# Association of male fatty acid intake with fecundability among couples planning pregnancy

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## ABSTRACT

STUDY QUESTION: To what extent is male fatty acid intake associated with fecundability among couples planning pregnancy?

SUMMARY ANSWER: We observed weak positive associations of male dietary intakes of total and saturated fatty acids with fecundability; no other fatty acid subtypes were appreciably associated with fecundability.

WHAT IS KNOWN ALREADY: Male fatty acid intake has been associated with semen quality in previous studies. However, little is known about the extent to which male fatty acid intake is associated with fecundability among couples attempting spontaneous conception.

STUDY DESIGN, SIZE, DURATION: We conducted an internet-based preconception prospective cohort study of 697 couples who enrolled during 2015–2022. During 12 cycles of observation, 53 couples (7.6%) were lost to follow-up.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Participants were residents of the USA or Canada, aged 21–45 years, and not using fertility treatment at enrollment. At baseline, male participants completed a food frequency questionnaire from which we estimated intakes of total fat and fatty acid subtypes. We ascertained time to pregnancy using questionnaires completed every 8 weeks by female participants until conception or up to 12 months. We used proportional probabilities regression models to estimate fecundability ratios (FRs) and 95% CIs for the associations of fat intakes with fecundability, adjusting for male and female partner characteristics. We used the multivariate nutrient density method to account for energy intake, allowing for interpretation of results as fat intake replacing carbohydrate intake. We conducted several sensitivity analyses to assess the potential for confounding, selection bias, and reverse causation.

MAIN RESULTS AND THE ROLE OF CHANCE: Among 697 couples, we observed 465 pregnancies during 2970 menstrual cycles of follow-up. The cumulative incidence of pregnancy during 12 cycles of follow-up after accounting for censoring was 76%. Intakes of total and saturated fatty acids were weakly, positively associated with fecundability. Fully adjusted FRs for quartiles of total fat intake were 1.32 (95% CI 1.01–1.71), 1.16 (95% CI 0.88–1.51), and 1.43 (95% CI 1.09–1.88) for the second, third, and fourth vs the first quartile, respectively. Fully adjusted FRs for saturated fatty acid intake were 1.21 (95% CI 0.94–1.55), 1.16 (95% CI 0.89–1.51), and 1.23 (95% CI 0.94–1.62) for the second, third, and fourth vs the first quartile, respectively. Intakes of monounsaturated, polyunsaturated, trans-, omega-3, and omega-6 fatty acids were not strongly associated with fecundability. Results were similar after adjustment for the female partner's intakes of trans- and omega-3 fats.

LIMITATIONS, REASONS FOR CAUTION: Dietary intakes estimated from the food frequency questionnaire may be subject to non-differential misclassification, which is expected to bias results toward the null in the extreme categories when exposures are modeled as quartiles. There may be residual confounding by unmeasured dietary, lifestyle, or environmental factors. Sample size was limited, especially in subgroup analyses.

WIDER IMPLICATIONS OF THE FINDINGS: Our results do not support a strong causal effect of male fatty acid intakes on fecundability among couples attempting to conceive spontaneously. The weak positive associations we observed between male dietary fat intakes and fecundability may reflect a combination of causal associations, measurement error, chance, and residual confounding.

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Keywords: diet / fatty acids / fecundability / fertility / prospective cohort study

## Introduction

Infertility, defined as the inability to conceive after 12 months of unprotected intercourse, affects ~15% of North American couples (Thoma *et al.*, 2013). Factors related to the male partner contribute to infertility in up to 50% of affected couples (Irvine, 1998), yet male factors remain understudied in infertility research (Schilit, 2019). Identification of modifiable risk factors for male infertility has important public health implications.

