

## ORIGINAL ARTICLE

# THE ASSOCIATION BETWEEN N-3 FATTY ACIDS IN ERYTHROCYTE MEMBRANES AND INSULIN RESISTANCE: THE INUIT HEALTH IN TRANSITION STUDY

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## ABSTRACT

**Objectives.** To examine the association between the content of n-3 fatty acids and insulin resistance in an Inuit population.

**Study design.** The Inuit Health in Transition Study was carried out between 2003 and 2007 in Greenland as a cross-sectional study. Our preliminary results are based on the first 452 participants aged 18 and above. Only participants with at least 1 Inuit grandparent and without diabetes were included.

**Methods.** The contents of n-3 fatty acids and the n-3/n-6 ratio were measured in the erythrocyte membrane phospholipids. BMI was calculated and questions concerning diabetes and ethnicity were answered. Insulin resistance was estimated using the HOMA-IR index based on fasting-glucose and fasting-insulin.

**Results.** We found an inverse association between C20:5 n-3 (EPA), C22:3 n-3, the n-3/n-6 ratio and HOMA-IR and a positive association between C18:3 n-3 *cis* and HOMA-IR. When adjusted for age, gender, BMI and ethnicity, the association remained statistically significant for C20:5 n-3 (EPA), C22:3 n-3 and C18:3 n-3 *cis*.

**Conclusions.** Our findings suggest that some types of n-3 fatty acids may have a protective effect against insulin resistance. The role of potential confounders such as physical activity, diet, energy intake, socio-economic status and contaminants deserves further exploration.

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**Keywords:** n-3 fatty acids, insulin resistance, Greenland

## INTRODUCTION

Studies of the Greenland population before the 1980s indicated a low prevalence of type 2 diabetes when compared to Western European populations (1,2). However, a recent study in 1999–2001, which collected data from 3 areas in Greenland, showed a high prevalence of diabetes, 8.8% among women and 10.8% among men (3).

Since the 1950s, substantial lifestyle changes have occurred in Greenland, including a change from a traditional marine diet to a more Western-inspired diet (4–6). Despite the transition to a more Westernized diet, the intake of sea mammals and fish and hence the intake of n-3 fatty acids remains much higher among Greenlanders as compared to most other populations. The level of adaptation to the Westernized lifestyle varies between the different regions in Greenland, and a higher intake of fish and sea mammals is still prevalent in the villages as compared to the towns (6). Thus, there is a great variation in the intake of n-3 fatty acids within the Inuit population, which makes this population very suitable for investigations into the association between n-3 fatty acids and insulin resistance.

N-3 fatty acids are mainly found in fish, shellfish and sea mammals, where decosahexaenoic acid (C22:6 n-3) (DHA) and eicosapentaenoic acid (C20:5 n-3) (EPA) are most abundant. Further, some n-3 fatty acids, especially alpha-linolenic acid (C18:3 n-3) (ALA), are found in plant oils and certain vegetables. N-6 fatty acids are mainly found in plant oils. Review articles have emphasized the importance of increasing the proportion of n-3 fatty acids in the diet, which would imply that a high n-3/n-6 ratio should be beneficial (7,8). Fatty

acids from the diet are incorporated into the body's cell membranes. Studies have shown that the fatty acid composition of erythrocyte membranes mirrors the fatty acid pattern of the diet over the past few months (9–13).

The hypothesis of a connection between n-3 fatty acids and diabetes arose from diet studies that linked a high fish or marine diet with a lower prevalence of diabetes (14–16). This finding has not been universally replicated (17,18). Evidence based on direct measurements of n-3 fatty acids has also been inconsistent (19–21).

Studies so far have been based on the measurement of n-3 fatty acids in serum or plasma. With the aim of clarifying the relation between n-3 fatty acids and insulin resistance, we set out to study the association between the content of n-3 fatty acids measured in erythrocyte membranes and insulin resistance in an Inuit population.

## MATERIAL AND METHODS

### Study population and design

The Inuit Health in Transition Study was carried out in the period 2003–2007 in West Greenland as a cross-sectional study with 2,345 participants aged 18 and above. The subjects were selected randomly from the central population registers of 8 regions in West Greenland. From the participating settlements in these regions, 11.4% of the adult population took part in the Inuit Health in Transition Study.

