Dietary Fat Intake and Development of Specific Breast Cancer Subtypes.

Sieri, Sabina; Chiodini, Paolo; Agnoli, Claudia; Pala, Valeria; Berrino, Franco; Trichopoulou, Antonia; Benetou, Vassiliki; Vasilopoulou, Effie; Sánchez, María-José; Chirlaque, Maria-Dolores; Amiano, Pilar; Quirós, J Ramón; Ardanaz, Eva; Buckland, Genevieve; Masala, Giovanna; Panico, Salvatore; Grioni, Sara; Sacerdote, Carlotta; Tumino, Rosario; Boutron-Ruault, Marie-Christine; Clavel-Chapelon, Françoise; Fagherazzi, Guy; Peeters, Petra H M; van Gils, Carla H; Bueno-de-Mesquita, H Bas; van Kranen, Henk J; Key, Timothy J; Travis, Ruth C; Khaw, Kay Tee; Wareham, Nicholas J; Kaaks, Rudolf; Lukanova, Annekatrin; Boeing, Heiner; Schütze, Madlen; Sonestedt, Emily; Wirfält, Elisabet; Sund, Malin; Andersson, Anne; Chajes, Veronique; Rinaldi, Sabina; Romieu, Isabelle; Weiderpass, Elisabete; Skeie, Guri; Dagrun, Engeset; Tjønneland, Anne; Halkjær, Jytte; Overvard, Kim; Merritt, Melissa A; Cox, David; Riboli, Elio

Published in:
Journal of the National Cancer Institute

DOI:
10.1093/jnci/dju068

2014

JNCI 13-1931R

Brief Communication

DIETARY FAT INTAKE AND DEVELOPMENT OF SPECIFIC BREAST CANCER SUBTYPES

Sabina Sieri[1], Paolo Chiodini[2], Claudia Agnoli[1], Valeria Pala[1] , Franco Berrino[1], Antonia Trichopoulou[3], Vassiliki Benetou[4], Effie Vasilopoulou[4], María-José Sánchez[5,6,7], Maria-Dolores Chirlaque[6,8], Pilar Amiano[6,9],J Ramón Quirós[10], Eva Ardanaz[6,11], Genevieve Buckland[12], Giovanna Masala[13], Salvatore Panico[14], Sara Grioni[1], Carlotta Sacerdote[15,16] , Rosario Tumino[17, 18], Marie-Christine Boutron-Ruault[19], Françoise Clavel-Chapelon[20], Guy Fagherazzi[21], Petra H.M Peeters[22], Carla H van Gils[22], H.Bas Bueno-de-Mesquita[23,24,25], Henk J. van Kranen[23], Timothy J Key[26], Ruth C Travis[26], Kay Tee Khaw[27], Nicholas J Wareham[28], Rudolf Kaaks[29], Annekatrin Lukanova[29], Heiner Boeing[30] , Schütze M[30], Emily Sonestedt[31], Elisabeth Wirfält[31], Malin Sund[32], Anne Andersson[33] ,Veronique Chajes[34], Sabina Rinaldi[34], Isabelle Romieu[34] , Elisabete Weiderpass[35,36;37,38], Guri Skeie[35], Engeset Dagrun[35], Anne Tjønneland[39], Jytte Halkjær[39], Kim Overvard[40], Melissa A Merritt[41], David Cox[34,41], Elio Riboli[41] and Vittorio Krogh[1]

[1]Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

[2] Medical Statistics Unit, Second University of Naples, Naples, Italy

[3] Hellenic Health Foundation, Athens, Greece

[4]Department of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Athens, Greece

[5] Escuela Andaluza de Salud Pública, Granada, Spain

[6]CIBER de Epidemiología y Salud Pública (CIBERESP), Spain

[7]Instituto de Investigación Biosanitaria de Granada (Granada.bs), Granada, Spain

[8]Department of Epidemiology, Murcia Health Authority, Murcia, Spain

[9]Public Health Division of Gipuzkoa, BioDonostia Research Institute, Health Department of Basque Region, San Sebastian, Spain

[10]Health Information Unit, Public Health and Health Planning Directorate, Asturias, Spain

[11]Navarre Public Health Institute, Pamplona, Spain.

[12]Unit of Nutrition, Environment and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO-IDIBELL), Barcelona, Spain

[13]Molecular and Nutritional Epidemiology Unit, ISPO-Cancer Research and Prevention Institute, Florence, Italy

[14]Department of Clinical and Experimental Medicine, University of Naples Federico II, Naples, Italy

[15]Center for Cancer Prevention (CPO-Piemonte), Turin, Italy

[16]Human Genetics Foundation (HuGeF), Turin, Italy

[17] Department of Oncology, Histopathology Unit, Ospedale Civile "M.P. Arezzo", Ragusa, Italy

[18]Cancer Registry, Ospedale Civile "M.P. Arezzo", Ragusa, Italy.

[19]INSERM, Centre for research in Epidemiology and Population Health (CESP), U1018, Nutrition, Hormones and Women's Health team, F-94805, Villejuif, France

[20]Univ Paris Sud, UMRS 1018, F-94805, Villejuif, France

[21]IGR, F-94805, Villejuif, France

[22]Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

[23]National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

[24]Department of Gastroenterology and Hepatology, University Medical Centre, Utrecht, The Netherlands

[25] The School of Public Health, Imperial College London, London, United Kingdom

[26]Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, United Kingdom

[27]Dunn Human Nutrition Unit, Medical Research Council, Cambridge, United Kingdom

[28]Epidemiology Unit, Institute of Metabolic Science, Medical Research Council, Cambridge, United Kingdom

[29]Department of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany

[30]Department of Epidemiology, German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany

[31] Department of Clinical Sciences in Malmö, Lund University, Sweden

[32]Department of Surgical and Perioperative Sciences/ Surgery, Umeå University, Sweden

[33] Department of Radiation Sciences, Oncology, Umeå University, Sweden

[34]International Agency for Research on Cancer (IARC), Lyon, France

[35]Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway
[36] Department of Research, Cancer Registry of Norway, Oslo, Norway
[37]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
[38]Samfundet Folkhälsan, Helsinki, Finland
[39]Danish Cancer Society Research Center, Copenhagen, Denmark
[40]Department of Clinical Epidemiology Aarhus University Hospital, Aalborg, Denmark
[41]Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK

