Ala12 variant of the peroxisome proliferator-activated receptor-γ gene (*PPARG*) is associated with higher polyunsaturated fat in adipose tissue and attenuates the protective effect of polyunsaturated fat intake on the risk of myocardial infarction[1–3]

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ABSTRACT

Background: Intake of polyunsaturated fat is protective against the development of coronary heart disease. Less is known about the genetic variation modulating this association. The Ala12 allele of the peroxisome proliferator-activated receptor-γ gene (*PPARG*) decreases the lipolysis of triacylglycerols in adipose tissue, which results in the accumulation of fatty acids in adipocytes.

Objective: We aimed to determine whether the Pro12Ala polymorphism interacts with polyunsaturated fat intake to affect the risk of myocardial infarction (MI).

Design: Cases (n = 1805) with a first nonfatal acute MI and population-based controls matched by age, sex, and area of residence (n = 1805) living in Costa Rica were genotyped for the *PPARG* Pro12Ala genetic polymorphism. Polyunsaturated fat intake was determined by use of a validated food-frequency questionnaire and by gas chromatography analysis of adipose tissue. Odds ratios and 95% CIs for MI were estimated by use of logistic regression.

Results: The relative allele frequencies of the Ala12 allele were 10% in controls and 11% in cases. Odds ratios (95% CI) for MI per each 5% increase in energy from polyunsaturated fat were 0.66 (0.53, 0.82) in Pro12/Pro12 subjects and 0.93 (0.61, 1.42) in carriers of the Ala12 allele (P for interaction = 0.03). Increments (95% CI) of polyunsaturated fat in adipose tissue per 5% increment in dietary intake were 5.4% (4.9%, 5.9%) in Pro12/Pro12 homozygotes, 6.9% (6.0%, 7.9%) in Pro12/Ala12 heterozygotes, and 7.7% (3.2%, 12.2%) in Ala12/Ala12 homozygotes (P for interaction = 0.016).

Conclusions: The protective effect of polyunsaturated fat intake on MI is attenuated in carriers of the Ala12 allele of *PPARG*. *Am J Clin Nutr* 2007;86:1238–42.

KEY WORDS Cardiovascular disease, peroxisome proliferator-activated receptor-γ, *PPARG*, polyunsaturated fatty acids, genetics, epidemiology, risk factors

INTRODUCTION

Although intake of polyunsaturated fat is clearly inversely associated with coronary heart disease (CHD), as reviewed in reference 1, the mechanisms mediating this protection are not completely understood. The best established cardioprotective mechanism of polyunsaturated fat intake is its strong effect on total and LDL-cholesterol lowering (2, 3). Other potential mechanisms include protection against arrhythmias (4, 5), reduction of inflammation (6, 7), and lowering of plasma triacylglycerols (8).

A deeper understanding of the mechanisms involved in the protective effects of polyunsaturated fat intake requires knowledge of the genetic control of fatty acid metabolism as well as the effect that genetic variation may have over this control. One key regulator of lipid metabolism is the peroxisome proliferator-activated receptor-γ gene (*PPARG*) (9). Two different protein isoforms, PPARγ1 and PPARγ2, are produced from the *PPARG* gene. The PPARγ1 isoform shows widespread expression, and the PPARγ2 isoform is mostly expressed in adipose tissue (10). Compared with the Pro12 wild-type allele, the Ala12 variant of the PPARγ2 isoform has been consistently associated with decreased risk of type 2 diabetes in different ethnic groups (11–13). A meta-analysis showed a 20% decrease in the risk of type 2 diabetes in carriers of the Ala12 allele compared with Pro12/Pro12 homozygotes (14). Because of the protective association between the Ala12 variant and risk of type 2 diabetes, it has been proposed that this allele may also affect the risk of CHD (15). Although some studies have reported that carriers of the Ala12 variant have a decreased risk of CHD (15, 16), other studies have not found such association (17, 18). At present, there is no consensus on whether the Pro12Ala polymorphism affects risk of CHD.

