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# Fish and marine fatty acids, genetic variant of FADS gene, and long-term weight gain



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| Complete List of Authors: | Huang, Tao<br>Wang, Tiange ; Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University<br>Heianza, Yoriko ; Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University<br>Wiggs, Janey ; Harvard Medical School, Massachusetts Eye and Ear Infirmary<br>Sun, Dianjianyi ; School of Public Health and Tropical Medicine, Tulane University<br>Han, Liyuan ; School of Public Health and Tropical Medicine, Tulane University<br>Choi, Hyon-Kyoo ; Massachusetts General Hospital - Harvard Medical School Center for Nervous System Repair<br>Chai, Jin Fang ; National University Singapore Yong Loo Lin School of Medicine<br>Sim, Xueling ; Epidemiology Domain Saw Swee Hock School of Public Health, National University of Singapore<br>Khor, Chiea Chuen ; Epidemiology Domain Saw Swee Hock School of Public Health, National University of Singapore<br>Friedlander, Yechiel ; Hebrew University-Hadassah Braun School of Public Health<br>Chan, Andrew T. ; Division of Gastroenterology, Massachusetts General Hospital<br>Curhan, Gary ; Department of Epidemiology, Harvard School of Public Health<br>Vivo, Immaculata De ; Department of Epidemiology, Harvard School of Public Health<br>van Dam, Rob Martinu. ; Saw Swee Hock School of Public Health, National University of Singapore<br>Heng, Chew Kiat ; Department of Paediatrics National University of Singapore<br>Fuchs, Charles S. ; Brigham and Women’s Hospital and Harvard Medical School<br>Pasquale, Louis R. ; Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School<br>Yuan, Jian-min ; Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute<br>Hu, Frank B. ; Department of Nutrition, Harvard School of Public Health<br>Koh, Woon Puay ; Duke-NUS Medical School<br>Qi, Lu; Tulane University, Department of Epidemiology, School of Public Health and Tropical Medicine, |

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**Fish and marine fatty acids, genetic variant of *FADS* gene, and long-term weight gain**

*Tao Huang[1,2,3,4*], Tiange Wang[3], Yoriko Heianza[3], Janey Wiggs[5], Dianjianyi Sun[3], Liyuan Han[3], Hyon-Kyoo Choi[6], Jin Fang Chai[2], Xueling Sim[2], Chiea Chuen Khor[7,8], Yechiel Friedlander[9], Andrew T. Chan[10], Gary Curhan[11], Immaculata De Vivo[11], Rob Martinu. van Dam[2,4], Chew Kiat Heng [12], Charles S. Fuchs[13,14], Louis R. Pasquale[15], Jian-min Yuan[16,17], Frank B. Hu[4,13], Woon Puay Koh[2,18], Lu Qi[3,4,19*]*

[1]*Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, China*

[2]*Epidemiology Domain Saw Swee Hock School of Public Health, National University of Singapore, Singapore, 117549.*

[3]*Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1724 New Orleans, LA 70112;*

[4]*Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA;*

[5]*Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02115, USA;*

[6]*Department of Rheumatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA;*

[7]*Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.*

[8]*Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

[9]*Unit of Epidemiology, Hebrew University-Hadassah Braun School of Public Health, POB 12272, Jerusalem 91120, Israel.*

[10]*Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114, USA;*

[11]*Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA;*

[12]*Department of Paediatrics National University of Singapore NUHS Tower Block, Level 12, 1E Kent Ridge Road Singapore 119228*

[13]*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

*02115, USA;*

*[14]The Center for Gastrointestinal Cancer, Dana-Farber Cancer Institute*

*[15]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA;*

*[16]Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.*

*[17]Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

*[18]Duke-NUS Medical School, Singapore, Singapore.*

*[19]Tulane University Obesity Research Center, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1724 New Orleans, LA 70112;*

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***Correspondence and requests for reprint:**

Dr. Tao Huang. Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China. Email: huangtao@bjmu.edu.cn

Dr. Lu Qi. Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University. 1440 Canal Street, Suite 1724, New Orleans, LA 70112

Telephone: 504-988-3549;

Email: lqi1@tulane.edu; luqi@hsph.harvard.edu

**Abstract**

**Objective:** We tested whether genetic variants near fatty acid desaturases gene (*FADS*) cluster, which were recently identified to be signatures of adaptation to fish- and n-3 PUFAs-rich diet, interacted with these dietary factors on change in body mass index (BMI).

**Design:** Three *FADS* variants were examined for gene-diet interactions on long-term (~10 years) changes in BMI and body weight were tested in three prospective cohort studies.

**Setting:** Population based study

**Participants:** 11,323 women from the Nurses' Health Study (NHS), 6,833 men from the Health Professionals Follow-up Study (HPFS), and replicated in 6,254 women from the Women's Health Initiative (WHI), and 5,264 Chinese from the Singapore Chinese Health Study (SCHS).

**Main outcomes:** Long-term (~10 years) changes in BMI and body weight

**Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS* rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 $kg/m^2$ per g, $P = 3\times10^{-5}$) than the rs174570 non-T carriers (beta=0.16 $kg/m^2$ per g, P = 0.08). Similar results were observed for fish intake.

**Conclusions:** Our analyses provide replicable evidence that long chain n-3 PUFAs and fish intakes may interact with the FADS variant on long-term weight gain.

**Article summary**

**Strengths and limitations of this study**

- This is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians with European ancestry and Singapore Chinese.
- The consistent results from these independent cohorts demonstrated the robustness of our findings.
- Unlike cross-sectional studies, our prospective analysis minimized the potential reverse causa.

## Introduction

Diet rich in fish and marine fatty acids especially long chain n-3 polyunsaturated fatty acids (PUFAs) has shown beneficial effects on cardiometabolic health (1, 2), however, data from population studies on the associations between such diet and body weight are inconsistent (3, 4). Emerging evidence suggests genetic variations may play a role in modifying the relation between dietary factors and body weight (5-7).

A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs. The strong signals locate in a cluster of fatty acid desaturases gene (*FADS*) that determine PUFAs levels (8). People living in the Arctic region have been found to be genetically prone to develop obesity (9, 10) as survival strength for energy storage(11, 12). Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in the Inuit population (8). Of note, due to long-standing selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various populations such as Europeans and Asians (13), in coincidence with varying levels of fish/marine fatty acids consumption, and adiposity patterns in these populations (14). We therefore hypothesized that the genetic signatures might interact with fish and marine PUFAs intakes on body weight (13).

The present study tested the interactions between n-3 PUFA and fish intakes and variants in *FADS* gene cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts the Women's Health Initiative (WHI) and the Singapore Chinese Health Study (SCHS).

## Methods

### Discovery cohorts

#### *The Nurses' Health Study*

The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were recruited to complete a baseline questionnaire about their lifestyle and medical history (15). The current analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere (16-22). All participants with genotyping data available based on previous GWASs were included (16-21). The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

#### *The Health Professionals Follow-up Study*

The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians, optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet (23). In the current analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere (16-22). All participants with genotyping data available based on previous GWASs were included (16-21). The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

### Replication cohorts

#### *The Women's Health Initiative (WHI)*

The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI study is presented elsewhere (24, 25). For the analyses, we included 6,254 Caucasians women with European ancestry who participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each participating institution reviewed and approved the study, and all women gave written informed consent.

***The Singapore Chinese Health Study (SCHS) cohort***

The design of Singapore Chinese Health Study (SCHS) has been previously described in detail (26). Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study (27). Two follow-up interviews were conducted via telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update information on body weight, selected lifestyle factors and medical history. All participants have given informed consent. The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved guidelines. Genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome Institute of Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation (28). Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip (29).

Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had both weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.

**Assessment of measures of body mass index**

Height and body weight were assessed by questionnaire at baseline, and weight information was requested on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly measured values (r=0.97 in HPFS and NHS) in a validation study (30). BMI was calculated as body weight (kg)/height ($m^2$). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS and HPFS cohorts (31), and from baseline (1993) to sixth year follow-up in the WHI (24, 25), and from baseline (1998) to second follow-up (2004) in the SCHS.

**Assessment of diets and other covariates**

Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires (FFQ) in the NHS and HPFS (32, 33). A 165-item validated semi-quantitative FFQ was used to collect dietary data and supplemental use of fish oil in the SCHS (27). Dietary data and supplemental use of fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI (34). Alternate health eating index was previously calculated in the NHS, HPFS (35), WHI, and SCHS respectively. Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities, and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously in the NHS and HPFS (36). In the WHI, an estimated metabolic equivalent (MET) level for each type of activity was assigned from a compendium of activities (37). Physical activity was assessed using eight continuous categories ranging from never to 31 hours or more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS (26).

**The *FADS* variants selection and genotyping**

Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes for signatures of adaptation (8) were derived from genome-wide scans available in the NHS, HPFS. We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication (**Supplemental Table 1**).

**Patient and Public Involvement**

patients and or public were not involved.

**Statistical analyses**

We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity measures and long-term changes in BMI using general linear models. Interactions between the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if available). We further tested the genetic associations with long-term changes in BMI according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to the *FADS* genotypes using general linear models after adjustment of potential confounders. Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models (if $P \geq 0.05$ for heterogeneity between studies) or random effects models (if $P < 0.05$ for

heterogeneity between studies). All reported P values are nominal and two sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

**Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts. The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The distribution of the *FADS* genetic variants in the 4 cohorts was shown in **Supplemental table 1**. We did not observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, BMI at endpoint, and long-term changes in BMI in three US cohorts ($P > 0.05$), however, we found that the *FADS* genotype was significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2)**.

### Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes

We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes of various sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the SCHS cohort (P for interaction = 0.02).

The pooled analyses of the 3 US (Caucasian) populations or all 4 cohorts showed that high intakes of food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly

accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No significant heterogeneity in the interaction effect was observed among these cohorts. Differences in long-term changes of BMI per T allele were -0.105 (SE 0.067), 0.027 (SE 0.064), and 0.120 (SE 0.067) kg/m$^2$ across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for interaction=0.003) showed similar interaction patterns; and the interactions remained significant when supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS* genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071), 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m$^2$ across three categories (≤ 1 serving/week, 1~6 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed (**Supplemental table 3 & 4**).

**Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had significantly greater increase of BMI (mean ± SE = 0.74±0.06, kg/m$^2$) than did those who consumed the lowest (T1) (mean ± SE = 0.39±0.07, kg/m$^2$) among the T allele carriers, whereas the corresponding BMI changes were 0.68±0.03 kg/m$^2$ and 0.49±0.03 kg/m$^2$, respectively, among the non-T carriers in 4 cohorts

combined (**Table 2 & Supplemental table 5** ). Similarly, we observed different associations between fish intake and BMI changes among the T allele carriers ($P = 1.5\times10^{-6}$) and non-carriers ($P = 0.01$) in the pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed among the cohorts.

**Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled results, the beta ± SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**) with long-term changes in BMI were 0.79 ± 0.19 kg/m$^2$ per g ($P = 0.000003$) and 0.64 ± 0.16 kg/m$^2$ per serving ($P = 0.00002$) among the T carriers, and whereas the corresponding beta ± SE were 0.16 ± 0.10 kg/m$^2$ per g ($P = 0.08$) and 0.18 ± 0.08 kg/m$^2$ per serving ($P = 0.01$) among the non-T carriers.

## Discussion

In 4 large prospective cohorts of the US and Chinese populations, we found reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS* rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.

Compelling evidence has shown that fish- and long chain n-3 PUFAs-rich diet are beneficial on improvement of cardiometabolic health (1, 2). However, large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight and obesity risk generated conflicting results (3, 4). In addition, several randomized controlled trials (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss (38-40), but the benefit was not evident in other trials (41-43). The

results from the current study lent support to our hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.

We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle, the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit, who had high fish/n-3 PUFAs intakes (8). The high frequency of T allele in Inuit reflects genetic adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that the signature allele (T) was related differently with weight changes (decrease or increase), depending on the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body weight when they are exposed to diet low in fish and n-3 PUFAs.

We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction patterns in relation to long-term changes in BMI; and the interactions also remained significant when supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is robust for fatty acids from various sources.

The mechanisms underlying the observed gene-diet interactions remain unclear, however, such interactions are biologically plausible. It has long been known that the *FADS* genes such as *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic

acid (n-6) and α-linolenic acid (n-3) (44). The selected allele of *FADS* rs174570 is significantly associated with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway (44). In addition, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function (45). In addition, storage of energy and body fat is very important for the Arctic population, who are regularly exposed to the extreme low temperature and fishes rich in n-3 PUFAs (11, 12). Under natural selection, these people are genetically prone to high fish intake to keep body fat (9, 10). Therefore, it's not surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying selective FADS signature (46, 47). Our findings support the view that extra n-3 PUFAs may not do much benefit at all for Europeans with selective FADS signature(8, 13).

**Strengths**

Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians with European ancestry and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings. Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results. Unlike cross-sectional studies, our prospective analysis minimized the potential reverse causality (48).

**Limitations**

However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable; however, the food frequency questionnaires and adiposity measures data have been well validated (27, 30, 32-34). Second, confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might

introduce bias in the present analyses. Finally, the participants included in our study were middle aged and older adults of Caucasians with European ancestry in the US and Chinese in Singapore, and it is unknown whether our findings could be generalized to other demographic or ethnic groups.

**Conclusions**

In summary, our data provides reproducible evidence from 4 multiethnic cohorts that high long chain n-3 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings emphasize the importance of considering precision nutritional interventions on prevention and treatment of obesity.

**Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH provided statistical expertise. TW, YH, DS, LH, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM, HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

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**Table 1 Baseline characteristics of all participants in the NHS, HPFS, WHI, and SCHS cohorts.**

| | NHS[1] | HPFS | WHI | SCHS |
|---|---|---|---|---|
| | n=11,323 | n=6,833 | n=6,254 | n=5,264 |
| Age (year) | 57 ± 9 | 57 ± 11 | 68 ± 5 | 56 ± 7 |
| Female (%) | 100 | 0 | 100 | 58.7 |
| Body weight (kg) | 70.1 ± 14.9 | 82.8 ± 12.5 | 73.7 ± 15.0 | 60.3 ± 9.8 |
| Body mass index (kg/m$^2$) | 26.2 ± 5.1 | 25.9 ± 3.3 | 28.3 ± 5.5 | 23.4 ± 3.3 |
| Alcohol consumption (g/day) | 5.14 ± 9.23 | 10.97 ± 15.05 | 6.00 ± 11.96 | 1.97 ± 8.02 |
| Physical activity (MET-h/week) | 19.3 ± 22.1 | 36.9 ± 39.5 | 11.6 ± 13.1 | 0.5 ± 1.0[2] |
| Television watching (h/week) | 17.5 ± 14.8 | 10.5 ± 8.2 | / | 2.2 ± 0.8 |
| Current smokers (n, (%)) | 1557(13.8) | 493(7.3) | 407(15.0) | 1364(20.0) |
| Total energy intake (kcal/day) | 1766 ± 502 | 1949 ± 578 | 1602 ± 654 | 1606 ± 573 |
| Alternative health eating index score | 53.4 ± 10.8 | 53.8 ± 11.4 | 53.5 ± 10.6 | 55.8 ± 8.2 |
| Sugar sweetened beverage intake (servings/day) | 0.13 ± 0.39 | 0.23 ± 0.48 | 0.39 ± 0.82 | 0.69 ± 2.40[3] |
| Total fried food (servings/day) | 0.12 ± 0.20 | 0.22 ± 0.28 | / | / |
| Fish intake (servings/day) | 0.31 ± 0.29 | 0.33 ± 0.30 | 0.23 ± 0.20 | 0.16 ± 0.07 |
| EPA (g/day) | 0.08 ± 0.14 | 0.12 ± 0.20 | 0.04 ± 0.04 | / |
| DHA (g/day) | 0.17 ± 0.14 | 0.22 ± 0.19 | 0.07 ± 0.07 | / |
| Food-sourced EPA+DHA (g/day) | 0.23 ± 0.19 | 0.31 ± 0.25 | 0.11 ± 0.10 | 0.33 ± 0.20 |
| Total EPA+DHA (g/day) | 0.26 ± 0.27 | 0.35 ± 0.37 | 0.38 ± 0.48 | / |

[1]Plus-minus values are means ± SD. [2]Hours per week of moderate activity in the SCHS. [3]Glasses per week of soda intake in the SCHS.

EPA: 20:5n-3; DHA: 22:6n-3; MET denotes metabolic equivalents.

The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively. Television watching assessed in 1992 for Nurses' Health Study and in 1990 for Health Professionals Follow-Up Study.

**Table 2 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| Diets | *FADS* genotypes | Long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| | | Categories of diets | | | | |
| **Total fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | | |
| NHS | Non-T carriers | 0.82±0.06 | 0.98±0.04 | 1.15±0.13 | 0.006 | 0.03 |
| | T carriers | 0.73±0.11 | 0.95±0.08 | 1.55±0.25 | 0.0007 | |
| HPFS | Non-T carriers | 0.43±0.05 | 0.52±0.04 | 0.59±0.12 | 0.73 | 0.03 |
| | T carriers | 0.21±0.11 | 0.52±0.07 | 0.79±0.22 | 0.02 | |
| WHI | Non-T carriers | 0.11±0.08 | 0.28±0.06 | 0.28±0.34 | 0.04 | 0.09 |
| | T carriers | 0.02±0.15 | 0.29±0.11 | 0.94±0.67 | 0.01 | |
| SCHS | Non-T carriers | -3.08±0.19 | -3.00±0.17 | -3.35±0.18 | 0.32 | 0.01 |
| | T carriers | -3.61±0.17 | -3.10±0.15 | -3.25±0.17 | 0.13 | |
| Pooled[1] | Non-T carriers | 0.50±0.03 | 0.67±0.03 | 0.81±0.08 | 0.01 | 0.0007 |
| | T carriers | 0.38±0.07 | 0.63±0.05 | 1.11±0.16 | $2\times10^{-4}$ | |
| **Food-sourced EPA+DHA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.79±0.06 | 0.92±0.05 | 1.11±0.06 | 0.01 | 0.005 |
| | T carriers | 0.71±0.10 | 0.84±0.11 | 1.19±0.11 | 0.0001 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.53±0.05 | 0.49±0.05 | 0.79 | 0.02 |
| | T carriers | 0.23±0.11 | 0.48±0.10 | 0.58±0.09 | 0.02 | |
| WHI | Non-T carriers | 0.02±0.08 | 0.21±0.08 | 0.41±0.08 | 0.06 | 0.04 |
| | T carriers | -0.03±0.15 | 0.29±0.14 | 0.35±0.15 | 0.004 | |
| SCHS | Non-T carriers | -3.32±0.17 | -3.15±0.18 | -2.99±0.17 | 0.16 | 0.035 |
| | T carriers | -3.55±0.16 | -3.34±0.16 | -3.05±0.16 | 0.02 | |
| Pooled[1] | Non-T carriers | 0.49±0.03 | 0.64±0.03 | 0.68±0.03 | 0.01 | 0.0003 |
| | T carriers | 0.39±0.07 | 0.57±0.06 | 0.74±0.06 | $1.5\times10^{-6}$ | |
| **Total EPA+DHA, g/day** | | T1 | T2 | T3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| NHS | Non-T carriers | 0.79±0.06 | 0.94±0.05 | 1.08±0.06 | 0.8 | 0.01 |
| | T carriers | 0.72±0.11 | 0.87±0.10 | 1.16±0.11 | 0.02 | |
| HPFS | Non-T carriers | 0.47±0.05 | 0.53±0.05 | 0.49±0.05 | 0.88 | 0.13 |
| | T carriers | 0.23±0.10 | 0.50±0.09 | 0.57±0.10 | 0.16 | |
| WHI | Non-T carriers | 0.39±0.10 | 0.04±0.08 | 0.23±0.10 | 0.42 | 0.27 |
| | T carriers | 0.04±0.18 | 0.28±0.15 | 0.28±0.16 | 0.84 | |
| Pooled[1] | Non-T carriers | 0.57±0.04 | 0.62±0.03 | 0.67±0.04 | 0.65 | 0.005 |
| | T carriers | 0.39±0.07 | 0.60±0.06 | 0.74±0.07 | 0.01 | |

Data are means ± SE.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Registration: www. clinicaltrials.gov. Registration ID: NCT03348566

**Figure Legends**

**Figure 1 Genetic variant of *FADS* rs174570, long chain n-3 PUFAs and fish intakes and long-term BMI changes**

Effect size (ES) (95% CI) values are β coefficients for interaction between the *FADS* variant rs174570 (additive model) and diets from results of the NHS, HPFS, WHI, and SCHS cohorts.

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the *FADS* variant-diets interaction by including a multiplicative interaction term in the models after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure 2 Genetic association of *FADS* variant rs174570 with long-term BMI change according to long chain n-3 PUFAs and fish intakes**

Pooled-EUR: data from NHS, HPFS, and WHI were pooled.

Pooled Multiethnic: data from NHS, HPFS, WHI and SCHS were pooled.

Data are β coefficients ± SE.

