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Associations between serum insulin and homocysteine in a Swedish population—a potential link between the metabolic syndrome and hyperhomocysteinemia: The Skaraborg project

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Abstract

The objective of this study is to examine the association between serum levels of insulin and homocysteine (Hcy) in a population-based sample of Swedish men and women. Men and women (537 and 571, respectively) 40 years or older, who were randomly selected from the population in Skara, southwestern Sweden, with valid information on serum levels of Hcy and insulin, were subject to a physical examination, including anthropometric measurement. Lifestyle factors were assessed by a questionnaire, and venous blood samples were drawn after an overnight fast. Insulin resistance was estimated by the homeostasis model assessment index. Homocysteine was higher in men (11.0 μmol/L) than in women (9.7 μmol/L) ($P < .001$) and was positively associated with age ($P < .001$ in both sexes) and serum creatinine ($P = .009$ in men, $P < .001$ in women), but inversely associated with leisure time physical activity ($P = .012$ in men, $P = .001$ in women). There was a positive association between serum insulin and serum Hcy independent of age and sex ($P = .004$). Upon exclusion of patients with diabetes and individuals with serum creatinine level greater than 130 μcat/L, this association was significant in the remaining 999 individuals also after adjustment for age, sex, serum creatinine, leisure time physical activity, body mass index, and smoking status ($P = .003$). A 1 SD difference in serum insulin corresponded to a difference of 0.5 μmol/L in serum Hcy. A similar association was found between insulin resistance and serum Hcy. In conclusion, there is an association between serum insulin and Hcy that may constitute a link between the metabolic syndrome and Hcy, either unilaterally or as part of a vicious circle.

1. Introduction

Over the past decade, the amino acid homocysteine (Hcy) has attracted much medical attention. It has been established as an independent risk factor or risk marker [1] for several illnesses, such as atherosclerosis [2,3], neural tube defects of the unborn [4], Alzheimer's disease, and other neurologic pathologies [5]. It has been suggested that hyperhomocysteinemia confers an independent risk for cardiovascular disease comparable to that of smoking and hyperlipidemia [6].

Normally, serum levels of Hcy are kept low by continuous turnover to either methionine or cysteine [7] (Fig. 1). Known risk factors for hyperhomocysteinemia are poor diet (lack of folate, vitamin B_{12}, or vitamin B_6), genetic variations (default in cystatin B synthetase or 5,10-methylene tetrahydrofolate reductase), impaired kidney function, certain carcinomas, hypothyroidism, certain medications, and smoking [8]. The atheropathology of hyperhomocysteinemia has been proposed to result from oxidizing effects on low-density lipoprotein (LDL) [8] as well as auto-oxidation of Hcy, leading to increases in endothelium-damaging hydrogen peroxide [9-11]. Many studies have investigated the physiologic background of serum Hcy levels. In addition to inadequate nutrition and deficiencies in vitamins, also kidney function, smoking, and coffee consumption have been proved determinants of high Hcy [12]. A Norwegian epidemiological study showed an inverse association between Hcy and leisure time physical activity (LTPA) [13], a finding that was recently confirmed by a study in patients with type 2 diabetes mellitus within our project [14].

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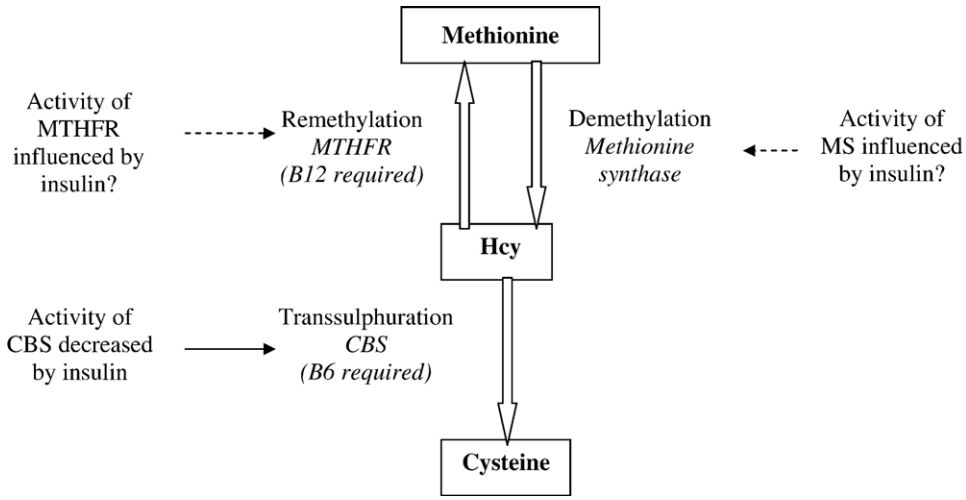

