Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 77–86
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Nontraditional atherosclerotic risk factors and extent of coronary atherosclerosis in patients with combined impaired fasting glucose and impaired glucose tolerance

Andrzej Surdacki[c,*], Ewa Stochmal[a], Magdalena Szurkowska[a], Stefanie M. Bode-Böger[d], Jens Martens-Lobenhoffer[d], Anna Stochmal[b], Artur Klecha[b], Kalina Kawecka-Jaszcz[b], Jacek S. Dubiel[c], Bohdan Huszno[a], Zbigniew Szybiński[a]

[a]*Department of Endocrinology, Jagiellonian University, 31-501 Cracow, Poland*
[b]*1st Department of Cardiology, Jagiellonian University, 31-501 Cracow, Poland*
[c]*2nd Department of Cardiology, Jagiellonian University, 31-501 Cracow, Poland*
[d]*Institute of Clinical Pharmacology, Otto-von-Guericke University, Magdeburg, Germany*

Received 2 May 2006; accepted 30 August 2006

Abstract

Partially inconsistent data exist on mutual relations between nontraditional atherosclerotic risk factors, including the magnitude of insulin resistance (IR), as well as on their relevance for atherogenesis in the metabolic syndrome. Subjects exhibiting combined impaired fasting glucose and impaired glucose tolerance (IFG/IGT) are exposed to an exceptionally high risk for atherogenesis and development of type 2 diabetes mellitus. Because of islet β-cell dysfunction, the usefulness of commonly used indices of IR is limited in IFG/IGT. Our aim was to assess the relationship between extent of angiographic coronary artery disease (CAD) and nontraditional atherosclerotic risk factors (including IR by a clamp-based golden standard method) in IFG/IGT. Fifty-three subjects (32 men, 21 women; mean age, 55 ± 11 years) with stable angina, preserved left ventricular systolic function, and IFG/IGT were divided into 3 groups: group A (no coronary stenoses >50%, n = 22), group B (1-vessel CAD, n = 15), and group C (2/3-vessel CAD, n = 16). Insulin sensitivity was quantified by a hyperinsulinemic euglycemic clamp technique and expressed as *M*. *M* value, plasma homocysteine (Hcy) level, and asymmetric dimethyl-L-arginine (ADMA)/L-arginine ratio were independent determinants of CAD extent as shown by forward stepwise discriminant function analysis. Compared with group A (M = 32.7 ± 9.3 μmol/kg fat-free mass [FFM] per minute; Hcy, 8.1 ± 1.4 μmol/L), lower M and higher Hcy levels were found in group B (M = 16.9 ± 8.2 μmol/kg FFM per minute, $P < .001$; Hcy, 11.2 ± 2.9 μmol/L, $P = .003$) and C (M = 16.4 ± 7.8 μmol/kg FFM per minute, $P < .001$; Hcy, 12.8 ± 3.9 μmol/L, $P < .001$). The ADMA/L-arginine ratio was increased in group C (0.0078 ± 0.0011) compared with group A (0.0063 ± 0.0013, $P = .03$) and B (0.0058 ± 0.0012, $P = .01$). Multivariate correlates ($P < .05$) of plasma Hcy concentrations were M (β = −.34 ± .12, $P = .008$), creatinine clearance (β = −.23 ± .10, $P = .03$), and fasting insulin (β = .25 ± .12, $P = .04$). This indicates an additive contribution of IR, plasma Hcy, and elevated ADMA/L-arginine ratio to the extent of angiographic CAD in combined IFG/IGT.

1. Introduction

Insulin resistance (IR), the underlying cause of the metabolic syndrome, is a predictor of cardiovascular disease [1]. Large prospective studies have shown that the incidence of coronary artery disease (CAD) or risk of major coronary events positively correlated with fasting insulinemia, or insulinemia during an oral glucose load, in both univariate and multivariate analysis [2-4]. These data argue in favor of a link between IR and atherogenesis, as fasting and postchallenge insulinemia are largely determined by the magnitude of IR in subjects without diabetes [5].

IR, when combined with impaired insulin secretion due to islet β-cell dysfunction, leads to impaired glucose tolerance (IGT) and type 2 diabetes mellitus [6,7]. IGT is associated with accelerated atherosclerosis. Indeed, in consecutive nondiabetic patients referred to coronary angiography, the prevalence of IGT was considerably elevated in comparison with the general population, ranging

* Corresponding author. Fax: +48 424 7180.
E-mail address: surdacki.andreas@gmx.net (A. Surdacki).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.08.023

from 25% to 50% [8-10]. IGT and postload glycemia appear to exhibit closer relationship with the magnitude of coronary and carotid atherosclerosis and cardiovascular mortality risk than impaired fasting glucose (IFG) and fasting glycemia [11-15]. On the other hand, Drexel et al [16] have shown an increased prevalence of significant coronary stenoses in IFG, even assuming a newly lowered cut point for IFG (the 2003 American Diabetes Association criterion) [17]; however, the occurrence of IGT has not been determined in their study. Nevertheless, not only is the risk of the development of type 2 diabetes mellitus particularly elevated in subjects with combined IFG/IGT [11], but they are also exposed to accelerated atherogenesis due to pronounced clustering of atherosclerotic risk factors [18,19]. Although partially inconsistent data exist on the relative contribution of IR and β-cell dysfunction to IFG and IGT [11,18,20-23], it is beyond doubt that subjects with IFG/IGT exhibit a combination of IR and impaired insulin secretion [11,18,20,23]. Therefore, the ability of common indices to predict the magnitude of IR is limited in IGT [24,25], IFG [26], and IFG/IGT [26], so that a "gold standard" for quantification of IR, a hyperinsulinemic euglycemic clamp, would be particularly advisable for investigating a link between the magnitude of IR and atherogenesis in IFG/IGT. However, because of obvious methodological problems, only a few studies attempted to relate clamp-assessed IR to the extent of angiographically defined CAD [14,27].

Recent analyses derived from the Third National Health and Nutrition Examination Survey have shown increased prevalence of some nontraditional cardiovascular risk factors (ie, not included in the Framingham Heart Study) including blood concentration of C-reactive protein (CRP) and IR index (derived from the homeostasis model assessment [HOMA] formula) in subjects with combined IFG/IGT vs IFG and those with normal fasting and postchallenge glucose [28]. Moreover, results of the Hoorn study point to more potent deleterious cardiovascular effects of another nontraditional risk factor, elevated homocysteine (Hcy) levels in patients with type 2 diabetes mellitus compared with nondiabetic subjects [29,30].

In addition, it has been reported that the magnitude of IR in subjects free of diabetes is associated with accumulation of an endogenous inhibitor of nitric oxide (NO) synthesis, asymmetric dimethyl-L-arginine (ADMA) [31]. Increased plasma levels of ADMA, initially described in advanced chronic renal failure [32], were later reported in atherosclerosis, in subjects with atherosclerotic risk factors without clinical evidence of atherosclerosis [33], being also linked to the risk of cardiovascular events [34,35].

