Title:

Higher dietary intake of alpha-linolenic acid is associated with lower insulin resistance in middle-aged Japanese.

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ABSTRACT

Objective. To investigate the associations between dietary intake of n-3 polyunsaturated fatty acids (plant-derived alpha-linolenic acid: ALA, and marine-derived eicosapentaenoic and docosahexaenoic acid: EPA+DHA) and insulin resistance (IR) in a lean population with high n-3 PUFA intake.

Method. We cross-sectionally studied 3383 Japanese local government workers aged 35-66 in 2002. IR was defined as the highest quartile of homeostasis model assessment, and nutrient intake was estimated from a diet history questionnaire. The odds ratios (ORs) of IR taking the lowest quartile of ALA or EPA+DHA intake as the reference were calculated by logistic regression analysis.

Results. Mean age, body mass index (BMI), and dietary ALA, and median of dietary EPA +DHA were 47.9 years, 22.9 kg/m^2, and 1.90 g/day (0.88 %E) and 0.77 g/day (0.36 %E), respectively. The ORs of IR decreased across the quartiles of ALA intake (multivariate-adjusted OR for Q4 versus Q1 = 0.74, *P* for trend = 0.01) and the association was observed only in subjects with a BMI < 25 kg/m^2 (*P* for interaction = 0.033). However EPA+DHA showed no such associations consistently.

Conclusion. Higher ALA intake was significantly associated with a lower prevalence of IR in normal weight individuals of middle-aged Japanese men and women.

Keywords. Nutritional sciences; Polyunsaturated fatty acids; alpha-Linolenic acid; Insulin resistance; Epidemiologic studies

Introduction

Insulin resistance (IR) is a state in which insulin cannot function sufficiently in skeletal muscle, liver and adipocytes (Haag and Dippenaar, 2005). Long-chain (C20-22) polyunsaturated fatty acids (PUFA) have been postulated to improve insulin sensitivity (Borkman et al., 1993) by regulating the cell membrane composition of fatty acids (Hulbert et al., 2005; Orton et al., 2008) which may affect cellular functions such as the translocation of glucose transporters (Storlien et al., 1996) and insulin signaling (Taouis et al., 2002). An animal study demonstrated that replacement of dietary saturated fatty acids (SFA) by either marine-derived n-3 PUFA (eicosapentaenoic acid: EPA and docosahexaenoic acid: DHA) or plant-derived n-3 PUFA (alpha-linolenic acid: ALA) restored once decreased insulin sensitivity (Storlien et al., 1991). However, EPA and DHA initially studied in patients with diabetes for their deleterious effect on insulin sensitivity (Borkman et al., 1989). The studies conducted thereafter are still showing inconsistent associations with impaired insulin sensitivity or DM incidence, varying from positive (Meyer et al., 2001; Mostad et al., 2006), null (Marshall et al., 1997; Hodge et al., 2007), to rather inverse (Salmeron et al., 2001). The associations of ALA, an inefficient precursor of EPA and DHA in humans (Burdge, 2006), also remain unclear (Barre, 2007). For example, intervention studies failed to find associations between dietary ALA and fasting glucose or insulin concentrations (Finnegan et al., 2003; Egert et al., 2008). While ALA content in adipose tissue or plasma phospholipid was inversely associated with glucose intolerance (Carlson and Walldius, 1975) or DM incidence (Wang et al., 2003), a cohort study found that a high ALA intake was significantly associated with increased DM incidence (Hodge et al., 2007).

One of the explanations for the discrepancies may be the degree of obesity of the

study population. For example, higher total PUFA intake was significantly associated with IR in the obese but not in normal weight subjects, where the association was rather inverse (Mayer-Davis et al., 1997). Similarly, total PUFA intake had a tendency to be inversely associated with DM incidence only in subjects with a body mass index (BMI) < 29 kg/m^2 (Colditz et al., 1992).

Our current understanding is based on the studies among Western populations with a relatively high BMI and low marine- and plant-derived n-3 PUFA intake. Only a few studies have been conducted in lean populations with high marine- and plant-derived n-3 PUFA intake (Sugano and Hirahara, 2000). Therefore, to examine the associations of EPA+DHA and ALA intake with IR, the present cross-sectional study was carried out to include apparently healthy and relatively lean Japanese subjects.

