Apolipoprotein E genotype and sex influence C-reactive protein levels regardless of exercise training status

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Abstract

C-reactive protein (CRP) is a marker for systemic inflammation and increased cardiovascular disease risk. Regular exercise may decrease CRP. Apolipoprotein E (apo E) has 3 common genotype variants—E2/3, 3/3, and 3/4—that modulate lipid metabolism and may have other metabolic physiologic roles, including some evidence that the genotype affects CRP levels. We assessed fasting serum CRP in 117 (male = 51, female = 66) healthy adults who volunteered for a 6-month aerobic exercise program. Both pre- and posttraining measurements were available in 71 (male = 31, female = 40) subjects. At baseline and follow-up, the numbers of subjects in the 3 groups were approximately equal: 2/3, n = 33 and 20; 3/3, n = 41 and 26; and 3/4, n = 43 and 25. At baseline, CRP levels differed by apo E genotype: means ± SD were 2.84 ± 2.18, 2.59 ± 2.34, and 1.90 ± 2.13 mg/L for E2/3, 3/3, and 3/4 subjects, respectively (3/4 vs 2/3, $P < .05$). In women, CRP was higher than that in men (3.14 ± 2.49 vs 2.12 ± 2.13 mg/L, $P < .006$). Exercise failed to affect CRP in the entire cohort (2.68 ± 2.38 vs 2.52 ± 2.48 mg/L) or in any apo E genotype group, and the apo E genotype effect observed at baseline persisted after training. In a largely white study cohort, CRP is higher in apo E3/3 than in 3/4 subjects and in women compared with men, but remains unchanged by 6 months of standard aerobic exercise training of the volume and higher intensity promoted by national organizations to reduce cardiovascular disease risk. How apo E genotype affects CRP is not known.

1. Introduction

Recent evidence suggests that inflammation contributes to the atherosclerotic process and that markers of inflammation including C-reactive Protein (CRP) are also risk markers for future atherosclerotic events [1]. C-reactive protein is one of several "acute phase reactants" that include other markers such as fibrinogen and serum amyloid A that increase with inflammatory stress [2]. Increased frequency

of physical activity [3] and higher aerobic fitness [4] are associated with lower CRP concentrations. These observations suggest that regular physical activity potentially diminishes the systemic inflammatory response and is postulated to delay atherosclerosis.

Despite the strong evidence of a cross-sectional association between physical activity/fitness and CRP, few prospective longitudinal studies have determined if CRP decreases with aerobic exercise training (ET). Exercise training was seen to reduce CRP levels in previously trained individuals during preparation for a marathon [5], but this volume of ET is far greater than the minimum required for

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health benefits [6]. The effect of such an exercise program with a lower volume of exercise on CRP levels is equivocal. One study observed a small, nonsignificant decrease (6%) in CRP in a group of weight-stable individuals who had completed a supervised 6-month ET program [7]. Smith et al [8] showed a much larger decrease in CRP over 6 months (35%), but high baseline variability may have contributed to this change not being statistically different. In contrast, data from the HERITAGE study were stratified according to baseline levels of CRP and showed that ET reduced CRP in those with high baseline (>3.0 mg/L) CRP [9]. Therefore, grouping individuals according to baseline levels of CRP and thus minimizing baseline variation might facilitate the observation of ET-induced changes in CRP levels.

Little is also known about the effect of specific genetic variants on CRP levels. For example, apolipoprotein E (apo E) has 3 common protein-coding allelic variants—E2, E3, and E4—that have been demonstrated to modulate lipoprotein metabolism in sedentary populations [10,11] and the response to training [12]. The presence of the apo E4 allele itself is a significant genetic risk factor for the early development of coronary artery disease [13], much of which has been ascribed to its effect on lipid metabolism causing higher low-density lipoprotein levels [14]. However, recently, additional mechanisms have been suggested including possible anti-inflammatory properties of apo E [15].

