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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-022877
Article Type:	Research
Date Submitted by the Author:	13-Mar-2018
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Keywords:	NUTRITION & DIETETICS, GENETICS, EPIDEMIOLOGY, obesity, gene-diet interaction

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

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

Running title: Genetic adaptation, fish, and weight change

Word count: 3420

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1 Abstract

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

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

7 **Setting:** Population based study

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

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

12 **Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the
13 *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in
14 combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for
15 interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS*
16 rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake
17 also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P
18 for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association
19 with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 kg/m² per g, P = 3×10⁻⁵) than
20 the rs174570 non-T carriers (beta=0.16 kg/m² per g, P = 0.08). Similar results were observed for fish
21 intake.

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

24 Article summary

25 Strengths and limitations of this study

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3 27 • This is the first study with consistent results from 4 well-established prospective cohorts of different
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5 28 racial populations such as Caucasians with European ancestry and Singapore Chinese.
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7 29 • The consistent results from these independent cohorts demonstrated the robustness of our findings.
8
9 30 • Unlike cross-sectional studies, our prospective analysis minimized the potential reverse causa.
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13 14 32 **Introduction**

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16 33 Diet rich in fish and marine fatty acids especially long chain n-3 polyunsaturated fatty acids (PUFAs) has
17
18 34 shown beneficial effects on cardiometabolic health (1, 2), however, data from population studies on the
19
20 35 associations between such diet and body weight are inconsistent (3, 4). Emerging evidence suggests genetic
21
22 36 variations may play a role in modifying the relation between dietary factors and body weight (5-7).
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26 38 A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs. The
27
28 39 strong signals locate in a cluster of fatty acid desaturases gene (*FADS*) that determine PUFAs levels (8).
29
30 40 People living in the Arctic region have been found to be genetically prone to develop obesity (9, 10) as
31
32 41 survival strength for energy storage(11, 12). Interestingly, the identified *FADS* genetic signatures of diet
33
34 42 adaptation have been also related to adiposity in the Inuit population (8). Of note, due to long-standing
35
36 43 selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various
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38 44 populations such as Europeans and Asians (13), in coincidence with varying levels of fish/marine fatty
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40 45 acids consumption, and adiposity patterns in these populations (14). We therefore hypothesized that the
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42 46 genetic signatures might interact with fish and marine PUFAs intakes on body weight (13).
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47 48 The present study tested the interactions between n-3 PUFA and fish intakes and variants in *FADS* gene
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49 49 cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in
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51 50 body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health
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53 51 Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts
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55 52 the Women's Health Initiative (WHI) and the Singapore Chinese Health Study (SCHS).
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45 54 **Methods**6
7 55 **Discovery cohorts**8
9 56 *The Nurses' Health Study*

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11 57 The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were
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13 58 recruited to complete a baseline questionnaire about their lifestyle and medical history (15). The current
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15 59 analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed
16
17 60 consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs
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19 61 genotyping and imputation when performed have been described in detail elsewhere (16-22). All
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21 62 participants with genotyping data available based on previous GWASs were included (16-21). The study
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23 63 protocol was approved by the institutional review boards of Brigham and Women's Hospital and Harvard
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25 64 School of Public Health.

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2930 66 *The Health Professionals Follow-up Study*

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32 67 The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians,
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34 68 optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants
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36 69 returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet (23). In the current
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38 70 analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our
39
40 71 analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all
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42 72 participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation
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44 73 when performed have been described in detail elsewhere (16-22). All participants with genotyping data
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46 74 available based on previous GWASs were included (16-21). The study protocol was approved by the
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48 75 institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.

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5253 77 **Replication cohorts**54 78 *The Women's Health Initiative (WHI)*
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3 79 The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National Heart,
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5 80 Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast and
6
7 81 colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI study
8
9 82 is presented elsewhere (24, 25). For the analyses, we included 6,254 Caucasians women with European
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11 83 ancestry who participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year
12
13 84 follow-up and for whom DNA was measured. The genomic DNA samples were processed according to
14
15 85 standard Affymetrix procedures for processing of the assay. The Affymetrix Human SNP Array 6.0
16
17 86 (Affymetrix®, Inc Santa Clara, CA) was used for genome wide SNP genotyping. Human subjects review
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19 87 committees at each participating institution reviewed and approved the study, and all women gave written
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21 88 informed consent.
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26 90 ***The Singapore Chinese Health Study (SCHS) cohort***

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28 91 The design of Singapore Chinese Health Study (SCHS) has been previously described in detail (26).
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30 92 Briefly, between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in
31
32 93 Singapore were enrolled into the cohort study (27). Two follow-up interviews were conducted via
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34 94 telephone among surviving participants between 1999 and 2004, and again between 2006 and 2010 to
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36 95 update information on body weight, selected lifestyle factors and medical history. All participants have
37
38 96 given informed consent. The study was approved by the Institutional Review Boards of the National
39
40 97 University of Singapore and the University of Pittsburgh, and the study was carried out in accordance with
41
42 98 the approved guidelines. Genome-wide genotyping for 2615 incident diabetes cases and 2615 matched
43
44 99 controls was performed at the Genome Institute of Singapore according to the manufacturer's
45
46 100 recommendations using an Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the
47
48 101 Affymetrix Corporation (28). Genome-wide genotyping for 717 incident myocardial infarction (MI) cases
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50 102 and 644 controls was performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip
51
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53 103 (29).
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3 104 Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had
4
5 105 both weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.
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9 107 **Assessment of measures of body mass index**

11 108 Height and body weight were assessed by questionnaire at baseline, and weight information was requested
12
13 109 on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly
14
15 110 measured values ($r=0.97$ in HPFS and NHS) in a validation study (30). BMI was calculated as body weight
16
17 111 (kg)/height (m^2). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS
18
19 112 and HPFS cohorts (31), and from baseline (1993) to sixth year follow-up in the WHI (24, 25), and from
20
21 113 baseline (1998) to second follow-up (2004) in the SCHS.
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26 115 **Assessment of diets and other covariates**

28 116 Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4
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30 117 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried
31
32 118 food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires
33
34 119 (FFQ) in the NHS and HPFS (32, 33). A 165-item validated semi-quantitative FFQ was used to collect
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36 120 dietary data and supplemental use of fish oil in the SCHS (27). Dietary data and supplemental use of fish
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38 121 oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI (34). Alternate
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40 122 health eating index was previously calculated in the NHS, HPFS (35), WHI, and SCHS respectively.
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42 123 Physical activity was expressed as metabolic equivalents per week by incorporating the reported time spent
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44 124 on various activities, and the intensity level of each activity. The validity of the self-reported physical
45
46 125 activity data has been described previously in the NHS and HPFS (36). In the WHI, an estimated metabolic
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48 126 equivalent (MET) level for each type of activity was assigned from a compendium of activities
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50 127 (37). Physical activity was assessed using eight continuous categories ranging from never to 31 hours or
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52 128 more in an average week spent doing strenuous sports; vigorous work; and moderate activities in the
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54 129 SCHS (26).
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131 **The *FADS* variants selection and genotyping**

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

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139 **Patient and Public Involvement**

140 patients and or public were not involved.

141

142 **Statistical analyses**

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

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3 156 heterogeneity between studies). All reported P values are nominal and two sided. Statistical analyses were
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5 157 performed in SAS 9.3 (SAS Institute, Cary, NC, USA).
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9 159 **Results**

11 160 **Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts**

13 161 **Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts.

15 162 The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic
17 163 data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The
19 164 distribution of the *FADS* genetic variants in the 4 cohorts was shown in **Supplemental table 1**. We did not
21 165 observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, BMI
23 166 at endpoint, and long-term changes in BMI in three US cohorts ($P > 0.05$), however, we found that the
25 167 *FADS* genotype was significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental**
27 168 **table 2**).
29

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32 170 **Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes**

34 171 We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes
36 172 of various sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS*
38 173 rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed
40 174 significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid
42 175 (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with
44 176 long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined cohorts)
46 177 (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the
48 178 SCHS cohort (P for interaction = 0.02).
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53 180 The pooled analyses of the 3 US (Caucasian) populations or all 4 cohorts showed that high intakes of
55 181 food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly

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3 182 accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No
4
5 183 significant heterogeneity in the interaction effect was observed among these cohorts. Differences in
6
7 184 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)
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9 185 kg/m² across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

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13 187 Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for
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15 188 interaction=0.003) showed similar interaction patterns; and the interactions remained significant when
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17 189 supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

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22 191 In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS*
23
24 192 genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for
25
26 193 interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results
27
28 194 (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071),
29
30 195 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m² across three categories (≤ 1 serving/week, 1~6
31
32 196 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

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35 197
36
37 198 In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster
38
39 199 (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI
40
41 200 in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed
42
43 201 (**Supplemental table 3 & 4**).

202 203 **Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

204 We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had
205 significantly greater increase of BMI (mean \pm SE = 0.74 \pm 0.06, kg/m²) than did those who consumed the
206 lowest (T1) (mean \pm SE = 0.39 \pm 0.07, kg/m²) among the T allele carriers, whereas the corresponding BMI
207 changes were 0.68 \pm 0.03 kg/m² and 0.49 \pm 0.03 kg/m², respectively, among the non-T carriers in 4 cohorts

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3 208 combined (**Table 2 & Supplemental table 5**). Similarly, we observed different associations between fish
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5 209 intake and BMI changes among the T allele carriers ($P = 1.5 \times 10^{-6}$) and non-carriers ($P = 0.01$) in the
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7 210 pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed
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9 211 among the cohorts.
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12 212
13 213 **Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake
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15 214 according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts
16
17 215 consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term
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19 216 changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled
20
21 217 results, the beta \pm SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**)
22
23 218 with long-term changes in BMI were 0.79 ± 0.19 kg/m² per g ($P = 0.000003$) and 0.64 ± 0.16 kg/m² per
24
25 219 serving ($P = 0.00002$) among the T carriers, and whereas the corresponding beta \pm SE were 0.16 ± 0.10
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27 220 kg/m² per g ($P = 0.08$) and 0.18 ± 0.08 kg/m² per serving ($P = 0.01$) among the non-T carriers.
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32 222 **Discussion**

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34 223 In 4 large prospective cohorts of the US and Chinese populations, we found reproducible evidence that long
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36 224 chain n-3 PUFAs and fish intakes accentuated the genetic association of the *FADS* genotypes with
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38 225 long-term changes in BMI. In addition, our results showed that the *FADS* rs174570 T allele carriers gained
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40 226 more weight than the non-carriers when they had higher long chain n-3 PUFAs and fish intakes.
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45 228 Compelling evidence has shown that fish- and long chain n-3 PUFAs-rich diet are beneficial on
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47 229 improvement of cardiometabolic health (1, 2). However, large prospective cohort studies examining the
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49 230 associations of fish or n-3 PUFAs with body weight and obesity risk generated conflicting results (3, 4).
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51 231 In addition, several randomized controlled trials (RCTs) supported the protective effects of fish, fish oils,
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53 232 or/and n-3 PUFAs intake on weight-loss (38-40), but the benefit was not evident in other trials (41-43). The
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3 233 results from the current study lent support to our hypothesis that the heterogeneous associations between
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5 234 fish or n-3 PUFAs and body weight might be at least partly due to gene-diet interactions.
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9 236 We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were
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11 237 stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle,
12
13 238 the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among
14
15 239 individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit,
16
17 240 who had high fish/n-3 PUFAs intakes (8). The high frequency of T allele in Inuit reflects genetic adaptation
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19 241 to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet
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21 242 adaptation have been also related to adiposity in this population. Our data indicated that the signature allele
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23 243 (T) was related differently with weight changes (decrease or increase), depending on the levels of fish/n-3
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25 244 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to
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27 245 greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body
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29 246 weight when they are exposed to diet low in fish and n-3 PUFAs.
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34 248 We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction
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36 249 patterns in relation to long-term changes in BMI; and the interactions also remained significant when
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38 250 supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of
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40 251 fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as
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42 252 Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is
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44 253 robust for fatty acids from various sources.
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49 255 The mechanisms underlying the observed gene-diet interactions remain unclear, however, such
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51 256 interactions are biologically plausible. It has long been known that the *FADS* genes such as
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53 257 *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important
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55 258 rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic
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3 259 acid (n-6) and α -linolenic acid (n-3) (44). The selected allele of *FADS* rs174570 is significantly associated
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5 260 with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway (44). In
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7 261 addition, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and
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9 262 function (45). In addition, storage of energy and body fat is very important for the Arctic population, who
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11 263 are regularly exposed to the extreme low temperature and fishes rich in n-3 PUFAs (11, 12). Under natural
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13 264 selection, these people are genetically prone to high fish intake to keep body fat (9, 10). Therefore, it's not
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15 265 surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people
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17 266 carrying selective *FADS* signature (46, 47). Our findings support the view that extra n-3 PUFAs may not
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19 267 do much benefit at all for Europeans with selective *FADS* signature(8, 13).
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22 268 **Strengths**

23
24 269 Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results
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26 270 from 4 well-established prospective cohorts of different racial populations such as Caucasians with
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28 271 European ancestry and Singapore Chinese. The consistent results from these independent cohorts
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30 272 demonstrated the robustness of our findings. Other major strengths include the prospective design, the large
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32 273 sample size, use of long-term change of BMI, and replication of the results. Unlike cross-sectional studies,
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34 274 our prospective analysis minimized the potential reverse causality (48).
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37 275 **Limitations**

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39 276 However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity
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41 277 measures were self-reported, measurement errors in these variables are inevitable; however, the food
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43 278 frequency questionnaires and adiposity measures data have been well validated (27, 30, 32-34). Second,
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45 279 confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for
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47 280 multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish
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49 281 consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic
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51 282 data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control
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53 283 studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the
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55 284 different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might
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3 285 introduce bias in the present analyses. Finally, the participants included in our study were middle aged and
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5 286 older adults of Caucasians with European ancestry in the US and Chinese in Singapore, and it is unknown
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7 287 whether our findings could be generalized to other demographic or ethnic groups.
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9 288 **Conclusions**

10
11 289 In summary, our data provides reproducible evidence from 4 multiethnic cohorts that high long chain n-3
12
13 290 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings
14
15 291 emphasize the importance of considering precision nutritional interventions on prevention and treatment
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17 292 of obesity.
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22 294 **Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH
23
24 295 provided statistical expertise. TW, YH, DS, LH, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM,
25
26 296 HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors
27
28 297 contributed to the interpretation of the results and critical revision of the manuscript for important
29
30 298 intellectual content and approved the final version of the manuscript.

