Does Peroxisome Proliferator–Activated Receptor γ Genotype (Pro12ala) Modify the Association of Physical Activity and Dietary Fat With Fasting Insulin Level?

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Peroxisome proliferator–activated receptor γ (PPARγ) has a role in controlling adipogenesis and insulin sensitivity. Previous studies have suggested that a common polymorphism (Pro12Ala) in the PPARγ-2 isoform of this gene may be associated with markers of insulin resistance. We have previously shown that in combination, the relationships with fasting insulin of dietary polyunsaturated to saturated fatty acid ratio (P:S ratio) and physical activity are additive. We have also demonstrated that the association between P:S ratio and fasting insulin level is modified by the Pro12Ala genotype. The purpose of the present study was to investigate whether the Pro12Ala genotype modified the combined relationships of P:S ratio and physical activity level (PAL) on fasting insulin concentration. A population-based cohort of 506 Caucasian men and women aged 31 to 71 years was genotyped for the Pro12Ala polymorphism. P:S ratio was assessed by food-frequency questionnaire (FFQ) and PAL was estimated from 4 days of free-living heart rate monitoring following individual calibration of heart rate against energy expenditure during an exercise stress test. The combined associations of PAL and P:S ratio on fasting insulin level were examined stratified by Pro12Ala genotypes in a dominant model for the Ala allele. Among Pro allele homozygotes, there was no interaction between PAL and P:S ratio on fasting insulin ($P = .929$). However, in carriers of the Ala allele the association of P:S ratio with fasting insulin was modified by activity level (interaction $P = 0.038$). In those who were inactive and carried the Ala allele, the age-, sex-, and body mass–adjusted relationship between P:S ratio and log insulin was not significant ($\beta = -0.03$, $P = .93$). In contrast, in physically active Ala carriers, the association of P:S ratio with log fasting insulin was highly significant ($\beta = -0.93$, $P = .004$). In conclusion, this study examined the modification by PPARγ genotype of the association between energy expenditure, P:S ratio, and fasting insulin level, a measure of insulin resistance. These data show that in Pro allele homozygotes the combined associations of P:S ratio and PAL are additive. In contrast, in Ala allele carriers, PAL modifies the association between P:S ratio and fasting insulin level in a multiplicative manner.

THE FUNCTIONAL Pro12Ala polymorphism of the peroxisome proliferator–activated receptor γ (PPARγ) gene has been shown to be associated with lower risk of type 2 diabetes compared with the common Pro12Pro variant.[1] PPARγ is expressed at high levels in white adipose tissue and is part of a subfamily of nuclear hormone receptors. It is involved in the control of adipogenesis and insulin sensitivity, as demonstrated by null murine models[2] and in humans with mutations,[3] and mediates energy storage. The synthetic ligands of PPARγ are the thiazolidinediones, which are a powerful class of drugs that act through PPARγ to increase insulin sensitivity, whereas the natural ligands include polyunsaturated fatty acids (PUFA).[4] The affinity of a fatty acid to the PPARγ receptor increases with chain length and desaturation.[4] Thus, diets rich in PUFAs may upregulate the expression of PPARγ to a greater extent compared with diets composed largely of saturated fatty acids. The association between dietary fat composition and insulin resistance in animal and human models has been widely reported.[5-7] Typically diets high in saturated fats are positively associated with insulin resistance, whereas diets high in polyunsaturated fat improve insulin sensitivity.[8-10] Luan et al recently demonstrated that the Pro12Ala polymorphism modifies the relationship between polyunsaturated to saturated fatty acid ratio (P:S ratio) and fasting insulin in a large UK population.[11] It is through higher expression of PPARγ[4] that diets rich in fish oils may facilitate the lower rates of diabetes and coronary heart disease typical of polar regions.