There are inconsistent data on mutual relationships between Hcy, ADMA, and IR. Contradictory reports exist on the effect of a Hcy-lowering therapy on plasma ADMA [36-38], on the association between ADMA and Hcy in atherosclerosis [37-39], and on the ability of acute hyperhomocysteinemia to induce elevation of circulating ADMA [40-42]. Similarly, levels of Hcy were found positively [43-45], inversely [46,47], or not significantly [48,49] correlated with the magnitude of IR in healthy individuals. Results of the Hypercoagulability and Impaired Fibrinolytic Function MECHanisms Predisposing to Myocardial Infarction study suggest association of low-grade inflammation with components of metabolic syndrome in subjects with a history of myocardial infarction [50]. Therefore, our aim was to investigate correlates of the extent of angiographically defined CAD in subjects with stable angina exhibiting both IFG and IGT. We assessed 2 hypotheses: first, that CAD extent may be related to such nontraditional risk factors as the clamp-assessed magnitude of IR, plasma levels of CRP and Hcy, and a balance between ADMA and L-arginine; second, that some of these nontraditional risk factors can be interrelated.

2. Subjects and methods

2.1. Patients

We studied 53 subjects (32 men, 21 women; age, 35 to 74 years; mean, 55 ± 11 years) with combined IFG/IGT and stable angina who were referred to planned coronary angiography. The patients had been selected on the basis of results of a standard 75-g oral glucose tolerance test. IFG was defined as fasting glucose in the venous plasma between 6.1 and 6.9 mmol/L (the previous American Diabetes Association criterion adopted by the World Health Organization in 1999) and IGT as 2-hour postload glucose level between 7.8 and 11.0 mmol/L [51]. Of the 53 study subjects, 30 patients exhibited a history of myocardial infarction, 50 patients had arterial hypertension, 19 subjects were obese (body mass index [BMI] $\geq$30 kg/m^2), and 29 were overweight (BMI = 25-29.9 kg/m^2). All the patients were treated with low-dose aspirin and β-blockers; angiotensin-converting enzyme inhibitors (ACEIs) and statins were used by 50 and 51 of the patients, respectively. Exclusion criteria were a history of acute coronary syndrome within the past 6 months, clinical evidence of heart failure, left ventricular ejection fraction less than 50% (by ultrasound), significant valvular heart disease, creatinine clearance less than 60 mL/min (by the Cockroft-Gault formula using serum creatinine), and clinical or biochemical evidence of any other serious disorders in addition to those remaining within the frame of the metabolic syndrome.

2.2. Assessment of IR by the clamp technique

A 2-hour hyperinsulinemic euglycemic clamp was performed after informed consent was obtained. The protocol was approved by the ethical committee of our institution. Therapy was kept unchanged during the preceding days due to ethical reasons, especially bearing in mind a potential risk of precipitating myocardial ischemia, ventricular arrhythmia, and/or hypertension crisis through the withdrawal of drugs. The clamp was performed in the morning after an overnight fast as described previously [52].

During a primed continuous infusion of insulin, steady euglycemia (5.0 mmol/L) was maintained by adjusting the rate of 20% glucose infusion according to measurements of glucose (by the glucose oxidase reaction) in the arterialized venous blood drawn at 5-minute intervals. A measure of insulin sensitivity (*M* value) was quantified as the rate of glucose infusion during the last 30 minutes of the clamp and expressed in micromoles of utilized glucose per kilogram of fat-free mass (FFM) per minute [14]. FFM was estimated by a bioelectric impedance analysis (Maltron Body Fat 905 Analyzer, Maltron International Ltd, Raleigh, Essex, UK).

2.3. Biochemical assays

A sample of blood was drawn from the fasting subjects between 7:30 and 8:30 AM into EDTA tubes; plasma was separated and frozen below −20°C for further assays. Insulin levels were estimated by a radioimmunoassay method (Polatom, Otwock-Swierk, Poland). CRP and Hcy concentrations were assessed by chemiluminescent immunoassay systems (Immulite 1000 and Immulite 2000, DPC, Los Angeles, CA). L-Arginine and its methylated analogs (ADMA and symmetric dimethyl-L-arginine [SDMA]) were measured by a newly described and validated liquid chromatographic/mass spectrometric method with an isotope-labeled internal standard [53]. Precision and accuracy tests for ADMA assay revealed intraday and interday relative SDs of 2.2% and 5.35%, respectively, whereas respective inaccuracies were 3.32% and 0.03%. As for L-arginine and SDMA, all relative SDs and inaccuracies were also below 10%.

2.4. Statistical analysis

The subjects were divided into 3 groups on the basis of the extent of CAD as described previously [54]: group A (no internal luminal narrowings >50% in major coronary arteries or their major branches, $n = 22$), group B (1-vessel disease, $n = 15$), and group C (2- or 3-vessel disease, $n = 16$).

Intergroup comparisons of individual continuous variables were performed by one-way analysis of variance (ANOVA) after logarithmic transformation when necessary (fasting insulin, Hcy, CRP, and *M* value). Post hoc comparisons were done by the Scheffe test. As to dichotomous variables (expressed as percentages), intergroup differences in frequencies were calculated by the χ^2 test with Yates correction when needed.

To identify a set of variables with optimal power of discrimination of the groups defined on the basis of the CAD extent, forward stepwise discriminant function anal-

Table 1
Demographic, clinical, and biochemical parameters vs angiographic CAD extent

	Group A, 0-vessel CAD (n = 22)	Group B, 1-vessel CAD (n = 15)	Group C, 2/3-vessel CAD (n = 16)	*P* by ANOVA (χ^2 for percentages)
Age (y)	53 ± 10	53 ± 9	57 ± 11	NS
Sex (male, %)	64	60	56	NS
Hypertension (%)	98	100	95	NS
Smoking habit (%)	14	20	19	NS
Drugs (%)				
Low-dose aspirin	100	100	100	NS
β-Blockers	100	100	100	NS
ACEIs	100	95	98	NS
Statins	95	100	100	NS
BMI (kg/m^2)	29.8 ± 3.4	29.3 ± 3.9	28.5 ± 3.1	NS
Waist-to-hip ratio	1.06 ± 0.16	1.05 ± 0.20	1.05 ± 0.17	NS
Creatinine clearance (mL/min)	103 ± 20	98 ± 23	88 ± 21	NS
Fasting glucose (mmol/L)	6.25 ± 0.30	6.41 ± 0.26	6.43 ± 0.30	NS
Fasting insulin (pmol/L)	65 ± 25	157 ± 119***	130 ± 80**	<.001
M (μmol/kg FFM per minute)	32.7 ± 9.3	16.9 ± 8.2****	16.4 ± 7.8****	<.001
LDL cholesterol (mmol/L)	3.3 ± 0.9	3.1 ± 0.8	3.2 ± 0.8	NS
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	NS
Triglycerides (mmol/L)	1.85 ± 0.8	1.6 ± 1.0	1.6 ± 0.6	NS
CRP (mg/L)	3.1 ± 3.5	3.2 ± 2.0	3.6 ± 3.2	NS
Hcy (μmol/L)	8.1 ± 1.4	11.2 ± 2.9***	12.8 ± 3.9****	<.001
L-Arginine (μmol/L)	70.5 ± 13.8	76.6 ± 25.0	59.1 ± 14.2†	.09
ADMA (μmol/L)	0.43 ± 0.07	0.45 ± 0.09	0.43 ± 0.06	NS
SDMA (μmol/L)	0.41 ± 0.07	0.47 ± 0.15	0.45 ± 0.11	NS
ADMA/L-arginine	0.0063 ± 0.0013	0.0058 ± 0.0012	0.0078 ± 0.0011*‡	.007

Data are the mean ± SD.

