# Correlation between Change in Serum Homocysteine Levels During Hyperinsulinemia and Insulin Sensitivity

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***Objective:*** *To study the correlation between the changes in homocysteine (Hcy) levels during hyperinsulinemia and insulin sensitivity.*

***Material and Method:*** *Forty-five subjects who underwent hyperinsulinemic euglycemic clamp were studied. Twenty-five subjects had normal glucose tolerance, seven had impaired glucose tolerance, and 13 had type 2 diabetes mellitus. Serum Hcy was measured before (Hcy 0) and at 120 minutes (Hcy 120) of glucose clamp. The change in Hcy levels during hyperinsulinemia was expressed as absolute difference between Hcy 0 and Hcy 120 (ΔHcy) and percentage difference over Hcy 0 (%ΔHcy). Insulin sensitivity index (ISI) was used to correlate with variables of interest.*

***Results:*** *The ISI was not correlated with Hcy 0 and Hcy 120 but was correlated with ΔHcy and %ΔHcy. The ΔHcy and %ΔHcy were not significantly different between subjects with normal and abnormal glucose tolerance, whereas they were significantly different between subjects whose ISI were above and below the mean value.*

***Conclusion:*** *Although the change in Hcy levels during hyperinsulinemia was correlated with insulin sensitivity, the Hcy levels per se were not found to be correlated with insulin sensitivity. The change in Hcy levels during hyperinsulinemia was significantly different in subjects whose ISI was above and below the mean value but not in subjects with normal and abnormal glucose tolerance. This indicated that insulin resistance, not the glucose tolerance status, affected Hcy metabolism.*

***Keywords:*** *Homocysteine, Hyperinsulinemia, Insulin sensitivity*

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Homocysteine (Hcy) is a thiol-containing amino acid, which originates from demethylation of dietary methionine. Data from both population-based and prospective studies found that Hcy is an independent risk factor for cardiovascular disease[1-7]. Insulin resistance is another well-established risk factor for cardiovascular disease[8,9]. Although hyperhomocysteinemia is associated with insulin resistance syndrome (impaired glucose tolerance, hypertension, obesity, dyslipidemia, and hyperinsulinemia)[10], the association between Hcy levels and insulin sensitivity from previous studies was conflicting[11-17]. A plausible explanation is that several factors including genetic, metabolic, nutritional, and physiological factors could potentially affect Hcy levels[18]. Previous studies demonstrated that acute hyperinsulinemia decreases Hcy levels in normal subjects[19,20], but not in insulin-resistance type 2 diabetic subjects[19]. Since it is well recognized that insulin has effects on amino acid metabolism, resistance to the effects of insulin on glucose uptake in peripheral tissues may also be associated with resistance to the suppressive effect of insulin on Hcy levels as well. In order to clarify these aspects, the present study was designed to correlate the change in Hcy levels during hyperinsulinemia and other markers of insulin resistance with insulin sensitivity in subjects with varying degrees of insulin resistance.

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## Material and Method

### *Subjects*

Forty-five subjects who underwent hyperinsulinemic euglycemic clamp for assessing insulin sensitivity were included in the present study. Using the WHO criteria, 25 of the presented subjects had normal glucose tolerance, seven had impaired glucose tolerance, and 13 had type 2 diabetes mellitus. None of the subjects with normal and impaired glucose tolerance was taking any drugs. Subjects with type 2 diabetes were treated with oral hypoglycemic agents. All type 2 diabetic subjects had fasting plasma glucose levels of less than 7.8 mmol/L. The present study protocol was approved by the Ethics Committee of Faculty of Medicine, Prince of Songkla University. Written informed consent was obtained from each subject before participation.

Blood samples for measurement of Hcy were collected before and at 120 minutes of hyperglycemic euglycemic clamp. Blood samples for measurements of vitamin B12, folate, creatinine, uric acid, total cholesterol, HDL cholesterol, and triglyceride were obtained during the fast on the day of the clamp study. All blood samples were immediately placed on ice. Serum was stored at –80 C until assays.

