In Vivo Endothelial Dysfunction Characterizes Patients With Impaired Fasting Glucose

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OBJECTIVE — The American Diabetes Association has recently defined a new category of abnormal glucose homeostasis called "impaired fasting glucose" (IFG), where glucose levels do not meet the criteria of diabetes but are too high to be considered normal. We determined whether endothelial dysfunction is a characteristic of subjects with IFG.

RESEARCH DESIGN AND METHODS — In vivo vasodilatory responses to intra-arterial infusions of endothelium-dependent (acetylcholine [ACh]) and -independent (sodium nitroprusside [SNP]) vasoactive agents were determined in 17 IFG subjects (age 63 ± 1 years, BMI 26.5 ± 0.8 kg/m^2, serum LDL cholesterol 3.5 ± 0.2 mmol/l) with fasting plasma glucose levels of 117 ± 1 mg/dl and in 12 subjects with normal fasting plasma glucose concentrations.

RESULTS — The blood-flow response to the low dose of ACh was 46% (5.9 ± 0.7 vs. 10.9 ± 1.3 ml · dl^{-1} · min^{-1}, IFG vs. normal, $P < 0.01$) and to the high dose was 31% (9.1 ± 1.2 vs. 13.2 ± 1.5 ml · dl^{-1} · min^{-1}, $P < 0.05$, respectively) lower in the IFG than in the normal subjects. In contrast, blood-flow responses to both low (7.8 ± 0.5 vs. 9.0 ± 0.9 ml · dl^{-1} · min^{-1}, IFG vs. normal, NS) and high (11.6 ± 1.2 vs. 12.3 ± 1.3 ml · dl^{-1} · min^{-1}, NS, respectively) doses of SNP were comparable. The ratio of endothelium-dependent to -independent blood flow was 40% lower in the IFG (0.75 ± 0.1) than in the normal (1.24 ± 0.1, $P < 0.001$) subjects. Both fasting plasma glucose ($r = -0.48$, $P < 0.01$) and glycosylated hemoglobin ($r = -0.42$, $P < 0.05$) were inversely correlated with endothelium-dependent vasodilatation but not with other parameters, such as weight, blood pressure, or lipids.

CONCLUSIONS — We conclude that vascular dysfunction is associated with abnormal, although nondiabetic, glucose homeostasis.

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Approximately 20% of patients with type 2 diabetes already have some evidence of coronary heart disease at the time of diagnosis (1). Epidemiological data, such as those generated in the U.K. Prospective Diabetes Study (UKPDS), have shown that the risk of cardiovascular disease increases linearly with increasing glycemia (2). Intima media thickness (IMT) is increased in individuals with impaired glucose tolerance (IGT) (3) and correlates positively with fasting plasma glucose concentrations (4). These results suggest that even relatively small increases in blood glucose concentrations can increase the risk of cardiovascular disease. On the other hand, the UKPDS did not convincingly demonstrate that an intensive glucose-control treatment policy significantly reduces the frequency of myocardial infarction in all treatment groups (5). These data raise the possibility that abnormal vascular function is an inherent feature of type 2 diabetes and not simply a consequence of chronic hyperglycemia.

Endothelial dysfunction, measured as the vasodilatory response of forearm resistance vessels to endothelium-dependent vasoactive agents such as acetylcholine (ACh), characterizes patients with atherosclerotic vascular disease (6,7). In patients with established type 2 diabetes, a defect in the vasodilatory response to ACh has been a consistent finding (8–13). Responses to sodium nitroprusside (SNP) have been found to be impaired in some (8,11,13) but not all (9,14) studies.

Recently, the American Diabetes Association proposed new diagnostic criteria for diabetes (15). It recognizes an intermediate category called "impaired fasting glucose" (IFG), in which fasting glucose concentrations do not meet the criteria for diabetes but are nevertheless too high to be considered normal. In subjects with IFG, fasting plasma glucose concentrations range between 110 and 126 mg/dl. It is unknown whether endothelial dysfunction characterizes such individuals. For this purpose, we studied a group of individuals found to have IFG concentrations and a group of subjects with normal fasting glucose concentrations. Endothelial function was determined by measuring forearm blood-flow responses to intra-arterial infusions of endothelium-dependent (ACh) and -independent (SNP) vasodilators.