Differential flux of fatty acids through adipocytes could mediate, in part, the association between the Pro12Ala polymorphism, the risk of type 2 diabetes, and the risk of CHD. Activation of PPARγ by thiazolidinedione antidiabetic drugs results in higher uptake of fatty acids by adipocytes (19, 20), with the

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[2] Supported by grants HL49086 and HL60692 from the National Institutes of Health. EAR-N was supported as a Roadmap Fellow at the Harvard School of Public Health by grants T90 DK070078 and R90 DK071507 from the National Institutes of Health.

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Received February 26, 2007.

Accepted for publication June 27, 2007.

subsequent lowering of circulating free fatty acids (21–23) and improved insulin sensitivity (21). Although few studies have evaluated the effect of the Ala12 variant in adipose tissue fatty acids, some have reported decreased lipolysis of triacylglycerols in adipose tissue in carriers of the Ala12 allele and therefore higher accumulation of fatty acids in adipocytes (24, 25). If carriers of the Ala12 variant indeed tend to accumulate more fatty acids in adipose tissue than do Pro12/Pro12 homozygotes, then the Ala12 allele could modify the effect of specific dietary fatty acids on the risk of CHD.

We hypothesize that the Ala12 variant would modify the beneficial effect of polyunsaturated fat intake on the risk of CHD, because fatty acids would tend to be sequestered in adipocytes rather than being released and exert their beneficial effects over other tissues. We conducted a case-control study of 1805 survivors of a first acute myocardial infarction (MI) and 1805 population-based controls to test whether presence of the Ala12 variant of *PPARG* attenuates the protective effect of polyunsaturated fat intake on the risk of MI.

SUBJECTS AND METHODS

Study population

Participants were adult patients who were survivors of a first acute MI as diagnosed by a cardiologist at any of the recruiting hospitals in the Central Valley of Costa Rica between 1994 and 2004. A study cardiologist confirmed all cases according to the World Health Organization criteria for MI, which requires typical symptoms plus either elevation in cardiac enzymes levels or diagnostic changes in the electrocardiogram. Enrollment was carried out in the step-down unit of the recruiting hospitals. Cases were not eligible if they *1*) died during hospitalization, *2*) were over 75 y of age on the day of their first MI, or *3*) were physically or mentally unable to answer the questionnaire and *4*) had a previous hospital admission related to CHD. For each case, one population-based control subject, matched for age (±5 y), sex, and area of residence (county) was recruited. The controls were randomly selected by using data from the National Census and Statistics Bureau of Costa Rica. Because of the nationwide health system in Costa Rica, in which all the persons have access to medical care regardless of income, the control subjects represent the base population that gave rise to the cases. Control subjects were ineligible if they had ever had an MI or if they were physically or mentally unable to answer the questionnaires.

Trained personnel visited all study participants at their homes for data collection, biological specimen collection, and anthropometric measurements. Sociodemographic characteristics, medical history, and lifestyle habits were collected by using a general questionnaire. Dietary intake was collected by using a food-frequency questionnaire that was developed and validated specifically to assess fatty acid intake among the Costa Rican population (26, 27). Physical activity was determined as previously described (28). Briefly, subjects were asked the average frequency and time spent on several occupational and leisure-time activities during the past year. The activities were grouped into 6 categories based on their intensity, or METS (metabolic equivalents). One MET is defined as the energy expenditure for sitting quietly or $\approx$1 kcal · kg body wt^{-1} · h^{-1} (29). Biological samples were collected in the morning after the subjects had fasted overnight. Subcutaneous adipose tissue biopsy samples were collected from the upper buttock with a 16-gauge needle by using a modified version of the method of Beynen et al (30). In this study, there were 1805 case-control pairs with genotype information and complete data on all the descriptive variables and potential confounders. Participation was 97% for cases and 89% for controls. All subjects gave informed consent on documents approved by the Human Subjects Committee of both Harvard School of Public Health and the University of Costa Rica.

Fatty acid analysis

Fatty acids from adipose tissue were quantified by gas-liquid chromatography (27). Peak retention times and area percentages of total fatty acids were identified by using known standards (NuCheck Prep, Elysium, MN) and were analyzed with the Agilent Technologies ChemStation A.08.03 software. Twelve duplicate samples, which were indistinguishable from the others, were analyzed throughout the study for quality control purposes. The CV was 3.2% for polyunsaturated fatty acids.

