Diabetologia (1996) 39: 1477–1482

Diabetologia

Dissociation between insulin sensitivity of glucose uptake and endothelial function in normal subjects

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Summary Insulin increases limb blood flow in a time- and dose-dependent manner. This effect can be blocked by inhibiting nitric oxide synthesis. These data raise the possibility that insulin resistance is associated with endothelial dysfunction. To examine whether endothelial function and insulin sensitivity are interrelated we quantitated in vivo insulin-stimulated rates of whole body and forearm glucose uptake at a physiological insulin concentration (euglycaemic hyperinsulinaemic clamp, 1 mU · kg^{-1} · min^{-1} insulin infusion for 2 h) and on another occasion, in vivo endothelial function (blood flow response to intrabrachial infusions of sodium nitroprusside, acetylcholine, and *N*-monomethyl-L-arginine) in 30 normal male subjects. Subjects were divided into an insulin-resistant (IR) and an insulin-sensitive (IS) group based on the median rate of whole body glucose uptake (31 ± 2 vs 48 ± 1 μmol · kg^{-1} · min^{-1}, $p < 0.001$). The IR and IS groups were matched for age, but the IR group had a slightly higher body mass index, percentage of body fat and blood pressure compared to the IS group. The IR group also had diminished insulin-stimulated glucose extraction ($p < 0.05$) compared to the IS group, while basal and insulin-stimulated forearm blood flow rates were identical. There was no difference between the IR and IS groups in the forearm blood flow response to endothelium-dependent (acetylcholine and *N*-monomethyl-L-arginine) or -independent (sodium nitroprusside) vasoactive drugs. In conclusion, the ability of insulin to stimulate glucose uptake at physiological insulin concentrations and endothelium-dependent vasodilatation are distinct phenomena and do not necessarily coexist. [Diabetologia (1996) 39: 1477–1482]

Keywords Blood flow, nitric oxide, vasodilatation, endothelium.

Insulin increases limb blood flow in a dose- and time-dependent manner [1, 2]. This effect of insulin can be blocked by inhibiting nitric oxide synthesis using *N*-monomethyl-L-arginine (L-NMMA) [3, 4]. Epidemiological studies have demonstrated an independent association between hyperinsulinaemia and increased cardiovascular mortality in non-diabetic men [5, 6] as well as in patients with non-insulin-dependent diabetes mellitus (NIDDM) [7]. The mechanisms explaining this association are unclear. Given the knowledge that insulin is an endothelium-dependent vasodilator, it is possible that insulin resistance is associated with endothelial dysfunction. Blunted endothelium-dependent vasodilatation has been consistently found in patients with atherosclerotic vascular disease [8, 9] and in subjects with increased risk of developing atherosclerosis [10, 11]. Hypercholesterolaemic individuals however, exhibit normal sensitivity to insulin [12, 13]. Since hypercholesterolaemia appears to be invariably associated with endothelial dysfunction [14–16], these data raise the possibility that insulin resistance is not necessarily accompanied

Received: 31 May 1996 and in revised form: 10 July 1996

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Abbreviations: NIDDM, Non-insulin-dependent diabetes mellitus; L-NMMA, *N*-monomethyl-L-arginine; SNP, sodium nitroprusside; ACh, acetylcholine; FFM, fat-free mass; IDDM, insulin-dependent diabetes mellitus.

by endothelial dysfunction. Data are conflicting regarding the presence of endothelial dysfunction in patients with essential hypertension [17–19] and NIDDM [20, 21], although resistance to the hypoglycaemic effect of insulin uniformly characterizes conditions such as hypertension [22, 23] and NIDDM [24].

In none of the studies cited above, have in vivo insulin sensitivity and endothelial function been quantitated in the same subjects. In the present study we measured both parameters to examine whether endothelial function and insulin sensitivity are interrelated. For this purpose, we determined, in 30 normal male subjects, insulin-stimulated rates of glucose uptake at the level of the whole body and across forearm tissues and, on another occasion, the forearm blood flow response to endothelium-dependent (acetycholine, ACh; L-NMMA) and -independent (sodium nitroprusside, SNP) vasoactive drugs.

