during a 3-hour hyperinsulinemic isoglycemic clamp. *$P < .05$. Values are mean ± SEM.

(0.85 ± 0.09) to hyperinsulinemia (0.78 ± 0.07) in FDR, whereas a significant increase was seen in CON (from 0.67 ± 0.09 to 0.77 ± 0.04 [$P < .05$]).

4. Discussion

The major findings of the present study are as follows: (1) FDR had diminished forearm vasodilatation response to ADN and ACH, and insulin did not add to the vasodilator effect of ADN; (2) the insulin-mediated increase in FBF was absent in FDR; (3) hsCRP and homocysteine were elevated in FDR, whereas the concentrations of ICAM-1, VCAM-1, vWF, and E-selectin did not differ between the groups; and (4) the reduced insulin-mediated glucose uptake found at whole-body level was also detected in the forearm, where reduced basal as well as insulin-mediated glucose clearance was found.

It is important to distinguish between different methodologies for examining FBF. The frequently used combination of ultrasound Doppler technique with FMD [4-8] primarily evaluates blood flow in the conduit vessels [10]. In the present study, we examined the resistance vessels and found impairment in the vasodilator responses in FDR. Both the resistance vessels and the conduit vessels control total blood flow. However, the resistance vessels are located in proximity to the terminal arterioles and may therefore also affect the microcirculation. Although the mechanism is not fully understood [24], insulin-mediated capillary recruitment is necessary for an optimal metabolic insulin response [25], suggesting that resistance vessel endothelial dysfunction could have consequences not only for insulin-mediated increase in bulk flow but also insulin-mediated capillary recruitment.

The FDR might also have some degree of impaired glucose tolerance. However, fasting blood glucose and HbA_{1c} were similar between groups; therefore, impaired glucose tolerance hardly can be a major contributor to the FDR endothelial dysfunction.

The finding of diminished vasodilator response to ACH stimulation extends similar findings in the conduit vessels [4,8,26] and is in line with findings in 7 obese, insulin-resistant women with previous gestational diabetes [27]. In the present study, we found no additional effect of hyperinsulinemia on the ACH response in either FDR or CON. This is in contrast to findings in 18 [19] and 10 [20] healthy subjects in whom insulin had a marked added effect to ACH-stimulated flow. Notable differences between these 2 aforementioned studies and the present study are that our healthy subjects were 20 to 30 years younger and that we used systemic hyperinsulinemia, whereas the previous studies used a local intrabrachial insulin infusion [19,20]. In addition, the blood flow response to identical ACH stimulation (eg, 60 μg min^{-1}) alone was twice as high in the present study (17.5 ± 2.6 mL 100 mL^{-1}·min^{-1}) as in the study by Rask-Madsen et al (≈8 mL 100 mL^{-1} min^{-1}) [20], which limits further increases in FBF in the present study.

To our knowledge, an impairment of ADN-stimulated FBF in FDR has not been shown before. Hyperinsulinemia significantly added to the vasodilatation effect of ADN in the healthy control subjects, whereas no effect was seen in FDR subjects, agreeing with our findings of a generally decreased insulin action in these subjects. A diminished coronary response to ADN simultaneous with decreased FMD of the forearm has been reported in FDR [7], supporting our findings and underlining the dissemination of the FDR endothelial dysfunction.

Distinct differences in insulin action were also found between FDR and CON.

In both arms, the vascular responsiveness to insulin was impaired in FDR compared with CON, a finding that has also been observed in obese individuals [28], where a reduced insulin-mediated capillary recruitment was also seen. A reduced responsiveness of the vascular bed in FDR has been demonstrated in adipose tissue, where a blunted adipose tissue blood flow after a meal was reported [29]. The data suggest that FDR may have an overall decreased ability to increase bulk blood flow in response to food intake/insulin release, whereas FDR insulin microcirculation response is still uninvestigated.

