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C-reactive protein genotype affects exercise training–induced changes in insulin sensitivity

Thomas O. Obisesan[a,b,*], Christiaan Leeuwenburgh[c], Robert E. Ferrell[d], Dana A. Phares[a], Jennifer A. McKenzie[a], Steven J. Prior[a], James M. Hagberg[a]

[a]*Department of Kinesiology, University of Maryland College Park, MD 20742, USA*
[b]*Section of Geriatrics, Department of Medicine, Howard University Hospital, Washington DC 20060, USA*
[c]*Biochemistry of Aging Laboratory, College of Medicine and Health and Human Performance, University of Florida, Gainesville, FL 32611, USA*
[d]*Department of Human Genetics, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA 15261, USA*

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Abstract

An etiologic role for chronic inflammation in the development of insulin resistance has been hypothesized. We determined whether the −732A/G and +219G/A C-reactive protein (CRP) gene variants affect insulin and glucose measures and whether these variants affect training-related changes in insulin sensitivity and glucose measures. Men and women 50 to 75 years old (n = 61) underwent baseline testing that included glucose tolerance, maximal oxygen consumption, body composition, CRP levels, and genotyping assessments. Tests were repeated after 24 weeks of aerobic exercise training. In bivariate analyses, CRP −732A/G G allele carriers had significantly lower baseline postprandial plasma glucose and after-training CRP levels. After exercise training, the −732A/G G allele carriers had ~28% increase in insulin sensitivity index (ISI) and ~26% reduction in insulin area under the curve (AUC), compared with the ~7% increase in ISI and ~15% reduction in insulin AUC in the A allele homozygotes ($P = .03$). The significant enhancement of ISI in −732A/G G allele carriers remained evident in analyses limited to those with normal glucose tolerance. Multivariate analyses adjusted for demographic and biologic variables confirmed the significant enhancement of training-induced improvement in ISI by the CRP gene variant. In addition, the CRP −732A/G and +219G/A haplotype significantly associated with training-induced improvements in ISI and insulin AUC in separate multivariate models. In conclusion, the CRP −732A/G variant modulates exercise training–related improvements in ISI and glucose AUC, and the haplotype of the CRP −732A/G and +219G/A variants significantly affected training-induced changes in ISI and insulin AUC.
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1. Introduction

Type 2 diabetes mellitus is a heterogeneous disorder of glucose metabolism characterized by insulin resistance and beta-cell dysfunction [1]. Epidemiological studies indicate that diabetes prevalence may reach 30% by the next decade, making it a major public health problem in the United States and worldwide [1,2].

The familial clustering of type 2 diabetes mellitus, together with a higher concordance rate in monozygotic vs dizygotic twins and a recurrence risk of ~3.5 in the first-degree relatives of patients with diabetes, suggests a strong genetic influence on the development of type 2 diabetes mellitus [3,4]. In several genome-wide scans, a diabetic susceptibility locus has been identified on chromosome 1q21-q23 [1,5,6]. The extent to which this putative type 2 diabetes mellitus susceptibility locus influences metabolic aspects of type 2 diabetes mellitus, such as insulin resistance, is not completely understood. Interestingly, the C-reactive protein (CRP) gene is located in the same general chromosomal region as this type 2 diabetes mellitus susceptibility locus. Recently, Wolford et al [7] identified several single nucleotide polymorphisms (SNPs) located in the putative promoter region of the CRP gene and found that a haplotype containing the low-risk alleles was associated with a surrogate measure of insulin secretion in Pima Indians. These findings suggest that CRP gene variations may influence insulin sensitivity and glucose metabolism either directly or indirectly through alterations in plasma CRP levels.

* Corresponding author. Section of Geriatrics, Department of Medicine, Howard University Hospital, Washington DC 20060, USA. Tel.: +1 202 865 3397; fax: +1 202 865 3777.
E-mail address: tobisesan@howard.edu (T.O. Obisesan).