Subjects and methods

Subjects

Thirty normal male subjects volunteered for the studies. In each subject in vivo insulin sensitivity (euglycaemic insulin clamp combined with measurement of forearm glucose uptake) and, on another occasion, in vivo endothelial function (blood flow response of forearm resistance vessels to ACh, SNP and L-NMMA) were measured with at least a 1-week interval between the studies. The subjects were healthy as judged by history and physical examination, and did not use any drugs known to affect glucose metabolism. Each subject had a normal fasting plasma glucose and glycosylated hemoglobin A_{1C} concentration (Table 1). For data analysis, the subjects were ranked according to their rate of whole body glucose uptake, and divided into two subgroups of equal size based on the median rate of whole body glucose uptake (43 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, 7.7 $mg \cdot kg^{-1} \cdot min^{-1}$): an insulin-resistant (IR) and an insulin-sensitive (IS) group (Table 1). There was one smoker in the IR and three smokers in the IS group. For 2 days before the insulin sensitivity measurements, the subjects ingested a weight-maintaining diet containing at least 200 g of carbohydrate per day. Written informed consent was obtained after the purpose, nature and potential risks were explained to the subjects. The experimental protocol was approved by the ethical committee of the Department of Medicine, Helsinki University Central Hospital.

Methods

Whole body glucose uptake. The study was begun at 07.30 hours after an overnight fast. Three 18 gauge catheters (Venflon, Viggo-Spectramed, Helsingborg, Sweden) were inserted as previously described [2]. Insulin and glucose were infused via a catheter inserted in the left antecubital vein. The left hand was kept in a heated box (65 °C) for sampling of arterialized venous blood from a heated dorsal hand vein. The deep branch of the right medial cubital vein draining forearm muscles was cannulated retrogradely so that the tip of the cannula could not be palpated superficially. The euglycaemic hyperinsulinaemic clamp technique was used to assess tissue sensitivity to insulin [25]. Insulin (Actrapid Human; Novo Nordisk, Copenhagen, Denmark) was infused in a primed continuous manner at a rate of 1 $mU \cdot kg^{-1} \cdot min^{-1}$ for 2 h. Normoglycaemia was maintained by adjusting the rate of a 20 % glucose infusion based on plasma glucose measurements performed at 5-min intervals. As hepatic glucose production is completely suppressed in normal subjects at the insulin concentrations employed in the present study [26], whole body glucose uptake was calculated from the glucose infusion rate after correcting for changes in the glucose pool size [25]. The reproducibility of whole body glucose uptake measurements with this technique in our laboratory is approximately 7 % [27].

Table 1. Physical characteristics of the subjects

	Insulin-resistant subjects (n = 15)	Insulin-sensitive subjects (n = 15)
Age (years)	28 ± 2	25 ± 1
Height (cm)	180 ± 1	180 ± 2
Weight (kg)	80.1 ± 3.2	73.2 ± 1.7
Body mass index (kg/m^2)	24.7 ± 0.9	22.5 ± 0.4[a]
Body fat (%)	16.1 ± 1.5	12.2 ± 1.0[a]
Fat free mass (kg)	66.6 ± 1.7	64.1 ± 1.1
Systolic blood pressure (mm Hg)	130 ± 4	114 ± 3[b]
Diastolic blood pressure (mm Hg)	75 ± 3	63 ± 2[b]
Mean arterial blood pressure (mm Hg)	94 ± 3	80 ± 2[b]
Fasting plasma glucose (mmol/l)	5.1 ± 0.1	5.1 ± 0.1
Fasting serum insulin (pmol/l)	39 ± 4	27 ± 4[a]
HbA_{1c} (%)	4.9 ± 0.1	5.1 ± 0.1
Serum cholesterol (mmol/l)	4.3 ± 0.3	4.1 ± 0.2
Serum HDL cholesterol (mmol/l)	1.1 ± 0.1	1.1 ± 0.1
Serum LDL cholesterol (mmol/l)	2.8 ± 0.2	2.5 ± 0.2
Serum triglycerides (mmol/l)	1.0 ± 0.1	0.7 ± 0.1[b]