Fasting insulin level is a proxy indicator of insulin resistance,[13-15] a key step in the development of type 2 diabetes mellitus.[12] Physical activity improves glucose disposal through numerous mechanisms. These involve weight loss,[16] improved lipid profiles,[16] improved glucose trafficking across the cell membrane,[17] hyperemia,[18] increased skeletal muscle surface area,[19] and an increased ratio of oxidative to nonoxidative muscle fiber.[19] The consumption of a high P:S ratio diet is positively correlated with physical activity level (PAL), suggesting that different aspects of a healthy lifestyle tend to be related. However, Harding et al recently demonstrated that after adjustment for body mass index, PAL and P:S ratio are independently associated with lower fasting insulin levels and are of additive benefit.[20] Thus far, the examination of the combined relationships of Pro12Ala genotype, habitual PAL, and dietary fatty acid ratio has not been reported. However, in a recent randomized controlled trial from Finland involving either an intensive diet and exercise intervention or a control intervention, Ala12 homozygotes lost more weight during the intervention compared with Pro12Pro homozygotes, and none of the Ala12 homozygotes developed diabetes during the trial.[21]

The purpose of this present study was to examine the combined associations of physical activity, dietary fatty acid ratio,

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and PPARγ genotype on fasting insulin levels in a large population-based cohort of Caucasian men and women.

MATERIALS AND METHODS

Study Population

A cohort of 506 nondiabetic Caucasian participants (mean age, 53.0 $\pm$ 10.8 years; 226 men and 281 women) was genotyped for the Pro12Ala polymorphism in the PPARγ gene. The volunteers were randomly selected from a cohort of 1,122 men and women from the Medical Research Council (MRC) Ely Study, a prospective population-based cohort study of the etiology and pathogenesis of type 2 diabetes and related metabolic disorders. Participant selection criteria has been described in detail elsewhere.[22,23] In brief, participants were randomly selected from a general practice register listing virtually all of the inhabitants of the City of Ely in Cambridgeshire and surrounding villages (N = 15,920). Seventy-four percent agreed to take part in an initial study that did not include the assessment of energy expenditure. Ninety percent of this cohort returned 4.5 years later for the current study. At this point complete anthropometric, dietary, and biochemical data were obtained in approximately 1,000 people. From this cohort a random sample of 598 individuals were selected and genotyped for an earlier study of interaction that did not include physical activity.[11] The present study is an extension of this previous study with the objective of ascertaining whether physical activity accounts for any additional modification of fasting insulin level. Data on physical activity in this earlier cohort was available in 506 individuals (85%). These individuals constituted the sample for the present study. The reasons for missing physical activity data were: abnormal electrocardiogram, and hence exclusion from the exercise stress test; incomplete heart rate data due to equipment malfunction; incomplete exercise stress test data; or refusal to undertake exercise stress test.

All participants underwent a standard oral glucose tolerance test to determine diabetic status, for which the methods have also been described previously.[22,23] As the Ala homozygotes were uncommon, all analyses were conducted comparing Pro homozygotes to Ala allele carriers. Anthropometric measurements were undertaken by trained observers using a rigid stadiometer and calibrated scales. Participants wore indoor clothing. Blood samples were taken after a 10-hour overnight fast, and were immediately placed on ice and centrifuged on site. Serum samples were aliquoted, packed in ice, and transferred to the University department of clinical biochemistry where they were stored at -70°C within 4 hours of withdrawal. Fasting plasma insulin was measured using 2-site immunometric assays with either ^{125}I or alkaline phosphatase labels.[22,23]

Assessment of Dietary Fats

Habitual diet during the previous year was assessed by means of a self-completion semiquantitative food-frequency questionnaire (FFQ). The FFQ is based on the questionnaire from the US Nurses' Health Study. The FFQ was developed and validated for use in a UK population on 127 women by comparison with 16-day weighed food records and 7-day diet diaries.[24] The frequency categories remained unchanged from the questionnaire used in the US Nurses Health Study, but, taking information from the British Food Survey, the lists of foods were changed to reflect the average British diet.

Assessment of Resting and Physical Activity Energy Expenditure

Following the measurement of anthropometry and blood pressure, a standard protocol for individually calibrating heart rate and energy expenditure was used. This method has been described in detail elsewhere.[25-27] In summary, this method relies on the computation for each individual of resting energy expenditure (REE), measured through indirect calorimetry, and the slope and intercept of the regression line describing the linear relationship between heart rate and energy expenditure during exercise. Following individual calibration, the volunteers wore a Polar heart rate monitor (Polar Electro, Kempele, Finland) continuously during the ensuing 4 days. Because heart rate is approximately linear to energy expenditure, the derivation of a regression coefficient for these 2 factors during the direct assessment of energy expenditure and heart rate permits the precise estimation of energy expenditure from heart rate data alone.[28]

Heart rate readings were directly downloaded onto a computer via a serial interface and the individual calibration data were used to predict minute energy expenditure for each person. Sleeping energy expenditure was calculated as 95% of basal metabolic rate (BMR). BMR was derived from published prediction equations.[29] PAL, which is the ratio of total energy expenditure to REE, was computed for each day and averaged over the 4-day period.