Corresponding author:
Sabina Sieri, PhD
Epidemiology and Prevention Unit,
Department of Preventive & Predictive Medicine,
Fondazione IRCCS Istituto Nazionale dei Tumori,
Via Venezian 1,
I-20133 Milan, Italy.
Tel: +39 02 23903506; Fax: +39 02 23903510;
E-mail: sabina.sieri@istitutotumori.mi.it

Abstract

We prospectively evaluated fat intake as predictor of developing breast cancer (BC) subtypes defined by ER, PR and HER2, in a large (n=337,327) heterogeneous cohort of women, with 10,062 BC cases after 11.5 years, estimating BC hazard ratios (HR) by Cox proportional hazard modeling. High total and saturated fat were associated with greater risk of ER+PR+ disease (HR:1.20; 95%CI:1.00 -1.45; HR:1.28; 95%CI:1.09-1.52, highest vs. lowest quintiles) but not ER-PR- disease. High saturated fat was statistically significantly associated with greater risk of HER2- disease. High saturated fat intake particularly increases risk of receptor-positive disease, suggesting saturated fat involvement in the etiology of this BC subtype.

The hypothesis that high fat intake increases breast cancer (BC) risk dates back to the 1970s (1), but has been persistently controversial. An extensive 2007 review (2) concluded that evidence from prospective epidemiological studies was inconsistent, while case-control studies indicate a statistically significant positive association between fat intake and BC. Our recent EPIC study (European Investigation into Cancer and Nutrition), found weak but statistically significant positive associations of saturated fat intake with BC risk (3). The conflicting results of earlier studies are likely due to difficulties in obtaining precise estimates of fat intake, and also to limited heterogeneity of intake within geographically confined populations (4).

BC is now classified into subtypes determined clinically by the expression of receptors for estrogen (ER), progesterone receptor (PR) and human epidermal growth factor (HER2) (5;6): the subtypes differ in prognoses and factors influencing their occurrence (5), which may have confounded associations between fat intake and BC. The association of fat intake with risk of BC subtypes has been little studied and with conflicting results (7-11).

To further investigate the effect of dietary fat on BC, we expanded the follow-up of our EPIC study (3), prospectively evaluating associations of dietary fat with BC subtypes defined by ER, PR and HER2 status. EPIC is a prospective cohort study conducted in 10 European countries (12) which recruited volunteers after informed consent and completion of dietary and lifestyle questionnaires. The study was approved by the ethical committees of the International Agency for Research on Cancer and participating centers.

The present EPIC study was conducted on 337,327 women and used multivariate Cox proportional hazard modeling to estimate hazard ratios (HRs) with 95% confidence intervals (CIs) for developing

BC in relation to fat intake (as quintiles and continuous variables), with stratification by center and age. Non-alcohol energy, energy from alcohol, smoking, education, age at menarche, full-term pregnancy, hormone therapy use, and BMI/menopausal status interaction, were covariates.

The proportional hazards assumption for each fat and fat subtype in relation to breast cancer risk was tested using the Grambsch and Therneau method (13). In all cases, the proportional hazards assumption was satisfied. The form of the predictor in the Cox regression is linear and was tested by means of a restricted cubic spline with 5 knots (14)**.**

To correct the dietary questionnaires for measurement errors, intake data were calibrated against highly standardized 24-hour dietary recall interviews on a random sample (8.0%) of the cohort (15;16) (See Supplementary Methods, available online, for more details). All tests of statistical significance were two-sided and a P value of less or equal to 0.05 was considered statistically significant.

After a mean of 11.5 years (359,814 person-years) 10,062 incident cases were identified. ER, PR and HER2 status, obtained from pathology reports, were available for 70.6%, 59.0%, and 22.9% of cases, respectively.

Women in the highest quintile of saturated fat intake had a statistically significantly greater risk of BC than those in the lowest quintile. Increases in total and monounsaturated fat intake (continuous variables) were also associated with greater BC risk (Supplementary Table 1). The association between fat intake and BC did not vary with menopausal status at baseline or at diagnosis (data not shown).

High total fat intake was positively associated with development of ER+PR+ disease (HR:1.20; 95%CI:1.00-1.45 highest vs. lowest quintile), but not ER-PR- disease, with statistically significant (P=0.05) heterogeneity between ER+PR+ and ER-PR- cancers (**Table 1**).

Women with highest quintile of saturated fat consumption had a statistically significantly greater risk of ER+PR+ BC than those in the lowest quintile (HR:1.28;95%CI:1.09-1.52), with a statistically significant trend (P=0.009). Increasing saturated fat intake (continuous variable) was also associated with greater risk of ER+PR- BC. Heterogeneity tests were not statistically significant for saturated fat. No association of any fat type with ER-PR- disease was found. Risk estimates for ER+, ER-, PR+ and PR- BC are presented separately in Supplementary-Table 2.

No association of any fat with HER2+ BC was found (**Table 2**). For saturated fat, all intake quintiles were associated with a statistically significantly greater risk of HER2- BC than reference (HR: 1.29; 95%CI:1.01-1.64 highest vs. lowest), with a statistically significant trend (P=0.04). Increase in monounsaturated fat intake (continuous variable) was also associated with greater risk of HER2-

disease. Furthermore, heterogeneity tests comparing HER2+ with HER2- cancer were always statistically non-significant.

The results of this study support our original finding (3) that high saturated fat intake is statistically significantly associated with increased BC risk, but indicate that excess dietary fat is more strongly associated with hormone-sensitive than receptor-negative disease. Similar findings have been reported previously (7,9,17), although other studies on postmenopausal women (10,11) found no evidence that the association between dietary fat and BC varied with ER or PR status.

High lifetime exposure to estrogen (early menarche, late menopause, postmenopausal hormone therapy and postmenopausal adiposity) is more strongly associated with ER+PR+ than ER-PR- BC(5); while high endogenous sex hormone levels have also been related to the development of receptor-positive BC (18-22). It is unclear whether high dietary fat increases sex hormone levels, but this is one mechanism by which is fat could increase susceptibility to receptor-positive BC (23).

Fat intake and BC HER2 status appear not to have been investigated previously. We found positive associations between high saturated and monounsaturated fat intake and HER- BC, but no relation to HER2+ disease. HER2+ BC is aggressive and seems little influenced by hormone-related risk factors (24). Furthermore our finding of no association between any type of fat intake and HER2+ disease is consistent with the fact that HER2+ cancers typically do not express ER or PR, and do not respond to tamoxifen (25;26). Evidence suggests that factors influencing hormonal status (e.g. parity, age at menarche, age at menopause) only influence the risk of developing HER2- disease (24;26).

