

# Fasting Plasma Homocysteine Levels in the Insulin Resistance Syndrome

## The Framingham Offspring Study

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**OBJECTIVE** — Insulin resistance, associated metabolic abnormalities, and elevated homocysteine levels are risk factors for cardiovascular disease (CVD). We examined relationships between homocysteine levels and features of insulin resistance syndrome (IRS).

**RESEARCH DESIGN AND METHODS** — We measured clinical characteristics, plasma levels of fasting homocysteine, folate, B vitamins, creatinine, and fasting and 2-h insulin and glucose levels after a 75-g oral glucose tolerance test in 2,214 subjects without CVD at the fifth examination (1991–1995) of the Framingham Offspring Study. After excluding 203 subjects with diabetes, the remaining 2,011 subjects were categorized as having none, one, two, or all three of the phenotypes of IRS: impaired glucose tolerance, hypertension, and/or a central metabolic syndrome (two or more traits: obesity, dyslipidemia, or hyperinsulinemia). In addition, in 1,592 subjects attending the sixth examination (1995–1998), we measured the urine albumin/creatinine ratio (UACR). Age-, sex-, creatinine-, vitamin-, and UACR-adjusted mean homocysteine levels or proportions with homocysteine >14  $\mu\text{mol/l}$  in each phenotypic category and differences between categories were assessed with regression models.

**RESULTS** — The mean age of the subjects was 54 years (range 28–82); 55% were women, 12.3% had hyperinsulinemia, and 15.9% had two or more of the IRS phenotypes. Adjusted mean homocysteine levels were higher comparing those with hyperinsulinemia (9.8  $\mu\text{mol/l}$ ) and those without (9.4  $\mu\text{mol/l}$ ,  $P = 0.04$ ) and were higher among subjects with two or more IRS phenotypes (9.9  $\mu\text{mol/l}$ ) compared with those with 1 or no phenotype (9.3  $\mu\text{mol/l}$ ,  $P = 0.003$ ). Mean UACR levels were also higher among subjects with two or more IRS phenotypes (7.2 mg/g) compared with those with 1 or no phenotype (5.5 mg/g,  $P = 0.007$ ).

**CONCLUSIONS** — Hyperhomocysteinemia and abnormal urinary albumin excretion are both associated with hyperinsulinemia and may partially account for increased risk of CVD associated with insulin resistance. Because hyperhomocysteinemia and microalbuminuria also reflect endothelial injury, these observations also support the hypothesis that endothelial dysfunction is associated with expression of the IRS.

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**Abbreviations:** CBS, cystathione  $\beta$ -synthase; CVD, cardiovascular disease; IGT, impaired glucose tolerance; IRS, insulin resistance syndrome; MTHFR, 5,10-methylenetetrahydrofolate reductase; OGTT, oral glucose tolerance test; PLP, plasma pyridoxal 5'-phosphate; UACR, urine albumin/creatinine ratio.

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

Insulin resistance is a fundamental abnormality in the pathogenesis of type 2 diabetes and may also play a role in the development of atherosclerotic cardiovascular disease (CVD) (1,2). Insulin resistance may be an expression of diffuse arterial endothelial dysfunction contributing to atherosclerosis (3), may lead directly to arterial damage through toxic effects of hyperinsulinemia (4), or may act indirectly through atherogenic effects of the constellation of risk factors associated with the insulin resistance syndrome (IRS) (5,6). Insulin resistance or hyperinsulinemia has been associated with CVD in some but not all studies, suggesting that previously unmeasured factors may be involved in the link between hyperinsulinemia and atherosclerosis (7–12). One potential factor may be variation in plasma levels of homocysteine.

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Elevated levels of homocysteine are toxic to vascular endothelium (13), inducing endothelial dysfunction and contributing to development of atherosclerosis independent of standard CVD risk factors in diabetic (14–17) and nondiabetic subjects (18–20). Plasma levels of insulin seem to influence homocysteine metabolism, possibly through effects on glomerular filtration or by influencing activity of key enzymes in homocysteine metabolism, including 5,10-methylenetetrahydrofolate reductase (MTHFR) or cystathione  $\beta$ -synthase (CBS). (21–25) However, most data suggest that fasting homocysteine levels are similar in subjects with type 2 diabetes and nondiabetic control subjects (26–30).