Apolipoprotein E appears to protect vessels from atherosclerotic development induced by inflammation, as inferred from accelerated atherosclerosis in apo E–null mice [16,17]. In humans, CRP and plasma apo E concentrations are negatively related [18]. Apolipoprotein E genotype has been shown to partly determine plasma levels of apo E, with E2 carriers having higher levels than E4 carriers [19]. Therefore, it is possible that apo E genotype might influence CRP levels. Contrary to the negative relationship between CRP and plasma apo E concentration, some epidemiologic studies have shown higher CRP in individuals carrying the E2 allele or lower CRP in individuals carrying the E4 allele [20-26]. Other studies show no effect of genotype [27-29].

Therefore, the purpose of this study was to examine the effect of apo E genotype on CRP levels in sedentary adults. Secondly, because longitudinal studies measuring the response of CRP to ET are inconsistent, a further purpose was to examine the effect of supervised ET on CRP. Finally, because there is evidence that ET may decrease CRP specifically in those individuals with higher pretraining levels [9] and that apo E genotype may affect CRP levels, we examined the effect of apo E genotype on the response of CRP to 6 months of supervised ET.

2. Methods

2.1. Study overview

This study was conducted by the Exercise and Genetics Collaborative Research Group, a consortium of investigators at 7 institutions. We recently completed a 6-month aerobic ET study in volunteers recruited to create equal-sized cohorts of the most common apo E genotypes, which are E2/3, E3/3, and E3/4, and showed that both the increase in maximal oxygen uptake and the decrease in the ratio of low- to high-density lipoproteins in response to ET varied by apo E genotype. The same study population was used to collect these data, the methods for which have previously been reported [12].

Institutional review board approval was granted at all institutions, and written informed consent was obtained from all participants. In total, 566 individuals were screened by genotype to create 3 cohorts equal for sex and for the 3 most common apo E genotypes: apo E2/3, 3/3, and 3/4. Investigators were unaware of subjects' genotype and were only informed whether or not a subject qualified by genotype for inclusion. A total of 174 subjects initiated ET, with 120 completing the 6-month program. The CRP measurements were only available on 117 of the 174 subjects at baseline and 73 of the 120 finishers, two of whom had levels high enough to indicate acute infection and so were not included in the analysis. Therefore, baseline data were analyzed on 117 participants, and the effect of ET was analyzed on 71 of the finishers.

2.2. Subjects

Subjects were recruited if they were healthy and without orthopedic problems, nonsmokers, physically inactive, between 18 and 70 years of age, and consumed <2 alcoholic beverages daily. Subjects were considered physically inactive if they participated in vigorous activity <4 times per month for the prior 6 months. Subjects with diagnosed metabolic abnormalities or who were taking medications known to affect CRP levels were excluded from the study. Subjects underwent a medical history, physical examination, and a maximal exercise test to detect unreported abnormalities and

Table 1
Baseline characteristics of all subjects at baseline and those who completed the 6 months of aerobic training (finishers)

	n	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist Circumference (cm)
All subjects	117	37.4 ± 11.6 (18-62)	170.4 ± 9.8 (152-193)	81.7 ± 17.1 (47.7-138.6)	28.0 ± 4.7 (17.3-40.3)	88.8 ± 14.3 (59.7-134.6)
Women	66	38.2 ± 11.6 (18-62)	164.3 ± 6.6 (152-185)	73.8 ± 13.4 (47.7-101.8)	27.3 ± 4.5 (18.3-38.0)	82.6 11.7 (59.7-120.1)
Men	51	36.4 ± 11.5 (18-56)	178.3 ± 7.2 (152-193)	92.0 ± 15.9 (62.7-138.6)	28.9 ± 4.7 (17.3-40.3)	97.0 ± 29.2 (67.056-134.6)
Finishers	71	40.0 ± 11.5 (18-62)	171.6 ± 1.0 (152-193)	83.4 ± 18.5 (50.0-138.6)	28.1 ± 4.7 (19.7-40.3)	91.2 ± 14.7 (64.3-134.6)
Women	40	40.0 ± 11.9 (20-62)	165.0 ± 0.07 (152-185)	73.7 ± 13.0 (50.0-101.8)	27.0 ± 4.2 (19.7-37.4)	83.8 ± 11.7 (64.3-120.1)
Men	31	40.1 ± 11.3 (18-56)	180.1 ± 6.0 (169-193)	96.0 ± 17.0 (65.0-138.6)	29.6 ± 4.9 (20.9-40.3)	100.7872 ± 2.3 (77.5-134.6)

Data are expressed as means ± SD (range).

occult coronary artery disease. Race has been shown to affect CRP levels [30,31], so it is important to state that the study population was largely white. The characteristics of the subjects are shown in Table 1.