Data are mean ± SEM
[a] $p < 0.05$, [b] $p < 0.01$

Forearm glucose uptake. Forearm glucose uptake was calculated by multiplying the glucose arteriovenous difference by forearm blood flow [28]. Plasma glucose concentrations were converted to whole blood values by multiplying the plasma glucose concentrations by 1-(0,30 × hematocrit). Forearm blood flow was measured by venous occlusion plethysmography using a mercury in silastic rubber strain-gauge apparatus (Hokanson Plethysmograph Model EC4; Bellevue, Wash., USA) [29]. Circulation to the hand was interrupted 2 min before blood sampling and flow measurements, by inflating a paediatric blood pressure cuff around the wrist above systolic blood pressure. Venous return was occluded by inflating a sphygmomanometer cuff around the upper arm to 50 mmHg using a rapid cuff inflator (Hokanson Rapid Cuff Inflator Model E20, Hokanson). The reproducibility of the measurement of the blood flow using this technique is approximately 13 % [2].

Endothelial function test. The study was performed after a 12-h fast. A 27 gauge needle (Cooper's Needle Works, Birmingham, UK) was inserted into the left brachial artery. The needle was kept patent by a 1 ml/min infusion of 154 mmol/1 NaCl. After 18 min, drugs were infused intrabrachially in the following sequence: SNP (Nipride; Roche, Basel, Switzerland) 3 and 10 μg/min, 6 min per dose, 154 mmol/1 NaCl 18 min, ACh chloride (Miochol; Iolab Corp., Claremont, Calif., USA) 7.5 and 15 μg/min, 6 min per dose, 154 mmol/1 NaCl 18 min, L-NMMA (Clinalfa Ag, Läufelfingen, Switzerland) 4 μmol/min for 6 min. The intra-arterial infusion rate was held constant (1 ml/min) throughout the study. Forearm blood flow was measured at 15-s intervals during the last 3 min of each drug and

saline infusion period. Blood flow was recorded in both the infused (experimental) and non-infused (control) forearms simultaneously using strain-gauge plethysmography as described above. An analogue-to-digital converter (MacLab/4e; AD Instruments Pty Ltd, Castle Hill, Australia) connected to a personal computer was used for recording and analysis of the blood flow data during endothelial function tests.

Other measurements. Blood pressure was measured using a mercury sphygmomanometer. Fat free mass (FFM) and the percentage of body fat were determined using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A; RJL Systems, Detroit, Mich., USA) [30]. The plasma glucose concentration was measured in duplicate with the glucose oxidase method [31] using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, Calif., USA). Serum free insulin was determined by double antibody radioimmunoassay (Pharmacia Insulin RIA kit; Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol [32]. The serum concentrations of cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol were determined by enzymatic colourimetric assays with an automated Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) [33]. Low-density lipoprotein (LDL) cholesterol concentration was calculated by the formula of Friedewald.

Statistical analysis. Student's unpaired *t*-test was used to compare mean values of the IR and IS groups. Comparison of blood flow responses to vasoactive drugs during the endothelial function test were performed by repeated measures analysis of variance followed by pairwise comparison using the Bonferroni test. All calculations were made using the SYSTAT statistical package (SYSTAT Inc., Evanston, Ill., USA). Data are expressed as mean ± SEM. *P*-values less than 0.05 were considered to be statistically significant.

