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Plasma homocysteine concentrations in healthy volunteers are not related to differences in insulin-mediated glucose disposal

Fahim Abbasi [a,b,c], Francesco Facchini [a,b,c], Michael H. Humphreys [a,b,c], Gerald M. Reaven [d,*]

[a] *Department of Medicine, Stanford University School of Medicine, Stanford, CA 94305, USA*
[b] *San Francisco General Hospital, San Francisco, CA, USA*
[c] *University of California San Francisco, San Francisco, CA 94110, USA*
[d] *Shaman Pharmaceuticals, Inc., 213 East Grand Avenue, South San Francisco, CA 94080, USA*

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Abstract

This study was initiated to test the hypothesis that plasma homocysteine concentrations are increased in insulin resistant individuals. For this purpose, the relationship between insulin resistance, as assessed by the steady-state plasma glucose (SSPG) concentration during the insulin suppression test, and fasting plasma homocysteine concentration was defined in 55 healthy volunteers. The results indicated that homocysteine concentrations did not vary as a function of SSPG concentrations ($r = 0.02$, $P = 0.88$). Furthermore, mean ($\pm$S.E.M.) plasma homocysteine concentrations were similar (8.2 ± 0.4 vs 8.7 ± 0.7 μmol/l) in individuals classified as being either insulin sensitive (SSPG < 100 mg/dl) or insulin resistant (SSPG > 180 mg/dl). On the other hand, SSPG concentration was significantly correlated with fasting plasma insulin ($r = 0.58$, $P < 0.001$), triglycerides ($r = 0.34$, $P < 0.05$), and HDL-cholesterol ($r = -0.36$, $P = 0.04$) concentrations. These data strongly suggest that the increased risk of atherosclerosis associated with increased plasma homocysteine concentrations is unrelated to insulin resistance and/or the metabolic abnormalities associated with it.

Keywords: Plasma homocysteine; Insulin resistance; Steady-state plasma glucose (SSPG)

1. Introduction

Evidence from several epidemiological studies has suggested that elevated levels of homocysteine are an independent risk factor for atherosclerosis [1–3]. It is also well known that combination of resistance to insulin-mediated glucose disposal and compensatory hyperinsulinemia predisposes individuals to develop a cluster of metabolic abnormalities, including glucose intolerance, a high triglyceride (TG) and a low high density lipoprotein (HDL) cholesterol concentration, and elevated blood pressure, all of which increase risk of coronary heart disease [4–6]. If increases in homocysteine concentrations were related to insulin resistance, it would provide another explanation for the link between insulin resistance and coronary heart disease. This possibility has received support in the form of a recent Letter to the Editor [7]. These results seemed somewhat surprising in light of previous publications showing that plasma homocysteine concentrations were only elevated in patients with type 2 diabetes when associated with vascular complications [8,9]. Since the vast majority of patients with type 2 diabetes are insulin resistant [4,6,10], if insulin resistance played an important role in regulation of plasma homocysteine concentrations as suggested by Giltay et al. [7], an increase in plasma homocysteine concentrations in patients with type 2 diabetes might have been anticipated. Given this apparent paradox, we felt it important to re-examine the relationship of insulin resistance to plasma homocysteine concentrations in a non-diabetic population.

* Corresponding author. Tel.: +1-650-952-7070; fax: +1-650-873-8377.
E-mail address: greaven@shaman.com (G.M. Reaven)

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2. Methods

Our study population consisted of 55 volunteers (28 men and 27 women), recruited from the San Francisco Bay Area through advertisements in the local newspapers. Their mean ± S.E.M. age was 49 ± 11 years (range 23–67), and body mass index (BMI) 28.0 ± 3.8 kg/m^2 (range 20.0–35.0). All participants were healthy as determined by history, physical examination, complete blood count, and chemistry panel, and without clinical evidence of cardiovascular disease. Fourteen individuals had a diagnosis of hypertension, defined as resting blood pressure > 140/90 mmHg, and/or treatment with antihypertensive medications. All subjects were determined to be non-diabetic on the basis of at least two fasting plasma glucose values < 126 mg/dl [11], had normal serum creatinine concentrations, and were free of albuminuria.

Informed consent was obtained before admission to the General Clinical Research Center. After an overnight fast, insulin-mediated glucose disposal was assessed by a modification [12] of the insulin sensitivity test as described by our laboratory [13]. Steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations after a 180-min infusion of somatostatin, insulin and glucose were used to quantitate insulin resistance. As steady-state plasma insulin (SSPI) levels were similar during the infusion, SSPG concentrations provided a direct estimate of insulin-mediated glucose disposal: the higher the SSPG concentration, the more resistant the individual. On the same day, fasting blood samples were obtained for measurement of homocysteine [14]. In order to define the relationship between SSPG and associated abnormalities, fasting plasma insulin, triglyceride, and high density lipoprotein (HDL) cholesterol concentrations were measured as described previously [15] in 34 individuals. Data are expressed as mean ± S.E.M. Pearson correlations were calculated to demonstrate relationships between variables of interest, and means were compared with Mann–Whitney test.