31
32 299 We acknowledge Dr. Gary C. Curhan's contribution to the genetic data.
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35 300

36 301 **Funding**

37
38
39 302 This study was supported by grants HL126024, HL034594, DK100383, DK091718, HL071981,
40
41 303 HL073168, CA87969, CA49449, CA055075, HL34594, HL088521, U01HG004399, DK080140,
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43 304 P30DK46200, U01CA137088, U54CA155626, DK58845, DK098311, U01HG004728, EY015473,
44
45 305 CA134958, DK70756 and DK46200 from the National Institutes of Health, with additional support for
46
47 306 genotyping from Merck Research Laboratories, North Wales, PA. LQ is a recipient of the American Heart
48
49 307 Association Scientist Development Award (0730094N). LRP is supported by the Arthur Ashley Williams
50
51 308 Foundation and a Harvard Ophthalmology Scholar Award (Harvard Medical School) from the Harvard
52
53 309 Glaucoma Center of Excellence. ATC is a Damon Runyon Cancer Foundation Clinical Investigator. The
54
55 310 SCHS study is supported by the National Institutes of Health, USA (NCI R01 CA144034, UM1

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3 311 CA182876, R01 DK080720), the Singapore National Medical Research Council (NMRC 1270/2010), the
4
5 312 HUI-CREATE Programme of the National Research Foundation, Singapore (Project Number 370062002)
6
7 313 and Biomedical Research Council, Singapore. The funding sources had no role in the design or conduct of
8
9 314 the study; collection, management, analysis, and interpretation of the data; or preparation, review, or
10
11 315 approval of the manuscript.
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13 316
14
15 317 **Declaration of interests:** All authors have no conflict of interest to declare. No support from any
16
17 318 organization for the submitted work; no financial relationships with any organizations that might have an
18
19 319 interest in the submitted work in the previous three years, no other relationship or activity that could
20
21 320 appear to have influenced the submitted work.
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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	/

¹Plus-minus values are means ± SD. ²Hours per week of moderate activity in the SCHS. ³Glasses 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	<i>FADS</i> 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	2×10 ⁻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	1.5×10 ⁻⁶	
Total EPA+DHA, g/day		T1	T2	T3		

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2							
3	NHS	Non-T carriers	0.79±0.06	0.94±0.05	1.08±0.06	0.8	0.01
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5		T carriers	0.72±0.11	0.87±0.10	1.16±0.11	0.02	
6							
7	HPFS	Non-T carriers	0.47±0.05	0.53±0.05	0.49±0.05	0.88	0.13
8							
9		T carriers	0.23±0.10	0.50±0.09	0.57±0.10	0.16	
10							
11	WHI	Non-T carriers	0.39±0.10	0.04±0.08	0.23±0.10	0.42	0.27
12							
13		T carriers	0.04±0.18	0.28±0.15	0.28±0.16	0.84	
14							
15	Pooled ¹	Non-T carriers	0.57±0.04	0.62±0.03	0.67±0.04	0.65	0.005
16							
17		T carriers	0.39±0.07	0.60±0.06	0.74±0.07	0.01	

Data are means ± SE.

¹P 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 \pm SE.

Numbers of participants across three categories ($\leq 1/\text{wk}$ / $1\sim 6/\text{wk}$ / $\geq 1/\text{d}$) 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.

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

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

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

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15 The general linear model was used to test the genetic association of the *FADS* variant (additive model)
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17 with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment
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19 for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy
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21 intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food
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23 consumption. The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for
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25 heterogeneity between studies).
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31 **Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS***
32
33 **genotypes**

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35 Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and
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37 876/5378, respectively.

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

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41 The general linear model was used to test the associations of long chain n-3 PUFAs intake with long-term
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43 changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data,
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45 baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index,
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47 television watching, sugar sweetened beverage, fried food consumption.
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50 The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts
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52 was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).
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3 **Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**
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5 Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and
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7 876/5378, respectively.
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9 Black circles for T allele carriers and open circle for non-T-carriers.
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11 The general linear model was used to test the associations of and fish intake with long-term changes in
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13 BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI,
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15 smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television
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17 watching, sugar sweetened beverage, fried food consumption.
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19 The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts
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21 was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).
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Figure 1

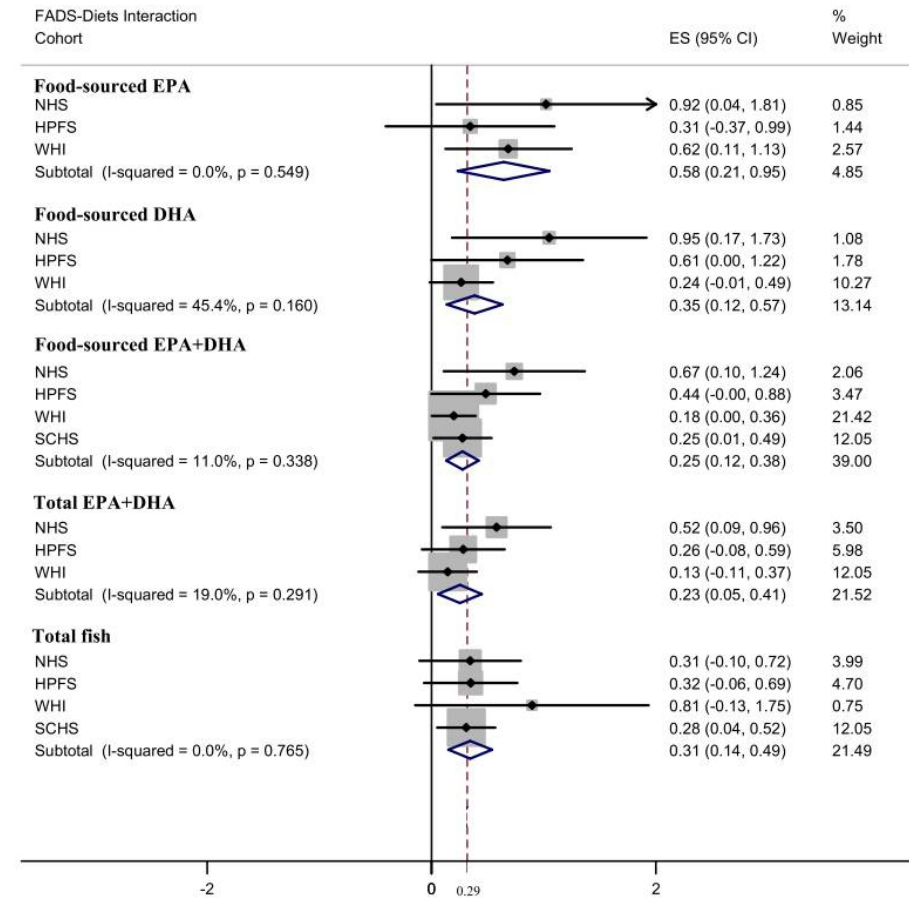


Figure 1

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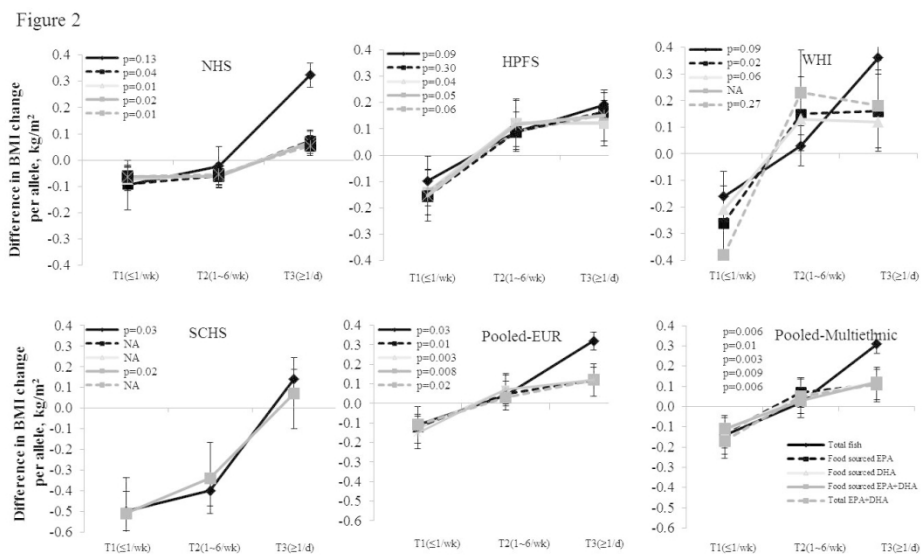


Figure 1

245x138mm (150 x 150 DPI)

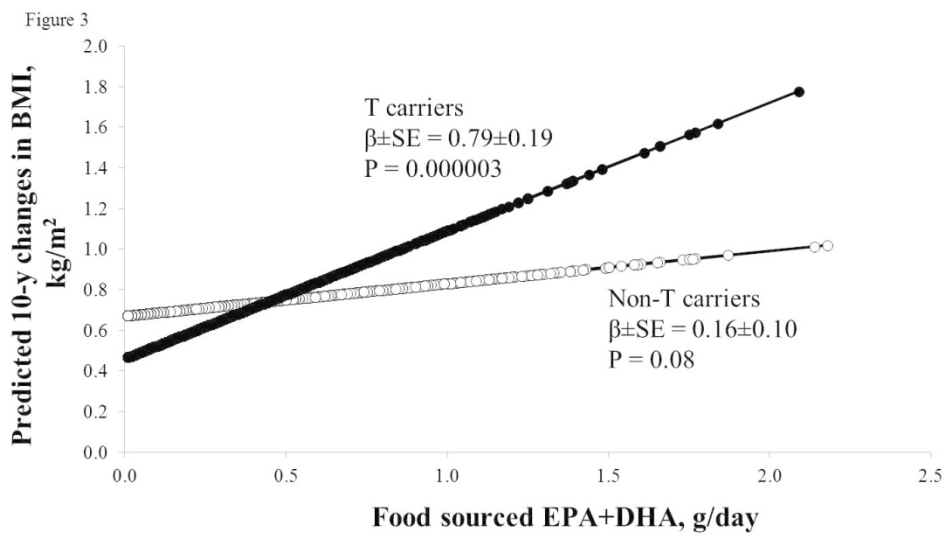


Figure 3

248x140mm (150 x 150 DPI)

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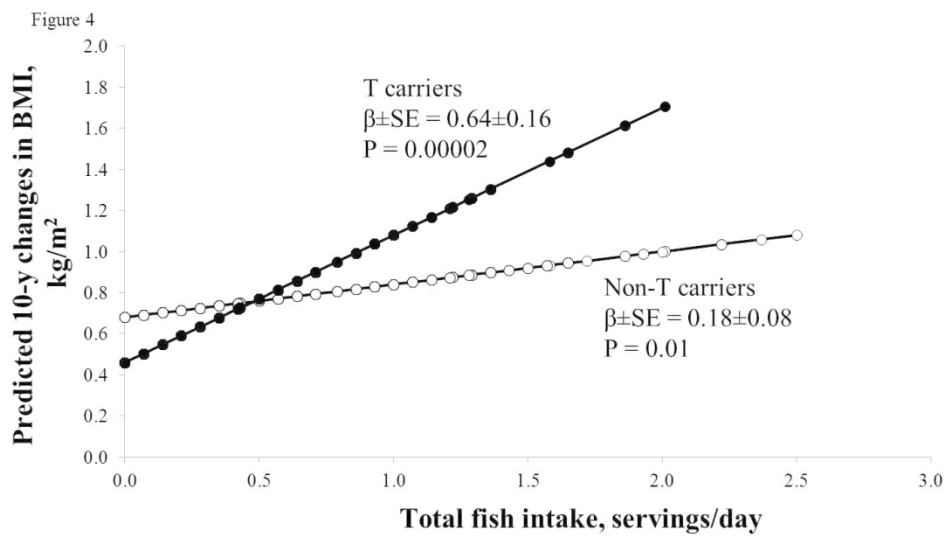


Figure 4
249x142mm (150 x 150 DPI)

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

Position ¹	Reference		DAF							PBS
	SNP identification number	Alleles ²	CEU	CHB	GI	NHS	HPFS	WHI	SCHS	
			chr11:61627960	rs74771917	C/T	0.025	0.16	0.98	/	
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

¹Positions refer to human genome assembly hg19.

²Alleles 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)	<i>FADS</i> 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,			P for interaction
	kg			
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 \pm 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: \leq 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 $P \geq 0.05$ 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	<i>FADS</i> 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	7×10 ⁻⁴	

Data are means ± SE.

¹P 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

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

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

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

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

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24 Registration: [www. clinicaltrials.gov](http://www.clinicaltrials.gov). Registration ID: NCT03348566
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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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (p. 5) (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount) (p. 10)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time (p. 10) <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure (p. 10) <i>Cross-sectional study</i> —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)
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*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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-022877.R1
Article Type:	Research
Date Submitted by the Author:	17-Aug-2018
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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

SCHOLARONE™
Manuscripts

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3 **Fish and marine fatty acids interacted with genetic variants of *FADS* gene in influencing long-term**
4 **weight gain**
5

6
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21 Registration: www.clinicaltrials.gov. Registration ID: NCT03348566

22
23 **Running title:** Genetic adaptation, fish, and weight change

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25 **Word count:** 3420

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1 Abstract

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

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

7 **Setting:** Population based study

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

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

12 **Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the
13 *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in
14 combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for
15 interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS*
16 rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake
17 also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P
18 for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association
19 with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 kg/m² per g, P = 3×10⁻⁵) than
20 the rs174570 non-T carriers (beta=0.16 kg/m² per g, P = 0.08). Similar results were observed for fish
21 intake.

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

24 Article summary

25 Strengths and limitations of this study

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

32 **Introduction**

33 Diet rich in fish and marine fatty acids especially long chain n-3 polyunsaturated fatty acids (PUFAs) has
34 shown beneficial effects on cardiometabolic health^{1,2}, however, data from population studies on the
35 associations between such diet and body weight are inconsistent^{3,4}. Emerging evidence suggests genetic
36 variations may play a role in modifying the relation between dietary factors and body weight⁵⁻⁷.

37
38 A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs⁸. The
39 strong signals locate in a cluster of fatty acid desaturases gene (*FADS*) that determine PUFAs levels⁸.
40 People living in the Arctic region have been found to be genetically prone to develop obesity^{9,10} as survival
41 strength for energy storage^{11,12}. Interestingly, the identified *FADS* genetic signatures of diet adaptation have
42 been also related to adiposity in the Inuit population⁸. Of note, due to long-standing selection pressure, the
43 identified *FADS* signatures differ in frequency of selective allele across various populations such as
44 Europeans and Asians¹³, in coincidence with varying levels of fish/marine fatty acids consumption, and
45 adiposity patterns in these populations¹⁴. We therefore hypothesized that the genetic signatures might
46 interact with fish and marine PUFAs intakes on body weight¹³.

47
48 The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene
49 cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes in
50 body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health
51 Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective cohorts
52 the Women's Health Initiative (WHI) and the Singapore Chinese Health Study (SCHS).