Although insulin resistance (or hyperinsulinemia) and hyperhomocysteinemia may both be associated with endothelial dysfunction and CVD, associations between fasting levels of insulin and homocysteine in nondiabetic or prediabetic subjects are not well characterized. In this study, we assessed the relationship between hyperinsulinemia, phenotypes of

IRS, and levels of fasting homocysteine in the population-based Framingham Offspring Study. In addition, we examined associations between hyperinsulinemia, levels of homocysteine, and renal function as measured by urinary albumin excretion.

## RESEARCH DESIGN AND METHODS

### Study population

Participants were subjects of the Framingham Offspring Study, a community-based observational study of risk factors for CVDs (31). From January 1991 through June 1995 (examination cycle 5), 3,799 participants fasted overnight and underwent a standardized clinical examination; those without diagnosed diabetes underwent an oral glucose tolerance test (OGTT). Diabetes was defined as a fasting plasma glucose level  $\geq 7.0$  mmol/l at any two previous examinations, use of hypoglycemic drug therapy at any examination, or as a current fasting plasma glucose level  $\geq 7.0$  mmol/l or 2-h postchallenge glucose level  $\geq 11.1$  mmol/l (32). Prevalent CVD (coronary heart disease, peripheral vascular disease, and stroke) was defined as described previously (33).

Of 3,799 participants, we excluded 385 with prevalent CVD, 162 missing fasting insulin levels, and 1,038 missing fasting homocysteine or vitamin levels. These levels were missing because homocysteine and vitamin sample collection began after examination cycle 5 was well underway. Subjects with missing data were younger than those for whom data were not missing (53 vs. 55 years,  $P = 0.002$ ), but there was no difference in the distributions of sex or IRS phenotypes comparing subjects with and without study data. We further excluded 203 subjects with diabetes because diabetes is a consequence, not a feature, of IRS; a total of 2,011 subjects remained for the main analysis. An additional 419 subjects were missing levels of urine albumin/creatinine ratio (UACR), resulting in a total of 1,592 subjects for analyses that included UACR. There were no differences in age, plasma homocysteine level, or distributions of sex or IRS phenotypes comparing those with to those without UACR data.

### Laboratory methods

The total fasting homocysteine concentration in plasma was determined by high-performance liquid chromatography with fluorometric detection (34); plasma folate was measured by a microbial assay (35,36); plasma pyridoxal 5'-phosphate (PLP) was measured by the tyrosine decarboxylase apoenzyme method (37); and plasma vitamin B<sub>12</sub> was measured by radioimmunoassay (Quantaphase II; Bio-Rad, Hercules, CA). Coefficients of variation for these assays were 8% for homocysteine, 13% for folate, 16% for PLP, and 7% for vitamin B<sub>12</sub>. Total homocysteine was measured in specimens frozen for up to 4 years at  $-70^{\circ}\text{C}$ ; homocysteine levels are stable over time under these conditions (38). Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (A-gent Glucose Test; Abbott, South Pasadena, CA). Glucose assays were run in duplicate; the intra-assay coefficient of variation was  $< 3\%$ . Fasting plasma triglyceride and total cholesterol levels were measured enzymatically (39), and the HDL cholesterol fraction was measured after precipitation of LDLs and VLDLs with dextran sulfate magnesium (40). The Framingham laboratory participates in the lipoprotein cholesterol laboratory standardization program administered by the Centers for Disease Control and Prevention (Atlanta, GA). Fasting insulin was measured in EDTA plasma as total immunoreactive insulin (Coat-A-Count Insulin; Diagnostic Products, Los Angeles, CA) and calibrated to serum levels for reporting purposes. Cross-reactivity of this assay with proinsulin at midcurve is  $\sim 40\%$ ; the intra- and interassay coefficient of variation ranged from 5.0 to 10.0% for concentrations reported here, and the lower limit of sensitivity was 8 pmol/l.