Genetic Analyses

To detect the Pro12Ala variant, which can also be identified as a C>G substitution at nucleotide 34, 2 primers, PPAR forward (5′-GCCAATTCAAGCCCAGTG-3′) and the mutagenic PPAR reverse (5′-GATATGTTTGCAGACAGTGTATCAGTGAAGGAATCGCTTTCCG-3′) (mutagenic position is underlined), were used in a polymerase chain reaction (PCR) reaction to amplify the fragment containing the C34G substitution from genomic DNA isolated from whole blood using a QIAamp blood kit (Qiagen, London, UK) and amplified 50-fold using the N*rich* kit (GENPAK Ltd, Brighton, UK). PCR was performed using Bio-Taq (Bioline, London, UK) and carried out under standard conditions. Thirty-five cycles (30 seconds at 96°C, 30 seconds at 55°C, and 60 seconds at 72°C) were performed using a GeneAmp 2400 PCR System (Perkin-Elmer, Beaconsfield, UK). The mutagenic PCR reaction introduced a *BstU*-1 restriction site when the C34G substitution was present. Restriction digest was performed using *BstU*-1 (New England Biolabs, Beverly, MA) and carried out under standard conditions for 120 minutes at 60°C. Gel electrophoresis was performed using 2% (wt/vol) agarose gels (Gibco BRL, Paisley, UK.) and the restriction digest products were subsequently detected with ethidium bromide under ultraviolet illumination.

Statistical Analyses

Fasting insulin was skewed and was normalized by logarithmic transformation for subsequent analysis. The unadjusted means and SEMs of anthropometric data were calculated stratified by sex, PAL, and genotype. PAL was stratified above and below its sex-specific median. One-way analysis of variance (ANOVA) was employed to test for differences between these strata. The unadjusted relationship between variables was estimated using Pearson correlation stratified by Pro12Ala genotype. In multiple linear regression models, the interaction between P:S ratio and PAL on log fasting insulin was initially explored in the whole dataset with adjustment for age and sex, and with and without additional adjustment for BMI. The same models were then tested stratified by Pro12Ala genotype (Pro/Pro *v* Pro/Ala + Ala/Ala). The interaction term in all models was fitted as PAL $\times$ P:S ratio. PAL and P:S ratio were analyzed as continuous data in all analyses. Finally, the association within genotype and PAL strata between P:S ratio and fasting insulin was assessed using multiple linear regression adjusted for age, sex, and BMI.

RESULTS

The frequency of Pro allele homozygotes in this cohort was 79.8%, and the frequencies of the Pro12Ala and Ala12Ala

Table 1. Participant Anthropometric, Biochemical, and Dietary Fatty Acid Ratio Data Stratified by Pro12Ala Genotype, Physical Activity Level, and Sex (N = 506)

	Low PAL (M/F)		High PAL (M/F)	
	Pro12Ala + Ala/Ala (27/26)	Pro12Pro (86/114)	Pro12Ala + Ala/Ala (22/32)	Pro12Pro (91/108)
Age (yr)				
Male	54.6 (2.39)	51.7 (1.26)	53.5 (2.63)	56.2 (1.01)[f]
Female	51.6 (1.79)	53.9 (0.99)	48.8 (1.64)	51.7 (1.00)[c]
BMI (kg/m^2)				
Male	27.8 (0.64)	27.0 (0.44)	26.5 (0.81)	26.9 (0.36)
Female	25.5 (0.90)[b]	26.9 (0.52)	25.9 (0.68)	25.6 (0.42)[b,e]
Fasting insulin (pmol · L^{-1})*				
Male	43.1 (34.5–53.8)	43.3 (37.5–50.0)	38.0 (29.4–49.1)	39.2 (34.4–44.6)
Female	38.6 (30.1–49.3)	40.7 (36.6–45.3)	35.7 (29.8–42.9)	33.3 (29.8–36.7)[b,f]
PAL (TEE/BMR)				
Male	1.62 (0.04)	1.65 (0.02)	2.40 (0.06)	2.23 (0.03)[a]
Female	1.50 (0.03)[b]	1.49 (0.01)[d]	1.99 (0.04)	1.99 (0.03)[d]
P:S ratio				
Male	0.55 (0.04)	0.57 (0.02)	0.53 (0.04)	0.54 (0.02)
Female	0.61 (0.04)	0.56 (0.02)	0.51 (0.04)	0.54 (0.02)