Methods

Subjects

The present cross-sectional study was based on a Japanese workers' cohort that was organized among civil servants in Aichi Prefecture in 1997 and again in 2002. Study subjects have been followed ever since for monitoring the occurrence of cardiovascular diseases by the Department of Public Health of the Nagoya University Graduate School of Medicine. The participants consisted of 5179 male and 1472 female workers aged 35-66 years in 2002. The survey included a physical examination, medical histories, collection of fasting blood samples, and a self-administered questionnaire.

We excluded subjects with missing values in all the variables used for the present analysis; a history of cardiovascular disease or cancer, or prevalent diabetes; and an estimated energy intake of < 500 kcal/day or > 3500 kcal/day, leaving 2652 men and

731 women for the analysis. A sensitivity analysis excluding subjects who theoretically underreported total energy intake based on a formula using sex- and age-specific basal metabolic rate (Goldberg et al., 1991) will not be shown since it did not materially change the results.

All subjects gave written consent, and the study protocol was approved by the Ethics Review Committee of the Nagoya University School of Medicine, Nagoya, Japan.

Questionnaire

The questionnaire was used to assess smoking status, physical activity, and alcohol consumption. Physically active subjects were defined as those who engaged in moderate or vigorous exercise of more than 60 minutes per week (Laaksonen et al., 2002). Alcohol consumption habit was defined as non-drinker, light (≤ 22.9 g/day of ethanol), moderate (> 22.9 and ≤ 45.8 g/day), and heavy (> 45.8 g/day).

Diet was assessed by a brief-type self-administered diet history questionnaire (BDHQ) that required recalling dietary habits over a 1-month period. The BDHQ is a questionnaire that inquires about the consumption frequency of a total 56 food and beverage items, with specified serving sizes described in terms of the natural portion or the standard weight and volume measurement of servings commonly consumed in the general Japanese populations. For each food item, the participants indicated their mean frequency of consumption in terms of the specified serving size by checking 1 of the 7 frequency categories ranging from “almost never” to “2 or more times/day.” Estimates of nutrients and energy were calculated using an ad-hoc computer algorithm for the BDHQ which was based on the Standard Tables of Food Composition in Japan, fifth

revised edition (Japan Science and Technology Agency, 2005), and assessments of fatty acid intakes were used as the nutrient database (Sasaki et al., 1999). The reproducibility and validity of the BDHQ using 16-d weighed dietary records in a different sample have been described elsewhere (Sasaki, 2004). Briefly, Pearson correlation coefficients in 92 Japanese men and 92 Japanese women aged 31-76 years were 0.42 and 0.45 for n-3 PUFA, 0.30 and 0.32 for ALA, 0.27 and 0.37 for EPA, and 0.26 and 0.27 for DHA, respectively. Furthermore, the Pearson correlation coefficients between the BDHQ and serum concentrations in the same men and women were 0.38 and 0.33 for EPA, and 0.36 and 0.27 for DHA, respectively (Sasaki, 2004).

Assessment of other variables

Anthropometric measurements were recorded under a fasting state with subjects wearing light clothing and no shoes. BMI was calculated as body weight in kilograms divided by the square of height in meters. Blood samples were drawn from the subjects after 8 hours or overnight fasting. Fasting blood glucose (FBG) was enzymatically determined by the hexokinase method, and fasting immunoreactive insulin (FIRI) was measured by solid-phase radioimmunoassay (RIABEAD II; Dinabot Co., Ltd., Chiba, Japan). Homeostasis model assessment of insulin resistance (HOMA-R), which was calculated as FBG (mg/dL) × FIRI (μU/mL) / 405, was used as an index of IR (Matthews et al., 1985). We defined the subjects with IR as those in the highest quartile of HOMA-R similarly to the method employed by the European Group for the Study of Insulin Resistance (Balkau and Charles, 1999). Our cut-off HOMA-R (2.15) was comparable to that suggested by the result of an oral glucose tolerance test in a healthy Japanese population (1.97) (Matsumoto et al., 1997).