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C-reactive protein is an acute-phase reactant, the elevation of which is indicative of acute or chronic inflammation. An etiologic role for chronic inflammation in the development of insulin resistance [6,8-13] has been hypothesized. Physical activity has been shown to reduce CRP levels [14,15], and we recently showed that 6 months of aerobic exercise training was associated with reduced CRP levels [16]. Given the association of inflammatory markers with the prediabetic state, the significant chromosomal overlap of diabetes susceptibility and CRP gene loci, and the effects of genetic variations on other cardiovascular disease (CVD) risk factor responses to exercise training [17,18], it is possible that CRP gene polymorphisms may interact with exercise training to differentially affect insulin sensitivity and glucose metabolism. We hypothesized that fasting and oral glucose tolerance test (OGTT) plasma glucose and insulin concentrations would associate with plasma CRP levels and variants of the CRP gene. More importantly, we hypothesized that the −732A/G and +219G/A CRP gene variants and their haplotypes would differentially influence insulin sensitivity and glucose concentration responses to aerobic exercise training.

2. Methods

Sedentary white and nonwhite men and women aged 50 to 75 years were screened via telephone to ascertain interest, suitability, and ability to participate in an exercise training intervention. The study protocol was approved by the institutional review boards at the University of Maryland College Park and the Howard University. Written informed consent was obtained during the participants' first laboratory visit. Eligible volunteers were nondiabetic, normotensive, or hypertensive with blood pressure (BP) controlled with medication (systolic BP <160 mm Hg, diastolic BP <90 mm Hg), nonsmokers, had body mass index (BMI) of less than 37 kg/m^2, did not have a regular aerobic exercise, and had no prior history of CVD. All women were postmenopausal and maintained the same hormone replacement therapy (HRT), either on or not on hormone replacement therapy, throughout the study.

During the participant's first laboratory visit, medical histories were reviewed to ensure subjects met the inclusion criteria, and BMI of less than 37 was ascertained. Participants had blood chemistry and fasting plasma glucose levels determined and underwent a standard OGTT. Those with fasting glucose of more than 126 or 2-hour glucose of more than 200 mg/dL were excluded. Participants had to have 1 or more National Cholesterol Education Program lipid abnormality as this study was part of a larger trial assessing training-induced plasma lipoprotein-lipid changes. Maximal treadmill exercise tests were performed; participants whose exercise test was terminated for CVD signs or symptoms or showed other evidence of CVD were excluded.

Participants then completed 6 weeks of instruction on the principles of an American Heart Association (AHA) step 1 diet (<30% energy from fat, ~55% from carbohydrates, ~15% from protein, cholesterol intake <300 mg/d) [19]. Participants completed a 7-day food record and adhered to the diet for more than 3 weeks before baseline testing. Participants maintained this diet throughout the study. A registered dietician analyzed all food records (Computrition, Chatsworth, CA). Participants completed food records during the exercise training intervention to ensure adherence to the diet.

Participants then completed baseline testing consisting of body composition, maximal oxygen consumption ($\dot{V}O_2$max), CRP level, and oral glucose tolerance test assessments. Body composition was assessed by dual-energy x-ray absorptiometry (DPX-L; Lunar, Madison, WI). $\dot{V}O_2$max was measured using a graded treadmill protocol [20]. On the day of blood sampling to determine CRP levels, participants must have had no alcohol or antiinflammatory medications for 24 hours, no exercise for 24 to 36 hours, and no infections in the preceding week. C-reactive protein was measured using an enzyme-linked immunosorbent assay system (minimum detectable CRP level, 0.35 mg/L; inter- and intra-assay coefficient of variation, 3%-7% and 2%-4%, respectively) (Alpha Diagnostic International, San Antonio TX). Before- and after-training samples for each participant were analyzed in the same assay to minimize interassay variation.

A 3-hour OGTT was performed in the morning after a 12-hour overnight fast. Participants were asked to consume more than 250 g of carbohydrates per day for 3 days before the OGTT and record all food consumed. Dietary records were collected and examined to ensure adequate carbohydrate intake; compliance with all other preparatory instructions was ascertained through questioning. A catheter was placed in an antecubital vein, and blood samples were drawn before and 30, 60, 90, 120, and 180 minutes after the ingestion of a 75-g glucose solution. The blood samples were centrifuged, and plasma samples were separated and stored at −80°C until assayed for glucose and insulin concentrations. Each subject's samples from before and after training were analyzed in the same assay to eliminate the effect of interassay variation. Plasma glucose levels were analyzed with a glucose analyzer (2300 STAT Plus, YSI, Yellow Springs, OH). Plasma insulin levels were determined by radioimmunoassay (HI-14K, Linco Research, St Charles, MO). Glucose and insulin total area under the curve (AUC) was calculated using the trapezoidal method, and insulin sensitivity index (ISI) was calculated using the method of Matsuda and DeFronzo [21] based on OGTT data.