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45 54 **Methods**6
7 55 **Discovery cohorts**8
9 56 *The Nurses' Health Study*

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11 57 The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were
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13 58 recruited to complete a baseline questionnaire about their lifestyle and medical history¹⁵. The current
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15 59 analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry. Informed
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17 60 consent was obtained from all participants. The DNA extraction methods, quality control measures, SNPs
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19 61 genotyping and imputation when performed have been described in detail elsewhere¹⁶⁻²². All participants
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21 62 with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI
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23 63 data, and genotyping data available based on previous GWASs were included¹⁶⁻²¹. The study protocol was
24
25 64 approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of
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27 65 Public Health.
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32 67 *The Health Professionals Follow-up Study*

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34 68 The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians,
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36 69 optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants
37
38 70 returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet²³. In the current
39
40 71 analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected. Our
41
42 72 analysis included 6,833 men whose genotype data were available. Informed consent was obtained from all
43
44 73 participants. The DNA extraction methods, quality control measures, SNPs genotyping and imputation
45
46 74 when performed have been described in detail elsewhere¹⁶⁻²². All participants with baseline long chain n-3
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48 75 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and genotyping data
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50 76 available based on previous GWASs were included¹⁶⁻²¹. The study protocol was approved by the
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52 77 institutional review boards of Brigham and Women's Hospital and Harvard School of Public Health.
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79 **Replication cohorts**

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

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

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

94 The design of Singapore Chinese Health Study (SCHS) has been previously described in detail²⁶. Briefly,
95 between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in
96 Singapore were enrolled into the cohort study²⁷. Two follow-up interviews were conducted via telephone
97 among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update
98 information on body weight, selected lifestyle factors and medical history. All participants have given
99 informed consent. The study was approved by the Institutional Review Boards of the National University of
100 Singapore and the University of Pittsburgh, and the study was carried out in accordance with the approved
101 guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data,
102 baseline and endpoint BMI data available were included. Among these participants, genome-wide
103 genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at the Genome
104 Institute of Singapore according to the manufacturer's recommendations using an Affymetrix ASI (Asian)

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2
3 105 Axiom array. Genotype calling was performed by the Affymetrix Corporation²⁸. Genome-wide
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5 106 genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was performed for SCHS
6
7 107 samples using the Illumina HumanOmni ZhongHua-8 Bead Chip²⁹.

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9 108 Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had
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11 109 both weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.
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15 111 **Assessment of measures of body mass index**

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17 112 Height and body weight were assessed by questionnaire at baseline, and weight information was requested
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19 113 on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly
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21 114 measured values ($r=0.97$ in HPFS and NHS) in a validation study³⁰. BMI was calculated as body weight
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23 115 (kg)/height (m²). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS
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25 116 and HPFS cohorts³¹, and from baseline (1993) to sixth year follow-up in the WHI^{24,25}, and from baseline
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27 117 (1998) to second follow-up (2004) in the SCHS.
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31 119 **Assessment of diets and other covariates**

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33 120 Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4
34
35 121 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried
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37 122 food intakes, and other dietary factors at baseline were assessed by validated food frequency questionnaires
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39 123 (FFQ) in the NHS and HPFS^{32,33}. A 165-item validated semi-quantitative FFQ was used to collect dietary
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41 124 data and supplemental use of fish oil in the SCHS²⁷. Dietary data and supplemental use of fish oil were
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43 125 obtained from a self-administered baseline 122-items validated FFQ in the WHI³⁴. Alternate health eating
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45 126 index was previously calculated in the NHS, HPFS³⁵, WHI, and SCHS respectively. Physical activity was
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47 127 expressed as metabolic equivalents per week by incorporating the reported time spent on various activities,
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49 128 and the intensity level of each activity. The validity of the self-reported physical activity data has been
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51 129 described previously in the NHS and HPFS³⁶. In the WHI, an estimated metabolic equivalent (MET) level
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53 130 for each type of activity was assigned from a compendium of activities³⁷. Physical activity was assessed
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3 131 using eight continuous categories ranging from never to 31 hours or more in an average week spent doing
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5 132 strenuous sports; vigorous work; and moderate activities in the SCHS ²⁶.

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9 134 **The *FADS* variants selection and genotyping**
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11 135 Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes
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13 136 for signatures of adaptation ⁸ were derived from genome-wide scans available in the NHS, HPFS. We
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15 137 assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as
16
17 138 following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1,
18
19 139 GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for replication
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21
22 140 **(Supplemental Table 1).**
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25 26 142 **Patient and Public Involvement**

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28 143 patients and or public were not involved.
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31 32 145 **Statistical analyses**

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34 146 We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity
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36 147 measures and long-term changes in BMI using general linear models. Interactions between the *FADS*
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38 148 variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long chain n-3
39
40 149 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative interaction term in
41
42 150 the models in the NHS and HPFS. The significant results for rs174570 were replicated in the WHI and
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44 151 SCHS. Potential confounders considered in multivariable models were age, baseline physical activity,
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46 152 baseline television watching, baseline smoking, baseline alcohol intake, baseline alternate healthy eating
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48 153 index, and baseline total energy intake, sugar sweetened beverages (if available), fried food intake (if
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50 154 available). We further tested the genetic associations with long-term changes in BMI according to long
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52 155 chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish intakes with long-term
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54 156 changes in BMI according to the *FADS* genotypes using general linear models after adjustment of potential
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3 157 confounders. Results across cohorts were pooled with inverse variance weighted meta-analyses by fixed
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5 158 effects models (if $P \geq 0.05$ for heterogeneity between studies) or random effects models (if $P < 0.05$ for
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7 159 heterogeneity between studies). Hardy-Weinberg equilibrium was tested using Chi-square test. All reported
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9 160 P values are nominal and two sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary,
10
11 161 NC, USA).

12 162

13 14 15 16 163 **Results**

17 18 164 **Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts**

19
20 165 **Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts.
21
22 166 The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic
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24 167 data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The
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26 168 distribution of the *FADS* genetic variants in the 4 cohorts was shown in **Supplemental table 1**. We did not
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28 169 observe any significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and
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30 170 long-term changes in BMI in three US cohorts ($P > 0.05$), however, we found that the *FADS* genotype was
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32 171 significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2**).

33 172

34 35 36 37 173 **Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes**

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39 174 We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and intakes
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41 175 of various sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that only *FADS*
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43 176 rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians) showed
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45 177 significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs (Eicosapentaenoic acid
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47 178 (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic association with
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49 179 long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in combined cohorts)
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51 180 (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction = 0.04) and the
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53 181 SCHS cohort (P for interaction = 0.02).

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3 183 The pooled analyses of the 3 US (Caucasian) populations or all 4 cohorts showed that high intakes of
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5 184 food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly
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7 185 accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No
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9 186 significant heterogeneity in the interaction effect was observed among these cohorts. Differences in
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11 187 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)
12
13 188 kg/m² across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

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18 190 Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for
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20 191 interaction=0.003) showed similar interaction patterns; and the interactions remained significant when
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22 192 supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

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26 194 In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS*
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28 195 genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for
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30 196 interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results
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32 197 (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071),
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34 198 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m² across three categories (≤ 1 serving/week, 1~6
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36 199 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

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41 201 In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster
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43 202 (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI
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45 203 in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed
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47 204 (**Supplemental table 3 & 4**).

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51 206 **Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

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53 207 We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had
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55 208 significantly greater increase of BMI (mean \pm SE = 0.74 \pm 0.06, kg/m²) than did those who consumed the

lowest (T1) (mean \pm SE = 0.39 ± 0.07 , kg/m²) among the T allele carriers, whereas the corresponding BMI changes were 0.68 ± 0.03 kg/m² and 0.49 ± 0.03 kg/m², 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 ($P = 1.5 \times 10^{-6}$) 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 \pm 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/m² per g ($P = 0.000003$) and 0.64 ± 0.16 kg/m² per serving ($P = 0.00002$) among the T carriers, and whereas the corresponding beta \pm SE were 0.16 ± 0.10 kg/m² per g ($P = 0.08$) and 0.18 ± 0.08 kg/m² 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-loss³⁸⁻⁴⁰, but the benefit was not evident in other trials⁴¹⁻⁴³. The results from the current study lent support to our

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3 235 hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at
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5 236 least partly due to gene-diet interactions.
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9 238 We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were
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11 239 stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle,
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13 240 the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among
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15 241 individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the Inuit,
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17 242 who had high fish/n-3 PUFAs intakes⁸. The high frequency of T allele in Inuit reflects genetic adaptation to
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19 243 the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic signatures of diet
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21 244 adaptation have been also related to adiposity in this population. Our data indicated that the signature allele
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23 245 (T) was related differently with weight changes (decrease or increase), depending on the levels of fish/n-3
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25 246 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature allele predisposed to
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27 247 greater weight gain and an increased risk of obesity; while carriers of this allele tended to have less body
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29 248 weight when they are exposed to diet low in fish and n-3 PUFAs.
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34 250 We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction
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36 251 patterns in relation to long-term changes in BMI; and the interactions also remained significant when
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38 252 supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of
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40 253 fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as
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42 254 Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype is
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44 255 robust for fatty acids from various sources.
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49 257 The mechanisms underlying the observed gene-diet interactions remain unclear, however, such
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51 258 interactions are biologically plausible. It has long been known that the *FADS* genes such as
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53 259 *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important
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55 260 rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic
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3 261 acid (n-6) and α -linolenic acid (n-3)⁴⁴. The selected allele of *FADS* rs174570 is significantly associated
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5 262 with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway⁴⁴. In
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7 263 addition, it has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and
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9 264 function⁴⁵. In addition, storage of energy and body fat is very important for the Arctic population, who are
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11 265 regularly exposed to the extreme low temperature and fishes rich in n-3 PUFAs^{11 12}. Under natural
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13 266 selection, these people are genetically prone to high fish intake to keep body fat^{9 10}. Therefore, it's not
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15 267 surprising that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people
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17 268 carrying selective *FADS* signature^{46 47}. Our findings support the view that extra n-3 PUFAs may not do
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19 269 much benefit at all for Europeans with selective *FADS* signature^{8 13}.

22 270 **Strengths**

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24 271 Several strengths of this study merit mention. To our knowledge, this is the first study with consistent results
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26 272 from 4 well-established prospective cohorts of different racial populations such as Caucasians with
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28 273 European ancestry and Singapore Chinese. The consistent results from these independent cohorts
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30 274 demonstrated the robustness of our findings. Other major strengths include the prospective design, the large
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32 275 sample size, use of long-term change of BMI, and replication of the results. Although we prospectively
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34 276 analyzed the data, we cannot exclude the possibility of reverse causality as this is a study on dietary intake
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36 277 and BMI or weight change from the baseline, which by default builds in the starting point (i.e. the cross
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38 278 sectional association).

41 279 **Limitations**

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43 280 However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity
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45 281 measures were self-reported, measurement errors in these variables are inevitable; however, the food
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47 282 frequency questionnaires and adiposity measures data have been well validated^{27 30 32-34}. Second,
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49 283 confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted for
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51 284 multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish
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53 285 consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with genetic
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55 286 data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort, case control
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3 287 studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge that the
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5 288 different methods in measuring anthropometric traits, genetic variants and food intake across cohorts might
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7 289 introduce bias in the present analyses. Finally, the participants included in our study were middle aged and
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9 290 older adults of Caucasians with European ancestry in the US and Chinese in Singapore, and it is unknown
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11 291 whether our findings could be generalized to other demographic or ethnic groups.

13 292 **Conclusions**

15 293 In summary, our data provides reproducible evidence from 4 multiethnic cohorts that high long chain n-3
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17 294 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings
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19 295 emphasize the importance of considering precision nutritional interventions on prevention and treatment
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21 296 of obesity.
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24 297
26 298 **Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH
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28 299 provided statistical expertise. TW, YH, DS, LH, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM,
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30 300 HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors
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32 301 contributed to the interpretation of the results and critical revision of the manuscript for important
33
34 302 intellectual content and approved the final version of the manuscript.

35
36 303 We acknowledge Dr. Gary C. Curhan's contribution to the genetic data.
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39 304

41 305 **Funding**

42
43 306 This study was supported by grants HL126024, HL034594, DK100383, DK091718, HL071981,
44
45 307 HL073168, CA87969, CA49449, CA055075, HL34594, HL088521, U01HG004399, DK080140,
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47 308 P30DK46200, U01CA137088, U54CA155626, DK58845, DK098311, U01HG004728, EY015473,
48
49 309 CA134958, DK70756 and DK46200 from the National Institutes of Health, with additional support for
50
51 310 genotyping from Merck Research Laboratories, North Wales, PA. LQ is a recipient of the American Heart
52
53 311 Association Scientist Development Award (0730094N). LRP is supported by the Arthur Ashley Williams
54
55 312 Foundation and a Harvard Ophthalmology Scholar Award (Harvard Medical School) from the Harvard
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3 313 Glaucoma Center of Excellence. ATC is a Damon Runyon Cancer Foundation Clinical Investigator. The
4
5 314 SCHS study is supported by the National Institutes of Health, USA (NCI R01 CA144034, UM1
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7 315 CA182876, R01 DK080720), the Singapore National Medical Research Council (NMRC 1270/2010), the
8
9 316 HUI-CREATE Programme of the National Research Foundation, Singapore (Project Number 370062002)
10
11 317 and Biomedical Research Council, Singapore. The funding sources had no role in the design or conduct of
12
13 318 the study; collection, management, analysis, and interpretation of the data; or preparation, review, or
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15 319 approval of the manuscript.
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20 321 **Declaration of interests:** All authors have no conflict of interest to declare. No support from any
21
22 322 organization for the submitted work; no financial relationships with any organizations that might have an
23
24 323 interest in the submitted work in the previous three years, no other relationship or activity that could
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26 324 appear to have influenced the submitted work.
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32 327 **Data sharing**

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34 328 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	/

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

<i>FADS</i> genotypes		Three categories of long chain n-3 PUFAs and fish intakes			P for trend	P for interaction*
		≤1/wk	1~6/wk	≥1/d		
Total fish, serving/day						
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	2×10 ⁻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	1.5×10 ⁻⁶	
Total EPA+DHA, g/day		Tertile1	Tertile2	Tertile3		

1							
2							
3	NHS	Non-T carriers	0.79±0.06	0.94±0.05	1.08±0.06	0.8	0.01
4							
5		T carriers	0.72±0.11	0.87±0.10	1.16±0.11	0.02	
6							
7	HPFS	Non-T carriers	0.47±0.05	0.53±0.05	0.49±0.05	0.88	0.13
8							
9		T carriers	0.23±0.10	0.50±0.09	0.57±0.10	0.16	
10							
11	WHI	Non-T carriers	0.39±0.10	0.04±0.08	0.23±0.10	0.42	0.27
12							
13		T carriers	0.04±0.18	0.28±0.15	0.28±0.16	0.84	
14							
15	Pooled ¹	Non-T carriers	0.57±0.04	0.62±0.03	0.67±0.04	0.65	0.005
16							
17		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.

¹P 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 \pm SE.

Numbers of participants across three categories ($\leq 1/\text{wk}$ / $1\sim 6/\text{wk}$ / $\geq 1/\text{d}$) 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.

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

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

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

14
15 The general linear model was used to test the genetic association of the *FADS* variant (additive model)
16
17 with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after adjustment
18
19 for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity, total energy
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21 intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried food
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23 consumption. The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for
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25 heterogeneity between studies).
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31 **Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS***
32
33 **genotypes**

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35 Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and
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37 876/5378, respectively.