* $P = .03$ vs group A.

** $P = .01$ vs group A.

*** $P = .003$ vs group A.

**** $P < .001$ vs group A.

† $P = .09$ vs group B.

‡ $P = .01$ vs group B by the Scheffe test. NS indicates nonsignificant.

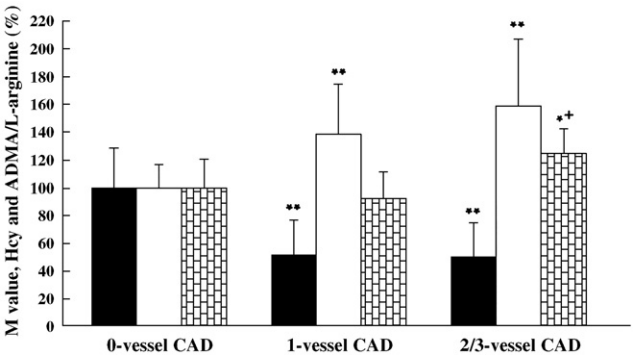

Fig. 1. Relative percent M values (solid bars), plasma Hcy levels (open bars), and ADMA/L-arginine ratio (hatched bars). Data are shown as mean ± SD and computed with reference to mean 0-vessel CAD values assumed to be 100%. *P = .03 vs 0-vessel CAD; **$P \leq .003$ vs 0-vessel CAD; ^{+}P = .01 vs 1-vessel CAD by the Scheffe test.

ysis was used. This method identifies a set of variables (a final "model") that contributes to predicting to which group a case belongs. Wilks λ for the final model and partial λ for individual variables were computed. Wilks λ can assume values from 0 (perfect discrimination) to 1 (no discrimination), whereas partial λ represents Wilks λ after adding the respective variable into the model divided by the Wilks λ before adding the variable. From partial λ and given numbers of groups, cases, and variables, F statistic and corresponding P value were calculated.

In an attempt to determine univariate correlates of variables representing nontraditional risk factors, Pearson (r) and Spearman correlation coefficients were computed to test their relationship with continuous variables and the severity of hypertension, respectively, whereas Student t test was used to estimate effects of dichotomous variables (sex, smoking habit, history of hypertension). In multivariate analysis, forward stepwise multiple linear regression was performed with a ridge regression approach to control for mutual correlations of independent variables. Adjusted coefficients of multiple determination (R^2) were presented as well as standardized mean regression coefficients (β) and their standard errors (SEM) for individual variables entering the final regression model. In search of determinants of CAD extent and of correlates of nontraditional risk factors, effects of numerous variables were tested including demographic, clinical, and biochemical parameters representing traditional and nontraditional risk factors. Statistical significance was assumed at P less than .05.

3. Results

3.1. Intergroup comparisons: demographic, clinical, and biochemical parameters vs extent of coronary atherosclerosis

Characteristics of subjects assigned to groups A, B, and C are presented in Table 1. Those with significant CAD exhibited higher Hcy and fasting insulin levels but lower M compared with those without coronary stenoses greater than 50%. However, when comparing subjects with 1-vessel disease vs more advanced CAD, the above mentioned parameters were similar and the only significant difference was higher ADMA/L-arginine ratio, largely due to a tendency (P = .09) to lower L-arginine levels in patients with 2- and 3-vessel disease (Table 1). No significant intergroup differences were found in sex, BMI, creatinine clearance, fasting glucose, lipid, and CRP levels, history of myocardial infarction, history and severity of hypertension, incidence and extent (pack-years) of smoking as well as drug therapy.

3.2. Discriminant function analysis: determinants of the extent of coronary atherosclerosis

Forward stepwise discriminant function analysis (Wilks λ = 0.33, $P < .001$) identified log M ($P < .005$), log(Hcy) ($P < .02$), and ADMA/L-arginine ratio ($P < .04$) as independent determinants of the extent of angiographically defined CAD. Among these variables, M and Hcy (entered as their logarithmic derivatives) differentiated those with and without significant CAD, whereas ADMA/L-arginine ratio discriminated between 1-vessel and 2/3-vessel CAD (Fig. 1).

3.3. Univariate and multivariate correlations

Considering the study group as a whole (n = 53), in univariate analysis plasma Hcy levels (after logarithmic transformation) were correlated with log M ($r = -0.62$, $P < .001$), log(fasting insulin) ($r = 0.61$, $P < .001$), creatinine clearance ($r = -0.40$, $P = .003$), and log(CRP) ($r = 0.28$, $P = .04$). Plasma Hcy level tended to be higher in smoking subjects (12.5 ± 4.2 vs 10.0 ± 3.1 μmol/L for smokers and nonsmokers, respectively; $P = .05$ for log-transformed data). By stepwise multiple regression (adjusted $R^2 = 0.41$, $P < .001$), Hcy concentrations were related to log M ($\beta = -.34 \pm .12$, $P = .008$), creatinine clearance ($\beta = -.23 \pm .10$, $P = .03$), log(fasting insulin) ($\beta = .25 \pm .12$, $P = .04$), and smoking habit ($\beta = .18 \pm .10$, $P = .08$) (Table 2).

Because altered ADMA/L-arginine ratio in group C subjects resulted from lower plasma L-arginine at comparable ADMA levels, we estimated correlates of plasma L-arginine and ADMA/L-arginine ratio identifying high-density lipoprotein (HDL) cholesterol as their only correlate in univariate approach ($r = 0.41$, $P = .002$ and $r = -0.37$, $P = .006$ for L-arginine and ADMA/L-arginine ratio, respectively) and in stepwise multiple regression (L-arginine: adjusted $R^2 = 0.13$, $\beta = .36 \pm .12$, $P = .0045$;

Table 2
Multivariate determinants of plasma Hcy levels (adjusted $R^2 = 0.41$, $P < .001$)

Variable	β ± SEM	P
Log M	−.34 ± .12	.008
Creatinine clearance	−.23 ± .10	.03
Log(fasting insulin)	.25 ± .12	.04
Smoking habit	.18 ± .10	.08

Standardized mean regression coefficients (β) ± SEM are presented.