Diet is a hypothesized modifiable risk factor for male infertility. Fatty acids are particularly relevant to male fertility because dietary fatty acid intake influences the fatty acid composition of spermatozoa and seminal fluids (Collodel *et al.*, 2020). Omega-3 fatty acids, a type of polyunsaturated fatty acid, are incorporated in the structure and function of spermatozoa cell membranes and tails (Collodel *et al.*, 2020). Additionally, omega-3 fatty acids contribute to the antioxidant activity of seminal plasma, preventing oxidative damage to spermatozoa (Safarinejad and Safarinejad, 2012). The fatty acid composition of seminal fluid differs between normozoospermic individuals and those with abnormal seminal conditions (Zerbinati *et al.*, 2016), and correlates with semen quality parameters (Chavarro *et al.*, 2011; Zerbinati *et al.*, 2016).

Observational studies have reported inverse associations of intakes of total (Attaman *et al.*, 2012; Eslamian *et al.*, 2015), saturated (Attaman *et al.*, 2012; Eslamian *et al.*, 2015; Jensen *et al.*, 2013), and trans-fatty acids (Chavarro *et al.*, 2014; Eslamian *et al.*, 2015), and positive associations of omega-3 fatty acids (Eslamian *et al.*, 2015) and fish oil supplement use (a source of omega-3 fatty acids) (Jensen *et al.*, 2020), with semen quality. Randomized controlled trials of the effects of fatty acid supplementation on semen quality have largely focused on the omega-3 fatty acids eicosapentanenoic acid (EPA) and docosahexaenoic acid (DHA) and dietary supplementation with walnuts (which are rich in a-linolenic acid, an omega-3 fatty acid). Randomized controlled trials of omega-3 fatty acid supplementation among infertile men have yielded varying results but generally support a benefit of supplementation. Mart ́ınez-Soto *et al.* (2016) observed that supplementation with DHA-enriched oil (vs 1.5 g/day of sunflower oil) for 10 weeks improved seminal plasma antioxidant capacity and reduced DNA damage in sperm, but did not meaningfully improve traditional semen quality parameters. Gonza ́lez-Ravina *et al.* (2018) found that supplementation with 2 g/day DHA (vs 0.5 g/day of primrose oil) improved sperm progressive motility among asthenozoospermic patients at 1 and 3 months. Moreover, two randomized studies of healthy men who consumed a Western diet found that dietary supplementation with nuts improved semen quality (Robbins *et al.*, 2012; Salas-Huetos *et al.*, 2018). These results have led to interest in using supplementation with fish oil or omega-3-rich foods to improve male fertility.

However, findings from semen quality studies may not translate into associations with fecundability, i.e. the per-cycle probability of conception, for several reasons. Semen quality is an imperfect predictor of male reproductive potential (Bonde *et al.*, 1998; Buck Louis *et al.*, 2014; Del Giudice *et al.*, 2022). Other barriers to fertility exist, including female factors and male factors not reflected in semen analysis. Previous work has identified predictors of semen quality that were not associated with fertility (e.g. soy intake (Chavarro *et al.*, 2008; Mumford *et al.*, 2014) and cannabis use (Wise *et al.*, 2018a; Payne *et al.*, 2019)), and predictors of fertility that were unrelated to semen quality in the same cohort (e.g. caffeine and alcohol intake (Karmon *et al.*, 2017)). Indeed, recent observational studies conducted among couples using IVF have consistently demonstrated no meaningful association of male dietary factors with implantation, clinical pregnancy or live birth (Mitsunami *et al.*, 2021; Salas-Huetos *et al.*, 2022a,b). These findings underscore the need for studies of male dietary factors in relation to fecundability, beyond semen quality (Chavarro, 2017).

Previous observational research in Pregnancy Study Online (PRESTO) found that higher trans-fatty acid intake and lower omega-3 fatty acid intake of the female partner were associated with lower fecundability (Wise *et al.*, 2018b). No previous study has investigated the association of male fatty acid intake with fecundability outside the setting of couples undergoing fertility treatment. Our objective was to estimate the associations of male fatty acid intake with fecundability in a cohort of couples attempting to conceive without use of ART.