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

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41 The general linear model was used to test the associations of long chain n-3 PUFAs intake with long-term
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43 changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data,
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45 baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index,
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47 television watching, sugar sweetened beverage, fried food consumption.
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50 The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts
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52 was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).
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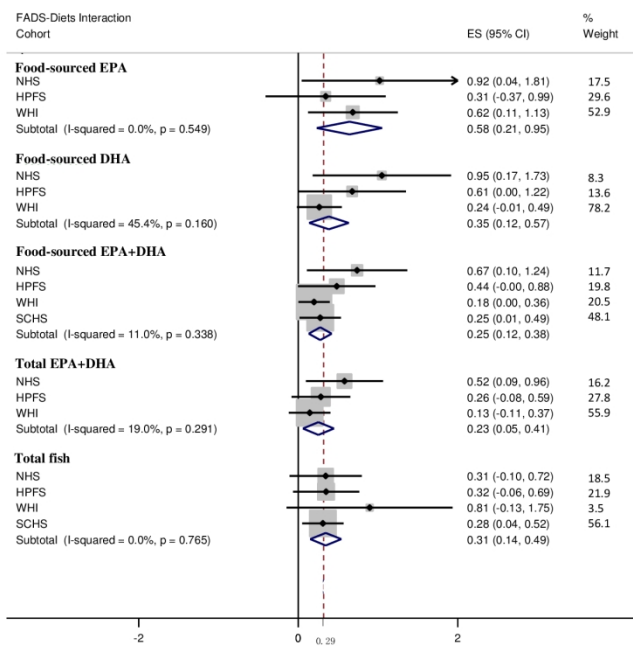
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3 **Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**
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5 Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and
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7 876/5378, respectively.
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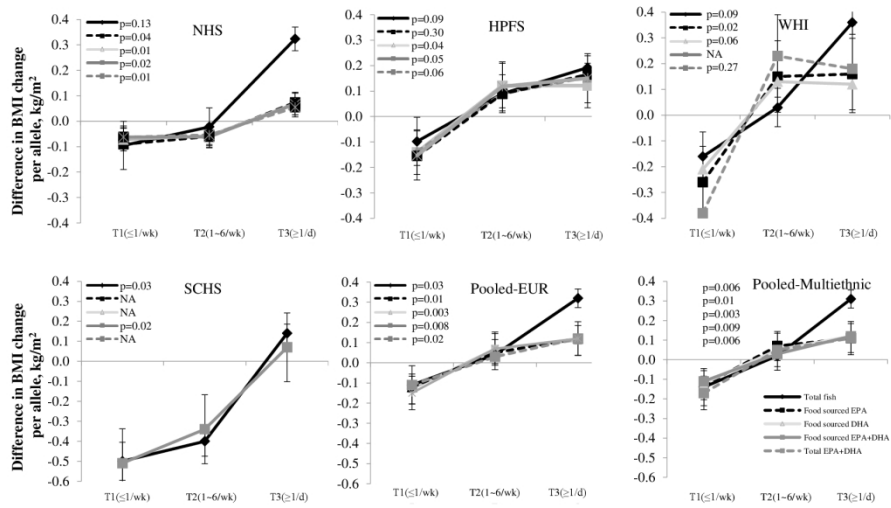
9 Black circles for T allele carriers and open circle for non-T-carriers.
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11 The general linear model was used to test the associations of and fish intake with long-term changes in
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13 BMI according to *FADS* genotypes after adjustment for age, source of genotyping data, baseline BMI,
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15 smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index, television
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17 watching, sugar sweetened beverage, fried food consumption.
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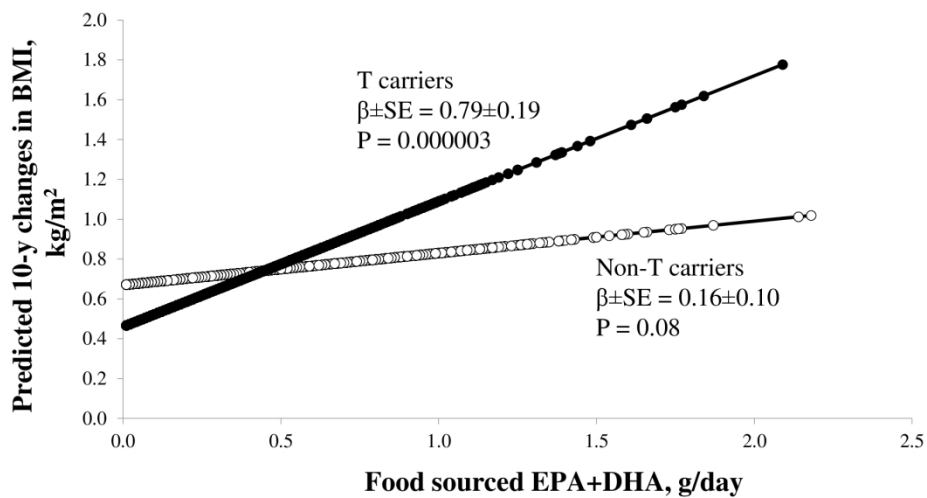
19 The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts
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21 was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).
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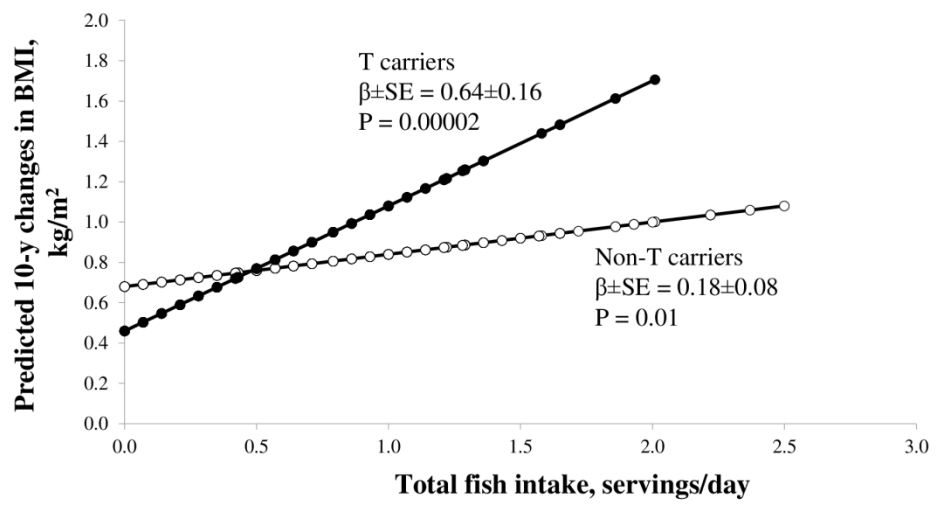


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Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit

Position ¹	Reference		DAF							PBS
	SNP identification number	Alleles ²	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

¹Positions refer to human genome assembly hg19.

²Alleles 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)	<i>FADS</i> 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,			P for interaction
	kg			
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 \pm 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 $P \geq 0.05$ 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	<i>FADS</i> 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	7×10 ⁻⁴	

Data are means ± SE.

¹P 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

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

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

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12 The general linear model was used to test the associations of long chain n-3 PUFAs and fish intakes with
13
14 long-term changes in BMI by *FADS* genotypes after adjustment for age, source of genotyping data,
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16 baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating index,
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18 television watching, sugar sweetened beverage, fried food consumption.

19
20 The results were pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between
21
22 studies).

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24 Registration: [www. clinicaltrials.gov](http://www.clinicaltrials.gov). Registration ID: NCT03348566
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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			
<i>Background rationale</i>	2	Explain the scientific background and rationale for the investigation being reported. (p. 4)	
<i>Objectives</i>	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			
<i>Study design</i>	4	Present key elements of study design early in the paper. (p. 5)	

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Setting</i>	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. (p. 3)	
<i>Participants</i>	6	<p>(a) Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. (p. 5)</p> <p>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.</p> <p>Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants.</p> <hr/> <p>(b) Cohort study – For matched studies, give matching criteria and number of exposed and unexposed.</p> <p>Case-control study – For matched studies, give matching criteria and the number of controls per case.</p>	<i>Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant. (p. 5)</i>
<i>Variables</i>	7	<i>(a)</i> Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. (p. 5)	<i>(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)</i>

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Data sources measurement</i>	8*	<i>(a)</i> 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)	<i>(b)</i> 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)
<i>Bias</i>	9	<i>(a)</i> Describe any efforts to address potential sources of bias. (p. 5 &6)	<i>(b)</i> 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

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

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)

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

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Outcome data</i>	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
<i>Main results</i>	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)	(d) Report results of any adjustments for multiple
		(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)	

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
			<i>comparisons. (p. 10)</i>
<i>Other analyses</i>	17	(a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. (p. 10)	<i>(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken. (p. 10)</i>
			<i>(c) If detailed results are available elsewhere, state how they can be accessed. (p. 10)</i>
Discussion			
<i>Key results</i>	18	Summarize key results with reference to study objectives. (p. 11)	
<i>Limitations</i>	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)
<i>Interpretation</i>	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. (p. 11)	
<i>Generalizability</i>	21	Discuss the generalizability (external validity) of the study results. (p. 11)	
Other Information			
<i>Funding</i>	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 = STrengthening 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-022877.R2
Article Type:	Research
Date Submitted by the Author:	13-Mar-2019
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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

SCHOLARONE™
Manuscripts

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

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23 **Running title:** Genetic adaptation, fish, and weight change

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25 **Word count:** 3420

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1 Abstract

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

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

7 **Setting:** Population based study

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

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

12 **Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the
13 *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in
14 combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for
15 interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS*
16 rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake
17 also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P
18 for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association
19 with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 kg/m² per g, P = 3×10⁻⁵) than
20 the rs174570 non-T carriers (beta=0.16 kg/m² per g, P = 0.08). Similar results were observed for fish
21 intake.

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

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3 27 **Article summary**
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5 28 **Strengths and limitations of this study**
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- 7 29 ● This is the first study with consistent results from 4 well-established prospective cohorts of different
8 racial populations such as Caucasians and Singapore Chinese. The consistent results from these
9 independent cohorts demonstrated the robustness of our findings.
10
11 30
12 31
13 32 ● Other major strengths include the prospective design, the large sample size, use of long-term change
14 of BMI, and replication of the results.
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16 33
17 34 ● Dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these
18 variables are inevitable.
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21 36 ● Confounding by other unmeasured or unknown factors might exist, although we have carefully
22 adjusted for multiple dietary and lifestyle factors.
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24 37
25 38 ● We acknowledge that the different methods in measuring anthropometric traits, genetic variants and
26 food intake across cohorts might introduce bias in the present analyses.
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42 **Introduction**

43 Diets rich in fish and marine fatty acids, especially long chain n-3 polyunsaturated fatty acids (PUFAs)
44 has shown beneficial effects on cardiometabolic health^{1,2}. However, data from population studies on the
45 associations between such diet and body weight are inconsistent^{3,4}. Emerging evidence suggests genetic
46 variations may play a role in modifying the relation between dietary factors and body weight⁵⁻⁷.

47
48 A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs⁸.
49 The strong signals locate in a cluster of fatty acid desaturases genes (*FADS*) that determine PUFAs levels
50 ⁸. People living in the Arctic region have been found to be genetically prone to develop obesity^{9,10} as
51 survival strength for energy storage^{11,12}. Interestingly, the identified *FADS* genetic signatures of diet
52 adaptation have been also related to adiposity in the Inuit population⁸. Of note, due to long-standing
53 selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various
54 populations such as Europeans and Asians¹³, in coincidence with varying levels of fish/marine fatty acids
55 consumption, and adiposity patterns in these populations¹⁴. We therefore hypothesized that the genetic
56 signatures might interact with fish and marine PUFAs intakes on body weight¹³.

57
58 The present study tested the interactions between n-3 PUFAs and fish intakes and variants in *FADS* gene
59 cluster, genetic signatures of adaptation to fish- and n-3 PUFAs-rich diet, in relation to long-term changes
60 in body mass index (BMI) in two US prospective cohorts: the Nurses' Health Study (NHS) and the Health
61 Professionals Follow-up Study (HPFS). We replicated the findings in two independent, prospective
62 cohorts the Women's Health Initiative (WHI, US) and the Singapore Chinese Health Study (SCHS).

64 **Methods**

65 **Discovery cohorts**

66 *The Nurses' Health Study*

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3 67 The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were
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5 68 recruited to complete a baseline questionnaire about their lifestyle and medical history ¹⁵. The current
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7 69 analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry.
8
9 70 Informed consent was obtained from all participants. The DNA extraction methods, quality control
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11 71 measures, SNPs genotyping and imputation when performed have been described in detail elsewhere ¹⁶⁻²².
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13 72 All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline
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15 73 and endpoint BMI data, and genotyping data available based on previous GWASs were included ¹⁶⁻²¹. The
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17 74 study protocol was approved by the institutional review boards of Brigham and Women's Hospital and
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19 75 Harvard School of Public Health.
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23 24 77 ***The Health Professionals Follow-up Study***

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26 78 The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians,
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28 79 optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants
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30 80 returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet ²³. In the current
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32 81 analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected.
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34 82 Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained
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36 83 from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and
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38 84 imputation when performed have been described in detail elsewhere ¹⁶⁻²². All participants with baseline
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40 85 long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and
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42 86 genotyping data available based on previous GWASs were included ¹⁶⁻²¹. The study protocol was also
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44 87 approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of
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50 51 90 **Replication cohorts**

52 53 91 ***The Women's Health Initiative (WHI)***

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3 92 The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National
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5 93 Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast
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7 94 and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI
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9 95 study is presented elsewhere^{24 25}. For the analyses, all participants with baseline long chain n-3 PUFAs
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11 96 and fish consumption and covariate data, baseline and endpoint BMI data, and genotyping data available
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13 97 based on previous GWASs were included. Finally, we included 6,254 Caucasians women who
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15 98 participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for
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17 99 whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix
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19 100 procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa
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21 101 Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each
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23 102 participating institution reviewed and approved the study, and all women gave written informed consent.
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104 ***The Singapore Chinese Health Study (SCHS) cohort***

105 The design of Singapore Chinese Health Study (SCHS) has been previously described in detail²⁶. Briefly,
106 between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in
107 Singapore were enrolled into the cohort study²⁷. Two follow-up interviews were conducted via telephone
108 among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update
109 information on body weight, selected lifestyle factors and medical history. All participants have given
110 informed consent. The study was approved by the Institutional Review Boards of the National University
111 of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the
112 approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and
113 covariates data, baseline and endpoint BMI data available were included. Among these participants,
114 genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at
115 the Genome Institute of the Singapore according to the manufacturer's recommendations using an
116 Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation²⁸.

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3 117 Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was
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5 118 performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip ²⁹.

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7 119 Among these two case-control studies nested within the cohort, 5,264 subjects with genotyping data had
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9 120 weight reported at both baseline and follow-up 2 interviews, and were included in this analysis.

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13 **122 Assessment of measures of body mass index**
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15 123 Height and body weight were assessed by questionnaire at baseline, and weight information was requested
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17 124 on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly
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19 125 measured values ($r=0.97$ in HPFS and NHS) in a validation study ³⁰. BMI was calculated as body weight
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21 126 (kg)/height (m²). We defined long-term changes in BMI as changes in BMI from 1990 to 2000 in the NHS
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23 127 and HPFS cohorts ³¹, and from baseline (1993) to sixth year follow-up in the WHI ^{24,25}, and from baseline
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25 128 (1998) to second follow-up (2004) in the SCHS.