Numbers of participants across three categories (≤1/wk/1~6/wk/≥1/d) of fish intake in the NHS, HPFS, WHI, and SCHS are 1618/8465/1239, 977/5108/748, 894/4675/684, and 752/3935/576, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

The general linear model was used to test the genetic association of the *FADS* variant (additive model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The general linear model was used to test the associations of long chain n-3 PUFAs intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The general linear model was used to test the associations of and fish intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Figure 1

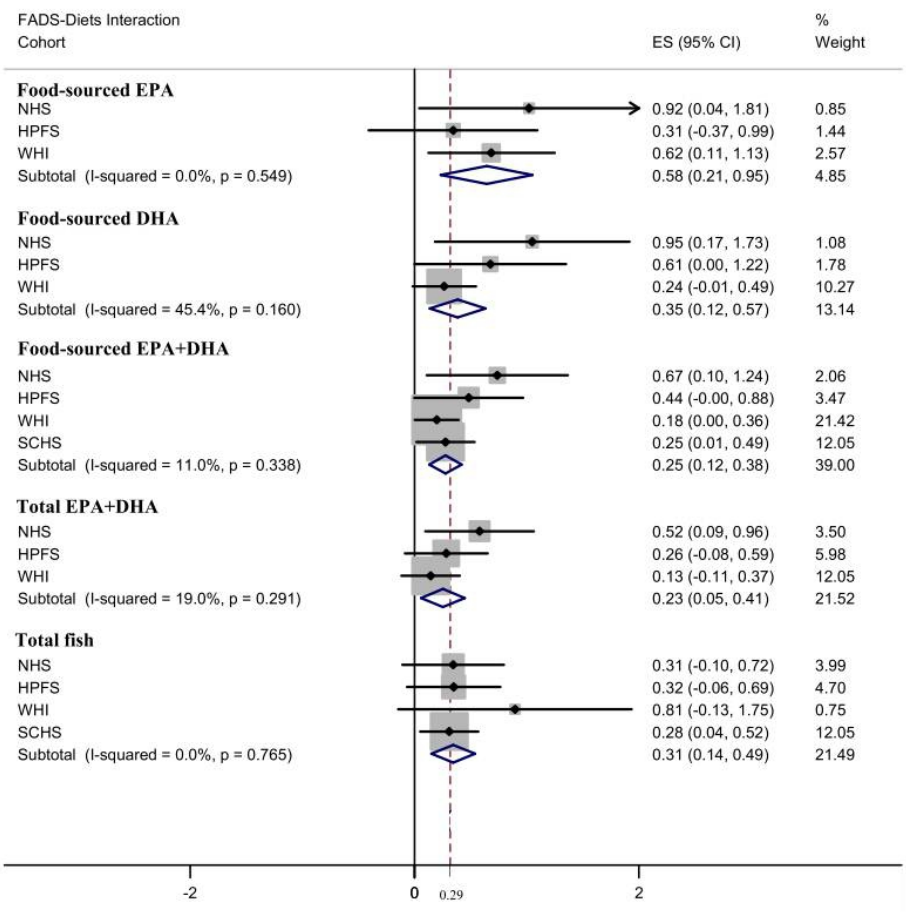


Figure 1

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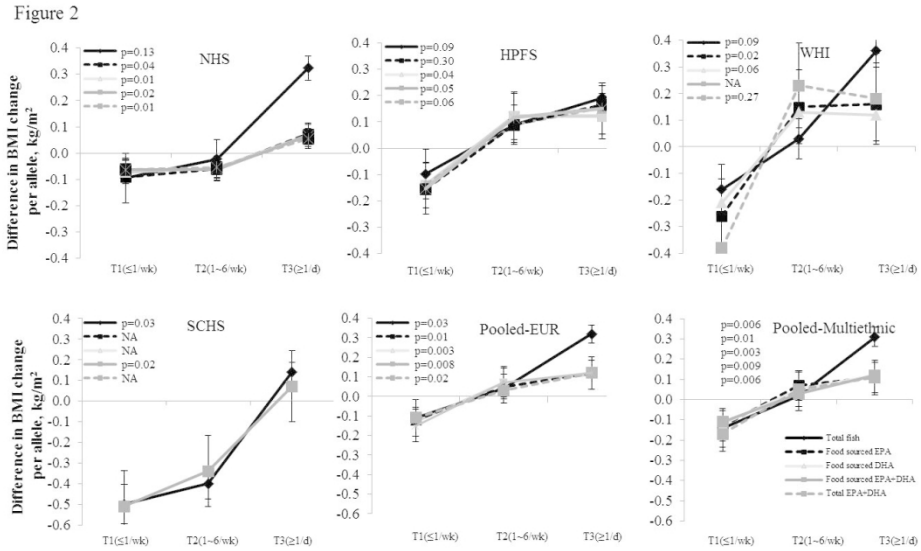


Figure 1

245x138mm (150 x 150 DPI)

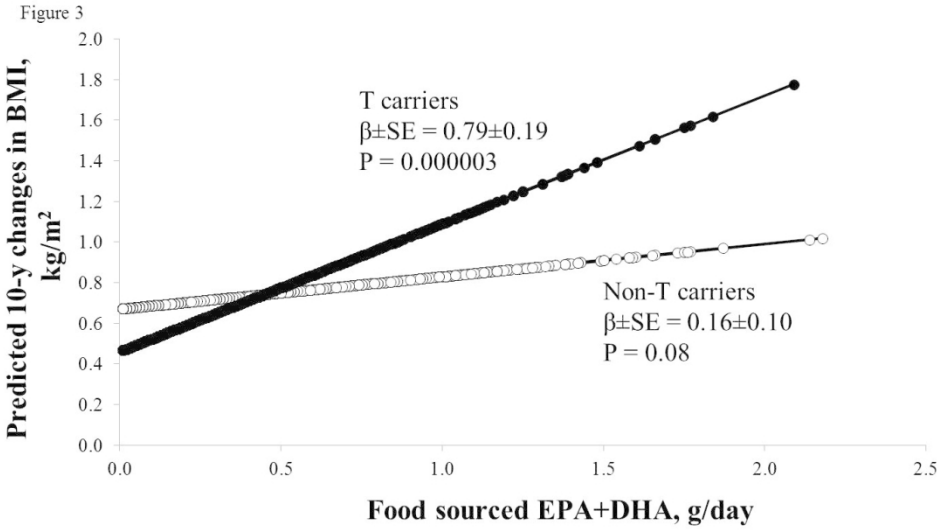


Figure 3

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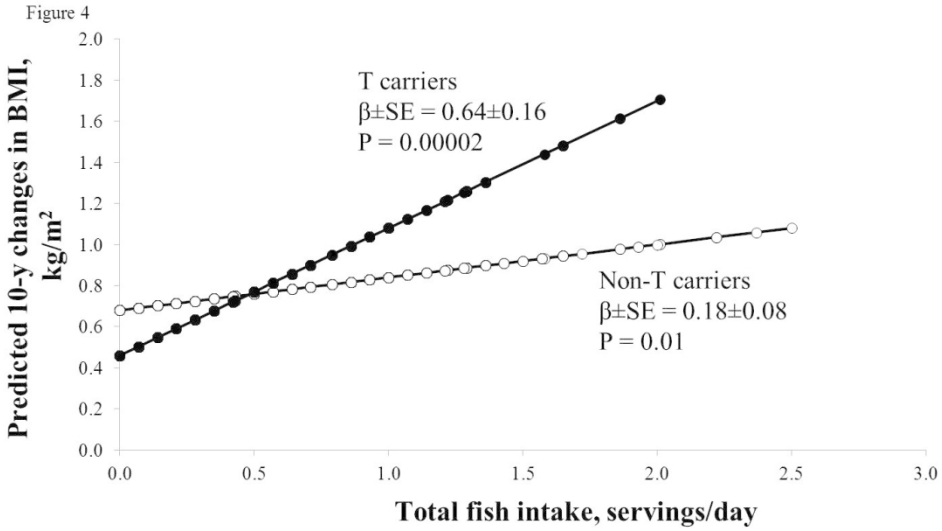


Figure 4

249x142mm (150 x 150 DPI)

**Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit**

| Position[1] | Reference SNP identification number | Alleles[2] | DAF | | | | | | | PBS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CEU | CHB | GI | NHS | HPFS | WHI | SCHS | |
| chr11:61627960 | rs74771917 | C/T | 0.025 | 0.16 | 0.98 | / | / | / | / | 2.67 |
| chr11:61631510 | rs3168072 | A/T | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61632310 | rs12577276 | A/G | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61641717 | rs7115739 | G/T | 0.017 | 0.22 | 0.98 | 0.004 | 0.004 | / | / | 2.54 |
| chr11:61624414 | rs174602 | C/T | 0.80 | 0.73 | 0.01 | 0.82 | 0.81 | / | / | 2.11 |
| chr11:61597212 | rs174570 | C/T | 0.16 | 0.34 | 0.99 | 0.15 | 0.15 | 0.14 | 0.35 | 2.06 |

[1]Positions refer to human genome assembly hg19.

[2]Alleles are coded as ancestral/derived states.

PBS, the population branch statistic; DAF, derived allele frequency; CEU, European ancestry; CHB, an Chinese; GI, Greenlandic Inuit

DAFs for each population (CEU, CHB, and GI) and PBS values are reported, along with the genomic position for each SNP.

**Supplemental table 2 Main effect of the *FADS* variants on adiposity in the four cohorts**

| Adiposity (kg/m$^2$) | *FADS* SNPs | NHS | | HPFS | | WHI | | SCHS | | Pooled | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P |
| Baseline BMI | rs174570 | 0.03 ± 0.10 | 0.733 | -0.05 ± 0.09 | 0.538 | -0.06±0.17 | 0.72 | 0.24±0.08 | 0.002 | 0.08±0.05 | 0.06 |
| Baseline BMI | rs174602 | 0.08 ± 0.10 | 0.418 | -0.05 ± 0.08 | 0.536 | / | | / | | 0.00 ± 0.03 | 0.559 |
| Baseline BMI | rs7115739 | 0.25 ± 0.52 | 0.634 | -0.77 ± 0.43 | 0.077 | / | | / | | -0.35 ± 0.14 | 0.196 |
| Long-term BMI change | rs174570 | -0.05 ± 0.06 | 0.401 | 0.01 ± 0.05 | 0.917 | -0.02±0.09 | 0.77 | -0.02±0.08 | 0.85 | -0.02±0.03 | 0.94 |
| Long-term BMI change | rs174602 | -0.14 ± 0.06 | 0.009 | 0.04 ± 0.05 | 0.413 | / | | / | | -0.04 ± 0.01 | 0.025 |
| Long-term BMI change | rs7115739 | 0.45 ± 0.29 | 0.124 | -0.23 ± 0.26 | 0.359 | / | | / | | 0.06 ± 0.08 | 0.183 |

Long-term BMI change: BMI change from 1990 to 2000.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Effect size (ES) values are β coefficients for relationship between the *FADS* variant rs174570 (additive model) and adiposity.

The general linear model was used to test the genetic association of *FADS* variants with long-term changes in BMI after adjustment for age, source of genotyping data.

**Supplemental table 3 Genetic association of *FADS* variant with long-term changes in body weight according to long chain n-3 PUFAs and fish intakes**

| Cohorts | Difference in long-term changes in weight, kg | | | P for interaction |
|---|---|---|---|---|
| **Total Fish, serving/day** | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | -0.69±0.64 | -0.13±0.49 | 1.78±1.64 | 0.05 |
| HPFS | -0.99±0.85 | 0.54±0.53 | 1.52±1.69 | 0.12 |
| WHI | -0.22±0.42 | 0.16±0.34 | 1.26±1.57 | 0.13 |
| SCHS | -0.42±0.29 | -0.44±0.28 | 0.20±0.29 | 0.08 |
| Pooled | -0.44±0.22 | -0.10±0.18 | 0.31±0.28 | 0.01 |
| **Food-sourced EPA, g/day** | T1 | T2 | T3 | |
| NHS | -0.77±0.62 | -0.25±0.72 | 0.53±0.64 | 0.06 |
| HPFS | -1.19±0.82 | 0.75±0.73 | 0.72±0.74 | 0.41 |
| WHI | -0.19±0.42 | 0.24±0.47 | 0.14±0.48 | 0.20 |
| Pooled | -0.50±0.32 | 0.24±0.34 | 0.37±0.34 | 0.10 |
| **Food-sourced DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.53±0.62 | -0.39±0.70 | 0.53±0.65 | 0.01 |
| HPFS | -1.06±0.82 | 0.49±0.71 | 0.89±0.76 | 0.09 |
| WHI | -0.20±0.43 | 0.22±0.42 | 0.30±0.50 | 0.26 |
| Pooled | -0.43±0.32 | 0.15±0.32 | 0.49±0.35 | 0.01 |
| **Food-sourced EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.56±0.63 | -0.32±0.68 | 0.49±0.66 | 0.01 |
| HPFS | -1.25±0.83 | 0.68±0.73 | 0.84±0.74 | 0.09 |
| WHI | -0.02±0.43 | 0.16±0.44 | 0.14±0.49 | 0.23 |
| SCHS | -0.47±0.29 | -0.16±0.28 | -0.03±0.29 | 0.10 |

| | | | | |
|---|---|---|---|---|
| Pooled | -0.56±0.25 | -0.09±0.24 | 0.14±0.25 | 0.005 |
| **Total EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.58±0.63 | -0.30±0.68 | 0.46±0.66 | 0.02 |
| HPFS | -1.20±0.82 | 0.75±0.70 | 0.86±0.77 | 0.18 |
| WHI | -0.48±0.47 | 0.64±0.43 | 0.04±0.47 | 0.15 |
| Pooled | -0.64±0.34 | 0.45±0.32 | 0.32±0.34 | 0.02 |

Data are β coefficients ± SE.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the genetic association with long-term changes in body weight according to baseline long chain n-3 PUFAs and fish intakes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from three or four cohorts pooled by means of fixed effects meta-analyses (if P ≥ 0.05 for heterogeneity between studies).

**Supplemental table 4 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight according to *FADS* genotypes**

| Cohorts | | Long-term changes in weight, kg | | | P for trend |
|---|---|---|---|---|---|
| **Total Fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | Non T carriers | 4.91±0.34 | 5.78±0.24 | 7.00±0.79 | 0.008 |
| | T carriers | 4.45±0.61 | 5.64±0.46 | 9.26±1.44 | 0.001 |
| HPFS | Non T carriers | 0.44±0.06 | 0.52±0.04 | 0.56±0.12 | 0.99 |
| | T carriers | 0.25±0.10 | 0.53±0.07 | 0.76±0.21 | 0.08 |
| WHI | Non T carriers | -0.25±0.23 | -0.43±0.18 | -0.91±0.93 | 0.50 |
| | T carriers | -0.56±0.37 | -0.25±0.28 | 1.30±1.71 | 0.13 |
| SCHS | Non T carriers | -3.15±0.23 | -3.50±0.21 | -3.38±0.21 | 0.48 |
| | T carriers | -3.68±0.20 | -3.41±0.19 | -3.34±0.20 | 0.16 |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.89±0.33 | 5.89±0.33 | 5.95±0.33 | 0.24 |
| | T carriers | 4.45±0.59 | 5.52±0.63 | 6.46±0.61 | 0.34 |
| HPFS | Non T carriers | 0.50±0.05 | 0.54±0.05 | 0.45±0.05 | 0.15 |
| | T carriers | 0.29±0.10 | 0.54±0.09 | 0.52±0.09 | 0.66 |
| WHI | Non T carriers | -0.30±0.25 | -0.54±0.24 | -0.29±0.24 | 0.42 |
| | T carriers | -0.51±0.39 | -0.36±0.37 | -0.15±0.38 | 0.14 |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.78±0.33 | 5.56±0.34 | 6.32±0.33 | 0.14 |
| | T carriers | 4.50±0.60 | 5.07±0.63 | 6.77±0.61 | 0.004 |
| HPFS | Non T carriers | 0.48±0.05 | 0.54±0.05 | 0.46±0.06 | 0.40 |
| | T carriers | 0.27±0.10 | 0.50±0.09 | 0.59±0.09 | 0.15 |
| WHI | Non T carriers | -0.41±0.25 | -0.25±0.24 | -0.45±0.25 | 0.51 |

| | | | | | |
|---|---|---|---|---|---|
| | T carriers | -0.71±0.39 | -0.15±0.37 | -0.16±0.39 | 0.18 |
| **Food-sourced EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.69±0.34 | 5.45±0.33 | 6.51±0.33 | 0.02 |
| | T carriers | 4.44±0.61 | 5.00±0.61 | 6.92±0.61 | 0.0003 |
| HPFS | Non T carriers | 0.48±0.05 | 0.53±0.05 | 0.47±0.05 | 0.93 |
| | T carriers | 0.26±0.10 | 0.49±0.09 | 0.59±0.09 | 0.08 |
| WHI | Non T carriers | -0.44±0.24 | -0.23±0.23 | -0.43±0.24 | 0.47 |
| | T carriers | -0.52±0.38 | -0.15±0.37 | -0.33±0.38 | 0.15 |
| SCHS | Non T carriers | -3.44±0.21 | -3.58±0.22 | -3.05±0.21 | 0.89 |
| | T carriers | -3.73±0.19 | -3.57±0.19 | -3.12±0.19 | 0.12 |
| **Total EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.74±0.34 | 5.55±0.32 | 6.36±0.34 | 0.81 |
| | T carriers | 4.49±0.61 | 5.16±0.60 | 6.70±0.61 | 0.03 |
| HPFS | Non T carriers | 0.49±0.05 | 0.53±0.05 | 0.47±0.06 | 0.24 |
| | T carriers | 0.26±0.10 | 0.51±0.09 | 0.58±0.09 | 0.33 |
| WHI | Non T carriers | 0.32±0.27 | -0.84±0.23 | -0.60±0.28 | 0.19 |
| | T carriers | -0.26±0.45 | -0.21±0.37 | -0.02±0.11 | 0.08 |

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight by *FADS* genotypes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from two cohorts pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies) or random effects meta-analyses (if $P < 0.05$ for heterogeneity between studies).

**Supplemental Table 5 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| Diets | *FADS* genotypes | Long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| | | Categories of diets | | | | |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.82±0.06 | 1.00±0.06 | 1.01±0.06 | 0.24 | 0.05 |
| | T carriers | 0.72±0.10 | 0.94±0.11 | 1.10±0.11 | 0.29 | |
| HPFS | Non-T carriers | 0.48±0.05 | 0.54±0.05 | 0.47±0.05 | 0.72 | 0.37 |
| | T carriers | 0.23±0.11 | 0.54±0.10 | 0.52±0.09 | 0.45 | |
| WHI | Non-T carriers | 0.10±0.09 | 0.09±0.09 | 0.45±0.09 | 0.21 | 0.02 |
| | T carriers | -0.08±0.15 | 0.23±0.15 | 0.46±0.15 | 0.003 | |
| Pooled | Non-T carriers | 0.54±0.04 | 0.63±0.04 | 0.65±0.04 | 0.35 | 0.01 |
| | T carriers | 0.39±0.07 | 0.63±0.07 | 0.70±0.06 | 0.01 | |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.80±0.06 | 0.94±0.06 | 1.08±0.06 | 0.14 | 0.009 |
| | T carriers | 0.74±0.10 | 0.83±0.11 | 1.17±0.10 | 0.002 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.54±0.05 | 0.49±0.05 | 0.99 | 0.05 |
| | T carriers | 0.24±0.10 | 0.49±0.10 | 0.58±0.09 | 0.05 | |
| WHI | Non-T carriers | 0.03±0.09 | 0.20±0.09 | 0.42±0.09 | 0.03 | 0.06 |
| | T carriers | -0.10±0.15 | 0.33±0.15 | 0.39±0.15 | 0.006 | |
| Pooled | Non-T carriers | 0.51±0.04 | 0.63±0.04 | 0.68±0.04 | 0.1 | 0.002 |
| | T carriers | 0.38±0.06 | 0.58±0.07 | 0.77±0.06 | $7\times10^{-4}$ | |

Data are means ± SE.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the

WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Registration: www. clinicaltrials.gov. Registration ID: NCT03348566

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract **(p. 3)** |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **(p. 3)** |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported **(p. 4)** |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses **(p. 4)** |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper **(p. 5)** |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **(p. 5)** |
| Participants | 6 | (*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up<br>*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls<br>*Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **(p. 5)** |
| | | (*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed<br>*Case-control study*—For matched studies, give matching criteria and the number of controls per case **(p. 5)** |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **(p. 5)** |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **(p. 5)** |
| Bias | 9 | Describe any efforts to address potential sources of bias **(p. 5 &6)** |
| Study size | 10 | Explain how the study size was arrived at **(p. 6 &7)** |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **(p. 7)** |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding **(p. 7-9)** |
| | | (*b*) Describe any methods used to examine subgroups and interactions **(p. 9)** |
| | | (*c*) Explain how missing data were addressed **(p. 19)** |
| | | (*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed<br>*Case-control study*—If applicable, explain how matching of cases and controls was addressed<br>*Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy **(p. 9)** |
| | | (*e*) Describe any sensitivity analyses **(p. 9)** |

Continued on next page

| | | |
|---|---|---|
| **Results** | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **(p. 10)** |
| | | (b) Give reasons for non-participation at each stage **(p. 10)** |
| | | (c) Consider use of a flow diagram **(p. 10)** |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **(p. 10)** |
| | | (b) Indicate number of participants with missing data for each variable of interest **(p. 10)** |
| | | (c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **(p. 10)** |
| Outcome data | 15* | *Cohort study*—Report numbers of outcome events or summary measures over time **(p. 10)** |
| | | *Case-control study*—Report numbers in each exposure category, or summary measures of exposure **(p. 10)** |
| | | *Cross-sectional study*—Report numbers of outcome events or summary measures **(p. 10)** |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **(p. 10)** |
| | | (*b*) Report category boundaries when continuous variables were categorized **(p. 10)** |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **(p. 10)** |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **(p. 10)** |
| **Discussion** | | |
| Key results | 18 | Summarise key results with reference to study objectives **(p. 11)** |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **(p. 115)** |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **(p. 11)** |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results **(p. 11)** |
| **Other information** | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **(p. 14)** |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

# Fish and marine fatty acids interacted with genetic variants of FADS gene in influencing long-term weight gain



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| | |
|---|---|
| | Health and Tropical Medicine, |
| Primary Subject Heading: | Nutrition and metabolism |
| Secondary Subject Heading: | Diabetes and endocrinology, Epidemiology, Genetics and genomics, Public health |
| Keywords: | NUTRITION & DIETETICS, GENETICS, EPIDEMIOLOGY, obesity, gene-diet interaction |
| | |

**Fish and marine fatty acids interacted with genetic variants of *FADS* gene in influencing long-term weight gain**

Tao Huang[1,2,3*], Tiange Wang[4], Yoriko Heianza[5], Janey Wiggs[6], Dianjianyi Sun[5], Liyuan Han[5], Hyon-Kyoo Choi[7], Jin Fang Chai[8], Xueling Sim[8], Chiea Chuen Khor[9,10], Yechiel Friedlander[11], Andrew T. Chan[12], Gary Curhan[13], Immaculata De Vivo[13], Rob Martinu. van Dam[8,14], Chew Kiat Heng[15], Charles S. Fuchs[16,17], Louis R. Pasquale[18], Jian-min Yuan[19,20], Frank B. Hu[14, 16], Woon Puay Koh[8, 21], Lu Qi[5, 14, 16]*

[1.] *Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, China.*

[2.] *Department of Global Health, School of Public Health, Peking University, China.*

[3.] *Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, China.*

[4.] *Shanghai Institute of Endocrine and Metabolic Diseases, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

[5.] *Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1724 New Orleans, LA 70112.*

[6.] *Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02115, USA.*

[7.] *Department of Rheumatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[8.] *Epidemiology Domain, Saw Swee Hock School of Public Health, National University of Singapore, Singapore, 117549.*

[9.] *Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.*

[10.] *Department of Biochemistry, Yong Loo Lin School of Medicine, , National University of Singapore, Singapore.*

[11.] *Unit of Epidemiology, Hebrew University-Hadassah Braun School of Public Health, POB 12272, Jerusalem 91120, Israel.*

[12.] *Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114, USA.*

[13.] *Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA.*

[14.] *Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA.*

[15.] *Department of Paediatrics, National University of Singapore NUHS Tower Block, Level 12, 1E Kent Ridge Road Singapore 119228.*

[16.] *Department of Medicine, Brigham and Women's Hospital and Harvard Medical School Boston, MA 02115, USA.*

[17.] *The Center for Gastrointestinal Cancer, Dana-Farber Cancer Institute, Boston, USA.*

[18.] *Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[19.] *Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.*

[20.] *Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

[21.] *Duke-NUS Medical School, Singapore, Singapore.*

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***Correspondence and requests for reprint:**

Dr. Tao Huang. Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China. Email: huangtao@bjmu.edu.cn

Dr. Lu Qi. Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University. 1440 Canal Street, Suite 1724, New Orleans, LA 70112

Telephone: 504-988-3549;

Email: lqi1@tulane.edu; luqi@hsph.harvard.edu

**Abstract**

**Objective:** We tested whether genetic variants near fatty acid desaturases gene (*FADS*) cluster, which were recently identified to be signatures of adaptation to fish- and n-3 PUFAs-rich diet, interacted with these dietary factors on change in body mass index (BMI).

