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Comparison of insulin resistance and serum high-sensitivity C-reactive protein levels according to the fasting blood glucose subgroups divided by the newly recommended criteria for fasting hyperglycemia in 10 059 healthy Koreans

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Abstract

The increment for the prevalence of diabetes mellitus and impaired glucose tolerance warrants lowering the cutoffs of normoglycemia to help predict the future development of diabetes. The aim of this study was to find out whether insulin resistance and high-sensitivity C-reactive protein (hsCRP), a nontraditional cardiovascular risk factor, were related to the fasting glucose level, even in normoglycemic range that was categorized by the newly recommended criteria by the American Diabetes Association. Among the participants undergoing medical checkup program at Kangbuk Samsung Hospital, 10 059 subjects (5535 men and 4524 women; mean age, 45 years) with normal fasting glucose levels, as defined by the newly recommended criteria (<5.6 mmol/L), were enrolled in this study. The blood pressures, body mass index (BMI), fasting blood glucose, fasting insulin, lipid batteries, and hsCRP levels were checked. The homeostatic model assessment–insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check indexes (QUICKI) were calculated. All subjects were subdivided into 4 groups according to the fasting glucose level. The HOMA-IR, QUICKI, and log-transformed (log) hsCRP, or log(hsCRP), level significantly increased according to the increment in fasting glucose, and these associations were consistent after adjustment for age and BMI, except for the log(hsCRP) ($P = .124$ after adjustment). Log(hsCRP) increased as the HOMA-IR increased and as the QUICKI decreased, and when multiple regression analysis was done with log(hsCRP) as the dependent variable, age, high BMI, male sex, high HOMA-IR, hypertriglyceridemia, and low high-density lipoprotein cholesterol were the significant predictor for log(hsCRP). In conclusion, the insulin resistance indexes and hsCRP increased gradually even in the normal fasting glucose range, as categorized by the newly recommended criteria for abnormal fasting glucose levels, supporting the rationale for expanding the range of fasting hyperglycemia.

1. Introduction

Hyperglycemia and insulin resistance are recognized as strong risk factors for cardiovascular diseases. Several population studies have definitely shown that a 2-hour postchallenge glucose level of 11.1 mmol/L (200 mg/dL) or higher during an oral glucose tolerance test clearly identifies those individuals who are at high risk for microvascular complications such as retinopathy and nephropathy. The current diabetic cutoff for fasting plasma glucose, which is defined as a fasting glucose level of higher than 7.0 mmol/L (126 mg/dL), has been selected because this level has a very high specificity for a 2-hour glucose of 11.1 mmol/L (200 mg/dL), which is the cutoff for the diagnosis of diabetes mellitus [1,2]. Because high death rates due to cardiovascular diseases have been reported for the patients with prediabetic conditions such as impaired glucose

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tolerance or impaired fasting hyperglycemia, it s reasonable that these conditions be considered as independent risk factors for cardiovascular diseases [3,4].

In November 2003, the American Diabetes Association (ADA) expanded the range of impaired fasting glucose by lowering the previous normal fasting plasma glucose level from 6.1 mmol/L (110 mg/dL) to levels lower than 5.6 mmol/L (100 mg/dL), and they also emphasized the importance of earlier intervention to the prediabetic stage [5]. Although the importance of early intervention and the prevention of prediabetic conditions are now being emphasized, there are scant studies concerning insulin resistance and the risk of cardiovascular diseases in the prediabetic range of the blood glucose level.

Numerous studies have been recently performed on the association of inflammation with atherosclerosis, as inflammatory reaction has been proposed to play a central role in the pathogenesis of arteriosclerosis [6,7]. High-sensitivity C-reactive protein (hsCRP) is an acute-phase reactant, and it is traditionally considered as a marker for systemic inflammatory reaction. It has recently been revealed that hsCRP not only reflects the degree of inflammation that induces arteriosclerosis, but that it also plays a direct role in the formation and rupture of atherosclerotic plaques [8,9]. In addition, recent reports have shown the importance of hsCRP as a predictor for future cardiovascular events in the patients with risk factors, even in healthy individuals, and it can predict the risk of acute myocardial infarction [10,11].