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30 **130 Assessment of diets and other covariates**
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32 131 Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4
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34 132 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried
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36 133 food intakes, and other dietary factors at baseline were assessed by validated food frequency
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38 134 questionnaires (FFQ) in the NHS and HPFS ^{32,33}. A 165-item validated semi-quantitative FFQ was used to
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40 135 collect dietary data and supplemental use of fish oil in the SCHS ²⁷. Dietary data and supplemental use of
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42 136 fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI ³⁴. Alternate
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44 137 health eating index was previously calculated in the NHS, HPFS ³⁵, WHI, and SCHS. Physical activity
45
46 138 was expressed as metabolic equivalents per week by incorporating the reported time spent on various
47
48 139 activities, and the intensity level of each activity. The validity of the self-reported physical activity data
49
50 140 has been described previously in the NHS and HPFS ³⁶. In the WHI, an estimated metabolic equivalent
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52 141 (MET) level for each type of activity was assigned from a compendium of activities ³⁷. Physical activity
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3 142 was assessed using eight continuous categories ranging from never to 31 hours or more in an average
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5 143 week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS ²⁶.

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9 145 **The *FADS* variants selection and genotyping**
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11 146 Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes
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13 147 for signatures of adaptation ⁸ were derived from genome-wide scans available in the NHS, HPFS. We
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15 148 assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as
16
17 149 following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1,
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19 150 GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for
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21 151 replication (**Supplemental Table 1**).

22 152 23 24 25 26 153 **Patient and Public Involvement**

27
28 154 Neither patients nor public were involved.
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32 33 156 **Statistical analyses**

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35 157 We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity
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37 158 measures and long-term changes in BMI using multiple linear regression model. Interactions between
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39 159 the *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced
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41 160 long chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative
42
43 161 interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated
44
45 162 in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline
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47 163 physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline
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49 164 alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available),
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51 165 fried food intake (if available). We further tested the genetic associations with long-term changes in BMI
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53 166 according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish

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3 167 intakes with long-term changes in BMI according to the *FADS* genotypes using multiple linear
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5 168 regression model after adjustment of potential confounders. Linear trend across categories of long chain
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7 169 n-3 PUFAs and fish intakes was quantified with a Wald test for linear trend by assigning the median value
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9 170 to each category and modeling it as a continuous variable. Results across cohorts were pooled with inverse
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11 171 variance weighted meta-analyses by fixed effects models ($P \geq 0.05$ for heterogeneity between studies)³⁸.
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13 172 Hardy-Weinberg equilibrium was tested using Chi-square test. All reported P values are nominal and two
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15 173 sided. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC, USA).
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175 **Results**

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

177 **Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts.
178 The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic
179 data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The
180 distribution of the *FADS* genetic variants in the 4 cohorts is shown in **Supplemental table 1**. Chi-square
181 test showed that the *FADS* rs174570 is in Hardy-Weinberg equilibrium. We did not observe any
182 significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term
183 changes in BMI in the three US cohorts ($P > 0.05$). However, we found that the *FADS* genotype was
184 significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2**).
185

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

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

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3 193 combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction
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5 194 = 0.04) and the SCHS cohort (P for interaction = 0.02).
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9 196 The pooled analyses of the 3 US (Caucasian) samples or all 4 cohorts showed that high intakes of
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11 197 food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly
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13 198 accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No
14
15 199 significant heterogeneity in the interaction effect was observed among these cohorts. Differences in
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17 200 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)
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19 201 kg/m² across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.
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24 203 Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for
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26 204 interaction=0.003) showed similar interaction patterns; and the interactions remained significant when
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28 205 supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).
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32 207 In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS*
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34 208 genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for
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36 209 interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results
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38 210 (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071),
39
40 211 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m² across three categories (≤ 1 serving/week, 1~6
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42 212 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.
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47 214 In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster
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49 215 (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI
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51 216 in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed
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53 217 (**Supplemental table 3 & 4**).
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219 **Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

220 We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had
221 significantly greater increase of BMI (mean \pm SE = 0.74 \pm 0.06, kg/m²) than did those who consumed the
222 lowest (T1) (mean \pm SE = 0.39 \pm 0.07, kg/m²) among the T allele carriers, whereas the corresponding BMI
223 changes were 0.68 \pm 0.03 kg/m² and 0.49 \pm 0.03 kg/m², respectively, among the non-T carriers in 4 cohorts
224 combined (**Table 2 & Supplemental table 5**). Similarly, we observed different associations between fish
225 intake and BMI changes among the T allele carriers ($P = 1.5 \times 10^{-6}$) and non-carriers ($P = 0.01$) in the
226 pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed
227 among the cohorts.

228
229 **Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake
230 according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts
231 consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term
232 changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled
233 results, the beta \pm SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**)
234 with long-term changes in BMI were 0.79 \pm 0.19 kg/m² per g ($P = 0.000003$) and 0.64 \pm 0.16 kg/m² per
235 serving ($P = 0.00002$) among the T carriers, and whereas the corresponding beta \pm SE were 0.16 \pm 0.10
236 kg/m² per g ($P = 0.08$) and 0.18 \pm 0.08 kg/m² per serving ($P = 0.01$) among the non-T carriers.

238 **Discussion**

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

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3 245 Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight
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5 246 and obesity risk have generated conflicting results^{3 4}. In addition, several randomized controlled trials
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7 247 (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss³⁹⁻⁴¹, but
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9 248 the benefit was not evident in other trials⁴²⁻⁴⁴. The results from the current study lend support to our
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11 249 hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at
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13 250 least partly due to gene-diet interactions.

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16 252 We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were
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18 253 stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle,
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20 254 the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among
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22 255 individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the
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24 256 Inuit, who had high fish/n-3 PUFAs intakes⁸. The high frequency of the T allele in Inuit reflects genetic
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26 257 adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic
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28 258 signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that
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30 259 the signature allele (T) was related differently to weight changes (decrease or increase), depending on
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32 260 the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature
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34 261 allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele
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36 262 tended to have less body weight when they are exposed to a diet low in fish and n-3 PUFAs.

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39 264 We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction
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41 265 patterns in relation to long-term changes in BMI; and the interactions also remained significant when
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43 266 supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of
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45 267 fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as
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47 268 Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype
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49 269 is robust for fatty acids from various sources.

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3 271 The mechanisms underlying the observed gene-diet interactions remain unclear. However, such
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5 272 interactions are biologically plausible. It has long been known that the *FADS* genes such as
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7 273 *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important
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9 274 rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic
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11 275 acid (n-6) and α -linolenic acid (n-3)⁴⁵. The selected allele of *FADS* rs174570 is significantly associated
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13 276 with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway⁴⁵. Further, it
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15 277 has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function⁴⁶. In
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17 278 addition, storage of energy and body fat is very important for the Arctic population, who are regularly
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19 279 exposed to extremely low temperatures and fishes rich in n-3 PUFAs^{11 12}. Under natural selection,
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21 280 these people are genetically prone to high fish intake to keep body fat^{9 10}. Therefore, it's not surprising
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23 281 that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying
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25 282 selective *FADS* signature^{47 48}. Our findings support the view that extra n-3 PUFAs may not have much
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27 283 benefit for Europeans with selective *FADS* signature^{8 13}.

284 **Strengths**

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

292 **Limitations**

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

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3 296 confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted
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5 297 for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish
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7 298 consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with
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9 299 genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort,
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11 300 case control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge
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13 301 that the different methods in measuring anthropometric traits, genetic variants and food intake across
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15 302 cohorts might introduce bias in the present analyses. Finally, the participants included in our study were
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17 303 middle aged and older adults of Caucasians in the US and Chinese in Singapore, and it is unknown
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19 304 whether our findings could be generalized to other demographic or ethnic groups.
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22 305 **Conclusions**

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24 306 In summary, our data provide reproducible evidence from 4 multiethnic cohorts that high long chain n-3
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26 307 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings
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28 308 emphasize the importance of considering precision nutritional interventions on prevention and treatment
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30 309 of obesity. We acknowledge that these results are hypothesis-generating and need to be confirmed in
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32 310 additional cohorts.
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36
37 312 **Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH
38
39 313 provided statistical expertise. TW, YH, DS, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM,
40
41 314 HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors
42
43 315 contributed to the interpretation of the results and critical revision of the manuscript for important
44
45 316 intellectual content and approved the final version of the manuscript.

46
47 317 We acknowledge Dr. Gary C. Curhan's contribution to the genetic data.
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49 318

51 319 **Funding**

52
53 320 This study was supported by grants HL126024, HL034594, DK100383, DK091718, HL071981,
54
55 321 HL073168, CA87969, CA49449, CA055075, HL34594, HL088521, U01HG004399, DK080140,
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3 322 P30DK46200, U01CA137088, U54CA155626, DK58845, DK098311, U01HG004728, EY015473,
4
5 323 CA134958, DK70756 and DK46200 from the National Institutes of Health, with additional support for
6
7 324 genotyping from Merck Research Laboratories, North Wales, PA. LQ is a recipient of the American Heart
8
9 325 Association Scientist Development Award (0730094N). LRP is supported by the Arthur Ashley Williams
10
11 326 Foundation and a Harvard Ophthalmology Scholar Award (Harvard Medical School) from the Harvard
12
13 327 Glaucoma Center of Excellence. ATC is a Damon Runyon Cancer Foundation Clinical Investigator. The
14
15 328 SCHS study is supported by the National Institutes of Health, USA (NCI R01 CA144034, UM1
16
17 329 CA182876, R01 DK080720), the Singapore National Medical Research Council (NMRC 1270/2010), the
18
19 330 HUI-CREATE Programme of the National Research Foundation, Singapore (Project Number 370062002)
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21
22 331 and Biomedical Research Council, Singapore. The funding sources had no role in the design or conduct of
23
24 332 the study; collection, management, analysis, and interpretation of the data; or preparation, review, or
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26 333 approval of the manuscript.

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30 335 **Declaration of interests:** All authors have no conflict of interest to declare. No support from any
31
32 336 organization for the submitted work; no financial relationships with any organizations that might have an
33
34 337 interest in the submitted work in the previous three years, no other relationship or activity that could
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36 338 appear to have influenced the submitted work.

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43 341 **Data sharing**
44
45 342 Data are available in Harvard school of public health, NHS and HPFS cohorts. All data relevant to the
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47 343 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	/

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

<i>FADS</i> genotypes		Three categories of long chain n-3 PUFAs and fish intakes			P for trend	P for interaction*
		≤1/wk	1~6/wk	≥1/d		
Total fish, serving/day						
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	2×10 ⁻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	1.5×10 ⁻⁶	
Total EPA+DHA, g/day		Tertile1	Tertile2	Tertile3		

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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.

¹P 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 \pm SE.

Numbers of participants across three categories ($\leq 1/\text{wk}$ / $1\sim 6/\text{wk}$ / $\geq 1/\text{d}$) 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.

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

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7 supplemental use)) and fish consumptions were assessed at baseline in the NHS (1990), the HPFS (1990),
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9 the WHI (1994-1998), and the SCHS (1993-1998), respectively.

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

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15 The multiple linear regression model was used to test the genetic association of the *FADS* variant (additive
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17 model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after
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19 adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity,
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23 food consumption. The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for
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25 heterogeneity between studies).
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31 **Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS***
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33 **genotypes**

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35 Numbers of T carriers/Non-T carriers in the NHS, HPFS, and WHI are 1698/9625, 1025/5808, and
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37 876/5378, respectively.

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

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

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49 The data on food-sourced EPA+DHA was pooled from the NHS and HPFS cohorts. Data from US cohorts
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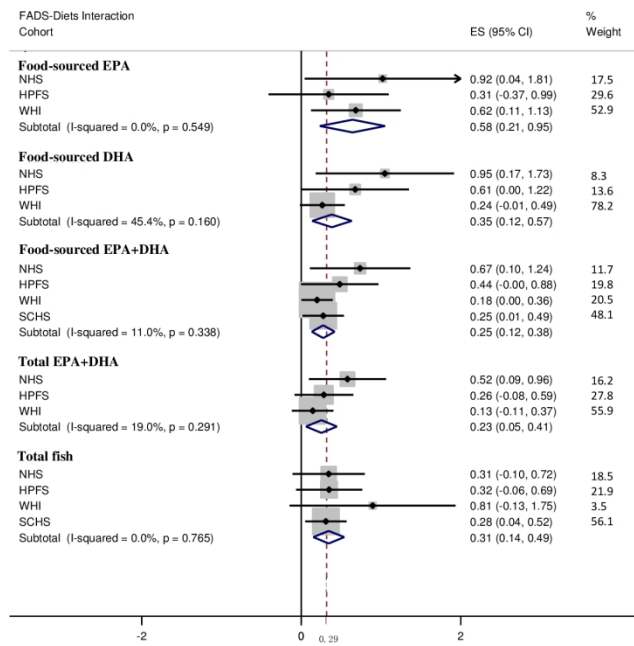
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3 **Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**
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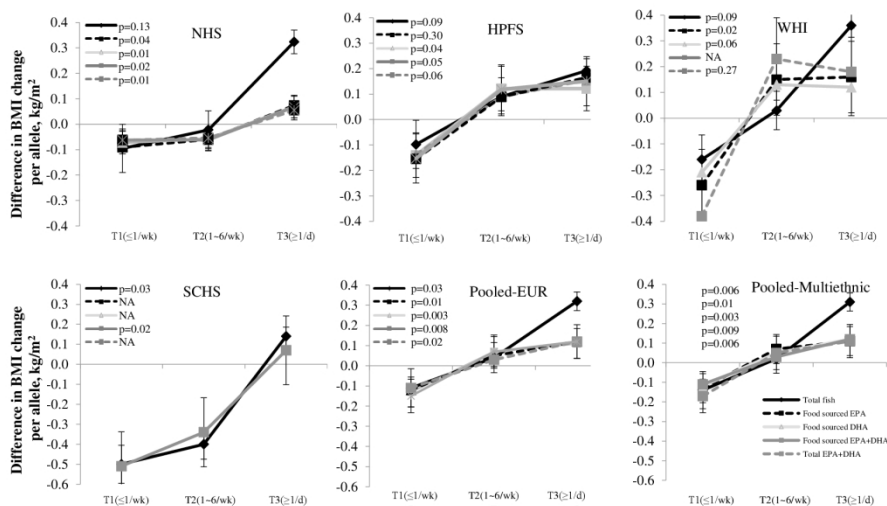
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17 index, television watching, sugar sweetened beverage, fried food consumption.
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19 The data on total fish intake was pooled from the NHS, HPFS, and WHI cohorts. Data from US cohorts
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21 was pooled by means of fixed effects meta-analyses (if $P \geq 0.05$ for heterogeneity between studies).
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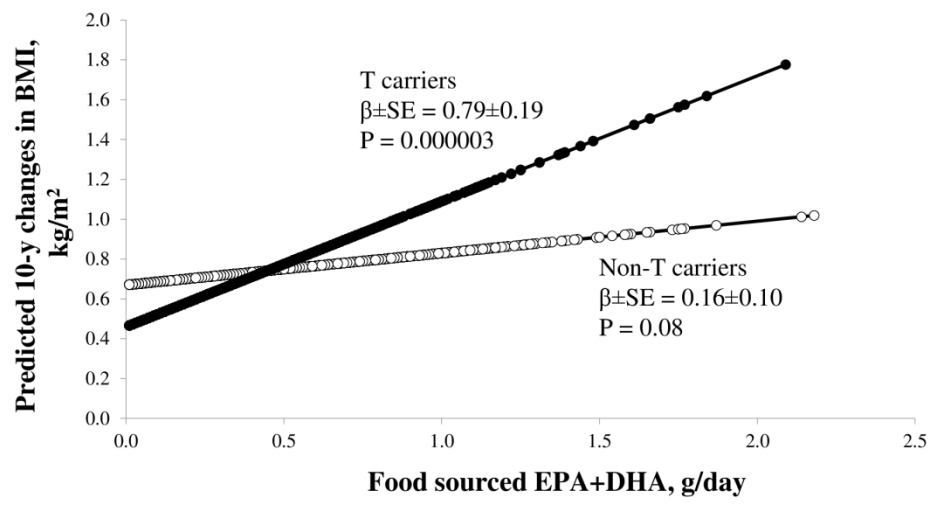