RESEARCH DESIGN AND METHODS

Study design

A total of 29 normal men were studied (Table 1). All men were retired (retirement age: 45 years) airline pilots who attended an annual health exam. We

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Abbreviations: AAPH, 2,2′-diazobis-(2-amidinopropane) dihydrochloride; ACh, acetylcholine; ANOVA, analysis of variance; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; FFA, free fatty acid; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IMT, intima media thickness; NO, nitric oxide; SH, sulfhydryl; SNP, sodium nitroprusside; UKPDS, U.K. Prospective Diabetes Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Physical and biochemical characteristics of the study groups*

	IFG	Normal
n	17	12
Fasting plasma glucose (mg/dl)	117 ± 1*	101 ± 1
HbA_{1c} (%)	5.8 ± 0.1	5.7 ± 0.1
Fasting serum insulin (mU/l)	9 ± 2	7 ± 1
Age (years)	63 ± 1	60 ± 1
BMI (kg/m^2)	26.5 ± 0.8	26.2 ± 0.6
Mean arterial pressure (mmHg)	101 ± 3	108 ± 2
Family history (positive/negative)	7/17†	1/12
Smokers (current/ex/never)	2/6/9	1/3/8
Serum total cholesterol (mmol/l)	5.4 ± 0.3	5.9 ± 0.2
Serum LDL cholesterol (mmol/l)	3.5 ± 0.2	3.8 ± 0.2
Serum HDL cholesterol (mmol/l)	1.4 ± 0.1	1.7 ± 0.1
Serum triglycerides (mmol/l)	1.2 ± 0.1	1.1 ± 0.1
Serum FFA (μmol/l)	944 ± 45	852 ± 38
Plasma TRAP (μmol/l)	923 ± 30	829 ± 40
Serum uric acid (μmol/l)	374 ± 15	349 ± 24
Plasma SH groups (μmol/l)	271 ± 5	273 ± 10
Plasma ascorbate (μmol/l)	57 ± 3	57 ± 4

Data are means ± SEM. For family history, positive includes diabetes in first-degree relatives. *$P < 0.001$ and †$P < 0.05$ for IFG vs. normal.

wished to perform an endothelial function test in all men who had IFG but were otherwise healthy (15) as judged by history and physical examination, a normal resting electrocardiogram, and normal results of standard laboratory tests. The latter included measurement of blood counts, serum creatinine and electrolyte concentrations, and the urinary albumin excretion rate. The subjects were not taking any medications. Twelve retired pilots also attending the annual health exam with normal fasting glucose concentrations were recruited as a control group. Four pilots in IFG and five in the control group were hypertensive (systolic blood pressure ≥160 mmHg or diastolic ≥90 mmHg) at the time of the endothelial function test. Ambulatory blood pressure measurements of these men did not, however, confirm the diagnosis of essential hypertension. None of the pilots were engaged in competitive sports. Habitual physical activity (light to moderate activity) habits were comparable between the groups and averaged 2.5 ± 0.5 and 2.0 ± 0.5 h/week in the control and IFG groups, respectively (NS). All patients gave their written informed consent before participation. The experimental protocol was designed and performed according to the principles of the Helsinki Declaration and was approved by the Ethical Committee of the Helsinki University Central Hospital.