Urinary albumin excretion was assessed at examination 6 (1995–1998) by the UACR. Subjects provided a single-void, early morning "spot" urine sample. The urine albumin concentration was measured by immunoturbimetry (Tinaquant Albumin Assay; Roche Diagnostics, Indianapolis, IN) and the plasma and urine creatinine concentrations using a modified Jaffe method. Coefficients of variation were 7.2% for the urine albumin assay and 2.3% for the urine creatinine assay; the lower limit of detection was 3 mg/l for albumin and 2.0 mg/dl for creatinine. The UACR is a validated, reliable,

single-sample measure of urinary albumin excretion that is highly correlated with albumin excretion rates assessed by 24-h urine collection (41–43). We classified subjects with a UACR  $< 30$  or  $\geq 30$  mg/g.

### IRS phenotype definitions

Subjects were classified with each of three basic phenotypes of the IRS using combinations of individual traits, as described previously, and then further categorized with none, one, any two, or all three of the IRS phenotypes (5). The hypertension phenotype was defined as a blood pressure  $> 140/90$  mmHg on both of two measurements or report of use of antihypertensive medication (44). The impaired glucose tolerance (IGT) phenotype was defined as impaired fasting glucose (fasting plasma glucose level  $\geq 6.1$  and  $< 7.0$  mmol/l) or impaired glucose tolerance (2-h postchallenge glucose level  $\geq 7.8$  and  $< 11.1$  mmol/l) (32). The central metabolic syndrome phenotype was defined as the presence of at least two of three central traits: obesity (either overall or central obesity), dyslipidemia (either a low HDL cholesterol level or an elevated triglyceride level), or hyperinsulinemia. Overall obesity was defined as a BMI  $\geq 27.8$  kg/m<sup>2</sup> in men or  $\geq 27.3$  kg/m<sup>2</sup> in women (the National Health and Nutrition Examination Survey II 85th percentiles), corresponding to  $\sim 120\%$  ideal body weight (45). Central obesity was defined as a waist-to-hip ratio  $> 1.0$  in men or  $> 0.9$  in women, corresponding to  $> 85\%$ th percentile in this population. Elevated triglyceride levels were defined as a fasting level  $\geq 2.51$  mmol/l, and low HDL cholesterol levels as  $\leq 0.91$  mmol/l in men or  $\leq 1.16$  mmol/l in women (46). Hyperinsulinemia was defined as a fasting insulin level exceeding the 90th percentile of its distribution among subjects with normal glucose tolerance (corresponding to a fasting serum insulin level  $> 94$  pmol/l). Elevated levels of fasting insulin serve as reasonably reliable single-sample measures of insulin resistance in nondiabetic populations as compared with insulin resistance assessed using clamp or minimal model methods (47–49).

Additional clinical characteristics assessed included cigarette smoking, defined as smoking at least one cigarette per day during the year before the examination; physical activity, assessed as a weighted sum of the proportion of a typ-

**Table 1—Study subject characteristics**

<i>n</i>	2,011
Women	54.7
Age (years)	54 (28–82)
Fasting plasma homocysteine (μmol/l)	9.5 (1.4)
Homocysteine >14 μmol/l	10.2
Folate (ng/ml)	5.6 (2.0)
Vitamin B <sub>12</sub> (pmol/l)	396.8 (1.7)
PLP (nmol/l)	61.0 (1.8)
Plasma creatinine (mg/dl)	1.0 (1.2)
UACR*	5.7 (4.5)
<30	90.4
30–300	8.9
>300	0.7
Hyperinsulinemia trait†	12.3
IRS phenotype	
None	56.3
Hypertension	12.5
IGT	4.9
CMS	10.3
Hypertension and CMS	6.0
Impaired glucose tolerance and CMS	3.3
Hypertension, IGT, and CMS	6.6

Data are inverse transformations of log (mean), log (SD), or %. \*A total of 1,592 subjects contributed UACR data; †>90th percentile fasting insulin in normal glucose tolerance. CMS, central metabolic syndrome.

ical day spent sleeping and performing sedentary, slight, moderate, or heavy physical activities (50); and extent of regular alcohol use.