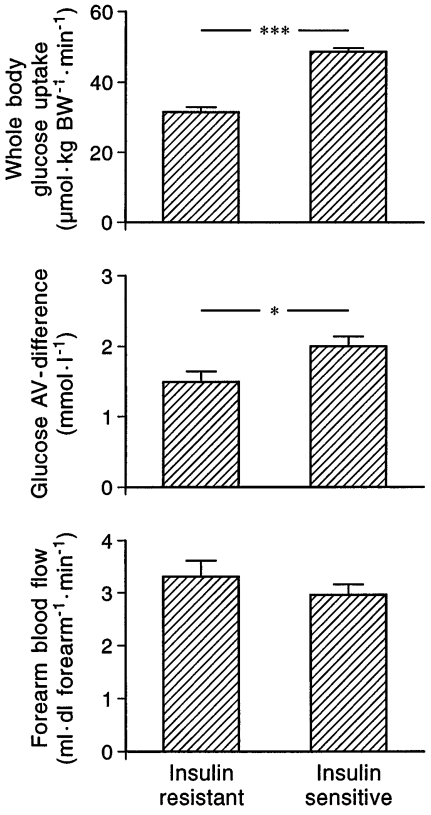

Fig. 1. Whole body glucose uptake (upper panel) calculated per kg body weight (BW), glucose arteriovenous difference (middle panel) and insulin-stimulated blood flow (lower panel) across the forearm in insulin-resistant and insulin-sensitive subjects. * $p < 0.05$; *** $p < 0.001$

Results

Characteristics of the insulin-resistant (IR) and insulin-sensitive (IS) groups (Table 1). The IR and IS groups were matched for age, but the IR group had slightly higher body mass index (BMI), percentage of body fat, arterial blood pressure and serum triglyceride concentration. Serum total and LDL-cholesterol concentrations were comparable between groups.

Insulin sensitivity. During the insulin infusion, normoglycaemia was maintained in both the IR (5.2 ± 0.1 mmol/l) and IS groups (5.2 ± 0.1 mmol/l). Serum free insulin concentrations averaged 381 ± 12 pmol/l and 365 ± 14 pmol/l (NS), respectively. Whole body glucose uptake, expressed per kg body weight (BW) was, by definition, 36 % lower in the IR (31 ± 2 μmol · kg $BW^{-1} \cdot min^{-1}$) than the IS (48 ± 1 μmol · kg BW^{-1} min^{-1}, $p < 0.001$) group. The rate of glucose uptake, expressed per kg FFM, was also significantly lower in the IR (37 ± 3 μmol · kg $FFM^{-1} \cdot min^{-1}$) than the IS (55 ± 1 μmol · kg $FFM^{-1} \cdot min^{-1}$, $p < 0.001$) group (Fig. 1).

Forearm glucose uptake was significantly lower in the IR (3.9 ± 0.4 μmol · dl $forearm^{-1} \cdot min^{-1}$) than the IS (5.0 ± 0.4 μmol · dl $forearm^{-1} \cdot min^{-1}$, $p < 0.05$) group. The defect in forearm glucose uptake was localized to glucose extraction, since the glucose arterio-venous difference was significantly lower in the IR (1.5 ± 0.1 mmol/l) than the IS (2.0 ± 0.1 mmol/l, $p < 0.05$) group (Fig. 1). Insulin-stimulated forearm blood flow was similar in both groups (3.3 ± 0.3 vs 2.9 ± 0.2 ml · dl $forearm^{-1} \cdot min^{-1}$, IR vs IS, NS) (Fig. 1). The increases in blood flow above basal (0.4 ± 0.2 vs 0.4 ± 0.2 ml · dl $forearm^{-1} \cdot min^{-1}$, respectively) were also comparable between the groups. The increase in blood flow was statistically significant in both the IR ($p < 0.05$) and IS groups ($p < 0.05$).

Endothelial function. The blood flow responses in the experimental forearm to SNP, ACh and L-NMMA are shown in Figure 2. There were no differences in the blood flow responses to any of the drugs between the IR and IS groups. This remained true even if the ratio of blood flow between the experimental and control forearm, or forearm vascular resistance were used in data analysis (data not shown). Neither were the percentage increases in forearm blood flow in response to two doses of SNP (279 ± 31 %, 464 ± 45 % vs 285 ± 23 %, 524 ± 49 %, SNP 3, 10 μg/min in IR vs IS, NS) or ACh (200 ± 32 %, 280 ± 40 % vs 230 ± 32 %, 323 ± 49 %, ACh 7.5, 15 μg/min in IR