NOTE. Data are mean (SEM) or *geometric mean (95% confidence interval).

Independent samples *t* tests for differences between Ala carriers *v* Pro12Pro homozygotes: [a]$P < .01$; men *v* women: [b]$P < .05$, [c]$P < .01$, [d]$P < .001$; above *v* below the sex-specific median for PAL: [e]$P < .05$, [f]$P < .01$

genotypes were 19.4% and 1.8%, respectively. These genotype frequencies did not deviate significantly from Hardy-Weinberg predictions ($P > .05$) assessed by chi-square statistic. Owing to the infrequency of the Ala12 homozygotes, we combined Ala12 homozygotes with Pro12Ala heterozygotes for all subsequent analyses.

The characteristics of the cohort are shown in Table 1 stratified by genotype, sex, and PAL above and below the sex-specific median. Fasting insulin data are presented as geometric means and 95% confidence interval. In the high-activity group, women carrying the Pro12Pro genotype were younger ($P < .01$), had a lower BMI ($P < .05$) and lower log fasting insulin ($P < .05$), and were less active ($P < .001$) compared with men in the same genotype group. In the low-activity group, women with the Pro12Pro genotype were less active than their male counterparts ($P < .001$), whereas female Ala12 allele carriers had lower BMI ($P < .05$) and were less active than men in the same group ($P < .05$). In the high-activity group, men homozygous for the Pro12 allele were less active than male Ala12 carriers ($P < .01$). Table 2 shows correlation coefficients between log fasting insulin and other key variables stratified by Pro12Ala genotype. In Pro12 homozygotes, age and BMI were significantly correlated with fasting insulin ($P = .02$ and $P < .0001$, respectively), and P:S ratio was inversely correlated at a borderline level of significance ($P = .08$). In Ala allele carriers, BMI was strongly positively correlated ($P < .0001$) and P:S ratio inversely correlated ($P = .02$) with fasting insulin.

Regression models were initially analyzed stratified by sex. However, since the direction of effect was the same for men and women, data were combined for all subsequent analyses. When PAL and P:S ratio were considered as continuous data in the same model without stratifying by Pro12Ala genotype, they were both significantly associated with log fasting insulin (PAL $\beta = -0.13$ $P = .005$; P:S ratio $\beta = -0.22$ $P = .005$) and did not interact. However, when data were stratified by Pro12Ala genotype, the combined relationship of P:S ratio and PAL with fasting insulin was additive in Pro homozygotes, but was multiplicative in Ala carriers. For ease of illustration, Figs 1 and 2 show data dichotomized above and below the sex-specific medians for PAL and P:S ratio for Pro homozygotes and Ala carriers, respectively. However, in interaction analyses, PAL and P:S ratio were analyzed in their continuous form to preserve statistical power. Table 3 illustrates the adjusted interaction models for PAL by P:S ratio with fasting insulin level as the outcome variable. In Pro homozygotes, the combined association of physical activity level and P:S ratio on log fasting insulin was not multiplicative ($P = .61$) (Table 3 and Fig 1). However, in carriers of the Ala allele, the associations of P:S ratio and PAL on log fasting insulin were multiplicative (interaction $P = .038$) (Table 3 and Fig 2). The interaction meant that in physically inactive Ala carriers, there was no association

Table 2. Correlation Coefficients Between Independent Variables and Log Fasting Insulin for Men and Women (N = 506)

PPARγ Genotype	Log Fasting Insulin (pmol/L)
Pro/Pro (n = 399)	
Age (yr)	0.12*
BMI (kg/m^2)	0.54†
P:S fatty acid ratio	−0.09
PAL (TEE/BMR)	−0.07
Ala carriers (n = 107)	
Age (yr)	0.07
BMI (kg/m^2)	0.53†
P:S fatty acid ratio	−0.22*
PAL (TEE/BMR)	−0.08

NOTE. Data are Pearson correlation coefficients.