## Materials and methods

### Study design

We analyzed data from PRESTO, an internet-based prospective cohort study of North American couples planning pregnancy. Data were collected during June 2013–June 2022. Methods have been described in detail elsewhere (Wise *et al.*, 2015). Eligibility criteria for female-identified participants were residence in the USA or Canada, age 21–45 years, in a relationship with a male partner, and trying to conceive without the use of fertility treatment. Female participants could opt to invite their male partner (eligibility criterion: age 2:21 years). Participation of male partners in PRESTO was optional. Participants provided online informed consent.

We collected self-reported data through online questionnaires. At baseline, female participants completed a detailed questionnaire related to socio-demographics, lifestyle factors, and reproductive and medical history. Male participants provided detailed self-reported data through a separate baseline questionnaire. Participants were invited to complete an online food frequency questionnaire (FFQ) 10 days after completing baseline. We started inviting male participants to complete the FFQ in October 2015. Female participants completed follow-up questionnaires every 8 weeks for up to 12 months. The follow-up questionnaires elicited data on pregnancy status, method of pregnancy confirmation, and changes in time-varying variables such as medication use, physical activity, and caffeine and alcohol intakes.

### Ethical approval

PRESTO was approved by the Institutional Review Board at Boston Medical Center.

### Exposure

Primary exposures of interest included the male partner's dietary intakes of total fat and fat subtypes (saturated, monounsaturated, polyunsaturated, trans-, omega-3, and omega-6) estimated from the male partner's FFQ. We used the Diet History Questionnaire (DHQ), an FFQ developed by the National Cancer Institute to collect data on past-year diet (Subar *et al.*, 2001). We used Diet*Calc software developed by the National Cancer Institute to calculate participants' fat intakes in grams/day and total energy intake in kcal/day (National Cancer Institute, 2012). Diet*Calc software uses nutrient data from the US Department of Agriculture's Food and Nutrient Databases for Dietary Studies, the US Department of Agriculture's Food Pattern Equivalents Databases, and the University of Minnesota's Nutrient Database for Research (National Cancer Institute, 2022). The original DHQ was validated against repeated 24-h dietary recalls among the US adults and demonstrated deattenuated correlation coefficients of 0.62 for total fat, 0.68 for saturated fatty acids, 0.60 for monounsaturated fatty acids, and 0.61 for polyunsaturated fatty acids among men (Subar *et al.*, 2001).

From the beginning of the study in 2013 until June 2020, we administered Version II of the DHQ. Starting in June 2020, we implemented Version III of the DHQ, which was updated to account for secular trends in diets and food products. The DHQ III includes more food and beverage items and more details about food preparation; a complete list of differences between Versions II and III of the DHQ is available at https://epi.grants.cancer.gov/dhq3/changes.html.

## Covariates

Covariate data were obtained from the baseline questionnaires. Potential confounders reported by the female partner included their age, educational attainment, height, weight, and last method of contraception used, and the couple's marital status, household income, intercourse frequency, cycles of attempt time at study entry, and whether they were doing something to improve chances of conceiving (e.g. menstrual cycle charting, ovulation testing). Potential confounders reported by the male partner included their age, educational attainment, height, weight, physical activity, smoking status, alcohol intake, caffeine intake, sugar-sweetened beverage intake, and use of fish oil supplements. We calculated BMI as weight (kilograms) divided by height (meters) squared. We calculated total metabolic equivalent (MET)-hours per week by multiplying the average number of hours per week engaged in activities by METs derived from the Compendium of Physical Activities (McKinnon *et al.*, 2016; Ainsworth *et al.*, 2000). We used the male partner's DHQ data to calculate the Healthy Eating Index-2010 (HEI-2010), a measure of dietary quality (Guenther *et al.*, 2013), for use in descriptive statistics and sensitivity analyses. We did not control for HEI-2010 in the main analysis because dietary sources of fatty acids contribute to several HEI-2010 components, and as such its inclusion in adjusted models would reduce the interpretability of estimands.