### Statistical analysis

We log-transformed positively skewed levels of homocysteine, insulin, triglycerides, plasma creatinine, vitamin levels, and UACR to improve normality for

regression modeling, correlations, and statistical testing. We then inverse-transformed logged means and SEs to report mean (SE) levels of these analytes. We used Student's *t* test or the  $\chi^2$  test to compare characteristics between men and women and those with or without study data and Spearman correlation coefficients to assess crude relationships between levels of log(homocysteine) and individual IRS traits. We used linear regression models to calculate least-squares mean log (homocysteine) levels and logistic regression models (51) to predict proportions with elevated homocysteine levels (defined as a plasma level >14 μmol/l, corresponding to a level previously associated with increased risk for CVD) (52) among the various IRS phenotype combinations. Nested regression models were adjusted for age, sex, plasma levels of creatinine, folate, vitamin B<sub>12</sub>, PLP, further adjusted for levels of UACR, and further adjusted for smoking, alcohol use, and physical activity. In alternative regression models, we adjusted levels of homocysteine for sex, vitamins, and creatinine clearance using the Cockcroft-Gault equation (53). All regression models included terms for each IRS phenotype or phenotype combination; in logistic regression models, the referent group included subjects with none or one of the IRS phenotypes, and in linear regression models, type 1 error in pairwise comparisons was controlled using Tukey's test (54). The same approach was used to predict hyperinsulinemia, UACR levels, and proportions with UACR ≥30 mg/g. Analyses were performed using SAS (55). Statistical significance was defined as a two-tailed *P* value <0.05.

**RESULTS**— Framingham Offspring Study subjects are of mixed European Caucasian ethnicity; this study sample was middle-aged and comprised similar proportions of men and women (Table 1). Men had higher mean levels of homocysteine than women (10.4 vs. 8.8 μmol/l, *P* ≤ 0.0001), but the distribution of IRS phenotypes among men and women was similar (*P* = 0.2). There was no interaction by sex on the effect of IRS phenotype predicting homocysteine levels (*P* = 0.3 for first-order interaction terms); therefore, overall sex-adjusted results are presented.

In this nondiabetic population, 15.9% of subjects had at least two IRS phenotypes, and 6.6% had all three phenotypes (Table 1). The overall prevalence of hyperinsulinemia was 12.3%, and subjects with the central metabolic syndrome alone or in combination with other phenotypes had higher fasting insulin levels than those without the central metabolic syndrome phenotype (Table 2). Subjects with ≥2 phenotypes had significantly higher fasting insulin levels (76 pmol/l) compared with those with ≤1 phenotype (31 pmol/l, *P* < 0.0001).

Correlations between levels of individual IRS traits and homocysteine, in descending order of magnitude, were modest overall: waist-to-hip ratio *r* = 0.24 (*P* = 0.0001), systolic blood pressure *r* = 0.16 (*P* = 0.0001), BMI *r* = 0.12 (*P* = 0.0001), HDL cholesterol *r* = -0.11 (*P* = 0.0001), fasting glucose *r* = 0.11 (*P* = 0.0001), fasting insulin *r* = 0.07 (*P* = 0.001), diastolic blood pressure *r* = 0.07 (*P* = 0.001), fasting triglycerides *r* = 0.03 (*P* = 0.3), and 2-h postchallenge glucose *r* = 0.01 (*P* = 0.6). Fasting homo-

