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Plasma Arachidonic Acid Influences Insulin-Stimulated Glucose Uptake in Healthy Adult Women

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Key Words
Fatty acid · Insulin resistance · Fuel oxidation · Arachidonic acid · Docosahexaenoic acid · Physical fitness

Abstract
Background: Fatty acids can modulate lipid metabolism, this is related to insulin resistance (IR). This study evaluated the relationship of plasma fatty acid profile with IR, fuel oxidative metabolism and plasma lipid concentration in 'healthy' women. ***Methods:*** Sixteen 'healthy', sedentary and non-obese women were evaluated under fasting conditions for fuel oxidation, plasma fatty acid profile, free fatty acids, triglycerides, glucose and insulin concentrations. IR, fuel oxidation and plasma lipids were measured under insulin-stimulated conditions. Using the Spearman test the correlation between relevant variables was assessed. Stepwise multiple regression analysis was done to identify the main clinical/metabolic and fatty acid determinants of IR. ***Results:*** Plasma arachidonic acid content (%) determined IR, and in combination with insulin-stimulated plasma triglyceride concentration explained 45% of the IR variance. IR was inversely related to physical fitness ($r_s = -0.48$, $p = 0.01$). The latter was inversely associated to plasma saturated fatty acid content (%) ($r_s = -0.48$, $p < 0.01$), but directly associated to plasma docosahexaenoic acid content (%) ($r_s = 0.40$, $p = 0.04$). ***Conclusions:*** Support for the hypothesis that specific fatty acids influence IR is provided. Plasma arachidonic acid was associated to IR, independent on clinical/metabolic study variables. Docosahexaenoic and saturated fatty acids could potentially affect insulin action through modulating mitochondrial oxidative function.

Background

Insulin resistance (IR) is induced by fat overload in healthy, lean humans whether by parenteral lipid infusion [1–3] or consumption of a high-energy diet [4]. Animal studies indicate that the effect is dependent on fat source: very-long-chain n–3 fatty acids have a protective effect, whereas saturated and n–6 polyunsaturated fatty acids impair insulin sensitivity [5, 6]. In humans, descriptive studies have reported that dietary or plasma content of saturated fat is directly, and polyunsaturated fat is in-

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Table 1. Metabolic characteristics of the subjects and time-dependent variability

	0 week	4 weeks to 0 week (%)	R
Body weight, kg	58.7 (54.7–64.1)	–0.1 (–0.4 to 0.5)	0.98[a]
Body mass index, kg/m^2	22.7 (21.6–24.0)	–0.1 (–0.4 to 0.5)	0.97[a]
Body fat, %	31.6 (29.0–34.9)	0.4 (–3.4 to 4.8)	0.87[a]
Fasting glucose, mg/dl	92.1 (87.7–96.3)	–2.3 (–5.9 to 1.0)	0.55[b]
Fasting insulin, μIU/ml	15.0 (11.8–32.2)	–2.6 (–44.2 to 9.6)	0.71[a]
Fasting FFA, μmol/l	403 (358–561)	–5.1 (–10.6 to 6.7)	0.82[a]
Fasting triglycerides, mg/dl	75.0 (66.5–85.9)	1.2 (–22.0 to 9.9)	0.79[a]
SSPG, mg/dl	145.6 (105.1–155.7)	–10 (–24 to –3)	0.78[a]
SSPI, μIU/ml	70.9 (55.3–91.7)	–4.6 (–9.9 to 0.3)	0.82[a]
SSPF, μmol/l	63.3 (34.4–87.4)	–9.4 (–29.1 to –1.4)	0.92[a]
IST-triglycerides, mg/dl[1]	42.4 (33.0–50.0)	–3.4 (–13.1 to 3.6)	0.85[a]
Basal metabolic rate, kcal/day	1,331 (1,261–1,457)	–8.9 (–11.7 to –1.8)	0.71[a]
Fasting RQ	0.88 (0.87–0.91)	–0.8 (–1.7 to 0.0)	0.80[a]
IST-RQ AUC, 3 h	112 (111–114)	–1.3 (–2.0 to 0.2)	0.55[b]

FFA = Free-fatty acids; SSPG = steady-state plasma glucose; SSPI = steady-state plasma insulin; SSPF = steady-state plasma free-fatty acids; IST-triglycerides = plasma TG concentration at the end of the insulin suppression test; RQ = respiratory quotient; IST-RQ AUC = RQ area under the curve during the insulin suppression test; R = Spearman value for 0 week vs. 4 week measurement.