The markedly higher plasma CRP in FDR compared with CON indicates the presence of a subinflammatory state in the FDR subjects. High concentrations of CRP have been associated with increased risk of future cardiovascular events and atherosclerosis [30,31]; endothelial dysfunction is generally regarded as an early sign of atherosclerosis [32]. Collectively ,these data are in accordance with our finding of a small but significantly higher total cholesterol and LDL cholesterol in FDR subjects (Table 1). Other well-established markers of endothelial dysfunction, ICAM-1, VCAM-1, and E-selectin, were not significantly elevated in FDR subjects. This is in accordance with a large study in a similar population; but in that study, endothelial vasoreactivity was not addressed [33]. Thus, it appears that impaired endothelial vasoreactivity occurs before an increase in markers of endothelial dysfunction. Concentrations of vWF were similar between groups. This is supported by a study of individuals with type 2 diabetes mellitus with endothelial dysfunction but unaffected vWF concentrations [34]. We found elevated homocysteine in the FDR subjects compared with CON, which agrees with studies on other prediabetic individuals [35] with endothelial dysfunction.

The groups in the present study differed in respect of percentage of body fat, central adiposity, total cholesterol, and HDL cholesterol. We cannot exclude the possibility that our findings can be attributed to adiposity and hyperlipidemia because these conditions are associated with endothelial dysfunction, insulin resistance, and atherosclerosis [36,37]. However, the phenotype of central adiposity is characteristic of the FDR population; and we therefore find the ambiguity inevitable.

The FDR subjects were as expected less insulin sensitive as documented by significantly lower M-values compared with those of CON. A novel, interesting, and potentially significant finding was reduced forearm (muscle) glucose extraction and clearance in the basal state in the FDR, indicating that muscle tissue plays a role in the abnormal glucose homeostasis eventually leading to overt type 2 diabetes mellitus even during low fasting plasma insulin concentrations. Furthermore, whole-body insulin-mediated glucose clearance rate (per LBM) correlated significantly with forearm glucose clearance (r^2 = 0.537, P = .005). The increased adiposity in FDR was not reflected in the fat content of the arms. This means that the reduced vasodilator responses found in the FDR compared with CON subjects cannot be attributed to a different distribution of muscle and fat tissue in the forearm between the 2 groups. However, it cannot a priori be excluded that cross talk from the central adipose tissue in the FDR mediated the reduced forearm vascular response.

The demonstration of reduced insulin-mediated glucose clearance in FDR confirms previous findings [1-3]. In addition, in the present study, we tried to delineate the relative influence of the 2 major components in glucose clearance: glucose delivery (blood flow) and extraction of glucose from the blood. Our results clearly show an inability of insulin to increase FBF in FDR (Figs. 2 and 3); and whereas glucose extraction at baseline was lower in FDR compared with CON (Fig. 3), glucose extraction increased in response to hyperinsulinemia in FDR, resulting in similar percentage of extraction compared with CON. In view of the lower FBF in FDR, glucose extraction should theoretically have been even higher than seen in CON because a lower blood flow allows for a longer transit time of glucose molecules in the capillaries. A major aspect of metabolic insulin resistance in FDR may therefore lie in the fact that the glucose extraction capacity is suboptimal. This is unmasked by exposing the skeletal muscle to conditions that are otherwise favorable for extraction of glucose from the blood, which has been demonstrated previously [2,38]. The net result of the defect flow and extraction responses to

hyperinsulinemia is a diminished glucose uptake in FDR. The independent measure of local forearm RQ, which increased with hyperinsulinemia in CON but not in FDR, supports the finding of diminished glucose clearance and uptake in FDR.

To summarize, we documented endothelial dysfunction of the resistance vessels as decreased forearm vasodilatation response to ADN and ACH in a young, healthy group of FDR compared with a well-matched group of CON, confirming and expanding previous findings of endothelial dysfunction of the conduit vessels in FDR. In addition, we found compromised insulin-mediated increase in bulk flow as well as diminished whole-body and forearm insulin-stimulated glucose uptake. Furthermore, forearm glucose clearance was significantly reduced in the FDR even during low basal plasma insulin levels. In conclusion, our data suggest that vascular insulin action is affected; however, insulin-mediated capillary recruitment remains to be examined.