Hence, we investigated the degree of insulin resistance and hsCRP in a normoglycemic groups composed of 10 059 apparently healthy Koreans who were subdivided by the newly recommended definition of the fasting hyperglycemia.

2. Materials and methods

2.1. Study subjects enrollment

Among those who underwent routine medical checkups from March 2003 to December 2003, in Kangbuk Samsung Hospital, Sungkyunkwan University, Seoul, South Korea, only those people with normal fasting plasma glucose levels, as defined by the newly recommended criteria of the ADA (<5.6 mmol/L), were enrolled [5]. A total of 10 059 subjects (5535 men and 4524 women; mean age, 44.6 ± 11 years) were selected as the study population. The subjects were divided in quartile groups according to their fasting blood glucose.

Those subjects with diabetes mellitus, acute infection, severe cardiovascular diseases, thyroid diseases, chronic renal diseases or malignancy, and those subjects taking medications such as oral hypoglycemic agents and corticosteroids that could affect the blood glucose levels were excluded from the studies. The protocol used was approved by the Institutional Review Board of Kangbuk Samsung Hospital, and informed consent was obtained from all participants.

2.2. Anthropometric measurement and blood chemistry

Height, weight, and the systolic and diastolic blood pressures were measured in duplicate, and the results were averaged. Weight and height were measured in kilograms and centimeters, respectively, to the second decimal points. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters.

Blood was sampled after 12 hours of fasting, and the fasting blood glucose, insulin, total cholesterol (TC), triglyceride (TG), and high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) were measured. The hexokinase method was used to measure the blood glucose levels, and an enzymatic calorimetric test was used to measure the TC and TG levels. The selective inhibition method was used to measure the level of HDL-C, and a homogeneous enzymatic calorimetric test was used to measure the level of LDL-C.

The fasting serum insulin level was measured by immunoradiometric assay (RIABEAD II, Abbott, Tokyo, Japan) having an intra-assay coefficient of variance of 1.2% to 1.9% and an inter-assay coefficient of variance of 1.4% to 3.3%.

2.3. hsCRP measurement

Serum hsCRP levels were measured by using a nephelometric assay and using a BNII nephelometer (Dade Behring, Deerfield, IL). The results were presented as milligrams per liter, and the limit of measurement was 0.175 mg/L with a sample dilution of 1:20.

2.4. Assessment of insulin resistance status

The subjects' insulin resistance status was calculated by using the homeostatic model assessment–insulin resistance (HOMA-IR) [12] and the quantitative insulin sensitivity check index (QUICKI) [13]. The formulas used are as follows:

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting blood glucose (mmol/L)}]/22.5$$

$$\text{QUICKI} = 1/[\log\{\text{fasting plasma insulin } (\mu\text{U/mL})\} + \log\{\text{fasting blood glucose (mg/dL)}\}]$$

2.5. Statistical analysis

All values were presented as mean ± SD. Statistical analyses were performed with the SPSS version 11.0 (Chicago, IL). The assessment for the normality of the variables was done with Kolmogorov-Smirnov test, and the hsCRP values were log-transformed as they did not follow normal distribution. Pearson correlation and partial correlation analyses were performed to obtain the association between the fasting blood glucose levels, and the log-transformed hsCRP [log(hsCRP)] values after an adjustment were made for age and BMI. The comparisons of mean

Table 1
General characteristics of the subjects

Variables (N = 10059)	Mean ± SD
Age (y)	44.6 ± 10.8
Male sex (%)	5535 (55)
Fasting glucose (mmol/L)	4.9 ± 0.3
BMI (kg/m^2)	23.6 ± 2.9
Fasting insulin (μIU/mL)	7.3 ± 2.7
Systolic BP (mm Hg)	113 ± 15
Diastolic BP (mm Hg)	73 ± 10
TC (mmol/L)	5.35 ± 0.9
TG (mmol/L)	1.47 ± 0.9
HDL-C (mmol/L)	1.47 ± 0.3
LDL-C (mmol/L)	3.06 ± 0.8
hsCRP (mg/L)	0.10 ± 0.17