Our study strengths are prospective design, large proportion of cases with receptor information, and wide variation in fat intake. The main source of hormone receptor data was medical records. Although receptor status was usually determined immunohistochemically, methods used varied across laboratories resulting in some misclassification. Another concern is that women with hormone receptor information may differ from those without this information. However findings for sub-groups without ER, PR or HER2 information were similar to these with this information, suggesting no selection bias related to receptor status information.

All dietary assessment methods involve measurement error. Although the dietary questionnaires used in the various centers were similar, they differed in detail because each was designed to capture local eating habits. To compensate for errors generated by differences in dietary assessment, we corrected (calibrated) dietary data, using data ‘predicted’ by 24-h dietary recall.

To conclude, the results of this prospective study on a large heterogeneous population of European women indicate that a high fat diet increases BC risk and, most conspicuously, that high saturated fat

intake increases risk of receptor-positive disease, suggesting saturated fat involvement in the etiology of receptor-positive BC.

Funding

EPIC is supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. National cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, and Institut National de la Santé et de la Recherche Médicale (France); Deutsche Krebshilfe, Deutsches Krebsforschungszentrum, and Federal Ministry of Education and Research (Germany); Hellenic Health Foundation (Greece); Italian Association for Research on Cancer, National Research Council, and Associazione Iblea per la Ricerca Epidemiologica (AIRE-ONLUS) Ragusa, Associazione Volontari Italiani Sangu Ragusa, Sicilian Government (Italy); Dutch Ministry of Public Health, Welfare and Sports, Netherlands Cancer Registry, LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund, and Statistics Netherlands (the Netherlands); European Research Council (grant number ERC-2009-AdG 232997) and Nordforsk, and Nordic Center of Excellence Programme on Food, Nutrition and Health (Norway); Health Research Fund, Regional Governments of Andalucía, Asturias, Basque Country, Murcia (No. 6236) and Navarra, the Centro de Investigación Biomédica en Red en Epidemiología y Salud Pública and Instituto de Salud Carlos II (RD06/0020) the Regional Government the Spanish Ministry of Health (FIS) and CIBERESP, San Sebastian (Spain); The Spanish Ministry of Health (ISCIII RETICC RD06/0020/0091) and the Catalan Institute of Oncology; Swedish Cancer Society, Swedish Scientific Council, and Regional Government of Skåne and Västerbotten (Sweden); Cancer Research UK, Medical Research Council, Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency, and Wellcome Trust (UK).

Note

The study sponsors had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; and the decision to submit the manuscript for publication.

References

(1) Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int J Cancer.* 1975;15(4):617-631.

(2) World Cancer Research Fund and American Institute for Cancer Research. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington, DC: AICR; 2007.

(3) Sieri S, Krogh V, Ferrari P et al. Dietary fat and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr.* 2008;88(5):1304-1312.

(4) Prentice RL. Measurement error and results from analytic epidemiology: dietary fat and breast cancer. *J Natl Cancer Inst.* 1988;88:1738-1747.

(5) Althuis MD, Fergenbaum JH, Garcia-Closas M et al. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. *Cancer Epidemiol Biomarkers Prev.* 2004;13(10):1558-1568.

(6) Rakha EA, El-Sayed ME, Green AR et al. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *J Clin Oncol.* 2007;25(30):4772-4778.

(7) Kushi LH, Potter JD, Bostick RM et al. Dietary fat and risk of breast cancer according to hormone receptor status.

(8) Lof M, Sandin S, Lagiou P et al. Dietary fat and breast cancer risk in the Swedish women's lifestyle and health cohort. *Br J Cancer.* 2007.

(9) Cho E, Spiegelman D, Hunter DJ et al. Premenopausal fat intake and risk of breast cancer. *J Natl Cancer Inst.* 2003;95(14):1079-1085.

(10) Park SY, Kolonel LN, Henderson BE, Wilkens LR. Dietary fat and breast cancer in postmenopausal women according to ethnicity and hormone receptor status: the Multiethnic Cohort Study. *Cancer Prev Res (Phila).* 2012;5(2):216-228.

(11) Kim EH, Willett WC, Colditz GA et al. Dietary fat and risk of postmenopausal breast cancer in a 20-year follow-up. *Am J Epidemiol.* 2006;164(10):990-997.

(12) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol.* 1997;26 Suppl 1:S6-14.

(13) Grambsch PM , Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika.* 1994;81(3):515-526.

(14) Harrell FE. Regression Modelling Strategies: with applications to linear models, logistic regression and survival analysis. New York: Springer-Verlag; 2001.

(15) Slimani N, Ferrari P, Ocke M et al. Standardization of the 24-hour diet recall calibration method used in the european prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr.* 2000;54(12):900-917.

(16) Ferrari P, Day NE, Boshuizen HC et al. The evaluation of the diet/disease relation in the EPIC study: considerations for the calibration and the disease models. *Int J Epidemiol.* 2008;37(2):368-378.

(17) Chlebowski RT, Blackburn GL, Thomson CAet al. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J Natl Cancer Inst.* 2006;98(24):1767-1776.

(18) James RE, Lukanova A, Dossus L et al. Postmenopausal serum sex steroids and risk of hormone receptor-positive and -negative breast cancer: a nested case-control study. *Cancer Prev Res (Phila).* 2011;4(10):1626-1635.

(19) Sieri S, Krogh V, Bolelli G et al. Sex hormone levels, breast cancer risk, and cancer receptor status in postmenopausal women: the ORDET cohort. *Cancer Epidemiol Biomarkers Prev.* 2009;18(1):169-176.

(20) Zhang X, Tworoger SS, Eliassen AH, Hankinson SE. Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up. *Breast Cancer Res Treat.* 2013;137(3):883-892.

(21) Zeleniuch-Jacquotte A, Afanasyeva Y, Kaaks R et al. Premenopausal serum androgens and breast cancer risk: a nested case-control study. *Breast Cancer Res.* 2012;14(1):R32.

(22) Fortner RT, Eliassen AH, Spiegelman D et al. Premenopausal endogenous steroid hormones and breast cancer risk: results from the Nurses' Health Study II. *Breast Cancer Res.* 2013;15(2):R19.