This report is based on data for the first 527 participants, some of whom were examined during the pilot study in 3 villages (Saqqaq, Qeqertaq and Ilimanaq) in the Ilulissat district in 2003, and others of whom were examined

in the towns of Aasiaat and Qasigiannnguit in 2005. In the villages of Saqqaq, Qeqertaq and Ilimanaq, 35% of the adult population participated in the study.

Participants were excluded if they did not have Inuit ethnicity, if they knew they had diabetes based on self-reporting, if they had diabetes diagnosed by an oral glucose tolerance test (OGTT), if they had not fasted for a minimum of 8 hours before the blood sample, if data for height and weight and hence BMI were missing, and if fasting-glucose or fasting-insulin were not available.

After exclusions were made, the study population consisted of 452 participants, with 90 living in villages and 362 living in towns. Thirty-nine were excluded because of having diabetes, 2 because they had not fasted, 3 because of non-Inuit ethnicity and 31 because of missing data on fasting-insulin, fasting-glucose or BMI.

The project was reviewed by the commission for Scientific Research in Greenland. All subjects were informed about the study in writing and orally, and gave their informed consent.

## Data collection

### *Laboratory measurements*

A sample of fasting blood was taken and participants without previously known diabetes received a standard 75 g oral glucose tolerance test (OGTT) with 2-hour blood sampling.

Within 1.5 hours of sample collections, the tubes were labelled and refrigerated at 4°C prior to centrifuging. Tubes were stored frozen at -20°C and specimens for fatty acids measurement were stored frozen at -80°C.

Plasma glucose and plasma insulin were

analysed at Steno Diabetes Center, Denmark, using the hexokinase/G6P-DH method on Hitaschi 912 system and the enzyme-linked 2-site immunoassay, respectively.

Fatty acids were analysed at Université Laval, Quebec. The composition of phospholipids of erythrocyte membranes was measured after total lipid extraction with chloroform/methanyl mixture, phospholipid separation by thin layer chromatography (22) and methylation of fatty acids (23), followed by capillary GLC (gas-liquid chromatographic) using a DB-23 column in an HP-Packard gas chromatograph. The n-3 fatty acids comprised C18:3, C18:4, C20:3, C20:4, C20:5, C22:3, C22:5 and C22:6. The ratio of n-3 and n-6 fatty acids was measured as well.

C20:4 n-3, C18:3 n-3 *trans* and C20:3 n-3 were only detectable in 76, 6 and 3 participants, respectively, and were therefore not included in the analyses presented here.

### *Anthropometric measurements*

Weight and height were measured with the participants wearing underwear, and BMI was calculated as weight divided by the squared height (kg/m<sup>2</sup>). With the participant standing, waist circumference was measured midway between the iliac crest and the costal margin.

### *Interview and questionnaire*

Known diabetes was assessed by a “yes” to one or both of the following questions: “Has a doctor ever told you that you had diabetes?” and/or “Do you have diabetes?”

The consumption of alcohol was assessed by questionnaire and classified into 4 subgroups: “3–7 days per week,” “1–2 days per week,” “1–3 days per month” and “less or never.”

The participants' smoking status was categorized into 3 groups: "current smoker," "former smoker" and "never smoked."

Heritage was determined based on questions about the ethnicity of the 4 grandparents, and Inuit ethnicity was defined as having at least 1 grandparent with Inuit ethnicity. Ethnicity was divided into full Inuit heritage (all 4 grandparents had Inuit ethnicity) and part Inuit heritage (1, 2 or 3 grandparents had Inuit ethnicity).

## Definitions

### *Insulin resistance*

Insulin resistance was estimated using the HOMA-IR index as  $\text{HOMA-IR} = (\text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting plasma glucose (mmol/l)}) / 22.5$ .