Genotyping

The Pro12Ala single-nucleotide polymorphism was genotyped by using a variation of the allele-specific assay. The single-nucleotide polymorphism genotyping procedure consisted of 3 steps: in step one, DNA fragments were obtained by using polymerase chain reaction primers designed according to the single-nucleotide polymorphism's vicinity sequence. The reverse primers contained an artificially introduced sequence (derived from the bacteriophage M13) at the 5' end. In step 2, the allele-specific assay, the single-nucleotide polymorphism was genotyped with allele-specific forward primers. The reverse primer was labeled at the 5' end with the fluorescent dye fluorescein. In step 3, allele-specific assay products were separated by capillary electrophoresis with the ABI Prism 310 genetic analyzer (Applied Biosystems, Perkin-Elmer, Foster City, CA) and were analyzed by using the GENOTYPER software (Applied Biosystems). Eight control samples were genotyped for each plate throughout the study to assess genotyping reproducibility. Reproducibility was 99.9%, and <1% of the control and unknown samples had missing values. All samples, cases and controls, were double-blinded.

Statistical analysis

All data were analyzed with the STATISTICAL ANALYSIS SYSTEMS software version 9 (SAS Institute Inc, Cary, NC). Differences in health characteristics and potential confounders between cases and controls were assessed by Wilcoxon's rank-sum tests for continuous variables and with chi-square tests for categorical variables. Allele frequencies were estimated by the gene-counting method, and an exact test was performed to identify departures from Hardy-Weinberg proportions.

Odds ratios (ORs) and 95% CIs for the Ala12 variant (carriers or noncarriers) and total dietary intake of polyunsaturated fat (quartiles of percentage of total energy intake) were estimated by using logistic regression. All models were adjusted for sex, age (± 5 y), county of residence, BMI (quintiles), physical activity measured in METS (quintiles), income (quintiles), smoking (never smoker, past smoker, or current smoker of <10 cigarettes/d, ≥10 to <20 cigarettes/d, or ≥20 cigarettes/d), alcohol consumption (never, past, or 3 tertiles of current drinkers), history of hypertension (no or yes), and history of diabetes (no or

TABLE 1
General characteristics in myocardial infarction cases and in population-based controls from the Central Valley of Costa Rica[1]

Characteristics	Controls (n = 1805)	Cases (n = 1805)
Age (y)	58 ± 11[2]	58 ± 11
Sex (% female)	26	26
Waist-to-hip ratio	0.95 ± 0.07	0.97 ± 0.07[3]
BMI (kg/m^2)	26.5 ± 4.2	26.0 ± 4.1[3]
Physical activity (METS)	1.56 ± 0.68	1.51 ± 0.67[3]
History of diabetes (%)	14.5	25.5[3]
History of hypertension (%)	30.4	38.5
Current smokers (%)	20.9	39.7[3]
Total energy intake (kcal/d)	2443 ± 756	2696 ± 936[3]
Saturated fat (% of energy)	10.4 ± 2.7	11.0 ± 2.9[3]
Monounsaturated fat (% of energy)	11.9 ± 3.9	11.9 ± 3.4
Polyunsaturated fat (% of energy)	6.2 ± 2.0	6.0 ± 2.0[3]
trans Fat (% of energy)	1.16 ± 0.62	1.19 ± 0.56[3]
Polyunsaturated fat in adipose tissue (% of total fat)	18.4 ± 4.0	18.1 ± 4.0[3]
PPARG Pro12Ala genotype		
Pro12/Pro12 (n)	1470	1440
Pro12/Ala12 (n)	310	341
Ala12/Ala12 (n)	25	24
Ala12 relative frequency	0.10	0.11

[1] METS, metabolic equivalent tasks; *PPARG,* gene encoding peroxisome proliferator-activated receptor-γ.
[2] $\bar{x}$ ± SD (all such values).
[3] Significantly different from controls, $P < 0.05$ (Wilcoxon rank-sum test for continuous variables and chi-square test for categorical variables).