### *Hyperinsulinemic euglycemic clamp*

Hyperinsulinemic euglycemic clamp was carried out as described originally by DeFronzo[21]. The subjects were studied in the supine position at 9 AM after a 10-hour overnight fast. An intravenous catheter was placed in an antecubital vein for infusion of insulin and glucose. Another catheter was placed in the contralateral hand for blood sampling. This hand was placed in a warming box thermostatically controlled at 55-60 C to arterialize the blood. An insulin solution (Actrapid , Novo Nordisk) was prepared with normal saline at a concentration of 0.3 U/mL. A 10-minute priming insulin infusion was followed by a constant infusion of 50 mU/$m^2$ surface area/min for 110 minutes. Plasma glucose concentration was measured at the bedside every 5 minutes and an infusion of 20% dextrose was adjusted to maintain the plasma glucose concentration of 5 mmol/L according to the computerized algorithm with a coefficient of variation < 5%. Blood samples were also collected at the beginning and every 10 minutes during the last hour of the present study for determination of serum insulin concentrations. Insulin sensitivity was expressed as insulin sensitivity index (ISI), calculated by dividing the glucose disposal rate by steady stated serum insulin levels during the last 60 minutes of the glucose clamp.

### *Assays*

Glucose concentrations were analyzed by glucose oxidase method using Synchron CX-3 Delta (Beckman Coulter Inc, Fullerton, CA, USA). Serum insulin concentrations were determined by radioimmunoassay (Diagnostic Products Corporation, CA, USA). Cholesterol, triglyceride, and uric acid concentrations were measured using enzymatic method. HDL cholesterol was determined in the supernatant of serum after magnesium chloride-phosphotungstic precipitation of apoprotein B-containing lipoproteins. LDL cholesterol was calculated by using the Friedewald formula. Serum creatinine was measured by modified Jaffe method. Hcy

**Table 1.** Subjects' characteristics and biochemical parameters classified by glucose tolerance status

| | Normal glucose tolerance | Impaired glucose tolerance | Type 2 diabetes mellitus |
|---|---|---|---|
| Number (male/female) | 25 (11/14) | 7 (2/5) | 13 (6/7) |
| Age (year) | 33±2* | 42±3 | 51±2 |
| Body mass index (kg/$m^2$) | 22.8±1.0* | 28.3±2.0 | 27.4±1.1 |
| Waist to hip ratio | 0.83±0.01* | 0.90±0.03 | 0.96±0.02 |
| Fasting plasma glucose (mmol/L) | 4.6±0.1* | 5.3±0.1 | 6.6±0.2 |
| Serum creatinine (μmol/L) | 83.9±2.1 | 80.9±4.5 | 84.1±3.9 |
| Serum folate (nmol/L) | 17.6±1.2 | 16.1±1.8 | 23.9±2.9 |
| Serum vitamin B12 (pmol/L) | 516.3±35.4 | 482.0±64.0 | 568.4±59.5 |
| Hcy 0 (μmol/L) | 8.4±0.5 | 9.0±0.6 | 9.4±0.9 |
| ISI ( mol/kg/min/pmol/L) | 6.05±0.66* | 3.28±0.82 | 2.36±0.49 |

* $p < 0.05$ compared with abnormal glucose tolerance and type 2 diabetes mellitus
$p < 0.05$ compared with type 2 diabetes mellitus

was determined by micropartical enzyme immunoassay, AxSYM system (Abbott. Abbott Park, IL). Serum folate and vitamin B12 were determined using an electrochemiluminescence assay, Elecsys system 2010 (Roche Diagnostics). Hcy was expressed as the following: 1) basal homocysteine (Hct 0); 2) Hcy at 120 minutes of hyperinsulinemic euglycemic clamp (Hcy 120); 3) absolute difference between Hcy 0 and Hcy 120 (ΔHcy); 4) percentage difference over Hcy 0 (%ΔHcy), (Hcy 0-Hcy 120) x 100 / Hcy 0.