**Design:** Three *FADS* variants were examined for gene-diet interactions on long-term (~10 years) changes in BMI and body weight were tested in three prospective cohort studies.

**Setting:** Population based study

**Participants:** 11,323 women from the Nurses' Health Study (NHS), 6,833 men from the Health Professionals Follow-up Study (HPFS), and replicated in 6,254 women from the Women's Health Initiative (WHI), and 5,264 Chinese from the Singapore Chinese Health Study (SCHS).

**Main outcomes:** Long-term (~10 years) changes in BMI and body weight

**Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS* rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 $kg/m^2$ per g, $P = 3\times10^{-5}$) than the rs174570 non-T carriers (beta=0.16 $kg/m^2$ per g, P = 0.08). Similar results were observed for fish intake.

**Conclusions:** Our analyses provide replicable evidence that long chain n-3 PUFAs and fish intakes may interact with the *FADS* variant on long-term weight gain.

**Article summary**

**Strengths and limitations of this study**

- This is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians with European ancestry and Singapore Chinese.
- The consistent results from these independent cohorts demonstrated the robustness of our findings.
- Unlike cross-sectional studies, our prospective analysis minimized the potential reverse causation.

## Introduction

Diet rich in fish and marine fatty acids especially long chain n-3 polyunsaturated fatty acids (PUFAs) has shown beneficial effects on cardiometabolic health [1 2], however, data from population studies on the associations between such diet and body weight are inconsistent [3 4]. Emerging evidence suggests genetic variations may play a role in modifying the relation between dietary factors and body weight [5-7].

A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs [8]. The strong signals locate in a cluster of fatty acid desaturases gene (*FADS*) that determine PUFAs levels [8]. People living in the Arctic region have been found to be genetically prone to develop obesity [9 10] as survival strength for energy storage[11 12]. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in the Inuit population [8]. Of note, due to long-standing selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various populations such as Europeans and Asians [13], in coincidence with varying levels of fish/marine fatty acids consumption, and adiposity patterns in these populations [14]. We therefore hypothesized that the genetic signatures might interact with fish and marine PUFAs intakes on body weight [13].

The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts the Women's Health Initiative (WHI) and the Singapore Chinese Health Study (SCHS).

## Methods

### Discovery cohorts

#### *The Nurses' Health Study*

The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were recruited to complete a baseline questionnaire about their lifestyle and medical history [15]. The current analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [16-22]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [16-21].. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

#### *The Health Professionals Follow-up Study*

The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians, optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet [23]. In the current analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [16-22]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [16-21]. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

## Replication cohorts

### *The Women's Health Initiative (WHI)*

The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI study is presented elsewhere [24 25]. For the analyses, all participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included. Finally, we included 6,254 Caucasians women with European ancestry who participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each participating institution reviewed and approved the study, and all women gave written informed consent.

### *The Singapore Chinese Health Study (SCHS) cohort*

The design of Singapore Chinese Health Study (SCHS) has been previously described in detail [26]. Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study [27]. Two follow-up interviews were conducted via telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update information on body weight, selected lifestyle factors and medical history. All participants have given informed consent. The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data available were included. Among these participants, genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome Institute of Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian)

Axiom array. Genotype calling was performed by the Affymetrix Corporation [28]. Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip [29].

Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had both weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.

**Assessment of measures of body mass index**

Height and body weight were assessed by questionnaire at baseline, and weight information was requested on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly measured values (r=0.97 in HPFS and NHS) in a validation study [30]. BMI was calculated as body weight (kg)/height ($m^2$). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS and HPFS cohorts [31], and from baseline (1993) to sixth year follow-up in the WHI [24 25], and from baseline (1998) to second follow-up (2004) in the SCHS.

**Assessment of diets and other covariates**

Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires (FFQ) in the NHS and HPFS [32 33]. A 165-item validated semi-quantitative FFQ was used to collect dietary data and supplemental use of fish oil in the SCHS [27]. Dietary data and supplemental use of fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI [34]. Alternate health eating index was previously calculated in the NHS, HPFS [35], WHI, and SCHS respectively. Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities, and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously in the NHS and HPFS [36]. In the WHI, an estimated metabolic equivalent (MET) level for each type of activity was assigned from a compendium of activities [37]. Physical activity was assessed

using eight continuous categories ranging from never to 31 hours or more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS [26].

**The *FADS* variants selection and genotyping**

Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes for signatures of adaptation [8] were derived from genome-wide scans available in the NHS, HPFS. We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication (**Supplemental Table 1**).

**Patient and Public Involvement**

patients and or public were not involved.

**Statistical analyses**

We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity measures and long-term changes in BMI using general linear models. Interactions between the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if available). We further tested the genetic associations with long-term changes in BMI according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to the *FADS* genotypes using general linear models after adjustment of potential

confounders. Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models (if $P \geq 0.05$ for heterogeneity between studies) or random effects models (if $P < 0.05$ for heterogeneity between studies). Hardy-Weinberg equilibrium was tested using Chi-square test. All reported P values are nominal and two sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

**Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts. The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The distribution of the *FADS* genetic variants in the 4 cohorts was shown in **Supplemental table 1**. We did not observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term changes in BMI in three US cohorts ($P > 0.05$), however, we found that the *FADS* genotype was significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2)**.

### Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes

We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes of various sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the SCHS cohort (P for interaction = 0.02).

The pooled analyses of the 3 US (Caucasian) populations or all 4 cohorts showed that high intakes of food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No significant heterogeneity in the interaction effect was observed among these cohorts. Differences in long-term changes of BMI per T allele were -0.105 (SE 0.067), 0.027 (SE 0.064), and 0.120 (SE 0.067) $kg/m^2$ across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for interaction=0.003) showed similar interaction patterns; and the interactions remained significant when supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS* genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071), 0.041 (SE 0.052), and 0.251 (SE 0.151) $kg/m^2$ across three categories (≤ 1 serving/week, 1~6 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed (**Supplemental table 3 & 4**).

**Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had significantly greater increase of BMI (mean ± SE = 0.74±0.06, $kg/m^2$) than did those who consumed the

lowest (T1) (mean ± SE = 0.39±0.07, kg/m$^2$) among the T allele carriers, whereas the corresponding BMI changes were 0.68±0.03 kg/m$^2$ and 0.49±0.03 kg/m$^2$, respectively, among the non-T carriers in 4 cohorts combined (**Table 2 & Supplemental table 5** ). Similarly, we observed different associations between fish intake and BMI changes among the T allele carriers ($P = 1.5\times10^{-6}$) and non-carriers (P = 0.01) in the pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed among the cohorts.

**Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled results, the beta ± SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**) with long-term changes in BMI were 0.79 ± 0.19 kg/m$^2$ per g (P = 0.000003) and 0.64 ± 0.16 kg/m$^2$ per serving (P = 0.00002) among the T carriers, and whereas the corresponding beta ± SE were 0.16 ± 0.10 kg/m$^2$ per g (P = 0.08) and 0.18 ± 0.08 kg/m$^2$ per serving (P = 0.01) among the non-T carriers.

## Discussion

In 4 large prospective cohorts of the US and Chinese populations, we found reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS* rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.

Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight and obesity risk generated conflicting results [3 4]. In addition, several randomized controlled trials (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss [38-40], but the benefit was not evident in other trials [41-43]. The results from the current study lent support to our

hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.

We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle, the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit, who had high fish/n-3 PUFAs intakes [8]. The high frequency of T allele in Inuit reflects genetic adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that the signature allele (T) was related differently with weight changes (decrease or increase), depending on the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body weight when they are exposed to diet low in fish and n-3 PUFAs.

We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction patterns in relation to long-term changes in BMI; and the interactions also remained significant when supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is robust for fatty acids from various sources.

The mechanisms underlying the observed gene-diet interactions remain unclear, however, such interactions are biologically plausible. It has long been known that the *FADS* genes such as *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic

acid (n-6) and α-linolenic acid (n-3) [44]. The selected allele of *FADS* rs174570 is significantly associated with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway [44]. In addition, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function [45]. In addition, storage of energy and body fat is very important for the Arctic population, who are regularly exposed to the extreme low temperature and fishes rich in n-3 PUFAs [11 12]. Under natural selection, these people are genetically prone to high fish intake to keep body fat [9 10]. Therefore, it's not surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying selective *FADS* signature [46 47]. Our findings support the view that extra n-3 PUFAs may not do much benefit at all for Europeans with selective *FADS* signature[8 13].

**Strengths**

Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians with European ancestry and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings. Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results. Although we prospectively analyzed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change from the baseline, which by default builds in the starting point (i.e. the cross sectional association).

**Limitations**

However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable; however, the food frequency questionnaires and adiposity measures data have been well validated [27 30 32-34]. Second, confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control

studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses. Finally, the participants included in our study were middle aged and older adults of Caucasians with European ancestry in the US and Chinese in Singapore, and it is unknown whether our findings could be generalized to other demographic or ethnic groups.

**Conclusions**

In summary, our data provides reproducible evidence from 4 multiethnic cohorts that high long chain n-3 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings emphasize the importance of considering precision nutritional interventions on prevention and treatment of obesity.

**Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH provided statistical expertise. TW, YH, DS, LH, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM, HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

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**Data sharing**

No additional data available.

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**Table 1 Baseline characteristics of all participants in the NHS, HPFS, WHI, and SCHS cohorts.**

| | NHS[1] | HPFS | WHI | SCHS |
|---|---|---|---|---|
| | n=11,323 | n=6,833 | n=6,254 | n=5,264 |
| Age (year) | 57 ± 9 | 57 ± 11 | 68 ± 5 | 56 ± 7 |
| Female (%) | 100 | 0 | 100 | 58.7 |
| Body weight (kg) | 70.1 ± 14.9 | 82.8 ± 12.5 | 73.7 ± 15.0 | 60.3 ± 9.8 |
| Body mass index (kg/m$^2$) | 26.2 ± 5.1 | 25.9 ± 3.3 | 28.3 ± 5.5 | 23.4 ± 3.3 |
| Alcohol consumption (g/day) | 5.14 ± 9.23 | 10.97 ± 15.05 | 6.00 ± 11.96 | 1.97 ± 8.02 |
| Physical activity (MET-h/week) | 19.3 ± 22.1 | 36.9 ± 39.5 | 11.6 ± 13.1 | 0.5 ± 1.0[2] |
| Television watching (h/week) | 17.5 ± 14.8 | 10.5 ± 8.2 | / | 2.2 ± 0.8 |
| Current smokers (n, (%)) | 1557(13.8) | 493(7.3) | 407(15.0) | 1364(20.0) |
| Total energy intake (kcal/day) | 1766 ± 502 | 1949 ± 578 | 1602 ± 654 | 1606 ± 573 |
| Alternative health eating index score | 53.4 ± 10.8 | 53.8 ± 11.4 | 53.5 ± 10.6 | 55.8 ± 8.2 |
| Sugar sweetened beverage intake (servings/day) | 0.13 ± 0.39 | 0.23 ± 0.48 | 0.39 ± 0.82 | 0.69 ± 2.40[3] |
| Total fried food (servings/day) | 0.12 ± 0.20 | 0.22 ± 0.28 | / | / |
| Fish intake (servings/day) | 0.31 ± 0.29 | 0.33 ± 0.30 | 0.23 ± 0.20 | 0.16 ± 0.07 |
| Food-sourced EPA (g/day) | 0.08 ± 0.14 | 0.12 ± 0.20 | 0.04 ± 0.04 | / |
| Food-sourced DHA (g/day) | 0.17 ± 0.14 | 0.22 ± 0.19 | 0.07 ± 0.07 | / |
| Food-sourced EPA+DHA (g/day) | 0.23 ± 0.19 | 0.31 ± 0.25 | 0.11 ± 0.10 | 0.33 ± 0.20 |
| Total EPA+DHA (g/day) | 0.26 ± 0.27 | 0.35 ± 0.37 | 0.38 ± 0.48 | / |

[1]Plus-minus values are means ± SD. [2]Hours per week of moderate activity in the SCHS. [3]Glasses per week of soda intake in the SCHS.

EPA: 20:5n-3; DHA: 22:6n-3; MET denotes metabolic equivalents. Total EPA+DHA includes food-sourced and supplemental EPA+DHA.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively. Television watching assessed in 1992 for NHS and in 1990 for HPFS.

**Table 2 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| | *FADS* genotypes | Three categories of long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| **Total fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | | |
| NHS | Non-T carriers | 0.82±0.06 | 0.98±0.04 | 1.15±0.13 | 0.006 | 0.03 |
| | T carriers | 0.73±0.11 | 0.95±0.08 | 1.55±0.25 | 0.0007 | |
| HPFS | Non-T carriers | 0.43±0.05 | 0.52±0.04 | 0.59±0.12 | 0.73 | 0.03 |
| | T carriers | 0.21±0.11 | 0.52±0.07 | 0.79±0.22 | 0.02 | |
| WHI | Non-T carriers | 0.11±0.08 | 0.28±0.06 | 0.28±0.34 | 0.04 | 0.09 |
| | T carriers | 0.02±0.15 | 0.29±0.11 | 0.94±0.67 | 0.01 | |
| SCHS | Non-T carriers | -3.08±0.19 | -3.00±0.17 | -3.35±0.18 | 0.32 | 0.01 |
| | T carriers | -3.61±0.17 | -3.10±0.15 | -3.25±0.17 | 0.13 | |
| Pooled[1] | Non-T carriers | 0.50±0.03 | 0.67±0.03 | 0.81±0.08 | 0.01 | 0.0007 |
| | T carriers | 0.38±0.07 | 0.63±0.05 | 1.11±0.16 | $2\times10^{-4}$ | |
| **Food-sourced EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |
| NHS | Non-T carriers | 0.79±0.06 | 0.92±0.05 | 1.11±0.06 | 0.01 | 0.005 |
| | T carriers | 0.71±0.10 | 0.84±0.11 | 1.19±0.11 | 0.0001 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.53±0.05 | 0.49±0.05 | 0.79 | 0.02 |
| | T carriers | 0.23±0.11 | 0.48±0.10 | 0.58±0.09 | 0.02 | |
| WHI | Non-T carriers | 0.02±0.08 | 0.21±0.08 | 0.41±0.08 | 0.06 | 0.04 |
| | T carriers | -0.03±0.15 | 0.29±0.14 | 0.35±0.15 | 0.004 | |
| SCHS | Non-T carriers | -3.32±0.17 | -3.15±0.18 | -2.99±0.17 | 0.16 | 0.035 |
| | T carriers | -3.55±0.16 | -3.34±0.16 | -3.05±0.16 | 0.02 | |
| Pooled[1] | Non-T carriers | 0.49±0.03 | 0.64±0.03 | 0.68±0.03 | 0.01 | 0.0003 |
| | T carriers | 0.39±0.07 | 0.57±0.06 | 0.74±0.06 | $1.5\times10^{-6}$ | |
| **Total EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| NHS | Non-T carriers | 0.79±0.06 | 0.94±0.05 | 1.08±0.06 | 0.8 | 0.01 |
| | T carriers | 0.72±0.11 | 0.87±0.10 | 1.16±0.11 | 0.02 | |
| HPFS | Non-T carriers | 0.47±0.05 | 0.53±0.05 | 0.49±0.05 | 0.88 | 0.13 |
| | T carriers | 0.23±0.10 | 0.50±0.09 | 0.57±0.10 | 0.16 | |
| WHI | Non-T carriers | 0.39±0.10 | 0.04±0.08 | 0.23±0.10 | 0.42 | 0.27 |
| | T carriers | 0.04±0.18 | 0.28±0.15 | 0.28±0.16 | 0.84 | |
| Pooled[1] | Non-T carriers | 0.57±0.04 | 0.62±0.03 | 0.67±0.04 | 0.65 | 0.005 |
| | T carriers | 0.39±0.07 | 0.60±0.06 | 0.74±0.07 | 0.01 | |

Data are means ± SE for long term changes in BMI.

Total EPA+DHA include food-sourced and supplemental EPA+DHA.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure Legends**

**Figure 1 Genetic variant of *FADS* rs174570, long chain n-3 PUFAs and fish intakes and long-term BMI changes**

Effect size (ES) (95% CI) values are β coefficients for interaction between the *FADS* variant rs174570 (additive model) and diets from results of the NHS, HPFS, WHI, and SCHS cohorts.

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the *FADS* variant-diets interaction by including a multiplicative interaction term in the models after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure 2 Genetic association of *FADS* variant rs174570 with long-term BMI change according to long chain n-3 PUFAs and fish intakes**

Pooled-EUR: data from NHS, HPFS, and WHI were pooled.

Pooled Multiethnic: data from NHS, HPFS, WHI and SCHS were pooled.

Data are β coefficients ± SE.

Numbers of participants across three categories (≤1/wk/1~6/wk/≥1/d) of fish intake in the NHS, HPFS, WHI, and SCHS are 1618/8465/1239, 977/5108/748, 894/4675/684, and 752/3935/576, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

The general linear model was used to test the genetic association of the *FADS* variant (additive model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

**Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The general linear model was used to test the associations of long chain n-3 PUFAs intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

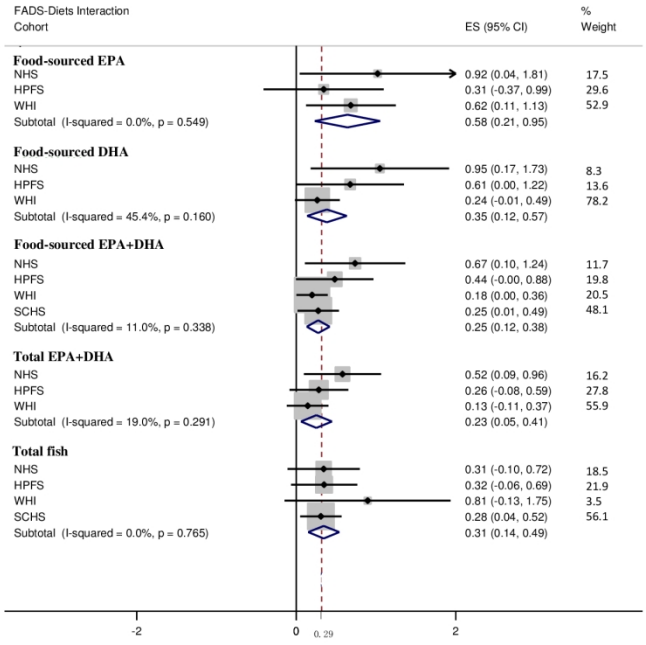
**Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The general linear model was used to test the associations of and fish intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

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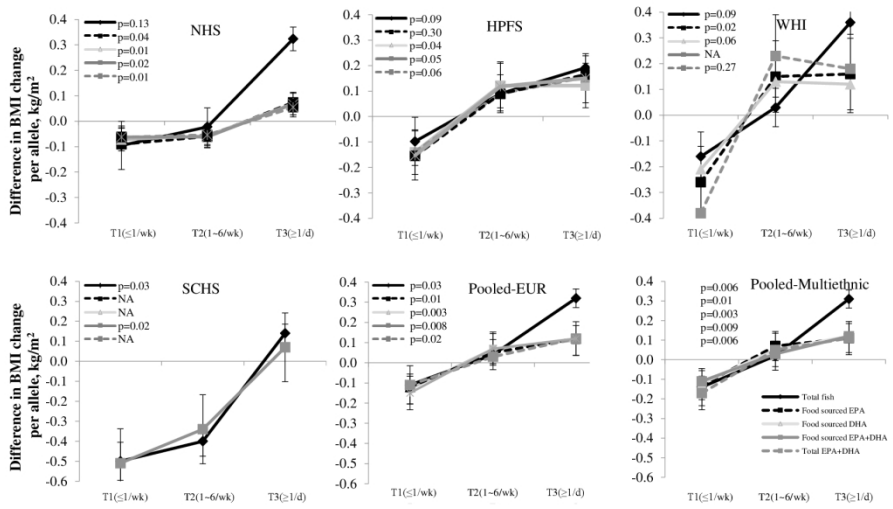


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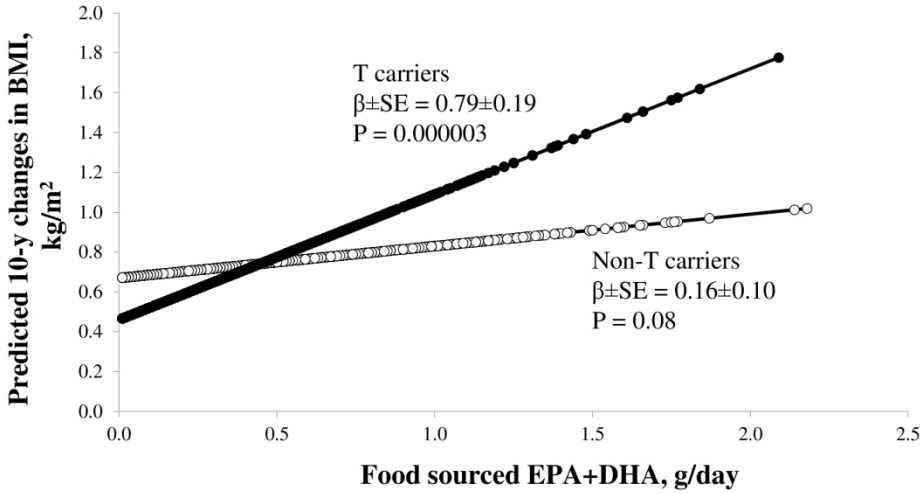


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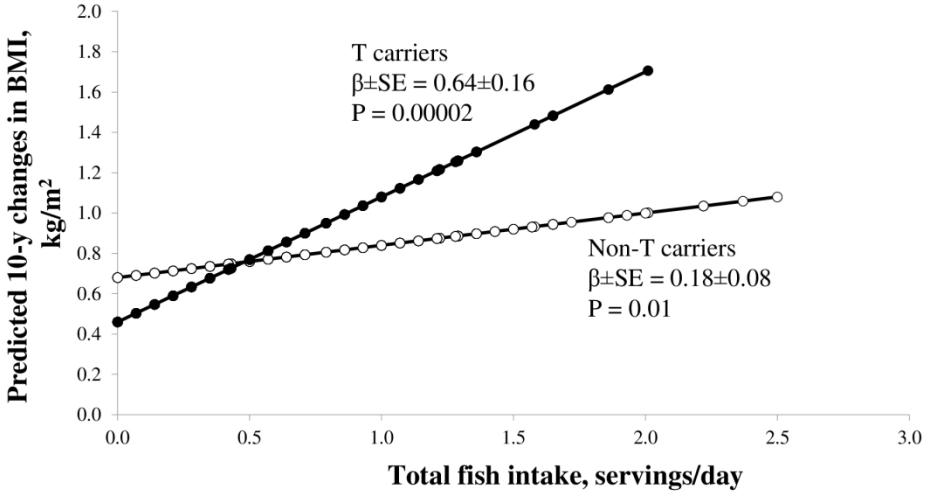


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**Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit**

| Position[1] | Reference SNP identification number | Alleles[2] | DAF | | | | | | | PBS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CEU | CHB | GI | NHS | HPFS | WHI | SCHS | |
| chr11:61627960 | rs74771917 | C/T | 0.025 | 0.16 | 0.98 | / | / | / | / | 2.67 |
| chr11:61631510 | rs3168072 | A/T | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61632310 | rs12577276 | A/G | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61641717 | rs7115739 | G/T | 0.017 | 0.22 | 0.98 | 0.004 | 0.004 | / | / | 2.54 |
| chr11:61624414 | rs174602 | C/T | 0.80 | 0.73 | 0.01 | 0.82 | 0.81 | / | / | 2.11 |
| chr11:61597212 | rs174570 | C/T | 0.16 | 0.34 | 0.99 | 0.15 | 0.15 | 0.14 | 0.35 | 2.06 |

[1]Positions refer to human genome assembly hg19.