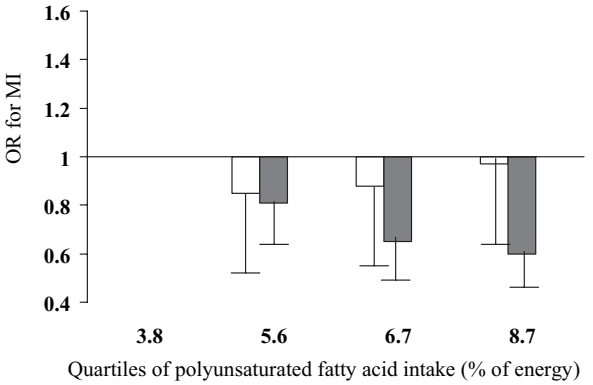

FIGURE 1. Odds ratios (ORs) for risk of myocardial infarction (MI) by polyunsaturated fat intake and Pro12Ala polymorphism genotype [□, carriers (P for trend = 0.99); ■, noncarriers (P for trend < 0.01)]. ORs were estimated by logistic regression by using as the reference the lowest quartile of polyunsaturated fat intake within each genotype. Error bars indicate 95% CI. Models were adjusted for sex, age, county of residence, BMI, physical activity measured in METS (metabolic equivalent tasks), income, smoking, alcohol consumption, history of hypertension, and history of diabetes. Interaction between the Pro12Ala polymorphism and dietary intake of polyunsaturated fat was assessed by likelihood ratio test. P for interaction = 0.03.

yes). Interaction between the Pro12Ala polymorphism and dietary intake of total polyunsaturated fat (quartiles of percentage of total energy intake) was assessed by use of the likelihood ratio test.

General linear models, with interaction terms, were used among controls to determine regression coefficients for polyunsaturated fatty acids in adipose tissue (% or total fatty acids) for each 5% increase in polyunsaturated fat intake (as percentage of total energy intake) by Pro12Ala genotype. The significance of the global interaction between the Pro12Ala polymorphism and polyunsaturated fat intake was assessed by use of the likelihood ratio test, comparing models with and without interaction terms. Models were adjusted for age, sex, county of residence, smoking status, and total energy intake.

RESULTS

The general characteristics of the study participants are shown in **Table 1**. Traditional risk factors were more frequent in the cases than in the population-based controls. The relative frequency of the Ala12 allele did not differ significantly between the cases and the controls. Multivariate ORs for the risk of MI by genotype (Pro12/Ala12 heterozygotes and Ala12/Ala12 homozygotes versus Pro12/Pro12 homozygotes as the reference group) did not show an association (OR = 1.10; 95% CI: 0.90, 1.33).

Polyunsaturated fat intake was significantly associated with decreased risk of MI among the Pro12/Pro12 homozygous subjects, who made up the majority of the population (**Figure 1**). Compared with the lowest quartile of polyunsaturated fat intake, the ORs (95% CI) for MI were 0.81 (0.63, 1.04) for the second, 0.65 (0.50, 0.84) for the third, and 0.60 (0.48, 0.76) for the fourth quartiles (P for trend < 0.01). In contrast, no significant association between polyunsaturated fat intake and MI was observed among carriers of the Ala12 allele. Compared with the lowest quartile of polyunsaturated fat intake, the ORs (95% CI) were 0.85 (0.52, 1.38) for the second, 0.88 (0.54, 1.41) for the third, and 0.97 (0.64, 1.48) for the fourth quartiles (P for trend = 0.99). We estimated that among the Pro12/Pro12 homozygous individuals, each 5% increment in energy from polyunsaturated fat was associated with an OR (95% CI) for MI of 0.66 (0.53, 0.82). No such protection was observed among carriers of the Ala12 allele. The estimated OR (95% CI) for MI for each 5% energy increase in polyunsaturated fat was 0.93 (0.61, 1.42). Thus, the association between polyunsaturated fat intake and risk of MI was modified by the presence of the Ala12 variant (P for interaction = 0.03).