Statistical analysis

Descriptive data were presented as means (standard deviations) for continuous variables or percentages for categorical variables. FIRI, HOMA-R, and EPA+DHA intake were presented as medians (interquartile ranges) because of their skewed distributions, and natural log-transformed values were used for the subsequent statistical analyses. Each nutrient intake was adjusted for total energy intake by the nutrient density method, and presented as a percentage of total energy (%E).

Unpaired *t*-tests were used for comparison of the continuous variables and Chi-squared tests for the categorical variables. The odds ratios (ORs) and 95% confidence intervals (CIs) of having IR according to quartile of dietary ALA or EPA+DHA taking the lowest quartile as the reference were calculated by logistic regression analysis. Model 1 adjusted for age (continuous), sex, BMI (continuous), physical activity (yes/no), alcohol consumption (non-drinker/light/moderate/heavy), current smoking (yes/no), and receipt of hormone replacement therapy (yes/no). We further adjusted for carbohydrate, protein, SFA, and monounsaturated fatty acids (MUFA) intake (Model 2). ALA and EPA+DHA intakes were simultaneously included in this model. However, we could not include ALA and n-6 PUFA in the same statistical model because of their high correlation (r = 0.96). Instead, we first stratified the sample by median intake of n-6 PUFA to address potential confounding by n-6 PUFA. We also ran a substitution model (Willett, 1998) that included total energy intake (kcal/d) and total fat intake (%E) in addition to the variables in Model 1. The regression coefficient can be interpreted as the effect of substituting energy intake from ALA for fat other than ALA while keeping total energy intake and total fat constant. Testing of linear trend was

performed by applying the median of ALA or EPA+DHA intake to each quartile and analyzed as continuous variables. To examine the effect modification by the level of obesity, we performed the stratified analyses and calculated the *P* for interaction of BMI categories, lean (< 25 kg/m^2) and overweight (≥ 25 kg/m^2), with dietary ALA quartiles.

All analyses were performed using the SPSS statistical package for Windows version 16.0J (SPSS Inc.). *P* value < 0.05 was considered statistically significant.

Results

Mean age and BMI of subjects were 47.9 years and 22.9 kg/m^2, respectively (Table 1). Mean dietary intake of ALA and median of EPA+DHA were 1.90 g/day (0.88 %E) and 0.77 g/day (0.36 %E), respectively. Subjects with IR were more likely to be male and older, had significantly higher BMI, less likely to be physically active, and also consumed significantly less total PUFA and ALA than those without IR. However, EPA+DHA intake and the ratio of n-6 to n-3 PUFA did not differ by IR.

Higher ALA intake was significantly associated with a lower prevalence of IR (OR for Q4 versus Q1 in the crude model = 0.73, *P* for trend = 0.005), and this linear association remained significant even in adjusted models (*P* for trend in model 1 = 0.010 and *P* for trend in model 2 = 0.046) (Table 2). Stratified analysis by n-6 PUFA intake showed similar inverse associations between ALA intake and IR in both lower and higher intake strata, however the association was statistically borderline significant only in the lower intake stratum (*P* for trend = 0.056). Substitution model also indicated that replacing energy from fat other than ALA by ALA would be inversely associated with IR (OR for Q4 versus Q1 = 0.58, *P* for trend = 0.001).

In contrast, EPA+DHA intake showed no significant association with IR in any

models. Even after comparing the highest decile to the lowest decile of EPA+DHA intake, there was no association with IR (data not shown).

ALA intake was inversely and significantly associated with IR only in lean subjects (OR for Q4 versus Q1 = 0.66, *P* for trend = 0.002), and there was a statistically significant effect modification (*P* for interaction = 0.033) (Figure 1).

Discussion

To our knowledge, the present study is the largest-scale cross-sectional study investigating the associations between dietary marine- or plant-derived n-3 PUFA and IR ever conducted in a non-Western population. The subjects in the present study were characterized by a lower BMI, lower intake of SFA, and higher intake of n-3 PUFA compared with those in previous studies conducted in Western populations (Marshall et al., 1997; Brunner et al., 2001). Our results provided the following insights: 1) ALA intake was inversely associated with the risk of incurring IR independently of potential confounding factors including SFA and MUFA; and 2) this association was modified by the degree of obesity; whereas 3) EPA+DHA intake showed no significant association with IR.