ADMA/L-arginine ratio: adjusted $R^2 = 0.11$, $\beta = -.33 \pm .12$, $P = .009$).

ADMA concentrations correlated with fasting glucose ($r = 0.31$, $P = .02$) and weakly with log(CRP) ($r = 0.24$, $P = .08$). However, in multivariate regression (adjusted $R^2 = 0.10$, $P < .03$) these associations were considerably attenuated (fasting glucose: $\beta = .26 \pm .13$, $P = .05$; log(CRP): $\beta = .19 \pm .13$, $P = .14$). SDMA levels were related exclusively to creatinine clearance ($r = -0.38$, $P = .005$).

CRP levels (after logarithmic transformation) were correlated with log(fasting insulin) ($r = 0.40$, $P = .003$), log M ($r = -0.30$, $P = .03$), log(Hcy) ($r = 0.28$, $P = .04$), HDL cholesterol ($r = -0.27$, $P = .05$) and weakly with ADMA ($r = 0.24$, $P = .08$). Multivariate analysis (adjusted $R^2 = 0.15$, $P < .01$) revealed log(fasting insulin) ($\beta = .34 \pm .13$, $P = .01$) and HDL cholesterol ($\beta = -.19 \pm .13$, $P = .15$) as independent predictors of CRP concentrations entering the final regression equation.

4. Discussion

Our data suggest that in nondiabetic subjects exhibiting combined elevation of fasting and postchallenge glucose levels (IFG/IGT), fasting concentrations of Hcy not only correlate with the degree of IR and fasting hyperinsulinemia, but are also associated with the extent of CAD independently of the degree of metabolic sensitivity to insulin. In addition, an altered balance between the substrate and endogenous inhibitor of NO synthesis in nonsmoking subjects with more advanced CAD suggests participation of diminished availability of L-arginine in later stages of atherogenesis.

4.1. Magnitude of IR vs angiographic extent of CAD

The relationship between the extent of angiographic CAD and the degree of IR is compatible with other data. In 1996, using a clamp technique, Bressler et al [27] showed an inverse relationship between the number of stenotic coronary arteries and insulin-mediated whole-body glucose disposal in 13 patients with normal glucose tolerance. In addition, Kanauchi et al [14], who studied 48 nondiabetic subjects (including 28 with IGT), demonstrated a positive correlation between the extent of CAD and serum levels of advanced glycation end products whose concentrations were negatively related to the M value. However, the authors [14] focused on the role of advanced glycation end products as independent determinants of atherosclerosis without entering M into the multiple regression with the number of critical coronary narrowings as a dependent variable. More importantly, Shinozaki et al [55] reported that a marker of IR, steady-state plasma glucose during infusion of insulin, glucose, and octreotide, was increased by about 100% in subjects with significant coronary narrowings and IGT compared with IGT without such narrowings. Moreover, in 1482 subjects recruited from the Insulin Resistance Atherosclerosis Study group (22% with IGT and 32% with diabetes), an inverse association between the clinical evidence of CAD and the minimal model-derived insulin sensitivity index was maintained (although weakened) in multiple logistic regression after adjustment for classical risk factors and insulinemia, whereas an association between coronary heart disease and insulinemia totally disappeared on multivariate adjustment [56].

It is noteworthy that in our study neither fasting nor postload hyperglycemia was associated with the number of stenotic coronary arteries in IFG/IGT subjects, which is apparently contradictory to the results of Sasso et al [13] and Kowalska et al [10], who reported a link between postload glucose and magnitude of coronary atherosclerosis. However, Sasso et al [13] limited their analysis to men with normal glucose tolerance, and in the study by Kowalska et al [10] (in which the percentages of IGT and type 2 diabetes mellitus were 36% and 16%, respectively) the significance of correlation between the respective variables disappeared on multivariate adjustment. Accordingly, different characteristics of patients studied was likely to account for the discrepancy, all the more because Fujiwara et al [8] reported no differences in fasting and postchallenge glycemia between men with IGT with 75% or greater coronary stenoses vs IGT and less than 25% stenoses, whereas postload insulinemia was elevated by about 60% in the former group, which suggests a link between IR and the presence of significant coronary narrowings in IGT. Finally, it is to be kept in mind that a considerable limitation of studies focused on correlates of CAD in IGT, IFG/IGT, or diabetes is that risk factors are presented as single measures, whereas atherosclerosis is a long-term process and significant coronary narrowings could already be present before the development of a given category of glucose intolerance.

4.2. Correlates of plasma Hcy levels

Positive correlation between IR and Hcy levels observed in our IFG/IGT patients, a prediabetic state, adds to a report on 24 healthy nonobese subjects studied with a glucose clamp technique [43]. In addition, in 2011 participants of the Framingham Offspring Study free of cardiovascular disease at fifth examination (of whom 14.8% exhibited IFG or IGT), Hcy levels were positively correlated with fasting insulinemia, and elevated Hcy was found in subjects exhibiting simultaneous presence of at least 2 IR syndrome phenotypes [44]. However, other authors have not confirmed positive association between IR and Hcy or even observed inverse relationship in healthy nonobese or obese subjects [46-49]. As to studies dealing with established type 2 diabetes mellitus, the independent contribution of IR to accumulation of circulating Hcy was reported by Emoto et al [57], who found a negative independent correlation of Hcy levels with a clamp-derived insulin sensitivity index by means of multiple regression in 59 patients with type 2 diabetes mellitus and creatinine clearance greater than 60 mL/min. In

addition, Araki et al [58] reported an independent positive relationship between plasma Hcy and serum C-peptide (an index of IR) in 223 elderly patients with type 2 diabetes mellitus and serum creatinine level of 1.3 mg/dL or higher. The negative correlations between renal excretory function and Hcy observed in the present study and by other authors [57-60] are obvious, keeping in mind the predominant role of the kidney in Hcy removal from the blood [61]. In contrast to the findings of some authors [57,58] and to our data, Buysschaert et al [59] observed the lack of association of Hcy levels with IR (calculated according to the HOMA formula) in subjects with type 2 diabetes mellitus. However, similarly to our findings, Buysschaert et al [59] found a higher prevalence of macroangiopathy (in particular, CAD) in subjects with hyperhomocysteinemia, which was maintained after controlling for renal function. Okumura and Aso [60] reported that exclusion of subjects with creatinine clearance less than 60 mL/min abolished significant Hcy elevations in macroangiopathic patients with type 2 diabetes mellitus, yet they found independent association of Hcy with plasma thrombomodulin, a glycoprotein whose soluble form is linked to endothelial injury.

Therefore, it can be proposed that apart from the contribution of renal dysfunction and/or IR to elevated circulating Hcy, it seems that hyperhomocysteinemia promotes atherogenesis and its antecedent, endothelial dysfunction, in type 2 diabetes mellitus, a process that may already occur in the prediabetic state—IFG/IGT. As to a tendency to higher Hcy concentrations in smokers, this finding is consistent with previous data and might hypothetically be linked to increased folate catabolism and lower pyridoxal phosphate synthesis [62].