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References

[1] Kriketos AD, Greenfield JR, Peake PW, Furler SM, Denyer GS, Charlesworth JA, et al. Inflammation, insulin resistance, and adiposity: a study of first-degree relatives of type 2 diabetic subjects. Diabetes Care 2004;27:2033-40.

[2] Price TB, Perseghin G, Duleba A, Chen W, Chase J, Rothman DL, et al. NMR studies of muscle glycogen synthesis in insulin-resistant offspring of parents with non–insulin-dependent diabetes mellitus immediately after glycogen-depleting exercise. Proc Natl Acad Sci U S A 1996;93:5329-34.

[3] Vaag A, Henriksen JE, Beck-Nielsen H. Decreased insulin activation of glycogen synthase in skeletal muscles in young nonobese Caucasian first-degree relatives of patients with non–insulin-dependent diabetes mellitus. J Clin Invest 1992;89:782-8.

[4] Balletshofer BM, Rittig K, Enderle MD, Volk A, Maerker E, Jacob S, et al. Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation 2000;101:1780-4.

[5] Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type 2 diabetes. Diabetes 1999;48:1856-62.

[6] Goldfine AB, Beckman JA, Betensky RA, Devlin H, Hurley S, Varo N, et al. Family history of diabetes is a major determinant of endothelial function. J Am Coll Cardiol 2006;47:2456-61.

[7] Hirata K, Kadirvelu A, Di TM, Homma S, Choy AM, Lang CC. Coronary vasomotor function is abnormal in first-degree relatives of patients with type 2 diabetes. Diabetes Care 2007;30:150-3.

[8] Scuteri A, Tesauro M, Rizza S, Iantorno M, Federici M, Lauro D, et al. Endothelial function and arterial stiffness in normotensive normoglycemic first-degree relatives of diabetic patients are independent of the metabolic syndrome. Nutr Metab Cardiovasc Dis 2007.

[9] Tesauro M, Rizza S, Iantorno M, Campia U, Cardillo C, Lauro D, et al. Vascular, metabolic, and inflammatory abnormalities in normoglycemic offspring of patients with type 2 diabetes mellitus. Metabolism 2007;56:413-9.

[10] Barac A, Campia U, Panza JA. Methods for evaluating endothelial function in humans. Hypertension 2007;49:748-60.

[11] Higashi Y, Yoshizumi M. New methods to evaluate endothelial function: method for assessing endothelial function in humans using a strain-gauge plethysmography: nitric oxide–dependent and –independent vasodilation. J Pharmacol Sci 2003;93:399-404.

[12] Eskurza I, Seals DR, DeSouza CA, Tanaka H. Pharmacologic versus flow-mediated assessments of peripheral vascular endothelial vasodilatory function in humans. Am J Cardiol 2001;88:1067-9.

[13] Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888-904.

[14] Newman JM, Ross RM, Richards SM, Clark MG, Rattigan S. Insulin and contraction increase nutritive blood flow in rat muscle in vivo determined by microdialysis of L-[14C]glucose. J Physiol 2007;585:217-29.

[15] Rattigan S, Bradley EA, Richards SM, Clark MG. Muscle metabolism and control of capillary blood flow: insulin and exercise. Essays Biochem 2006;42:133-44.

[16] Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, et al. Insulin sensitivity of muscle capillary recruitment in vivo. Diabetes 2004;53:447-53.

[17] Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. J Clin Invest 1994;94:2511-5.

[18] Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. J Clin Invest 1994;94:1172-9.