[2]Alleles are coded as ancestral/derived states.

PBS, the population branch statistic; DAF, derived allele frequency; CEU, European ancestry; CHB, an Chinese; GI, Greenlandic Inuit

DAFs for each population (CEU, CHB, and GI) and PBS values are reported, along with the genomic position for each SNP.

**Supplemental table 2 Main effect of the *FADS* variants on adiposity in the four cohorts**

| Outcomes (kg/m$^2$) | *FADS* SNPs | NHS | | HPFS | | WHI | | SCHS | | Pooled | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P |
| Baseline BMI | rs174570 | 0.03 ± 0.10 | 0.733 | -0.05 ± 0.09 | 0.538 | -0.06±0.17 | 0.72 | 0.24±0.08 | 0.002 | 0.08±0.05 | 0.06 |
| Baseline BMI | rs174602 | 0.08 ± 0.10 | 0.418 | -0.05 ± 0.08 | 0.536 | / | | / | | 0.00 ± 0.03 | 0.559 |
| Baseline BMI | rs7115739 | 0.25 ± 0.52 | 0.634 | -0.77 ± 0.43 | 0.077 | / | | / | | -0.35 ± 0.14 | 0.196 |
| Long-term BMI change | rs174570 | -0.05 ± 0.06 | 0.401 | 0.01 ± 0.05 | 0.917 | -0.02±0.09 | 0.77 | -0.02±0.08 | 0.85 | -0.02±0.03 | 0.94 |
| Long-term BMI change | rs174602 | -0.14 ± 0.06 | 0.009 | 0.04 ± 0.05 | 0.413 | / | | / | | -0.04 ± 0.01 | 0.025 |
| Long-term BMI change | rs7115739 | 0.45 ± 0.29 | 0.124 | -0.23 ± 0.26 | 0.359 | / | | / | | 0.06 ± 0.08 | 0.183 |

Long-term BMI change: BMI change from 1990 to 2000.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Effect size (ES) values are β coefficients for relationship between the *FADS* variant rs174570 (additive model) and adiposity.

The general linear model was used to test the genetic association of *FADS* variants with long-term changes in BMI after adjustment for age, source of genotyping data.

**Supplemental table 3 Genetic association of *FADS* variant with long-term changes in body weight according to long chain n-3 PUFAs and fish intakes**

| | Difference in long-term changes in weight, kg | | | P for interaction |
|---|---|---|---|---|
| **Total Fish, serving/day** | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | -0.69±0.64 | -0.13±0.49 | 1.78±1.64 | 0.05 |
| HPFS | -0.99±0.85 | 0.54±0.53 | 1.52±1.69 | 0.12 |
| WHI | -0.22±0.42 | 0.16±0.34 | 1.26±1.57 | 0.13 |
| SCHS | -0.42±0.29 | -0.44±0.28 | 0.20±0.29 | 0.08 |
| Pooled | -0.44±0.22 | -0.10±0.18 | 0.31±0.28 | 0.01 |
| **Food-sourced EPA, g/day** | T1 | T2 | T3 | |
| NHS | -0.77±0.62 | -0.25±0.72 | 0.53±0.64 | 0.06 |
| HPFS | -1.19±0.82 | 0.75±0.73 | 0.72±0.74 | 0.41 |
| WHI | -0.19±0.42 | 0.24±0.47 | 0.14±0.48 | 0.20 |
| Pooled | -0.50±0.32 | 0.24±0.34 | 0.37±0.34 | 0.10 |
| **Food-sourced DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.53±0.62 | -0.39±0.70 | 0.53±0.65 | 0.01 |
| HPFS | -1.06±0.82 | 0.49±0.71 | 0.89±0.76 | 0.09 |
| WHI | -0.20±0.43 | 0.22±0.42 | 0.30±0.50 | 0.26 |
| Pooled | -0.43±0.32 | 0.15±0.32 | 0.49±0.35 | 0.01 |
| **Food-sourced EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.56±0.63 | -0.32±0.68 | 0.49±0.66 | 0.01 |
| HPFS | -1.25±0.83 | 0.68±0.73 | 0.84±0.74 | 0.09 |
| WHI | -0.02±0.43 | 0.16±0.44 | 0.14±0.49 | 0.23 |
| SCHS | -0.47±0.29 | -0.16±0.28 | -0.03±0.29 | 0.10 |

| | | | | |
|---|---|---|---|---|
| Pooled | -0.56±0.25 | -0.09±0.24 | 0.14±0.25 | 0.005 |
| **Total EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.58±0.63 | -0.30±0.68 | 0.46±0.66 | 0.02 |
| HPFS | -1.20±0.82 | 0.75±0.70 | 0.86±0.77 | 0.18 |
| WHI | -0.48±0.47 | 0.64±0.43 | 0.04±0.47 | 0.15 |
| Pooled | -0.64±0.34 | 0.45±0.32 | 0.32±0.34 | 0.02 |

Data are β coefficients ± SE.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the genetic association with long-term changes in body weight according to baseline long chain n-3 PUFAs and fish intakes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from three or four cohorts pooled by means of fixed effects meta-analyses (if P ≥ 0.05 for heterogeneity between studies).

**Supplemental table 4 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight according to *FADS* genotypes**

| Cohorts | | Long-term changes in weight, kg | | | P for trend |
|---|---|---|---|---|---|
| **Total Fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | Non T carriers | 4.91±0.34 | 5.78±0.24 | 7.00±0.79 | 0.008 |
| | T carriers | 4.45±0.61 | 5.64±0.46 | 9.26±1.44 | 0.001 |
| HPFS | Non T carriers | 0.44±0.06 | 0.52±0.04 | 0.56±0.12 | 0.99 |
| | T carriers | 0.25±0.10 | 0.53±0.07 | 0.76±0.21 | 0.08 |
| WHI | Non T carriers | -0.25±0.23 | -0.43±0.18 | -0.91±0.93 | 0.50 |
| | T carriers | -0.56±0.37 | -0.25±0.28 | 1.30±1.71 | 0.13 |
| SCHS | Non T carriers | -3.15±0.23 | -3.50±0.21 | -3.38±0.21 | 0.48 |
| | T carriers | -3.68±0.20 | -3.41±0.19 | -3.34±0.20 | 0.16 |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.89±0.33 | 5.89±0.33 | 5.95±0.33 | 0.24 |
| | T carriers | 4.45±0.59 | 5.52±0.63 | 6.46±0.61 | 0.34 |
| HPFS | Non T carriers | 0.50±0.05 | 0.54±0.05 | 0.45±0.05 | 0.15 |
| | T carriers | 0.29±0.10 | 0.54±0.09 | 0.52±0.09 | 0.66 |
| WHI | Non T carriers | -0.30±0.25 | -0.54±0.24 | -0.29±0.24 | 0.42 |
| | T carriers | -0.51±0.39 | -0.36±0.37 | -0.15±0.38 | 0.14 |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.78±0.33 | 5.56±0.34 | 6.32±0.33 | 0.14 |
| | T carriers | 4.50±0.60 | 5.07±0.63 | 6.77±0.61 | 0.004 |
| HPFS | Non T carriers | 0.48±0.05 | 0.54±0.05 | 0.46±0.06 | 0.40 |
| | T carriers | 0.27±0.10 | 0.50±0.09 | 0.59±0.09 | 0.15 |
| WHI | Non T carriers | -0.41±0.25 | -0.25±0.24 | -0.45±0.25 | 0.51 |

| | | | | | |
|---|---|---|---|---|---|
| | T carriers | -0.71±0.39 | -0.15±0.37 | -0.16±0.39 | 0.18 |
| **Food-sourced EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.69±0.34 | 5.45±0.33 | 6.51±0.33 | 0.02 |
| | T carriers | 4.44±0.61 | 5.00±0.61 | 6.92±0.61 | 0.0003 |
| HPFS | Non T carriers | 0.48±0.05 | 0.53±0.05 | 0.47±0.05 | 0.93 |
| | T carriers | 0.26±0.10 | 0.49±0.09 | 0.59±0.09 | 0.08 |
| WHI | Non T carriers | -0.44±0.24 | -0.23±0.23 | -0.43±0.24 | 0.47 |
| | T carriers | -0.52±0.38 | -0.15±0.37 | -0.33±0.38 | 0.15 |
| SCHS | Non T carriers | -3.44±0.21 | -3.58±0.22 | -3.05±0.21 | 0.89 |
| | T carriers | -3.73±0.19 | -3.57±0.19 | -3.12±0.19 | 0.12 |
| **Total EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.74±0.34 | 5.55±0.32 | 6.36±0.34 | 0.81 |
| | T carriers | 4.49±0.61 | 5.16±0.60 | 6.70±0.61 | 0.03 |
| HPFS | Non T carriers | 0.49±0.05 | 0.53±0.05 | 0.47±0.06 | 0.24 |
| | T carriers | 0.26±0.10 | 0.51±0.09 | 0.58±0.09 | 0.33 |
| WHI | Non T carriers | 0.32±0.27 | -0.84±0.23 | -0.60±0.28 | 0.19 |
| | T carriers | -0.26±0.45 | -0.21±0.37 | -0.02±0.11 | 0.08 |

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight by *FADS* genotypes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from two cohorts pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies) or random effects meta-analyses (if $P < 0.05$ for heterogeneity between studies).

**Supplemental Table 5 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| Diets | *FADS* genotypes | Long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| | | Categories of diets | | | | |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.82±0.06 | 1.00±0.06 | 1.01±0.06 | 0.24 | 0.05 |
| | T carriers | 0.72±0.10 | 0.94±0.11 | 1.10±0.11 | 0.29 | |
| HPFS | Non-T carriers | 0.48±0.05 | 0.54±0.05 | 0.47±0.05 | 0.72 | 0.37 |
| | T carriers | 0.23±0.11 | 0.54±0.10 | 0.52±0.09 | 0.45 | |
| WHI | Non-T carriers | 0.10±0.09 | 0.09±0.09 | 0.45±0.09 | 0.21 | 0.02 |
| | T carriers | -0.08±0.15 | 0.23±0.15 | 0.46±0.15 | 0.003 | |
| Pooled | Non-T carriers | 0.54±0.04 | 0.63±0.04 | 0.65±0.04 | 0.35 | 0.01 |
| | T carriers | 0.39±0.07 | 0.63±0.07 | 0.70±0.06 | 0.01 | |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.80±0.06 | 0.94±0.06 | 1.08±0.06 | 0.14 | 0.009 |
| | T carriers | 0.74±0.10 | 0.83±0.11 | 1.17±0.10 | 0.002 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.54±0.05 | 0.49±0.05 | 0.99 | 0.05 |
| | T carriers | 0.24±0.10 | 0.49±0.10 | 0.58±0.09 | 0.05 | |
| WHI | Non-T carriers | 0.03±0.09 | 0.20±0.09 | 0.42±0.09 | 0.03 | 0.06 |
| | T carriers | -0.10±0.15 | 0.33±0.15 | 0.39±0.15 | 0.006 | |
| Pooled | Non-T carriers | 0.51±0.04 | 0.63±0.04 | 0.68±0.04 | 0.1 | 0.002 |
| | T carriers | 0.38±0.06 | 0.58±0.07 | 0.77±0.06 | $7\times10^{-4}$ | |

Data are means ± SE.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the

WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Registration: www. clinicaltrials.gov. Registration ID: NCT03348566

**TABLE 1. STREGA reporting recommendations, extended from STROBE Statement**

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Title and Abstract** | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract. **(p. 3)** | |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found. **(p. 3)** | |
| **Introduction** | | | |
| *Background rationale* | 2 | Explain the scientific background and rationale for the investigation being reported. **(p. 4)** | |
| *Objectives* | 3 | State specific objectives, including any pre-specified hypotheses. | ***State if the study is the first report of a genetic association, a replication effort, or both.*** **(p. 3)** |
| **Methods** | | | |
| *Study design* | 4 | Present key elements of study design early in the paper. **(p. 5)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Setting* | 5 | Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. **(p. 3)** | |
| *Participants* | 6 | (a) **Cohort study –** Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **(p. 5)**<br><br>**Case-control study –** Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls.<br><br>**Cross-sectional study –** Give the eligibility criteria, and the sources and methods of selection of participants. | ***Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant***. **(p. 5)** |
| | | (b) **Cohort study –** For matched studies, give matching criteria and number of exposed and unexposed.<br><br>**Case-control study –** For matched studies, give matching criteria and the number of controls per case. | |
| *Variables* | 7 | *(a)* Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. **(p. 5)** | ***(b) Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).*** **(p. 5)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Data sources measurement* | 8* | *(a)* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. **(p. 5)** | ***(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory/centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.*** **(p. 5)** |
| *Bias* | 9 | *(a)* Describe any efforts to address potential sources of bias. **(p. 5 &6)** | ***(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***with this.*** **(p. 5 &6)** |
| *Study size* | 10 | Explain how the study size was arrived at. **(p. 5 &6)** | |
| *Quantitative variables* | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. **(p. 7)** | ***If applicable, describe how effects of treatment were dealt with.*** **(p. 7)** |
| *Statistical methods* | 12 | (a) Describe all statistical methods, including those used to control for confounding. **(p. 7-9)** | ***State software version used and options (or settings) chosen.*** **(p. 9)** |
| | | (b) Describe any methods used to examine subgroups and interactions. **(p. 9)** | |
| | | (c) Explain how missing data were addressed. **(p. 9)** | |
| | | (d) **Cohort study –** If applicable, explain how loss to follow-up was addressed. **(p. 9)**<br><br>**Case-control study –** If applicable, explain how matching of cases and controls was addressed.<br><br>**Cross-sectional study –** If applicable, describe analytical methods taking account of sampling strategy. | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | (e) Describe any sensitivity analyses. **(p. 9)** | |
| | | Hardy-Weinberg equilibrium was tested using Chi-square test. **(p. 9)** | ***(f) State whether Hardy-Weinberg equilibrium was considered and, if so, how.*** |
| | | We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). **(p. 8&9)** | ***(g) Describe any methods used for inferring genotypes or haplotypes.*** |
| | | | ***(h) Describe any methods used to assess or address population stratification.*** **(p. 9)** |
| | | | ***(i) Describe any methods used to address multiple comparisons or to control risk of false positive findings.*** **(p. 9)** |
| | | | ***(j) Describe any methods used to address and correct for relatedness among subjects*****(p. 9)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Results** | | | |
| *Participants* | 13* | (a) Report the numbers of individuals at each stage of the study – e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. **(p. 10)** | ***Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.*** **(p. 10)** |
| | | (b) Give reasons for non-participation at each stage. **(p. 10)** | |
| | | (c) Consider use of a flow diagram. **(p. 10)** | |
| *Descriptive data* | 14* | (a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. **(p. 10)** | ***Consider giving information by genotype.*** **(p. 10)** |
| | | (b) Indicate the number of participants with missing data for each variable of interest. **(p. 10)** | |
| | | (c) **Cohort study –** Summarize follow-up time, e.g. average and total amount. **(p. 10)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Outcome data* | 15 * | **Cohort study-**Report numbers of outcome events or summary measures over time. | ***Report outcomes (phenotypes) for each genotype category over time*** |
| | | **Case-control study –** Report numbers in each exposure category, or summary measures of exposure. | ***Report numbers in each genotype category*** |
| | | **Cross-sectional study –** Report numbers of outcome events or summary measures. | ***Report outcomes (phenotypes) for each genotype category*** |
| *Main results* | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence intervals). Make clear which confounders were adjusted for and why they were included. **(p. 10)** | |
| | | (b) Report category boundaries when continuous variables were categorized. **(p. 10)** | |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. **(p. 10)** | |
| | | | ***(d) Report results of any adjustments for multiple*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***comparisons.* (p. 10)** |
| *Other analyses* | 17 | (a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. **(p. 10)** | |
| | | | ***(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken.* (p. 10)** |
| | | | ***(c) If detailed results are available elsewhere, state how they can be accessed.* (p. 10)** |
| **Discussion** | | | |
| *Key results* | 18 | Summarize key results with reference to study objectives. **(p. 11)** | |
| *Limitations* | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. **(p. 11)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Interpretation* | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. **(p. 11)** | |
| *Generalizability* | 21 | Discuss the generalizability (external validity) of the study results. **(p. 11)** | |
| **Other Information** | | | |
| *Funding* | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. **(p. 14)** | |

STREGA = STrengthening the REporting of Genetic Association studies; STROBE = STtrengthening the Reporting of Observational Studies in Epidemiology.

* Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

BMJ Open

# Fish and marine fatty acids intakes modified the genetic effects of the FADS gene on long-term weight gain: a gene-diet interaction analysis



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| Complete List of Authors: | Huang, Tao; Peking University, Department of Epidemiology and Biostatistics<br>Wang, Tiange ; Shanghai Jiao Tong University, Shanghai Institute of Endocrine and Metabolic Diseases<br>Heianza, Yoriko ; Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University<br>Wiggs, Janey ; Harvard Medical School, Department of Ophthalmology<br>Sun, Dianjianyi ; School of Public Health and Tropical Medicine, Tulane University<br>Choi, Hyon-Kyoo ; Massachusetts General Hospital - Harvard Medical School Center for Nervous System Repair<br>Chai, Jin Fang ; National University Singapore Yong Loo Lin School of Medicine<br>Sim, Xueling ; National University of Singapore, Epidemiology Domain, Saw Swee Hock School of Public Health<br>Khor, Chiea Chuen ; National University of Singapore, Department of Biochemistry, Yong Loo Lin School of Medicine<br>Friedlander, Yechiel ; Hebrew University, Unit of Epidemiology<br>Chan, Andrew T. ; Massachusetts General Hospital, Division of Gastroenterology<br>Curhan, Gary ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>Vivo, Immaculata De ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>van Dam, Rob Martinu. ; National University of Singapore, Department of Epidemiology<br>Heng, Chew Kiat ; National University of Singapore, Department of Paediatrics<br>Fuchs, Charles ; Harvard Medical School, Department of Medicine, Brigham and Women's Hospital<br>Pasquale, Louis R. ; Harvard Medical School, Channing Division of Network Medicine, Department of Medicine<br>Yuan, Jian-min ; University of Pittsburgh, Division of Cancer Control and Population Sciences<br>Hu, Frank B. ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>Koh, Woon Puay ; National University of Singapore, Department of Epidemiology<br>Qi, Lu; Tulane University, Department of Epidemiology, School of Public |

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**Fish and marine fatty acids intakes modified the genetic effects of the *FADS* gene on long-term weight gain: a gene-diet interaction analysis**

Tao Huang[1,2,3*], Tiange Wang[4], Yoriko Heianza[5], Janey Wiggs[6], Dianjianyi Sun[5], Hyon-Kyoo Choi[7], Jin Fang Chai[8], Xueling Sim[8], Chiea Chuen Khor[9,10], Yechiel Friedlander[11], Andrew T. Chan[12], Gary Curhan[13], Immaculata De Vivo[13], Rob Martinu. van Dam[8,14], Chew Kiat Heng[15], Charles S. Fuchs[16,17], Louis R. Pasquale[18], Jian-min Yuan[19,20], Frank B. Hu[14, 16], Woon Puay Koh[8, 21], Lu Qi[5, 14, 16*]

[1.] *Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, China.*

[2.] *Department of Global Health, School of Public Health, Peking University, China.*

[3.] *Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, China.*

[4.] *Shanghai Institute of Endocrine and Metabolic Diseases, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

[5.] *Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1724 New Orleans, LA 70112.*

[6.] *Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02115, USA.*

[7.] *Department of Rheumatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[8.] *Epidemiology Domain, Saw Swee Hock School of Public Health, National University of Singapore, Singapore, 117549.*

[9.] *Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.*

[10.] *Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

[11.] *Unit of Epidemiology, Hebrew University-Hadassah Braun School of Public Health, POB 12272, Jerusalem 91120, Israel.*

[12.] *Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114, USA.*

[13.] *Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA.*

[14.] *Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA.*

[15.] *Department of Paediatrics, National University of Singapore NUHS Tower Block, Level 12, 1E Kent Ridge Road Singapore 119228.*

[16.]*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School Boston, MA 02115, USA.*

[17.]*The Center for Gastrointestinal Cancer, Dana-Farber Cancer Institute, Boston, USA.*

[18.]*Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[19.]*Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.*

[20.]*Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

[21.]*Duke-NUS Medical School, Singapore, Singapore.*

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***Correspondence and requests for reprint:**

Dr. Tao Huang. Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China. Email: huangtao@bjmu.edu.cn

Dr. Lu Qi. Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University. 1440 Canal Street, Suite 1724, New Orleans, LA 70112

Telephone: 504-988-3549;

Email: lqi1@tulane.edu; luqi@hsph.harvard.edu

**Abstract**

**Objective:** We tested whether genetic variants near fatty acid desaturases gene (*FADS*) cluster, which were recently identified to be signatures of adaptation to fish- and n-3 PUFAs-rich diet, interacted with these dietary factors on change in body mass index (BMI).