Level of significance within genotypes: *$P < 0.05$, †$P < .0001$.

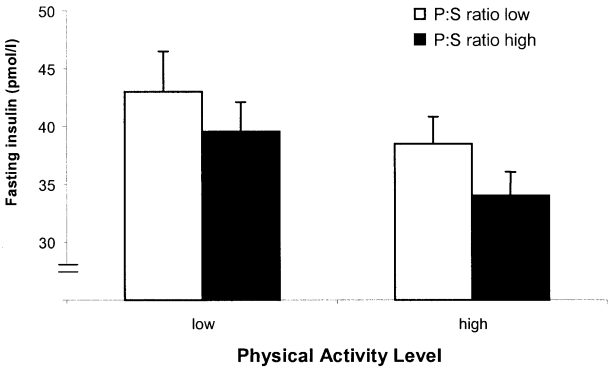

Fig 1. The interaction of PAL and P:S ratio on fasting insulin level in Pro allele homozygotes for the PPARγ Pro12Ala polymorphism (n = 399). (*P* for interaction in the model where PAL and P:S ratio were entered as continuous data = .610)

between P:S ratio and fasting insulin level ($\beta = -0.02$, $P = .96$). In contrast, in physically active Ala carriers the association between P:S ratio and fasting insulin was highly significant ($\beta = -0.95$, $P = .003$). For Pro allele homozygotes, the magnitude of the slopes for PAL ($\beta = -0.15$) and P:S ratio ($\beta = -0.24$) with log fasting insulin were similar to those observed in the nongenetic model for PAL ($\beta = -0.13$) and P:S ratio ($\beta = -0.22$), but they were not significant. The amount of variance in fasting insulin level explained by BMI in the model was substantially greater than that explained by the exposures of PAL and P:S ratio combined ($r^2 = 22.5\%$ *v* 5.85%). The removal of BMI from the model resulted in a marginal reduction in the explained variance of fasting insulin by PAL and P:S ratio combined ($r^2 = 3.71\%$).

DISCUSSION

This study investigated the interaction between physical activity energy expenditure, fatty acid composition of the diet, and PPARγ genotype on fasting plasma insulin levels in 506 men and women from the MRC Ely Study, Cambridgeshire, England. These results, when adjusted for age, sex, and BMI, demonstrate associations of diet and activity level on fasting insulin, which differ between Pro12Ala genotypes. The association between consumption of a high P:S ratio diet and increased physical activity on fasting insulin level is additive in homozygotes for the Pro12 allele. In individuals who are inactive and consume a diet that has a low ratio of P:S, fasting insulin level is highest. In contrast, fasting insulin level is lowest in Pro homozygotes who consume both high P:S ratio diets and who are physically active. When either P:S ratio or activity level is elevated and the other suppressed, the magnitude of change in fasting insulin is approximately half that when comparing the difference between the previous 2 exposure groups. These relationships are of similar magnitude to those observed in the dataset as a whole, but do not reach a level of statistical significance in the subgroup of individuals who are homozygous for the Pro12 allele. This lack of statistical significance is probably due to the smaller sample size. Diet and activity level do not relate in the same additive manner in Ala carriers. Instead the relationship of diet and activity with fasting insulin level in Ala carriers is multiplicative, and fasting insulin level is only attenuated when both exposures of diet and activity are simultaneously elevated. Those who are both inactive and have low P:S ratio diets, or who consume high P:S ratio diets, but are inactive, or who are active but consume low P:S ratio, all have similar fasting insulin levels. These levels are comparable to the highest levels seen in carriers of the Pro12Pro genotype (ie, those who are inactive and consume low P:S ratio diets). However, when the dietary ratio of P:S and activity level are both elevated, fasting insulin level in Ala carriers is marginally lower than the lowest level seen in the Pro/Pro genotype.