4.3. Possible mechanisms of the observed association between Hcy levels and IR

Positive correlations between Hcy levels, IR, and hyperinsulinemia might have been due to insulin-mediated decreases in the activity of key enzymes regulating the activity of the transsulfuration pathway (governed by cystathionine β-synthase) and/or remethylation pathway (controlled by N^5,N^{10}-methylenetetrahydrofolate reductase) [63-66]. In addition, because the ability of acute hyperinsulinemia to decrease circulating Hcy [67] (probably due to stimulation of methionine uptake by the skeletal muscle [68]) had been demonstrated to disappear in insulin-resistant patients with type 2 diabetes mellitus [67], this could also contribute to elevated Hcy in patients with more marked IR. However, it cannot be excluded that elevated Hcy levels might also contribute to IR not being solely an effect of IR. Indeed, contrary to data on animals [64], thiazolidinedione therapy for 26 weeks was not associated with Hcy lowering despite an attenuation of IR (as quantified by the HOMA formula) by about 30% in type 2 diabetes mellitus [69]. Moreover, Hcy thiolactone has been shown to inhibit insulin signaling via increased oxidative stress [70], and decreases in Hcy were associated with attenuation of IR and endothelial dysfunction in subjects with metabolic syndrome treated with folic acid and vitamin B_{12} [36]. In addition, activation of the interleukin 6 (IL-6)–dependent innate immunity axis might link Hcy to IR. Indeed, Hcy has been shown to stimulate secretion of IL-6 by endothelial cells and monocytes [71,72], whereas IL-6, in turn, is likely to both inhibit insulin signaling [73] and increase Hcy probably via pyridoxal phosphate depletion [74,75]. Araki et al [58] found that both IR and IL-6 (but not CRP) were independently related to plasma Hcy in type 2 diabetes mellitus. However, in the present study, a univariate positive correlation between Hcy and CRP disappeared after multivariate adjustment and IL-6 concentrations have not been measured.

4.4. Relevance of elevated Hcy for atherogenesis in the presence of IR and hyperglycemia

It may be hypothesized that the proatherosclerotic effect of IR was operating by mechanisms not approached in the present study design. Indeed, a number of abnormalities have been reported as a possible explanation of IR-induced atherogenesis, such as endothelial dysfunction, oxidative stress, increased expression of adhesion molecules, abnormal low-density lipoprotein (LDL) particles (exhibiting lower diameter and increased degree of oxidation), decreased fibrinolytic activity [76], most of which are at least potentiated (or may be even caused) by deficiency of NO, an endogenous antiatherogenic molecule. As to Hcy, there is strong evidence that generation of reactive oxygen species largely contributes to its deleterious cardiovascular effects [77]. In the presence of elevated Hcy levels, endothelial NO synthase may be transformed from the source of NO into a superoxide-generating enzyme [78]. The equilibrium between endothelial NO and superoxide formation in favor of the latter is also shifted during hyperglycemia [79], known to augment the ability of Hcy to produce endothelial dysfunction [80,81]. In addition, results of the Hoorn study provide evidence that diabetes poses a particular opportunity for the effects of hyperhomocysteinemia to translate into accelerated atherogenesis [29] and risk of acute coronary events [30]. Moreover, Okada et al [82] have found an independent correlation between the severity of coronary atherosclerosis and Hcy levels in type 2 diabetics in contrast to nondiabetics. Finally, Gardemann et al [83] observed an association of the magnitude of coronary atherosclerosis with a thermolabile variant of N^5,N^{10}-methylenetretrahydrofolate reductase (associated with a lower activity of the remethylation pathway and, consequently, higher Hcy levels) exclusively in those with fasting glucose level greater than 112 mg/dL (6.22 mmol/L).

4.5. Relevance of elevated ADMA/L-arginine ratio for atherogenesis in the presence of increased Hcy and IR

In the present study IFG/IGT subjects with more advanced CAD exhibited increased ADMA/L-arginine ratio, a factor known to impair NO bioavailability. L-Arginine

deficiency [84] and/or accumulation of ADMA [85] accelerate superoxide generation by endothelial NO synthase at the cost of NO formation. Because NO exerts multiple antiatherogenic activities including protection against oxidative stress, the coincidence of elevated Hcy with an altered balance between the endogenous substrate and inhibitor of NO generation in insulin-resistant IFG/IGT patients (ie, with fasting and postchallenge hyperglycemia) was likely to create a particularly detrimental milieu, thus explaining increased ADMA/L-arginine ratio in our subjects with 2- and 3-vessel CAD. It is to be mentioned that the majority of our study group (83%) constituted nonsmoking subjects, which might have facilitated the detection of a link between ADMA and atherosclerosis, as Valkonen et al [35] related ADMA to future coronary events only in nonsmokers, possibly due to an interference of ADMA-independent pathways of atherogenesis in smokers.

4.6. Correlates of plasma levels of L-arginine and its dimethylated analogs

L-Arginine levels were found to be independently positively correlated with HDL cholesterol. There are few data on determinants of circulating L-arginine, yet some observations suggest that concentrations of this amino acid may decrease in states associated with inflammatory activation. Mittermayer et al [86] have reported that plasma L-arginine decreased by 27% 3.5 hours after intravenous administration of *Escherichia coli* endotoxin in healthy volunteers. According to the Insulin Resistance Atherosclerosis Study investigators, in 1008 nondiabetic subjects CRP levels were inversely related to HDL cholesterol and a minimal model-derived insulin sensitivity index both in IGT and normal glucose tolerance [87]. In the present study, we have also found significant relationships among CRP, *M* value, and fasting insulin levels, although only a marginally significant negative correlation between CRP and HDL. Thus, keeping in mind the association of acute-phase reactants with IR and features of the metabolic syndrome across a wide range of glucose tolerance [50,87,88], generation of proinflammatory cytokines (from the adipose tissue and, possibly, the vascular wall) seems a possible reason for the decrease in both HDL cholesterol and plasma L-arginine levels, thus explaining their correlation in our study. Indeed, endotoxin and tumor necrosis factor α are known to stimulate arginase activity in endothelial cells [89]. In addition, in a recent placebo-controlled study, simultaneous increases in both L-arginine and HDL cholesterol were observed in hyperlipidemic men after micronized fenofibrate therapy [90], a therapy that can reduce CRP levels and improve endothelial function [91].

The absence of significant correlations between ADMA concentrations and the analyzed variables (with the exception of fasting glucose) could be at least partially due to the influence of pharmacologic therapy. ACEIs administered to almost all our subjects were previously shown to decrease plasma ADMA levels [92]. A weakly significant correlation between ADMA and fasting glucose might reflect the ability of hyperglycemia to inhibit the activity of dimethylarginine dimethylaminohydrolase, an enzyme that degrades ADMA [93]. An insignificant tendency to positive correlation between ADMA and CRP in our IFG/IGT patients might be due to the capability of ADMA to induce inflammatory processes within the vascular wall [85]. The significant negative correlation between creatinine clearance and SDMA, but not ADMA, is compatible with the findings of Marescau et al [94], who found relatively higher increases in SDMA than in ADMA in mild renal insufficiency, presumably due to the lack of ability of dimethylarginine dimethylaminohydrolase to degrade SDMA, which is thus more dependent on urinary excretion compared with ADMA.