Fig. 1. The metabolism of Hcy. CBS indicates cystathionine β-synthase; MTHFR, 5,10-methylene tetrahydrofolate reductase; MS, methionine synthase.

Several, although not all, earlier studies have also found associations between diabetes itself and Hcy [15-18]. Research in rat models has proposed that insulin alters the activity of metabolic enzymes involved in the turnover of Hcy [19]. In 1998, the first study in humans exploring such a connection showed a positive association between serum insulin and Hcy levels [20]. Since then, several similar studies have been performed; however, most have been based on small population samples or have been restricted to diabetic patients. To our knowledge, only 2 other large-scale epidemiological studies exist to date. Of these, one in Mexican American men failed to show an association between Hcy and diabetes status or fasting serum insulin [21], whereas the other, from the Framingham Offspring Study, did show a moderate association between components of the metabolic syndrome and Hcy levels [22]. In the current study, we thus aimed to investigate the association between serum insulin and plasma levels of Hcy in a Swedish community, and to explore the impact of lifestyle factors and kidney function on this association.

2. Research design and methods

2.1. Subjects

The Skara Population Study was conducted in 1993 to 1994 as an age-stratified randomized sample of both men and women 40 years or older [23]. Of all the invited, 1109 (80%) accepted to join the study. Of these, 1 man lacked sample for serum insulin and serum homocysteine, and he was excluded from the present study population, leaving 537 men and 571 women to be further explored. Mean age was 63.0 years (range, 40-90 years) in men and 62.7 years (range, 40-90 years) in women.

The study protocol was approved by the regional ethical review board at the University of Göteborg, Göteborg, Sweden. All participants gave informed consent before enrolment.

2.2. Methods

In the morning, after an overnight 10-hour fast, all individuals were seen at the Skara Health Care Centre, Skara, Sweden, and examined by specially trained nurses. The survey has been presented in more detail previously [24]. A standard physical examination recorded standing heart rate, and systolic and diastolic (phase V) blood pressure to the closest 2 mm Hg. Measurements were made in the right brachial artery with the arm at heart level using a cuff with automatic adjustment for arm circumference [25]. Height (no shoes, nearest centimeters), weight (light indoor clothing, nearest 0.1 kg), and waist-hip ratio (nearest centimeters) were also recorded.

Serum creatinine was analyzed at the local hospital laboratory (Kärnsjukhuset, Skövde, Sweden). Serum lipid analyses were performed at the Lipids Laboratory, Lund University Hospital, Lund, Sweden. Serum insulin and plasma Hcy were analyzed at the Wallenberg Laboratory, Malmö University Hospital, Malmö, Sweden. Serum insulin was analyzed using enzyme-linked immunosorbent assay with less than 0.3% cross-reactivity for proinsulin (kit from DRACO Diagnostics, Ely, Cambridgeshire, UK) [26]. Analysis of plasma Hcy measured the total concentration of all Hcy forms, using high-performance liquid chromatography with fluorescence detection (reference range < 18 μmol/L) [27].

Diagnostic criteria for diabetes mellitus followed World Health Organization recommendations [28], and this procedure has been described in detail previously [24]. Homeostasis model assessment (HOMA) of insulin resistance (IR) was computed from fasting glucose and insulin concentrations using the HOMA method [29]. Because the HOMA method is not applicable to insulin-treated subjects, 13 individuals receiving insulin treatment were excluded from HOMA analyses.