Because the *PPARG* gene regulates the flux of fatty acids through adipose tissue, we postulated that the interaction between polyunsaturated fat intake, the Pro12Ala polymorphism, and risk of MI could be mediated by differential flux of fatty acids from and into adipocytes. The effect of a 5% increment in polyunsaturated fat intake on the difference in polyunsaturated fat in adipose tissue by genotype of the Pro12Ala polymorphism is shown in **Figure 2**. Each 5% energy increase in polyunsaturated intake was associated with a 5.4% (95% CI: 4.9%, 5.9%) increase in the proportion of polyunsaturated fat in adipose tissue in Pro12/Pro12 homozygotes, 6.9% (95% CI: 6.0%, 7.9%) in Pro12/Ala12 heterozygotes, and 7.7% (95% CI: 3.2%, 12.2%) in Ala12/Ala12 homozygotes. The likelihood ratio test comparing the models with and without interaction terms showed heterogeneity of slopes by Pro12Ala genotype (P for interaction = 0.016). These data suggest that compared with Pro12/Pro12 homozygotes, carriers of the Ala12 allele tend to accumulate more polyunsaturated fat in adipose tissue in response to the intake of polyunsaturated fat.

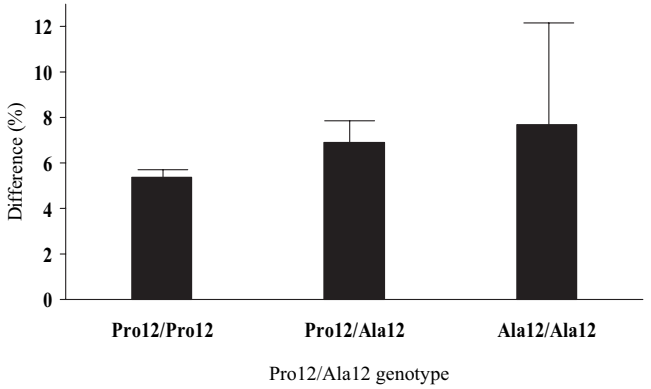

FIGURE 2. Difference in adipose tissue polyunsaturated fat associated with a 5% increase in polyunsaturated fat intake by Pro12Ala genotype. Error bars indicate 95% CIs. Regression coefficients were estimated by using general linear models adjusted for age, sex, county of residence, smoking status, and total energy intake. Significance of the global interaction between the Pro12Ala polymorphism and polyunsaturated fat intake was assessed by likelihood ratio test, comparing models with and without interaction terms. P for interaction = 0.016.

DISCUSSION

Although it is clear that intake of polyunsaturated fat is associated with protection against CHD (31–33), the mechanisms mediating this effect have not been completely elucidated. The *PPARG* gene plays a major role as a regulator of energy homeostasis and lipid metabolism (9, 34). Furthermore, carriers of Ala12, a common variant of the *PPARG* gene, may have higher accumulation of fatty acids in adipose tissue than do Pro12/Pro12 homozygous subjects (24, 25). In this study, we evaluated whether the Ala12 variant modifies the association between polyunsaturated fat intake and risk of MI and whether the diet–adipose tissue correlations differ by *PPARG* genotypes. The data show that the Ala12 variant of the *PPARG* gene attenuates the inverse association between polyunsaturated fat intake and risk of MI that was observed in the majority of the population. In addition, we found that a 5% increase in polyunsaturated fat intake was associated with a 20% higher increase in adipose tissue polyunsaturated fat in carriers of the Ala12 allele than in noncarriers of the variant. Our data suggest that in response to dietary intake of polyunsaturated fat, adipose tissue tends to preferentially accumulate polyunsaturated fat in carriers of the Ala12 variant compared with Pro12/Pro12 homozygotes. The greater accretion of polyunsaturated fat in adipocytes in carriers of the Ala12 allele could explain the lack of association between polyunsaturated fat intake and risk of MI observed among carriers of the Ala12 allele in the present study. Polyunsaturated fat may reduce the risk of CHD through a variety of mechanisms, including lowering of serum cholesterol (2, 3) and triacylglycerols (8), protection against arrhythmia (4, 5), and reduction of inflammation (6, 7); thus, it is possible that the trapping of polyunsaturated fat into adipose tissue would reduce the efficacy of these mechanisms.