5. Conclusions

Our data indicate additive and independent contributions of the magnitude of IR, plasma Hcy levels, and elevated ADMA/L-arginine ratio to the extent of angiographic CAD in combined IFG/IGT, a prediabetic state with accelerated atherogenesis. This might suggest a need for interventional studies focused on nontraditional atherosclerotic risk factors in IFG/IGT and, preferentially, also at earlier phases of the metabolic syndrome. Simultaneous targeting of multiple risk factors may be necessary to produce desirable effects. In fact, as suggested by Loscalzo [95], recently published results of 3 prospective trials (the Vitamin Intervention for Stroke Prevention trial, the Norweigian Vitamin trial, and the Heart Outcomes Evaluation 2 trial) showing the lack of clinical benefits despite folic acid/vitamins B_6/B_{12}–induced Hcy lowering may reflect an adverse interference of such therapy with other factors, eg, methylation potential and ADMA formation, which has previously been suggested [96].

References

[1] Yip J, Facchini FS, Reaven GM. Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. J Clin Endocrinol Metab 1998;83:2773-6.

[2] Fontbonne A, Charles MA, Thibult N, Richard JL, Claude JR, Warnet JM, et al. Hyperinsulinemia as a predictor of coronary heart disease mortality in a healthy population: the Paris Prospective Study, 15-year follow-up. Diabetologia 1991;34:356-61.

[3] Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 1996;334:952-7.

[4] Pyorala M, Miettinen H, Laakso M, Pyorala K. Hyperinsulinemia predicts coronary heart disease risk in healthy middle-aged men. The 22-year follow-up results of the Helsinki Policemen Study. Circulation 1998;98:398-404.

[5] Yeni-Komshian H, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. Diabetes Care 2000;23:171-5.

[6] Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, et al. Insulin resistance and insulin secretory dysfunction as precursors of non–insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. N Engl J Med 1993;329:1988-92.

[7] Weyer C, Tataranni PA, Bogardus C, Pratley RE. Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. Diabetes Care 2001;24:89-94.
[8] Fujiwara R, Kutsumi Y, Hayashi T, Nishio H, Koshino Y, Shimada Y, et al. Relation of angiographically defined coronary artery disease and plasma concentrations of insulin, lipid, and apolipoprotein in normolipidemic subjects with varying degrees of glucose tolerance. Am J Cardiol 1995;75:122-6.
[9] Seibaek M, Sloth C, Vallebo L, Hansen T, Urhammer SA, Burchardt H, et al. Glucose tolerance status and severity of coronary artery disease in men referred to coronary arteriography. Am Heart J 1997; 133:622-9.
[10] Kowalska I, Prokop J, Bachorzewska-Gajewska H, Telejko B, Kinalska I, Kochman W, et al. Disturbances of glucose metabolism in men referred for coronary arteriography. Postload glycemia as predictor for coronary atherosclerosis. Diabetes Care 2001; 24:897-901.
[11] Unwin N, Shaw J, Zimmet P, Alberti KGMM. Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. Diabet Med 2002;19:708-23.
[12] Hanefeld M, Temelkova-Kurktschiev T, Schaper F, Henkel E, Siegert G, Koehler C. Impaired fasting glucose is not a risk factor for atherosclerosis. Diabet Med 1999;16:212-8.
[13] Sasso FC, Carbonara O, Nasti R, Campana B, Marfella R, Torella M, et al. Glucose metabolism and coronary heart disease in patients with normal glucose tolerance. JAMA 2004;291:1857-63.
[14] Kanauchi M, Tsujimoto N, Hashimoto T. Advanced glycation end products in nondiabetic patients with coronary artery disease. Diabetes Care 2001;24:1620-3.
[15] DECODE Study Group. European Diabetes Epidemiology Group. Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? Diabetes Care 2003;26:688-96.
[16] Drexel H, Aczel S, Marte T, Benzer W, Langer P, Moll W, et al. Is atherosclerosis in diabetes and impaired fasting glucose driven by elevated LDL cholesterol or by decreased HDL cholesterol? Diabetes Care 2005;28:101-17.
[17] The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up Report on the Diagnosis of Diabetes Mellitus. Diabetes Care 2003;26:3160-7.
[18] Festa A, D'Agostino Jr R, Hanley AJ, Karter AJ, Saad MF, Haffner SM. Differences in insulin resistance in nondiabetic subjects with isolated impaired glucose tolerance or isolated impaired fasting glucose. Diabetes 2004;53:1549-55.
[19] Blake DR, Meigs JB, Muller DC, Najjar SS, Andres R, Nathan DM. Impaired glucose tolerance, but not impaired fasting glucose, is associated with increased levels of coronary heart disease risk factors: results from the Baltimore Longitudinal Study on Aging. Diabetes 2004;53:2095-100.
[20] Weyer C, Bogardus C, Pratley RE. Metabolic characteristics of individuals with impaired fasting glucose and/or impaired glucose tolerance. Diabetes 1999;48:2197-203.
[21] Hanefeld M, Koehler C, Fuecker K, Henkel E, Schaper F, Temelkova-Kurktschiev T. Insulin secretion and insulin sensitivity pattern is different in isolated impaired glucose tolerance and impaired fasting glucose. Diabetes Care 2003;26:868-74.
[22] Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. β-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J Clin Endocrinol Metab 2005;90:453-500.
[23] Carnevale G, Schianca P, Rossi A, Sainaghi PP, Maduli E, Bartoli E. The significance of impaired fasting glucose versus impaired glucose tolerance: importance of insulin secretion and resistance. Diabetes Care 2003;26:1333-7.
[24] Laakso M. How good a marker is insulin level for insulin resistance? Am J Epidemiol 1993;137:959-65.
[25] Ferrara CM, Goldberg AP. Limited value of the homeostasis model assessment to predict insulin resistance in older men with impaired glucose tolerance. Diabetes Care 2001;24:245-9.
[26] Tripathy D, Almgren P, Tuomi T, Groop L. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. Diabetes Care 2004;27:2204-10.
[27] Bressler P, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. Diabetologia 1996;39:1345-50.
[28] Muntner P, He J, Chen J, Fonseca V, Whelton PK. Prevalence of non-traditional cardiovascular disease risk factors among persons with impaired fasting glucose, impaired glucose tolerance, diabetes, and the metabolic syndrome: analysis of the Third National Health and Nutrition Examination Survey (NHANES III). Ann Epidemiol 2004;14:686-95.
[29] Hoogeveen EK, Kostense PJ, Beks PJ, Mackaay AJC, Jakobs C, Bouter LM, et al. Hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, especially in non–insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1998;18:133-8.
[30] Becker A, Kostense PJ, Bos G, Heine RJ, Dekker JM, Nijpels G, et al. Hyperhomocysteinaemia is associated with coronary events in type 2 diabetes. J Int Med 2003;253:293-300.
[31] Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, et al. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. JAMA 2002;287:1420-6.
[32] Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339:572-5.
[33] Fliser D, Kielstein JT, Haller H, Bode-Böger SM. Asymmetric dimethylarginine: a cardiovascular risk factor in renal disease? Kidney Int Suppl 2003;84:S37-S40.
[34] Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto FA, Tripepi G, Malatino LS, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001;358:2113-7.
[35] Valkonen V-P, Paiva H, Salonen JT, Lakka TA, Lehtimaki T, Laakos J, et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001;358:2127-8.
[36] Setola E, Monti LD, Galluccio E, Palloshi A, Fragasso G, Paroni R, et al. Insulin resistance and endothelial function are improved after folate and vitamin B_{12} therapy in patients with metabolic syndrome: relationship between homocysteine levels and hyperinsulinemia. Eur J Endocrinol 2004;151:483-9.
[37] Jonasson TF, Hedner T, Hultberg B, Ohlin H. Hyperhomocysteinaemia is not associated with increased levels of asymmetric dimethylarginine in patients with ischaemic heart disease. Eur J Clin Invest 2003;33:543-9.
[38] Ziegler S, Mittermayer F, Plank C, Minar E, Wolzt M, Schernthaner G-H. Homocysteine-lowering therapy does not affect plasma ADMA concentrations in patients with peripheral artery disease. J Clin Endocrinol Metab 2005;90:2175-8.
[39] Sydow K, Hornig B, Arakawa N, Bode-Böger SM, Tsikas D, Munzel T, et al. Endothelial dysfunction in patients with peripheral arterial disease and chronic hyperhomocysteinemia: potential role of ADMA. Vasc Med 2004;9:93-101.
[40] Stuhlinger MC, Oka RK, Graf EE, Schmolzer I, Upson BM, Kapoor O, et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. Circulation 2003;108:933-8.
[41] Wanby P, Brattstrom L, Brudin L, Hultberg B, Teerlink T. Asymmetric dimethylarginine and total homocysteine in plasma after oral methionine loading. Scand J Clin Lab Invest 2003;63:347-53.
[42] Doshi S, McDowell I, Goodfellow J, Stabler S, Boger R, Allen R, et al. Relationship between *S*-adenosylmethionine, *S*-adenosylhomocysteine, asymmetric dimethylarginine, and endothelial function in healthy human subjects during experimental hyper- and hypohomocysteinemia. Metabolism 2005;54:351-60.
[43] Giltay EJ, Hoogeveen EK, Elbers JM, Gooren LJ, Asscheman H, Stehouwer CD. Insulin resistance is associated with elevated plasma