(23) Prentice RL, Caan B, Chlebowski RTet al. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. JAMA. 2006;295(6):629-642.

(24) Balsari A, Casalini P, Bufalino R, Berrino F, Menard S. Role of hormonal risk factors in HER2-positive breast carcinomas. *Br J Cancer.* 2003;88(7):1032-1034.

(25) Pietras RJ, Arboleda J, Reese DM et al. HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. *Oncogene.* 1995;10(12):2435-2446.

(26) Carlomagno C, Perrone F, Gallo C et al. c-erb B2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. *J Clin Oncol.* 1996;14(10):2702-2708.

Table 1. Multivariable-adjusted hazard ratios (HRs)* with 95% confidence intervals (CIs) for developing breast cancer subtypes defined by hormone receptor status, according to quintiles of fat intake (5601 cases)

	Cases/ person-year	**HR (95% CI)**	Cases/ person-year	**HR (95% CI)**	Cases/ person-year	**HR (95% CI)**	Cases/ person-year	**HR (95% CI)**
		ER + PR+		**ER + PR -**		**ER- PR-**		**ER PR unknown**
Total fat (g/day)								
1 (43.2)	644/646061	1	185/646061	1	196/646061	1	632/646061	1
2 (59.8)	694/646187	1.05(0.94-1.18)	205/646187	1.07(0.87-1.33)	200/646187	0.96(0.78-1.18)	646/646187	1.01(0.90-1.14)
3 (72.6)	689/646722	1.03(0.91-1.17)	208/646722	1.04(0.83-1.32)	196/646722	0.89(0.71-1.12)	666/646722	1.09(0.96-1.24)
4 (87.4)	703/649001	1.03(0.90-1.19)	244/649001	1.19(0.92-1.55)	204/649001	0.84(0.64-1.09)	644/649001	1.12(0.96-1.30)
5 (117.3)	810/644992	**1.20(1.00-1.45)**	230/644992	1.11(0.79-1.56)	222/644992	0.79(0.56-1.11)	567/644992	1.15(0.94-1.40)
P for trend†		0.21		0.35		0.13		0.09
Intake as continuous variable‡		1.03(0.99-1.07)		1.05(0.98-1.12)		0.96(0.90-1.03)		1.03(0.99-1.07)
P for heterogeneity§			0.71		**0.05**			
Calibrated data‖		**1.10(1.01-1.20)**		**1.18(1.01-1.39)**		0.87(0.74-1.02)		1.07(0.98-1.17)
Saturated fat (g/day)								
1 (15.4)	584/644252	1	175/644252	1	166/644252	1	614/644252	1
2 (22.2)	683/645600	1.10(0.98-1.24)	179/645600	0.99(0.80-1.23)	193/645600	1.04(0.84-1.30)	616/645600	0.98(0.87-1.10)
3 (27.6)	674/648063	1.07(0.95-1.21)	232/648063	**1.26(1.01-1.57)**	230/648063	1.17(0.93-1.46)	644/648063	1.01(0.89-1.15)
4 (33.9)	734/648537	**1.15(1.01-1.32)**	219/648537	1.16(0.91-1.48)	183/648537	0.86(0.67-1.11)	678/648537	1.09(0.95-1.25)
5 (47.5)	865/646512	**1.28(1.09-1.52)**	267/646512	1.31(0.97-1.77)	246/646512	0.96(0.70-1.31)	603/646512	1.07(0.90-1.27)
P for trend†		**0.009**		**0.05**		0.39		0.19
Intake as continuous variable‡		**1.03(1.01-1.06)**		**1.06(1.01-1.11)**		0.99(0.94-1.04)		1.02(0.99-1.05)
P for heterogeneity§			0.44		0.08			
Calibrated data‖		**1.09(1.03-1.16)**		**1.16(1.04-1.29)**		0.96(0.86-1.06)		1.03(0.97-1.09)
Monounsaturated fat (g/day)								

1 (14.2)	655/645501	1	181/645500	1	187/645501	1	669/645501	1
2 (20.2)	646/644536	0.94(0.84-1.06)	202/644536	1.07(0.87-1.33)	209/644536	1.04(0.85-1.29)	652/644536	0.98(0.86-1.08)
3 (25.2)	737/645323	1.04(0.92-1.17)	230/645323	1.14(0.91-1.44)	209/645323	0.97(0.76-1.21)	682/645323	1.06(0.94-1.21)
4 (31.6)	783/649092	1.06(0.92-1.22)	244/649092	1.14(0.88-1.48)	207/649092	0.89(0.69-1.16)	668/649092	**1.17(1.01-1.35)**
5 (46.4)	719/648512	1.09(0.91-1.30)	215/648512	1.16(0.83-1.61)	206/648512	0.95(0.68-1.34)	484/648512	1.06(0.87-1.30)
P for trend†		0.17		0.34		0.44		0.07
Intake as continuous variable‡		1.02(0.99-1.06)		1.04(0.98-1.10)		0.97(0.92-1.03)		1.03(0.99-1.06)
P for heterogeneity§			0.77		0.06			
Calibrated data‖		**1.08(1.01-1.17)**		1.14(1.00-1.30)		0.92(0.81-1.05)		1.07(1.00-1.15)
Polyunsaturated fat (g/day)								
1 (6.6)	714/655117	1	230/655117	1	230/655117	1	588/655117	1
2 (9.3)	729/646944	1.01(0.90-1.12)	218/646944	1.00(0.82-1.21)	171/646944	0.80(0.65-0.98)	638/646944	1.01(0.89-1.13)
3 (11.6)	704/643652	0.96(0.85-1.08)	198/643652	0.90(0.73-1.12)	188/643652	0.87(0.70-1.08)	672/643652	1.04(0.92-1.18)
4 (14.6)	669/643236	0.89(0.78-1.01)	223/643236	0.98(0.78-1.22)	225/643236	1.01(0.80-1.27)	642/643236	1.02(0.89-1.16)
5 (21.6)	724/644016	0.98(0.85-1.13)	203/644016	0.90(0.69-1.16)	204/644016	0.91(0.70-1.19)	615/644016	1.03(0.89-1.20)
P for trend†		0.28		0.45		0.77		0.68
Intake as continuous variable‡		0.98(0.96-1.00)		0.97(0.93-1.00)		0.98(0.94-1.02)		1.00(0.98-1.03)
P for heterogeneity§			0.79	0.92(0.83-1.01)	0.49			
Calibrated data‖		0.96(0.91-1.01)				0.96(0.87-1.05)		1.02(0.97-1.07)

*Stratified by center, age and adjusted for non-alcohol energy, educational attainment, smoking status, BMI/menopausal status interaction, energy from alcohol, full term pregnancy and hormone replacement therapy use.