**Design:** Three *FADS* variants were examined for gene-diet interactions on long-term (~10 years) changes in BMI and body weight in four prospective cohort studies.

**Setting:** Population based study

**Participants:** 11,323 women from the Nurses' Health Study (NHS), 6,833 men from the Health Professionals Follow-up Study (HPFS), and replicated in 6,254 women from the Women's Health Initiative (WHI), and 5,264 Chinese from the Singapore Chinese Health Study (SCHS).

**Main outcomes:** Long-term (~10 years) changes in BMI and body weight

**Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS* rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 $kg/m^2$ per g, P = $3\times10^{-5}$) than the rs174570 non-T carriers (beta=0.16 $kg/m^2$ per g, P = 0.08). Similar results were observed for fish intake.

**Conclusions:** Our hypothesis-driven analyses provide replicable evidence that long chain n-3 PUFAs and fish intakes may interact with the *FADS* variant on long-term weight gain. Further investigation is needed to confirm our findings in other cohorts.

## Article summary

### Strengths and limitations of this study

- This is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings.
- Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results.
- Dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable.
- Confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors.
- We acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses.

## Introduction

Diets rich in fish and marine fatty acids, especially long chain n-3 polyunsaturated fatty acids (PUFAs) has shown beneficial effects on cardiometabolic health [1 2]. However, data from population studies on the associations between such diet and body weight are inconsistent [3 4]. Emerging evidence suggests genetic variations may play a role in modifying the relation between dietary factors and body weight [5-7].

A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs [8]. The strong signals locate in a cluster of fatty acid desaturases genes (*FADS*) that determine PUFAs levels [8]. People living in the Arctic region have been found to be genetically prone to develop obesity [9 10] as survival strength for energy storage[11 12]. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in the Inuit population [8]. Of note, due to long-standing selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various populations such as Europeans and Asians [13], in coincidence with varying levels of fish/marine fatty acids consumption, and adiposity patterns in these populations [14]. We therefore hypothesized that the genetic signatures might interact with fish and marine PUFAs intakes on body weight [13].

The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts the Women's Health Initiative (WHI, US) and the Singapore Chinese Health Study (SCHS).

## Methods

### Discovery cohorts

#### *The Nurses' Health Study*

The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were recruited to complete a baseline questionnaire about their lifestyle and medical history [15]. The current analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [16-22]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [16-21].. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

***The Health Professionals Follow-up Study***

The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians, optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet [23]. In the current analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [16-22]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [16-21]. The study protocol was also approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

**Replication cohorts**

***The Women's Health Initiative (WHI)***

The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI study is presented elsewhere [24 25]. For the analyses, all participants with baseline long chain n-3 PUFAs and fish consumption and covariate data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included. Finally, we included 6,254 Caucasians women who participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each participating institution reviewed and approved the study, and all women gave written informed consent.

***The Singapore Chinese Health Study (SCHS) cohort***

The design of Singapore Chinese Health Study (SCHS) has been previously described in detail [26]. Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study [27]. Two follow-up interviews were conducted via telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update information on body weight, selected lifestyle factors and medical history. All participants have given informed consent. The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data available were included. Among these participants, genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome Institute of the Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation [28].

Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip [29].

Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.

**Assessment of measures of body mass index**

Height and body weight were assessed by questionnaire at baseline, and weight information was requested on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly measured values (r=0.97 in HPFS and NHS) in a validation study [30]. BMI was calculated as body weight (kg)/height ($m^2$). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS and HPFS cohorts [31], and from baseline (1993) to sixth year follow-up in the WHI [24 25], and from baseline (1998) to second follow-up (2004) in the SCHS.

**Assessment of diets and other covariates**

Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires (FFQ) in the NHS and HPFS [32 33]. A 165-item validated semi-quantitative FFQ was used to collect dietary data and supplemental use of fish oil in the SCHS [27]. Dietary data and supplemental use of fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI [34]. Alternate health eating index was previously calculated in the NHS, HPFS [35], WHI, and SCHS. Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities, and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously in the NHS and HPFS [36]. In the WHI, an estimated metabolic equivalent (MET) level for each type of activity was assigned from a compendium of activities [37]. Physical activity

was assessed using eight continuous categories ranging from never to 31 hours or more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS [26].

**The *FADS* variants selection and genotyping**

Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes for signatures of adaptation [8] were derived from genome-wide scans available in the NHS, HPFS. We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication (**Supplemental Table 1**).

**Patient and Public Involvement**

Neither patients nor public were involved.

**Statistical analyses**

We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity measures and long-term changes in BMI using multiple linear regression model. Interactions between the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if available). We further tested the genetic associations with long-term changes in BMI according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish

intakes with long-term changes in BMI according to the *FADS* genotypes using multiple linear regression model after adjustment of potential confounders. Linear trend across categories of long chain n-3 PUFAs and fish intakes was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable. Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models ($P \geq 0.05$ for heterogeneity between studies) [38]. Hardy-Weinberg equilibrium was tested using Chi-square test. All reported P values are nominal and two sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

**Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts. The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The distribution of the *FADS* genetic variants in the 4 cohorts is shown in **Supplemental table 1**. Chi-square test showed that the *FADS* rs174570 is in Hardy-Weinberg equilibrium. We did not observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term changes in BMI in the three US cohorts ($P > 0.05$). However, we found that the *FADS* genotype was significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2)**.

### Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes

We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes of variously sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in

combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the SCHS cohort (P for interaction = 0.02).

The pooled analyses of the 3 US (Caucasian) samples or all 4 cohorts showed that high intakes of food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No significant heterogeneity in the interaction effect was observed among these cohorts. Differences in long-term changes of BMI per T allele were -0.105 (SE 0.067), 0.027 (SE 0.064), and 0.120 (SE 0.067) kg/m$^2$ across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for interaction=0.003) showed similar interaction patterns; and the interactions remained significant when supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS* genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071), 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m$^2$ across three categories (≤ 1 serving/week, 1~6 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed (**Supplemental table 3 & 4**).

**Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had significantly greater increase of BMI (mean ± SE = 0.74±0.06, kg/m$^2$) than did those who consumed the lowest (T1) (mean ± SE = 0.39±0.07, kg/m$^2$) among the T allele carriers, whereas the corresponding BMI changes were 0.68±0.03 kg/m$^2$ and 0.49±0.03 kg/m$^2$, respectively, among the non-T carriers in 4 cohorts combined (**Table 2 & Supplemental table 5** ). Similarly, we observed different associations between fish intake and BMI changes among the T allele carriers ($P = 1.5\times10^{-6}$) and non-carriers (P = 0.01) in the pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed among the cohorts.

**Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled results, the beta ± SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**) with long-term changes in BMI were 0.79 ± 0.19 kg/m$^2$ per g (P = 0.000003) and 0.64 ± 0.16 kg/m$^2$ per serving (P = 0.00002) among the T carriers, and whereas the corresponding beta ± SE were 0.16 ± 0.10 kg/m$^2$ per g (P = 0.08) and 0.18 ± 0.08 kg/m$^2$ per serving (P = 0.01) among the non-T carriers.

## Discussion

In 4 large prospective cohorts of the US and Chinese populations, our hypothesis-driven analyses showed reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS* rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.

Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight and obesity risk have generated conflicting results [3 4]. In addition, several randomized controlled trials (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss [39-41], but the benefit was not evident in other trials [42-44]. The results from the current study lend support to our hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.

We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle, the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit, who had high fish/n-3 PUFAs intakes [8]. The high frequency of the T allele in Inuit reflects genetic adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that the signature allele (T) was related differently to weight changes (decrease or increase), depending on the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body weight when they are exposed to a diet low in fish and n-3 PUFAs.

We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction patterns in relation to long-term changes in BMI; and the interactions also remained significant when supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is robust for fatty acids from various sources.

The mechanisms underlying the observed gene-diet interactions remain unclear. However, such interactions are biologically plausible. It has long been known that the *FADS* genes such as *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic acid (n-6) and α-linolenic acid (n-3) [45]. The selected allele of *FADS* rs174570 is significantly associated with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway [45]. Further, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function [46]. In addition, storage of energy and body fat is very important for the Arctic population, who are regularly exposed to extremely low temperatures and fishes rich in n-3 PUFAs [11 12]. Under natural selection, these people are genetically prone to high fish intake to keep body fat [9 10]. Therefore, it's not surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying selective *FADS* signature [47 48]. Our findings support the view that extra n-3 PUFAs may not have much benefit for Europeans with selective *FADS* signature[8 13].

**Strengths**

Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings. Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results. Although we prospectively analyzed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change from the baseline, which by default builds in the starting point (i.e. the cross sectional association).

**Limitations**

However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable. Despite this, the food frequency questionnaires and adiposity measures data have been well validated [27 30 32-34]. Second,

confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses. Finally, the participants included in our study were middle aged and older adults of Caucasians in the US and Chinese in Singapore, and it is unknown whether our findings could be generalized to other demographic or ethnic groups.

**Conclusions**

In summary, our data provide reproducible evidence from 4 multiethnic cohorts that high long chain n-3 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings emphasize the importance of considering precision nutritional interventions on prevention and treatment of obesity. We acknowledge that these results are hypothesis-generating and need to be confirmed in additional cohorts.

**Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH provided statistical expertise. TW, YH, DS, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM, HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

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**Data sharing**

Data are available in Harvard school of public health, NHS and HPFS cohorts. All data relevant to the study are included in the article or uploaded as supplementary information.

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**Table 1 Baseline characteristics of all participants in the NHS, HPFS, WHI, and SCHS cohorts.**

| | NHS[1] | HPFS | WHI | SCHS |
|---|---|---|---|---|
| | n=11,323 | n=6,833 | n=6,254 | n=5,264 |
| Age (year) | 57 ± 9 | 57 ± 11 | 68 ± 5 | 56 ± 7 |
| Female (%) | 100 | 0 | 100 | 58.7 |
| Body weight (kg) | 70.1 ± 14.9 | 82.8 ± 12.5 | 73.7 ± 15.0 | 60.3 ± 9.8 |
| Body mass index (kg/m$^2$) | 26.2 ± 5.1 | 25.9 ± 3.3 | 28.3 ± 5.5 | 23.4 ± 3.3 |
| Alcohol consumption (g/day) | 5.14 ± 9.23 | 10.97 ± 15.05 | 6.00 ± 11.96 | 1.97 ± 8.02 |
| Physical activity (MET-h/week) | 19.3 ± 22.1 | 36.9 ± 39.5 | 11.6 ± 13.1 | 0.5 ± 1.0[2] |
| Television watching (h/week) | 17.5 ± 14.8 | 10.5 ± 8.2 | / | 2.2 ± 0.8 |
| Current smokers (n, (%)) | 1557(13.8) | 493(7.3) | 407(15.0) | 1364(20.0) |
| Total energy intake (kcal/day) | 1766 ± 502 | 1949 ± 578 | 1602 ± 654 | 1606 ± 573 |
| Alternative health eating index score | 53.4 ± 10.8 | 53.8 ± 11.4 | 53.5 ± 10.6 | 55.8 ± 8.2 |
| Sugar sweetened beverage intake (servings/day) | 0.13 ± 0.39 | 0.23 ± 0.48 | 0.39 ± 0.82 | 0.69 ± 2.40[3] |
| Total fried food (servings/day) | 0.12 ± 0.20 | 0.22 ± 0.28 | / | / |
| Fish intake (servings/day) | 0.31 ± 0.29 | 0.33 ± 0.30 | 0.23 ± 0.20 | 0.16 ± 0.07 |
| Food-sourced EPA (g/day) | 0.08 ± 0.14 | 0.12 ± 0.20 | 0.04 ± 0.04 | / |
| Food-sourced DHA (g/day) | 0.17 ± 0.14 | 0.22 ± 0.19 | 0.07 ± 0.07 | / |
| Food-sourced EPA+DHA (g/day) | 0.23 ± 0.19 | 0.31 ± 0.25 | 0.11 ± 0.10 | 0.33 ± 0.20 |
| Total EPA+DHA (g/day) | 0.26 ± 0.27 | 0.35 ± 0.37 | 0.38 ± 0.48 | / |

[1]Plus-minus values are means ± SD. [2]Hours per week of moderate activity in the SCHS. [3]Glasses per week of soda intake in the SCHS.

EPA: 20:5n-3; DHA: 22:6n-3; MET denotes metabolic equivalents. Total EPA+DHA includes food-sourced and supplemental EPA+DHA.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively. Television watching assessed in 1992 for NHS and in 1990 for HPFS.

**Table 2 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| | *FADS* genotypes | Three categories of long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| **Total fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | | |
| NHS | Non-T carriers | 0.82±0.06 | 0.98±0.04 | 1.15±0.13 | 0.006 | 0.03 |
| | T carriers | 0.73±0.11 | 0.95±0.08 | 1.55±0.25 | 0.0007 | |
| HPFS | Non-T carriers | 0.43±0.05 | 0.52±0.04 | 0.59±0.12 | 0.73 | 0.03 |
| | T carriers | 0.21±0.11 | 0.52±0.07 | 0.79±0.22 | 0.02 | |
| WHI | Non-T carriers | 0.11±0.08 | 0.28±0.06 | 0.28±0.34 | 0.04 | 0.09 |
| | T carriers | 0.02±0.15 | 0.29±0.11 | 0.94±0.67 | 0.01 | |
| SCHS | Non-T carriers | -3.08±0.19 | -3.00±0.17 | -3.35±0.18 | 0.32 | 0.01 |
| | T carriers | -3.61±0.17 | -3.10±0.15 | -3.25±0.17 | 0.13 | |
| Pooled[1] | Non-T carriers | 0.50±0.03 | 0.67±0.03 | 0.81±0.08 | 0.01 | 0.0007 |
| | T carriers | 0.38±0.07 | 0.63±0.05 | 1.11±0.16 | $2\times10^{-4}$ | |
| **Food-sourced EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |
| NHS | Non-T carriers | 0.79±0.06 | 0.92±0.05 | 1.11±0.06 | 0.01 | 0.005 |
| | T carriers | 0.71±0.10 | 0.84±0.11 | 1.19±0.11 | 0.0001 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.53±0.05 | 0.49±0.05 | 0.79 | 0.02 |
| | T carriers | 0.23±0.11 | 0.48±0.10 | 0.58±0.09 | 0.02 | |
| WHI | Non-T carriers | 0.02±0.08 | 0.21±0.08 | 0.41±0.08 | 0.06 | 0.04 |
| | T carriers | -0.03±0.15 | 0.29±0.14 | 0.35±0.15 | 0.004 | |
| SCHS | Non-T carriers | -3.32±0.17 | -3.15±0.18 | -2.99±0.17 | 0.16 | 0.035 |
| | T carriers | -3.55±0.16 | -3.34±0.16 | -3.05±0.16 | 0.02 | |
| Pooled[1] | Non-T carriers | 0.49±0.03 | 0.64±0.03 | 0.68±0.03 | 0.01 | 0.0003 |
| | T carriers | 0.39±0.07 | 0.57±0.06 | 0.74±0.06 | $1.5\times10^{-6}$ | |
| **Total EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| NHS | Non-T carriers | 0.79±0.06 | 0.94±0.05 | 1.08±0.06 | 0.8 | 0.01 |
| | T carriers | 0.72±0.11 | 0.87±0.10 | 1.16±0.11 | 0.02 | |
| HPFS | Non-T carriers | 0.47±0.05 | 0.53±0.05 | 0.49±0.05 | 0.88 | 0.13 |
| | T carriers | 0.23±0.10 | 0.50±0.09 | 0.57±0.10 | 0.16 | |
| WHI | Non-T carriers | 0.39±0.10 | 0.04±0.08 | 0.23±0.10 | 0.42 | 0.27 |
| | T carriers | 0.04±0.18 | 0.28±0.15 | 0.28±0.16 | 0.84 | |
| Pooled[1] | Non-T carriers | 0.57±0.04 | 0.62±0.03 | 0.67±0.04 | 0.65 | 0.005 |
| | T carriers | 0.39±0.07 | 0.60±0.06 | 0.74±0.07 | 0.01 | |

Data are means ± SE for long term changes in BMI.

Total EPA+DHA include food-sourced and supplemental EPA+DHA.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The multiple linear regression model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure Legends**

**Figure 1 Genetic variant of *FADS* rs174570, long chain n-3 PUFAs and fish intakes and long-term BMI changes**

Effect size (ES) (95% CI) values are β coefficients for interaction between the *FADS* variant rs174570 (additive model) and diets from results of the NHS, HPFS, WHI, and SCHS cohorts.

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The multiple linear regression model was used to test the *FADS* variant-diets interaction by including a multiplicative interaction term in the models after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure 2 Genetic association of *FADS* variant rs174570 with long-term BMI change according to long chain n-3 PUFAs and fish intakes**

Pooled-EUR: data from NHS, HPFS, and WHI were pooled.

Pooled Multiethnic: data from NHS, HPFS, WHI and SCHS were pooled.

Data are β coefficients ± SE.

Numbers of participants across three categories (≤1/wk/1~6/wk/≥1/d) of fish intake in the NHS, HPFS, WHI, and SCHS are 1618/8465/1239, 977/5108/748, 894/4675/684, and 752/3935/576, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

The multiple linear regression model was used to test the genetic association of the *FADS* variant (additive model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The multiple linear regression model was used to test the associations of long chain n-3 PUFAs intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

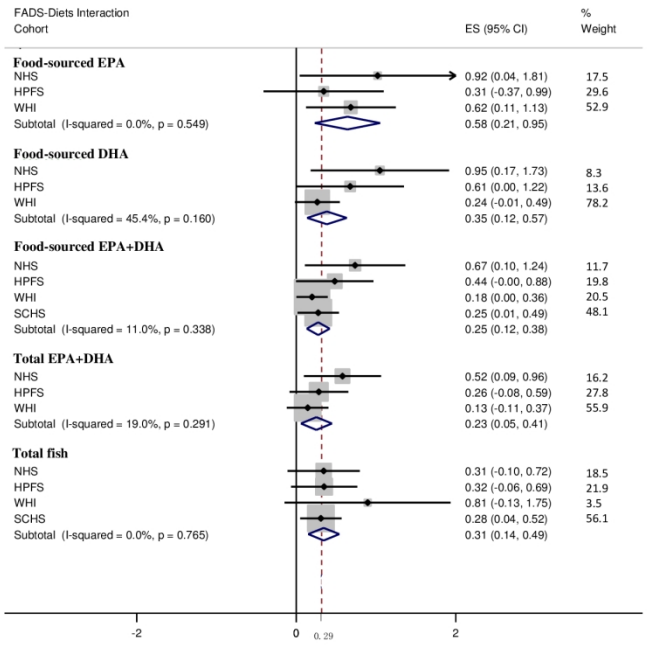
**Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and 876/5378, respectively.

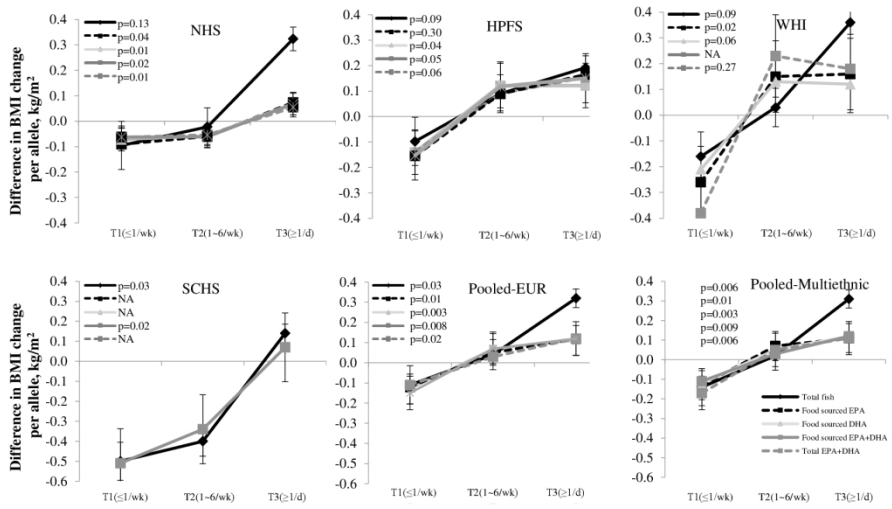
Black circles for T allele carriers and open circle for non-T-carriers.

The multiple linear regression model was used to test the associations of and fish intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).