**Table 2—Fasting insulin and urine albumin/creatinine levels by IRS phenotype**

	IRS phenotypes						
	None	HTN only	IGT only	CMS only	CMS and HTN	CMS and IGT	CMS, HTN, and IGT
<i>n</i>	1,133	252	99	208	120	67	132
Mean fasting serum insulin (pmol/l)	26 (6.1) 6	35 (6.3)* 6	31 (6.5) 6	64 (6.3)* 6	83 (6.4)* 6	85 (6.6)* 6	67 (6.4)*
Hyperinsulinemia	2.2	3.6	2.2	30.2*	50.1*	56.6*	37.0*
Number with UACR	900	195	73	167	95	53	109
Mean UACR (mg/g)	5.3 (1.1)	7.0 (1.1)	6.1 (1.2)	4.5 (1.1)	6.2 (1.1)	7.0 (1.2)	8.5 (1.2)††
UACR >30 mg/g	7.6	11.3	8.4	8.0	13.8†	16.1†	16.9§†

Data are inverse transformations of log (mean), log (SD), or % adjusted for age and sex. HTN, hypertension; CMS, central metabolic syndrome (any two or all of obesity, dyslipidemia, or hyperinsulinemia). Hyperinsulinemia = fasting insulin >90th percentile in normal glucose tolerance. Pairwise statistical comparisons with subjects with no IRS phenotypes as the reference group are shown for insulin and UACR; other pairwise comparisons are shown for UACR only \**P* < 0.0001; †*P* ≤ 0.05 compared with no IRS phenotypes; ‡*P* ≤ 0.05 compared with CMS only; §*P* < 0.001.

Table 3—Plasma homocysteine levels by IRS phenotype

	IRS phenotypes						
	None	HTN only	IGT only	CMS only	CMS and HTN	CMS and IGT	CMS, HTN, and IGT
<i>n</i>	1,133	252	99	208	120	67	132
Mean homocysteine levels							
Age- and sex-adjusted	9.3	10.2‡	9.4	9.4	9.8	9.9	9.7
Age-, sex-, creatinine-, and vitamin-adjusted	9.3	9.9†	9.4	9.4	10.0	9.9	9.8
Sex-, vitamin-, and Cockcroft-Gault equation-adjusted	9.1	10.1§	9.6	9.5	10.3§	10.2*	10.2§
Percentage with homocysteine >14 μmol/l							
Age- and sex-adjusted	8.8	14.2†	7.9	8.3	13.8	17.6*	11.1
Age-, sex-, creatinine-, and vitamin-adjusted	8.9	12.6*	8.1	8.2	14*	17.9†	13.2
Sex-, vitamin-, and Cockcroft-Gault equation-adjusted	8.0	13.4†	8.5	9.3	17.0†	20.2†	16.6†

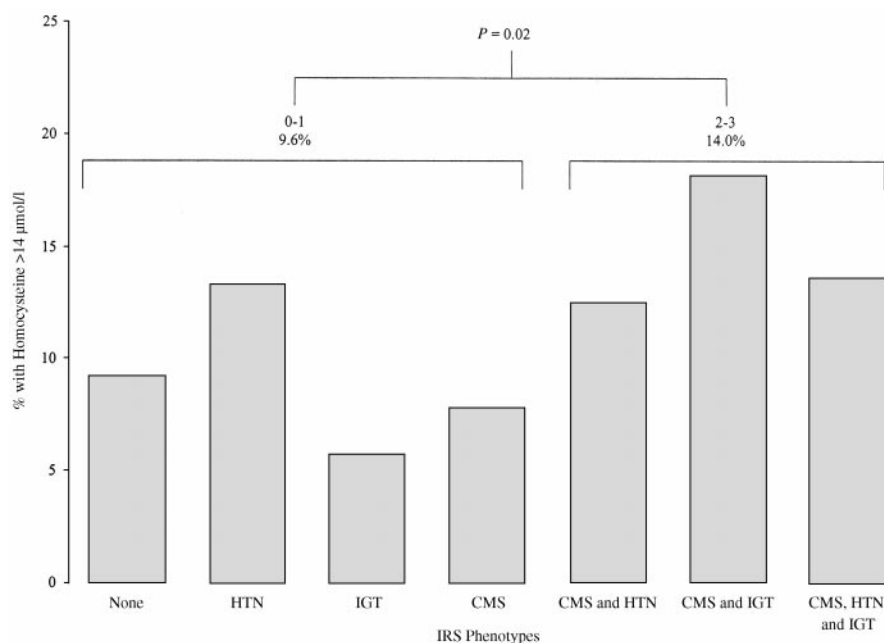
Data are inverse transformations of log (mean), log (SD), or % adjusted as indicated (vitamins = folate, B<sub>12</sub>, and PLP levels). HTN, hypertension; CMS, central metabolic syndrome (any two or all of obesity, dyslipidemia, or hyperinsulinemia). Pairwise statistical comparisons are shown. \**P* ≤ 0.05 compared with no IRS phenotypes; †*P* < 0.01; ‡*P* < 0.001; §*P* < 0.0001; ||*P* ≤ 0.05 compared with CMS only; ¶*P* ≤ 0.05 compared with IGT only.