†Tests of linear trend were performed by modeling the variable whose value was the number of the quintile to which the subject belonged. All statistical tests were two-sided.

‡$\text{Log}_{1.2}$ transformed (so HRs represent the risk associated with a 20% increase in fat intake).

[§]ER+ PR+ vs. ER+ PR-, ER+ PR+ vs. ER- PR-. Test for Heterogeneity.

[‖] Calibrated data were obtained by linear regression models that compared observed nutrient questionnaire measurements with 24-hour dietary recall.

ER=estrogen receptor; PR=progesterone receptor

Table 2. Multivariable-adjusted hazard ratios (HRs)* with 95% confidence intervals (CIs) for developing breast cancer subtypes defined by HER2 status, according to quintiles of fat intake (2259 cases)						
	Cases/person-year	**HR (95% CI)**	Cases/person-year	**HR (95% CI)**	Cases/person-year	**HR (95% CI)**
		HER2 positive		**HER2 negative**		**HER2 unknown**
Total fat (g/day)						
1 (44.5)	116/576095	1	343/576095	1	961/576096	1
2 (61.7)	109/571176	1.01 (0.76-1.34)	357/571176	1.11 (0.94-1.30)	1049/571176	1.03 (0.94-1.12)
3 (74.6)	96/567416	0.93 (0.68-1.29)	352/567416	1.15 (0.96-1.38)	1173/567416	1.03 (0.94-1.14)
4 (89.5)	94/566802	0.94 (0.65-1.37)	330/566803	1.13 (0.92-1.40)	1214/566803	1.05 (0.94-1.17)
5 (119.6)	124/559834	1.34 (0.84-2.14)	338/559835	1.28 (0.98-1.68)	1359/559835	1.06 (0.92-1.22)
P for trend†		0.59		0.14		0.42
Intake as continuous variable‡		0.99 (0.91-1.09)		1.05 (1.00-1.11)		1.02 (0.99-1.05)
P for heterogeneity§			0.30		0.94	
Calibrated data‖		1.09(0.87-1.36)		**1.13(1.00-1.28)**		1.03(0.97-1.10)
Saturated fat (g/day)						
1 (15.7)	111/571950	1	298/571950	1	1110/571950	1
2 (22.9)	105/570260	0.90 (0.68-1.19)	370/570260	**1.19 (1.01-1.39)**	1201/570260	1.01 (0.92-1.10)
3 (28.3)	115/569843	1.01 (0.75-1.37)	359/569843	**1.20 (1.01-1.43)**	1265/569843	1.08 (0.98-1.19)
4 (34.8)	101/567160	0.91 (0.64-1.28)	359/567160	**1.27 (1.04-1.54)**	1261/567160	1.07 (0.97-1.19)
5 (48.6)	107/562112	0.95 (0.62-1.46)	334/562112	**1.29 (1.01-1.64)**	919/562112	**1.14 (1.01-1.30)**
P for trend†		0.86		**0.04**		**0.03**
Intake as continuous variable‡		0.98 (0.92-1.05)		**1.04 (1.00-1.09)**		**1.03 (1.01-1.05)**
P for heterogeneity§			0.14		0.53	
Calibrated data‖		1.00(0.86-1.15)		**1.11(1.03-1.21)**		**1.05(1.00-1.09)**
Monounsaturated fat (g/day)						
1 (14.7)	99/577228	1	325/577228	1	1040/577228	1
2 (21.0)	101/571405	1.02 (0.76-1.37)	322/571405	1.02 (0.87-1.20)	1101/571405	1.00 (0.92-1.09)
3 (26.1)	101/567034	1.01 (0.73-1.40)	344/567034	1.11 (0.93-1.33)	1239/567034	1.04 (0.94-1.14)
4 (32.6)	108/565210	0.99 (0.69-1.43)	368/565210	1.15 (0.94-1.41)	1179/565210	1.07 (0.96-1.19)

5 (47.4)	130/560448	1.11 (0.70-1.76)	361/560448	1.07 (0.82-1.40)	1197/560448	0.98 (0.85-1.13)
P for trend[†]		0.80		0.28		0.54
Intake as continuous variable[‡]		1.02 (0.94-1.10)		**1.05 (1.01-1.09)**		1.01 (0.99-1.04)
P for heterogeneity[§]			0.52		0.42	
Calibrated data[‖]		1.15(0.96-1.38)		**1.13(1.02-1.25)**		1.01(0.96-1.07)
Polyunsaturated fat (g/day)						
1 (6.6)	133/579794	1	428/579794	1	1040/579794	1
2 (9.5)	112/569599	1.01 (0.77-1.32)	392/569599	1.02 (0.88-1.18)	1101/569599	0.96(0.88-1.05)
3 (11.9)	101/566179	1.02 (0.76-1.38)	307/566179	0.90 (0.76-1.06)	1239/566179	1.01 (0.92-1.11)
4 (15.0)	106/563959	1.22 (0.88-1.68)	322/563959	1.04 (0.86-1.24)	1179/563959	0.91 (0.83-1.04)
5 (22.1)	87/561794	1.12 (0.77-1.62)	271/561794	1.00 (0.81-1.23)	1197/561794	0.93 (0.84-1.04)
P for trend[†]		0.33		0.98		0.13
Intake as continuous variable[‡]		1.01 (0.95-1.06)		0.98 (0.95-1.01)		0.99 (0.97-1.01)
P for heterogeneity[§]			0.45		0.73	
Calibrated data[‖]		1.01(0.88-1.16)		0.95(0.88-1.02)		0.98(0.94-1.02)

[*]Stratified by center, age and adjusted for non-alcohol energy, educational attainment, smoking status, BMI/menopausal status interaction, energy from alcohol, full term pregnancy and hormone replacement therapy use.

†Two-sided tests of linear trend were performed by modeling the variable whose value was the number of the quintile to which the subject belonged.

[‡]$\mathrm{Log}_{1.2}$ transformed (so HRs represent the risk associated with a 20% increase in fat intake).