254x190mm (200 x 200 DPI)

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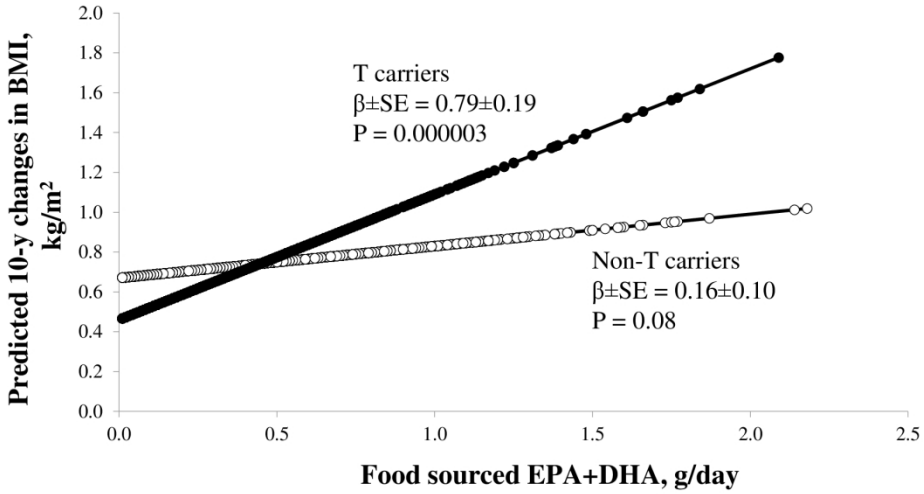


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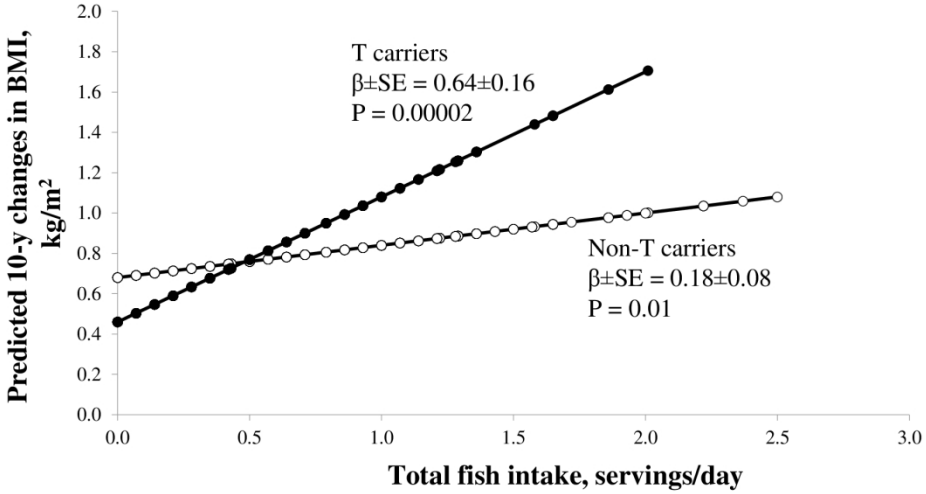


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**Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit**

| Position[1] | Reference SNP identification number | Alleles[2] | DAF | | | | | | | PBS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CEU | CHB | GI | NHS | HPFS | WHI | SCHS | |
| chr11:61627960 | rs74771917 | C/T | 0.025 | 0.16 | 0.98 | / | / | / | / | 2.67 |
| chr11:61631510 | rs3168072 | A/T | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61632310 | rs12577276 | A/G | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61641717 | rs7115739 | G/T | 0.017 | 0.22 | 0.98 | 0.004 | 0.004 | / | / | 2.54 |
| chr11:61624414 | rs174602 | C/T | 0.80 | 0.73 | 0.01 | 0.82 | 0.81 | / | / | 2.11 |
| chr11:61597212 | rs174570 | C/T | 0.16 | 0.34 | 0.99 | 0.15 | 0.15 | 0.14 | 0.35 | 2.06 |

[1]Positions refer to human genome assembly hg19.

[2]Alleles are coded as ancestral/derived states.

PBS, the population branch statistic; DAF, derived allele frequency; CEU, European ancestry; CHB, an Chinese; GI, Greenlandic Inuit

DAFs for each population (CEU, CHB, and GI) and PBS values are reported, along with the genomic position for each SNP.

**Supplemental table 2 Main effect of the *FADS* variants on adiposity in the four cohorts**

| Outcomes (kg/m$^2$) | *FADS* SNPs | NHS | | HPFS | | WHI | | SCHS | | Pooled | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P |
| Baseline BMI | rs174570 | 0.03 ± 0.10 | 0.733 | -0.05 ± 0.09 | 0.538 | -0.06±0.17 | 0.72 | 0.24±0.08 | 0.002 | 0.08±0.05 | 0.06 |
| Baseline BMI | rs174602 | 0.08 ± 0.10 | 0.418 | -0.05 ± 0.08 | 0.536 | / | | / | | 0.00 ± 0.03 | 0.559 |
| Baseline BMI | rs7115739 | 0.25 ± 0.52 | 0.634 | -0.77 ± 0.43 | 0.077 | / | | / | | -0.35 ± 0.14 | 0.196 |
| Long-term BMI change | rs174570 | -0.05 ± 0.06 | 0.401 | 0.01 ± 0.05 | 0.917 | -0.02±0.09 | 0.77 | -0.02±0.08 | 0.85 | -0.02±0.03 | 0.94 |
| Long-term BMI change | rs174602 | -0.14 ± 0.06 | 0.009 | 0.04 ± 0.05 | 0.413 | / | | / | | -0.04 ± 0.01 | 0.025 |
| Long-term BMI change | rs7115739 | 0.45 ± 0.29 | 0.124 | -0.23 ± 0.26 | 0.359 | / | | / | | 0.06 ± 0.08 | 0.183 |

Long-term BMI change: BMI change from 1990 to 2000.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Effect size (ES) values are β coefficients for relationship between the *FADS* variant rs174570 (additive model) and adiposity.

The general linear model was used to test the genetic association of *FADS* variants with long-term changes in BMI after adjustment for age, source of genotyping data.

**Supplemental table 3 Genetic association of *FADS* variant with long-term changes in body weight according to long chain n-3 PUFAs and fish intakes**

| | Difference in long-term changes in weight, kg | | | P for interaction |
|---|---|---|---|---|
| **Total Fish, serving/day** | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | -0.69±0.64 | -0.13±0.49 | 1.78±1.64 | 0.05 |
| HPFS | -0.99±0.85 | 0.54±0.53 | 1.52±1.69 | 0.12 |
| WHI | -0.22±0.42 | 0.16±0.34 | 1.26±1.57 | 0.13 |
| SCHS | -0.42±0.29 | -0.44±0.28 | 0.20±0.29 | 0.08 |
| Pooled | -0.44±0.22 | -0.10±0.18 | 0.31±0.28 | 0.01 |
| **Food-sourced EPA, g/day** | T1 | T2 | T3 | |
| NHS | -0.77±0.62 | -0.25±0.72 | 0.53±0.64 | 0.06 |
| HPFS | -1.19±0.82 | 0.75±0.73 | 0.72±0.74 | 0.41 |
| WHI | -0.19±0.42 | 0.24±0.47 | 0.14±0.48 | 0.20 |
| Pooled | -0.50±0.32 | 0.24±0.34 | 0.37±0.34 | 0.10 |
| **Food-sourced DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.53±0.62 | -0.39±0.70 | 0.53±0.65 | 0.01 |
| HPFS | -1.06±0.82 | 0.49±0.71 | 0.89±0.76 | 0.09 |
| WHI | -0.20±0.43 | 0.22±0.42 | 0.30±0.50 | 0.26 |
| Pooled | -0.43±0.32 | 0.15±0.32 | 0.49±0.35 | 0.01 |
| **Food-sourced EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.56±0.63 | -0.32±0.68 | 0.49±0.66 | 0.01 |
| HPFS | -1.25±0.83 | 0.68±0.73 | 0.84±0.74 | 0.09 |
| WHI | -0.02±0.43 | 0.16±0.44 | 0.14±0.49 | 0.23 |
| SCHS | -0.47±0.29 | -0.16±0.28 | -0.03±0.29 | 0.10 |

| | | | | |
|---|---|---|---|---|
| Pooled | -0.56±0.25 | -0.09±0.24 | 0.14±0.25 | 0.005 |
| **Total EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.58±0.63 | -0.30±0.68 | 0.46±0.66 | 0.02 |
| HPFS | -1.20±0.82 | 0.75±0.70 | 0.86±0.77 | 0.18 |
| WHI | -0.48±0.47 | 0.64±0.43 | 0.04±0.47 | 0.15 |
| Pooled | -0.64±0.34 | 0.45±0.32 | 0.32±0.34 | 0.02 |

Data are β coefficients ± SE.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the genetic association with long-term changes in body weight according to baseline long chain n-3 PUFAs and fish intakes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from three or four cohorts pooled by means of fixed effects meta-analyses (if P ≥ 0.05 for heterogeneity between studies).

**Supplemental table 4 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight according to *FADS* genotypes**

| Cohorts | | Long-term changes in weight, kg | | | P for trend |
|---|---|---|---|---|---|
| **Total Fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | Non T carriers | 4.91±0.34 | 5.78±0.24 | 7.00±0.79 | 0.008 |
| | T carriers | 4.45±0.61 | 5.64±0.46 | 9.26±1.44 | 0.001 |
| HPFS | Non T carriers | 0.44±0.06 | 0.52±0.04 | 0.56±0.12 | 0.99 |
| | T carriers | 0.25±0.10 | 0.53±0.07 | 0.76±0.21 | 0.08 |
| WHI | Non T carriers | -0.25±0.23 | -0.43±0.18 | -0.91±0.93 | 0.50 |
| | T carriers | -0.56±0.37 | -0.25±0.28 | 1.30±1.71 | 0.13 |
| SCHS | Non T carriers | -3.15±0.23 | -3.50±0.21 | -3.38±0.21 | 0.48 |
| | T carriers | -3.68±0.20 | -3.41±0.19 | -3.34±0.20 | 0.16 |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.89±0.33 | 5.89±0.33 | 5.95±0.33 | 0.24 |
| | T carriers | 4.45±0.59 | 5.52±0.63 | 6.46±0.61 | 0.34 |
| HPFS | Non T carriers | 0.50±0.05 | 0.54±0.05 | 0.45±0.05 | 0.15 |
| | T carriers | 0.29±0.10 | 0.54±0.09 | 0.52±0.09 | 0.66 |
| WHI | Non T carriers | -0.30±0.25 | -0.54±0.24 | -0.29±0.24 | 0.42 |
| | T carriers | -0.51±0.39 | -0.36±0.37 | -0.15±0.38 | 0.14 |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.78±0.33 | 5.56±0.34 | 6.32±0.33 | 0.14 |
| | T carriers | 4.50±0.60 | 5.07±0.63 | 6.77±0.61 | 0.004 |
| HPFS | Non T carriers | 0.48±0.05 | 0.54±0.05 | 0.46±0.06 | 0.40 |
| | T carriers | 0.27±0.10 | 0.50±0.09 | 0.59±0.09 | 0.15 |
| WHI | Non T carriers | -0.41±0.25 | -0.25±0.24 | -0.45±0.25 | 0.51 |

| | | | | | |
|---|---|---|---|---|---|
| | T carriers | -0.71±0.39 | -0.15±0.37 | -0.16±0.39 | 0.18 |
| **Food-sourced EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.69±0.34 | 5.45±0.33 | 6.51±0.33 | 0.02 |
| | T carriers | 4.44±0.61 | 5.00±0.61 | 6.92±0.61 | 0.0003 |
| HPFS | Non T carriers | 0.48±0.05 | 0.53±0.05 | 0.47±0.05 | 0.93 |
| | T carriers | 0.26±0.10 | 0.49±0.09 | 0.59±0.09 | 0.08 |
| WHI | Non T carriers | -0.44±0.24 | -0.23±0.23 | -0.43±0.24 | 0.47 |
| | T carriers | -0.52±0.38 | -0.15±0.37 | -0.33±0.38 | 0.15 |
| SCHS | Non T carriers | -3.44±0.21 | -3.58±0.22 | -3.05±0.21 | 0.89 |
| | T carriers | -3.73±0.19 | -3.57±0.19 | -3.12±0.19 | 0.12 |
| **Total EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.74±0.34 | 5.55±0.32 | 6.36±0.34 | 0.81 |
| | T carriers | 4.49±0.61 | 5.16±0.60 | 6.70±0.61 | 0.03 |
| HPFS | Non T carriers | 0.49±0.05 | 0.53±0.05 | 0.47±0.06 | 0.24 |
| | T carriers | 0.26±0.10 | 0.51±0.09 | 0.58±0.09 | 0.33 |
| WHI | Non T carriers | 0.32±0.27 | -0.84±0.23 | -0.60±0.28 | 0.19 |
| | T carriers | -0.26±0.45 | -0.21±0.37 | -0.02±0.11 | 0.08 |

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight by *FADS* genotypes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from two cohorts pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies) or random effects meta-analyses (if $P < 0.05$ for heterogeneity between studies).

**Supplemental Table 5 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| Diets | *FADS* genotypes | Long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| | | Categories of diets | | | | |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.82±0.06 | 1.00±0.06 | 1.01±0.06 | 0.24 | 0.05 |
| | T carriers | 0.72±0.10 | 0.94±0.11 | 1.10±0.11 | 0.29 | |
| HPFS | Non-T carriers | 0.48±0.05 | 0.54±0.05 | 0.47±0.05 | 0.72 | 0.37 |
| | T carriers | 0.23±0.11 | 0.54±0.10 | 0.52±0.09 | 0.45 | |
| WHI | Non-T carriers | 0.10±0.09 | 0.09±0.09 | 0.45±0.09 | 0.21 | 0.02 |
| | T carriers | -0.08±0.15 | 0.23±0.15 | 0.46±0.15 | 0.003 | |
| Pooled | Non-T carriers | 0.54±0.04 | 0.63±0.04 | 0.65±0.04 | 0.35 | 0.01 |
| | T carriers | 0.39±0.07 | 0.63±0.07 | 0.70±0.06 | 0.01 | |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.80±0.06 | 0.94±0.06 | 1.08±0.06 | 0.14 | 0.009 |
| | T carriers | 0.74±0.10 | 0.83±0.11 | 1.17±0.10 | 0.002 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.54±0.05 | 0.49±0.05 | 0.99 | 0.05 |
| | T carriers | 0.24±0.10 | 0.49±0.10 | 0.58±0.09 | 0.05 | |
| WHI | Non-T carriers | 0.03±0.09 | 0.20±0.09 | 0.42±0.09 | 0.03 | 0.06 |
| | T carriers | -0.10±0.15 | 0.33±0.15 | 0.39±0.15 | 0.006 | |
| Pooled | Non-T carriers | 0.51±0.04 | 0.63±0.04 | 0.68±0.04 | 0.1 | 0.002 |
| | T carriers | 0.38±0.06 | 0.58±0.07 | 0.77±0.06 | $7\times10^{-4}$ | |

Data are means ± SE.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the

WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Registration: www. clinicaltrials.gov. Registration ID: NCT03348566

**TABLE 1. STREGA reporting recommendations, extended from STROBE Statement**

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Title and Abstract** | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract. **(p. 3)** | |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found. **(p. 3)** | |
| **Introduction** | | | |
| *Background rationale* | 2 | Explain the scientific background and rationale for the investigation being reported. **(p. 4)** | |
| *Objectives* | 3 | State specific objectives, including any pre-specified hypotheses. | ***State if the study is the first report of a genetic association, a replication effort, or both.*** **(p. 3)** |
| **Methods** | | | |
| *Study design* | 4 | Present key elements of study design early in the paper. **(p. 5)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Setting* | 5 | Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. **(p. 3)** | |
| *Participants* | 6 | (a) **Cohort study –** Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **(p. 5)**<br><br>**Case-control study –** Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls.<br><br>**Cross-sectional study –** Give the eligibility criteria, and the sources and methods of selection of participants. | ***Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant***. **(p. 5)** |
| | | (b) **Cohort study –** For matched studies, give matching criteria and number of exposed and unexposed.<br><br>**Case-control study –** For matched studies, give matching criteria and the number of controls per case. | |
| *Variables* | 7 | *(a)* Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. **(p. 5)** | ***(b) Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).*** **(p. 5)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Data sources measurement* | 8* | *(a)* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. **(p. 5)** | ***(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory/centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.*** **(p. 5)** |
| *Bias* | 9 | *(a)* Describe any efforts to address potential sources of bias. **(p. 5 &6)** | ***(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***with this.*** **(p. 5 &6)** |
| *Study size* | 10 | Explain how the study size was arrived at. **(p. 5 &6)** | |
| *Quantitative variables* | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. **(p. 7)** | ***If applicable, describe how effects of treatment were dealt with.*** **(p. 7)** |
| *Statistical methods* | 12 | (a) Describe all statistical methods, including those used to control for confounding. **(p. 7-9)** | ***State software version used and options (or settings) chosen.*** **(p. 9)** |
| | | (b) Describe any methods used to examine subgroups and interactions. **(p. 9)** | |
| | | (c) Explain how missing data were addressed. **(p. 9)** | |
| | | (d) **Cohort study –** If applicable, explain how loss to follow-up was addressed. **(p. 9)**<br><br>**Case-control study –** If applicable, explain how matching of cases and controls was addressed.<br><br>**Cross-sectional study –** If applicable, describe analytical methods taking account of sampling strategy. | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | (e) Describe any sensitivity analyses. **(p. 9)** | |
| | | Hardy-Weinberg equilibrium was tested using Chi-square test. **(p. 9)** | ***(f) State whether Hardy-Weinberg equilibrium was considered and, if so, how.*** |
| | | We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). **(p. 8&9)** | ***(g) Describe any methods used for inferring genotypes or haplotypes.*** |
| | | | ***(h) Describe any methods used to assess or address population stratification.*** **(p. 9)** |
| | | | ***(i) Describe any methods used to address multiple comparisons or to control risk of false positive findings.*** **(p. 9)** |
| | | | ***(j) Describe any methods used to address and correct for relatedness among subjects*****(p. 9)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Results** | | | |
| *Participants* | 13* | (a) Report the numbers of individuals at each stage of the study – e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. **(p. 10)** | ***Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.*** **(p. 10)** |
| | | (b) Give reasons for non-participation at each stage. **(p. 10)** | |
| | | (c) Consider use of a flow diagram. **(p. 10)** | |
| *Descriptive data* | 14* | (a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. **(p. 10)** | ***Consider giving information by genotype.*** **(p. 10)** |
| | | (b) Indicate the number of participants with missing data for each variable of interest. **(p. 10)** | |
| | | (c) **Cohort study –** Summarize follow-up time, e.g. average and total amount. **(p. 10)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Outcome data* | 15 * | **Cohort study-**Report numbers of outcome events or summary measures over time. | ***Report outcomes (phenotypes) for each genotype category over time*** |
| | | **Case-control study –** Report numbers in each exposure category, or summary measures of exposure. | ***Report numbers in each genotype category*** |
| | | **Cross-sectional study –** Report numbers of outcome events or summary measures. | ***Report outcomes (phenotypes) for each genotype category*** |
| *Main results* | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence intervals). Make clear which confounders were adjusted for and why they were included. **(p. 10)** | |
| | | (b) Report category boundaries when continuous variables were categorized. **(p. 10)** | |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. **(p. 10)** | |
| | | | ***(d) Report results of any adjustments for multiple*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***comparisons.* (p. 10)** |
| *Other analyses* | 17 | (a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. **(p. 10)** | |
| | | | ***(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken.* (p. 10)** |
| | | | ***(c) If detailed results are available elsewhere, state how they can be accessed.* (p. 10)** |
| **Discussion** | | | |
| *Key results* | 18 | Summarize key results with reference to study objectives. **(p. 11)** | |
| *Limitations* | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. **(p. 11)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Interpretation* | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. **(p. 11)** | |
| *Generalizability* | 21 | Discuss the generalizability (external validity) of the study results. **(p. 11)** | |
| **Other Information** | | | |
| *Funding* | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. **(p. 14)** | |

STREGA = STrengthening the REporting of Genetic Association studies; STROBE = STtrengthening the Reporting of Observational Studies in Epidemiology.

* Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

BMJ Open

# Fish and marine fatty acids intakes, the FADS genotypes and long-term weight gain: a prospective cohort study



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|---|---|
| Complete List of Authors: | Huang, Tao; Peking University, Department of Epidemiology and Biostatistics<br>Wang, Tiange ; Shanghai Jiao Tong University, Shanghai Institute of Endocrine and Metabolic Diseases<br>Heianza, Yoriko ; Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University<br>Wiggs, Janey ; Harvard Medical School, Department of Ophthalmology<br>Sun, Dianjianyi ; School of Public Health and Tropical Medicine, Tulane University<br>Choi, Hyon-Kyoo ; Massachusetts General Hospital - Harvard Medical School Center for Nervous System Repair<br>Chai, Jin Fang ; National University Singapore Yong Loo Lin School of Medicine<br>Sim, Xueling ; National University of Singapore, Epidemiology Domain, Saw Swee Hock School of Public Health<br>Khor, Chiea Chuen ; National University of Singapore, Department of Biochemistry, Yong Loo Lin School of Medicine<br>Friedlander, Yechiel ; Hebrew University, Unit of Epidemiology<br>Chan, Andrew T. ; Massachusetts General Hospital, Division of Gastroenterology<br>Curhan, Gary ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>Vivo, Immaculata De ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>van Dam, Rob Martinu. ; National University of Singapore, Department of Epidemiology<br>Heng, Chew Kiat ; National University of Singapore, Department of Paediatrics<br>Fuchs, Charles ; Harvard Medical School, Department of Medicine, Brigham and Women's Hospital<br>Pasquale, Louis R. ; Harvard Medical School, Channing Division of Network Medicine, Department of Medicine<br>Yuan, Jian-min ; University of Pittsburgh, Division of Cancer Control and Population Sciences<br>Hu, Frank B. ; Harvard University T H Chan School of Public Health, Department of Epidemiology<br>Koh, Woon Puay ; National University of Singapore, Department of Epidemiology<br>Qi, Lu; Tulane University, Department of Epidemiology, School of Public Health and Tropical Medicine |

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# Fish and marine fatty acids intakes, the *FADS* genotypes and long-term weight gain: a prospective cohort study

Tao Huang[1,2,3*], Tiange Wang[4], Yoriko Heianza[5], Janey Wiggs[6], Dianjianyi Sun[5], Hyon-Kyoo Choi[7], Jin Fang Chai[8], Xueling Sim[8], Chiea Chuen Khor[9,10], Yechiel Friedlander[11], Andrew T. Chan[12], Gary Curhan[13], Immaculata De Vivo[13], Rob Martinu. van Dam[8,14], Chew Kiat Heng[15], Charles S. Fuchs[16,17], Louis R. Pasquale[18], Jian-min Yuan[19,20], Frank B. Hu[14, 16], Woon Puay Koh[8, 21], Lu Qi[5, 14, 16*]

[1.] *Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing 100191, China.*

[2.] *Department of Global Health, School of Public Health, Peking University, China.*

[3.] *Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, China.*

[4.] *Shanghai Institute of Endocrine and Metabolic Diseases, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.*

[5.] *Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Suite 1724 New Orleans, LA 70112.*

[6.] *Department of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA 02115, USA.*

[7.] *Department of Rheumatology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[8.] *Epidemiology Domain, Saw Swee Hock School of Public Health, National University of Singapore, Singapore, 117549.*

[9.] *Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore.*

[10.] *Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.*

[11.] *Unit of Epidemiology, Hebrew University-Hadassah Braun School of Public Health, POB 12272, Jerusalem 91120, Israel.*

[12.] *Division of Gastroenterology, Massachusetts General Hospital, Boston, MA 02114, USA.*

[13.] *Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA.*

[14.] *Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA.*

[15.] *Department of Paediatrics, National University of Singapore NUHS Tower Block, Level 12, 1E Kent Ridge Road Singapore 119228.*

[16.]*Department of Medicine, Brigham and Women's Hospital and Harvard Medical School Boston, MA 02115, USA.*

[17.]*The Center for Gastrointestinal Cancer, Dana-Farber Cancer Institute, Boston, USA.*

[18.]*Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA.*

[19.]*Division of Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA.*

[20.]*Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA, USA.*

[21.]*Duke-NUS Medical School, Singapore, Singapore.*

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***Correspondence and requests for reprint:**

Dr. Tao Huang. Department of Epidemiology and Biostatistics, School of Public Health, Peking University, Beijing, China. Email: huangtao@bjmu.edu.cn

Dr. Lu Qi. Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University. 1440 Canal Street, Suite 1724, New Orleans, LA 70112

Telephone: 504-988-3549;

Email: lqi1@tulane.edu; luqi@hsph.harvard.edu

**Abstract**

**Objective:** We tested whether genetic variants near fatty acid desaturases gene (*FADS*) cluster, which were recently identified to be signatures of adaptation to fish- and n-3 PUFAs-rich diet, interacted with these dietary factors on change in body mass index (BMI).