### *Statistical analysis*

Data were expressed as mean ± SE. Differences in mean values between groups were tested by unpaired *t* test. Pearson's correlation coefficients were used for studying the strength of association. A *p*-value of less than 0.05 was considered as statistically significant. Statistical analysis was done with a SPSS 11.0 for Windows.

## Results

Subjects' characteristics and biochemical parameters classified by glucose tolerance status are shown in Table 1. Age, body mass index, waist to hip ratio, fasting plasma glucose, and ISI of subjects with normal glucose tolerance test are significantly lower than those with impaired glucose tolerance and diabetes mellitus. Correlation coefficients of ISI and Hcy levels as well as other markers of insulin resistance are shown in Table 2. The ISI was not correlated with Hcy 0 and Hcy 120 but was significantly correlated with ΔHcy and %ΔHcy. By using the mean value of ISI (4.55 μmol/kg/min/pmol/l), 10 of 25 of the normal glucose tolerance subjects had ISI below the mean, one from the impaired glucose tolerance group and one from the type 2 diabetic group had ISI above the mean (ISI = 9.94 and 13.35, respectively). Since the numbers of subjects with impaired glucose tolerance and diabetes mellitus were small and the ISI of these groups were similar, we combined these two groups as subjects with abnormal glucose tolerance. The ΔHcy and %ΔHcy were not significantly different between subjects with normal and abnormal glucose tolerance, whereas they were significantly different between subjects whose ISI was above and below the mean value (Fig. 1).

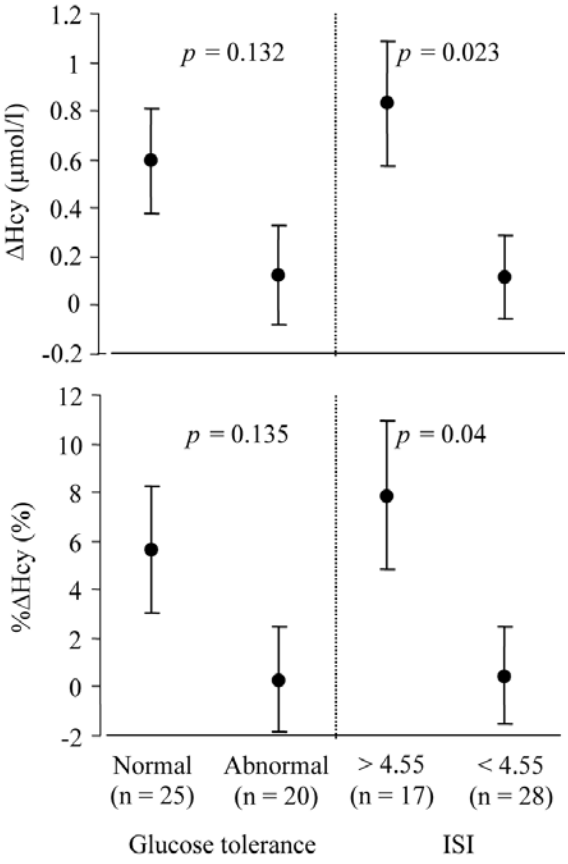


**Fig. 1** Mean (SE) of ΔHcy and %ΔHcy in subjects with normal and abnormal glucose tolerance (left panel) and those whose ISI were above and below the mean (4.55 μmol/ kg/min/pmol/L) (right panel)

**Table 2.** Correlation coefficients of ISI and Hcy levels as well as other markers of insulin resistance

| | Correlation coefficient (r) | p-value |
|---|---|---|
| Hcy 0 | 0.099 | 0.518 |
| Hcy 120 | -0.052 | 0.734 |
| ΔHcy | 0.360 | 0.015 |
| %ΔHcy | 0.319 | 0.033 |
| Body mass index | -0.744 | <0.0001 |
| Waist to hip ratio | -0.716 | <0.0001 |
| Systolic blood pressure | -0.368 | 0.013 |
| Diastolic blood pressure | -0.462 | 0.001 |
| HDL cholesterol | 0.395 | 0.007 |
| Triglyceride | -0.502 | <0.0001 |
| Serum uric acid | -0.401 | 0.006 |
| Fasting serum insulin | -0.656 | <0.0001 |