### Data analysis

Analyses were performed using SAS version 9.1. The difference in levels of fatty acids by gender, age, place of living, BMI, smoking status, alcohol consumption and ethnicity was tested in univariate regression models with fatty acids as outcome. The distribution of

HOMA-IR was skewed and was therefore log transformed before analysis. Multiple regression models were used with logHOMA-IR as outcome and the fatty acid as exposure. The analyses were progressively adjusted for age, sex, BMI and ethnicity (full or part Inuit heritage). Model 1 is adjusted for age and sex, model 2 for age, sex and BMI and model 3 for age, sex, BMI and ethnicity. Estimates were considered significant with a p-value <0.05.

## RESULTS

The characteristics of the subjects are shown in Table I by gender. Women were in the majority, representing 61% of the study population. Women were younger and had a higher BMI than men.

Table II shows the proportions of n-3 fatty acids and the ratio between n-3 fatty acids and n-6 fatty acids in the erythrocyte membranes.

The fatty acids with the highest relative percent are shown for gender and place of living in Figures 1 and 2.

**Table I.** Characteristics of subjects by gender.

	Women (n=281)	Men (n=171)
Age <sup>a</sup> (years)	45.8 (13.0)	48.3 (13.6)
BMI <sup>a</sup> (kg/m <sup>2</sup> )	27.0 (4.7)	25.9 (4.6)
Waist (cm)	93.2 (12.1)	92.8 (13.1)
Smokers % (95%CI)	68 (58-71)	60 (51-68)
Alcohol group % (95%CI)		
3-7 per week	0.9 (0.1-3.8)	1.4 (0.2-5.9)
1-2 per week	11.8 (8.8-19.1)	18.2 (14.4-30.1)
1-3 monthly	27.6 (21.5-34.8)	32.9 (23.5-40.8)
Less/never	59.7 (50.3-64.9)	47.6 (35.9-54.3)
Full Inuit % (95%CI)	93 (87-95)	84 (73-84)
HOMA-IR <sup>b</sup>	1.4 (1.0-2.0)	1.2 (0.7-2.0)

<sup>a</sup>Mean (SD).

<sup>b</sup>Median (Q1-Q3).

**Table II.** Composition of erythrocyte membrane phospholipids (as percentage of total fatty acids). Median (Q1–Q3).

Fatty acid	Relative percentage	Detectable values <sup>a</sup>
Total n-3	12.0 (9.2-14.9)	All
C22:6 n-3 (DHA)	6.4 (5.1-7.6)	All
C20:5 n-3 (EPA)	2.7 (1.6-4.2)	All
C22:5 n-3 (DPA)	2.1 (1.8-2.5)	All
C22:3 n-3	0.30 (0.21-0.42)	402
C18:3 n-3 <i>cis</i>	0.18 (0.00-0.23)	309
C18:4 n-3	0.13 (0.00-0.19)	402
n-3/n-6 ratio	0.57 (0.41-0.79) <sup>b</sup>	All

<sup>a</sup>Number of subjects with detectable fatty acid values in their erythrocyte membranes.

<sup>b</sup>Ratio between n-3 fatty acids and n-6 fatty acids instead of relative percentage.

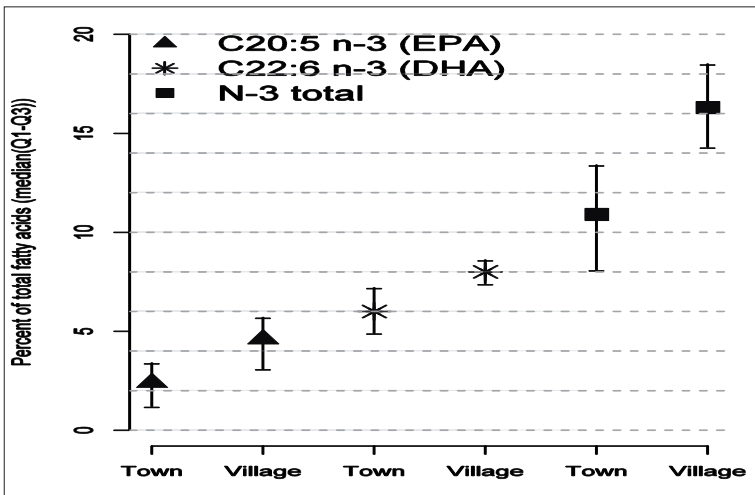


Figure 1. The content of total n-3 fatty acids, C22:6 n-3 (DHA) and C20:5 n-3 (EPA) in erythrocyte membrane phospholipids in towns and villages.