Genomic DNA was extracted from peripheral lymphocytes using standard methods [22]. Fluorescence polarization [23] was used to genotype the 2 CRP gene polymorphisms (−732A/G and +219 G/A). Haplotypes for the −732 and +219 loci were generated using PHASE v2 [24]. Unequivocal haplotypes were assigned to all subjects with the exception of double heterozygotes.

Participants underwent 3 supervised exercise training sessions per week for 6 months. Initial sessions consisted of

20 minutes of 50% $\dot{V}O_2$max exercise and progressed until 40 minutes of 70% $\dot{V}O_2$max exercise was completed during each session [16,20]. Exercise consisted of treadmill walking/jogging, stair stepping, and cycle and rowing ergometry. Participants added a lower-intensity unsupervised 45- to 60-minute walk on the weekend after 12 weeks of training. After exercise training, body composition, $\dot{V}O_2$max, CRP, and OGTT assessments were completed as before training. Participants' dietary compliance was determined before final testing. Samples for plasma CRP and OGTT measurements were drawn 24 to 36 hours after the subject's training session.

Statistical analyses were performed using the SAS statistical software system (SAS, Cary, NC) [25]. Participants with CRP levels of 10 mg/L or higher were excluded from our analyses based on the AHA CRP levels criteria [12]. C-reactive protein levels, ISI, and insulin and glucose AUC were transformed to achieve normalization for analyses. To determine the association of insulin and glucose indices with CRP levels, we categorized baseline plasma CRP levels into tertiles and used a *t* test to compare insulin, glucose, and body composition indices of the lower (first) and the upper (third) CRP tertiles.

Rare allele homozygotes for each SNP were combined with heterozygotes as carriers of the rare allele and then compared with the noncarrier group for all analyses. Only 3 haplotype groups were used for statistical analyses because of the limitation posed by small sample size and the apparently dominant effect of the −732A/G G allele: haplotype I, homozygotes for the common A and G alleles at the −732A/G and +219G/A loci, respectively, were combined as a double-homozygote haplotype group; haplotype II, homozygotes for the −732A/G A allele, and +219 locus heterozygotes or A allele homozygotes were combined as the A homozygote haplotype group; and haplotype III, −732A/G locus heterozygotes or G allele homozygotes, and all +219 locus genotypes were combined as the G allele carrier haplotype group. The CRP variants included in the analyses showed no evidence of linkage disequilibrium ($D' = 0.45$).

For each of the 2 SNPs, separate analysis of variance (ANOVA) was performed using the general linear models. At baseline, complete data on CRP −732A/G and +219G/A genotype were available on 61 and 59 subjects, respectively. For the after-training bivariate models, 55 and 53 subjects, respectively, had complete data on CRP −732A/G and +219G/A genotypes. To examine the potential effect of impaired glucose tolerance (IGT: 2-hour OGTT glucose 140-199 mg/dL) on insulin indices, we stratified the sample into IGT and normal glucose tolerance (NGT: 2-hour OGTT glucose <140 mg/dL) groups. Because very few participants had IGT, subgroup analysis for the effect of the −732A/G gene variant on insulin indices was performed in the NGT group, but not in the IGT group. Each model testing the influence of the −732A/G variant was first adjusted for demographic variables (age, sex, ethnicity). The final models included adjustment for demographic and biologic variables (body weight, percentage of total body fat). For each outcome variable, separate models were constructed for baseline levels and for training-induced changes. Because only the −732A/G variant showed consistent associations with ISI and glucose AUC changes, detailed analyses only focused on this variant; association with baseline and training-induced changes in ISI, and glucose and insulin AUC. Preplanned contrasts within an ANOVA framework, while covarying for age, sex, and ethnicity, were used for haplotype comparisons. For haplotype effects, all full models assessing the significance of ISI, and insulin and glucose AUC changes with exercise training were adjusted for corresponding baseline values of the dependent variables, CRP levels, and training-induced changes in biologic variables (body weight, percentage of total body fat). Statistical significance was accepted at $P \leq .05$.