In vivo endothelial function test

In vivo endothelial function was determined by measuring forearm blood flow responses to intra-arterial infusions of endothelium-dependent (ACh) and -independent (SNP) vasodilators. The study began after a 10–12 h fast at 7:30 A.M. Venous blood samples were withdrawn for measurement of plasma glucose and serum free insulin, HbA_{1c}, and triglyceride, HDL, and total cholesterol concentrations. A 27-gauge unmounted steel cannula (Coopers Needle Works, Birmingham, U.K.), connected to an epidural catheter (Portex, Hythe, Kent, U.K.), was inserted into the left brachial artery. Drugs were infused at a constant rate of 1 ml/min with infusion pumps (Braun, Mesungen, Germany). Subjects rested supine in a quiet environment for 30 min after needle placement before blood-flow measurements. Normal saline was first infused for 18 min. Drugs were then infused in the following sequence: SNP (Roche, Basel, Switzerland), 3 (low dose) and 10 (high dose) μg/min; ACh (Iolab, Claremont, CA), 7.5 (low dose) and 15 (high dose) μg/min. Each dose was infused for 6 min, and the infusion of each drug was separated by infusion of normal saline for 18 min, during which blood flow returned to basal values. Forearm blood flow was recorded for 10 s at 15-s intervals during the last 3 min of each drug and saline infusion period. It was recorded using mercury-in-rubber strain-gauge venous occlusion plethysmography (EC 4 Strain Gauge Plethysmograph, Hokanson, Bellevue, WA), which was connected to a rapid cuff inflator (E 20, Hokanson), an analog-to-digital converter (McLab/4e, AD Instruments, Castle Hill, Australia), and a personal computer, as previously described (16). Before the blood flow measurements, circulation to the hand was interrupted by inflating a pediatric blood pressure cuff around the wrist at 100 mmHg above systolic blood pressure. Blood flow measurements were performed simultaneously in the infused (experimental) and control arm. Means of the final five measurements of each recording period were used for analysis.

Measurement of plasma TRAP and water-soluble antioxidants

Total radical trapping capacity of plasma (TRAP) was determined spectrophotometrically using a recently validated method (17). This assay uses 2′,7′-dichlorofluorescein diacetate (DCFH-DA) to follow formation of free radicals during decomposition of 2,2′-diazobis-(2-amidinopropane) dihydrochloride (AAPH). The DCF formation was monitored at 504 nm using a Multiskan spectrophotometer (Labsystems, Helsinki, Finland). Plasma was mixed with phosphate-buffered saline to a final dilution of 1%, and DCFH-DA was added (final concentration 14 μmol/l). The reaction was started by adding AAPH (final concentration 56 mmol/l). Plasma ascorbic acid concentrations were measured using the spectrophotometric method of Denson and Bowers (18). Plasma protein-bound thiols (sulfhydryl [SH] groups) were determined as described by Ellman (19). Plasma uric acid concentrations were measured by an enzymatic colorimetric assay (Roche Unimate 5 UA, Roche).

Other measurements

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method (20) using the Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Glycosylated hemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography using a fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA). Serum free insulin concentrations were determined by double antibody radioimmunoassay (Pharmacia Insulin RIA kit; Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol (21). Serum free fatty acid (FFA) (22), serum total cholesterol

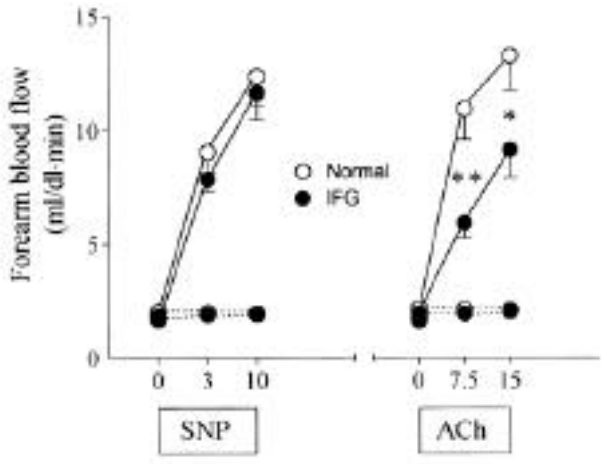

Figure 1—*Forearm blood-flow responses to intra-arterial SNP and ACh infusions in the IFG and the normal groups in the experimental (——) and control (– – –) arms.* *P < *0.05 and* **P < *0.01 for IFG vs. normal group.*

and triglyceride (23), and HDL cholesterol (24) concentrations were measured as previously described. Urinary albumin excretion was measured using Micral-Test II strips (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis

Group, dose, and group times dose effects of the vasoactive drugs on forearm blood flow were analyzed by repeated measures analysis of variance (ANOVA) as described by Ludbrook et al. (25). Vertical pairwise contrasts were performed using the unpaired Student's *t* test followed by the Bonferroni correction (25). Correlation analyses were performed using Spearman's nonparametric correlation coefficient. The calculations were made using the Systat statistical package (Systat version 5.0, Evanston, IL). All *P* values are two-tailed. A *P* value of <0.05 was considered statistically significant. Data are expressed as means ± SEM.