Values are given as mean ± SD or n (%). BP indicates blood pressure.

values of the variables among the subdivided groups were compared using 1-way analysis of variance testing, and multiple comparisons were performed with a post hoc analysis using the least significant difference method. The comparisons of the mean values among the subgroups, after adjustment was made for age and BMI, were performed with an analysis of covariance (ANCOVA) test. For the analysis of linear trends, the number of quartiles (coded 0, 1, 2, and 3) was used as a continuous variable and tested in this model. Multiple regression analysis was performed with log(hsCRP) as the dependent variable, with backward method, to find out the predictors for log(hsCRP). The variables were recoded dichotomously according to the criteria for the diagnosis of metabolic syndrome of Adult Treatment Panel III guidelines [14]. P values less than .05 were considered statistically significant.

Table 2
Comparison of mean values for HOMA-IR, QUICKI, and log(hsCRP) according to the groups by fasting glucose levels

Groups by fasting glucose	Mean ± SD
*HOMA-IR**,**	
1	1.358 ± 0.519
2	1.566 ± 0.563
3	1.706 ± 0.617
4	1.889 ± 0.696
Total	1.615 ± 0.631
*QUICKI**,**	
1	0.127 ± 0.025
2	0.120 ± 0.022
3	0.117 ± 0.022
4	0.113 ± 0.022
Total	0.119 ± 0.024
*log(hsCRP)**,***	
1	−2.968 ± 0.960
2	−2.957 ± 0.948
3	−2.877 ± 0.935
4	−2.789 ± 0.930
Total	−2.901 ± 0.947

Group 1, <4.72 mmol/L (n = 2799); group 2, 4.73 to 4.99 mmol/L (n = 2134); group 3, 5.0 to 5.22 mmol/L (n = 2313); group 4, 5.23 to 5.59 mmol/L (n = 2235). The significant differences seen among the mean values of HOMA-IR and QUICKI according to the glucose group were consistently significant after adjustment for age and BMI by the ANCOVA test (P < .01). The significant differences seen among the mean values of log(hsCRP) were lost after adjustment for age and BMI by the ANCOVA test (P = .124).

* P < .01 for the comparison of the mean values among different groups by 1-way analysis of variance test.

** P < .01 for multiple comparisons of the mean values among the individual groups by post hoc analysis.

*** P < .05 for multiple comparisons of the mean values among the individual groups by post hoc analysis, except between groups 1 and 2 (P = .68).

Table 3
Comparisons of mean values of log(hsCRP) according to quartiles of HOMA and QUICKI

Quartiles	Mean ± SD	P for trend[a]
HOMA-IR		
1	−3.01201 ± 0.965731	.018
2	−2.98599 ± 0.904495	
3	−2.88808 ± 0.947017	
4	−2.73473 ± 0.941214	
QUICKI		
1	−2.74710 ± 0.938086	.042
2	−2.89595 ± 0.950095	
3	−2.96889 ± 0.915514	
4	−3.01039 ± 0.959358	

The number of quartiles (coded 0, 1, 2, and 3) is used as a continuous variable and tested in a regression model with each variable as the dependent variable.

[a] P for trend was calculated with adjustment for age and BMI.

3. Results

The mean age of the 10059 participants (5535 men and 4524 women) was 44.6 ± 11 years. The mean values for the fasting plasma glucose, insulin, and hsCRP were 4.9 ± 0.3 mmol/L, 7.3 ± 2.7 μU/mL, and 0.10 ± 0.17 mg/L, respectively, and the mean systolic and diastolic blood pressures were 113 ± 15 and 73 ± 10 mm Hg, respectively (Table 1).