[a] $p < 0.05$; [b] $p = 0.06$.

[1] Insulin-stimulated plasma triglyceride concentration.

versely associated with the incidence of type 2 diabetes or the presence of IR [7–9]. Evidence of the potential mechanisms involved in this causative pathway is scarce. Moreover, changes in plasma/tissue fatty acid composition could be the result of IR rather than a potential cause, since activities of Δ^5- and Δ^6-desaturases, key enzymes in the formation of long-chain polyunsaturated derivatives, are insulin-dependent [10]. Thus, insulin-resistant subjects having a low dietary intake of arachidonic (AA) and docosahexaenoic (DHA) acid might have lower plasma/tissue content of these fatty acids since their formation from linoleic and α-linolenic acid, respectively, may be compromised. On the other hand, fatty acids are also known to have differential oxidation rates [11], variable capacity to influence fuel oxidative pathways [12] and regulate plasma triglyceride concentration [13]. These latter processes have additional implications affecting the pathogenesis of IR [14].

The present study assessed the relationship between plasma fatty acid profile with IR and some of the potential underlying mechanisms. Additionally, clinical/metabolic variables and specific plasma fatty acids were examined as putative determinant factors of IR using stepwise multiple regression analysis.

Variability in plasma fatty acid profile was reduced by measuring each subject twice 1 month apart and confounding variables as body mass index, body fat, physical fitness and age were controlled by design.

The main findings of the present study were an inverse correlation between plasma AA and AA/linoleic acid ratio with IR. No associations between IR and n–3 long-chain fatty acids were found; however, plasma DHA content was directly associated to peak aerobic capacity, which in turn was inversely related to IR.

Methods

Subjects

Sixteen healthy, young Hispanic [24.0 (21.5–26.0) years old], non-obese, sedentary women were recruited by massive e-mailing to students, employees and faculties of the University of Chile and by announcements on the local public radio. Volunteers were first evaluated by a physician to assess their overall health. Inclusion criteria were to have a normal glucose tolerance and plasma lipid profile within acceptable values, and a peak oxygen consumption <40 ml O_2/kg/min. Additionally, subjects were required to have had a stable body weight for the previous 3 months, regular menstrual cycles, not taking medications regularly, and no antecedents of type 2 diabetes in parents and siblings. The anthropometric and metabolic characteristics of the subjects are shown in table 1. INTA's Ethical Board for human research approved this study. Detailed information about the study was provided by the investigators and signed consent was obtained from each subject before admission.

Experimental Design

Subjects were evaluated under standardized conditions on two occasions with a 4-week period in between. Three days before admission to the INTA's metabolic facilities, subjects were instructed to maintain their usual dietary intake and to avoid intense physical activity. Subjects arrived the evening before and remained under supervision for 2 days on both occasions. A standardized dinner was provided after arrival [41 kJ (0.3 g fat, 1.4 g carbohydrate and 0.3 g protein) per kg of body weight]. The following day, a standardized diet supplied a 1.4-fold estimated basal metabolic rate according to published 2004 FAO/WHO/UNU equations [15]. Energy distribution for carbohydrate, fat and protein was 56, 30 and 14%. Dietary fatty acid composition was given as 24% saturated, 37% monounsaturated and 39% polyunsaturated fat. On the following day and after a 12-hour overnight fast, basal metabolic rate and body mass were determined. After that, a blood sample to measure the plasma fatty acid profile and metabolic profile was drawn. IR and fuel oxidation were assessed for 180 min under insulin-stimulated conditions. Body composition was measured after voiding and 3 h after the last meal. After completing the first study, subjects were instructed to follow their usual diet and physical activity pattern. To confirm that energy balance was maintained during the whole period, body weight, body composition and basal metabolic rate were determined again 4 weeks later. To avoid the influence of menstrual cycle on metabolic measurements, tests were scheduled between –13 and –1 day of the anticipated date of menstruation.