The present study elucidates some of the opposing results regarding the role of the Pro12Ala polymorphism on the risk of MI. It is clear from several lines of evidence that the Ala12 allele has a protective effect against the development of type 2 diabetes (11–13, 35). However, the evidence regarding the association between the Ala12 variant and risk of CHD is inconclusive (15–18). In US men in the Physicians' Health Study, the presence of the Ala12 allele was associated with a 23% decrease in risk of MI under a dominant genetic model (15). In individuals with type 2 diabetes in the Go-DARTS (Diabetes Audit and Research in Tayside Scotland) study, the Ala12 variant was associated with 79% reduction in the risk of MI in subjects <70 y old (16). In contrast, there was no global association between the Ala12 allele and decreased risk of CHD in US women in the Nurses' Health Study or in US men in the Health Professionals Follow-Up Study (18). In fact, the Ala12 variant was associated with higher risk of CHD among subjects with BMIs (in kg/m^2) $\geq$ 25 (18). In addition, no association was found in German individuals with type 2 diabetes (17). Although all these studies have adjusted for several traditional risk factors for CHD, they did not adjust for dietary variables, and no data on polyunsaturated fat intake were reported (15–18). It is possible that differences in intake of polyunsaturated fat in the populations studied accounted for some of these discrepant results, and that the assessment of the effect of the Ala12 variant on the risk of CHD must take into account dietary variables such as polyunsaturated fat intake. In fact, our data suggest that in the context of diets that are high in polyunsaturated fat, the Ala12 allele would be associated with increased risk of MI because carriers of the Ala12 variant would not get the benefit from these diets.

At present, few studies have evaluated the effect of the Ala12 variant on flux of fatty acids through adipose tissue. One study found decreased lipolysis of triacylglycerols in adipose tissue in carriers of the Ala12 allele (24), which suggests a reduced release of fatty acids from adipocytes. A recent report found higher insulin clearance in carriers of the Ala12 variant than in Pro12/Pro12 control subjects (25). This increased insulin clearance was associated with lower plasma concentrations of free fatty acids, which also suggests a greater suppression of lipolysis of triacylglycerols in adipose tissue in carriers of the Ala12 allele (25). Our results are consistent with these previous reports, and they suggest a differential flux of fatty acids through adipose tissue according to Pro12Ala genotype.

Although the Ala12 allele was initially associated with lower BMI (36), this association turned out to be inconsistent in further studies (37–40). Three different meta-analyses have been conducted to assess the relation between the Pro12Ala polymorphism and BMI. The first one reported that the Ala12 allele was in fact associated with higher BMI in persons with a BMI > 27 (41). The second meta-analysis found a small trend toward higher BMI in carriers of the Ala12 variant than in Pro12/Pro12 homozygotes (OR = 1.13; 95% CI = 0.98, 1.29) (42). A recent meta-analysis found a significant association between the Ala12 allele and increased BMI in whites (43). We did not find an association between the Ala12 allele and BMI.

In summary, the present study showed that the Ala12 allele of the *PPARG* gene is associated with preferential accumulation of polyunsaturated fat in adipose tissue in response to polyunsaturated fat intake, and that the protective effect of polyunsaturated fat intake over the risk of MI is attenuated among carriers of the Ala12 allele. It remains to be determined which downstream pathways are most affected by the differential trapping of polyunsaturated fat in adipose tissue.

We thank Frank Sacks for his comments on the manuscript. We also are grateful to Xinia Siles for data collection and study management in Costa Rica, to the study participants, and to the staff of Proyecto Salud Coronaria, San José, Costa Rica.

The contributions of the authors were as follows–EAR-N: designed and conducted the data analysis, performed the main aspects of data interpretation, and wrote the manuscript; HC: designed the study; HC and PK: contributed to the data analyses and proofread and edited the manuscript; and ER-N: conducted the genotyping. The authors had no conflicts of interest.

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