While ALA is known to be a precursor of EPA and DHA, only about 0.2% of plasma ALA is destined for the synthesis of EPA in healthy individuals (Pawlosky et al., 2001). In an animal study, ALA itself is considered to promote the secretion of a glucagon-like peptide-1 (GLP-1) by activating the extracellular signal-regulated kinase (ERK) pathway, thereby enhancing the secretion of insulin (Adachi et al., 2006). Furthermore, a human study demonstrated that plasma ALA is more rapidly beta-oxidized than other fatty acids, thus decreasing lipid accumulation in skeletal

muscle and liver, and consequently improving insulin sensitivity (Cunnane, 2003).

Although the major dietary source of ALA in Japan is reported to be edible vegetable oils, namely soybean and rapeseed oils (Sugano and Hirahara, 2000), the present finding is consistent with a recent double-blind randomized controlled trial which has demonstrated that a flaxseed-enriched diet containing a high level of ALA significantly reduced HOMA-R by 23.7% compared with wheat in hypercholesterolemic adults (Bloedon et al., 2008), or the Atherosclerosis Risk in Communities (ARIC) Study which has demonstrated that a higher proportion of phospholipid ALA was inversely associated with the incidence of DM after a 9-year follow-up (Wang et al., 2003). Although animal studies are also in line with our finding (Ghafoorunissa et al., 2005; Chicco et al., 2009), some human studies reported either no effect (Finnegan et al., 2003; Egert et al., 2008) or the opposite (Hodge et al., 2007). Some studies have stratified their population by the level of obesity, and reported that total PUFA intake was inversely associated with IR or DM incidence only in subjects with a lower degree of obesity (Colditz et al., 1992; Mayer-Davis et al., 1997; Salmeron et al., 2001). Therefore, the inconsistencies of association between ALA intake and IR may arise from the small sample size (Egert et al., 2008) or the relatively high BMI of subjects (Finnegan et al., 2003; Hodge et al., 2007). The latter is supported by our findings that demonstrated a statistically significant interaction and inverse association between ALA intake and IR only in normal weight subjects.

The present study also demonstrated that EPA+DHA intake showed no association with IR. Although the mean BMI of subjects in the present study was much lower and the estimated amount of EPA+DHA intake was much higher than those in previous observational studies in Western countries, our findings were actually consistent with

theirs (Marshall et al., 1997; Hodge et al., 2007). We have attempted to contrast individuals with very high EPA+DHA intake (the highest decile, median: 0.82 %E) and those with very low EPA+DHA intake (the lowest decile, median: 0.15 %E), but the null finding nevertheless remained.

Study limitations and strength

Our data have the strengths in investigating real-world diets as opposed to the artificially-imposed isocaloric substitutions or supplementations, however, several limitations warrant consideration. First, there may be imprecision and biases in estimating dietary intakes from a self-administered questionnaire; however, misclassification would likely to be non-differential to the presence of IR. High correlation between dietary ALA and n-6 PUFA was probably due to their common food sources; however, inability to isolate the effects of each fat in the present population using BDHQ should be overcome by future studies with different dietary assessments or by those in other population. Second, we used a surrogate measure of IR rather than reference methods such as euglycemic hyperinsulinemic clamp or glucose tolerance test. Although HOMA-R is often used in epidemiologic studies due to its practicability, the method precluded the subjects with prevalent diabetes, who would have various degrees of insulin resistance (Wallace et al., 2004). However, ALA or EPA+DHA intake of excluded subjects due to prevalent diabetes did not significantly differ from those of included subjects in our sample (data not shown). Next, there are several issues related to the validity of HOMA-R: It is a measure of basal, not of stimulated IR (Wallace and Matthews, 2002) although they are well correlated (Wallace et al., 2004); it may be exaggerated in a state where insulin clearance is impaired (Kotronen et al., 2007); and it