[§]HER2-positive vs. HER2-negative and HER2-positive vs. HER2-unknown. Two-sided test for Heterogeneity.

[‖]Calibrated data were obtained by linear regression models that compared observed nutrient questionnaire measurements with 24-hour dietary recall.

Supplementary Methods

Study Populations

EPIC is a large prospective cohort study conducted in 23 centers in Denmark (Aarhus, Copenhagen), France, Germany (Heidelberg, Potsdam), Greece, Italy (Florence, Varese, Ragusa, Turin, Naples), Norway, Spain (Asturias, Granada, Murcia, Navarra, San Sebastian), Sweden (Malmö, Umeå), The Netherlands (Bilthoven, Utrecht) and the UK (Cambridge, Oxford) (1).

Briefly, 519,978 volunteers were recruited after giving informed consent. They completed dietary and lifestyle questionnaires, and anthropometric measurements were recorded. The present study was conducted on 337,327 women after excluding those with: prevalent any site cancer at recruitment (n=19,853); lost to follow-up at time 0 (n=2,292); age outside 20-70 years (n=6,401); in situ breast cancer (n=1,398); diet and lifestyle questionnaires not completed (n=3,320); and ratio of total energy intake (determined from the questionnaire) to basal metabolic rate [determined by Harris-Benedict equation (2)] at either extreme of the distribution (cut-offs first and last percentiles) in order to reduce the impact of implausible extreme values (n=6,764).

The study was approved by the International Agency for Research on Cancer ethical committee and the local ethical committees of the participating centers.

Data Collection

Ascertainment of cancer cases. Cases were ascertained by population-based cancer registries in seven countries (Denmark, Italy, the Netherlands, Spain, Sweden, the UK, and Norway). In France, Germany, Greece, and the Italian center of Naples, various methods were used to identify cases, including consulting national health insurance records and regional or national pathology registries; and active follow-up (contacting participants or next-of-kin). Mortality data were obtained mostly from mortality registries at regional or national levels.

Subjects were followed-up from study entry to any cancer diagnosis (except non-melanoma skin cancer), death, emigration or end of follow-up, whichever occurred first. Follow-up ended at the end of: December 2004 in Asturias (Spain); December 2006 [Florence, Varese and Ragusa (Italy);

and Granada and San Sebastian (Spain)]; December 2007 [Murcia and Navarra (Spain), Oxford (UK), Bilthoven and Utrecht (The Netherlands), and Denmark]; June 2008 Cambridge (UK); and December 2008 [Turin (Italy), Malmö, Umeå (Sweden), and Norway]. For study centers with active follow-up, the end of follow-up was considered to be the last known contact with study participants: December 2006 for France and Naples (Italy); December 2008 for Potsdam (Germany); December 2009 for Greece; and June 2010 for Heidelberg (Germany).

The second edition of the International Classification of Diseases for Oncology was used to code cases. Information on ER and PR status was obtained from pathology reports. To standardize the quantification of receptor status, the following criteria for a positive receptor status were adopted: ≥10.0% cells stained, any 'positive' description, ≥20 fmol/mg, Allred score ≥3, immunoreactive score (IRS) ≥2, or H-score ≥10 (3). HER2 overexpression was considered positive for a score of +3 by immunohistochemistry or positive by FISH(4). Information on receptor status (ER, PR and HER2) was not available for any case from Granada (Spain), Malmö (Sweden). Turin (Italy) and Norway did not provide information on HER2 status.

Dietary Assessment Diet was assessed by using country-specific (or in some cases center-specific) dietary questionnaires designed to capture local dietary habits. Eight countries used self-administered dietary questionnaires, whereas, in Greece, Spain, and southern Italy (Naples and Ragusa), the questionnaires were administered by interviewers. In most countries, the questionnaires were extensive quantitative instruments (containing up to 260 food items). In Denmark, Norway, Umeå (Sweden), and Naples (Italy), semi-quantitative food-frequency questionnaires (FFQs) were administered. In Malmö (Sweden), an interview-based diet history method combining a questionnaire with a 7-day menu book was used. In the UK, an FFQ and a 7-day dietary record were used, but all results are from the FFQ (5). All dietary questionnaires were validated (6).

The EPIC Nutrient Database (7) was used to convert the quantities of food consumed into daily energy and total, saturated, monounsaturated, and polyunsaturated fat intakes.

Statistical Analyses

Multivariate Cox proportional hazard models were used to assess the association of fat intakes with breast cancer risk, with stratification by center to control for center effects, and age (1 year categories). In all models, age was the primary time variable. Because macronutrient intake correlates strongly with energy intake, we used a modified standard model (8), which includes absolute fat intake (g) and total non-alcohol energy intake (instead of total energy intake), to adjust for the confounding effect of energy intake. We subtracted energy from alcohol from total energy intake, and included in the model as a separated covariate, in order to better adjust the model for alcohol given it is a common risk factor for breast cancer. Fat intakes were analyzed as both categorical and continuous variables. For the former, quintiles of fat intake were determined from the distribution in each receptor subset included in the analysis. Linear trends were tested by modeling the variable whose value was the number of the quintile to which the subject belonged. When intakes of total fat and fat subtype were modeled as continuous variables, they were transformed to logarithms to the base 1.2, so that HRs represent the risk associated with a 20.0% increase in fat intake.

The following covariates were included in the models: total energy excluding energy from alcohol (continuous),energy from alcohol (continuous), smoking status (never, former, current, unknown), educational attainment (years of schooling), age at menarche (≤11, 12–14, >14 years, missing), full-term pregnancy (yes, no, missing) and hormone replacement therapy use (ever, never, missing). Missing values (generally <2.0%) were accounted for by creating an extra category for each categorical co-variable. In order to take into account the differing effect of body mass index (BMI) on breast cancer risk in relation to menopausal status, all models were also adjusted by an interaction term between these two variables (BMI/menopausal status interaction) Menopausal status at baseline was defined as described elsewhere (9) using an algorithm that accounts for information on menstrual status/history, type of menopause, use of oral contraceptives and

menopausal hormones. The cut-off of 50 years of age was used as an approximate indicator of menopausal status at diagnosis (women diagnosed at ≤50 years and >50 years).

To correct FFQs for measurement errors, the intake data were calibrated against highly standardized 24-hour dietary recall (24-HDR) interviews conducted using the EPIC-soft software on a random sample (8.0%) of the cohort: a fixed-effects linear model was used in which center and sex-specific 24-HDR data were regressed on FFQ intakes (10;11).