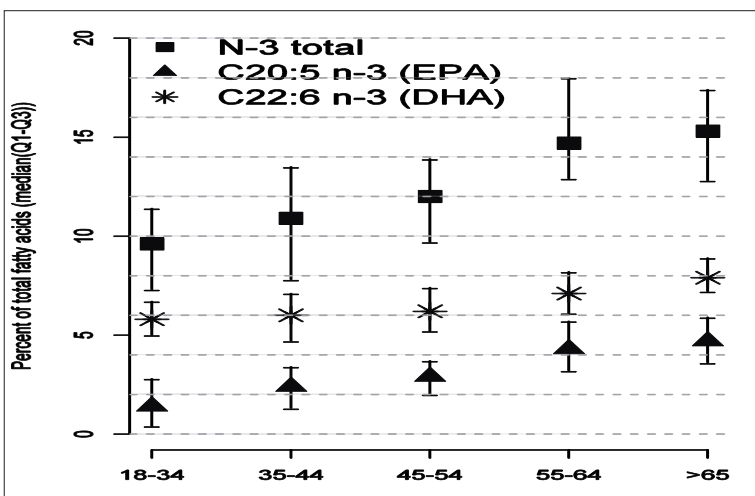


Figure 2. The content of total n-3 fatty acids, C22:6 n-3 (DHA) and C20:5 n-3 (EPA) in erythrocyte membrane phospholipids in the various age groups.

Results from the multiple regression analysis are shown in Table III. The table only shows the fatty acids, which had a significant univariate association with HOMA-IR. The  $\beta$  value expresses the relative change in the HOMA-IR value, when the fatty acid increases with 1 unit. As fatty acids are expressed in percent of total fatty acids, 1 unit corresponds to 1 percentage point.

The unadjusted models showed an inverse association between total n-3 fatty acids, C20:5 n-3 (EPA), C22:6 n-3 (DHA), C22:5 n-3 (DPA), C22:3 n-3, C20:4 n-3, the n-3/n-6 ratio and HOMA-IR. However, the association was only statistically significant for C20:5 n-3 (EPA), C22:3 n-3 and the n-3/n-6 ratio. A rise of 1 percentage point in these fatty acids was associated with a decrease of HOMA-IR of 4%, 46% and 21%, respectively. Conversely, a positive association between C18:3 n-3 *cis*, C18:4 n-3 and HOMA-IR was seen, and this at a statistically significant level for C18:3 n-3 *cis*. For C18:3 n-3 *cis* an increase of 1 percentage point was associated

with an increase in HOMA-IR of 124%.

After adjustment for age, gender, BMI and ethnicity (model 3), the association between C20:5 n-3 (EPA), C22:3 n-3, C18:3 n-3 *cis* and HOMA-IR remained present. However, the association was attenuated for C20:5 n-3 (EPA), when only adjusted for age, sex and BMI (models 1 and 2). The association between the n-3/n-6 ratio and HOMA-IR did not remain statistically significant after adjustments.

Adjusting for waist circumference instead of BMI did not change the associations (data not shown).

The results did not change if the study population was restricted to those with only full Inuit heritage.

When stratified by gender, the same directions of the associations were observed at a statistically significant level for women. When stratified by place of residence, we saw the same directions of the associations as well, but only a few of the associations remained statistically significant.

**Table III.** Multiple regression models with stepwise adjustments for age, gender, BMI and ethnicity. The  $\beta$  value expresses the relative change of HOMA-IR, when the fatty acid rises with 1 percentage point. Model 1: age and gender, model 2: age, gender and BMI, model 3: age, gender, BMI and ethnicity.