cysteine was more strongly correlated with plasma creatinine ( $r = 0.27$ ,  $P = 0.0001$ ) than with UACR ( $r = -0.03$ ,  $P = 0.3$ ).

Associations between IRS phenotypes and homocysteine levels or proportions with elevated levels are shown in Table 3. Subjects with the hypertension phenotype alone had higher age-, sex-, creatinine-, and vitamin-adjusted mean homocysteine levels than those without any IRS phenotype (9.9 vs. 9.3 μmol/l,  $P = 0.01$ ), but otherwise, there were no significant pairwise differences in mean levels across phenotypes. Groups of subjects with the hypertension phenotype alone, in combination with the central metabolic syndrome phenotype, or those with the central metabolic syndrome and IGT phenotype together had greater proportions with elevated homocysteine levels compared with those with no IRS phenotype (all  $P \leq 0.05$ ); groups with the central metabolic syndrome and IGT phenotypes together had greater proportions with an elevated homocysteine level than those with either phenotype in isolation (all  $P \leq 0.02$ ). Removal of terms for creatinine and vitamins from predicting models had a negligible effect on the results; alternative adjustment using the Cockcroft-Gault equation somewhat strengthened associations between more complete expression of the IRS and homocysteine levels (Table 3). Associations also remained significant after additional adjustment for UACR (Fig. 1); groups with two or three phenotypes of the IRS had greater proportions of subjects with elevated homocysteine levels compared

with groups with none or one of the phenotypes (14.0 vs. 9.6%,  $P = 0.02$ ), as well as higher mean homocysteine levels (9.9 vs. 9.3 μmol/l,  $P = 0.003$ ). There was no interaction by category of UACR on the association between IRS phenotypes and homocysteine levels ( $P = 0.8$  for first-order interaction term), and results were not different when models were further adjusted for cigarette smoking, alcohol use, and physical activity (not shown).

As an individual trait, hyperinsulinemia was weakly associated with variation in homocysteine levels. Subjects with a fasting insulin level >94 pmol/l had slightly higher adjusted mean homocysteine levels (9.8 μmol/l) compared with those below this threshold (9.4 μmol/l,  $P = 0.04$ ). We also compared homocysteine levels in the 113 excluded subjects with type 2 diabetes and UACR data with levels in the 1,592 nondiabetic subjects.



**Figure 1**—Age-, sex-, plasma creatinine-, vitamin-, and urine albumin/creatinine ratio-adjusted proportions with elevated homocysteine levels (>14 μmol/l) according to IRS phenotype. HTN, hypertension phenotype; CMS, central metabolic syndrome phenotype (≥2 of obesity, dyslipidemia, or hyperinsulinemia); IGT, impaired glucose tolerance phenotype.

Adjusted levels were similar comparing diabetic (9.6  $\mu\text{mol/l}$ ) with nondiabetic subjects (9.4  $\mu\text{mol/l}$ ,  $P = 0.5$ ).