The *Q* test statistic with 9 degrees of freedom was used to assess statistical heterogeneity and investigate the hypothesis that associations between dietary components and breast cancer risk were the same in all countries (12)

Models investigating associations of total fat and fat subtypes with all breast cancers and with breast cancer types defined by ER status (ER+, ER-), PR status (PR+, PR-), combined ER and PR status (ER+PR+, ER+PR-, ER-PR-, ER PR unknown), and also HER2 status (HER2+, HER2-, HER2 unknown) were run. The heterogeneity of associations according to receptor status was assessed using the data augmentation method (13), in which the difference in log likelihood between a model with receptor status-specific variables and a model with a single HR estimate for the 2 categories of receptor status was compared to a chi-square distribution with 1 degree of freedom (comparison between positive and negative receptor). In these analyses, women who developed a competing breast cancer subtype or had missing receptor status, were censored at the time of occurrence.

We also examined whether the association between fat and breast cancer risk was modified by menopausal status (post- vs. pre-menopause). This was achieved by modeling product terms of the dichotomized menopausal variable multiplied by the subject's fat intake considered as a continuous variable. The statistical significance of the interaction was assessed using a likelihood ratio test that compared the models with and without the product term, to a chi-square distribution with one degree of freedom.

In additional analyses we also examined the association between intake of total fat and fat subtypes and breast cancer risk, when breast cancer cases diagnosed in the two first years of follow up were excluded (to investigate a possible influence of subclinical disease on dietary fat). These analyses did not produce results differing from those reported in the tables and the results were not shown.

References

(1) Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. *Int J Epidemiol.* 1997;26 Suppl 1:S6-14.

(2) Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A.* 1918;4(12):370-373.

(3) Layfield LJ, Gupta D, Mooney EE. Assessment of Tissue Estrogen and Progesterone Receptor Levels: A Survey of Current Practice, Techniques, and Quantitation Methods. *Breast J.* 2000;6(3):189-196.

(4) Wolff AC, Hammond ME, Schwartz JNet al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med.* 2007;131(1):18-43.

(5) Riboli E, Hunt K, Slimani Net al. The EPIC study: study population and data collection. *Public Health Nutrition.* 2002;5(6b):1113-1124.

(6) Sieri S, Krogh V, Ferrari Pet al. Dietary fat and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr.* 2008;88(5):1304-1312.

(7) Slimani N, Deharveng G, Unwin Iet al. The EPIC nutrient database project (ENDB): a first attempt to standardize nutrient databases across the 10 European countries participating in the EPIC study. *Eur J Clin Nutr.* 2007;61(9):1037-10-56.

(8) Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124(1):17-27.

(9) Lahmann PH, Hoffmann K, Allen Net al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). *Int J Cancer.* 2004;111(5):762-771.

(10) Slimani N, Ferrari P, Ocke Met al. Standardization of the 24-hour diet recall calibration method used in the european prospective investigation into cancer and nutrition (EPIC): general concepts and preliminary results. *Eur J Clin Nutr.* 2000;54(12):900-917.

(11) Ferrari P, Day NE, Boshuizen HCet al. The evaluation of the diet/disease relation in the EPIC study: considerations for the calibration and the disease models. *Int J Epidemiol.* 2008;37(2):368-378.

(12) Cochran WG. The combination of estimate from different experiments. *Biometrics.* 1954;10:101-129.

(13) Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51(2):524-532.

Supplementary Table 1. **Multivariable-adjusted hazard ratios (HRs)[*] with 95% confidence intervals (CIs) for developing breast cancer according to quintiles of fat intake (10,062 cases).**

	Quintile of intake					P trend[†]	Intake as continuous variable [‡]	Calibrated data (Intake as continuous variable) [§]
Total fat								
	1	2	3	4	5			
Mean value (g /day)	43	60	72	87	117			
N cases/N person-years	1861/716963	1975/718600	2061/720744	2054/722719	2111/719119			
HR (95% CI)	1	1.02 (0.96-1.09)	1.05 (0.98-1.13)	1.04 (0.95-1.13)	1.08 (0.97-1.21)	0.22	**1.02 (1.00-1.04)**	**1.06(1.01-1.12)**
Saturated fat								
Mean value (g/day)	15	22	28	34	48			
N cases/N person-years	1717/714093	1893/717100	2047/720513	2114/723118	2291/723321			
HR (95% CI)	1	1.03 (0.97-1.11)	**1.08 (1.01-1.16)**	**1.09 (1.01-1.18)**	**1.14 (1.03-1.26)**	**0.006**	**1.02 (1.01-1.04)**	**1.05(1.02-1.08)**
Monounsaturated fat								
Mean value (g/day)	14	20	25	31	46			
N cases/N person-years	1910/715111	1964/717040	2142/720744	2156/724658	1890/720593			
HR (95% CI)	1	0.99 (0.93-1.06)	1.06 (0.98-1.14)	1.07 (0.98-1.16)	1.07 (0.96-1.20)	0.06	**1.02 (1.00-1.04)**	**1.06(1.02-1.11)**
Polyunsaturated fat								
Mean value (g /day)	7	9	12	15	22			
N cases/N person-years	1954/725946	2019/720236	2047/718025	2033/717894	2009/716043			
HR (95% CI)	1	1.00 (0.94-1.07)	0.99 (0.93-1.06)	0.97 (0.90-1.04)	0.99 (0.91-1.08)	0.57	0.99 (0.98-1.00)	0.98(0.95-1.01)

[*] Stratified by center, and age, and adjusted for non-alcohol energy, educational attainment, smoking status, BMI/menopausal status interaction term, energy from alcohol, full-term pregnancies and hormone replacement therapy use.

† Tests of linear trend were performed by modeling the variable whose value was the number of the quintile to which the subject belonged

‡$\log_{1.2}$ transformed (so HRs represent risk associated with 20.0% increase in fat intake).

§Calibrated data were obtained by linear regression models that compare observed nutrient questionnaire measurements with 24-hour dietary recall.

ER=estrogen receptor; PR=progesterone receptor

Supplementary Table 2. Multivariable-adjusted hazard ratios (HRs)* with 95% confidence intervals (CIs) of developing breast cancer subtypes defined by ER (70101 cases) and PR (5858 cases) status. according to quintiles of fat intake.