The variance in fasting insulin that is explained by the exposures of PAL and P:S ratio in the Ala carrier subgroup is low, relative to the variance explained by BMI. This would

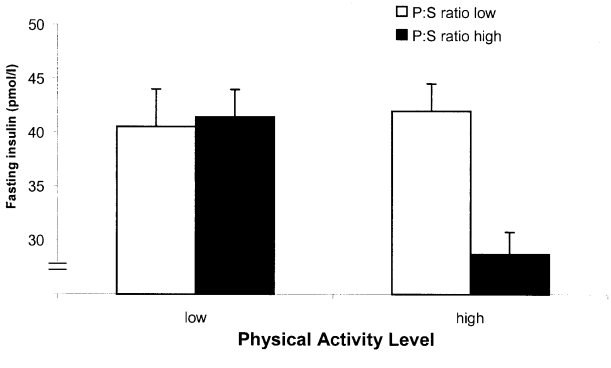

Fig 2. The interaction of PAL and P:S ratio on fasting insulin level in carriers of the Ala allele for the PPAR γ Pro12Ala polymorphism (n= 107). (*P* for interaction in the model where PAL and P:S ratio were entered as continuous data = 0.038).

Table 3. Linear Regression Model for the Interaction of PAL and P:S Ratio on Fasting Insulin Level Adjusted for Age, Sex, and BMI: Models Stratified by Pro/Pro and Pro/Ala + Ala/Ala Genotypes (N = 506)

	Log Fasting Insulin (pmol/L)	
PPARγ Genotype	β	95% CI
Pro/Pro (n = 399)		
Sex (men *v* women)	0.07	−0.04-0.17
P:S ratio	−0.24	−1.40-0.92
BMI (kg/m^2)	0.07†	0.06-0.08
Age (yr)	0.00*	0.00-0.01
PAL (TEE/BMR)	−0.15	−0.53-0.24
PAL * P:S ratio	0.04	−0.59-0.66
Ala carriers (n = 107)		
Sex (men *v* women)	0.01	−0.18-0.19
P:S ratio	1.56	−0.40-3.51
BMI (kg/m^2)	0.07†	0.05-0.10
Age (yrs)	0.00	−0.01-0.01
PAL (TEE/BMR)	0.45	−0.13-1.03
PAL * P:S ratio	−1.10*	−2.11-−0.09

NOTE: Data are adjusted β coefficients and 95% confidence intervals.

Level of significance: *$P < .05$, †$P < .0001$.

indicate that BMI may be more important in modifying fasting insulin level than P:S ratio and PAL. Nonetheless, understanding how diet, activity, and genotype affect sensitivity to insulin is of fundamental importance in preventing diabetes and insulin resistance, and understanding the underlying mechanisms that lead to metabolic dysfunction is clearly necessary. However, to determine the causal relationships of Pro12Ala genotype, diet, physical activity, and obesity with insulin resistance will require data from longitudinal cohort studies and randomized intervention trials.

In a recent publication from the Finnish Diabetes Prevention Study,[21] Lindi et al reported different responses to a 3-year exercise and diet intervention by genotype at the Pro12Ala locus of PPARγ. In the control group, Ala carriers were at 2.36-fold (95% confidence interval [CI], 1.21 to 4.60) increased risk of developing diabetes compared with Pro homozygotes. However, the Ala allele was not significantly associated with the risk of developing diabetes in the intervention group (odds ratio = 1.90; 95% CI, 0.70 to 5.18), and no Ala homozygotes in this group developed diabetes. Furthermore, although in the control group 3-year weight change did not differ between Pro12Ala genotypes ($P = .733$), in the intervention group Ala12 homozygotes lost significantly more weight across the 3 years than Pro12 allele homozygotes ($-8.3\% \pm 7.3\%$ *v* $-3.4\% \pm 5.7\%$; $P = .043$). These findings indicate that Ala12 homozygotes may benefit most from an intervention designed to increase physical activity and to improve diet. The data from our study concur to some extent with this observation, in that carriers of the Ala12 allele appear to derive at least the same benefit, perhaps more, in terms of their fasting insulin level, from being simultaneously physically activity and consuming diets high in P:S ratio, by comparison with Pro homozygotes. However, our observations also indicate that any reduction in insulin level in Ala carriers may require simultaneous changes in physical activity and P:S ratio.