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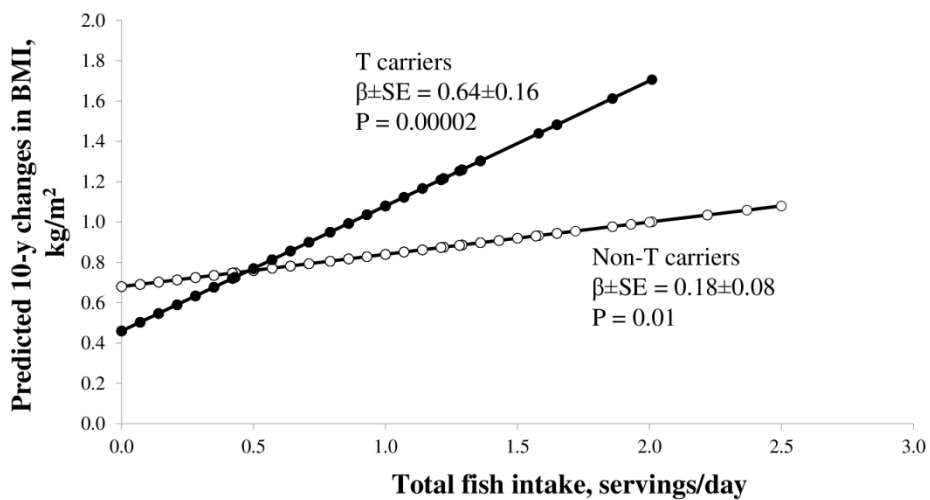


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Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit

Position ¹	Reference		DAF							PBS
	SNP identification number	Alleles ²	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

¹Positions refer to human genome assembly hg19.

²Alleles 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)	<i>FADS</i> 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,			P for interaction
	kg			
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 \pm 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: \leq 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 $P \geq 0.05$ 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	<i>FADS</i> 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	7×10 ⁻⁴	

Data are means ± SE.

¹P 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

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

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8 Long-term BMI changes were calculated based on the changes in BMI from baseline to follow-up year in
9
10 the four cohorts, respectively.

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

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

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24 Registration: [www. clinicaltrials.gov](http://www.clinicaltrials.gov). Registration ID: NCT03348566
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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			
<i>Background rationale</i>	2	Explain the scientific background and rationale for the investigation being reported. (p. 4)	
<i>Objectives</i>	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			
<i>Study design</i>	4	Present key elements of study design early in the paper. (p. 5)	

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Setting</i>	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. (p. 3)	
<i>Participants</i>	6	<p>(a) Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. (p. 5)</p> <p>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.</p> <p>Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants.</p> <hr/> <p>(b) Cohort study – For matched studies, give matching criteria and number of exposed and unexposed.</p> <p>Case-control study – For matched studies, give matching criteria and the number of controls per case.</p>	<i>Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant. (p. 5)</i>
<i>Variables</i>	7	(a) Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. (p. 5)	<i>(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)</i>

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Data sources measurement</i>	8*	<i>(a)</i> 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)	<i>(b)</i> 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)
<i>Bias</i>	9	<i>(a)</i> Describe any efforts to address potential sources of bias. (p. 5 &6)	<i>(b)</i> 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)
			<i>with this. (p. 5 &6)</i>
<i>Study size</i>	10	Explain how the study size was arrived at. (p. 5 &6)	
<i>Quantitative variables</i>	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. (p. 7)	<i>If applicable, describe how effects of treatment were dealt with. (p. 7)</i>
<i>Statistical methods</i>	12	<p>(a) Describe all statistical methods, including those used to control for confounding. (p. 7-9)</p> <hr/> <p>(b) Describe any methods used to examine subgroups and interactions. (p. 9)</p> <hr/> <p>(c) Explain how missing data were addressed. (p. 9)</p> <hr/> <p>(d) Cohort study – If applicable, explain how loss to follow-up was addressed. (p. 9)</p> <p>Case-control study – If applicable, explain how matching of cases and controls was addressed.</p> <p>Cross-sectional study – If applicable, describe analytical methods taking account of sampling strategy.</p>	<i>State software version used and options (or settings) chosen. (p. 9)</i>

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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			
<i>Participants</i>	13*	<p>(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)</p> <hr/> <p>(b) Give reasons for non-participation at each stage. (p. 10)</p> <hr/> <p>(c) Consider use of a flow diagram. (p. 10)</p>	<p><i>Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful. (p. 10)</i></p>
<i>Descriptive data</i>	14*	<p>(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. (p. 10)</p> <hr/> <p>(b) Indicate the number of participants with missing data for each variable of interest. (p. 10)</p> <hr/> <p>(c) Cohort study – Summarize follow-up time, e.g. average and total amount. (p. 10)</p>	<p><i>Consider giving information by genotype. (p. 10)</i></p>

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Outcome data</i>	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
<i>Main results</i>	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)	(d) Report results of any adjustments for multiple
		(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)	

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
			<i>comparisons. (p. 10)</i>
<i>Other analyses</i>	17	(a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. (p. 10)	
			<i>(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken. (p. 10)</i>
			<i>(c) If detailed results are available elsewhere, state how they can be accessed. (p. 10)</i>
Discussion			
<i>Key results</i>	18	Summarize key results with reference to study objectives. (p. 11)	
<i>Limitations</i>	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)	

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Interpretation</i>	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. (p. 11)	
<i>Generalizability</i>	21	Discuss the generalizability (external validity) of the study results. (p. 11)	
Other Information			
<i>Funding</i>	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 = STrengthening 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-022877.R3
Article Type:	Research
Date Submitted by the Author:	07-Apr-2019
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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



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3 **Fish and marine fatty acids intakes, the *FADS* genotypes and long-term weight gain: a prospective**
4 **cohort study**
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21 Registration: www.clinicaltrials.gov. Registration ID: NCT03348566

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23 **Running title:** Genetic adaptation, fish, and weight change

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25 **Word count:** 3420

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1 Abstract

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

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

7 **Setting:** Population based study

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

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

12 **Results:** In the NHS and HPFS cohorts, food-sourced n-3 PUFAs intake showed interactions with the
13 *FADS* rs174570 on changes of BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in
14 combined). Such interactions were replicated in two independent cohorts WHI and SCHS (P for
15 interaction = 0.04 in WHI, 0.02 in SCHS, and 0.001 in combined). The genetic associations of the *FADS*
16 rs174570 with changes in BMI increased across the tertiles of n-3 PUFAs in all the cohorts. Fish intake
17 also accentuated the genetic associations of the *FADS* rs174570 with long-term changes in BMI (pooled P
18 for interaction = 0.006). Viewed differently, long chain n-3 PUFAs intake showed stronger association
19 with long-term changes in BMI among the rs174570 T carriers (beta = 0.79 kg/m² per g, P = 3×10⁻⁵) than
20 the rs174570 non-T carriers (beta=0.16 kg/m² per g, P = 0.08). Similar results were observed for fish
21 intake.

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

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3 27 **Article summary**
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5 28 **Strengths and limitations of this study**
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- 7 29 ● This is the first study with consistent results from 4 well-established prospective cohorts of different
8 racial populations such as Caucasians and Singapore Chinese. The consistent results from these
9 independent cohorts demonstrated the robustness of our findings.
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13 32 ● Other major strengths include the prospective design, the large sample size, use of long-term change
14 of BMI, and replication of the results.
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16 33
17 34 ● Dietary fatty acids, fish, and adiposity measures were self-reported, measurement errors in these
18 variables are inevitable.
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21 36 ● Confounding by other unmeasured or unknown factors might exist, although we have carefully
22 adjusted for multiple dietary and lifestyle factors.
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24 37
25 38 ● We acknowledge that the different methods in measuring anthropometric traits, genetic variants and
26 food intake across cohorts might introduce bias in the present analyses.
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42 Introduction

43 Diets rich in fish and marine fatty acids, especially long chain n-3 polyunsaturated fatty acids (PUFAs)
44 has shown beneficial effects on cardiometabolic health^{1 2}. However, data from population studies on the
45 associations between such diet and body weight are inconsistent^{3 4}. Emerging evidence suggests genetic
46 variations may play a role in modifying the relation between dietary factors and body weight⁵⁻⁷. For
47 example, we previously found that high intakes of fish and long-chain n-3 PUFAs are associated with an
48 attenuation of the genetic association with long-term weight gain based on results from 3 prospective
49 cohorts of Caucasians.⁸

50
51 A recent study of Inuit identified genetic signatures of adaptation to diets rich in fish and n-3 PUFAs⁹.
52 The strong signals locate in a cluster of fatty acid desaturases genes (*FADS*) that determine PUFAs levels
53 ⁹. People living in the Arctic region have been found to be genetically prone to develop obesity^{10 11} as
54 survival strength for energy storage^{12 13}. Interestingly, the identified *FADS* genetic signatures of diet
55 adaptation have been also related to adiposity in the Inuit population⁹. Of note, due to long-standing
56 selection pressure, the identified *FADS* signatures differ in frequency of selective allele across various
57 populations such as Europeans and Asians¹⁴, in coincidence with varying levels of fish/marine fatty acids
58 consumption, and adiposity patterns in these populations¹⁵. We therefore hypothesized that the genetic
59 signatures might interact with fish and marine PUFAs intakes on body weight¹⁴.

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

67 Methods

68 **Discovery cohorts**

69 *The Nurses' Health Study*

70 The NHS began in 1976, when 121,700 female registered nurses aged 30-55 y residing in 11 states were
71 recruited to complete a baseline questionnaire about their lifestyle and medical history¹⁶. The current
72 analysis baseline was set in 1990 for the NHS. We included 11,323 women of European ancestry.
73 Informed consent was obtained from all participants. The DNA extraction methods, quality control
74 measures, SNPs genotyping and imputation when performed have been described in detail elsewhere¹⁷⁻²³.
75 All participants with baseline long chain n-3 PUFAs and fish consumptions and covariates data, baseline
76 and endpoint BMI data, and genotyping data available based on previous GWASs were included¹⁷⁻²². The
77 study protocol was approved by the institutional review boards of Brigham and Women's Hospital and
78 Harvard School of Public Health.

80 *The Health Professionals Follow-up Study*

81 The HPFS was initiated in 1986, and was composed of 51,529 male dentists, pharmacists, veterinarians,
82 optometrists, osteopathic physicians, and podiatrists, aged 40-75 y at baseline. The male participants
83 returned a baseline questionnaire about detailed medical history, lifestyle, and usual diet²⁴. In the current
84 analysis, we used 1990 as baseline in the HPFS, when the earliest complete dietary data were collected.
85 Our analysis included 6,833 men whose genotype data were available. Informed consent was obtained
86 from all participants. The DNA extraction methods, quality control measures, SNPs genotyping and
87 imputation when performed have been described in detail elsewhere¹⁷⁻²³. All participants with baseline
88 long chain n-3 PUFAs and fish consumptions and covariates data, baseline and endpoint BMI data, and
89 genotyping data available based on previous GWASs were included¹⁷⁻²². The study protocol was also
90 approved by the institutional review boards of Brigham and Women's Hospital and Harvard School of
91 Public Health.

93 **Replication cohorts**

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3 94 ***The Women's Health Initiative (WHI)***
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5 95 The Women's Health Initiative (WHI) is a large, multiethnic, 40-center study funded by the National
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7 96 Heart, Lung, and Blood Institute (NHLBI) that focuses on strategies for preventing heart disease, breast
8
9 97 and colorectal cancer, and osteoporotic fractures in postmenopausal women. A full description of the WHI
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11 98 study is presented elsewhere^{25 26}. For the analyses, all participants with baseline long chain n-3 PUFAs
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13 99 and fish consumption and covariate data, baseline and endpoint BMI data, and genotyping data available
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15 100 based on previous GWASs were included. Finally, we included 6,254 Caucasians women who
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17 101 participated in the WHI clinical trial studies at baseline (1994-1998) and at sixth-year follow-up and for
18
19 102 whom DNA was measured. The genomic DNA samples were processed according to standard Affymetrix
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21 103 procedures for processing of the assay. The Affymetrix Human SNP Array 6.0 (Affymetrix®, Inc Santa
22
23 104 Clara, CA) was used for genome wide SNP genotyping. Human subjects review committees at each
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25 105 participating institution reviewed and approved the study, and all women gave written informed consent.
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30 107 ***The Singapore Chinese Health Study (SCHS) cohort***
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32 108 The design of Singapore Chinese Health Study (SCHS) has been previously described in detail²⁷. Briefly,
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34 109 between 1993 and 1998, 63,257 Chinese men and women between ages of 45 and 74 years living in
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36 110 Singapore were enrolled into the cohort study²⁸. Two follow-up interviews were conducted via telephone
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38 111 among surviving participants between 1999 and 2004, and again between 2006 and 2010 to update
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40 112 information on body weight, selected lifestyle factors and medical history. All participants have given
41
42 113 informed consent. The study was approved by the Institutional Review Boards of the National University
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44 114 of Singapore and the University of Pittsburgh, and the study was carried out in accordance with the
45
46 115 approved guidelines. All participants with baseline long chain n-3 PUFAs and fish consumptions and
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48 116 covariates data, baseline and endpoint BMI data available were included. Among these participants,
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50 117 genome-wide genotyping for 2615 incident diabetes cases and 2615 matched controls was performed at
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52 118 the Genome Institute of the Singapore according to the manufacturer's recommendations using an
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54 119 Affymetrix ASI (Asian) Axiom array. Genotype calling was performed by the Affymetrix Corporation²⁹.
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3 120 Genome-wide genotyping for 717 incident myocardial infarction (MI) cases and 644 controls was
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5 121 performed for SCHS samples using the Illumina HumanOmni ZhongHua-8 Bead Chip³⁰. Among these
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7 122 two case-control studies nested within the cohort, 5,264 subjects with genotyping data had weight reported
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9 123 at both baseline and follow-up 2 interviews, and were included in this analysis.
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125 **Assessment of measures of body mass index**

126 Height and body weight were assessed by questionnaire at baseline, and weight information was requested
127 on follow-up questionnaire in all 4 cohorts. Self-reported weights were highly correlated with directly
128 measured values ($r=0.97$ in HPFS and NHS) in a validation study³¹. BMI was calculated as body weight
129 (kg)/height (m²). As defined previously,⁸ the long-term changes in BMI was calculated as changes in BMI
130 from 1990 to 2000 in the NHS and HPFS cohorts³², and from baseline (1993) to sixth year follow-up in
131 the WHI^{25,26}, and from baseline (1998) to second follow-up (2004) in the SCHS.
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134 **Assessment of diets and other covariates**

135 Questionnaires were used to collect information on a medical history and diet/lifestyle factors in all 4
136 cohorts. Total fish, n-3 PUFAs, supplemental use of fish oil, alcohol, sugar sweetened beverages, fried
137 food intakes, and other dietary factors at baseline were assessed by validated food frequency
138 questionnaires (FFQ) in the NHS and HPFS^{33,34}. A 165-item validated semi-quantitative FFQ was used to
139 collect dietary data and supplemental use of fish oil in the SCHS²⁸. Dietary data and supplemental use of
140 fish oil were obtained from a self-administered baseline 122-items validated FFQ in the WHI³⁵. Alternate
141 health eating index was previously calculated in the NHS, HPFS³⁶, WHI, and SCHS. Physical activity
142 was expressed as metabolic equivalents per week by incorporating the reported time spent on various
143 activities, and the intensity level of each activity. The validity of the self-reported physical activity data
144 has been described previously in the NHS and HPFS³⁷. In the WHI, an estimated metabolic equivalent
(MET) level for each type of activity was assigned from a compendium of activities³⁸. Physical activity

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3 145 was assessed using eight continuous categories ranging from never to 31 hours or more in an average
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5 146 week spent doing strenuous sports; vigorous work; and moderate activities in the SCHS ²⁷.