total homocysteine levels in healthy, non-obese subjects. Atherosclerosis 1998;139:197-8.
[44] Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N, et al. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham Offspring Study. Diabetes Care 2001;24:1403-10.
[45] Martos R, Valle M, Morales R, Canete R, Gavilan MI, Sanchez-Margalet V. Hyperhomocysteinemia correlates with insulin resistance and low-grade systemic inflammation in obese prepubertal children. Metabolism 2006;55:72-7.
[46] Fonseca VA, Fink LM, Kern PA. Insulin sensitivity and plasma homocysteine concentrations in non-diabetic obese and normal weight subjects. Atherosclerosis 2003;167:105-9.
[47] Rosolova H, Simon J, Mayer Jr O, Racek J, Dierze T, Jacobsen DW. Unexpected inverse relationship between insulin resistance and serum homocysteine in healthy subjects. Physiol Res 2002;51:93-8.
[48] Godsland IF, Rosankiewicz JR, Proudler AJ, Johnston DG. Plasma total homocysteine concentrations are unrelated to insulin sensitivity and components of the metabolic syndrome in healthy men. J Clin Endocrinol Metab 2001;86:719-23.
[49] Abbasi F, Facchini F, Humphreys MH, Reaven GM. Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal. Atherosclerosis 1999;146:175-8.
[50] Yudkin JS, Juhan-Vague I, Hawe E, Humphries SE, Di Minno G, Margaglione M, et al. Low-grade inflammation may play a role in the etiology of the metabolic syndrome in patients with coronary heart disease: the HIFMECH study. Metabolism 2004;53:852-7.
[51] World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: World Health Organization; 1999.
[52] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.
[53] Martens-Lobenhoffer J, Krug O, Bode-Böger SM. Determination of arginine and asymmetric dimethylarginine (ADMA) in human plasma by liquid chromatography/mass spectrometry with the isotope dilution technique. J Mass Spectrom 2004;39:1287-94.
[54] Austen WG, Edwards JE, Frye RL, Gensini GG, Gott VL, Griffith LS, et al. A reporting system on patients evaluated for coronary artery disease: report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. Circulation 1975;51(Suppl 4):5-40.
[55] Shinozaki K, Suzuki M, Ikebuchi M, Hara Y, Harano Y. Demonstration of insulin resistance in coronary artery disease documented with coronary angiography. Diabetes Care 1996;19:1-7.
[56] Rewers M, Zaccaro D, D'Agostino R, Haffner S, Saad MF, Selby JV, et al. Insulin sensitivity, insulinemia, and coronary artery disease: Insulin Resistance Atherosclerosis Study. Diabetes Care 2004;27:781-7.
[57] Emoto M, Kanda H, Shoji T, Kawagishi T, Komatsu M, Mori K, et al. Impact of insulin resistance and nephropathy on homocysteine in type 2 diabetes. Diabetes Care 2001;24:533-8.
[58] Araki A, Hosoi T, Orimo H, Ito H. Association of plasma homocysteine with serum interleukin-6 and C-peptide levels in patients with type 2 diabetes. Metabolism 2005;54:809-14.
[59] Buysschaert M, Dramasi A-S, Wallemacq PE, Hermans MP. Hyperhomocysteinemia in type 2 diabetes. Diabetes Care 2000;23:1816-22.
[60] Okumura K, Aso Y. High plasma homocysteine concentrations are associated with plasma concentrations of thrombomodulin in patients with type 2 diabetes and link diabetic nephropathy to macroangiopathy. Metabolism 2003;52:1517-22.
[61] Bostom A, Brosnan JT, Hall B, Nadeau MR, Selhub J. Net uptake of plasma homocysteine by the rat kidney in vivo. Atherosclerosis 1995;116:59-62.
[62] Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med 1998;338:1042-50.
[63] Fonseca V, Dicker-Brown A, Ranganathan S, Song W, Barnard RJ, Fink L, et al. Effects of a high-fat-sucrose diet on enzymes in homocysteine metabolism in the rat. Metabolism 2000;49:736-41.
[64] Fonseca V, Keebler M, Dicker-Brown A, DeSouza C, Poirier LA, Murthy SN, et al. The effect of troglitazone on plasma homocysteine, hepatic and red blood cell *S*-adenosyl methionine, and *S*-adenosyl homocysteine and enzymes in homocysteine metabolism in Zucker rats. Metabolism 2002;51:783-6.
[65] Poirier LA, Brown AT, Fink LM, Wise CK, Randolph CJ, Delongchamp RR, et al. Blood *S*-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. Metabolism 2001;50:1014-8.
[66] Dicker-Brown A, Fonseca VA, Fink LM, Kern PA. The effect of glucose and insulin on the activity of methylene tetrahydrofolate reductase and cystathionine-β-synthase: studies in hepatocytes. Atherosclerosis 2001;158:297-301.
[67] Fonseca VA, Mudaliar S, Schmidt B, Fink LM, Kern PA, Henry RR. Plasma homocysteine concentrations are regulated by acute hyperinsulinemia in nondiabetic but not type 2 diabetic subjects. Metabolism 1998;47:686-9.
[68] Arfvidsson B, Zachrisson H, Moller-Loswick ACV, Hyltander A, Sandstrom R, Lundholm K. Effect of systemic hyperinsulinemia on amino acid flux across human legs in postaborptive state. Am J Physiol 1991;260:E46-E52.
[69] Sarafidis PA, Lasaridis AN, Nilsson PM, Mouslech TF, Hitoglou-Makedou AD, Stafylas PC, et al. The effect of rosiglitazone on novel atherosclerotic risk factors in patients with type 2 diabetes mellitus and hypertension. An open-label observational study. Metabolism 2005;54:1236-42.
[70] Najib S, Sanchez-Margalet V. Homocysteine thiolactone inhibits insulin signaling, and glutathione has a protective effect. J Mol Endocrinol 2001;27:85-91.
[71] van Aken BE, Jansen J, van Deventer SJ, Reitsma PH. Elevated levels of homocysteine increase IL-6 production in monocytic Mono Mac 6 cells. Blood Coagul Fibrinolysis 2000;11:159-64.
[72] Dalal S, Parkin SM, Homer-Vanniasinkam S, Nicolaou A. Effect of homocysteine on cytokine production by human endothelial cells and monocytes. Ann Clin Biochem 2003;40(Pt 5):534-41.
[73] Cardellini M, Perego L, D'Adamo M, Marini MA, Procopio C, Hribal ML, et al. C-174G polymorphism in the promoter of the interleukin-6 gene is associated with insulin resistance. Diabetes Care 2005;28:2007-12.
[74] Friso S, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B_6 is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. Circulation 2001;103:2788-91.
[75] McCarty MF. Increased homocyst(e)ine associated with smoking, chronic inflammation, and aging may reflect acute-phase induction of pyridoxal phosphatase activity. Med Hypotheses 2000;55:289-93.
[76] Reaven GM. Multiple CHD risk factors in type 2 diabetes: beyond hyperglycaemia. Diabetes Obes Metab 2002;4(Suppl 1):S13-8.
[77] Weiss N, Heydrick SJ, Postea O, Keller C, Keaney Jr JF, Loscalzo J. Influence of hyperhomocysteinemia on the cellular redox state—impact on homocysteine-induced endothelial dysfunction. Clin Chem Lab Med 2003;41:1455-61.
[78] Zhang X, Li H, Jin H, Ebin Z, Brodsky S, Goligorsky MS. Effects of homocysteine on endothelial nitric oxide production. Am J Physiol 2000;279:F671-8.
[79] Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997;96:25-8.
[80] Hofmann MA, Kohl B, Zumbach MS, Borcea V, Bierhaus A, Henkels M, et al. Hyperhomocyst(e)inemia and endothelial dysfunction in IDDM. Diabetes Care 1998;21:841-8.
[81] Shukla N, Thompson CS, Angelini GD, Mikhailidis DP, Jeremy JY. Homocysteine enhances impairment of endothelium-dependent relax-