A previously tested self-administered questionnaire [24] filled out at the clinic provided information regarding smoking habits and LTPA. Regarding LTPA level, subjects

Table 1
Descriptive characteristics of the study population

Variables (units)	Men (n = 537)	Women (n = 571)	P
	Mean (SD)	Mean (SD)	
Age (y)	63.0 (13.0)	62.7 (13.0)	.707
Hcy (μmol/L)	11.0 (4.4)	9.7 (4.4)	<.001
Waist circumference (cm)	95 (10.1)	84 (10.1)	<.001
Waist-hip ratio (cm)	0.93 (0.1)	0.81 (0.1)	<.001
BMI (kg/m^2)	26.3 (4.1)	26.5 (4.1)	.588
Systolic blood pressure (mm Hg)	137 (18.4)	139 (18.4)	.074
Diastolic blood pressure (mm Hg)	78 (10.1)	75 (10.1)	<.001
Creatinine (μcat/L)	96 (12.8)	84 (12.8)	<.001
Triglycerides (mmol/L)[a]	1.3 (7.1)	1.1 (7.1)	<.001
Total cholesterol (mmol/L)	5.9 (1.1)	6.1 (1.1)	.020
HDL-C (mmol/L)	1.0 (0.3)	1.2 (0.2)	<.001
LDL-C (mmol/L)	4.3 (1.0)	4.3 (1.0)	.357
Serum insulin (mU/L)[a]	5.3 (7.6)	5.0 (7.6)	.134
HOMA-IR[a]	1.11 (1.3)	1.01 (1.4)	.055

Means were adjusted for age difference. Because of missing values, 0 to 17 men and women were excluded per analysis. HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] Geometric means used because of skewed distributions.

chose from 4 options: (1) leisure time mainly spent reading and watching television; (2) soft physical activity, such as walking or bicycling, at least 4 hours per week; (3) moderate physical exercise, such as running, swimming, playing tennis, or the equivalent, at least 2 h/wk; and (4) regular hard exercise or competitive sport activities. Because of the low number of subjects in group 4, this group was combined with group 3 for statistical analysis. Regarding smoking, 3 groups were posited: those never having been smokers, ex-smokers, and current smokers.

2.3. Statistical analyses

All statistical analyses were performed using the SPSS program (SPSS system for Windows 11.5; SPSS, Chicago, IL). Standard methods were used for the descriptive statistics. Proportions were standardized according to the complete population, 40 years and older, in Skara using 10-year age categories. Means were adjusted for differences in age between groups with general linear model (GLM). Differences in mean levels of continuous variables between groups were analyzed with GLM and trends across groups with linear regression analysis adjusting for differences in age and other variables as specified. Associations between Hcy and both serum insulin and HOMA-IR were computed with multiple linear regressions with outcomes presented using the regression coefficient and corresponding confidence interval (CI). Because of skewed distributions, serum insulin, HOMA-IR, and serum triglycerides were log transformed for analyses. Confounding from sex was accounted for by stratification or by adjustment for sex differences in multiple regression analyses. All tests were 2-sided, and statistical significance was assumed at $P < .05$.

3. Results

3.1. Characteristics of study group

Descriptive characteristics of the population sample are presented in Table 1. Plasma Hcy was significantly higher in men (11.0 ± 4.4 μmol/L) than in women (9.7 ± 4.4 μmol/L) ($P < .001$). Table 2 shows the successive increase in Hcy levels by 10-year age groups in men and in women, both P for trend of less than .001. A corresponding, but weaker, trend was seen for serum insulin in both sexes.

3.2. Mean Hcy levels in lifestyle subgroups

Table 3 presents Hcy means in subgroups according to lifestyle factors, serum creatinine, and obesity. In both sexes, there were significant dose-related associations between LTPA and plasma Hcy (inverse) and between serum creatinine levels and Hcy (positive). Body mass index (BMI) and other obesity markers (waist circumference and waist-hip ratio) were not significant determinators of Hcy levels. However, levels of Hcy were highest in obese subjects in both men and women.