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9 148 **The *FADS* variants selection and genotyping**

11 149 Three of the 6 *FADS* single-nucleotide polymorphisms (SNPs) reported in a recent scan of Inuit genomes
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13 150 for signatures of adaptation ⁹ were derived from genome-wide scans available in the NHS, HPFS. We
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15 151 assumed that each SNP in the panel acts independently in an additive manner. We coded the SNPs as
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17 152 following: rs174570 (TT=2, TC=1, CC=0); rs174602 (TT=2, TC=1, CC=0); rs7115739 (TT=2, TG=1,
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19 153 GG=0). The *FADS* rs174570 was extracted from GWAS data in the WHI and SCHS cohorts for
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21 154 replication (**Supplemental Table 1**).

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26 156 **Patient and Public Involvement**

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28 157 Neither patients nor public were involved.

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32 159 **Statistical analyses**

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34 160 We examined the associations of the *FADS* variants (rs174570, rs174602, rs7115739) with adiposity
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36 161 measures and long-term changes in BMI using multiple linear regression model. Interactions between the
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38 162 *FADS* variants (rs174570, rs174602, rs7115739) and baseline fish intake, and total or food-sourced long
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40 163 chain n-3 PUFAs intakes on long-term changes in BMI were tested by including a multiplicative
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42 164 interaction term in the models in the NHS and HPFS. The significant results for rs174570 were replicated
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44 165 in the WHI and SCHS. Potential confounders considered in multivariable models were age, baseline
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46 166 physical activity, baseline television watching, baseline smoking, baseline alcohol intake, baseline
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48 167 alternate healthy eating index, and baseline total energy intake, sugar sweetened beverages (if available),
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50 168 fried food intake (if available). We further tested the genetic associations with long-term changes in BMI
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52 169 according to long chain n-3 PUFAs and fish intakes, and associations of long chain n-3 PUFAs and fish

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3 170 intakes with long-term changes in BMI according to the *FADS* genotypes using multiple linear regression
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5 171 model after adjustment of potential confounders. Linear trend across categories of long chain n-3 PUFAs
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7 172 and fish intakes was quantified with a Wald test for linear trend by assigning the median value to each
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10 173 category and modeling it as a continuous variable³⁹. Results across cohorts were pooled with inverse
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12 174 variance weighted meta-analyses by fixed effects models ($P \geq 0.05$ for heterogeneity between studies)⁴⁰.
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14 175 The individual participant data from the NHS and HPFS cohorts were pooled to generate the predicted
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16 176 10-year changes in body weight according to the *FADS* genotypes. Hardy-Weinberg equilibrium was
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18 177 tested using Chi-square test. All reported P values are nominal and two sided. Statistical analyses were
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21 178 performed in SAS 9.3 (SAS Institute, Cary, NC, USA).
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23 179

25 180 **Results**

27 181 **Baseline characteristics of all participants in the NHS, HPFS, WHI and SCHS cohorts**

29 182 **Table 1** shows the baseline characteristics for all participants in the NHS, HPFS, WHI, and SCHS cohorts.
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31 183 The present study included 11,323 women with genetic data from the NHS cohort, 6,833 men with genetic
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33 184 data from the HPFS cohort, 6,254 women from the WHI, and 5,264 Chinese from the SCHS. The
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35 185 distribution of the *FADS* genetic variants in the 4 cohorts is shown in **Supplemental table 1**. Chi-square
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37 186 test showed that the *FADS* rs174570 is in Hardy-Weinberg equilibrium. We did not observe any
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39 187 significant genetic association between the *FADS* rs174570 genotype and baseline BMI, and long-term
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41 188 changes in BMI in the three US cohorts ($P > 0.05$). However, we found that the *FADS* genotype was
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43 189 significantly associated with baseline BMI in the SCHS ($P = 0.002$) (**Supplemental table 2**).
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48 191 **Genetic associations with long-term changes in BMI according to LC n-3 PUFAs/fish intakes**

50 192 We first tested interactions between the *FADS* genetic variants (rs174570, rs174602, rs7115739) and
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52 193 intakes of variously sourced long chain n-3 PUFAs and fish in the NHS and HPFS cohorts. We found that
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54 194 only *FADS* rs174570 (C/T, with T as the common allele in Inuit, but rare allele in Europeans and Asians)
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3 195 showed significant interaction with LC n-3 PUFAs/fish intakes. Food-sourced n-3 PUFAs
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5 196 (Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA)) intake consistently magnified the genetic
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7 197 association with long-term changes in BMI (P for interaction = 0.02 in NHS, 0.05 in HPFS, and 0.007 in
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9 198 combined cohorts) (**Figure 1**). We successfully replicated our results in the WHI cohort (P for interaction
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11 199 = 0.04) and the SCHS cohort (P for interaction = 0.02).

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16 201 The pooled analyses of the 3 US (Caucasian) samples or all 4 cohorts showed that high intakes of
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18 202 food-sourced n-3 PUFAs intake (P for interaction = 0.008 and 0.009, respectively) significantly
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20 203 accentuated the genetic association of the *FADS* genotypes with long-term changes in BMI (**Figure 2**). No
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22 204 significant heterogeneity in the interaction effect was observed among these cohorts. Differences in
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24 205 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)
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26 206 kg/m² across three tertiles of food-sourced n-3 PUFAs in pooled results from all the 4 cohorts.

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30 208 Individual food-sourced n-3 PUFAs such as EPA (pooled P for interaction=0.01) and DHA (pooled P for
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32 209 interaction=0.003) showed similar interaction patterns; and the interactions remained significant when
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34 210 supplemented n-3 PUFAs were considered (pooled P for interaction=0.007) (**Figure 2**).

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38 212 In addition, fish intake showed similar, though less significant, interaction patterns with the *FADS*
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40 213 genotype on long-term changes in BMI in the NHS (P for interaction=0.16), HPFS (P for
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42 214 interaction=0.09), WHI (P for interaction=0.09), SCHS (P for interaction=0.03) and combined results
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44 215 (pooled P for interaction=0.006), and the differences in BMI changes per T allele were -0.096 (SE 0.071),
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46 216 0.041 (SE 0.052), and 0.251 (SE 0.151) kg/m² across three categories (≤ 1 serving/week, 1~6
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48 217 servings/week, and ≥ 1 serving/day) of fish intake in combined results from all the 4 cohorts.

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52 219 In addition, we did not observe significant interaction between two other genetic variants in *FADS* cluster
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54 220 (rs174602 and rs7115739) and long chain n-3 PUFAs/fish intakes in relation to long-term changes in BMI

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3 221 in the NHS and HPFS cohorts. Similar interactions for long-term changes in body weight were observed
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5 222 (Supplemental table 3 & 4).

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9 224 **Long chain n-3 PUFAs/fish intakes and long-term changes in BMI according to the *FADS* genotype**

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11 225 We found that individuals who consumed the highest food-sourced n-3 PUFAs (EPA+DHA; T3) had
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13 226 significantly greater increase of BMI (mean \pm SE = 0.74 \pm 0.06, kg/m²) than did those who consumed the
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15 227 lowest (T1) (mean \pm SE = 0.39 \pm 0.07, kg/m²) among the T allele carriers, whereas the corresponding BMI
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17 228 changes were 0.68 \pm 0.03 kg/m² and 0.49 \pm 0.03 kg/m², respectively, among the non-T carriers in 4 cohorts
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19 229 combined (Table 2 & Supplemental table 5). Similarly, we observed different associations between fish
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21 230 intake and BMI changes among the T allele carriers ($P = 1.5 \times 10^{-6}$) and non-carriers ($P = 0.01$) in the
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23 231 pooled results from these US cohorts. No significant heterogeneity in the interaction effect was observed
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25 232 among the cohorts.
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30 234 **Figure 3** presents the predicted long-term changes in BMI from food-sourced n-3 PUFAs and fish intake
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32 235 according to the T carriers and the non-T carriers. Results from the NHS, HPFS and WHI cohorts
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34 236 consistently showed that the associations of food-sourced n-3 PUFAs and fish intakes with long-term
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36 237 changes in BMI were stronger among the T carriers than those among the non-T carriers. In the pooled
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38 238 results, the beta \pm SE for associations of food-sourced n-3 PUFAs (**Figure 3**) and fish intake (**Figure 4**)
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40 239 with long-term changes in BMI were 0.79 \pm 0.19 kg/m² per g ($P = 0.000003$) and 0.64 \pm 0.16 kg/m² per
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42 240 serving ($P = 0.00002$) among the T carriers, and whereas the corresponding beta \pm SE were 0.16 \pm 0.10
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44 241 kg/m² per g ($P = 0.08$) and 0.18 \pm 0.08 kg/m² per serving ($P = 0.01$) among the non-T carriers.
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47 243 **Discussion**

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50 244 In 4 large prospective cohorts of the US and Chinese populations, our hypothesis-driven analyses showed
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52 245 reproducible evidence that long chain n-3 PUFAs and fish intakes accentuated the genetic association of
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54 246 the *FADS* genotypes with long-term changes in BMI. In addition, our results showed that the *FADS*

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3 247 rs174570 T allele carriers gained more weight than the non-carriers when they had higher long chain n-3
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5 248 PUFAs and fish intakes.

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9 250 Large prospective cohort studies examining the associations of fish or n-3 PUFAs with body weight
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11 251 and obesity risk have generated conflicting results^{3,4}. In addition, several randomized controlled trials
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13 252 (RCTs) supported the protective effects of fish, fish oils, or/and n-3 PUFAs intake on weight-loss⁴¹⁻⁴³, but
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15 253 the benefit was not evident in other trials⁴⁴⁻⁴⁶. The results from the current study lend support to our
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17 254 hypothesis that the heterogeneous associations between fish or n-3 PUFAs and body weight might be at
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19 255 least partly due to gene-diet interactions.

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24 257 We found that the genetic associations between the *FADS* rs174570 and long-term BMI change were
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26 258 stronger along with increasing intakes of long chain n-3 PUFAs and fish. Viewed from a different angle,
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28 259 the magnitude of associations of fish and long chain n-3 PUFAs intakes with BMI changes varied among
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30 260 individuals with different genotypes. The *FADS* rs174570 was recently identified from a study of the
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32 261 Inuit, who had high fish/n-3 PUFAs intakes⁹. The high frequency of the T allele in Inuit reflects genetic
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34 262 adaptation to the special fish- and n-3 PUFA rich diet. Interestingly, the identified *FADS* genetic
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36 263 signatures of diet adaptation have been also related to adiposity in this population. Our data indicated that
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38 264 the signature allele (T) was related differently to weight changes (decrease or increase), depending on
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40 265 the levels of fish/n-3 PUFAs intakes. In people with high fish/n-3 PUFAs intakes, carrying the signature
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42 266 allele predisposed to greater weight gain and an increased risk of obesity; while carriers of this allele
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44 267 tended to have less body weight when they are exposed to a diet low in fish and n-3 PUFAs.

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49 269 We found that individual food-sourced n-3 PUFAs such as EPA and DHA showed similar interaction
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51 270 patterns in relation to long-term changes in BMI; and the interactions also remained significant when
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53 271 supplemented n-3 PUFAs were considered. In addition, our results indicated that the interactions of
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55 272 fish/n-3 PUFAs intakes and the *FADS* genotype were persistent across different racial populations such as
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3 273 Europeans and Asians. Our data suggest that the interactions between n-3 PUFAs and the *FADS* genotype
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5 274 is robust for fatty acids from various sources.
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9 276 The mechanisms underlying the observed gene-diet interactions remain unclear. However, such
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11 277 interactions are biologically plausible. It has long been known that the *FADS* genes such as
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13 278 *FADS1* and *FADS2* encode delta-5 and delta-6 desaturases respectively, which are the important
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15 279 rate-limiting steps in the endogenous formation of long-chain PUFA such as EPA and DHA from linoleic
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17 280 acid (n-6) and α -linolenic acid (n-3)⁴⁷. The selected allele of *FADS* rs174570 is significantly associated
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19 281 with an increase in the concentration of n-3 fatty acids upstream in the n-3 synthesis pathway⁴⁷. Further, it
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21 282 has been reported that dietary n-3 PUFAs might regulate adipocyte *FADS* expression and function⁴⁸. In
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23 283 addition, storage of energy and body fat is very important for the Arctic population, who are regularly
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25 284 exposed to extremely low temperatures and fishes rich in n-3 PUFAs^{12 13}. Under natural selection,
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27 285 these people are genetically prone to high fish intake to keep body fat^{10 11}. Therefore, it's not surprising
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29 286 that high fish or n-3 PUFAs intake accentuated genetic susceptibility to obesity among people carrying
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31 287 selective *FADS* signature^{49 50}. Our findings support the view that extra n-3 PUFAs may not have much
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33 288 benefit for Europeans with selective *FADS* signature^{9 14}.
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36 289 **Strengths**

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39 290 Several strengths of this study merit mention. To our knowledge, this is the first study with consistent
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41 291 results from 4 well-established prospective cohorts of different racial populations such as Caucasians and
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43 292 Singapore Chinese. The consistent results from these independent cohorts demonstrated the robustness of
44
45 293 our findings. Other major strengths include the prospective design, the large sample size, use of long-term
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47 294 change of BMI, and replication of the results. Although we prospectively analyzed the data, we cannot
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49 295 exclude the possibility of reverse causality as this is a study on dietary intake and BMI or weight change
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51 296 from the baseline, which by default builds in the starting point (i.e. the cross sectional association).
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54 297 **Limitations**