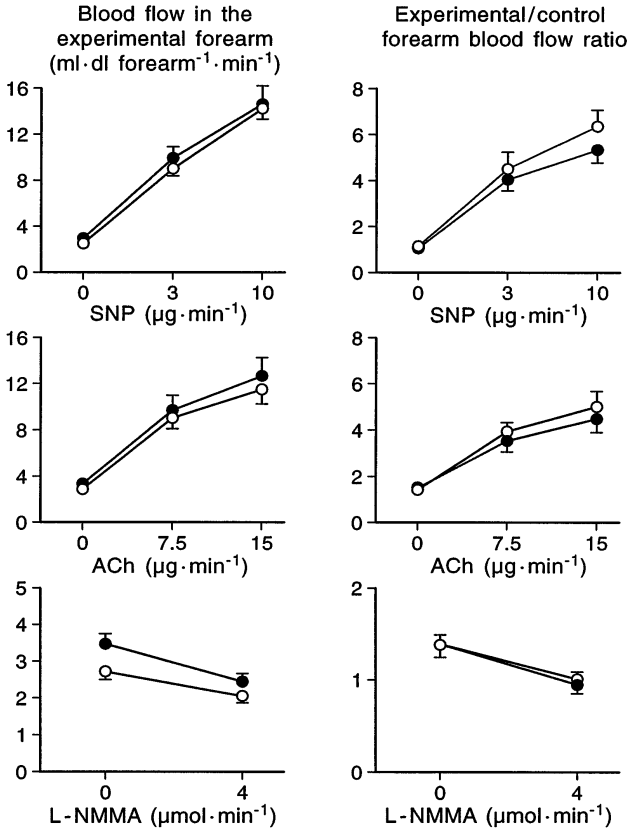

Fig. 2. The response of the experimental forearm blood flow (left panels) and the experimental/control forearm blood flow ratio (right panels) to increasing doses of SNP (upper panels), ACh (middle panels) and L-NMMA (lower panels) in the insulin-resistant (—●—) and insulin sensitive (—○—) groups

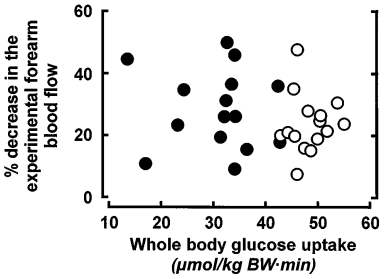

Fig. 3. A scatter plot showing the lack of association between whole body glucose uptake and the percent decrease in forearm blood flow in response to intrabrachial infusion of L-NMMA in insulin-resistant (●) and insulin-sensitive (○) subjects

vs IS, NS) or the percentage decrease in blood flow by L-NMMA (28 ± 3 % vs 24 ± 2 %, NS) (Fig. 3) statistically different between the groups. Responses to ACh and to SNP are known to be affected by forearm length [34]. However, forearm lengths (measured from medial epicondyle to ulnar styloid) were similar in both groups (28.2 ± 0.4 vs 27.8 ± 0.4 cm, NS).

In the entire study group, mean arterial blood pressure ($r = -0.53$, $p < 0.01$), BMI ($r = -0.43$, $p < 0.02$) and percentage body fat ($r = -0.43$, $p < 0.02$) were negatively correlated with whole body glucose uptake. In contrast, these parameters were not correlated with the blood flow response to either dose of ACh or SNP, or with the percentage decrease in flow induced by L-NMMA (Fig. 3).

Discussion

In the present study, insulin sensitivity of glucose uptake and the blood flow response of forearm resistance vessels to endothelium-dependent and -independent vasoactive agents were measured in a group of normal male subjects. The subjects were divided into two subgroups of equal size based on their insulin-stimulated rate of whole body glucose uptake. The insulin-resistant subgroup had, by definition, significantly lower whole body glucose uptake than the sensitive subgroup. This resistance could be attributed to a defect in glucose extraction across forearm tissues. Forearm blood flow was, however, similar both during infusion of insulin, and during infusion of endothelium-dependent (ACh, L-NMMA) and -independent (SNP) vasoactive agents. These data suggest that endothelial function and insulin stimulation of glucose uptake at physiological insulin concentrations are differently regulated phenomena.