	C20:5 n-3 (EPA)		C22:3 n-3		C18:3 n-3 <i>cis</i>		n-3/n-6	
	$\beta$	95%CI	$\beta$	95%CI	$\beta$	95%CI	$\beta$	95%CI
<b>Unadj.</b>	0.96	0.93-0.99	0.54	0.39-0.75	2.24	1.47-3.44	0.79	0.65-0.97
<b>Model 1</b>	0.97	0.93-1.00	0.56	0.38-0.83	2.23	1.46- 3.39	0.85	0.67-1.07
<b>Model 2</b>	0.97	0.94-1.00	0.68	0.49-0.93	1.55	1.09-2.19	0.85	0.70-1.03
<b>Model 3</b>	0.97	0.94-1.00	0.67	0.49-0.92	1.55	1.10- 2.20	0.85	0.70-1.02

## DISCUSSION

We found in an Inuit population in Greenland that 2 types of n-3 fatty acids, C20:5 n-3 (EPA) and C22:3 n-3, and the n-3/n-6 ratio were inversely associated with HOMA-IR, while 1 type of n-3 fatty acid, C18:3 n-3 *cis*, was positively associated with HOMA-IR. In the case of C20:5 n-3 (EPA), C22:3 n-3 and C18:3 n-3 *cis*, the relations were robust in their adjustment for age, gender, BMI and ethnicity.

The Inuit population is exceptionally suitable for a study of the association between n-3 fatty acids and insulin resistance. The intake of n-3 fatty acids is much higher in the Greenland Inuit population compared to other European populations. Comparison of the composition of n-3 fatty acids of the erythrocyte membrane phospholipids in this study with studies from other parts of Europe (9,24–26) confirms that the content of n-3 fatty acids in the erythrocyte membranes is considerably higher in the Inuit population. Furthermore, there is a great variation in the intake of n-3 fatty acids in the Inuit population with a higher intake in villages as compared to towns (6). In this study, people from both villages and towns were included to maximize the variation of n-3 fatty acid intake.

Another strength of this study is the measurement taken of the n-3 fatty acids in erythrocyte membranes. Studies have shown that fat intake tends to be underestimated, when assessed by dietary recall or records (27,28). Fat intake may be assessed more reliably when based on dietary biomarkers such as measurement of fatty acids in erythrocyte membranes. Studies have shown that

the fatty acid composition of erythrocyte membranes mirrors the fatty acid pattern of the diet over the past few months (9–13). A high proportion of n-3 fatty acids in erythrocyte membranes reflect a high dietary intake of n-3 fatty acids during the period before the sample was taken. Fatty acids measured in cholesterol esters in serum reflects the intake over a period limited to the past week or 2 (9,29).

Fewer men than women and fewer people from villages than towns participated in the study. When stratified by gender, we saw the same directions of the associations. However, the associations were only significant for women, which probably is due to the lower number of men in the study. Nothing in the literature indicates that there should be any gender difference in the association between n-3 fatty acids and insulin resistance in men and women. The lack of significant associations, when stratified by place of residence, is probably due to the limited number of participants in each group.

The gold standard for the assessment of insulin resistance is the euglycemic clamp technique. As this method is not practicable in epidemiological settings, we used HOMA-IR, which has been validated against the gold standard (30–32), to estimate insulin resistance in our study.

Previous studies examining the association between n-3 fatty acids and insulin resistance and glucose intolerance are inconsistent. Two longitudinal studies (15,16) and a cross-sectional study from Alaska of an Inuit population (14) have linked fish intake with protection against glucose intolerance, while another follow-up study did not find any association between fish intake and

the risk of diabetes (18). A cross-sectional study from Greenland even found a positive association between a marine diet and blood glucose (17). It is not known which components of the marine diet are responsible for these associations.

Two cross-sectional studies from Alaska, where n-3 fatty acids were measured in serum and plasma, have shown that reduced concentrations of some n-3 fatty acids were associated with glucose intolerance (19) and insulin resistance (20), while another study showed an inverse association with insulin resistance (21).

Most intervention studies with supplementation of fish oil capsules have been performed in type 2 diabetic patients and have not induced any change in fasting blood glucose or insulin sensitivity (33–35). Also, randomized controlled trials performed in healthy subjects with n-3 fatty acid supplementation have not shown any influence on insulin sensitivity (36,37). However, most of these studies are probably conducted for periods too short to allow a real change in the composition of cell membrane phospholipids. An intervention study among the Inuit in the Alaska Siberia project showed that an increased consumption of traditional foods rich in n-3 fatty acids resulted in a decrease in plasma glucose (38).