	Cases/person-year	HR (95% CI)	Cases/person-year	HR (95% CI)	Cases/person-year	HR (95% CI)	Cases/person-year	HR (95% CI)
		ER +		**ER -**		**PR +**		**PR -**
Total fat (g/day)								
1	1036/675397	1	258/675397	1	680/646061	1	385/646061	1
2	1098/670744	1.03 (0.94-1.13)	276/670744	1.00 (0.84-1.20)	733/646187	1.04 (0.93-1.16)	406/646187	1.01 (0.87-1.17)
3	1103/669741	1.02 (0.93-1.13)	278/669741	0.96 (0.78-1.17)	739/646722	1.04 (0.91-1.16)	404/646722	0.95 (0.81-1.12)
4	1152/671360	1.06 (0.95-1.19)	281/671360	0.89 (0.71-1.11)	748/649001	1.02 (0.88-1.17)	448/649001	1.00 (0.83-1.20)
5	1226/669147	1.16 (1.00-1.34)	302/669147	0.84 (0.63-1.13)	861/644992	1.17 (0.98-1.40)	454/644992	0.93 (0.73-1.19)
P for trend†		0.11		0.18		0.32		0.66
Intake as continuous variable‡		1.03 (1.00-1.06)		0.98 (0.93-1.04)		1.03 (0.99-1.06)		1.00 (0.96-1.05)
P for heterogeneity§			0.14				0.42	
Calibrated data‖		**1.11 (1.04-1.19)**		0.93(0.82-1.07)		1.08 (0.99-1.18)		1.02 (0.91-1.14)
Saturated fat (g/day)								
1	941/667940	1	224/667939	1	618/644252	1	343/644252	1
2	1071/676787	1.07 (0.97-1.17)	262/676787	1.04 (0.86-1.25)	719/645600	1.09 (0.98-1.22)	373/645600	1.01 (0.87-1.18)
3	1099/677429	1.07 (0.97-1.18)	308/677429	1.14 (0.94-1.39)	719/648062	1.07 (0.95-1.21)	464/648063	**1.21 (1.04-1.42)**
4	1163/672227	**1.12 (1.01-1.25)**	273/672227	0.94 (0.76-1.17)	785/648537	**1.14 (1.00-1.30)**	402/648537	1.00 (0.84-1.19)
5	1341/662007	**1.26 (1.11-1.44)**	328/662007	0.98 (0.75-1.27)	920/646512	**1.26 (1.07-1.48)**	515/646512	1.13 (0.91-1.40)
P for trend†		**0.001**		0.62		**0.01**		0.42
Intake as continuous variable‡		**1.03 (1.01-1.05)**		1.01 (0.97-1.06)		**1.04 (1.01-1.06)**		1.02 (0.99-1.06)
P for heterogeneity§			0.42				0.58	
Calibrated‖		**1.09(1.04-1.14)**		1.01(0.93-1.11)		**1.09 (1.03-1.16)**		1.05 (0.98-1.14)
Monounsaturated fat (g/day)								
1	1067/688342	1	251/688342	1	688/645501	1	373/645501	1
2	1070/675387	0.97 (0.89-1.06)	297/675387	1.11 (0.94-1.32)	687/644536	0.95 (0.85-1.06)	411/644536	1.04 (0.90-1.21)
3	1175/663058	1.05 (0.95-1.16)	280/663058	0.99 (0.81-1.20)	781/645323	1.03 (0.92-1.16)	439/645323	1.04 (0.89-1.23)
4	1232/659164	1.10 (0.99-1.23)	286/659164	0.95 (0.76-1.19)	833/649092	1.05 (0.91-1.20)	453/649093	1.01 (0.84-1.21)
5	1071/670438	1.11 (0.96-1.28)	281/670438	0.99 (0.74-1.33)	772/648512	1.07 (0.90-1.28)	421/648512	1.04 (0.82-1.32)

P for trend[†]		**0.03**		0.51		0.24		0.91
Intake as continuous variable[‡]		**1.02 (1.00-1.05)**		0.99(0.94-1.04)		1.02 (0.99-1.05)		1.01 (0.97-1.04)
P for heterogeneity[§]			0.17				0.54	
Calibrated[‖]		**1.09 (1.03-1.15)**		0.97(0.87-1.09)		1.07 (1.00-1.15)		1.03 (0.93-1.13)
Polyunsaturated fat (g/day)								
1	1123/670945	1	305/670944	1	766/655117	1	462/655117	1
2	1148/666689	0.99 (0.91-1.08)	253/666689	0.86 (0.72-1.02)	776/646944	1.00 (0.90-1.11)	391/646944	0.90 (0.78-1.03)
3	1094/665451	0.93 (0.85-1.02)	272/665451	0.91 (0.75-1.09)	748/643652	0.95 (0.85-1.07)	387/643652	0.88 (0.76-1.03)
4	1112/668132	0.92 (0.84-1.02)	288/668132	0.94 (0.77-1.14)	703/643236	**0.87 (0.77-0.98)**	448/643236	0.99 (0.84-1.16)
5	1138/685172	0.94 (0.84-1.06)	277/685172	0.88 (0.70-1.10)	768/644016	0.97 (0.84-1.11)	409/644016	0.91 (0.75-1.09)
P for trend[†]		0.15		0.58		0.18		0.72
Intake as continuous variable[‡]		**0.98 (0.96-1.00)**		0.97 (0.94-1.00)		0.97 (0.94-1.00)		0.97 (0.95-1.00)
P for heterogeneity[§]			0.54				0.68	
Calibrated[‖]		**0.95 (0.91-0.99)**		0.93 (0.86-1.02)		**0.95(0.90-1.00)**		0.94 (0.87-1.00)

[*] Stratified by center, age and adjusted for non-alcohol energy, educational attainment, smoking status, BMI/menopausal status interaction energy from alcohol, full term pregnancy and hormone replacement therapy use.

[†] Tests of linear trend performed by modeling the variable whose value was the number of the quintile to which the subject belonged

[‡] [$\text{Log}_{1.2}$ transformed (so HRs represent risk associated with 20.0% increase in fat intake).

[§] PR-positive vs. PR-negative.

[‖] Calibrated data were obtained by linear regression models that compare observed nutrient questionnaire measurements with 24-hour dietary recall.

ER=estrogen receptor; PR=progesterone receptor