3. Results

The relationship between SSPG and fasting homocysteine concentrations is depicted in Fig. 1. Despite considerable variation in both SSPG (~sixfold) and homocysteine (~threefold) concentrations, it is apparent that there was no correlation between these two variables ($r = 0.02$, $P = 0.88$). Because of a report that homocysteine concentrations are higher in patients with hypertension [16,17], the 14 individuals with high blood pressure are indicated by the open circles in Fig. 1. It is apparent that their inclusion was not the reason for the lack of a relationship between insulin resistance and plasma homocysteine concentration. Indeed in the absence of the patients with hypertension, the relationship between SSPG and homocysteine became somewhat negative ($r = -0.24$, $P = 0.12$). The correlation coefficient between SSPG and homocysteine was essentially unchanged, and remained negative, when log-transformed. Thus, if anything, the more insulin resistant a normotensive individual, the lower the plasma homocysteine concentration.

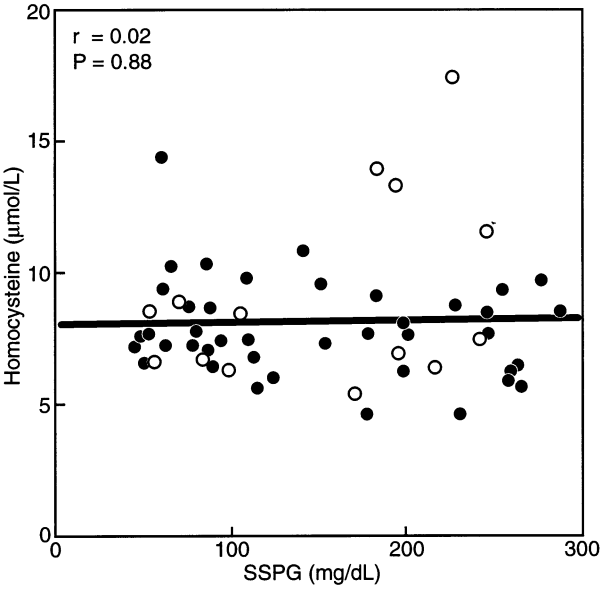

Fig. 1. Relationship between insulin resistance (SSPG) and plasma homocysteine concentrations. The open circles identify volunteers with high blood pressure.

Table 1 represents multiple regression analysis, with homocysteine as the dependent variable, and age, gender, body mass index, mean arterial pressure, and log SSPG as the independent variable. This approach further demonstrates that insulin resistance is not statistically associated with plasma homocysteine concentration. Indeed, the only relationship that was statistically significant was between homocysteine concentration and gender.

In an effort to search further for evidence of a relationship between insulin resistance and homocysteine, we performed an analysis similar to that of Giltay et al. [7] by creating an insulin resistant (SSPG > 180 mg/dl) and an insulin sensitive (< 100 mg/dl) group. It

Table 1
Multiple regression analysis of the relationship between plasma homocysteine concentration and several experimental variables

Variable	Standard coefficient	P-value
Age	0.007	0.96
Gender	0.62	<0.005
Body mass index (BMI)	0.21	0.23
Mean arterial pressure	−0.28	0.09
Log SSPG	−0.09	0.55

Table 2
Comparison of insulin sensitive and resistant volunteers

Variable	Insulin sensitive ($n = 21$)	Insulin resistant ($n = 22$)	P-value
SSPG (mg/dl)	70 ± 4 (45–98)	231 ± 7 (182–288)	<0.0001
Age	49 ± 3 (23–65)	52 ± 2 (29–67)	0.36
Gender (M/F)	11/10	12/10	0.89
Hypertension (yes/no)	5/16	7/15	0.56
BMI (kg/m^2)	27.5 ± 1.0 (20.0–34.5)	28.2 ± 0.7 (22.0–35)	0.53
Homocysteine (μmol/l)	8.2 ± 0.4 (6.4–14.4)	8.7 ± 0.7 (4.6–17.5)	0.99

can be seen from Table 2 that the two groups thus formed were similar in terms of age, body mass index, and gender. It is also apparent that plasma homocysteine concentrations were essentially identical in the two groups, despite the dramatic differences in SSPG concentration.

Finally, in order to make sure that the individuals included in this study were not atypical, we calculated the relationship between SSPG and the variables shown previously to be significantly related to insulin resistance [4–6]. These results documented significant relationships between SSPG concentration and fasting plasma insulin ($r = 0.58$, $P < 0.001$), triglyceride ($r = 0.34$, $P = 0.05$), and HDL-cholesterol ($r = -0.36$, $P = 0.04$). Thus, the lack of a relationship between SSPG and homocysteine concentrations was seen in a population that did demonstrate the typical relationship between SSPG and other variables [4–6], supporting the view that our findings could not be attributed to the possibility that the 55 individuals we studied were not representative of the world at large.

4. Discussion

It is apparent that our results are quite different from those of Giltay et al. [7]. In the group of healthy volunteers studied, we could not demonstrate any relationship between insulin resistance and plasma homocysteine concentration, and this remained the case, no matter how we analyzed the results. The most obvious difference between the results of the two studies is the fact that we enrolled more than twice as many subjects. In addition, inspection of the results of Giltay et al. [7] raises the possibility that one very insulin sensitive individual and one extremely insulin resistant subject were largely responsible for their conclusion.

