Hematocrit and Hemoglobin Are Independently Related to Insulin Resistance and Compensatory Hyperinsulinemia in Healthy, Non-Obese Men and Women

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In this study, we evaluated the relationship between resistance to insulin-mediated glucose disposal and hematocrit (Hct) and hemoglobin (Hgb) concentrations in 150 normal, healthy volunteers: 100 men and 50 women. Insulin resistance was defined as the steady-state plasma glucose (SSPG) concentration at the end of a 180-minute infusion of somatostatin, insulin, and glucose. Since the steady-state plasma insulin (SSPI) concentrations are similar in all individuals, the SSPG concentrations provide a direct measure of insulin resistance: the higher the SSPG, the more insulin-resistant the subject. The results indicated that SSPG was significantly ($P < .001$) related to Hct and Hgb in both men and women, with correlation coefficients (r) ranging from 0.38 to 0.43. A series of other variables were also related to Hct and Hgb, including blood pressure, plasma glucose and insulin responses to oral glucose, and plasma triglyceride and high-density lipoprotein (HDL) concentrations. When multiple regression analysis was used to evaluate these relationships, the only variables that were consistently found to be associated with Hct and Hgb were insulin resistance and plasma insulin response to oral glucose. Thus, these results suggest that Hct and Hgb concentrations be added to the cluster of variables related to insulin resistance and compensatory hyperinsulinemia.

TWO REPORTS have been recently published that are consistent with the view that hematocrit (Hct) is increased in insulin-resistant individuals.[1,2] Moan et al[1] initially demonstrated in 20 healthy, young males, that the higher the Hct/viscosity, the more insulin-resistant the individual. More recently, Wannawethee et al[2] demonstrated that there was a significant relationship in approximately 7,000 men between Hct and the development of non–insulin-dependent diabetes mellitus, independent of age, body-mass index (BMI), smoking, physical activity, high-density lipoprotein (HDL)-cholesterol, and systolic blood pressure. Since insulin resistance and/or hyperinsulinemia are metabolic characteristics of the prediabetic state,[3-6] the observations by Wannawethee et al provide further support for the view that there is an association between Hct and insulin resistance. The present study was initiated to extend these observations and represented an effort to answer the following questions: (1) can the relationship between a specific measure of insulin-mediated glucose disposal and both Hct and hemoglobin (Hgb) be discerned in a large number of subjects?; and (2) is the association between these two variables as strong in women as it appeared to be in men?[1,2] If the answers to both of these questions were positive, it would be possible to make the generalization that an association existed between insulin resistance and Hct and Hgb. Consequently, we measured insulin sensitivity and related variables, along with Hct and Hgb in 150 nondiabetic subjects (100 men and 50 women), aged 20 to 71 years. To avoid the confounding effect of obesity and hypertension, we only enrolled participants who were normotensive and had a BMI less than 30 kg/m^2.

METHODS

The study population included 100 men and 50 women with mean (±SE) ages of 49 ± 13 years (range, 20 to 71) and 43 ± 12 years (range, 21 to 71), respectively. They were considered to be healthy on the basis of a normal medical history, physical examination, hemogram, routine blood chemistry, and a standard oral glucose tolerance test.[7] Subjects were not taking any medication known to affect carbohydrate and lipoprotein metabolism or Hct and Hgb concentrations. Only 12 of the 150 were smokers—all women. After an initial screening visit, all subjects gave informed, written consent and were admitted to the Stanford University General Clinical Research Center. None of the subjects reported strenuous physical activity within 72 hours before being tested. Subjects were weighed in light clothing with an electronic scale. Obesity was estimated by the BMI. Blood pressure was the mean of two determinations taken by a trained member staff with a mercury sphygmomanometer, with subjects in the fasting state, and sitting at least 5 minutes in a quiet room. Disappearance of Korotkoff's sounds was used as a criterion for diastolic blood pressure.

An oral glucose tolerance test was performed by administering a 75-g oral glucose challenge after an overnight fast. Blood was drawn before and 30, 60, 120, and 180 minutes after the glucose load and measurements were made of plasma glucose[8] and insulin[9] concentrations. The area under the plasma glucose and insulin responses over time was calculated by the trapezoidal formula.