ation and guanosine cyclic monophosphate formation in aortae from diabetic rabbits. Diabetologia 2002;45:1325-31.

[82] Okada E, Oida K, Tada H, Asazuma K, Eguchi K, Tohda G, et al. Hyperhomocysteinemia is a risk factor for coronary arteriosclerosis in Japanese patients with type 2 diabetes. Diabetes Care 1999; 22:484-90.

[83] Gardemann A, Weidemann H, Philipp M, Katz N, Tillmanns H, Hehrlein FW, et al. The TT genotype of the methylenetetrahydrofolate reductase C677T gene polymorphism is associated with the extent of coronary atherosclerosis in patients at high risk for coronary artery disease. Eur Heart J 1999;20:584-92.

[84] Klatt P, Schmidt K, Uray G, Mayer B. Multiple catalytic functions of brain nitric oxide synthase. Biochemical characterization, cofactor-requirement, and the role of N^{ω}-hydroxy-L-arginine as an intermediate. J Biol Chem 1993;268:14781-7.

[85] Böger RH, Bode-Böger SM, Tsao PS, Lin PS, Chan JR, Cooke JP. An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. J Am Coll Cardiol 2000;36:2287-95.

[86] Mittermayer F, Namiranian K, Pleiner J, Schaller G, Wolzt M. Acute *Escherichia coli* endotoxaemia decreases the plasma L-arginine/asymmetrical dimethylarginine ratio in humans. Clin Sci 2004; 106:577-81.

[87] Festa A, D'Agostino Jr R, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42-7.

[88] Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.

[89] Bachetti T, Comini L, Francolini G, Bastianon D, Valetti B, Cadei M, et al. Arginase pathway in human endothelial cells in pathophysiological conditions. J Mol Cell Cardiol 2004;37:515-23.

[90] Dierkes J, Westphal S, Lobenhoffer-Martens J, Luley C, Bode-Böger SM. Fenofibrate increases the L-arginine:ADMA ratio by increase of L-arginine concentration but has no effect on ADMA concentration. Atherosclerosis 2004;173:239-44.

[91] Wang TD, Chen WJ, Lin JW, Cheng CC, Chen MF, Lee YT. Efficacy of fenofibrate and simvastatin on endothelial function and inflammatory markers in patients with combined hyperlipidemia: relations with baseline lipid profiles. Atherosclerosis 2003;170:315-23.

[92] Delles C, Schneider MP, John S, Gekle M, Schmieder RE. Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical dimethylarginine in human essential hypertension. Am J Hypertens 2002;15:590-3.

[93] Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, et al. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 2002;106:987-92.

[94] Marescau B, Nagels G, Possemiers I, De Broe ME, Becaus I, Billiouw JM, et al. Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. Metabolism 1997;46:1024-31.

[95] Loscalzo J. Homocysteine trials—clear outcomes for complex reasons. N Engl J Med 2006;354:1629-32.

[96] Böger RH, Bode-Böger SM, Sydow K, Heistad DD, Lentz SR. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia. Arterioscler Thromb Vasc Biol 2000;20:1557-64.