2.3. Apolipoprotein E genotype determination

The DNA was extracted from leukocytes, and apo E variants were determined using standard techniques [32].

2.4. Serum CRP measurements

One fasting serum sample was obtained after a 12-hour fast before the start and after 6 months of ET. Posttraining samples were obtained within 24 hours of the penultimate and final ET sessions. The CRP was assayed via rate nephelometry using a highly sensitive, 2-site chemiluminescent enzyme immunometric assay with 1 monoclonal and 1 polyclonal anti-CRP antibody. This method has been standardized against the World Health Organization International Reference Standard for CRP Immunoassay [33] and validated against the method approved by the Food and Drug Administration for use in assessing the risk of cardiovascular and peripheral vascular disease [34]. The CRP levels in women before and after training were obtained during the first 10 days of the menstrual cycle to avoid possible variations in values.

2.5. Anthropometric measurements

Body weight and height were measured using balance beam scales and wall-mounted tape measures. Waist girth measurements were taken using a cloth tape measure at the narrowest portion of the torso between the umbilicus and xiphoid process [35].

2.6. Insulin resistance

Insulin resistance was not directly measured, but was estimated from fasting glucose and insulin values using the homeostasis model assessment method [36].

2.7. Maximal exercise capacity

Subjects underwent 2 pretraining and 1 posttraining maximal treadmill exercise tests. The first pretraining test was designed to detect occult ischemia and to familiarize subjects with the measurement protocol, but was not used in data analysis. The second pretraining test and the posttest used the modified Åstrand protocol [37]. Blood pressure and 12-lead electrocardiogram, as well as expired oxygen, carbon dioxide, and ventilatory volume, were measured. Each test site used its own metabolic measurement system for cardiorespiratory measurements and followed manufacturers' calibration procedures. Maximal oxygen uptake was defined as the average of the 2 highest consecutive 30-second values at peak exercise.

2.8. Dietary control procedures

Tchernof et al [38] recently showed that CRP levels were reduced in response to weight loss from calorie restriction. Therefore, to asses the unmasked effects of ET, subjects were asked not to change their usual dietary composition throughout the study in an effort to maintain their original weight. Dietary calories and composition were assessed by random 24-hour dietary recall [39,40]. Trained dietitians called the subjects by telephone on 1 weekday and 1 weekend day before the start and during the last month of ET. Results from the 2 calls were averaged to estimate dietary intake.

2.9. Exercise program

Subjects underwent a 6-month, progressive, supervised exercise program. Exercise session duration was increased from 15 to 40 minutes during the first 4 weeks. Subjects exercised between 60% and 85% of their maximal exercise capacity based on pretraining maximal heart rate (HR). Once subjects could perform 40 minutes of exercise, they continued this duration of exercise 4 days a week for an additional 5 months. Subjects also participated in 5 minutes of warm-up and cooldown so that each workout required 50 minutes. The primary mode of exercise was performed on a treadmill; but a variety of other modalities including stationary cycles, cross-country ski machines, stair steppers, and rowing machines were also available.

All the subjects' exercise sessions were monitored by exercise physiologists to ensure that the appropriate levels of frequency, duration, and intensity were attained. Upon arriving at the training facility, all participants had to sign in at the beginning of each session. They were then given an HR monitor that was used to ensure that exercise was being performed at the appropriate intensity. Before signing out, each participant handed their HR monitor over to the exercise physiologist who recorded the average HR for that session. Failure to meet the stated limits resulted in removal from the study.