3. Results

Allele and genotype frequencies for the −732A/G and +219G/A CRP SNPs were in Hardy-Weinberg equilibrium (Table 1). In the total sample and within CRP −732A/G and +219 G/A genotype groups, there were approximately the same distributions of men and women, of whites and nonwhites (Table 2).

3.1. Baseline

At baseline, the lower CRP tertile group had ~31% higher ISI than the upper CRP tertile group ($P = .02$). In addition, lower CRP levels tended to associate with lower insulin AUC, with the lower CRP tertile group having ~22% lower insulin AUC than the upper CRP tertile group ($P = .06$). Although the CRP tertile groups were similar in body weight, the lower CRP tertile group had ~20% lower total body fat compared with the upper CRP tertile group ($P < .01$). All other variables were not significantly different between the lower and upper CRP tertile groups.

The significant association of ISI with CRP tertile was maintained in multivariate analyses adjusted for demographic variables (main effect $P = .01$), and remained significant with additional adjustment for biologic variables (main effect $P = .04$), with these models accounting for

Table 1
Allele and genotype frequencies for the intervention group

SNP	Allele frequency		Genotype frequency		
+219G/A (n = 53)	G = 0.69	A = 0.31	GG = 0.45 (n = 24)	GA = 0.47 (n = 25)	AA = 0.08 (n = 4)
−732A/G (n = 55)	A = 0.75	G = 0.25	AA = 0.58 (n = 32)	AG = 0.35 (n = 19)	GG = 0.07 (n = 4)

Table 2
Before- and after-training characteristics of the sample by −732A/G genotype

Parameter	Baseline		After training	
	AA (n = 35-36)	G allele carriers (n = 24-25)	AA (n = 29-32)	G allele carriers (n = 21-23)
Age (y)	57.3 ± 1.0	58.6 ± 1.3	57.7 ± 1.0	59.3 ± 1.3
Sex (%)				
Men	52	48	50	50
Women	64	36	65	36
Ethnicity (%)				
Whites	60	40	60	41
Nonwhites	57	43	54	46
NGT (%)	83	91	72	95
IGT (%)	17	9	28	5
Body weight (kg)	76.9 ± 2.3	81.4 ± 2.9	77.3 ± 2.4	80.2 ± 2.9
BMI (kg/m^2)	27.4 ± 0.7	27.4 ± 0.8	27.3 ± 0.7	26.9 ± 0.7
Total body fat (%)	35.7 ± 1.6	33.4 ± 2.0	33.8 ± 1.8	32.1 ± 2.1
$\dot{V}O_2$max (L/min)	1.9 ± 0.1	2.1 ± 0.1[a]	2.2 ± 0.1	2.5 ± 0.1
$\dot{V}O_2$max (mL/kg per min)	24.7 ± 0.8	26.4 ± 0.8	28.5 ± 1.3	30.8 ± 1.2
CRP (mg/L)	2.7 ± 0.4	2.1 ± 0.4	2.9 ± 0.5	1.5 ± 0.3[a]
Fasting glucose (mg/dL)	89.5 ± 2	90.7 ± 1.7	92.4 ± 2	90.2 ± 2
Postprandial glucose (mg/dL)	115.1 ± 5.5	106.6 ± 4.4	116.4 ± 7	101.4 ± 8
Fasting insulin (pmol/L)	75.1 ± 5	90.2 ± 6[a]	69.0 ± 4	72.3 ± 5[b]
Postprandial insulin (pmol/L)	401.7 ± 44	433.6 ± 59	350.4 ± 51	300.0 ± 47[a,b]

Data are means ± SE. P value was set at less than .05 significance level. Of the 61 persons with complete data on genotype, and glucose and insulin indices at baseline, 55 completed the intervention and final testing. $\dot{V}O_2$max (L/min) and $\dot{V}O_2$max (mL/kg per minute) indicate absolute and relative maximal oxygen consumption, respectively.

[a] Statistically significant between genotype comparison.

[b] Change within genotype groups.