RESULTS — The normal and IFG groups were comparable with respect to physical and biochemical parameters other than fasting plasma glucose concentrations (Table 1). The coefficient of variation between the two plasma glucose measurements performed at the time of the health exam and before the endothelial function test was 5 ± 1%. Also, first-degree relatives of IFG subjects had a greater clustering of type 2 diabetes than did relatives of subjects with normal fasting glucose concentrations (Table 1). None of the subjects had microalbuminuria.

Basal blood flows in the experimental arm were similar in the IFG and normal groups (Fig. 1). In ANOVA for repeated measures, group ($P = 0.01$), dose ($P < 0.001$), and dose times group ($P = 0.012$) effects for the blood-flow response to ACh were significant. For SNP, neither group nor dose times group effects were significant. Comparison of blood-flow responses during the different drug doses revealed that the blood-flow responses to the low (5.9 ± 0.7 vs. 10.9 ± 1.3 ml · dl^{-1} · min^{-1}, IFG vs. normal group, $P < 0.01$) and high (9.1 ± 1.2 vs. 13.2 ± 1.5 ml · dl^{-1} · min^{-1}, $P < 0.05$) doses of ACh were significantly and by 46 and 31% lower in the IFG than the normal group, respectively (Fig. 1). The percent increases in ACh-stimulated blood flows above basal were also significantly impaired in the IFG subjects (Fig. 2). The ratio of endothelium-dependent to -independent blood flow during the low-dose drug infusions (ACh, 7.5 µg/min; SNP, 3 µg/min) was 40% lower in the IFG (0.75 ± 0.1) than in the normal (1.24 ± 0.1, $P < 0.01$) group. The ratio of endothelium-dependent to -independent blood flow during the high-dose drug infusions (ACh, 15 µg/min; SNP, 10 µg/min) was 30% lower in the IFG (0.79 ± 0.1) than in the normal (1.10 ± 0.1) group ($P < 0.05$).

Interrelationships between various physical and biochemical characteristics and blood-flow responses to the vasoactive agents are shown in Table 2. Fasting plasma glucose was significantly inversely correlated with blood flow during the low-dose ACh infusion ($r = -0.48$, $P < 0.01$) and to the ratio of endothelium-dependent to -independent blood flow during both the low-dose ($r = -0.53$, $P < 0.01$, Fig. 3) and high-dose ($r = -0.40$, $P < 0.05$) infusions. The former correlation remained significant after adjustment for age ($P < 0.05$). The concentration of glycosylated hemoglobin was also significantly inversely correlated with blood flow during both the low-dose (Table 2) and the high-dose ($r = -0.34$, $P < 0.05$) infusions of ACh. Regarding antioxidant concentrations, concentrations of TRAP, uric acid, SH groups, and ascorbate were comparable between the groups. Serum uric acid was significantly correlated with TRAP in the entire group ($r = 0.66$, $P < 0.001$).

CONCLUSIONS — In the present study, in vivo endothelial function was determined in a group of retired pilots whose fasting plasma glucose concentration fell in the category of IFG (110–126

Table 2—*Interrelationships (Spearman's nonparametric correlation coefficient) between forearm blood flow during intrabrachial infusions of endothelium-dependent (ACh) and -independent (SNP) vasodilatators with other variables in all subjects*

Variable	ACh 7.5 (µg/min)	SNP 3 (µg/min)	ACh 7.5/SNP 3
Age	−0.39*	−0.03	−0.45*
Weight	−0.09	−0.24	0.08
BMI	0.03	−0.06	0.11
Mean arterial pressure	0.11	−0.13	0.24
Fasting plasma glucose	−0.48†	−0.11	−0.53†
HbA_{1c}	−0.42*	−0.33	−0.26
Fasting serum insulin	0.22	−0.07	0.30
Serum triglycerides	0.02	0.12	0.01
Serum LDL cholesterol	0.11	0.07	0.02

*$P < 0.05$; †$P < 0.01$.