Urinary albumin excretion was also related to combinations of IRS phenotypes (Table 2). Groups with all three phenotypes had higher age- and sex-adjusted UACRs than those with no phenotypes (8.5 vs. 5.3 mg/g,  $P = 0.03$ ) as well as a greater proportion with UACR  $>30$  mg/g (16.9 vs. 7.6%,  $P = 0.001$ ). Overall, groups with two or three phenotypes of the IRS had higher mean UACRs than groups with none or one of the phenotypes (7.2 vs. 5.5 mg/g,  $P = 0.007$ ) and a greater proportion with UACR  $>30$  mg/g (15.5 vs. 8.4%,  $P = 0.0003$ ). As an individual trait, groups with hyperinsulinemia tended to have a greater proportion of subjects with UACR  $>30$  mg/g (13.2%) compared with those without hyperinsulinemia (9.1%,  $P = 0.07$ ). Urinary albumin excretion was not significantly associated with variation in plasma homocysteine levels. Compared with subjects with UACR  $\leq 30$  mg/g, those with UACR  $\geq 30$  mg/g had similar multivariate-adjusted mean levels of homocysteine (9.4 vs. 9.7  $\mu\text{mol/l}$  with UACR  $>30$ ,  $P = 0.2$ ) and proportions with elevated homocysteine levels (10.2 vs. 11.4%,  $P = 0.6$ ).

**CONCLUSIONS**— Interest in associations between plasma homocysteine and insulin resistance has been spurred by the search for novel metabolic factors accounting for the disturbing excess burden of CVD in type 2 diabetes and IGT (56). Although elevated homocysteine levels increase risk for CVD in type 2 diabetes patients to a greater extent than among nondiabetic subjects (14), fasting levels of homocysteine per se do not seem to be different comparing subjects with type 2 diabetes to nondiabetic control subjects in our study and in others (26–30) but not in all studies (57). Similar homocysteine levels in diabetes could be due to methodological differences across studies in accounting for renal function. In type 2 diabetes, glomerular filtration rate varies substantially with duration of disease (58) and is a critical determinant of plasma homocysteine levels (59,60). Data are relatively sparse on variations in homocysteine levels among subjects at increased risk for type 2 diabetes or CVD.

In this analysis of nondiabetic participants without CVD in a large, communi-

ty-based population study, we found positive associations between fasting levels of plasma homocysteine and some individual traits associated with insulin resistance. Elevated levels of fasting insulin were modestly but significantly associated with fasting homocysteine, even after adjustment for several important confounders. After grouping related traits into clinical phenotypes of the IRS, mean homocysteine levels or proportions with elevated levels were not dramatically different comparing groups with different phenotypes, although groups with two or more phenotypes had significantly higher levels and greater proportions of subjects with elevated levels than groups with only one or no IRS phenotypes. Our findings suggest that insulin resistance itself was modestly associated with elevated homocysteine levels; moreover, the co-occurrence of specific features of the IRS, especially hypertension and central obesity, was associated with more marked elevations in homocysteine levels.

We also found abnormal levels of urinary albumin excretion to be a feature of the IRS, confirming findings in some (61–63) but not all (64) epidemiological studies. Elevated UACR was also positively correlated with serum creatinine (59,65), but control for UACR and serum creatinine did not alter associations between IRS phenotypes and homocysteine levels. However, an important limitation of our study is that UACR and serum creatinine are probably not adequately sensitive markers of true glomerular filtration rate among subjects with normal renal function (59,60). Alternative adjustment for creatinine clearance using the Cockcroft-Gault equation did not weaken but actually slightly strengthened associations between IRS phenotypes and homocysteine levels; however, validity of this equation to adjust for renal function in populations different from that in which it was originally developed is questionable (66). Also, we measured UACR  $\sim 4$  years after assessment of homocysteine levels and IRS phenotypes, further attenuating its ability to adequately control for renal dysfunction. It is possible that valid control for true glomerular filtration rate would eliminate any association between homocysteine levels and insulin resistance. This result would suggest an alternative hypothesis that insulin resistance is associated with subtle abnormalities in renal function and that modest elevations in

homocysteine levels reflect subclinical renal dysfunction. The recent demonstration that hyperhomocysteinemia predicts development of microalbuminuria supports this hypothesis (67).