This study is the first to assess n-3 fatty acids in erythrocyte membranes and to determine the association with insulin resistance in an Inuit population.

C18:3 n-3 *cis*, which is mainly found in plant oils, was associated with an increased risk of insulin resistance in this study. This could be because this fatty acid is related to a Westernized diet. However, this finding

is not consistent with other studies. Two studies, 1 from Alaska and 1 from Finland, found that low concentrations of C18:3 n-3 were associated with glucose intolerance (19,39).

Two of the fatty acids (C22:3 n-3 and C18:3 n-3) that are significantly associated with HOMA-IR are only present in small amounts. This could mean that even though the observed relations are statistically significant, it may have little physiological impact.

Our multivariate analyses showed that the associations between n-3 fatty acids and insulin resistance are complexly interrelated and they are probably likely to be affected by the interplay of diet, physical activity, energy intake, socio-economic status and contaminants. Physical activity could be an important confounder, because it is possible that people, who consume more traditional diets high in n-3 fatty acids, are more active.

The role of such potential confounders deserves further detailed exploration in future studies.

In conclusion, our findings suggest that some n-3 fatty acids, C20:5 n-3 (EPA) and C22:3 n-3, which are associated with a high intake of fish and sea mammals, and a high n-3/n-6 ratio may improve insulin sensitivity, while C18:3 n-3 *cis* may worsen insulin sensitivity.

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## REFERENCES

1. Sagild U, Littauer J, Jespersen CS, Andersen S. Epidemiological studies in Greenland 1962–1964. I. Diabetes mellitus in Eskimos. *Acta Med Scand* 1966;179: 29–39.
2. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950–1974. *Acta Med Scand* 1980; 208:401–406.
3. Jørgensen ME, Bjerregaard P, Borch-Johnsen K, Backer V, Becker U. Diabetes and impaired glucose tolerance among the Inuit population of Greenland. *Diabetes Care* 2002;25:1766–1771.
4. Deutch B, Dyerberg J, Pedersen HS, Aschlund E, Hansen JC. Traditional and modern Greenlandic food – dietary composition, nutrients and contaminants. *Sci Total Environ* 2007;384:106–119.
5. Deutch B, Dyerberg J, Pedersen HS, Asmund G, Møller P, Hansen JC. Dietary composition and contaminants in north Greenland, in the 1970s and 2004. *Sci Total Environ* 2006;370:372–381.
6. Pars T, Osler M, Bjerregaard P. Contemporary use of traditional and imported food among Greenlandic Inuit. *Arctic* 2001;54:22–31.
7. Harper CR, Jacobson TA. The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. *Arch Intern Med* 2001;161:2185–2192.
8. Nordic Nutrition Recommendations 2004. 4th ed. Copenhagen: Nordic Council of Ministers; 2004. 436 pp.
9. Katan MB, Deslypere JP, Birgelen APJM van, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res* 1997;38:2012–2022.
10. Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM. Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. *Am J Clin Nutr* 1987;45:443–455.
11. Glatz JF, Soffers AE, Katan MB. Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. *Am J Clin Nutr* 1989;49:269–276.
12. Romon M, Nuttens MC, Th  ret N, Delbart C, Lecerf JM, Fruchart JC, et al. Comparison between fat intake assessed by a 3-day food record and phospholipid fatty acid composition of red blood cells: results from the Monitoring of Cardiovascular Disease-Lille Study. *Metabolism* 1995;44:1139–1145.
13. Parra MS, Schnaas L, Meydani M, Perroni E, Mart  nez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. *Public Health Nutr* 2002;5:931–937.
14. Adler AI, Boyko EJ, Schraer CD, Murphy NJ. Lower prevalence of impaired glucose tolerance and diabetes associated with daily seal oil or salmon consumption among Alaska Natives. *Diabetes Care* 1994;17: 1498–1501.
15. Feskens EJ, Bowles CH, Kromhout D. Inverse association between fish intake and risk of glucose intolerance in normoglycemic elderly men and women. *Diabetes Care* 1991;14:935–941.
16. Feskens EJ, Virtanen SM, R  s  nen L, Tuomilehto J, Steng  rd J, Pekkanen J, et al. Dietary factors determining diabetes and impaired glucose tolerance. A 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study. *Diabetes Care* 1995; 18:1104–1112.
17. Bjerregaard P, Pedersen HS, Mulvad G. The associations of a marine diet with plasma lipids, blood glucose, blood pressure and obesity among the Inuit in Greenland. *Eur J Clin Nutr* 2000;54:732–737.
18. Dam RM van, Willett WC, Rimm EB, Stampfer MJ, Hu FB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. *Diabetes Care* 2002;25: 417–424.
19. Ebbesson SO, Kennish J, Ebbesson L, Go O, Yeh J. Diabetes is related to fatty acid imbalance in Eskimos. *Int J Circumpolar Health* 1999;58:108–119.
20. Ebbesson SO, Risica PM, Ebbesson LO, Kennish JM, Tejero ME. Omega-3 fatty acids improve glucose tolerance and components of the metabolic syndrome in Alaskan Eskimos: the Alaska Siberia project. *Int J Circumpolar Health* 2005;64:396–408.
21. Lovejoy JC, Champagne CM, Smith SR, DeLany JP, Bray GA, Lefevre M, et al. Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. *Metabolism* 2001;50:86–92.
22. Shaikh NA, Downar E. Time course of changes in porcine myocardial phospholipid levels during ischemia. A reassessment of the lysolipid hypothesis. *Circ Res* 1981;49:316–325.
23. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986;27:114–120.
24. Hoff S, Seiler H, Heinrich J, Kompauer I, Nieters A, Becker N, et al. Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes. *Eur J Clin Nutr* 2005;59:1071–1080.
25. Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry* 1998; 43:315–319.
26. Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord* 1998;48:149–155.
27. Brunner E, Stallone D, Juneja M, Bingham S, Marmot M. Dietary assessment in Whitehall II: comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. *Br J Nutr* 2001;86: 405–414.