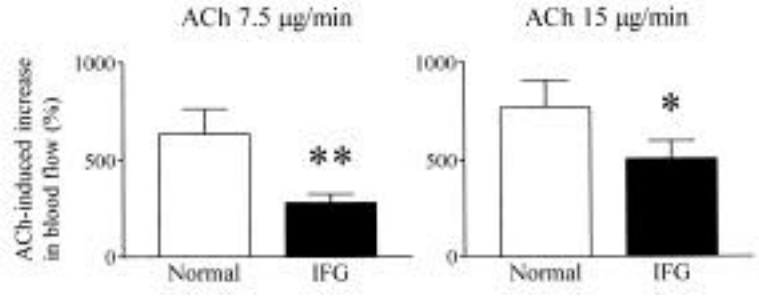

Figure 2—*ACh-induced increases in forearm blood flow during low-dose (ACh 7.5 µg/min) and high-dose (ACh 15 µg/min) infusions. *P < 0.05 and **P < 0.01 for IFG vs. normal group.*

mg/dl) and in normoglycemic pilots attending the same health examination. This rather homogenous group of elderly men was chosen because their family and medical histories had been well documented during annual surveys. Mild fasting hyperglycemia was associated with a highly significant impairment in the blood-flow response to endothelium-dependent but not -independent vasodilators.

We assessed endothelial function by measuring the vasodilator responses of forearm resistance vessels to ACh and SNP. Although clinically significant atherosclerotic changes do not develop in the brachial artery, a recent autopsy study showed that early atherosclerotic endothelial lesions occur commonly in this artery (26). The extent of lesions in the brachial artery correlated significantly with those in the carotid and coronary arteries (26). This suggests that peripheral arteries may be useful for studies predicting an individual's predisposition to atherosclerosis. Also, impaired endothelium-dependent vasodilatation of forearm resistance vessels has been shown to correlate with impaired endothelium-dependent vasodilatation in coronary arteries in several studies (6,7).

Regarding the mechanisms underlying endothelial dysfunction in subjects with IFG, acute hyperglycemia appears an unlikely candidate. First, data on effects of acute hyperglycemia have been inconsistent. Houben et al. (27,28) found no effects of local hyperglycemia (15 mmol/l for 24 h) induced by a 5% glucose infusion on either endothelium-dependent or -independent vasodilatation, whereas Williams et al. (29) found 6 h of local hyperglycemia (17 mmol/l) to decrease methacholine-induced vasodilatation. Second, the IFG group had on the average only 16 mg/dl (0.9 mmol/l) higher glucose concentrations than the normal group. This increase is trivial compared with that (17 mmol/l) reported to acutely induce endothelial dysfunction in humans (29). Regarding chronic hyperglycemia, in the Rancho Bernardo Study (30), an increase of fasting plasma glucose from 5 to 7 mmol/l was associated with a doubling of coronary heart disease mortality in men and a tripling in women. In two additional studies in nondiabetic subjects, carotid IMT has been shown to increase in parallel with fasting glucose (4) and with HbA_{1c} (31) concentrations. These data are consistent with the idea that even small chronic increases in fasting glucose may either predispose to vascular disease or serve as a marker of cardiovascular risk. In the UKPDS, fasting glucose was maintained ~36 mg/dl (2 mmol/l) lower in the intensively than conventionally treated patients for 10 years. This difference was not associated with a significant reduction in the incidence of myocardial infarction or other signs of macrovascular disease. These data do not, however, exclude the possibility that mild hyperglycemia has adverse effects on early vascular alterations such as impaired endothelium-dependent vasodilatation, which is thought to precede clinical manifestations of atherosclerosis.