14% and 28%, respectively, of the variance in ISI. Baseline insulin AUC significantly associated with CRP tertile (main effect P = .02) in an age-, sex-, and ethnicity-adjusted model, and this relationship trended toward significance (main effect P = .09) when body weight and percentage of total body fat were considered, with the model accounting for 25% of the variance.

Although −732A/G G allele carriers had ~22% lower CRP levels than A allele homozygotes, this difference was not statistically significant (Table 2). Absolute $\dot{V}O_2$max differed significantly between CRP genotype groups, with G allele carriers having significantly higher values (Table 2). Otherwise, baseline glucose and insulin concentrations, and body composition did not differ significantly between −732A/G genotype groups. None of the other independent variables (age, sex, ethnicity) contributed significantly to the models (P = .35, .29, and .72, respectively).

3.2. Training-induced changes

In the total population, there was a significant training-induced increase in ISI (3.69 ± 0.2 vs 4.44 ± 0.3; P < .001) and a reduction in insulin AUC (9114 ± 444 vs 7453 ± 480 pmol/L per minute; P < .001), but no significant change in glucose AUC (16041 ± 390 vs 15490 ± 545 mg/dL per minute; P = .73). Within genotype groups, the G allele carriers had significantly greater training-induced improvements in fasting and postprandial insulin levels compared with A homozygotes (both P < .05) (Table 2). Training-induced ISI and insulin AUC changes were also significantly influenced by CRP −732A/G genotype, with G allele carriers having ~3-fold greater improvement in ISI (1.0 ± 0.2 vs 0.3 ± 0.2; P = .03) and ~2-fold greater reduction in insulin AUC (−2688 ± 571 vs −1301 ± 306 pmol/L per minute; P = .03) than A homozygotes. These greater training-induced increases in ISI in the −732A/G G allele carriers compared with A homozygotes remained evident in subgroup analyses limited to the NGT (P < .001). Independently, the −732A/G genotype significantly influenced CRP levels, with G allele carriers having lower values (1.5 ± 0.3 vs 2.9 ± 0.5 mg/L; P = .03) compared with A homozygotes. The percentage of exercise attendance did not differ significantly between the genotype groups (difference = 2%; P = .30). The −732A/G genotype did not interact with exercise training to significantly affect changes in body composition indices.

In multivariate analysis covarying for baseline CRP levels and the baseline value of the dependent variable, the −732A/G variant had a significant influence on training-induced ISI changes (P = .03), contributing ~7%, whereas the total model accounted for ~26% of the variance in ISI change with training (P < .01) (Table 3). After accounting

Table 3
Multivariate ANOVA modeling of exercise training–induced changes in ISI, and insulin and glucose AUC by CRP −732A/G genotype

Dependent and independent variables	Model 1 (bivariate)	Model 2 (adjusted)	Model 3 (adjusted)	Model 4 (adjusted)
	R^2 (P)	R^2 (P)	R^2 (P)	R^2 (P)
Change in glucose AUC (%)	4	12.0	20.4	24.3
CRP −732A/G genotype	(.15)	(.10)	(.06)	(.04)[a]
Glucose AUC (baseline)		(.94)	(.58)	(.78)
CRP (baseline)		(.05)[a]	(.04)[a]	(.14)
Change in insulin AUC (%)	6	15.9	20.3	29.2
CRP −732A/G genotype	(.07)	(.09)	(.10)	(.08)
Insulin AUC (baseline)		(.07)	(.11)	(.99)
CRP (baseline)		(.66)	(.49)	(.56)
Change in ISI (%)	13	26.1	32.6	35.3
CRP −732A/G genotype	(<.01)[a]	(.03)[a]	(.02)[a]	(.02)[a]
ISI (baseline)		(.02)[a]	(.02)[a]	(.32)
CRP (baseline)		(.43)	(.30)	(.39)

Model 1 is bivariate with CRP −732A/G genotype as independent variable; model 2 is multivariate adjusted for baseline values of the dependent variable and CRP levels; model 3 is also adjusted for age, sex, and ethnicity; and model 4 is additionally adjusted for training-related changes in body weight and percentage of total body fat.

[a] Statistically significant effect at an α level of less than .05.