**Design:** Three *FADS* variants were examined for gene-diet interactions on long-term (~10 years) changes in BMI and body weight in four prospective cohort studies.

**Setting:** Population based study

**Participants:** 11,323 women from the Nurses' Health Study (NHS), 6,833 men from the Health Professionals Follow-up Study (HPFS), and replicated in 6,254 women from the Women's Health Initiative (WHI), and 5,264 Chinese from the Singapore Chinese Health Study (SCHS).

**Main outcomes:** Long-term (~10 years) changes in BMI and body weight

**Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS* rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 $kg/m^2$ per g, P = $3\times10^{-5}$) than the rs174570 non-T carriers (beta=0.16 $kg/m^2$ per g, P = 0.08). Similar results were observed for fish intake.

**Conclusions:** Our hypothesis-driven analyses provide replicable evidence that long chain n-3 PUFAs and fish intakes may interact with the *FADS* variant on long-term weight gain. Further investigation is needed to confirm our findings in other cohorts.

## Article summary

### Strengths and limitations of this study

- This is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings.
- Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results.
- Dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable.
- Confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors.
- We acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses.

## Introduction

Diets rich in fish and marine fatty acids, especially long chain n-3 polyunsaturated fatty acids (PUFAs) has shown beneficial effects on cardiometabolic health [1 2]. However, data from population studies on the associations between such diet and body weight are inconsistent [3 4]. Emerging evidence suggests genetic variations may play a role in modifying the relation between dietary factors and body weight [5-7]. For example, we previously found that high intakes of fish and long-chain n-3 PUFAs are associated with an attenuation of the genetic association with long-term weight gain based on results from 3 prospective cohorts of Caucasians.[8]

A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs [9]. The strong signals locate in a cluster of fatty acid desaturases genes (*FADS*) that determine PUFAs levels [9]. People living in the Arctic region have been found to be genetically prone to develop obesity [10 11] as survival strength for energy storage[12 13]. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in the Inuit population [9]. Of note, due to long-standing selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various populations such as Europeans and Asians [14], in coincidence with varying levels of fish/marine fatty acids consumption, and adiposity patterns in these populations [15]. We therefore hypothesized that the genetic signatures might interact with fish and marine PUFAs intakes on body weight [14].

The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts the Women's Health Initiative (WHI, US) and the Singapore Chinese Health Study (SCHS).

## Methods

**Discovery cohorts**

***The Nurses' Health Study***

The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were recruited to complete a baseline questionnaire about their lifestyle and medical history [16]. The current analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [17-23]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [17-22]. The study protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

***The Health Professionals Follow-up Study***

The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians, optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet [24]. In the current analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation when performed have been described in detail elsewhere [17-23]. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included [17-22]. The study protocol was also approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

**Replication cohorts**

***The Women's Health Initiative (WHI)***

The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI study is presented elsewhere [25 26]. For the analyses, all participants with baseline long chain n-3 PUFAs and fish consumption and covariate data, baseline and endpoint BMI data, and genotyping data available based on previous GWASs were included. Finally, we included 6,254 Caucasians women who participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each participating institution reviewed and approved the study, and all women gave written informed consent.

***The Singapore Chinese Health Study (SCHS) cohort***

The design of Singapore Chinese Health Study (SCHS) has been previously described in detail [27]. Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in Singapore were enrolled into the cohort study [28]. Two follow-up interviews were conducted via telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update information on body weight, selected lifestyle factors and medical history. All participants have given informed consent. The study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data available were included. Among these participants, genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome Institute of the Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation [29].

Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip [30]. Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.

**Assessment of measures of body mass index**

Height and body weight were assessed by questionnaire at baseline, and weight information was requested on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly measured values (r=0.97 in HPFS and NHS) in a validation study [31]. BMI was calculated as body weight (kg)/height ($m^2$). As defined previously,[8] the long-term changes in BMI was calculated as changes in BMI from 1990 to 2000 in the NHS and HPFS cohorts [32], and from baseline (1993) to sixth year follow-up in the WHI [25 26], and from baseline (1998) to second follow-up (2004) in the SCHS.

**Assessment of diets and other covariates**

Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires (FFQ) in the NHS and HPFS [33 34]. A 165-item validated semi-quantitative FFQ was used to collect dietary data and supplemental use of fish oil in the SCHS [28]. Dietary data and supplemental use of fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI [35]. Alternate health eating index was previously calculated in the NHS, HPFS [36], WHI, and SCHS. Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent on various activities, and the intensity level of each activity. The validity of the self-reported physical activity data has been described previously in the NHS and HPFS [37]. In the WHI, an estimated metabolic equivalent (MET) level for each type of activity was assigned from a compendium of activities [38]. Physical activity

was assessed using eight continuous categories ranging from never to 31 hours or more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS [27].

**The *FADS* variants selection and genotyping**

Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes for signatures of adaptation [9] were derived from genome-wide scans available in the NHS, HPFS. We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication (**Supplemental Table 1**).

**Patient and Public Involvement**

Neither patients nor public were involved.

**Statistical analyses**

We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity measures and long-term changes in BMI using multiple linear regression model. Interactions between the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if available). We further tested the genetic associations with long-term changes in BMI according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish

intakes with long-term changes in BMI according to the *FADS* genotypes using multiple linear regression model after adjustment of potential confounders. Linear trend across categories of long chain n-3 PUFAs and fish intakes was quantified with a Wald test for linear trend by assigning the median value to each category and modeling it as a continuous variable[39]. Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed effects models (P ≥ 0.05 for heterogeneity between studies) [40]. The individual participant data from the NHS and HPFS cohorts were pooled to generate the predicted 10-year changes in body weight according to the *FADS* genotypes. Hardy-Weinberg equilibrium was tested using Chi-square test. All reported P values are nominal and two sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).

## Results

### Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts

**Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts. The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The distribution of the *FADS* genetic variants in the 4 cohorts is shown in **Supplemental table 1**. Chi-square test showed that the *FADS* rs174570 is in Hardy-Weinberg equilibrium. We did not observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term changes in BMI in the three US cohorts (P > 0.05). However, we found that the *FADS* genotype was significantly associated with baseline BMI in the SCHS (P = 0.002) (**Supplemental table 2)**.

### Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes

We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes of variously sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians)

showed significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the SCHS cohort (P for interaction = 0.02).

The pooled analyses of the 3 US (Caucasian) samples or all 4 cohorts showed that high intakes of food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No significant heterogeneity in the interaction effect was observed among these cohorts. Differences in long-term changes of BMI per T allele were -0.105 (SE 0.067), 0.027 (SE 0.064), and 0.120 (SE 0.067) kg/m$^2$ across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for interaction=0.003) showed similar interaction patterns; and the interactions remained significant when supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS* genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071), 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m$^2$ across three categories (≤ 1 serving/week, 1~6 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI

in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed (**Supplemental table 3 & 4**).

**Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had significantly greater increase of BMI (mean ± SE = 0.74±0.06, kg/m$^2$) than did those who consumed the lowest (T1) (mean ± SE = 0.39±0.07, kg/m$^2$) among the T allele carriers, whereas the corresponding BMI changes were 0.68±0.03 kg/m$^2$ and 0.49±0.03 kg/m$^2$, respectively, among the non-T carriers in 4 cohorts combined (**Table 2 & Supplemental table 5** ). Similarly, we observed different associations between fish intake and BMI changes among the T allele carriers ($P = 1.5\times10^{-6}$) and non-carriers (P = 0.01) in the pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed among the cohorts.

**Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled results, the beta ± SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**) with long-term changes in BMI were 0.79 ± 0.19 kg/m$^2$ per g (P = 0.000003) and 0.64 ± 0.16 kg/m$^2$ per serving (P = 0.00002) among the T carriers, and whereas the corresponding beta ± SE were 0.16 ± 0.10 kg/m$^2$ per g (P = 0.08) and 0.18 ± 0.08 kg/m$^2$ per serving (P = 0.01) among the non-T carriers.

**Discussion**

In 4 large prospective cohorts of the US and Chinese populations, our hypothesis-driven analyses showed reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS*

rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.

Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight and obesity risk have generated conflicting results [3 4]. In addition, several randomized controlled trials (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss [41-43], but the benefit was not evident in other trials [44-46]. The results from the current study lend support to our hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.

We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle, the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit, who had high fish/n-3 PUFAs intakes [9]. The high frequency of the T allele in Inuit reflects genetic adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that the signature allele (T) was related differently to weight changes (decrease or increase), depending on the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body weight when they are exposed to a diet low in fish and n-3 PUFAs.

We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction patterns in relation to long-term changes in BMI; and the interactions also remained significant when supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as

Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is robust for fatty acids from various sources.

The mechanisms underlying the observed gene-diet interactions remain unclear. However, such interactions are biologically plausible. It has long been known that the *FADS* genes such as *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic acid (n-6) and α-linolenic acid (n-3) [47]. The selected allele of *FADS* rs174570 is significantly associated with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway [47]. Further, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function [48]. In addition, storage of energy and body fat is very important for the Arctic population, who are regularly exposed to extremely low temperatures and fishes rich in n-3 PUFAs [12 13]. Under natural selection, these people are genetically prone to high fish intake to keep body fat [10 11]. Therefore, it's not surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying selective *FADS* signature [49 50]. Our findings support the view that extra n-3 PUFAs may not have much benefit for Europeans with selective *FADS* signature[9 14].

**Strengths**

Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results from 4 well-established prospective cohorts of different racial populations such as Caucasians and Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of our findings. Other major strengths include the prospective design, the large sample size, use of long-term change of BMI, and replication of the results. Although we prospectively analyzed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change from the baseline, which by default builds in the starting point (i.e. the cross sectional association).

**Limitations**

However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these variables are inevitable. Despite this, the food frequency questionnaires and adiposity measures data have been well validated [28 31 33-35]. Second, confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might introduce bias in the present analyses. Finally, the participants included in our study were middle aged and older adults of Caucasians in the US and Chinese in Singapore, and it is unknown whether our findings could be generalized to other demographic or ethnic groups.

**Conclusions**

In summary, our data provide reproducible evidence from 4 multiethnic cohorts that high long chain n-3 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings emphasize the importance of considering precision nutritional interventions on prevention and treatment of obesity. We acknowledge that these results are hypothesis-generating and need to be confirmed in additional cohorts.

**Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH provided statistical expertise. TW, YH, DS, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM, HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors contributed to the interpretation of the results and critical revision of the manuscript for important intellectual content and approved the final version of the manuscript.

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**Data sharing**

Data are available in Harvard school of public health, NHS and HPFS cohorts. All data relevant to the study are included in the article or uploaded as supplementary information.

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**Table 1 Baseline characteristics of all participants in the NHS, HPFS, WHI, and SCHS cohorts.**

| | NHS[1] | HPFS | WHI | SCHS |
|---|---|---|---|---|
| | n=11,323 | n=6,833 | n=6,254 | n=5,264 |
| Age (year) | 57 ± 9 | 57 ± 11 | 68 ± 5 | 56 ± 7 |
| Female (%) | 100 | 0 | 100 | 58.7 |
| Body weight (kg) | 70.1 ± 14.9 | 82.8 ± 12.5 | 73.7 ± 15.0 | 60.3 ± 9.8 |
| Body mass index (kg/m$^2$) | 26.2 ± 5.1 | 25.9 ± 3.3 | 28.3 ± 5.5 | 23.4 ± 3.3 |
| Alcohol consumption (g/day) | 5.14 ± 9.23 | 10.97 ± 15.05 | 6.00 ± 11.96 | 1.97 ± 8.02 |
| Physical activity (MET-h/week) | 19.3 ± 22.1 | 36.9 ± 39.5 | 11.6 ± 13.1 | 0.5 ± 1.0[2] |
| Television watching (h/week) | 17.5 ± 14.8 | 10.5 ± 8.2 | / | 2.2 ± 0.8 |
| Current smokers (n, (%)) | 1557(13.8) | 493(7.3) | 407(15.0) | 1364(20.0) |
| Total energy intake (kcal/day) | 1766 ± 502 | 1949 ± 578 | 1602 ± 654 | 1606 ± 573 |
| Alternative health eating index score | 53.4 ± 10.8 | 53.8 ± 11.4 | 53.5 ± 10.6 | 55.8 ± 8.2 |
| Sugar sweetened beverage intake (servings/day) | 0.13 ± 0.39 | 0.23 ± 0.48 | 0.39 ± 0.82 | 0.69 ± 2.40[3] |
| Total fried food (servings/day) | 0.12 ± 0.20 | 0.22 ± 0.28 | / | / |
| Fish intake (servings/day) | 0.31 ± 0.29 | 0.33 ± 0.30 | 0.23 ± 0.20 | 0.16 ± 0.07 |
| Food-sourced EPA (g/day) | 0.08 ± 0.14 | 0.12 ± 0.20 | 0.04 ± 0.04 | / |
| Food-sourced DHA (g/day) | 0.17 ± 0.14 | 0.22 ± 0.19 | 0.07 ± 0.07 | / |
| Food-sourced EPA+DHA (g/day) | 0.23 ± 0.19 | 0.31 ± 0.25 | 0.11 ± 0.10 | 0.33 ± 0.20 |
| Total EPA+DHA (g/day) | 0.26 ± 0.27 | 0.35 ± 0.37 | 0.38 ± 0.48 | / |

[1]Plus-minus values are means ± SD. [2]Hours per week of moderate activity in the SCHS. [3]Glasses per week of soda intake in the SCHS.

EPA: 20:5n-3; DHA: 22:6n-3; MET denotes metabolic equivalents. Total EPA+DHA includes food-sourced and supplemental EPA+DHA.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively. Television watching assessed in 1992 for NHS and in 1990 for HPFS.

**Table 2 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| | *FADS* genotypes | Three categories of long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| **Total fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | | |
| NHS | Non-T carriers | 0.82±0.06 | 0.98±0.04 | 1.15±0.13 | 0.006 | 0.03 |
| | T carriers | 0.73±0.11 | 0.95±0.08 | 1.55±0.25 | 0.0007 | |
| HPFS | Non-T carriers | 0.43±0.05 | 0.52±0.04 | 0.59±0.12 | 0.73 | 0.03 |
| | T carriers | 0.21±0.11 | 0.52±0.07 | 0.79±0.22 | 0.02 | |
| WHI | Non-T carriers | 0.11±0.08 | 0.28±0.06 | 0.28±0.34 | 0.04 | 0.09 |
| | T carriers | 0.02±0.15 | 0.29±0.11 | 0.94±0.67 | 0.01 | |
| SCHS | Non-T carriers | -3.08±0.19 | -3.00±0.17 | -3.35±0.18 | 0.32 | 0.01 |
| | T carriers | -3.61±0.17 | -3.10±0.15 | -3.25±0.17 | 0.13 | |
| Pooled[1] | Non-T carriers | 0.50±0.03 | 0.67±0.03 | 0.81±0.08 | 0.01 | 0.0007 |
| | T carriers | 0.38±0.07 | 0.63±0.05 | 1.11±0.16 | $2\times10^{-4}$ | |
| **Food-sourced EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |
| NHS | Non-T carriers | 0.79±0.06 | 0.92±0.05 | 1.11±0.06 | 0.01 | 0.005 |
| | T carriers | 0.71±0.10 | 0.84±0.11 | 1.19±0.11 | 0.0001 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.53±0.05 | 0.49±0.05 | 0.79 | 0.02 |
| | T carriers | 0.23±0.11 | 0.48±0.10 | 0.58±0.09 | 0.02 | |
| WHI | Non-T carriers | 0.02±0.08 | 0.21±0.08 | 0.41±0.08 | 0.06 | 0.04 |
| | T carriers | -0.03±0.15 | 0.29±0.14 | 0.35±0.15 | 0.004 | |
| SCHS | Non-T carriers | -3.32±0.17 | -3.15±0.18 | -2.99±0.17 | 0.16 | 0.035 |
| | T carriers | -3.55±0.16 | -3.34±0.16 | -3.05±0.16 | 0.02 | |
| Pooled[1] | Non-T carriers | 0.49±0.03 | 0.64±0.03 | 0.68±0.03 | 0.01 | 0.0003 |
| | T carriers | 0.39±0.07 | 0.57±0.06 | 0.74±0.06 | $1.5\times10^{-6}$ | |
| **Total EPA+DHA, g/day** | | Tertile1 | Tertile2 | Tertile3 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| NHS | Non-T carriers | 0.79±0.06 | 0.94±0.05 | 1.08±0.06 | 0.8 | 0.01 |
| | T carriers | 0.72±0.11 | 0.87±0.10 | 1.16±0.11 | 0.02 | |
| HPFS | Non-T carriers | 0.47±0.05 | 0.53±0.05 | 0.49±0.05 | 0.88 | 0.13 |
| | T carriers | 0.23±0.10 | 0.50±0.09 | 0.57±0.10 | 0.16 | |
| WHI | Non-T carriers | 0.39±0.10 | 0.04±0.08 | 0.23±0.10 | 0.42 | 0.27 |
| | T carriers | 0.04±0.18 | 0.28±0.15 | 0.28±0.16 | 0.84 | |
| Pooled[1] | Non-T carriers | 0.57±0.04 | 0.62±0.03 | 0.67±0.04 | 0.65 | 0.005 |
| | T carriers | 0.39±0.07 | 0.60±0.06 | 0.74±0.07 | 0.01 | |

Data are means ± SE for long term changes in BMI.

Total EPA+DHA include food-sourced and supplemental EPA+DHA.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The multiple linear regression model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure Legends**

**Figure 1 Genetic variant of *FADS* rs174570, long chain n-3 PUFAs and fish intakes and long-term BMI changes**

Effect size (ES) (95% CI) values are β coefficients for interaction between the *FADS* variant rs174570 (additive model) and diets from results of the NHS, HPFS, WHI, and SCHS cohorts.

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The multiple linear regression model was used to test the *FADS* variant-diets interaction by including a multiplicative interaction term in the models after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure 2 Genetic association of *FADS* variant rs174570 with long-term BMI change according to long chain n-3 PUFAs and fish intakes**

Pooled-EUR: data from NHS, HPFS, and WHI were pooled.

Pooled Multiethnic: data from NHS, HPFS, WHI and SCHS were pooled.

Data are β coefficients ± SE.

Numbers of participants across three categories (≤1/wk/1~6/wk/≥1/d) of fish intake in the NHS, HPFS, WHI, and SCHS are 1618/8465/1239, 977/5108/748, 894/4675/684, and 752/3935/576, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on BMI, long chain n-3 PUFAs (food sourced EPA+ DHA and total EPA+ DHA (food and supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the WHI, and from 2006 to 2010 in the SCHS, respectively.

The multiple linear regression model was used to test the genetic association of the *FADS* variant (additive model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption. The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for heterogeneity between studies).

**Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS and HPFS are 1698/9625, and 1025/5808, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The multiple linear regression model was used to test the associations of long chain n-3 PUFAs intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The individual participant data from the NHS and HPFS cohorts were pooled.

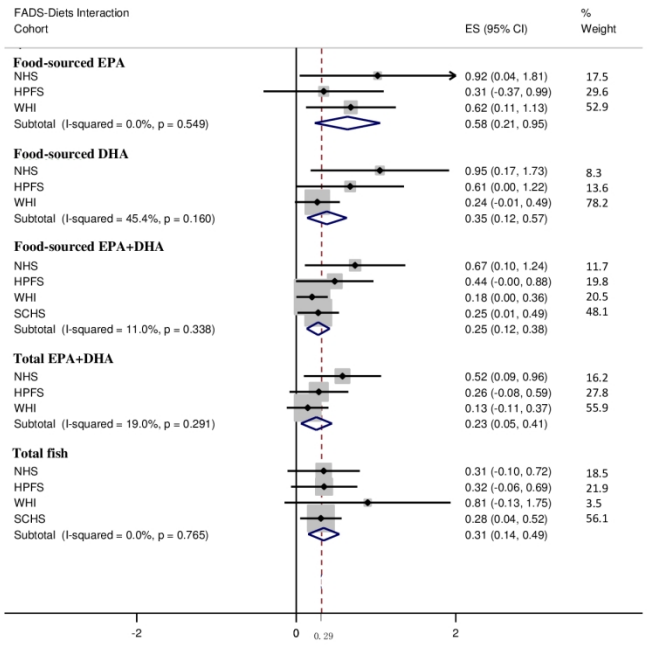
**Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**

Numbers of T carriers/Non-T carriers in the NHS and HPFS are 1698/9625 and 1025/5808, respectively.

Black circles for T allele carriers and open circle for non-T-carriers.

The multiple linear regression model was used to test the associations of and fish intake with long-term changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The individual participant data from the NHS and HPFS cohorts were pooled.