Insulin Resistance Test

IR was measured by the modified insulin suppression test [16]. Briefly, an intravenous cannula (BD Insyte) was inserted into a superficial vein in each arm. One arm was used to infuse a 10% glucose solution for 180 min at a rate of 330 mg glucose/m^2/min, 32 mIU/m^2/min of insulin (Humulin R, Eli Lilly, Santiago, Chile) and octreotide acetate (Sandostatin, Novartis, Chile) at 0.27 μg/m^2/min using an infusion pump (Infusomat fmS®, B. Braun, Santiago, Chile). Blood was sampled from the contralateral arm every 30 min until 150 min and then every 10 min to complete 180 min. Steady-state plasma glucose (SSPG), plasma insulin (SSPI) and plasma free-fatty acid (SSPF) concentrations were determined from the average of the last 4 blood samples. Additionally, the last blood sample was used to determine plasma triglyceride (TG) concentration. Since SSPI concentrations are similar between tests, the SSPG concentration provides a direct estimate of insulin-mediated glucose uptake. The higher the SSPG, the more insulin-resistant the individual is.

Basal Metabolic Rate and Fuel Oxidation

Basal metabolic rate and fuel oxidation were determined by indirect calorimetry using breath-by-breath gas analysis (MedGraphics CPX; Medical Graphics Co., St. Paul, Minn., USA). The oxygen and carbon dioxide sensors were calibrated before and as needed during the gas exchange measurement using gases with known oxygen, nitrogen, and carbon dioxide concentrations. The flow sensor was calibrated with the 3-liter syringe provided by the manufacturer. Energy expenditure and fuel oxidation were calculated from VO_2 and VCO_2 according to the method of Livesey and Elia [17]. The respiratory quotient (RQ) was calculated as the VCO_2/VO_2 ratio.

During the insulin suppression test, total area under the curve for RQ was calculated by the trapezoidal method, considering 6 periods of 30 min.

Blood Analysis

Blood samples for insulin, TGs and FFA were taken in EDTA-Vacutainer® tubes, whereas for glucose in BD Microtainer® tubes. These were immediately centrifuged and stored at –20°C for later analysis. To reduce variance between assays, blood samples from each subject were analyzed as one batch. Plasma glucose was assayed by the glucose oxidase method (GOD-PAP, QCA, Amposta, Spain). Plasma FFA by Wako NEFA-C test kit (Wako Chemicals, Richmond, Va., USA). Plasma TG determined with a colorimetric enzymatic kit (Dialab GmbH, Austria), and plasma insulin by ELISA (Dako Insulin, Dako Diagnostics, Ely, UK). Measurement errors for glucose, FFA, TG, insulin were 2, 5, 2 and 3%, respectively.

Plasma lipids were extracted according to Folch et al. [18]. Gas chromatography of fatty acid methyl esters was performed using a 5890 Series II Plus (Hewlett Packard, Palo Alto, Calif., USA), and a silica capillary column DB-FFAP (30 m × 0.25 mm ID) equipped with a flame-ionization detector. Methyltricosanoate (C23:0) was used as an internal standard. The analytical error for this determination is 0.17%. The detection limit for fatty acid concentration is 0.01%. Plasma fatty acids with a concentration below this value were assigned a value of 0.010%.

Body Weight and Body Composition

Body mass was measured under fasting conditions, after voiding with minimal clothing using a calibrated scale. Body composition was measured using the BOD-POD (Life Measurement, Inc., Concord, Calif., USA) and the Siri equation [19]. Fat-free mass (in kg) was calculated by subtracting fat mass from the total body mass. Measurement error for this determination is 3%.

Physical Fitness Measurement

Peak oxygen consumption was assessed using a graded ergometer bicycle (SECA Cardiotest Model 545, Germany) starting with 2 min at 50 W and increasing the load every 2 min by 25 W until exhaustion. The latter judged by one of the following criteria: subject could not maintain 60 rpm pedaling rate, the estimated maximum heart rate was reached [220 – age (years)] or a RQ >1.2. Oxygen consumption was continuously measured (CPX Express, MedGraphics Co., St. Paul, Minn., USA) during the whole period of exercise. The last minute of exercise was used to calculate the peak oxygen consumption (peak VO_2).

Data Analysis

All statistical analyses were done with the SAS software (Version 9.1, SAS Institute Inc., Cary, N.C., USA). Since several variables (i.e., fatty acid profile %, SSPG) were non-normally distributed (evaluated using the Shapiro-Wilk test), data were expressed as median and interquartile range and treated as such.