Although we could not discern a relationship between insulin resistance and homocysteine, the results in Table 1 demonstrate that homocysteine and gender were significantly related. This result was not unexpected, in light of the previous observation of sex differences in homocysteine concentrations in healthy volunteers [18]. Further evidence that our population was not atypical was the observation that insulin resistance correlated significantly with fasting plasma insulin, triglyceride, and HDL cholesterol concentrations—metabolic variables known to be associated with insulin resistance [4–6].

In conclusion, we could not demonstrate any relationship between insulin resistance and plasma homocysteine concentrations in a group of healthy volunteers. Given our inability to confirm the findings of Giltay et al. [7], caution shall be exercised before adopting the view that the relationship between increases in fasting plasma homocysteine concentrations and atherosclerosis is related to insulin resistance and/or its metabolic consequences. On the other hand, given the report by Munshi et al. [19], of an increase in plasma homocysteine concentrations following methionine loading in some patients with type 2 diabetes free of obvious vascular complications, it may be premature to exclude the possibility that homocysteine may be abnormal in insulin resistant individuals.

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References

[1] Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I. Hyperhomocysteinemia: an independent risk factor for vascular disease. New Engl J Med 1991;324:1149–55.
[2] Arneson E, Refusum H, Bonaa KH, Ueland PM, Rorede OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol 1995;24:704–9.
[3] Graham IM, Daly LE, Reefsum HM, et al. Plasma homocysteine as risk factor for vascular disease. The European Concerted Action Project. J Am Med Assoc 1997;227:1775–81.
[4] Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595–607.
[5] Laws A, Reaven GM. Insulin resistance and risk factors for coronary heart disease. Balliere's Clin Endocrinol Metab 1993;7:1063–78.
[6] Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75:473–86.
[7] Giltay EJ, Hoogeveen EK, Elbers JMH, Gooren LJG, Asscheman H, Stehouwer CDA. Insulin resistance is associated with

elevated plasma total homocysteine levels in healthy, non-obese subjects. Atherosclerosis 1998;139:197–8.

[8] Araki A, Sako Y, Ito H. Plasma homocysteine concentrations in Japanese patients with non-insulin-dependent diabetes mellitus: effect of parenteral methylcobalamin treatment. Atherosclerosis 1993;103:149–57.

[9] Chico A, Pérez A, Córdoba A, Arcelús R, Carreras G, de Leiva A, González-Sastre F, Blanco-Vaca F. Plasma homocysteine is related to albumin excretion rate in patients with diabetes mellitus: a new link between diabetic nephropathy and cardiovascular disease? Diabetologia 1998;41:684–93.

[10] Reaven GM. Insulin resistance in noninsulin-dependent diabetes mellitus: does it exist and can it be measured? Am J Med 1983;74(Suppl 1A):3–17.

[11] American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183–1197.

[12] Shen DC, Shieh SM, Fuh MT, Wu DA, Chen YDI, Reaven GM. Resistance to insulin-stimulated-glucose uptake in patients with hypertension. J Clin Endocrinol Metab 1988;66:580–3.

[13] Greenfield M, Doberne L, Kramer F, Tobey T, Reaven GM. Assessment of insulin resistance with insulin suppression test and the euglycemic clamp. Diabetes 1981;30:387–93.

[14] Dudman NP, Guo XW, Crooks R, Xie L, Silverberg JS. Assessment of plasma homocysteine: light sensitivity of the fluorescent 7-benzo-2-oxa-1, 3-diazole-4-sulfonic acid derivative, and use of appropriate calibrators. Clin Chem 1996;42:2028–32.

[15] Carantoni M, Abbasi F, Chu L, Chen Y-DI, Reaven GM, Tsao PS, Varasteh B, Cooke JP. Adherence of mononuclear cells to endothelium in vitro is increased in patients with NIDDM. Diabetes Care 1997;20:1462–5.

[16] Malinow MR, Levenson J, Giral P, Nieto FJ, et al. Role of blood pressure, uric acid, and hemorheological parameters on plasma homocyst(e)ine concentration. Atherosclerosis 1995;114:175–83.

[17] Sutton-Tyrrell K, Bostom A, Selhub J, Zeigler-Johnson C. High homocysteine levels are independently related to isolated systolic hypertension in older adults. Circulation 1997;96:1745–9.

[18] Jacobsen DW, Gatautis VJ, Green R, Robinson K, Savon SR, Secic M, Ji J, Otto JM, Taylor LM Jr. Rapid HPLC determination of total homocysteine and other thiols in serum and plasma: sex differences and correlation with cobalamin and folate concentrations in healthy subjects. Clin Chem 1994;40(6):873–81.

[19] Munshi MN, Stone A, Fink L, Fonseca V. Hyperhomocysteinemia following a methionine load in patients with non-insulin-dependent diabetes mellitus and macrovascular disease. Metabolism 1996;45:133–5.