The insulin suppression test was used to quantitate in vivo insulin-mediated glucose disposal.[10] After an overnight fast, subjects were given a continuous intravenous infusion of somatostatin (5 μg/min), glucose (240 mg/m^2/min), and insulin (25 mU/m^2/min) for 180 minutes, administered via a Harvard pump into an indwelling teflon catheter placed in a superficial antecubital vein. Venous blood samples were obtained from a similar catheter inserted in a contralateral antecubital vein and kept patent by a slow infusion of 0.9% NaCl. Blood was obtained every 60 minutes during the first 2 hours and every 10 minutes during the last half hour for measurement of plasma glucose and insulin concentrations. The mean value of the four measurements made during the last half hour were used to calculate the steady-state plasma insulin (SSPI) and the steady state plasma glucose (SSPG) concentrations. Under these circumstances, the higher the SSPG, the more insulin-resistant the individual.

Fasting venous blood was obtained on 2 separate days for measurement of plasma triglyceride[11] and total cholesterol[12] concentrations. In addition, HDL was separated by conventional ultracentrifugational techniques[13] and the cholesterol concentration of this fraction was also determined. Hct and Hgb were determined on a sample of venous whole

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blood by automated standard procedures (Coulter Counter S 4; Coulter Electronics, Hialeah, FL).

Data are expressed as the mean ± SE. Correlation coefficients between each set of two variables were assessed among all demographic and metabolic variables by Pearson product-moment analysis. Nonnormally distributed variables were normalized by logarithmic transformation. To adjust for potential covariates and confounders, eg, age, BMI, etc, multiple regression analysis was used. Statistical computations were performed by a microcomputer (Macintosh Quadra 650; Apple Computers, Cupertino, CA), using a commercial statistical package (Statview, 512; Abacus, Calabasas, CA), and P values are considered significant to reject the null hypothesis at a level of ≤5%.

RESULTS

Baseline clinical and metabolic characteristics are listed in Table 1, with the values for the men and women given separately. Hct and Hgb concentrations were higher ($P < .001$) in men as compared with women, which is consistent with the fact that gender is a major determinant of oxygen-carrying capacity in adults. In addition, these data show that there were multiple other differences between the men and women. Consequently, results for men and women will be presented separately.

The relationships between insulin resistance (log SSPG) and Hct and Hgb are shown in Figs 1 and 2, respectively. These data show that insulin resistance was significantly correlated ($P < .001$) with both Hct and Hgb, and this was true of males and females. Further, the correlation coefficients were similar for all of the relationships.

Similar data for the relationship between Hct and Hgb and the total integrated plasma insulin response during an oral glucose tolerance test are shown in Figs 3 and 4. As with SSPG, there are statistically significant ($P < .001$) relationships between the plasma insulin response and both Hct and Hgb, which are similar in magnitude and present in both sexes.

Correlation coefficients between Hct and Hgb and the other variables measured are listed in Table 2. It is apparent that age, BMI, and total cholesterol were the only variables that did not correlate with Hct or Hgb in either sex. At the other extreme, in addition to SSPG and insulin response, the total integrated plasma glucose and fasting plasma triglyceride concentration

Table 1. Baseline Characteristics (mean ± SEM)

Variable	Men (n = 100)	Women (n = 50)
Age (yr)	49 ± 1.3	43 ± 1.7‡
BMI (kg/m²)	24.4 ± 0.2	25.0 ± 0.7†
Systolic BP (mm Hg)	124 ± 2	114 ± 2‡
Diastolic BP (mm Hg)	76 ± 1	74 ± 1*
Glucose area (mmol/L · h¹)	18.7 ± .39	16.9 ± .50‡
Insulin area (pmol/L · h¹)	1024 ± 72	1,139 ± 100
SSPG (mmol/L)	7.0 ± .39	7.9 ± .61*
Cholesterol (mmol/L)	4.8 ± .10	4.6 ± .10*
Triglyceride (mmol/L)	1.27 ± .07	1.02 ± .06‡
HDL-cholesterol (mmol/L)	1.21 ± .02	1.45 ± .02‡
Hct (%)	41.5 ± 0.2	37.9 ± 0.4‡
Hgb (mmol/L)	8.81 ± .06	7.95 ± .06‡

Abbreviation: BP, blood pressure.
*$P < .05$.
†$P < .01$.
‡$P < .001$.