Intraindividual variability was calculated with the following formula [(final – initial/final value) × 100], and differences between measurement times were evaluated by the Wilcoxon test. Spearman correlation was used to assess the correspondence between values obtained in either measurement time.

Correlations between study variables were assessed by the Spearman test with and without adjustment for confounding

Table 2. Plasma fasting fatty acid profile and time-dependent variability

% of total fatty acids	0 week	4 weeks to 0 week (%)	R
14:0+16:0	20.1 (18.7–20.9)	–2.5 (–9.9 to –0.9)	0.15
18:0	7.0 (6.5–7.5)	0.6 (–8.1 to 5.0)	0.08
Saturated	27.6 (25.7–29.4)	–3.0 (–7.8 to –0.7)	0.25
18:1	21.1 (19.0–22.4)	–11 (–19 to –1)	–0.06
Monounsaturated	23.9 (22.9–26.0)	–12 (–20 to 4)	–0.20
18:2n–6	36.9 (33.3–41.1)	7.4 (–1.5 to 14.5)	0.13
20:4n–6	4.8 (4.1–6.0)	2.0 (–6.9 to 19.6)	0.65[a]
n–6 polyunsaturated	44.4 (40.2–46.9)	4.2 (–1.8 to 13.3)	0.21
18:3n–3	0.55 (0.40–0.69)	–12 (–49 to 7)	0.31
EPA	0.39 (0.35–0.47)	2.2 (–30.7 to 24.3)	–0.20
DHA	2.7 (1.8–3.0)	–0.4 (–22.2 to 19.8)	0.02
n–3 polyunsaturated	4.3 (3.4–4.8)	–6.6 (–37.3 to 27.8)	0.04
18:1/18:0	2.9 (2.8–3.2)	–4.8 (–25.7 to –0.9)	0.42
AA/LA	0.14 (0.11–0.18)	–6.3 (–8.3 to –1.6)	0.88[a]
EPA+DHA/α-linolenic	5.1 (3.9–7.1)	11 (–16 to 46)	0.36
DHA/EPA	7.3 (5.0–8.0)	–12 (–53 to 24)	0.01

R = Spearman value for 0 week vs. 4 week measurement.
[a] $p < 0.05$.

variables. The latter referred to variables showing a significant association to IR, which were controlled to isolate the effect of plasma fatty acid profile on IR. Finally, stepwise multiple regression analysis was done to identify the main plasma fatty acid determinants of IR. Clinical and metabolic variables were also included in the model as independent factors. A p value <0.05 was considered as statistically significant.

Results

Variability of Study Measurements

The subjects' anthropometric and metabolic characteristics and relative time-dependent variation for each variable are shown in table 1. No significant time-dependent changes were observed. Energy balance remained stable with a median variation for body weight, body fat and basal metabolic rate of –0.1, 0.4 and –8.9%, respectively. The SSPG (IR marker) had a –10% variation ($p > 0.05$). A similar or even lower variation was observed in plasma glucose and lipid concentrations ($p > 0.05$), whereas fuel oxidative metabolism showed a change close to –1% ($p > 0.05$). In general, high within-subject correlation between the two measurements was found (table 1).

Plasma fatty acid profiles revealed no significant time-dependent differences (table 2). However, significant within-subject correlations were found for plasma AA and AA/linoleic acid ratio (table 2). Median intraindividual variability for plasma saturated, monounsaturated, n–6 and n–3 polyunsaturated fatty acid concentration were –3, –12, 4 and –7%, respectively. Interindividual variability for the main IR determinant factors was low, since subjects by selection criteria were homogenous in body mass index, body fat, age and physical fitness (VO_2 peak = 27.4 (24.8–29.9) ml O_2/kg/min) (see table 1).

Insulin Resistance and Clinical Measurements

To reduce the influence of body mass, body fat, age and physical fitness on IR, subjects with similar characteristics were selected as previously stated. Thus, no significant relationship between variances in these variables and IR were expected. However, peak VO_2 was inversely related to IR ($rs = –0.48$, $p = 0.01$), suggesting that the individual fuel oxidative metabolism induced by exercise may have intrinsic relationships with IR beyond the variables used as part of our standardized selection process.