## Outcome

The outcome of the study was fecundability, defined as the per-cycle probability of conception. Female participants provided follow-up data on menstrual cycles and conception for up to 12 months. We estimated time-to-pregnancy in cycles as [(reported cycles of pregnancy attempt time at baseline) þ [(date of last menstrual period from most recent follow-up questionnaire − date of baseline questionnaire)/cycle length] þ 1], rounded to the nearest whole number (Wise *et al.*, 2018b).

## Participants

Among 16 630 female participants who completed the baseline questionnaire in June 2013–June 2022, we limited the analysis to couples who had been trying to conceive for :::6 cycles at study entry (n ¼ 12 856) to reduce the potential for selection bias and reverse causation (Supplementary Fig. S1). Among these, 6895 (54%) female participants invited their partners, of whom 3181 (46%) completed the male baseline questionnaire. Of the 3046 partners who were invited to complete the DHQ, 757 (25%) completed the DHQ. To reduce the potential for reverse causation (i.e. dietary changes resulting from prolonged attempt time), we excluded couples in which the male partner completed his DHQ 2:90 days after baseline (n ¼ 51). We also excluded couples (n ¼ 9) in which the male partner reported an implausible total energy intake (<600 kcal/day (n ¼ 8) or >5000 kcal/day (n ¼ 1)), yielding 697 couples for analysis. Participants contributed person-time from cohort entry until pregnancy (n ¼ 465, 67%) or the occurrence of a censoring event (initiation of fertility treatment (n ¼ 64, 9%), cessation of pregnancy attempts (n ¼ 16, 2%), loss to follow-up (n ¼ 53, 8%), or the end of follow-up at 12 cycles of total pregnancy attempts (n ¼ 94, 13%)), whichever came first. The remaining five couples (1%) were still participating at the time of data analysis.

## Statistical analysis

We calculated descriptive statistics for covariates according to quartiles of male dietary fat intakes, standardized to the age distribution of the cohort at baseline. We calculated pairwise Spearman correlation coefficients for intakes of fatty acid subtypes in grams per day, separately by DHQ version. We used life-table methods to estimate the percentage of couples who conceived during 12 cycles of attempt time, accounting for censoring events.

We used the multivariate nutrient density modeling approach to account for total energy intake. We calculated quartiles of percent total energy intake from total fat and fat subtypes, separately by DHQ version, and modeled quartiles of fat intakes as nominal categorical variables. In regression analyses, we adjusted for percent energy from total fat minus the respective fat subtype (continuous; not applicable to the model for total fat), percent energy from protein intake (continuous), percent energy from alcohol intake (continuous), and total energy intake (kcal/day, continuous). Results are interpreted as the association with fecundability of replacing carbohydrate intake with the respective quartile of fat intake, holding constant total energy intake, protein intake, alcohol intake, and the other covariates (Willett *et al.*, 1997). We also present results modeling the percent energy from total fat or fat subtypes as continuous variables.

We used a directed acyclic graph-based approach (Textor *et al.*, 2016) to identify a sufficient set of covariates to estimate the causal effect of male dietary fat intakes on fecundability: the male partner's age (<30, 30–34, 35–39, 2:40 years), BMI (<25, 25–29, 2:30 kg/m$^2$), educational attainment (<16, 16, 2:17 years), physical activity (<20, 20–39, 2:40 MET-hours/week), caffeine intake (<100, 100–199, 2:200 mg/day), and sugar-sweetened beverage intake (0, 1, 2–6, 2:7 drinks/week); the female partner's age (continuous) and BMI (<25, 25–29, 2:30 kg/m$^2$); and the couple's household income (<$50 000, $50 000–$99 999, $100 000–$149 999, 2:$150 000) (Supplementary Fig. S2). We adjusted for DHQ version (II or III) in all models. Additionally, we controlled for calendar year (ordinal categorical variable) in all trans-fat models to account for secular trends, because the US Food and Drug Administration ruled in 2015 that manufacturers must remove artificial trans-fats from food products by June 2018 (Dewey, 2018). In Canada, artificial trans-fats were banned from the food supply in 2017, and this regulation came into force in September 2018 (Franco-Arellano *et al.*, 2020). Categorical variables other than year were modeled as nominal categorical variables.