was reported to be more valid measure of IR relatively in overweight and obese subjects than in normal weight (Kim et al., 2004). However, it was considered unlikely that null association of IR with intakes of ALA or EPA+DHA in overweight subjects was due to misclassification of IR attributable to the HOMA-R characteristics. Nonetheless, further investigation that incorporated other measurement for IR is ideal in future. Finally, despite the associations were similar in both lower and higher n-6 PUFA intake strata, ALA intake had a tendency to be inversely associated with IR only in subjects with lower n-6 PUFA intake. Although previous studies did not generally show significant modification of association between ALA and cardiovascular outcomes by the level of n-6 PUFA intake (Mozaffarian et al., 2005; Djoussé et al., 2001), future studies using experimental design would be ideal to address the issue.

Conclusion

Higher dietary intake of ALA was associated with a lower prevalence of IR in normal weight individuals of middle-aged Japanese men and women, whereas EPA+DHA showed no such associations consistently.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Table 1

Baseline characteristics of the study sample according to the quartiles of homeostasis model assessment of insulin resistance : Aichi, Japan, 2002.

Characteristics	Quartiles of homeostasis model assessment of insulin resistance				*P* value
	Q1 (lowest)	Q2	Q3	Q4 (highest)	
N (sample)	851	842	848	842	
Age (years)	47.7 (7.2)	47.9 (7.2)	47.6 (7.1)	48.5 (7.1)	0.026
Male gender (%)	75.8	76.6	80.0	81.2	0.016
Body mass index (kg/m^2)	21.5 (2.3)	22.5 (2.3)	23.4 (2.4)	24.3 (3.0)	<0.001
Current smoker (%)	29.6	28.3	28.9	32.3	0.29
Physically active [a] (%)	25.0	18.8	20.4	15.7	<0.001
Receipt of hormone replacement therapy [b] (%)	13.6	13.2	14.7	12.7	0.96
Alcohol consumption (%)					0.006
Non-drinker	20.6	23.9	20.0	24.8	
Light drinker (> 0 and ≤ 22.9 g/d)	53.0	54.8	55.9	51.1	
Moderate drinker (> 22.9 g/d and ≤ 45.8 g/d)	18.4	13.5	17.0	13.9	
Heavy drinker (> 45.8 g/d)	8.0	7.8	7.1	10.2	
Fasting blood glucose (mg/dL)	84.8 (8.8)	89.2 (10.5)	92.7 (14.6)	105.6 (28.5)	<0.001
Fasting immunoreactive insulin (μU/mL)	3.0 (3.0-4.0)	5.0 (5.0-6.0)	7.0 (7.0-8.0)	14.0 (11.0-23.0)	<0.001
Homeostasis model assessment of insulin resistance	0.7 (0.5-0.8)	1.1 (1.0-1.3)	1.7 (1.5-1.9)	3.2 (2.5-5.9)	<0.001
Total energy intake (kcal/d)	1883 (520)	1921 (559)	1942 (525)	1957 (558)	0.032
Dietary intake of nutrients (% of total energy)					
Carbohydrate	52.9 (8.5)	52.9 (8.5)	53.4 (8.0)	53.2 (8.3)	0.56
Protein	14.3 (2.6)	14.4 (2.7)	14.2 (2.4)	14.3 (2.6)	0.49
Total fat	26.0 (6.0)	26.3 (6.2)	25.8 (5.7)	25.8 (6.0)	0.27
Saturated fatty acids	6.4 (1.8)	6.5 (1.9)	6.4 (1.7)	6.5 (1.9)	0.45
Monounsaturated fatty acids	9.3 (2.3)	9.4 (2.4)	9.3 (2.2)	9.2 (2.2)	0.37
Polyunsaturated fatty acids	6.9 (1.6)	7.0 (1.7)	6.9 (1.6)	6.8 (1.6)	0.023
alpha-Linolenic acid	0.88 (0.25)	0.89 (0.25)	0.88 (0.25)	0.86 (0.23)	0.026
Eicosapentaenoic acid and docosahexaenoic acid	0.35 (0.26-0.50)	0.36 (0.25-0.51)	0.36 (0.25-0.51)	0.36 (0.26-0.51)	0.41
n-3 series	1.39 (0.39)	1.41 (0.42)	1.37 (0.39)	1.37 (0.41)	0.15
ratio of n-6 to n-3	4.12 (0.83)	4.13 (0.84)	4.16 (0.79)	4.09 (0.79)	0.26

Data are presented as means (standard deviations) or medians (interquartile ranges) for continuous variables, and percentages for categorical variables.