2.10. Data analysis

The CRP data were log transformed to normalize the positively skewed distributions seen in men, women, and within apo E genotype groups. Lakka et al [9] showed that women have higher CRP levels than men; therefore, sex was also selected as a dependent variable. To test for apo E, sex, and a possible interaction effect at baseline, we applied a 3 (group) × 2 (sex) analysis of variance using the GLM model (SPSS version 10.1; SPSS, Chicago, IL) to accommodate different sample sizes across sex and apo E genotype. To test for the effect of exercise, a 3 (group) × 2 (sex) × 2 (exercise, pre and post) repeated-measures analysis of variance was used. Analysis of covariance was used to eliminate the effects of body mass index (BMI) and maximum oxygen consumption (VO_2max) on the CRP results. The Sidak adjusted post hoc tests were used when F ratios were significant. Significance levels were 2-sided with $\alpha = .05$.

3. Results

3.1. The effects of apo E genotype and sex on baseline CRP levels

Both sex (Fig. 1) and apo E genotype (Table 2) showed independent effects on baseline levels of CRP. Subjects with the apo E3/4 genotype had lower CRP values than either the apo E2/3 or 3/3 groups ($P \leq .05$). Fig. 1 shows CRP values for men and women and reveals a significant sex effect with women having higher CRP than men ($P < .05$). However, there was no sex by apo E genotype interaction ($P > .05$).

3.2. The effect of 6 months of ET on cardiovascular fitness and anthropometric measures

Six months of ET resulted in an overall increase in VO$_2$max (30.75 ± 7.09 vs 33.61 ± 8.32 mL/[kg min], $P < .001$). As has previously been reported from this cohort, apo E genotype did influence the magnitude of change in VO$_2$max [12]. There were also observed reductions in body mass (-1.24 ± 3.63 kg, $P < .01$) and waist circumference (-1.65 ± 4.27 cm, $P < .001$) and no change in insulin resistance, all of which were unaffected by apo E genotype (time × genotype interaction, $P > .05$). Diet composition was unchanged over the study period (data not shown).

3.3. The response of CRP to 6 months of ET: the effect of apo E genotype and sex

The results of the repeated-measures analysis of variance suggest that the rate of change in CRP values due to 6 months of ET was not statistically different for either apo E genotypes (2/3, 3/3, and 3/4) or sex groups ($P > .05$). Indeed, 6 months of ET, overall, was not observed to significantly lower CRP values to a statistically significant degree
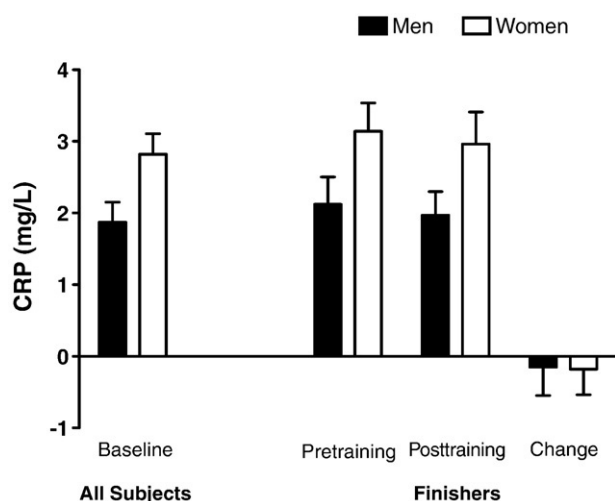

Fig. 1. Differences in CRP between men and women in a group of 117 participants who started the study (male = 51, female = 66) and in the group of 71 participants who completed the study (male = 31, female = 40). *Significantly different than men, $P < .05$.

Table 2
Data are presented for baseline CRP (in milligrams per liter) levels in all subjects and only those who completed 6 months of ET

	All subjects		Subjects who completed ET			
	n	Baseline	n	Baseline	Posttraining	Δ
E2/3	33	2.84 ± 2.18	20	3.09 ± 2.36	2.98 ± 2.43	-0.11 ± 1.84
E3/3	41	2.59 ± 2.34	26	3.05 ± 2.38	2.96 ± 2.69	-0.09 ± 2.63
E4/3	43	$1.90* \pm 2.13$	25	$1.99^{\dagger} \pm 2.34$	$1.71^{\dagger} \pm 2.15$	-0.28 ± 2.10
Total	117	2.40 ± 2.24	71	2.68 ± 2.38	2.52 ± 2.48	-0.17 ± 2.22

Posttraining CRP levels are presented only for the subsection of subjects who completed the training. The effect of apo E genotype is shown.