The fasting blood glucose level and the log(hsCRP) showed a weak positive correlation (r = 0.063, P < .01), and the significance was lost after an adjustment was made for age and BMI (P = .657).

The mean HOMA-IR levels increased, and the mean QUICKI levels decreased significantly as the fasting plasma glucose levels increased. On the post hoc analysis, the differences were significant among all groups (P < .01), and these significances were consistent even after an adjustment was made for age and BMI (Table 2).

The mean log(hsCRP) levels increased significantly as the fasting plasma glucose level increased, and on the post hoc analysis, the differences among the groups were significant, except for that between the first and the second quartile groups (P = .68, Table 2). These differences were lost after adjustment for age and BMI (P = .124).

The log(hsCRP) was positively correlated with the HOMA-IR (r = 0.123, P < .01), and the log(hsCRP)

Table 4
Multiple regression analyses with log(hsCRP) as the dependent variable

	β	SE	P
Age	.01055	.001	<.01
BMI ≥25 kg/m^2	.348	.021	<.01
Male sex	.189	.019	<.01
HOMA-IR ≥75 percentile	.09051	.022	<.01
BP ≥130/85 mm Hg	.05146	.030	.088
TG ≥1.695 mmol/L	.126	.021	<.01
HDL-C <1.034 mmol/L in men and <1.293 mmol/L in women	.184	.020	<.01

Multiple regression analysis was done with the backward method.

was inversely correlated with the QUICKI ($r = -0.094$, $P < .01$) on the bivariate analyses; after adjustment for age and BMI, the HOMA-IR showed a small but consistent correlation with the log(hsCRP) ($r = 0.027$, $P = .006$), and the correlation with QUICKI was lost ($r = -0.010$, $P = .326$). Mean values for log(hsCRP) according to quartiles of HOMA-IR and QUICKI showed a significant linear trend as the quartile values increased or decreased (Table 3).

To find out the significant predictors for log(hsCRP), multiple regression analysis was performed with log(hsCRP) as the dependent variable. Although the β values were small, age, high BMI(≥25 kg/m^2), male sex, high HOMA-IR (≥75 percentile), hypertriglyceridemia (≥1.695 mmol/L), and low HDL-C (<1.034 mmol/L in men, <1.293 mmol/L in women) were the significant predictors for log(hsCRP) (Table 4).

4. Discussion

In this study, the insulin resistance indexes and hsCRP levels were noted to increase in proportion to the difference of the subjects' fasting blood glucose within the normal fasting blood glucose range defined by the newly recommended definition of the ADA in 2003. This suggests that even in the newly recommended criteria for the normoglycemic range, the risk of cardiovascular diseases may be increased linearly, without definite cutoffs.

Insulin resistance and the accompanying metabolic derangements play an important role in the pathophysiology of not only diabetes, but also for coronary artery diseases [15,16]. Although the euglycemic hyperinsulinemic clamp test is an established gold standard method for the detection of insulin resistance, because of this method's invasiveness and high cost, its clinical utility is limited [17]. Thus, we applied a relatively simple calculation method for the insulin resistance, such as HOMA-IR and QUICKI, which are easy to apply to large-scale studies, known to be correlated well with euglycemic hyperinsulinemic clamp test [12,13]. The results of this study showed that insulin resistance significantly increased as the fasting blood glucose increased, even in the reference range as defined by the newly recommended criteria from the ADA. These facts imply that even at the normoglycemic range, the risk for atherosclerosis, which is the major cause for cardiovascular mortality, might increase linearly according to the glucose levels, without any significant cutoff.