[a] Defined as those who engaged in moderate or vigorous exercise of more than 60 minutes per week.

[b] Percentages among female subjects.

Table 2

Multivariate-adjusted odds ratios (95% CIs) of insulin resistance [a] according to the quartile of dietary n-3 polyunsaturated fatty acids.

Variables	Quartiles of dietary intake (% of total energy)				*P* for trend
	Q1 (lowest)	Q2	Q3	Q4 (highest)	
ALA					
Median (range) (% of total energy)	0.61 (0.16-0.71)	0.79 (0.71-0.87)	0.94 (0.87-1.04)	1.16 (1.04-2.02)	
N of subjects with insulin resistance	234	217	206	185	
Crude	1 (reference)	0.90 (0.73-1.12)	0.84 (0.68-1.05)	0.73 (0.59-0.91)	0.005
Model 1 [b]	1 (reference)	0.91 (0.73-1.15)	0.84 (0.67-1.06)	0.74 (0.58-0.94)	0.010
Model 2 [c]	1 (reference)	0.88 (0.68-1.13)	0.79 (0.59-1.06)	0.67 (0.45-0.99)	0.046
Substitution model [d]	1 (reference)	0.83 (0.65-1.06)	0.72 (0.55-0.95)	0.58 (0.41-0.81)	0.001
EPA+DHA					
Median (range) (% of total energy)	0.20 (0-0.26)	0.31 (0.26-0.36)	0.42 (0.36-0.51)	0.66 (0.51-1.95)	
N of subjects with insulin resistance	204	218	207	213	
Crude	1 (reference)	1.09 (0.88-1.36)	1.02 (0.82-1.27)	1.06 (0.85-1.32)	0.77
Model 1 [b]	1 (reference)	1.16 (0.92-1.46)	1.06 (0.84-1.34)	1.01 (0.80-1.28)	0.75
Model 2 [c]	1 (reference)	1.19 (0.94-1.52)	1.11 (0.85-1.44)	1.05 (0.76-1.44)	0.93
Substitution model [d]	1 (reference)	1.17 (0.92-1.48)	1.08 (0.85-1.37)	1.03 (0.80-1.33)	0.85

ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[a] Defined as the highest quartile of homeostasis model assessment of insulin resistance ($\geq$ 2.15 in this sample).

[b] Model 1 adjusted for age (continuous), sex , body mass index (continuous), physical activity (yes/no), alcohol consumption (non-drinker/light/moderate/heavy), current smoking (yes/no), and receipt of hormone replacement therapy (yes/no).

[c] Model 2 included variables in model 1 plus carbohydrate, protein, saturated fatty acids, monounsaturated fatty acids, ALA, and EPA+DHA (% of total energy).

[d] Substitution model included variables in model 1 plus total energy intake (kcal/d) and total fat (% of total energy).