28. Lissner L, Heitmann BL, Bengtsson C. Population studies of diet and obesity. *Br J Nutr* 2000;83 Suppl 1:S21–S24.
29. Arab L, Akbar J. Biomarkers and the measurement of fatty acids. *Public Health Nutr* 2002;5:865–871.
30. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997;20:1087–1092.
31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–419.
32. McAuley KA, Mann JI, Chase JG, Lotz TF, Shaw GM. Point: HOMA – satisfactory for the time being: HOMA: the best bet for the simple determination of insulin sensitivity, until something better comes along. *Diabetes Care* 2007;30:2411–2413.
33. Annuzzi G, Rivellesse A, Capaldo B, Di Marino L, Iovine C, Marotta G, et al. A controlled study on the effects of n-3 fatty acids on lipid and glucose metabolism in non-insulin-dependent diabetic patients. *Atherosclerosis* 1991;87:65–73.
34. McManus RM, Jumpson J, Finegood DT, Clandinin MT, Ryan EA. A comparison of the effects of n-3 fatty acids from linseed oil and fish oil in well-controlled type II diabetes. *Diabetes Care* 1996;19:463–467.
35. Luo J, Rizkalla SW, Vidal H, Oppert JM, Colas C, Boussairi A, et al. Moderate intake of n-3 fatty acids for 2 months has no detrimental effect on glucose metabolism and could ameliorate the lipid profile in type 2 diabetic men. Results of a controlled study. *Diabetes Care* 1998;21:717–724.
36. Giacco R, Cuomo V, Vessby B, Uusitupa M, Hermansen K, Meyer BJ, et al. Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? *Nutr Metab Cardiovasc Dis* 2007;17:572–580.
37. Vessby B, Uusitupa M, Hermansen K, Riccardi G, Rivellesse AA, Tapsell LC, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia* 2001;44:312–319.
38. Ebbesson SO, Ebbesson LO, Swenson M, Kennish JM, Robbins DC. A successful diabetes prevention study in Eskimos: the Alaska Siberia project. *Int J Circumpolar Health* 2005;64:409–424.
39. Salomaa V, Ahola I, Tuomilehto J, Aro A, Pietinen P, Korhonen HJ, et al. Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population-based study. *Metabolism* 1990;39:1285–1291.

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