It seems doubtful that the results we report in this present study could be due to confounding by other dietary factors, since the Pro12Ala genotype is unlikely to affect dietary preference. Previous studies have indicated that the effect of dietary fatty acid on insulin resistance may be modified by physical activity. In studies of female twins, Mayer et al showed that the positive association between total dietary fat and fasting insulin level was attenuated in moderately active compared with sedentary women.[30] Although this study addressed the effects of total fat, and not specifically P:S ratio, which may explain why the results are different to those reported in the present study, it does suggest that dietary fat consumption and physical activity affect insulin sensitivity in a synergistic manner.

The causal mechanisms underlying the observations reported here will require experimental models for clearer elucidation. Nonetheless, it is plausible that the interaction between activity level, P:S ratio and Pro12Ala genotype on fasting insulin is a result of improved transportation, via increased energy expenditure, of the ligand PUFA to the gene receptor. The outcome of improved ligand transport may be increased insulin sensitivity. There are 2 potential components to this mechanism. The first relates to fatty acid flux through the adipocyte. Aerobic exercise lasting more than 20 minutes or so promotes lipolysis.[31-33] In contrast to people who undertake exercise or dietary regimes for weight-loss purposes, people who are active across the lifespan are likely to experience less fluctuation in body mass. Relative to inactive weight-stable people, active weight-stable people will consume more food energy in order to maintain a neutral energy balance. Therefore, assuming similar P:S ratio diets between activity groups, the delivery of PPARγ ligands (ie, PUFAs) to the adipocyte, which is one of the main expression regions for PPARγ, is almost certainly greater in weight-stable active people compared with those who are inactive.

The second potential component to this mechanism relates to the differential channelling of fatty acid subtypes. PUFAs are preferentially metabolized and saturated fatty acids are preferentially stored,[34] an effect that is augmented by exercise.[35] In the postabsorptive state, fatty acids provide the predominant source of energy for resting muscle tissue.[36] Aerobic exercise training increases the rate of basal nonesterified fatty acid (NEFA0 metabolism,[37] but does not increase the ability to metabolize fatty acids per se when exercising. However, the ability to metabolize long-chain fatty acids (LCFA) does increase with training.[38] This is important, since saturated fatty acids tend to have the shortest chain length, whereas PUFAs, particularly those thought to be ligands for PPARγ, tend to have the longest. LCFAs bind to carnitine to produce fatty acylcarnitine, which is transported across the mitochondrial membrane via the carnitine-acylcarnitine translocase system. Sidossis et al conducted experiments to test whether training affects the rate of entry into the mitochondria of LCFA and medium chain fatty acids (MCFA) in 5 endurance-trained and 5 sedentary men.[38] Participants exercised at 40% and 80% peak oxygen consumption and LCFA and MCFA were infused throughout the course of the experiment. In trained participants, the percentage of LCFA oxidation was significantly higher than in untrained men ($P < .05$). However, there was no difference in the percentage of MCFA metabolized. These results indicate that endurance training increases the preferential metabolism of LCFA, which promotes the preferential storage of saturated fatty acids. Therefore, diets rich in saturated fats are likely to inhibit the delivery of PUFAs to the adipocyte, thus diminishing its effectiveness as a ligand for PPARγ.

The data presented here suggest that the beneficial additive effects of physical activity and dietary PUFAs on insulin sensitivity are restricted to Pro allele homozygotes. In contrast, the benefit of a high P:S ratio diet and of high activity levels are mutually dependent in carriers of the minor Ala allele. Unless physical activity and P:S ratio levels are simultaneously elevated, fasting insulin levels are equivalent to those seen in people whose diet and activity levels are deemed to be unhealthy, irrespective of genotype. Altshuler et al recently reported meta-analysis results where Ala12 allele was shown to protect against diabetes.[1] The results reported here suggest that although the Ala allele may confer protection from insulin resistance, it may be dependent on lifestyle behaviors, a finding that has important implications for our understanding of the pathogenesis of insulin resistance and for targeted health promotion.

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