## Discussion

Although markers of insulin resistance i.e. body mass index, waist to hip ratio, blood pressure, HDL cholesterol, triglyceride, serum uric acid, and

fasting serum insulin levels were correlated with insulin sensitivity, a correlation between Hcy levels and insulin sensitivity was not found in the present study. Previous studies had discrepant results for the correlation between Hcy levels and insulin sensitivity. A plausible explanation is that several factors including genetic, metabolic, nutritional, and physical factors could potentially affect Hcy levels[18]. Most of the studies in which Hcy levels and insulin sensitivity were correlated showed negative correlation between Hcy levels and insulin sensitivity[11-14]. Recently, Fonseca et al[22] demonstrated a positive correlation between Hcy levels and insulin sensitivity ($r = 0.53$, $p = 0.006$) in non-diabetes subjects. They also found a negative correlation between Hcy and vitamin B12 levels ($r = -0.44$, $p = 0.017$). Logically, vitamin B12 levels should be negatively correlated with insulin sensitivity. In contrast, they found a positive correlation between vitamin B12 levels and insulin sensitivity ($r = 0.4$, $p = 0.045$). Unexplained interrelationship between Hcy levels, vitamin B12, and insulin sensitivity in their study may be caused by statistical errors.

It is well recognized that insulin has important effects on amino acid metabolism. Hcy is a thiol-containing amino acid formed by demethylation of methionine, therefore insulin should have an effect on Hcy as well. Previous studies demonstrated that acute hyperinsulinemia decreases Hcy levels in normal subjects[19,20], but not in insulin-resistance type 2 diabetic subjects[19]. These data suggest that resistance to the effects of insulin on glucose disposal may be associated with resistance to the suppressive effect of insulin on Hcy levels. Based on a general rule for endocrinologic tests, when results of a test vary, a dynamic test is required to reduce the variation. As basal Hcy levels are affected by several factors, the correlation between insulin sensitivity and change in Hcy levels during hyperinsulinemia should give a better result than the correlation between insulin sensitivity and basal Hcy levels. This notion was demonstrated by the present study. Fonseca et al[19] found that acute hyperinsulinemia decreases Hcy levels in normal subjects but not in insulin-resistance type 2 diabetic subjects. However, they found no correlation between the change in Hcy levels during hyperinsulinemia and insulin sensitivity, expressed as the glucose disposal rate. The reason for this discrepancy may be due to two different rates of insulin infusion during hyperinsulinemic euglycemic clamp used in Fonseca's study. About half of their subjects received submaximal rate of insulin infusion (40 $mU/m^2/min$) and the other half received the maximal rate of insulin infusion (300 $mU/m^2/min$). Although they did not describe the serum insulin levels during the hyperinsulinemic euglycemic clamp, these two different rates of insulin infusion could make serum insulin levels different, 6 times or more. Since glucose disposal rate depends on the levels of serum insulin concentrations, the subjects with the same insulin sensitivity should have a different glucose disposal rate when the serum insulin levels vary. No correlation between the change in Hcy levels during hyperinsulinemia and the glucose disposal rate, demonstrated by Fonseca et al may be explained by an inappropriate study design. However, the variation of serum insulin levels during hyperinsulinemic euglycemic clamp still occurred when using the same infusion rate of insulin. To avoid these problems, we used the same infusion rate of insulin and expressed the insulin sensitivity as insulin sensitivity index. By these methods, the authors found a slightly better correlation between the change of Hcy levels during hyperinsulinemia and insulin sensitivity expressed as insulin sensitivity index ($r = 0.360$, $p = 0.015$ for $\Delta$Hcy and $r = 0.319$, $p = 0.033$ for $\%\Delta$Hcy) than those expressed as glucose disposal rate ($r = 0.342$, $p = 0.021$ for $\Delta$Hcy and $r = 0.315$, $p = 0.035$ for $\%\Delta$Hcy).