3.3. Associations between serum insulin and Hcy levels

There was a significant direct association between serum insulin and Hcy levels, independent of age and sex ($P = .007$). The association remained significant after further adjustment for serum creatinine, LTPA, BMI, and smoking status one by one; however, whenever 2 of these covariates were entered in the same model, the significance disappeared. However, upon exclusion of patients with diabetes

Table 2
Serum Hcy levels (μmol/L) and serum insulin levels (mU/L) in 10-year age groups in men and women, respectively

Age groups	n	%	Homocysteine	Serum insulin
			Mean (SD)	Mean (SD)
Men (n = 537)				
40 to <50	112	20.8	9.4 (3.7)	4.6 (6.0)
50 to <60	117	21.7	10.4 (6.5)	5.0 (6.0)
60 to <70	126	23.4	10.8* (4.6)	5.9** (6.0)
70 to <80	125	23.2	12.4*** (5.7)	5.8** (6.0)
≥80	58	10.8	13.5*** (4.8)	5.1 (6.0)
Test for trend			$P < .001$	$P = .033$
Women (n = 571)				
40 to <50	117	20.5	8.1 (2.6)	4.5 (8.9)
50 to <60	131	22.9	8.9 (3.0)	4.5 (9.0)
60 to <70	139	24.3	9.0* (2.1)	4.8 (8.9)
70 to <80	116	20.3	10.8*** (4.2)	6.0** (9.0)
≥80	68	11.9	13.6*** (5.4)	5.4 (8.9)
Test for trend			$P < .001$	$P = .002$

Differences in means between age groups were analyzed by GLM. Linear regression was used to determine trend across different age groups.
* $P < .05$ for difference compared with the reference group (aged 40 to <50 years).
** $P < .01$ for difference compared with the reference group (aged 40 to <50 years).
*** $P < .001$ for difference compared with the reference group (aged 40 to <50 years).

(n = 97) and individuals with serum creatinine level greater than 130 μcat/L (n = 19), this association was significant in both men (P = .041) and women (P = .002) and remained after adjustment for age, sex, creatinine, LTPA, BMI, and smoking status (P = .003; regression coefficient, 1.57; 95% CI, 0.54-2.59) (Table 4). In absolute terms, a difference of 1 SD in serum insulin corresponded to a difference of 0.5 μmol/L in serum Hcy. Similar results were obtained when only patients with diabetes but not those with serum creatinine greater than 130 μcat/L were excluded. When HOMA-IR was substituted for serum insulin, the same significant pattern was revealed.

Table 3
Mean serum Hcy levels (μmol/L) in subgroups according to LTPA, kidney function, smoking status, and BMI category in men and women