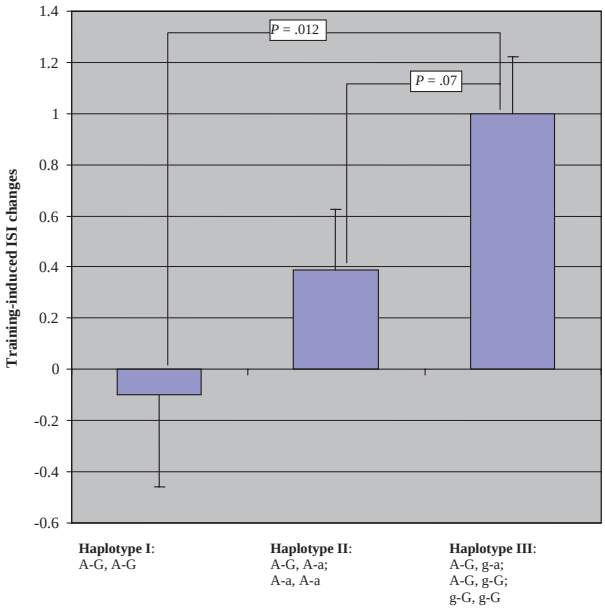

Fig. 1. Exercise training–induced changes in insulin sensitivity by haplotype of the CRP −732A/G and +219G/A gene variants (haplotype I: homozygotes for the common A and G alleles at the −732A/G and +219G/A loci, respectively [n = 9]; haplotype II: homozygote for the common A allele at the −732A/G locus, and heterozygotes or A homozygotes at +219 locus [n = 19]; haplotype III: heterozygotes or G allele homozygotes at −732A/G locus, and all genotypes at the +219 locus [n = 21]).

for demographic variables the model R^2 value increased to 33%, with the CRP −732A/G variant main effect remaining significant (P = .02), contributing ~7% to the variance in ISI changes with training. When the model was further adjusted for training-induced body weight and total body fat changes, the −732A/G variant influenced ISI, increasing the genotype main effect contribution to ~10% (P = .02), and the model contribution to ~35% (P = .01) of the variance in ISI changes with training. None of the other independent variables (age, sex, ethnicity) contribute significantly to the models (P = .18, .65, and .33, respectively). The full multivariate model restricted to those with NGT at baseline continued to show a significant association between the −732A/G variant and training-related improvements in ISI (P = .004).

Models equivalent to those for ISI changes with exercise training were also constructed for insulin and glucose AUC changes with training (Table 3). In the initial bivariate model, training-induced insulin AUC changes tended to associate with CRP genotype (P = .07), with the model accounting for ~6% of the variance. Separate adjustments for baseline values of the dependent variable and CRP levels and for demographic variables increased the model contribution to ~16% (genotype main effect P = .09) and ~20% (genotype main effect P = .10), respectively. With the addition of training-induced body weight and total percentage of body fat changes, the model's contribution increased to 29% and the genotype main effect trended toward significance (P = .08), contributing ~6% to the variance in insulin AUC change with training.

Although change in glucose AUC with training did not vary by −732A/G genotype on a bivariate basis, the genotype main effect trended toward significance (P = .06) when adjusted for demographic variables, with the model accounting for ~20% of the variance in glucose AUC change with training. With adjustment for training-induced body weight and percentage of body fat changes, the genotype main effect was significant (P = .04), explaining ~7% of the variance, whereas the model contribution increased to ~24% of the variance in glucose AUC change with training.

Independently, the CRP +219G/A variant did not affect training-induced ISI, or glucose or insulin AUC changes. In bivariate analyses, the −732A/G and +219G/A haplotypes were significantly related to training-induced ISI changes, with haplotype III having significantly greater training-induced ISI changes compared with haplotype I (P = .01), and trended toward significance when compared with haplotype II (P = .07) (Fig. 1). Haplotype III also had significantly greater improvement in training-induced insulin AUC changes compared with haplotype II (P = .007) and again trended toward significance when compared with haplotype I (P = .07) (Fig. 2). The association of training-induced glucose AUC changes with haplotype groups did not reach significance (P = .18) on a bivariate basis. In the fully adjusted multivariate model, ISI and insulin AUC was significantly associated with CRP gene haplotype (main effect P = .03 and .04, respectively), with the models accounting for ~41% and ~39%, respectively, of the variance. The haplotype of the CRP gene variants indepen-