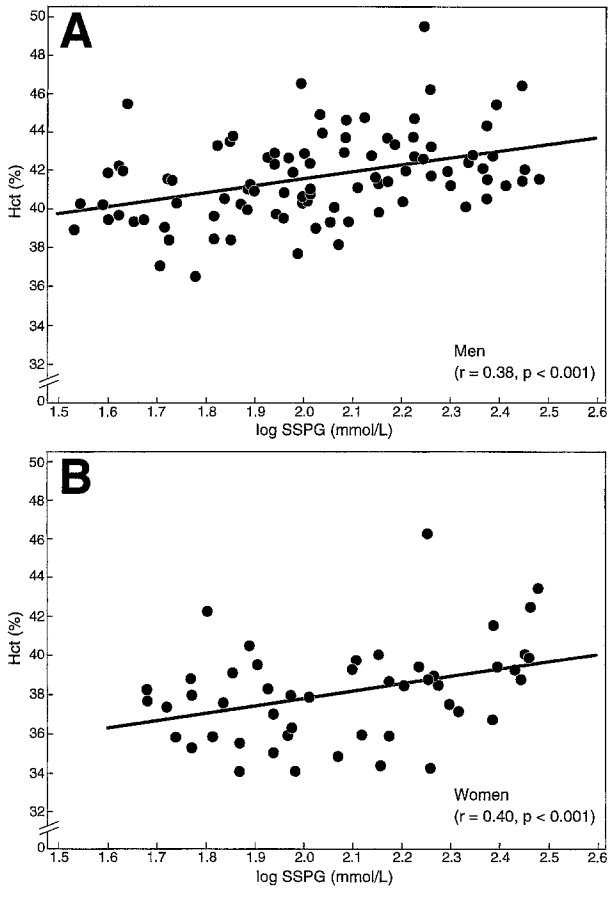

Fig 1. Relationship between insulin resistance (log SSPG) and Hct in 100 healthy men (A) and 50 healthy women (B).

were significantly correlated with both Hct and Hgb in both sexes. However, diastolic blood pressure and HDL-cholesterol were correlated with Hct and Hgb in men only, whereas only females showed a significant correlation between Hct and Hgb and systolic blood pressure.

Multiple regression analysis was used to assess the independence of the relationship between Hct and Hgb and the variables listed in Table 2. For this purpose, only those variables shown to have a significant univariate relationship to Hct or Hgb in Table 2 were entered into the model. Furthermore, since SSPG and insulin response are highly correlated, they were entered into the model separately. The results for men are listed in Table 3, and indicate that only diastolic blood pressure and SSPG were independently related to Hct and/or Hgb. Table 4 presents the analyses when SSPG was replaced with insulin response, and it is apparent that results in Table 3 and 4 are comparable.

Tables 5 and 6 display the results of a similar analysis performed in women. As described earlier, only variables with a significant univariate relationship to Hct and Hgb as seen in Table 2 were entered into the model, and SSPG and insulin response were entered separately. When comparing these results with those seen in men, it is clear that there are some gender-related differences. For example, no relationship was seen between blood pressure and either Hct or Hgb in women. It can also be seen by comparing Tables 3 and 5 that the

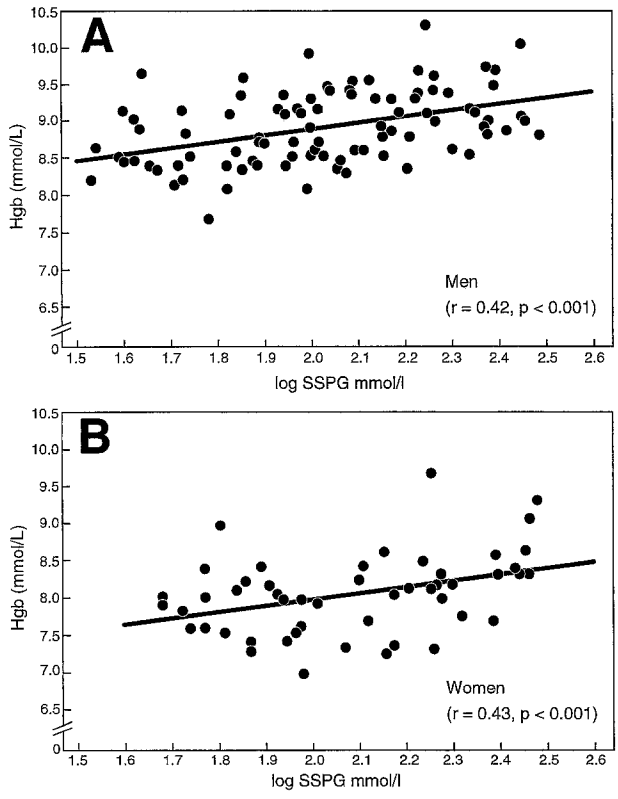

Fig 2. Relationship between insulin resistance (log SSPG) and Hgb concentration in 100 healthy men (A) and 50 healthy women (B).