[19] Taddei S, Virdis A, Mattei P, Natali A, Ferrannini E, Salvetti A. Effect of insulin on acetylcholine-induced vasodilation in normotensive subjects and patients with essential hypertension. Circulation 1995;92:2911-8.

[20] Rask-Madsen C, Ihlemann N, Krarup T, Christiansen E, Kober L, Nervil KC, et al. Insulin therapy improves insulin-stimulated endothelial function in patients with type 2 diabetes and ischemic heart disease. Diabetes 2001;50:2611-8.

[21] Gaenzer H, Neumayr G, Marschang P, Sturm W, Lechleitner M, Foger B, et al. Effect of insulin therapy on endothelium-dependent dilation in type 2 diabetes mellitus. Am J Cardiol 2002;89:431-4.

[22] Vehkavaara S, Makimattila S, Schlenzka A, Vakkilainen J, Westerbacka J, Yki-Jarvinen H. Insulin therapy improves endothelial function in type 2 diabetes. Arterioscler Thromb Vasc Biol 2000;20:545-50.

[23] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.

[24] Rattigan S, Bussey CT, Ross RM, Richards SM. Obesity, insulin resistance, and capillary recruitment. Microcirculation 2007;14:299-309.

[25] Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. Diabetes 2004;53:1418-23.

[26] Iellamo F, Tesauro M, Rizza S, Aquilani S, Cardillo C, Iantorno M, et al. Concomitant impairment in endothelial function and neural cardiovascular regulation in offspring of type 2 diabetic subjects. Hypertension 2006;48:418-23.

[27] Pleiner J, Mittermayer F, Langenberger H, Winzer C, Schaller G, Pacini G, et al. Impaired vascular nitric oxide bioactivity in women with previous gestational diabetes. Wien Klin Wochenschr 2007;119:483-9.

[28] Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes 2006;55:1436-42.

[29] Dimitriadis G, Lambadiari V, Mitrou P, Maratou E, Boutati E, Panagiotakos DB, et al. Impaired postprandial blood flow in adipose tissue may be an early marker of insulin resistance in type 2 diabetes. Diabetes Care 2007;30:3128-30.

[30] Elias-Smale SE, Kardys I, Oudkerk M, Hofman A, Witteman JC. C-reactive protein is related to extent and progression of coronary and extra-coronary atherosclerosis; results from the Rotterdam study. Atherosclerosis 2007;195:e195-e202.

[31] Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. Circulation 2001 3;103:1813-8.

[32] Biegelsen ES, Loscalzo J. Endothelial function and atherosclerosis. Coron Artery Dis 1999;10:241-56.

[33] Ruotsalainen E, Vauhkonen I, Salmenniemi U, Pihlajamaki J, Punnonen K, Kainulainen S, et al. Markers of endothelial dysfunction and low-grade inflammation are associated in the offspring of type 2 diabetic subjects. Atherosclerosis 2008;197:271-7.

[34] Ihlemann N, Stokholm KH, Eskildsen PC. Impaired vascular reactivity is present despite normal levels of von Willebrand factor in patients with uncomplicated type 2 diabetes. Diabet Med 2002;19: 476-81.

[35] Franco MC, Higa EM, D'Almeida V, de Sousa FG, Sawaya AL, Fortes ZB, et al. Homocysteine and nitric oxide are related to blood pressure and vascular function in small-for-gestational-age children. Hypertension 2007;50:396-402.

[36] D'Agostino Sr RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation 2008;117: 743-53.

[37] De FE, Cusi K, Ocampo G, Berria R, Buck S, Consoli A, et al. Exercise-induced improvement in vasodilatory function accompanies increased insulin sensitivity in obesity and type 2 diabetes mellitus. J Clin Endocrinol Metab 2006;91:4903-10.

[38] Karlsson HK, Ahlsen M, Zierath JR, Wallberg-Henriksson H, Koistinen HA. Insulin signaling and glucose transport in skeletal muscle from first-degree relatives of type 2 diabetic patients. Diabetes 2006;55:1283-8.