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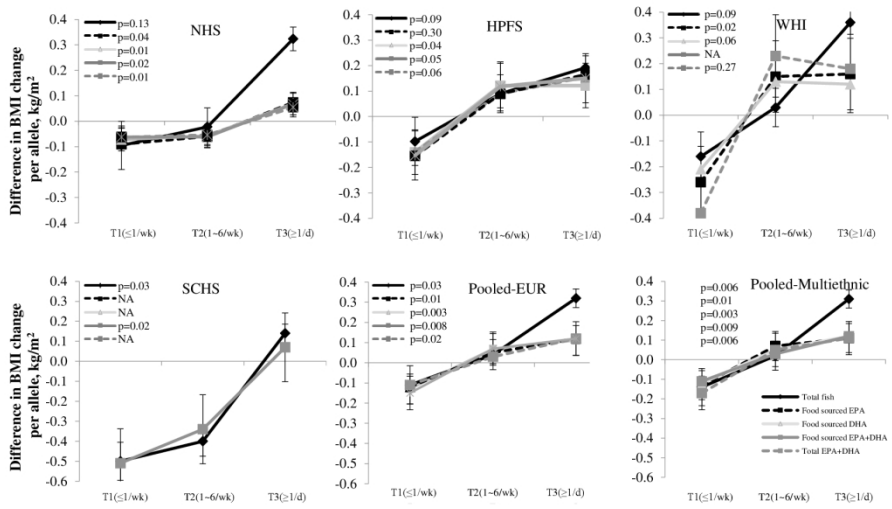


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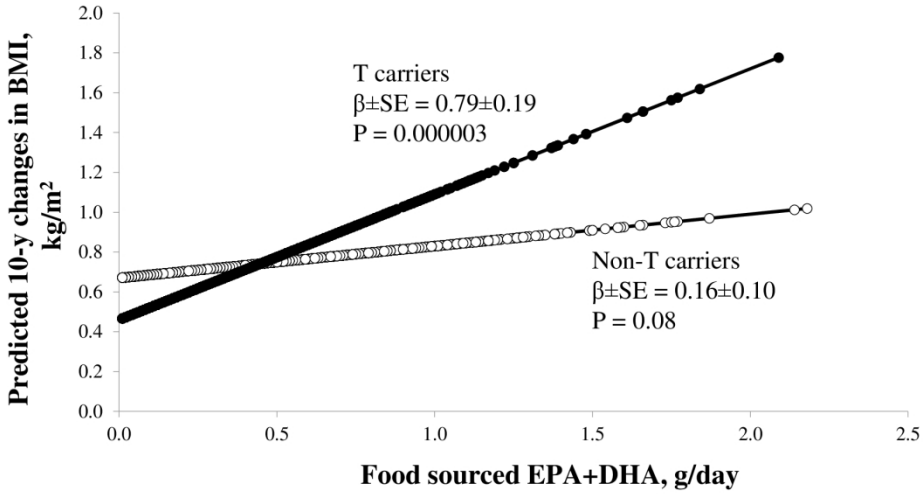


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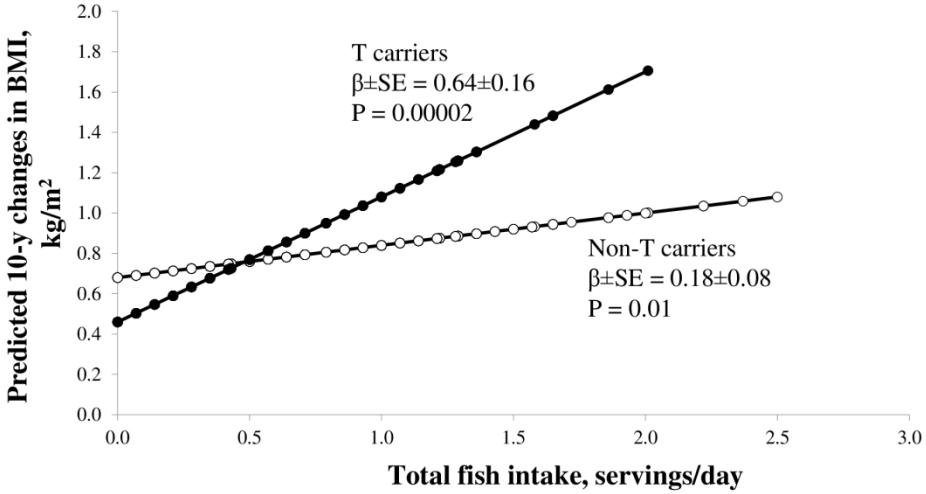


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**Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit**

| Position[1] | Reference SNP identification number | Alleles[2] | DAF | | | | | | | PBS |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | CEU | CHB | GI | NHS | HPFS | WHI | SCHS | |
| chr11:61627960 | rs74771917 | C/T | 0.025 | 0.16 | 0.98 | / | / | / | / | 2.67 |
| chr11:61631510 | rs3168072 | A/T | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61632310 | rs12577276 | A/G | 0.017 | 0.18 | 0.98 | / | / | / | / | 2.64 |
| chr11:61641717 | rs7115739 | G/T | 0.017 | 0.22 | 0.98 | 0.004 | 0.004 | / | / | 2.54 |
| chr11:61624414 | rs174602 | C/T | 0.80 | 0.73 | 0.01 | 0.82 | 0.81 | / | / | 2.11 |
| chr11:61597212 | rs174570 | C/T | 0.16 | 0.34 | 0.99 | 0.15 | 0.15 | 0.14 | 0.35 | 2.06 |

[1]Positions refer to human genome assembly hg19.

[2]Alleles are coded as ancestral/derived states.

PBS, the population branch statistic; DAF, derived allele frequency; CEU, European ancestry; CHB, an Chinese; GI, Greenlandic Inuit

DAFs for each population (CEU, CHB, and GI) and PBS values are reported, along with the genomic position for each SNP.

**Supplemental table 2 Main effect of the *FADS* variants on adiposity in the four cohorts**

| Outcomes (kg/m$^2$) | *FADS* SNPs | NHS | | HPFS | | WHI | | SCHS | | Pooled | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P | Beta ± SE | P |
| Baseline BMI | rs174570 | 0.03 ± 0.10 | 0.733 | -0.05 ± 0.09 | 0.538 | -0.06±0.17 | 0.72 | 0.24±0.08 | 0.002 | 0.08±0.05 | 0.06 |
| Baseline BMI | rs174602 | 0.08 ± 0.10 | 0.418 | -0.05 ± 0.08 | 0.536 | / | | / | | 0.00 ± 0.03 | 0.559 |
| Baseline BMI | rs7115739 | 0.25 ± 0.52 | 0.634 | -0.77 ± 0.43 | 0.077 | / | | / | | -0.35 ± 0.14 | 0.196 |
| Long-term BMI change | rs174570 | -0.05 ± 0.06 | 0.401 | 0.01 ± 0.05 | 0.917 | -0.02±0.09 | 0.77 | -0.02±0.08 | 0.85 | -0.02±0.03 | 0.94 |
| Long-term BMI change | rs174602 | -0.14 ± 0.06 | 0.009 | 0.04 ± 0.05 | 0.413 | / | | / | | -0.04 ± 0.01 | 0.025 |
| Long-term BMI change | rs7115739 | 0.45 ± 0.29 | 0.124 | -0.23 ± 0.26 | 0.359 | / | | / | | 0.06 ± 0.08 | 0.183 |

Long-term BMI change: BMI change from 1990 to 2000.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Effect size (ES) values are β coefficients for relationship between the *FADS* variant rs174570 (additive model) and adiposity.

The general linear model was used to test the genetic association of *FADS* variants with long-term changes in BMI after adjustment for age, source of genotyping data.

**Supplemental table 3 Genetic association of *FADS* variant with long-term changes in body weight according to long chain n-3 PUFAs and fish intakes**

| | Difference in long-term changes in weight, kg | | | P for interaction |
|---|---|---|---|---|
| **Total Fish, serving/day** | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | -0.69±0.64 | -0.13±0.49 | 1.78±1.64 | 0.05 |
| HPFS | -0.99±0.85 | 0.54±0.53 | 1.52±1.69 | 0.12 |
| WHI | -0.22±0.42 | 0.16±0.34 | 1.26±1.57 | 0.13 |
| SCHS | -0.42±0.29 | -0.44±0.28 | 0.20±0.29 | 0.08 |
| Pooled | -0.44±0.22 | -0.10±0.18 | 0.31±0.28 | 0.01 |
| **Food-sourced EPA, g/day** | T1 | T2 | T3 | |
| NHS | -0.77±0.62 | -0.25±0.72 | 0.53±0.64 | 0.06 |
| HPFS | -1.19±0.82 | 0.75±0.73 | 0.72±0.74 | 0.41 |
| WHI | -0.19±0.42 | 0.24±0.47 | 0.14±0.48 | 0.20 |
| Pooled | -0.50±0.32 | 0.24±0.34 | 0.37±0.34 | 0.10 |
| **Food-sourced DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.53±0.62 | -0.39±0.70 | 0.53±0.65 | 0.01 |
| HPFS | -1.06±0.82 | 0.49±0.71 | 0.89±0.76 | 0.09 |
| WHI | -0.20±0.43 | 0.22±0.42 | 0.30±0.50 | 0.26 |
| Pooled | -0.43±0.32 | 0.15±0.32 | 0.49±0.35 | 0.01 |
| **Food-sourced EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.56±0.63 | -0.32±0.68 | 0.49±0.66 | 0.01 |
| HPFS | -1.25±0.83 | 0.68±0.73 | 0.84±0.74 | 0.09 |
| WHI | -0.02±0.43 | 0.16±0.44 | 0.14±0.49 | 0.23 |
| SCHS | -0.47±0.29 | -0.16±0.28 | -0.03±0.29 | 0.10 |

| | | | | |
|---|---|---|---|---|
| Pooled | -0.56±0.25 | -0.09±0.24 | 0.14±0.25 | 0.005 |
| **Total EPA+DHA, g/day** | T1 | T2 | T3 | |
| NHS | -0.58±0.63 | -0.30±0.68 | 0.46±0.66 | 0.02 |
| HPFS | -1.20±0.82 | 0.75±0.70 | 0.86±0.77 | 0.18 |
| WHI | -0.48±0.47 | 0.64±0.43 | 0.04±0.47 | 0.15 |
| Pooled | -0.64±0.34 | 0.45±0.32 | 0.32±0.34 | 0.02 |

Data are β coefficients ± SE.

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Frequency of fish intake: ≤ 1 serving per week, 1~6 servings per week, and 1 serving per day

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the genetic association with long-term changes in body weight according to baseline long chain n-3 PUFAs and fish intakes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from three or four cohorts pooled by means of fixed effects meta-analyses (if P ≥ 0.05 for heterogeneity between studies).

**Supplemental table 4 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight according to *FADS* genotypes**

| Cohorts | | Long-term changes in weight, kg | | | P for trend |
|---|---|---|---|---|---|
| **Total Fish, serving/day** | | ≤1/wk | 1~6/wk | ≥1/d | |
| NHS | Non T carriers | 4.91±0.34 | 5.78±0.24 | 7.00±0.79 | 0.008 |
| | T carriers | 4.45±0.61 | 5.64±0.46 | 9.26±1.44 | 0.001 |
| HPFS | Non T carriers | 0.44±0.06 | 0.52±0.04 | 0.56±0.12 | 0.99 |
| | T carriers | 0.25±0.10 | 0.53±0.07 | 0.76±0.21 | 0.08 |
| WHI | Non T carriers | -0.25±0.23 | -0.43±0.18 | -0.91±0.93 | 0.50 |
| | T carriers | -0.56±0.37 | -0.25±0.28 | 1.30±1.71 | 0.13 |
| SCHS | Non T carriers | -3.15±0.23 | -3.50±0.21 | -3.38±0.21 | 0.48 |
| | T carriers | -3.68±0.20 | -3.41±0.19 | -3.34±0.20 | 0.16 |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.89±0.33 | 5.89±0.33 | 5.95±0.33 | 0.24 |
| | T carriers | 4.45±0.59 | 5.52±0.63 | 6.46±0.61 | 0.34 |
| HPFS | Non T carriers | 0.50±0.05 | 0.54±0.05 | 0.45±0.05 | 0.15 |
| | T carriers | 0.29±0.10 | 0.54±0.09 | 0.52±0.09 | 0.66 |
| WHI | Non T carriers | -0.30±0.25 | -0.54±0.24 | -0.29±0.24 | 0.42 |
| | T carriers | -0.51±0.39 | -0.36±0.37 | -0.15±0.38 | 0.14 |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.78±0.33 | 5.56±0.34 | 6.32±0.33 | 0.14 |
| | T carriers | 4.50±0.60 | 5.07±0.63 | 6.77±0.61 | 0.004 |
| HPFS | Non T carriers | 0.48±0.05 | 0.54±0.05 | 0.46±0.06 | 0.40 |
| | T carriers | 0.27±0.10 | 0.50±0.09 | 0.59±0.09 | 0.15 |
| WHI | Non T carriers | -0.41±0.25 | -0.25±0.24 | -0.45±0.25 | 0.51 |

| | | | | | |
|---|---|---|---|---|---|
| | T carriers | -0.71±0.39 | -0.15±0.37 | -0.16±0.39 | 0.18 |
| **Food-sourced EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.69±0.34 | 5.45±0.33 | 6.51±0.33 | 0.02 |
| | T carriers | 4.44±0.61 | 5.00±0.61 | 6.92±0.61 | 0.0003 |
| HPFS | Non T carriers | 0.48±0.05 | 0.53±0.05 | 0.47±0.05 | 0.93 |
| | T carriers | 0.26±0.10 | 0.49±0.09 | 0.59±0.09 | 0.08 |
| WHI | Non T carriers | -0.44±0.24 | -0.23±0.23 | -0.43±0.24 | 0.47 |
| | T carriers | -0.52±0.38 | -0.15±0.37 | -0.33±0.38 | 0.15 |
| SCHS | Non T carriers | -3.44±0.21 | -3.58±0.22 | -3.05±0.21 | 0.89 |
| | T carriers | -3.73±0.19 | -3.57±0.19 | -3.12±0.19 | 0.12 |
| **Total EPA+DHA, g/day** | | T1 | T2 | T3 | |
| NHS | Non T carriers | 4.74±0.34 | 5.55±0.32 | 6.36±0.34 | 0.81 |
| | T carriers | 4.49±0.61 | 5.16±0.60 | 6.70±0.61 | 0.03 |
| HPFS | Non T carriers | 0.49±0.05 | 0.53±0.05 | 0.47±0.06 | 0.24 |
| | T carriers | 0.26±0.10 | 0.51±0.09 | 0.58±0.09 | 0.33 |
| WHI | Non T carriers | 0.32±0.27 | -0.84±0.23 | -0.60±0.28 | 0.19 |
| | T carriers | -0.26±0.45 | -0.21±0.37 | -0.02±0.11 | 0.08 |

Data on baseline fish and fatty acids consumptions were assessed in 1990 (NHS) and 1990 (HPFS).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on body weight were assessed in 1990 and 2000 in NHS and 1990 and 2000 in HPFS.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in body weight by *FADS* genotypes after adjustment for age, source of genotyping data, baseline body weight, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

Data from two cohorts pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies) or random effects meta-analyses (if $P < 0.05$ for heterogeneity between studies).

**Supplemental Table 5 Associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI according to *FADS* genotypes**

| Diets | *FADS* genotypes | Long chain n-3 PUFAs and fish intakes | | | P for trend | P for interaction* |
|---|---|---|---|---|---|---|
| | | Categories of diets | | | | |
| **Food-sourced EPA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.82±0.06 | 1.00±0.06 | 1.01±0.06 | 0.24 | 0.05 |
| | T carriers | 0.72±0.10 | 0.94±0.11 | 1.10±0.11 | 0.29 | |
| HPFS | Non-T carriers | 0.48±0.05 | 0.54±0.05 | 0.47±0.05 | 0.72 | 0.37 |
| | T carriers | 0.23±0.11 | 0.54±0.10 | 0.52±0.09 | 0.45 | |
| WHI | Non-T carriers | 0.10±0.09 | 0.09±0.09 | 0.45±0.09 | 0.21 | 0.02 |
| | T carriers | -0.08±0.15 | 0.23±0.15 | 0.46±0.15 | 0.003 | |
| Pooled | Non-T carriers | 0.54±0.04 | 0.63±0.04 | 0.65±0.04 | 0.35 | 0.01 |
| | T carriers | 0.39±0.07 | 0.63±0.07 | 0.70±0.06 | 0.01 | |
| **Food-sourced DHA, g/day** | | T1 | T2 | T3 | | |
| NHS | Non-T carriers | 0.80±0.06 | 0.94±0.06 | 1.08±0.06 | 0.14 | 0.009 |
| | T carriers | 0.74±0.10 | 0.83±0.11 | 1.17±0.10 | 0.002 | |
| HPFS | Non-T carriers | 0.46±0.05 | 0.54±0.05 | 0.49±0.05 | 0.99 | 0.05 |
| | T carriers | 0.24±0.10 | 0.49±0.10 | 0.58±0.09 | 0.05 | |
| WHI | Non-T carriers | 0.03±0.09 | 0.20±0.09 | 0.42±0.09 | 0.03 | 0.06 |
| | T carriers | -0.10±0.15 | 0.33±0.15 | 0.39±0.15 | 0.006 | |
| Pooled | Non-T carriers | 0.51±0.04 | 0.63±0.04 | 0.68±0.04 | 0.1 | 0.002 |
| | T carriers | 0.38±0.06 | 0.58±0.07 | 0.77±0.06 | $7\times10^{-4}$ | |

Data are means ± SE.

[1]P for interaction was generated from dominant model of *FADS* rs174570 (CC vs CT+TT).

Numbers of T carriers/Non-T carriers in the NHS, HPFS, WHI, and SCHS are 1698/9625, 1025/5808, 876/5378, and 1842/3422, respectively.

Data on BMI, long chain n-3 PUFAs consumptions were assessed at baseline in the NHS (1990), the HPFS (1990), the WHI (1994-1998), and the SCHS (1993-1998), respectively.

Data on follow-up BMI was assessed in 2000 in the NHS and HPFS, in the sixth follow-up year in the

WHI, and from 2006 to 2010 in the SCHS, respectively.

Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in the four cohorts, respectively.

The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food consumption.

The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).

Registration: www. clinicaltrials.gov. Registration ID: NCT03348566

**TABLE 1. STREGA reporting recommendations, extended from STROBE Statement**

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Title and Abstract** | 1 | (a) Indicate the study's design with a commonly used term in the title or the abstract. **(p. 3)** | |
| | | (b) Provide in the abstract an informative and balanced summary of what was done and what was found. **(p. 3)** | |
| **Introduction** | | | |
| *Background rationale* | 2 | Explain the scientific background and rationale for the investigation being reported. **(p. 4)** | |
| *Objectives* | 3 | State specific objectives, including any pre-specified hypotheses. | ***State if the study is the first report of a genetic association, a replication effort, or both.*** **(p. 3)** |
| **Methods** | | | |
| *Study design* | 4 | Present key elements of study design early in the paper. **(p. 5)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Setting* | 5 | Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. **(p. 3)** | |
| *Participants* | 6 | (a) **Cohort study –** Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. **(p. 5)**<br><br>**Case-control study –** Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls.<br><br>**Cross-sectional study –** Give the eligibility criteria, and the sources and methods of selection of participants. | ***Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant***. **(p. 5)** |
| | | (b) **Cohort study –** For matched studies, give matching criteria and number of exposed and unexposed.<br><br>**Case-control study –** For matched studies, give matching criteria and the number of controls per case. | |
| *Variables* | 7 | *(a)* Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. **(p. 5)** | ***(b) Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin).*** **(p. 5)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Data sources measurement* | 8* | *(a)* For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. **(p. 5)** | ***(b) Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory/centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches.*** **(p. 5)** |
| *Bias* | 9 | *(a)* Describe any efforts to address potential sources of bias. **(p. 5 &6)** | ***(b) For quantitative outcome variables, specify if any investigation of potential bias resulting from pharmacotherapy was undertaken. If relevant, describe the nature and magnitude of the potential bias, and explain what approach was used to deal*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***with this.*** **(p. 5 &6)** |
| *Study size* | 10 | Explain how the study size was arrived at. **(p. 5 &6)** | |
| *Quantitative variables* | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. **(p. 7)** | ***If applicable, describe how effects of treatment were dealt with.*** **(p. 7)** |
| *Statistical methods* | 12 | (a) Describe all statistical methods, including those used to control for confounding. **(p. 7-9)** | ***State software version used and options (or settings) chosen.*** **(p. 9)** |
| | | (b) Describe any methods used to examine subgroups and interactions. **(p. 9)** | |
| | | (c) Explain how missing data were addressed. **(p. 9)** | |
| | | (d) **Cohort study –** If applicable, explain how loss to follow-up was addressed. **(p. 9)**<br><br>**Case-control study –** If applicable, explain how matching of cases and controls was addressed.<br><br>**Cross-sectional study –** If applicable, describe analytical methods taking account of sampling strategy. | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | (e) Describe any sensitivity analyses. **(p. 9)** | |
| | | Hardy-Weinberg equilibrium was tested using Chi-square test. **(p. 9)** | ***(f) State whether Hardy-Weinberg equilibrium was considered and, if so, how.*** |
| | | We assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1, GG=0). **(p. 8&9)** | ***(g) Describe any methods used for inferring genotypes or haplotypes.*** |
| | | | ***(h) Describe any methods used to assess or address population stratification.*** **(p. 9)** |
| | | | ***(i) Describe any methods used to address multiple comparisons or to control risk of false positive findings.*** **(p. 9)** |
| | | | ***(j) Describe any methods used to address and correct for relatedness among subjects*****(p. 9)** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| **Results** | | | |
| *Participants* | 13* | (a) Report the numbers of individuals at each stage of the study – e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. **(p. 10)** | ***Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful.*** **(p. 10)** |
| | | (b) Give reasons for non-participation at each stage. **(p. 10)** | |
| | | (c) Consider use of a flow diagram. **(p. 10)** | |
| *Descriptive data* | 14* | (a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. **(p. 10)** | ***Consider giving information by genotype.*** **(p. 10)** |
| | | (b) Indicate the number of participants with missing data for each variable of interest. **(p. 10)** | |
| | | (c) **Cohort study –** Summarize follow-up time, e.g. average and total amount. **(p. 10)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Outcome data* | 15 * | **Cohort study-**Report numbers of outcome events or summary measures over time. | ***Report outcomes (phenotypes) for each genotype category over time*** |
| | | **Case-control study –** Report numbers in each exposure category, or summary measures of exposure. | ***Report numbers in each genotype category*** |
| | | **Cross-sectional study –** Report numbers of outcome events or summary measures. | ***Report outcomes (phenotypes) for each genotype category*** |
| *Main results* | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence intervals). Make clear which confounders were adjusted for and why they were included. **(p. 10)** | |
| | | (b) Report category boundaries when continuous variables were categorized. **(p. 10)** | |
| | | (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. **(p. 10)** | |
| | | | ***(d) Report results of any adjustments for multiple*** |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| | | | ***comparisons.* (p. 10)** |
| *Other analyses* | 17 | (a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. **(p. 10)** | |
| | | | ***(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken.* (p. 10)** |
| | | | ***(c) If detailed results are available elsewhere, state how they can be accessed.* (p. 10)** |
| **Discussion** | | | |
| *Key results* | 18 | Summarize key results with reference to study objectives. **(p. 11)** | |
| *Limitations* | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. **(p. 11)** | |

| Item | Item number | STROBE Guideline | Extension for Genetic Association Studies (STREGA) |
|---|---|---|---|
| *Interpretation* | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. **(p. 11)** | |
| *Generalizability* | 21 | Discuss the generalizability (external validity) of the study results. **(p. 11)** | |
| **Other Information** | | | |
| *Funding* | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. **(p. 14)** | |

STREGA = STrengthening the REporting of Genetic Association studies; STROBE = STtrengthening the Reporting of Observational Studies in Epidemiology.

* Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.