	Serum Hcy	
	n (%)	Mean (SD)
Men (n = 537)		
LTPA		
Moderate to high	156 (31.7)	10.2 (5.23)
Light	303 (58.1)	11.4* (5.20)
None	54 (10.2)	11.9* (5.26)
Test for trend (adjusted for age)		P = .012
Test for trend (adjusted for age, creatinine)		P = .015
Serum creatinine (quartiles)		
≤80 μcat/L	43 (8.1)	10.5 (5.12)
81-88 μcat/L	101 (19.0)	10.6 (5.15)
89-98 μcat/L	186 (35.7)	10.4 (5.13)
>98 μcat/L	207 (37.2)	12.0 (5.18)
Test for trend (adjusted for age)		P = .009
Test for trend (adjusted for age, LTPA)		P = .012
Smoking status		
Never smoked	205 (37.2)	11.0 (5.19)
Ex-smoker	210 (39.2)	10.8 (5.17)
Current smoker	118 (23.6)	11.6 (5.21)
Test for trend (adjusted for age)		P = .598
BMI categories (WHO)		
<25 kg/m^2 (normal)	186 (35.3)	11.3 (5.12)
25 to <30 kg/m^2 (overweight)	285 (52.8)	10.6 (5.12)
≥30 kg/m^2 (obese)	62 (11.9)	12.4 (5.12)
Test for trend (adjusted for age)		P = .658
Women (n = 571)		
LTPA		
Moderate to high	99 (17.7)	9.4 (3.44)
Light	404 (71.5)	9.5 (3.41)
None	58 (10.8)	11.9** (3.59)
Test for trend (adjusted for age)		P = .001
Test for trend (adjusted for age, creatinine)		P = .003
Serum creatinine (quartiles)		
≤80 μcat/L	256 (45.0)	8.9 (3.29)
81-88 μcat/L	167 (29.8)	9.4 (3.25)
89-98 μcat/L	100 (16.8)	10.7** (3.27)
>98 μcat/L	48 (8.4)	13.3** (3.37)
Test for trend (adjusted for age)		P < .001
Test for trend (adjusted for age, LTPA)		P < .001
Smoking status		
Never smoked	350 (59.7)	9.5 (3.53)
Ex-smoker	117 (21.4)	9.9 (3.49)
Current smoker	103 (18.9)	10.1 (3.55)
Test for trend (adjusted for age)		P = .115
BMI categories (WHO)		
<25 kg/m^2 (Normal)	235 (42.5)	9.5 (3.47)
25 to <30 kg/m^2 (overweight)	221 (38.2)	9.9 (3.46)
≤30 kg/m^2 (obese)	112 (19.3)	10.0 (3.46)
Test for trend (adjusted for age)		P = .157

Differences in means were analyzed by GLM with age as covariate. Multiple linear regressions were used to test for trend of difference in Hcy across categories. WHO indicates World Health Organization.

* P < .05 for difference compared with the reference group (first group).

** P < .001 for difference compared with the reference group (first group).

Table 4
Associations between serum insulin (independent variable) and Hcy (dependent variable) after exclusion of subjects with diabetes and/or creatinine values greater than 130 μcat/L (n = 999)

	Regression coefficient[a] (95% CI)	P
Serum insulin (log)	2.02 (1.08-2.95)	.000
Adjusted for age and sex	1.58 (0.69-2.47)	.001
Adjusted for age, sex, and creatinine	1.45 (0.57-2.32)	.001
Adjusted for age, sex, and LTPA	1.43 (0.50-2.35)	.003
Adjusted for age, sex, and BMI	1.61 (0.60-2.62)	.002
Adjusted for age, sex, and smoking status	1.66 (0.76-2.55)	.000
Adjusted for age, sex, creatinine, and LTPA	1.57 (0.54-2.59)	.003

Associations between serum insulin and Hcy were estimated using multiple linear regressions. For this sample, the association was subsignificant whenever any 2 of the variables serum creatinine, LTPA, BMI, or smoking status were entered into the same model together with age and sex.

[a] Regression coefficient for the association between serum insulin (independent variable) and Hcy (dependent variable).

4. Discussion

The main finding of this study was the significant independent association between serum insulin and Hcy levels. Thus, Hcy may be dependent on serum insulin, providing a potential link between the metabolic syndrome and hyperhomocysteinemia.

First, we explored the association between serum insulin and Hcy in the full population sample. When this association was confirmed, we excluded subjects with diabetes and those with serum creatinine level greater than 130 μcat/L, and the association between serum insulin and Hcy was strengthened. Patients with diabetes were excluded also in the 2 previous large-scale epidemiological studies [21,22]. Physiologic associations between insulin and Hcy are disturbed by the diabetic condition and by its treatment [30]. The rationale for excluding individuals with high serum creatinine levels is that the impaired renal function indicated by high creatinine values tends to increase Hcy levels because of disruption of kidney metabolism and clearance of Hcy independent of insulin levels [31,32].