Fuel Oxidative Metabolism, Plasma Lipids and Insulin Resistance

The main fuel substrate oxidized by these subjects under fasting conditions was glucose, representing over 60% of energy expenditure based on measured RQ of ~0.88. We failed to demonstrate a relationship between fasting oxidative metabolism (RQ) and IR. On the other hand, during the glucose/insulin infusion period, RQ rapidly increased reaching values close to 1.00 around 2 h, and maximal values at 3 h (end of the period), which

indicated fully suppressed fat oxidation and exclusive dependence of glucose as a fuel. A significant inverse association between the RQ area under the curve and IR was observed (rs = –0.43, p = 0.03); indicating a relationship between increased glucose oxidation and higher insulin sensitivity. Plasma lipid concentrations (TG and FFA) were not correlated to IR, whether under fasting or insulin-stimulated conditions.

Fuel Oxidation, Physical Fitness and Plasma Fatty Acid Profile

Fuel oxidative metabolism was related to plasma fatty acid profile only under insulin-stimulated conditions, the latter evaluated as the RQ area under the curve. In this case, an inverse correlation with plasma α-linolenic acid content (%) (rs = –0.46, p = 0.01) was found. In addition, direct associations with plasma AA (%) (rs = 0.41, p = 0.03) and AA/linoleic acid ratio (rs = 0.38, $p < 0.05$) were observed. Plasma n–3 polyunsaturated fatty acids were not correlated to insulin-stimulated fuel oxidation, however direct associations were found when the product/precursor ratio EPA+DHA/α-linolenic acid ratio was used as an index of n–3 metabolism (rs = 0.54, $p < 0.01$).

Physical fitness measured by the peak VO_2 showed an inverse correlation with plasma saturated fatty acid content (rs = –0.46, p = 0.01), mostly explained by the sum of myristic and palmitic fatty acids (rs = –0.48, $p < 0.01$), whereas a direct correlation with plasma DHA concentration was noted (rs = 0.40, p = 0.04).

Plasma Lipids and Fatty Acid Profile

Plasma fasting fatty acid profile was differently correlated with plasma FFA concentration if studied under fasting or insulin-stimulated conditions. Thus, in the fasted state, plasma n–6 fatty acids and its major fatty acid (linoleic acid) were both inversely associated to plasma FFA concentration (rs = –0.53, $p < 0.01$), while at the end of the glucose/infusion period, plasma FFA concentration was directly related to plasma monounsaturated content (rs = 0.47, p = 0.02) and oleic/stearic acid ratio (rs = 0.48, p = 0.02), but not to plasma saturated content. No associations with other fatty acids were observed, however the EPA+DHA/α-linolenic acid ratio was inversely related to the insulin-stimulated plasma FFA concentration (rs = –0.39, $p < 0.05$).

In contrast to the observations in plasma FFA, plasma TG concentration was related to fatty acid profile equally under fasting and insulin-stimulated conditions. We found inverse associations between plasma stearic acid (rs = –0.49, p = 0.01) and plasma EPA+DHA/α-linolenic acid ratio (rs = –0.47, p = 0.02) with plasma TG, whereas a direct correlation with plasma monounsaturated fatty acids (rs = 0.38, p = 0.05) and oleic/stearic acid ratio (rs = 0.57, $p < 0.01$) was noted.

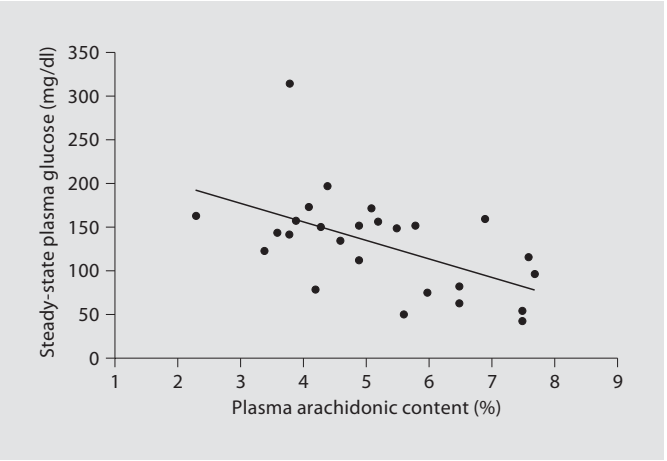

Fig. 1. Relationship between the steady-state plasma glucose and plasma arachidonic acid in healthy women. rs = –0.44, p = 0.03 controlled for physical fitness.