* $P < .05$, significantly different from E2/3.

† $P < .05$, significantly different from E2/3 and E3/3.

($P > .05$). Furthermore, the sex by apo E genotype interaction did not exhibit statistical significance ($P > .05$).

4. Discussion

In the present study, individuals with the apo E4/3 genotype had lower CRP at baseline before training. In addition, men had significantly lower CRP levels than women. In the entire cohort, 6 months of ET with minimal weight loss and no change in insulin resistance failed to reduce CRP levels. Furthermore, despite the baseline differences in CRP across apo E genotypes, no genotype effect was seen on the CRP response to training.

These results contrast with prior cross-sectional reports demonstrating that CRP is lower in more physically active or more physically fit individuals [3,4]. Although there is evidence that ET reduces CRP levels, it is questionable whether this was due to concomitant weight loss [41-43]. Body mass is a major determinant of CRP, with levels being increased in obesity, especially if the subjects are also insulin resistant [44,45]. The evidence of a causal relationship is strengthened by the observation that weight loss leads to reductions in CRP [38]. Therefore, it can be difficult to evaluate the impact of exercise, which is often accompanied by weight loss, on CRP levels. The present study was designed so that participants would remain weight stable over the 6-month training period, therefore allowing us to observe the effects of exercise per se along with apo E genotype. Despite this design, small but significant decreases in body weight and waist circumference were observed. However, these were likely to be metabolically insignificant as evidenced by the lack of change in insulin resistance.

There is considerable variability within the general population in CRP values, which poses a problem when investigating interventions designed to alter CRP levels. Specifically, an insufficiently small sample size given the variability may increase the risk of a type II error. In a group of recreational runners, a relatively homogenous population in terms of CRP, 9 months of marathon preparation resulted in a small absolute change in CRP (1.19 to 0.82 mg/L), but one that was statistically significant [5]. In contrast, the much larger decrease (although a similar percentage decrease)

observed by Smith et al [8] (4.18 to 3.13 mg/L) was not statistically significant in their more heterogeneous cohort. As such, we took measures to try and minimize variability in baseline CRP levels, such as exclusion of participants with diagnosed metabolic disorders such as diabetes and those who were on medications that have been shown to affect CRP, and finally eliminated all participants whose baseline or posttraining CRP values were greater than 10 mg/L, a value indicative of acute infection. That, coupled with our relatively large sample size, gives us confidence that the lack of change in CRP with 6 months of ET (2.68 to 2.52 mg/L) is real and not a statistical anomaly and is in agreement with some previous longitudinal studies [7,8].

The subjects in the previously cited study by Mattusch et al [5] were recreational runners who increased their running from 32 to 53 km/wk in preparation for participation in a marathon. Recreational runners typically exercise 5 to 6 times per week, sometimes at high intensity. Such intensity and volume, greater than in the present study, may be required to lower CRP and may explain the difference in results compared with the present study and suggest that a threshold may exist for ET-induced reductions in CRP to be observed. If true, these data suggest that an exercise program designed to meet the minimum levels required to deliver the health benefits as recommended by the Centers for Disease Control and Prevention is not sufficient to meet this threshold. However, we were able to collect training HR data on 66 of the 71 individuals with posttraining CRP data and investigated the possibility that there were different responses between those who habitually exercised at the lower end compared with the higher end of the intensity range. The bivariate correlation between change in CRP and average training HR was 0.009. We also split the cohort in half, into tertiles, and into quartiles according to training HR and in no case were within-group differences observed. In no instance was any statistical significance observed. Therefore, there is nothing to suggest that actual training intensity affected the CRP response in the present study.