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3 298 However, several limitations need to be acknowledged. First, dietary fatty acids, fish, and adiposity
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5 299 measures were self-reported, measurement errors in these variables are inevitable. Despite this, the food
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7 300 frequency questionnaires and adiposity measures data have been well validated^{28 31 33-35}. Second,
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9 301 confounding by other unmeasured or unknown factors might exist, although we have carefully adjusted
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11 302 for multiple dietary and lifestyle factors. Third, a causal relation among long chain n-3 PUFAs and fish
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13 303 consumption, and adiposity cannot be inferred from an observational study. Fourth, all subjects with
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15 304 genetic data were selected in each cohort. The source of genotyping data was diverse (e.g. sub-cohort,
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17 305 case control studies), therefore, subject selection might be a major source of bias. Fifth, we acknowledge
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19 306 that the different methods in measuring anthropometric traits, genetic variants and food intake across
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21 307 cohorts might introduce bias in the present analyses. Finally, the participants included in our study were
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23 308 middle aged and older adults of Caucasians in the US and Chinese in Singapore, and it is unknown
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25 309 whether our findings could be generalized to other demographic or ethnic groups.
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28 310 **Conclusions**

29
30 311 In summary, our data provide reproducible evidence from 4 multiethnic cohorts that high long chain n-3
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32 312 PUFAs and fish intakes accentuate the genetic association of the *FADS* with adiposity. These findings
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34 313 emphasize the importance of considering precision nutritional interventions on prevention and treatment
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36 314 of obesity. We acknowledge that these results are hypothesis-generating and need to be confirmed in
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38 315 additional cohorts.
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43 317 **Contributors:** TH and LQ designed the study and wrote the first draft. TH analyzed the data. FBH
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45 318 provided statistical expertise. TW, YH, DS, CSF, JW, LRP, AT, GC, IDV, HKC, JF, XS, CCK, YF, RM,
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47 319 HCK, JY, KWP, and LQ were involved in data collection. TH and LQ are guarantors. All authors
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49 320 contributed to the interpretation of the results and critical revision of the manuscript for important
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51 321 intellectual content and approved the final version of the manuscript.
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53 322 We acknowledge Dr. Gary C. Curhan's contribution to the genetic data.
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Funding

This study was supported by grants HL126024, HL034594, DK100383, DK091718, HL071981, HL073168, CA87969, CA49449, CA055075, HL34594, HL088521, U01HG004399, DK080140, P30DK46200, U01CA137088, U54CA155626, DK58845, DK098311, U01HG004728, EY015473, CA134958, DK70756 and DK46200 from the National Institutes of Health, with additional support for genotyping from Merck Research Laboratories, North Wales, PA. LQ is a recipient of the American Heart Association Scientist Development Award (0730094N). LRP is supported by the Arthur Ashley Williams Foundation and a Harvard Ophthalmology Scholar Award (Harvard Medical School) from the Harvard Glaucoma Center of Excellence. ATC is a Damon Runyon Cancer Foundation Clinical Investigator. The SCHS study is supported by the National Institutes of Health, USA (NCI R01 CA144034, UM1 CA182876, R01 DK080720), the Singapore National Medical Research Council (NMRC 1270/2010), the HUI-CREATE Programme of the National Research Foundation, Singapore (Project Number 370062002) and Biomedical Research Council, Singapore. The funding sources had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Declaration of interests: All authors have no conflict of interest to declare. No support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years, no other relationship or activity that could appear to have influenced the submitted work.

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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	/

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

<i>FADS</i> genotypes		Three categories of long chain n-3 PUFAs and fish intakes			P for trend	P for interaction*
		≤1/wk	1~6/wk	≥1/d		
Total fish, serving/day						
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	2×10 ⁻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	1.5×10 ⁻⁶	
Total EPA+DHA, g/day		Tertile1	Tertile2	Tertile3		

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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.

¹P 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 \pm SE.

Numbers of participants across three categories ($\leq 1/\text{wk}$ / $1\sim 6/\text{wk}$ / $\geq 1/\text{d}$) 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.

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

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

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

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15 The multiple linear regression model was used to test the genetic association of the *FADS* variant (additive
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17 model) with long-term changes in BMI by frequency of fish intake and tertiles of LC fatty acids after
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19 adjustment for age, source of genotyping data, baseline BMI, smoking, alcohol intake, physical activity,
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21 total energy intake, alternate healthy eating index, television watching, sugar sweetened beverage, fried
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23 food consumption. The results were pooled by means of fixed effects meta-analyses ($P \geq 0.05$ for
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25 heterogeneity between studies).
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31 **Figure 3 Predicted long-term changes in BMI from long chain n-3 PUFAs intake according to *FADS***
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33 **genotypes**

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

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

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

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47 The individual participant data from the NHS and HPFS cohorts were pooled.
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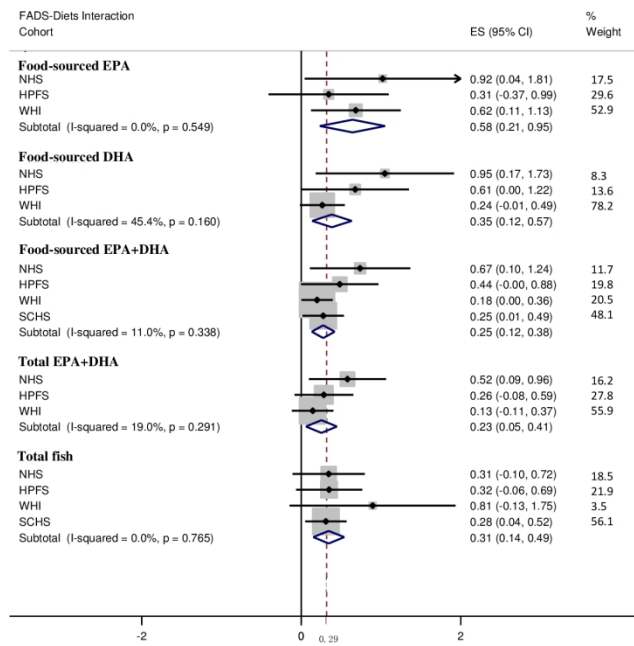
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3 **Figure 4 Predicted long-term changes in BMI from fish intake according to *FADS* genotypes**
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5 Numbers of T carriers/Non-T carriers in the NHS and HPFS are 1698/9625 and 1025/5808, respectively.
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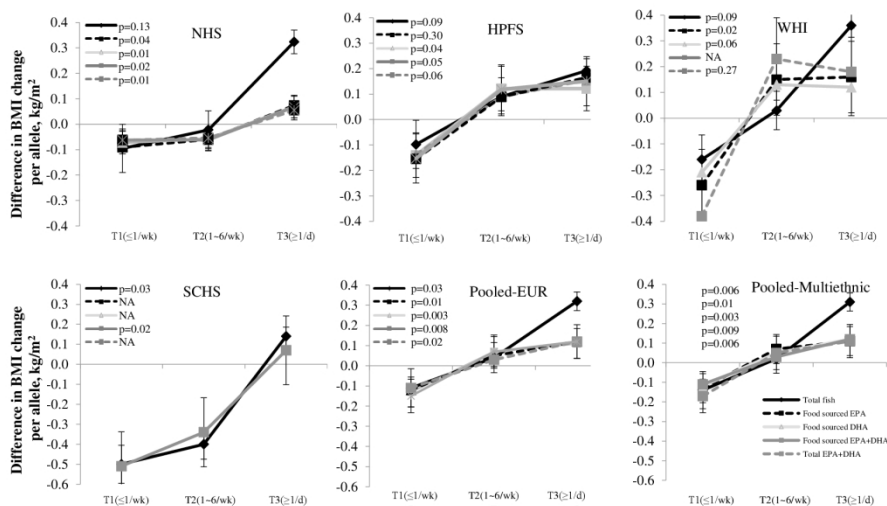
7 Black circles for T allele carriers and open circle for non-T-carriers.
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9 The multiple linear regression model was used to test the associations of and fish intake with long-term
10 changes in BMI according to *FADS* genotypes after adjustment for age, source of genotyping data,
11 baseline BMI, smoking, alcohol intake, physical activity, total energy intake, alternate healthy eating
12 index, television watching, sugar sweetened beverage, fried food consumption.
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18 The individual participant data from the NHS and HPFS cohorts were pooled.
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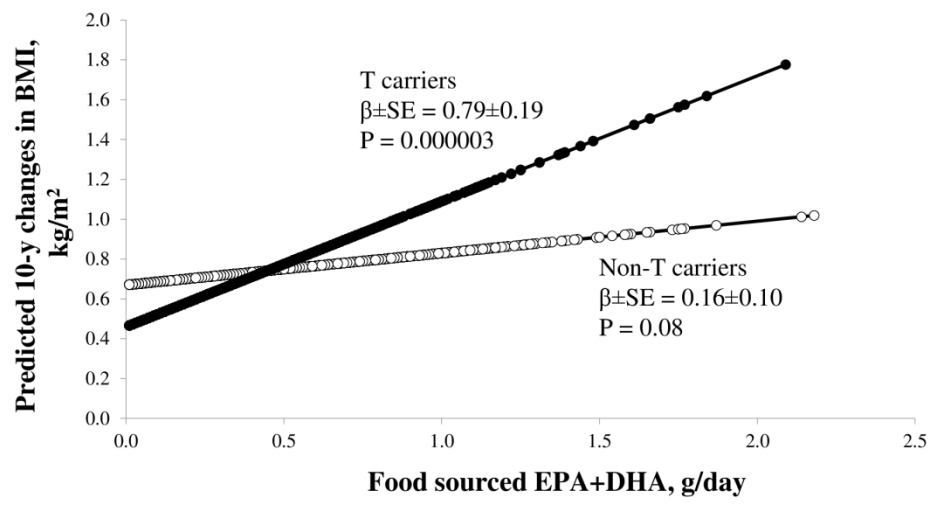


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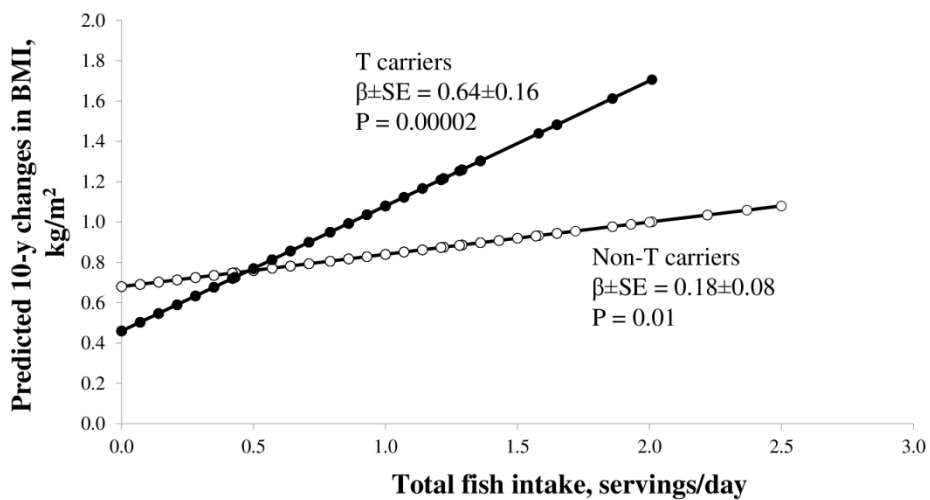


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Supplemental table 1. Annotation for the top six SNPs under positive selection in Greenlandic Inuit

Position ¹	Reference		DAF							PBS
	SNP identification number	Alleles ²	CEU	CHB	GI	NHS	HPFS	WHI	SCHS	
			chr11:61627960	rs74771917	C/T	0.025	0.16	0.98	/	
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

¹Positions refer to human genome assembly hg19.

²Alleles 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)	<i>FADS</i> 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,			P for interaction
	kg			
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 \pm 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 $P \geq 0.05$ 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	<i>FADS</i> 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	7×10 ⁻⁴	

Data are means ± SE.

¹P 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

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

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

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

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

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24 Registration: [www. clinicaltrials.gov](http://www.clinicaltrials.gov). Registration ID: NCT03348566
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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			
<i>Background rationale</i>	2	Explain the scientific background and rationale for the investigation being reported. (p. 4)	
<i>Objectives</i>	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			
<i>Study design</i>	4	Present key elements of study design early in the paper. (p. 5)	

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Setting</i>	5	Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up, and data collection. (p. 3)	
<i>Participants</i>	6	<p>(a) Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. (p. 5)</p> <p>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.</p> <p>Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants.</p> <hr/> <p>(b) Cohort study – For matched studies, give matching criteria and number of exposed and unexposed.</p> <p>Case-control study – For matched studies, give matching criteria and the number of controls per case.</p>	<i>Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant. (p. 5)</i>
<i>Variables</i>	7	<i>(a)</i> Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable. (p. 5)	<i>(b)</i> 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)

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Data sources measurement</i>	8*	<i>(a)</i> 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)	<i>(b)</i> 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)
<i>Bias</i>	9	<i>(a)</i> Describe any efforts to address potential sources of bias. (p. 5 &6)	<i>(b)</i> 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)
			<i>with this. (p. 5 &6)</i>
<i>Study size</i>	10	Explain how the study size was arrived at. (p. 5 &6)	
<i>Quantitative variables</i>	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. (p. 7)	<i>If applicable, describe how effects of treatment were dealt with. (p. 7)</i>
<i>Statistical methods</i>	12	<p>(a) Describe all statistical methods, including those used to control for confounding. (p. 7-9)</p> <hr/> <p>(b) Describe any methods used to examine subgroups and interactions. (p. 9)</p> <hr/> <p>(c) Explain how missing data were addressed. (p. 9)</p> <hr/> <p>(d) Cohort study – If applicable, explain how loss to follow-up was addressed. (p. 9)</p> <p>Case-control study – If applicable, explain how matching of cases and controls was addressed.</p> <p>Cross-sectional study – If applicable, describe analytical methods taking account of sampling strategy.</p>	<i>State software version used and options (or settings) chosen. (p. 9)</i>

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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			
<i>Participants</i>	13*	<p>(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)</p> <hr/> <p>(b) Give reasons for non-participation at each stage. (p. 10)</p> <hr/> <p>(c) Consider use of a flow diagram. (p. 10)</p>	<p>Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful. (p. 10)</p>
<i>Descriptive data</i>	14*	<p>(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders. (p. 10)</p> <hr/> <p>(b) Indicate the number of participants with missing data for each variable of interest. (p. 10)</p> <hr/> <p>(c) Cohort study – Summarize follow-up time, e.g. average and total amount. (p. 10)</p>	<p>Consider giving information by genotype. (p. 10)</p>

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Outcome data</i>	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
<i>Main results</i>	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)	(d) Report results of any adjustments for multiple
		(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)	

Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
			<i>comparisons. (p. 10)</i>
<i>Other analyses</i>	17	(a) Report other analyses done – e.g., analyses of subgroups and interactions, and sensitivity analyses. (p. 10)	
			<i>(b) If numerous genetic exposures (genetic variants) were examined, summarize results from all analyses undertaken. (p. 10)</i>
			<i>(c) If detailed results are available elsewhere, state how they can be accessed. (p. 10)</i>
Discussion			
<i>Key results</i>	18	Summarize key results with reference to study objectives. (p. 11)	
<i>Limitations</i>	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)	

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Item	Item number	STROBE Guideline	Extension for Genetic Association Studies (STREGA)
<i>Interpretation</i>	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. (p. 11)	
<i>Generalizability</i>	21	Discuss the generalizability (external validity) of the study results. (p. 11)	
Other Information			
<i>Funding</i>	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 = STrengthening 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.