relationship between SSPG and Hct and Hgb is weaker in women than in men. In contrast, comparison of Tables 4 and 6 shows that the relationship between the plasma insulin response to Hct and Hgb is independent of differences in gender. The analyses described in Tables 5 and 6 were repeated, excluding the 12 women who were smokers. Although the actual values changed somewhat, there were no changes in the statistical significance of the relationships described in Tables 5 and 6.

Finally, the multiple regression analysis were repeated, only in this case, the goal was to see which variables predicted insulin resistance and insulin response. In the case of men, several variables were significantly related to insulin resistance, including age ($P = .01$), diastolic blood pressure ($P = .06$), BMI ($P = .05$), and Hct/Hgb ($P = .01$). The situation was somewhat different in women, with only BMI and diastolic blood pressure showing a significant relationship ($P = .005$) to SSPG.

DISCUSSION

The goal of this study was to further evaluate the possibility that insulin resistance, and/or variables related to this defect, were also related to Hct and Hgb concentrations. To do this, we examined these relationships in a large number of healthy, non-obese, normotensive men (n = 100) and women (n = 50). In the most general sense, our findings support the results published by Moan et al in 21 men, aged 21 years,[1] which demonstrated a relationship between insulin resistance and Hct/viscosity. However, by (1) substantially enlarging the size of the study population, (2) broadening the age span to include individuals from 20 to 71 years, and (3) enrolling both men and women, we have considerably strengthened the notion that insulin resistance and/or compensatory hyperinsulinemia are independently related to Hct and Hgb. Insulin resistance and compensatory hyperinsulinemia are known to be related to a cluster of other metabolic and hemodynamic variables,[14] and it was of interest that neither the plasma glucose response to oral glucose, nor plasma triglyceride and HDL-cholesterol concentrations, were independently related to Hct and Hgb. In fact, the only other variable identified as having an independent relationship with Hct and Hgb was diastolic blood pressure, and that was only true in men.

Before speculating as to the pathophysiologic relationship between Hct and Hgb on one hand, and insulin resistance and compensatory hyperinsulinemia on the other, it seems necessary to address the quantitative relationships observed between the four variables in question, as well as some gender differences. Since the results in Tables 2 through 6 indicate that the relationships between Hct and Hgb, and the other variables measured, were similar, in the remainder of this discussion, we will refer to the hematologic end points as Hct/Hgb. The only consistent independent relationship defined between Hct/Hgb was with the total integrated insulin response to oral glucose, and this was true of both men and women. The relationship between Hct/Hgb and SSPG was not as robust, and this was true

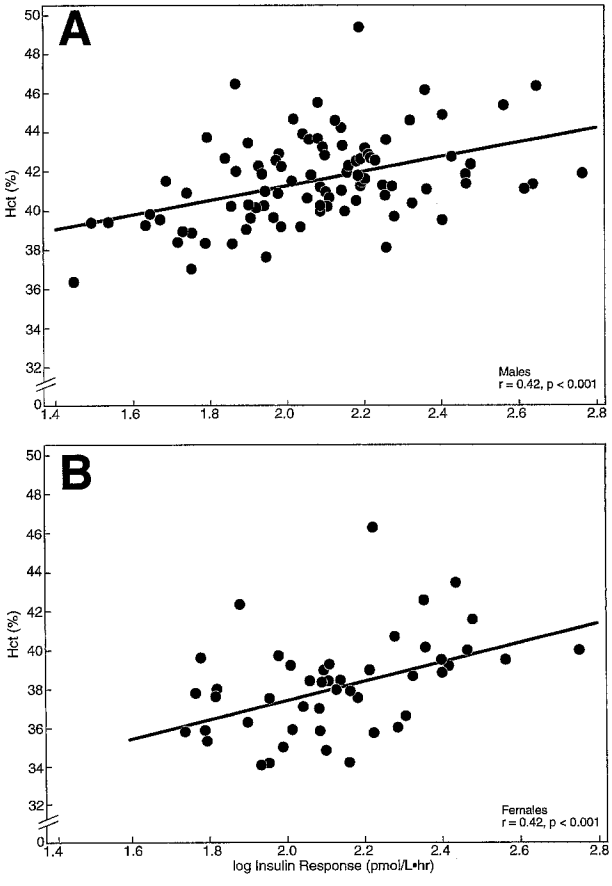

Fig 3. Relationship between log of the total integrated plasma insulin response to glucose and Hct in 100 healthy men (A) and 50 healthy women (B).