Abnormal renal function in insulin resistance may be a consequence of diffuse subclinical vascular injury and endothelial dysfunction (68). Elevated plasma levels of homocysteine may contribute to endothelial dysfunction by diverse mechanisms, including accelerated generation of reactive oxygen species that directly damage endothelial cells, exposing the subendothelial matrix and creating a prothrombotic environment; by impairing nitric oxide-dependent vasodilatation; and by enhancing oxidation of LDL cholesterol. (13) These mechanisms lead to atherosclerosis of the large arteries and (alone or in combination with the atherogenic effects of hypertension, smoking, and hyperlipidemia) to the subsequent expression of clinical CVD (18–20). Insulin resistance has been proposed to arise from similar pathogenic mechanisms in the peripheral arteriolar and capillary beds, with endothelial dysfunction in skeletal muscle, liver, and adipose tissue, and the kidney giving rise to insulin resistance and to diverse features of the IRS (3,69). This hypothesis provides a plausible mechanism directly linking hyperhomocysteinemia with insulin resistance. However, the direction of causality in this association is not clear. Hyperhomocysteinemia can induce insulin resistance (13), leading to compensatory hyperinsulinemia, which may impair activity of the MTHFR and CBS enzymes, leading to accumulation of homocysteine in plasma (25). Therefore, insulin resistance and hyperhomocysteinemia may create a deleterious feedback loop, each promoting the development and propagation of the other. Whereas the cross-sectional nature of our analysis precludes assigning cause or effect to insulin resistance or hyperhomocysteinemia, the observation that homocysteine levels are elevated in proportion to the number of phenotypes of the IRS (reflecting progressively greater insulin resistance) is consistent with a dose-response relationship between insulin resistance and homocysteine levels.

Another limitation of our study includes the lack of direct measurement of insulin resistance. Although elevated fasting insulin levels are highly correlated with insulin resistance assessed using

more direct measurements, especially among normal glucose-tolerant subjects (47,48), our approach may have produced some misclassification by insulin resistance status, which would weaken observed associations and produce an underestimate of the effect of insulin resistance on homocysteine levels. The few studies in which insulin resistance in nondiabetic subjects has been assessed directly have shown either moderate positive associations with homocysteine levels (21) or no association (21,70,71). We do not have data on postmethionine challenge plasma homocysteine levels; these may be higher in patients with type 2 diabetes than in nondiabetic control subjects (27). Postchallenge homocysteine levels might be more closely associated with insulin resistance than fasting levels, given that hyperinsulinemia may affect MTHFR and CBS activity (25); the influence on plasma homocysteine levels of activity of these enzymes is most apparent with methionine challenge (72). Also, we do not have data on genetic variation in MTHFR or CBS; variation in MTHFR has been associated with a greater degree of CVD in diabetes (73) and might modify the effect of insulin resistance or hyperinsulinemia on plasma levels of homocysteine.

In conclusion, we found modest associations between hyperinsulinemia, reflecting insulin resistance, and fasting levels of plasma homocysteine. Mean homocysteine levels, or the likelihood of clinically important elevations in homocysteine levels (52), were highest among subjects with fully expressed IRS (subjects with two or three of its component phenotypes). Elevated urinary albumin excretion was also a feature of the IRS, but control for renal function using UACR and serum creatinine did not weaken associations between insulin resistance and hyperhomocysteinemia. Our data suggest that clinically important hyperhomocysteinemia and subtly abnormal renal function are both features of the IRS, potentially accounting for some of the increased risk for CVD associated with insulin resistance. These observations are also consistent with the hypothesis that endothelial dysfunction is associated with expression of the IRS. Our findings may have implications for clinical prevention. In addition to regular physical exercise, diets or dietary supplements rich in folate and B vitamins may be beneficial for

lowering plasma homocysteine levels, improving insulin sensitivity, and reducing risk for development of type 2 diabetes or CVD. Controlled clinical trials are required to demonstrate definitively an association between reduced homocysteine levels, improved insulin sensitivity, and prevention of CVD outcomes.

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