Insulin Resistance and Plasma Fatty Acid Profile

The relationship between IR and specific plasma fatty acids was assessed for saturated, monounsaturated, n–6 and n–3 polyunsaturated fatty acids. No associations with saturated, monounsaturated or long-chain polyunsaturated fatty acid precursors were found. Plasma AA content (rs = –0.51, $p < 0.01$; fig. 1) and AA/linoleic acid ratio (rs = –0.42, p = 0.03) were related to IR; these remained significant after controlling for peak oxygen consumption. For plasma n–3 polyunsaturated fatty acids, IR tended to be associated to plasma DHA content (rs = –0.35, p = 0.08) and EPA+DHA/α-linolenic acid ratio (rs = –0.37, p = 0.06). This trend was weaker after controlling for peak VO_2.

In order to identify the main determinant factors of IR, study variables were included in two separated multiple regression (stepwise) models, one for clinical/metabolic variables and the other for the plasma fatty acid profile. Significant predictors of IR on each model and variables associated by simple correlation to IR were included in the final model. Independent variables were peak VO_2, plasma AA, AA/linoleic acid ratio, insulin-stimulated TG and RQ. The main determinant factors for IR were insulin-stimulated plasma TG concentration and plasma AA content. Both variables in combination explained close to one half of the variability in IR ($R^2 = 0.45$, p = 0.03; table 3).

Table 3. Multiple regression analysis for insulin resistance

Variables	Partial R^2	Model R^2	F value	p
Plasma IST triglycerides[a]	0.31	0.31	10.5	0.004
Plasma arachidonic acid	0.13	0.45	5.4	0.03
Physical fitness (peak VO_2)	0.07	0.52	3.1	0.09

Factors entered into the model: peak VO_2, insulin-stimulated plasma triglyceride concentration, insulin-stimulated RQ, plasma arachidonic acid and AA/linoleic acid ratio.

[a] Plasma TG concentration at the end of the insulin suppression test.

Discussion

The main finding of the present study was that fasting plasma AA content (%) was an important and significant determinant factor of IR; in combination with insulin-stimulated plasma TG concentration it accounted for about one half of the IR variance in healthy women. Additionally, saturated fatty acids and DHA were not found to be related to IR, however the former was inversely while DHA was directly related to physical fitness. The main limitation of this study is the observational nature of the findings. The strength of the design is the inclusion of several experimental approaches which when combined provides an integrative assessment of the relationship between plasma fatty acid profile with insulin-dependent glucose uptake and fuel oxidative metabolism. We considered this study as a necessary first step in our efforts to better define the diet- and nutrition-related determinants of IR in humans.

Plasma AA content was a key determinant of IR using multivariate analysis. This finding is in line with results of previous studies demonstrating that skeletal muscle phospholipid AA content (%) is closely associated to insulin sensitivity in healthy men [20]. Whether plasma/tissue AA content is the cause or consequence of IR remains an open question. Lower plasma/tissue AA and DHA content could be a consequence of IR, since the main responsible enzymes (Δ^5- and Δ^6-desaturases) for their formation are activated by insulin [10]. Thus, insulin-resistant subjects could have impaired conversion of linoleic acid to AA and from α-linolenic acid to DHA. Our results in part lend support to this hypothesis, since we observed an inverse association between IR and plasma AA content and AA/linoleic acid ratio. However, we did not observe a significant association with n–3 long-chain fatty acids (EPA and DHA). On the contrary, divergent results were found by Pelikánová et al. [8] who reported an increased proportion of AA and DHA in plasma phospholipid from diabetic versus healthy individuals. Since no direct assessment of Δ^5- and Δ^6-desaturase activity or conversion rate using labeled fatty acids have been conducted in diabetic subjects, we cannot conclude that IR or diabetes limit fatty acid desaturation in humans. Specific mechanisms to explain a potential beneficial influence of AA on insulin action are not clear from the literature, nor are they elucidated in the present study, since plasma AA content was not related to the clinical and metabolic variables assessed. On the contrary, a potential detrimental effect on insulin-stimulated glucose uptake can be construed, since AA is precursor for proinflammatory eicosanoids, and inflammation is strongly related to IR [21, 22]. This idea is not supported by our data; moreover, when the plasma AA/EPA ratio was used as a marker for inflammatory potential [23], no relation with IR was noted ($rs = -0.27$, $p = 0.18$).