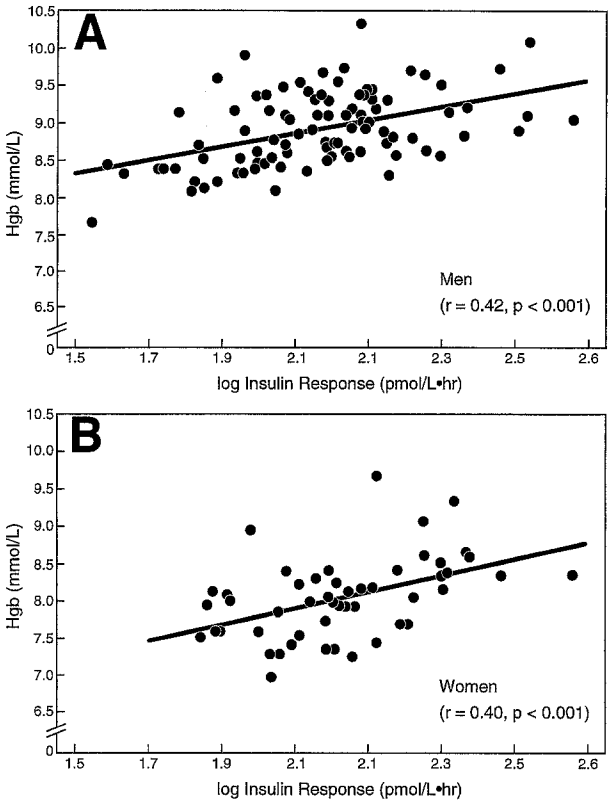

Fig 4. Relationship between log of the total integrated insulin response to glucose and Hgb concentration in 100 healthy men (A) and 50 healthy women (B).

of every comparison. On the other hand, insulin resistance and insulin response to glucose are known to be highly correlated,[15] and this was certainly true of the relationship between SSPG and insulin response in the men ($r = .75$, $P < .001$) and women ($r = .76$, $P < .001$) in the current study. Given the difficulty in defining the "independence" of two closely related variables with a third variable,[16] we do not believe our data should be interpreted as signifying that there is any fundamental difference in the relationship between Hct/Hgb and insulin resistance

Table 2. Correlation Coefficient Between Hct and Hgb and the Other Experimental Variables

	Men		Women	
Variable	Hct	Hgb	Hct	Hgb
Age (yr)	.05	.01	.12	.06
BMI (kg/m²)	.09	.11	.27	.26
Systolic BP	.18	.17	.28*	.25*
Diastolic BP	.32†	.32†	.23	.21
Glucose area	.22*	.24*	.28*	.24
Insulin area	.42‡	.42‡	.42‡	.40‡
SSPG	.38‡	.42‡	.40‡	.43‡
Cholesterol	.10	.09	.11	.05
Triglyceride	.23*	.26†	.31†	.41‡
HDL-cholesterol	−.27†	−.25†	−.24	−.23

*$P < .05$.
†$P < .01$.
‡$P < .001$.

Table 3. Multiple Regression Analysis of the Relationship Between Hct and Hgb and Diastolic Blood Pressure, Triglyceride, HDL-Cholesterol, Glucose Area, and SSPG in Men (n = 100)

	Hct		Hgb	
Independent Variable	$R^2 = .21$ Standard Value	$P < .001$ P	$R^2 = .22$ Standard Value	$P < .0001$ P
Diastolic BP	.23	.03	.21	.04
Glucose area	.07	.38	.08	.44
SSPG	.26	.07	.31	.03
Triglyceride	−.16	.19	−.13	.29
HDL-cholesterol	−.19	.10	−.13	.25

versus insulin response. In further support of this conclusion is the fact that the standard values were not that different. For example, the standard values between Hct and SSPG and Hct and insulin response was 0.26 versus 0.30 in men, and 0.21 versus 0.36 in women. The similarity in standard values between Hgb and SSPG and insulin response is even closer, ie, 0.31 for both insulin resistance and response in men and 0.38 and 0.44, respectively, in women. Consequently, we believe it is reasonable to conclude from our data that insulin resistance and compensatory hyperinsulinemia are independently related to Hct/Hgb in healthy, non-obese men and women. In addition, the converse is also true, ie, Hct/Hgb were found to be independently related to SSPG in this population.