Several studies have documented vasodilatation mediated by nitric oxide (NO) to be impaired in both patients with type 1 and those with type 2 diabetes (8,10–13,16,32). In human aortic endothelial cells, high glucose increases production of free radicals such as superoxide anions (33). The latter could inactivate NO in the absence of adequate antioxidant defense mechanisms. In human plasma, vitamin C and protein thiols (SH groups) serve as first-line defense mechanisms against increased oxidative stress (34). In the present study, neither the total peroxyl-trapping capacity of plasma nor the concentrations of ascorbate and SH-thiols were different between the IFG and the normal groups. This finding of unaltered antioxidant capacity, especially in plasma, which is exposed to hyperglycemia, argues against the possibility that hyperglycemia caused impaired endothelium-dependent vasodilatation via a mechanism involving increased oxidative stress. Of course, normal antioxidant capacity of plasma does not exclude the possibility of hyperglycemia-induced changes in intracellular production of free radicals (35). Regarding other possible causes of endothelial dysfunction, elevated circulating FFA levels impair endothelium-dependent vasodilatation (36), but in the present study the FFA concentrations were unaltered in the IFG as compared with the normal subjects. Whether individual differences in the profile of FFAs could have contributed to vari-

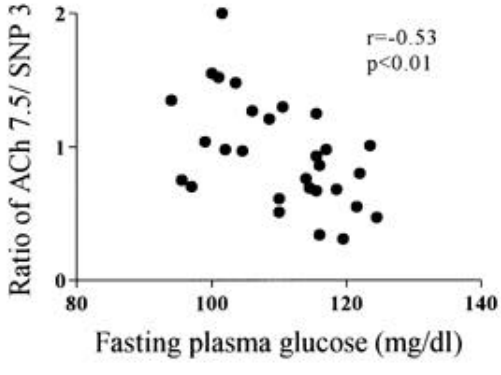

Figure 3—*The relationship (Spearman's nonparametric correlation coefficient) between the fasting plasma glucose concentration and the ratio of endothelium-dependent to -independent flow during infusion of low doses of ACh and SNP. ACh 7.5, infusion of ACh at a rate of 7.5 µg/min; SNP 3, infusion of SNP at a rate of 3.0 µg/min.*

ation in endothelial function (37,38) remains unclear because the total concentration of FFAs was determined.

It has recently been proposed that endothelial dysfunction could be a common antecedent of the insulin resistance syndrome or a closely associated feature of the syndrome (39). Anastasiou et al. (40) found flow-mediated but not nitrate-mediated vasodilatation to be impaired in women with previous gestational diabetes. This abnormality could not be attributed to body weight, fat distribution, or insulin resistance (40). The patients with former gestational diabetes had, however, significantly higher glucose (but not insulin) concentrations after an oral glucose load at 30, 60, and 90 min. In subjects with fasting hyperglycemia of similar magnitude as in the IFG group in the present study, the microvascular hyperemic response to local heating of the skin of the foot has been found to be blunted compared with subjects with normal fasting glucose concentrations (41). Finally, disturbed flow-mediated brachial artery dilatation characterizes glucose-tolerant first-degree relatives of patients with type 2 diabetes (42). The subjects in the present study did not seem to have features of the insulin resistance syndrome. Whether they represent individuals with endotheliopathy before the development of insulin resistance, as suggested by Tooke et al. (39), or were predisposed to cardiovascular disease for other reasons, is unknown. The stronger family history of diabetes (Table 1) in the IFG than in the normal group is consistent with both possibilities.

In conclusion, the present study adds to the growing evidence supporting the idea that even a mild derangement in glucose metabolism may be associated with, or serve as a marker of, endothelial dysfunction and an increased risk for atherosclerosis (43). The finding of clear endothelial dysfunction in vivo in individuals with IFG also supports use of fasting glucose measurements in the identification of individuals who should be targets for intensified lifestyle and possibly pharmaceutical interventions to prevent cardiovascular disease.

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