We classified the subjects as "insulin-resistant" and "insulin-sensitive" based on the measured rate of whole body glucose uptake. The subjects classified as insulin-resistant had a mean rate of whole body glucose uptake of 31 μmol · kg^{-1} · min^{-1} (5.6 mg · kg^{-1} · min^{-1}). This rate is clearly higher than the rates which characterize typical NIDDM patients [24], but lower than the rates characterizing average Finnish healthy subjects of the same age and BMI [35]. It may be noted that no prospective data regarding the effect of insulin sensitivity measured with the clamp technique as a predictor of NIDDM or cardiovascular mortality are available in Caucasians. It is therefore unclear, whether the degree of insulin resistance characterizing the "insulin-resistant" subjects in the present study is associated with a significant risk for the subject's subsequent health.

The ability of insulin to stimulate muscle blood flow depends both on the duration and dose of insulin [1, 2, 36, 37]. At physiological insulin concentrations, such as those employed in the present study, insulin increases glucose extraction maximally within 30–60 min [2]. In contrast, the effects on limb blood flow are controversial, some studies showing no or marginal increases [2, 36, 38–40], while others have found significant increases (20–60 %) [3, 41, 42] during this time period. During prolonged [1, 37], or high-dose [1, 2, 43] insulin infusions, blood flow increases markedly up to 80–110 % above basal. Under these conditions, glucose extraction remains constant [1, 2]. Consequently, one would expect

defects in glucose extraction to predominate when insulin-resistant and sensitive individuals are compared using physiological insulin concentrations, and defects in blood flow to distinguish between the groups in studies using supraphysiological insulin concentrations. In keeping with this postulate, defects in glucose extraction have explained insulin resistance in patients with essential hypertension [44], insulin-dependent diabetes (IDDM) [45] and NIDDM [24, 46] at physiological insulin concentrations. At high insulin concentrations, defects in blood flow have been found in subjects with marginally elevated blood pressure [43], and in patients with IDDM [47] and NIDDM [48], although these findings have not been uniform in either hypertensive subjects [49, 50] or in patients with NIDDM [51, 52]. In keeping with these data, the defect in glucose uptake between our insulin-resistant and sensitive individuals, in whom insulin sensitivity was measured using a physiological insulin concentration, was localized to glucose extraction.

The mechanism by which insulin and ACh induce endothelium-dependent vasodilatation differs in several respects. In vivo insulin-induced vasodilatation can be blocked by L-NMMA, an inhibitor of endothelial cell nitric oxide synthase [3, 4] and by blocking corticotropin-releasing hormone-release by dexamethasone [53]. However, the precise mechanism coupling nitric oxide to insulin-induced vasodilatation is presently unclear. Compared to ACh, the vasodilatory effect of insulin is slow. While ACh increases blood flow several-fold within minutes in normal subjects [54], doubling of blood flow with high insulin concentrations takes hours [2]. In support of differences in the mechanism of insulin vs ACh induced vasodilatation, Taddei et al. [55] observed blunted endothelium-dependent responses in hypertensive as compared to normotensive subjects, but normal potentiation of ACh-induced vasodilatation by insulin. After submission of the present manuscript, Petrie et al. [56] reported that basal endothelial nitric oxide synthesis, measured as the percent decrease in the forearm blood flow ratio during infusion of L-NMMA, was inversely correlated with whole body insulin sensitivity in 15 normotensive males (age 27 years, BMI 24.6 kg/m^2, blood pressure 130/69 mmHg). We could not, however, confirm this result in the group of 30 normotensive males (age 27 years, BMI 23.6 kg/m^2, blood pressure 122/69 mmHg) in the present study. We therefore conclude that endothelial function and insulin sensitivity of glucose uptake under physiological conditions do not necessarily change in parallel and are therefore likely to be differently regulated phenomena.

Acknowledgements. We thank Ms. S. Hämäläinen, R. T., for excellent technical assistance during the studies. The Academy of Finland (H. Y.), the Ahokas (H. Y.) and Sigrid Juselius (H. Y.) Foundations, and the M. D./Ph. D. program of the University of Helsinki (T. U.) are gratefully acknowledged for financial support.

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