Plasma TG concentration at the end of glucose/insulin infusion period was the main determinant factor for IR in the healthy women recruited in the present study. This was readily apparent using simple correlation analysis, but became evident when we controlled for potential confounders in the stepwise multiple regression analysis. The positive regression coefficient for plasma TG suggests a critical role played by adipose tissue in clearing plasma lipids under insulin-stimulated conditions [24]. If this capacity is impaired, ectopic lipid accumulation is stimulated as a consequence of higher plasma TG and FFA availability. This might explain the relationship between plasma TG and IR, since higher plasma/tissue lipid concentration is strongly and causally related to IR [25]. However, we did not observe a similar effect by analyzing plasma FFA concentration.

Plasma TG concentration was inversely related to the plasma EPA+DHA/α-linolenic acid ratio. The hypolipidemic effect of n–3 fatty acids is mainly given by EPA, which is able to stimulate hepatic lipid oxidation and inhibit TG formation and VLDL secretion [26]. We failed to find a relationship between EPA itself and plasma lipid TG concentration or fuel oxidation. One alternative explanation for the absence of an effect relates to the nature of the measurements we used (plasma TG and whole-body fuel oxidation); these may be not sensitive enough to detect the expected influence of EPA on liver metabolism, VLDL secretion and lipid oxidation. Plasma TG is determined by other mechanisms, as the adipose tissue clearance of TG [27], while the effect of EPA on liver metabolic rates may also be undetectable since the liver contributes by about 20% to whole-body energy flux [28].

In the present study, known IR determinant factors were controlled by recruiting subjects with similar body mass index, age, fatness, race and physical fitness in order to isolate the potential relationship between plasma fatty acid profile and IR. Despite these efforts, the influence of physical fitness on IR remained significant. This finding suggests that insulin-dependent glucose uptake is highly sensitive to changes in aerobic fitness under an exercised stress condition. Improved physical fitness serves as an indirect marker of mitochondrial function, specifically of the activity of the electron transport chain. The latter is reduced in diabetic individuals [29], which might be a precondition to develop IR, particularly related to impaired fatty acid oxidation [30, 31]. The role of specific fatty acids in regulating mitochondrial oxidative activity as a potential mechanism to explain their effect on IR is suggested by our data. We demonstrated that enhanced physical fitness was accompanied by higher plasma DHA and lower saturated fatty acid content. Oxygen consumption and energy production efficiency is a process sensitive to the mitochondrial membrane fatty acid composition [32]. Fatty acid may affect mitochondrial activity by changing membrane properties by themselves or by interacting with mitochondrial proteins. For instance, saturated fatty acids reduce membrane fluidity, whereas DHA has an opposing effect [33]. Thus, fatty acid composition of plasma under fasting as an index of tissue fatty acid composition may be marking the potential for mitochondrial oxidative activity under exercise. On the other hand, plasma fatty acid composition could be a consequence of differences in physical training as shown by Helge et al. [34]. They found in humans an increased DHA content in skeletal muscle phospholipids after a 4-week exercise program. In the present study, subjects recruited were sedentary and did not practice physical exercise on a regular basis, therefore we underscored a role of physical training as determinant of plasma fatty acid composition.

Fasting fuel oxidation was not related to IR as reported by other authors [35, 36]. However, under insulin-stimulated conditions, an inverse association with IR was found. The latter might be an expected finding, since enhanced insulin sensitivity will determine higher intracellular glucose to be oxidized; thus, we expected the increased glucose oxidation and lower lipid oxidation in comparison to insulin-resistant individuals. Similar results have previously been reported [37]. We anticipate that higher glucose oxidation is a consequence of improved insulin sensitivity. As a function of this, a significant association between plasma AA content and AA/linoleic acid ratio with insulin-stimulated fuel oxidation will be derived from the association with IR.

Because of the observational nature of the experimental design, the data presented in this paper have significant limitations in their interpretation. The associations found cannot be construed as causal relationships but rather should be considered in the generation of hypothesis, which will require experimental testing by appropriately designed randomized controlled intervention studies. The modification of plasma fatty acid concentrations by dietary means is the necessary next step to define whether the observations of this study have the potential to modify IR.

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