Although the results of this study demonstrate that insulin resistance, compensatory hyperinsulinemia, and Hct/Hgb are closely related, they do not provide any insight into the cause and effect nature of the association. Theoretically, it is possible that the increase in Hct/Hgb is due to insulin resistance and/or compensatory hyperinsulinemia, or vice versa. The two sets of variables could also be secondary to a third abnormality. One possible link between Hct/Hgb and insulin resistance is the fact that Hct is a major determinant of whole-blood viscosity, and that viscosity is directly related to increased peripheral resistance and blood pressure.[17-19] Thus, it could be argued that the insulin resistance was secondary to increased peripheral resistance and impaired capillary plasma flow and tissue delivery of insulin and glucose.[20] However, this seems somewhat unlikely, since after adjustment for gender, age and blood pressure, SSPG emerged as the strongest predictor of Hct/Hgb, independent of blood pressure. Further, in females the univariate relation between Hct/Hgb and blood pressure disappeared after adjusting for SSPG. Alternatively, insulin is an anabolic hormone, and may be directly stimulating hematopoiesis. Consistent with this possibility is the previously described association between

Table 4. Multiple Regression Analysis of the Relationship Between Hct and Hgb and Diastolic Blood Pressure, Triglyceride, HDL-Cholesterol, Glucose, and Insulin Area in Men (n = 100)

	Hct		Hgb	
Independent Variable	$R^2 = .24$ Standard Value	$P < .001$ P	$R^2 = .24$ Standard Value	$P < .0001$ P
Diastolic BP	.22	.03	.21	.04
Glucose area	.09	.36	.10	.36
Insulin area	.30	.01	.31	.01
Triglyceride	−.12	.29	−.07	.52
HDL-cholesterol	−.18	.10	.14	.21

Table 5. Multiple Regression Analysis of the Relationship Between Hct and Hgb and Systolic Blood Pressure, Triglyceride, Glucose Area, and SSPG in Women (n = 50)

	Hct		Hgb	
Independent Variable	$R^2 = .25$ Standard Value	$P < .01$ P	$R^2 = .24$ Standard Value	$P < .01$ P
Systolic BP	.05	.72	−.02	.86
Glucose area	.20	.22	—	—
SSPG	.21	.28	.38	.06
Triglyceride	.19	.28	.20	.26

Table 6. Multiple Regression Analysis of the Relationship Between Hct and Hgb and Systolic Blood Pressure, Triglyceride, Glucose, and Insulin Area in Women (n = 50)

	Hematocrit		Hemoglobin	
Independent Variable	$R^2 = .26$ Standard Value	$P < .001$ P	$R^2 = .30$ Standard Value	$P < .001$ P
Systolic BP	.03	.81	−.02	.83
Glucose area	.11	.49	—	—
Insulin area	.36	.03	.44	.009
Triglyceride	.24	.15	.19	.22

insulin resistance and white blood cell count.[21,22] A variant of this association is that the enhanced sympathetic nervous system activity, known to be related to insulin resistance and compensatory hyperinsulinemia,[23] could directly increase hematopoiesis[24] and/or increase Hct/Hgb by hemoconcentration. Perhaps the more likely possibility is that the increased hematopoiesis associated with insulin resistance is an epiphenomenon, secondary to the link between insulin resistance, compensatory hyperinsulinemia, and multiple risk factors for coronary heart disease (CHD). In this instance, the relationship between Hct/Hgb and insulin resistance would be similar to that between some common bacterial infections and C-reactive protein with CHD and cardiovascular risk factors.[25-27]

In conclusion, although the cause-and-effect relationships remain to be clarified, these results strongly suggest that an increase in Hct/Hgb be added to the long list of metabolic, hemodynamic, and hemostatic variables known to be associated with insulin resistance and compensatory hyperinsulinemia.

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