For regression analyses, we used an Andersen-Gill data structure with one row per menstrual cycle to account for left truncation. We used proportional probabilities regression models (Weinberg *et al.*, 1989) to estimate the fecundability ratios (FR) associated with quartiles of exposures and their 95% CI. An FR > 1 indicates shorter time-to-pregnancy (higher fecundability), and an FR <1 indicates longer time to pregnancy (lower fecundability). Models were adjusted for cycle number using indicator variables to account for the decrease in fecundability in the study population with increasing pregnancy attempt time. We ran a minimally adjusted model (adjusted for female age, male total energy intake, and DHQ version) and a fully adjusted model (adjusted for the covariates described above). We used restricted cubic spline models to flexibly model the fully adjusted associations of male dietary fat intakes with fecundability (Durrleman and Simon, 1989; Hertzmark *et al.*, 2014).

We conducted several sensitivity analyses in the fully adjusted models. First, we adjusted for the female partner's dietary intakes of trans- and omega-3 fats because previous research in PRESTO found that higher female trans-fat intake and lower female omega-3 fatty acid intake were associated with lower fecundability (Wise *et al.*, 2018b). The female partner's intakes of trans- and omega-3 fatty acids were modeled as continuous variables (percentage of total energy intake), and the female partner's total energy intake was included as a continuous covariate. Second, we further divided omega-3 and omega-6 fatty acids according to the number of carbons (18 vs 2:20) and separately estimated the associations of these fatty acid intakes with fecundability. Third, we stratified by male BMI (<25, 25–29, or 2:30 kg/m$^2$). Because male BMI may impact spermatogenesis and fertility (Palmer *et al.*, 2012), we hypothesized that BMI may modify the association of male dietary fat intake with fertility. Fourth, we restricted analysis to nulliparous couples, who had never tried to conceive before, with <3 cycles of pregnancy attempt time at study entry, to reduce the potential for reverse causation. Fifth, we truncated follow-up to the first three observed cycles of pregnancy attempts, to reduce the potential for differential measurement error. This analysis was limited to couples with <3 cycles of pregnancy attempt time at study entry to further reduce the potential for reverse causation. Sixth, we adjusted for the male partner's red meat intake (<4, 4 to <8, 2:8 oz./week) in the analyses of saturated and trans-fats; poultry intake (<4, 4 to <8, 2:8 oz./week) in the analysis of trans-fats; and fish intake (<2, 2 to <4, 2:4 oz./week) in the analysis of omega-3 fatty acids, to reduce confounding by other nutrients or environmental contaminants (e.g. polychlorinated biphenyls (Montano *et al.*, 2022), mercury (M'ınguez-Alarco´n *et al.*, 2018)) in major sources of dietary fat intakes. Dietary intakes of red meat, poultry and fish were estimated from the male partner's DHQ. Seventh, we adjusted for the male partner's intakes of total vegetables and fruit, measured using the respective components of the HEI-2010. These measures were chosen to control for confounding by overall dietary quality without changing the interpretability of our results with respect to fatty acid intakes. Eighth, we adjusted for parity given that participants' diets could have been influenced by children in the home. Ninth, we restricted analysis to male partners who did not use fish oil supplements, expecting the hypothesized effect of dietary omega-3 fat intake on fecundability to be greater among male participants who did not consume omega-3 fatty acids through supplement use. Tenth, we considered the male partner's use of fish oil supplements as the exposure of interest, and estimated the FR associated with fish oil supplement use, adjusted for the covariates in the fully adjusted model including total energy intake. Eleventh, we restricted the analysis of trans-fat intake to participants who enrolled during 2015–2018, because artificial trans-fats were removed from the USA and Canadian food supplies in 2018.