The HERITAGE study showed that CRP was not universally reduced as a result of a 20 week aerobic exercise intervention [9]. However, when participants were grouped according to baseline levels of CRP, those with the highest level significantly reduced CRP independent of changes in body weight. Abdominal and visceral fat is highly associated with the systemic inflammation associated with the metabolic syndrome [46]. Recent evidence suggests that exercise can reduce fat storage in this depot without an overall improvement in body weight [47,48]. Such observations provide a rationale for the sometimes-observed decrease in CRP independent of weight loss. Although not reported in the specific cohort, it stands to reason that the high-CRP group in the HERITAGE study may have had higher baseline abdominal and visceral fat levels than the other groups. It can therefore not be ruled out that the nonuniversal changes in CRP observed in this cohort could have been explained by differential changes in these 2 fat depots that were not manifested through measurement of body mass. In the present study, despite the statistically significant reduction in waist circumference, the practical decrease was negligible and was accompanied by no change in levels of insulin resistance.

It has been several years since Manttari et al [22] first showed the unexpected negative relationship between the apo E4 allele and CRP. Most subsequent reports have confirmed this finding [20,21,23-26], but not all [27-29]. The present study agrees with the original findings, but the reason for the negative relationship between the apo E4 allele and CRP is still unclear. Plasma apo E concentrations are genotype dependent, with E3/E3 being intermediate between the higher E3/E4 and lower E2/E3 [19]. Apolipoprotein E has been shown to specifically down-regulate tumor necrosis factor α production in the central nervous system [49] and modulate systemic levels of proinflammatory cytokines [50], which partly explains the relationship between the E4 allele and the development of atherosclerosis [13] and Alzheimer disease [51], both in part inflammatory processes. However, this might lead to a hypothesis that CRP would be lowest in those with E2 and highest in those with E4; but the present data suggest the contrary. This finding persisted even after adjustment for BMI, so it is unlikely to have been mediated by pathology linked to differences in body weight.

A limitation of the present study is the fact that apo E2/3 and E4/3 genotypes were overrepresented in the present study. Expected population frequencies of E3/3, E2/3, and E4/3 genotypes are 65% to 70%, 5% to 10%, and 10% to 15%, respectively. The genotype distribution in the present study was 28%, 35%, and 37%. Caution must be taken when generalizing our results to the population. Additional larger studies of this relationship are therefore required. Furthermore, there are no prospective studies of the effects of high-intensity, high-volume ET on CRP. The CRP levels have been shown to be affected by modifications of the endocrine environment, such as oral contraceptive use and hormone replacement therapy [21]. The number of women on hormone replacement therapy in our cohort was too small (n = 4) to perform any further analysis, but excluding their data from the analysis had no effect on the outcome. Furthermore, we only had incomplete data on oral contraceptive use, which could be seen as a limitation to the present study.

These data are from a primarily white cohort. However, a preliminary analysis of the data suggested that race interacted with apo E genotype to affect CRP values ($P \leq$ .05). Closer inspection of the results revealed that the CRP values of the 3 participants of color increased overall as a result of regular ET, whereas the CRP values of the white participants decreased. Because the number of participants of color was too small for the interaction to be studied more closely, their scores were not analyzed further. Although race has been shown to influence CRP levels [30,31] and to affect the relationship between physical fitness and CRP levels [52], no study has shown race to affect how CRP responds to

ET. This requires further study. However, these data do confirm the observation that CRP levels are higher in women than in men [9,26].

A strength of the present study was that every exercise session was monitored by an exercise physiologist to ensure that the exercise prescription was being met. This strict enforcement did result in a dropout rate of approximately 30% over the course of 6 months, but was required to ensure that only those who were doing the appropriate ET were included in the results of a study investigating the effects of ET per se. As such, these data suggest that 6 months of ET does not alter CRP levels in individuals who maintain baseline levels of insulin resistance despite improvements in cardiorespiratory fitness and modest reductions in body mass and waist circumference. Furthermore, these data confirm the findings that both the apo E4 allele and male sex are associated with lower values of CRP. Despite the previous observation that baseline CRP levels play a role in the response to aerobic ET, the baseline effect of either apo E genotype or sex was not sufficiently large to affect the response to exercise in this cohort.

Acknowledgment

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