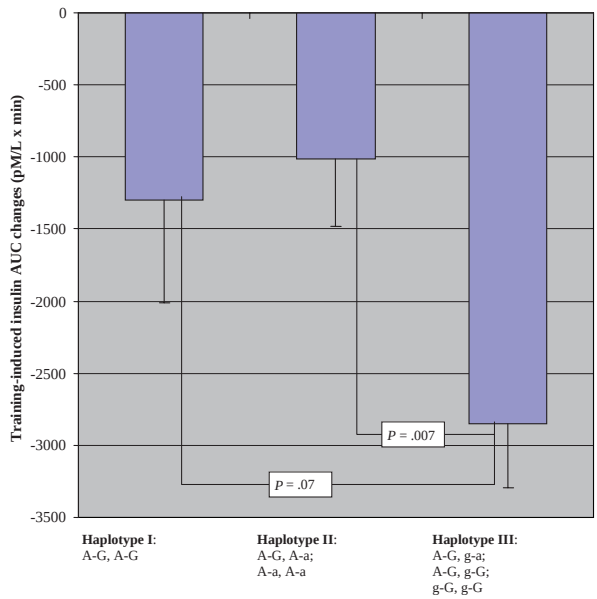

Fig. 2. Exercise training–induced changes in insulin AUC by haplotype of the CRP −732A/G and +219G/A gene variants (haplotypes as defined in the caption for Fig. 1).

dently accounted for 14% and 11% of the variation in training-induced ISI and insulin AUC changes, respectively.

4. Discussion

At baseline, ISI and insulin AUC associated with CRP tertiles, but not with CRP genotype. The most important finding of this study is that the −732A/G variant in the promoter region, but not the +219G/A variant, significantly modified exercise training-induced changes in ISI in our population. The haplotype of these variants also significantly influenced training-induced changes in ISI and insulin AUC. These data support earlier observations by others that a region on chromosome 1q21-q23, where the CRP gene is located, is linked to type 2 diabetes mellitus [1,7]. Thus, the CRP −732A/G variant in this region may have important clinical utility in identifying individuals at risk for type 2 diabetes mellitus who may be especially amenable to lifestyle intervention strategies.

C-reactive protein is one of the most stable inflammatory markers, and its levels have been shown to be higher in type 2 diabetic patients than in controls [26-28]. This led to the hypothesis that chronic inflammation may play an important role in the pathogenesis of type 2 diabetes mellitus [28]. Furthermore, a growing body of evidence indicates that high CRP levels are associated with insulin resistance in persons with increased propensity for type 2 diabetes mellitus, but who still have relatively normal blood glucose levels [29,30]. Festa et al [31] showed that prediabetic subjects with predominant insulin resistance had higher levels of inflammatory proteins than those with decreased insulin secretion. In our sample, the lower CRP tertile group had ~31% higher ISI and ~23% lower insulin AUC than the upper CRP tertile group at baseline. These observations support the association of low levels of CRP with enhanced ISI and a more favorable glucose profile.

Although the preponderance of evidence suggests that CRP may play an important etiologic role in the pathogenesis of type 2 diabetes mellitus, evidence is emerging on the contribution of the CRP gene to type 2 diabetes mellitus risk. In a genome-wide scan, Vionnet et al [1] reported a locus on chromosome 1q21-q24 to be in linkage with diabetes traits. In addition, Wolford et al [7] recently showed the same general locus, and specifically variants of the CRP gene, to be associated with type 2 diabetes mellitus susceptibility. In the present study, at baseline, the −732A/G G allele carriers had ~20% higher fasting plasma insulin levels, despite relatively similar glucose levels, compared with the A allele homozygotes, suggesting a more favorable metabolic profile in G allele carriers. It is, of course, possible that unknown confounders at baseline might have contributed to these differences. Nonetheless, these observations agree with reports of others suggesting a possible association of the CRP gene with glucose metabolism.