Glucose tolerance depends on a complex interaction between insulin secretion and insulin sensitivity. The glucose tolerance of individuals with low insulin sensitivity could be normal if insulin secretion were adequately compensated. On the other hand, the glucose intolerance could occur in individuals with normal insulin sensitivity if their $\beta$-cells function were impaired. Ten of 25 (40%) of the presented normal glucose tolerance subjects had ISI below the mean, while one from the impaired glucose tolerance group and one from the type 2 diabetic group had ISI above the mean. The change in Hcy levels during hyperinsulinemia in subjects whose ISI were above the mean, were significantly greater than those whose ISI were below the mean, while it was not significantly different between subjects with normal and abnormal glucose tolerance. This indicated that insulin resistance, not the glucose tolerance status, affected Hcy metabolism.

In conclusion, the present study demonstrated that the change in Hcy levels during hyperinsulinemia, not Hcy levels was positively correlated with insulin sensitivity. While the change of Hcy levels during hyperinsulinemia in subjects whose ISI were above the mean was significantly greater than those whose ISI were below the mean, it did not become significantly different between subjects with normal and abnormal

glucose tolerance. This indicated that insulin resistance, not the glucose tolerance status, affected Hcy metabolism. Larger scales studies are warranted to confirm these findings.

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## *ความสัมพันธ์ระหว่างการเปลี่ยนแปลงของระดับโฮโมซีสทีอินขณะมีระดับอินซูลินในเลือดสูงกับความไวอินซูลิน*

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***วัตถุประสงค์:*** *ศึกษาความสัมพันธ์ระหว่างการเปลี่ยนแปลงของระดับโฮโมซีสทีอินขณะมีระดับอินซูลินในเลือดสูงกับความไวอินซูลิน*

***วัสดุและวิธีการ:*** *อาสาสมัครที่ได้รับการทำ euglycemic hyperinsulinemic clamp จำนวน 45 คน ในจำนวนนี้มี glucose tolerance ปกติ 25 คน มี impaired glucose tolerance 7 คน และเป็นเบาหวาน 13 คน วัดระดับโฮโมซีสทีอินก่อนทำ euglycemic hyperinsulinemic clamp (Hcy0) และที่ 120 นาทีของการทำ euglycemic hyperinsulinemic clamp (Hcy120) การเปลี่ยนแปลงของระดับโฮโมซีสทีอินแสดงในรูปของผลต่างระหว่าง Hcy0 และ Hcy120 (ΔHcy) และร้อยละของความแตกต่าง (%ΔHcy) ความไวอินซูลินได้จากการทำ euglycemic hyperinsulinemic clamp แสดงในรูปดัชนีความไวอินซูลิน*

***ผลการศึกษา:*** *ดัชนีความไวอินซูลินไม่มีความสัมพันธ์กับ Hcy0 และ Hcy120 แต่มีความสัมพันธ์กับ ΔHcy และ %ΔHcy นอกจากนั้นยังพบว่า ΔHcy และ %ΔHcy ไม่แตกต่างกันระหว่างอาสาสมัครที่มี glucose tolerance ปกติและผิดปกติ แต่จะแตกต่างกันระหว่างอาสาสมัครที่มีดัชนีความไวอินซูลินสูงและต่ำกว่าค่าเฉลี่ย*

***สรุป:*** *การเปลี่ยนแปลงของระดับโฮโมซีสทีอินขณะมีระดับอินซูลินในเลือดสูงมีความสัมพันธ์กับความไวอินซูลิน แต่ระดับโฮโมซีสทีอินเองไม่มีความสัมพันธ์กับความไวอินซูลิน การเปลี่ยนแปลงของระดับโฮโมซีสทีอินแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างอาสาสมัครที่มีดัชนีความไวอินซูลินสูงและต่ำกว่าค่าเฉลี่ย แต่ไม่พบความแตกต่างระหว่างอาสาสมัครที่มี glucose tolerance ปกติและผิดปกติ แสดงว่า ภาวะดื้ออินซูลินมีผลต่อเมตาบอลิสมของโฮโมซีสทีอิน*