The cross-sectional nature of this study makes it impossible to determine any causal relationships, or the direction of any associations. However, 2 plausible theories could explain our findings. Experimental research on

induced diabetes in rat models or cell cultures [19,33-36] indicates that insulin levels affect the activity of enzymes that are critical in the metabolism of Hcy. This has been shown most consistently for the enzyme cystathionine β-synthase, which converts Hcy to cysteine (Fig. 1). In the rat models, insulin causes a reduction of cystathionine β-synthase, leading to Hcy accumulation. In addition, enzymes 5,10-methylene tetrahydrofolate reductase and methionine synthase have been shown to vary with insulin levels, although here, the evidence is controversial [19,34,36]. These experimental findings in rat models are consistent with our results. According to the alternative theory, interactions go the other way. Elevated levels of Hcy are damaging to the endothelium, through generation of reactive oxidative chemicals, hampering of the vasodilating function of nitric oxide, and oxidation of LDL cholesterol [8-11]. Possibly, this endothelial damage also increases IR, which is known to result from similar endothelial changes [37]. This IR will then lead to elevated insulin levels, still in accordance with our findings.

In both cases, IR seems to be an important factor. The increase in insulin after IR will affect Hcy metabolism according to the first model [36], or high Hcy levels will induce IR according to the second model [22]. In our study, an association between IR and Hcy similar to that of insulin was indeed found consistent with the existence of a vicious circle. Thus, part of the dangers of Hcy might be explained by its link with IR. Two American large-scale epidemiological studies, by Meigs et al [22] and by Gillum [21], have previously explored the issue. Like our study, Meigs et al found a significant association between serum insulin and Hcy levels. However, in his study of Mexican American men, Gillum found no such association. This might be explained by that study's smaller sample size or by the genetic, cultural, and socioeconomic differences between Mexican Americans and our Swedish rural population.

Other results in our study include the clear-cut, dose-response inverse relationship between Hcy and LTPA. This is well in keeping with earlier studies [13,14,38], but these offer no explanation for the finding. Along the lines of the hypothesis mentioned, focusing on IR giving high insulin levels that alter the metabolism of Hcy, the association to LTPA might be consistent with LTPA counteracting IR [39,40]. Furthermore, exercise-aware individuals also tend to keep a good nutrition, and it is well known that a high intake of folate and vitamins B_{12} and B_6 counteracts high Hcy levels [12]. An association between smoking and Hcy has been described by several research teams [12,41,42]. In our study, we found no such association. Partly, the reason could be that these studies graded smokers depending on daily cigarette consumption, whereas our study only discerned among smokers, ex-smokers, and nonsmokers. Furthermore, the prevalence of smoking in our study sample was comparatively low. These circumstances may have reduced the power to detect any differences associated with smoking, and the possibility of a type 2 error should be considered. Like in many previous studies, BMI and other markers for obesity were not significant determinants of Hcy [21,43-45]. We cannot, however, conclusively rule out an association because levels of Hcy were highest in obese subjects in both men and women, and the possibility of insufficient power should be considered.

The strength of this article is its community-based epidemiological setting. The population sample is larger and the participation rate higher than in many previous studies. In addition, whereas there are several studies examining Hcy levels in Swedish elderly individuals with regard to diet and medication [46-48], this is the first to show descriptive figures for Hcy levels in a defined Swedish population representing the whole adult population. One limitation is the lack of information on vitamin status. A high intake of folate and vitamins B_6 and B_{12} is known to correlate with low plasma Hcy [12] because these vitamins play a role in the metabolism of Hcy (Fig. 1). For the association between LTPA and Hcy, in particular, vitamin status could be a confounder because those with a healthy lifestyle also tend to eat healthier [49].

In conclusion, in this epidemiological study, we found a significant association between Hcy and both serum insulin and HOMA-IR, thus, linking Hcy and the metabolic syndrome. This linkage is also suggested by the inverse associations to LTPA. More research is needed to elucidate the physiologic mechanisms mediating these effects. This study is also consistent with the possibility that the atherogenic effects of Hcy are in fact attributable to IR. In future Hcy intervention studies, we suggest that insulin levels and IR should be monitored in addition to vitamins and exercise, and also in nondiabetic subjects.

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