Aerobic exercise training has been shown to improve ISI in both obese and nonobese individuals [32], and with and without changes in body composition [33]. However, training-related improvement in ISI is highly variable among individuals. To date, no study has specifically examined the clinical utility of the CRP locus or its variants in targeting those at risk for developing type 2 diabetes mellitus for lifestyle interventions. Because of the association of preclinical diabetes with CRP level and the susceptibility of other CVD risk factors to lifestyle changes, we sought to determine whether CRP −732A/G genotype is associated with training-induced changes in insulin sensitivity, and glucose and insulin AUC. We also determined the extent to which the −732A/G and +219G/A variants, or their haplotype, influence exercise training–induced changes in these variables. After 6 months of aerobic exercise training, −732A/G G allele carrier group increased ISI by ~28% and reduced insulin AUC by ~26% compared with ~7% and ~15% improvements, respectively, in A allele homozygotes. Restriction of the analyses to persons with NGT did not change the significance of this association, indicating that IGT does not account for the differences between the 2 genotype groups. In multivariate analyses, −732A/G genotype explained ~7% of the variance in exercise training–induced ISI changes after accounting for demographic variables, and this increased to ~10% after adjusting for biologic variables. In the final model, training-induced ISI changes were independent of baseline ISI and CRP values. To a lesser extent, −732A/G CRP genotype significantly associated with training-induced changes in glucose AUC, whereas insulin AUC trended toward significance in the full multivariate model. These observations suggest that the CRP −732A/G G allele interacted with aerobic exercise training to enhance ISI.

In multivariate analysis, the haplotype of the CRP −732A/G and +219G/A variants associated with training-induced ISI changes. Haplotype III (heterozygotes or G allele homozygotes at −732A/G locus, and all +219 locus genotypes combined as the G allele carrier haplotype group) had the largest exercise training–induced improvements in ISI, and insulin and glucose AUC, compared with haplotype I (homozygotes for the A and G alleles, at the −732A/G and +219G/A loci, respectively, combined as double-homozygote haplotype group). Our study was not designed to capture the tag SNP for the CRP gene haplotype and the diabetic susceptibility locus on chromosome 1q21-q23 [34]. Nevertheless, significant improvements in ISI and insulin AUC for haplotype III compared with haplotypes I and II, respectively, highlight the relative importance of the G allele to its haplotype block [35]. It appears that the −732A/G G allele most likely plays a dominant role in influencing training-induced improvements in insulin sensitivity, and may, in fact, play a similarly important role in identifying high-risk individuals for specific lifestyle interventions to reduce their CVD risk. Therefore, the significance of the G allele as a tag SNP for CRP gene haplotypes and the diabetes susceptibility locus on the CRP gene warrants further investigation.

In addition to the potential clinical utility of gene-based risk stratification, it has also been suggested that observations similar to ours may, in fact, facilitate the characterization of the physiologic relationships needed to validate the potential relevance of genetic polymorphisms [36]. In light of this, a potential explanation for our observation of training-induced ISI improvements in this study is that DNA variation in the CRP gene may induce CRP-mediated signaling pathway up-regulation and improve the peripheral effects of insulin. Otherwise, a favorable environment, created by lower CRP levels in G allele carriers at baseline, may have an augmenting effect on exercise training–induced ISI changes.

The longitudinal study design, adherence to an AHA step 1 diet, stable body weight, strict screening, and standardized intervention are the specific strengths of the study. However, our observations and conclusions are limited by a relatively small sample size. Even so, given the magnitude of ISI improvement, it is likely that a larger sample size would confirm our present observations. Nonetheless, it remains possible that other nearby known or yet to be identified SNPs could contribute to the association of CRP gene variant with insulin sensitivity.

Collectively, our findings suggest that 6 months of aerobic exercise training interacted with −732A/G genotype, or a locus nearby that may be in linkage disequilibrium, to significantly modify insulin and glucose metabolism. In addition, the haplotype of the −732A/G and +219G/A CRP gene variants significantly interacted with exercise training to influence ISI improvement. These results strengthen the association of the CRP genomic region on chromosome 1q21-q23 with the diabetes susceptibility locus. More importantly, our findings lend support to the use of genotyping to identify high-risk patients who may specifically benefit from primary prevention strategies. Collectively, these observations are consistent with a putative causal role for type 2 diabetes mellitus that may benefit from early lifestyle intervention.

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