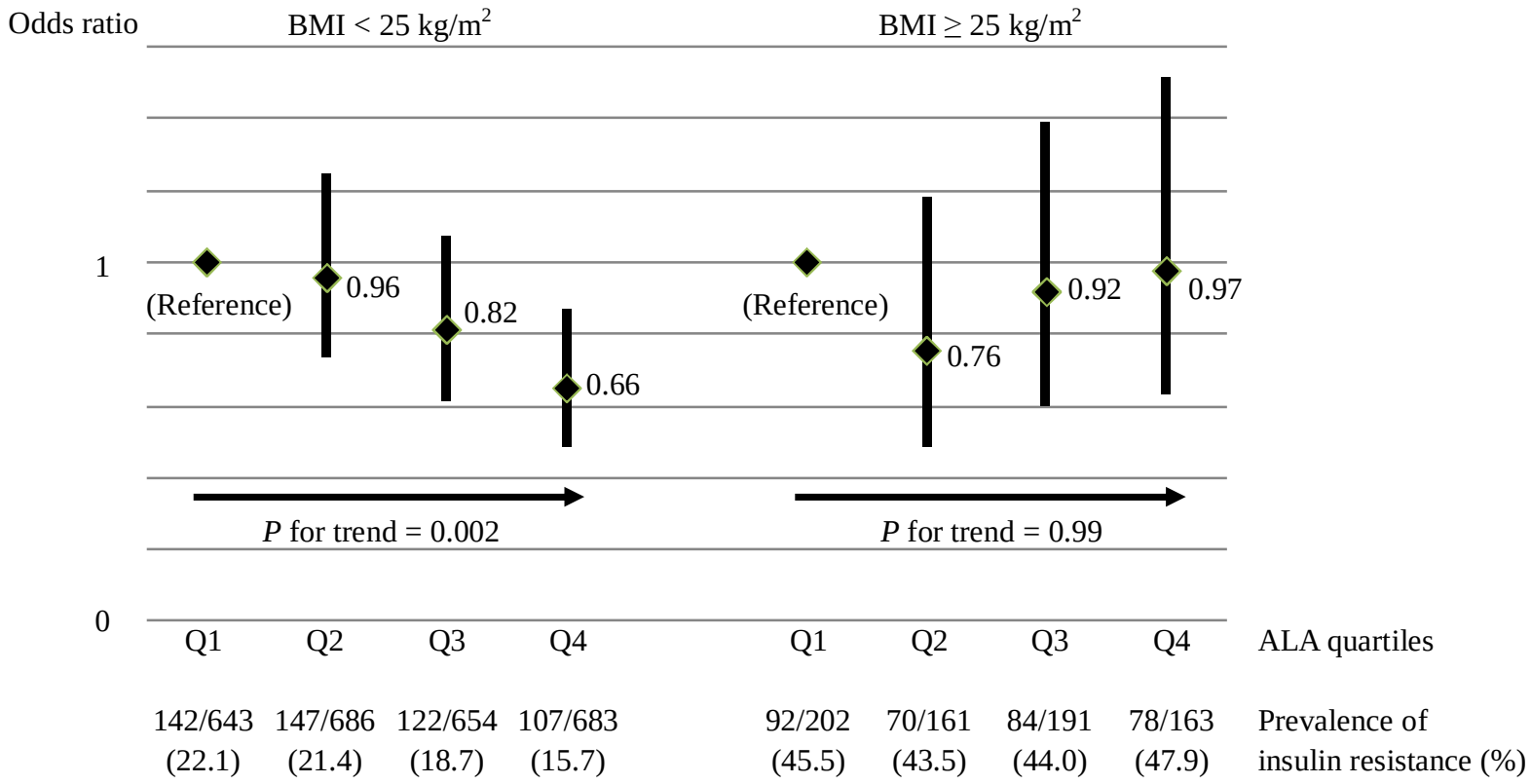
Figure legends

Figure 1

Title:
Multivariate-adjusted [a] odds ratios (95% CIs) of insulin resistance [b] according to the quartiles of ALA intake stratified by BMI.

Footnote:
BMI = body mass index; ALA = alpha-linolenic acid.
[a] Adjusted for age (continuous), sex, body mass index (continuous), physical activity (yes/no), alcohol consumption (non-drinker/light/moderate/heavy), current smoking (yes/no) and receipt of hormone replacement therapy (yes/no).
[b] Defined as the highest quartile of homeostasis model assessment of insulin resistance (≥ 2.15 in this sample).

Figure 1


Odds ratio
BMI < 25 kg/m2
BMI ≥ 25 kg/m2
1
0
(Reference)
0.96
0.82
0.66
(Reference)
0.76
0.92
0.97
P for trend = 0.002
P for trend = 0.99
Q1
Q2
Q3
Q4
Q1
Q2
Q3
Q4
ALA quartiles
142/643 (22.1)
147/686 (21.4)
122/654 (18.7)
107/683 (15.7)
92/202 (45.5)
70/161 (43.5)
84/191 (44.0)
78/163 (47.9)
Prevalence of insulin resistance (%)