Although CRP has been traditionally considered as a marker for a systemic inflammatory reaction, recent reports on the involvement of inflammation for the initiation of atherosclerosis support the newly recommended role of CRP as being the reflection and a measure of the degree of the inflammation that induces atherosclerosis, as well as its direct role in the formation and rupture of thrombotic plaques [6-9]. CRP has been suggested to be an important prognostic factor for the future clinical outcome of acute coronary syndrome and cardiovascular events [10,11,18]. The result of our study demonstrating that similar to the increase of insulin resistance the plasma levels of hsCRP also increased as the fasting plasma glucose increased suggests that even in the normoglycemic range defined by the newly lowered criteria for fasting hyperglycemia, the progression of cardiovascular diseases or atherosclerosis, as reflected by the degree of vascular inflammation, might already be progressing. Furthermore, age, male sex, dyslipidemia, and insulin resistance were the positive predictors for the hsCRP level, even in the normoglycemic subjects, which supports the notion of no existence of cutoff of blood glucose for the start of increment of cardiovascular risk.

It has been reported that cardiovascular diseases are a major cause of death for type 2 diabetes, reaching up to 40% to 50% of all causes of death in this population [19]. The notion that the risk of cardiovascular disease may initiate from the normoglycemic stage and will gradually progress as the plasma glucose levels increase has been suggested by the 22-year follow-up study by Bjornholt et al [4]. The authors of that study reported that the cardiovascular mortality noted in the nondiabetic group with fasting plasma glucose levels higher than 85 mg/dL was 1.4 times higher than the group with fasting glucose levels lower than 85 mg/dL. Balkau et al [20], in a prospective study done in 7018 men, suggested that there might not be a definite threshold for the fasting and 2-hour postprandial glucose levels that marks the rapid increase in mortality, and it might increase linearly depending on the fasting glucose and 2-hour postprandial glucose values. This may be because the diagnostic criterion for diabetes by the fasting plasma glucose was defined by the limit for the increasing microvascular complications such as diabetic retinopathy [1,2], and the importance of the risk for macrovascular diseases was not considered in the definition. Although impaired glucose tolerance is considered as a preliminary prerequisite for future diabetes and vascular diseases, the importance of prevention and the proper intervention has not been sufficiently defined or implemented in clinical practice. Therefore, the application of methods for attenuating insulin resistance or vascular inflammation, such as exercise and drugs like thiazolidinedione, metformin, or statin, even in the prediabetic stage that includes normoglycemia, should be considered more seriously and implemented aggressively as an easier

strategy for the early prevention of developing cardiovascular disease in the future.

Based on the notion that the prevention of diabetes itself may be more important rather than the treatment of the complications in the patients who have already developed diabetes, in December 2003, the ADA recommended lowering the cutoff for the normal fasting plasma glucose level from 6.1 mmol/L (110 mg/dL) to 5.6 mmol/L (100 mg/dL), expanding the range of the fasting hyperglycemia [5]. This recommendation is based upon the efforts to block the risk for developing diabetes in the earlier stage and to diagnose diabetes earlier by controlling the other risk factors and also by performing frequent glucose monitoring. Furthermore, this decision was based upon the epidemiological data from various ethnic groups to make an impaired fasting glucose threshold for predicting future diabetes, not on the precise results from the prospective studies predicting cardiovascular risk applying the threshold [21]. In this study, we applied the newly recommended cutoff for the normal fasting plasma glucose, and we observed that the insulin resistance and the subsequent cardiovascular risk might be increased even for the fasting plasma glucose level lower than 5.6 mmol/L (100 mg/dL). This supports the current rationale of the ADA for lowering the fasting blood glucose levels so as to render earlier diagnoses of diabetes and to lower the future prevalence of the vascular complication of diabetes, which are mostly caused by atherosclerosis.

There is a limitation in this study. Because only fasting blood glucose was measured and postprandial blood glucose was not measured, some subjects diagnosed as "normoglycemic" could have impaired glucose tolerance. These undetected prediabetic patients could have affected the significant relationship of fasting glucose with HOMA-IR or hsCRP.

In conclusion, insulin resistance and the subsequent cardiovascular risk increased continuously, not only in the hyperglycemic range, but also in the normoglycemic range, as defined by the newly recommended criteria. This supports the current rationale of the ADA to lower the cutoff value for the fasting hyperglycemia. Further extensive studies are needed to discover a definite theoretical basis for lowering the criteria for normoglycemia.

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