We used fully conditional specification methods to multiply impute missing covariate and outcome data, using PROC MI to create 20 imputed datasets from imputation models. We did not impute missing male dietary data. The prevalence of missing covariate data ranged from 0 (e.g. age) to 3% (household income). For participants who did not provide any follow-up data (n ¼ 27 couples, 4%), we assigned one menstrual cycle of follow-up and multiply imputed the outcome (pregnant or not pregnant). Participants who were lost to follow-up but provided at least one cycle of follow-up data (n ¼ 26 couples, 4%) were censored upon being lost to follow-up. For couples in which the female partner did not complete the DHQ (n ¼ 55) or had implausible total energy intake defined as <600 kcal/day or >5000 kcal/day (n ¼ 6), we multiply imputed the female partner's dietary intakes of trans and omega-3 fats and total energy intake. For descriptive statistics and restricted cubic spline models, we analyzed the first imputed dataset. For other regression analyses, we combined coefficients and standard errors across the 20 imputed datasets using PROC MIANALYZE. We conducted analyses using SAS Version 9.4 (SAS Institute, Inc., Cary, NC, USA).

## Results

Across stages of participation, included and excluded couples were similar in terms of male and female age, male and female BMI, female partner lifestyle characteristics, household income, and intercourse frequency (Supplementary Table S1). Included couples had higher male and female educational attainment, were more likely to be married, and were less likely to be smokers compared with excluded couples. Among couples in which the male partner participated in PRESTO, we evaluated additional lifestyle variables reported on the male baseline questionnaire, and these did not meaningfully differ by inclusion status (Supplementary Table S2). The cumulative incidence of pregnancy during 12 cycles of follow-up was similar among included and excluded couples (76% and 73%, respectively).

Among 697 couples, we observed 2970 menstrual cycles and 465 pregnancies. The duration of follow-up was: one to three cycles, 55%; four to six cycles, 20%; seven to nine cycles, 14%; 10–12 cycles, 11%. The mean SD age was 32 (4.6) years among male participants and 30 (3.7) years among female participants. The mean pregnancy attempt time at study entry was two cycles. Of the participants, 15% resided in Canada and 85% resided in the US Descriptive statistics for the percentage of total energy intake from total fat and fat subtypes, overall and by DHQ version, are shown in Supplementary Table S3. The overall mean (SD) percentages of total energy intake were 36.2 (6.6) for total fat, 11.6 (2.4) for saturated fat, 13.7 (3.0) for monounsaturated fat, 7.4 (1.9) for polyunsaturated fat, 1.6 (0.5) for trans-fat, 0.7 (0.3) for omega-3 fat, and 6.5 (1.7) for omega-6 fat. The top five dietary contributors to each fat subtype by DHQ version are listed in Supplementary Table S4. The top contributors to saturated fat intake were cheese, dairy milk, beef, eggs, butter, and nuts and seeds. The top contributors to trans-fat intake included fried potatoes, beef, poultry, macaroni and cheese, and popcorn. The top contributors to omega-3 intake included salad dressing, fish, corn/canola/vegetable oil, mayonnaise, and poultry. Pairwise Spearman correlations of fat intakes in grams per day were moderate-to-strong and positive, ranging from 0.45 (trans-fat and omega-3 fat, DHQ III) to 1.00 (polyunsaturated fat and omega-6 fat, DHQ II and DHQ III; Supplementary Table S5).

Total fat intake was positively associated with male and female educational attainment, the male partner's caffeine intake, red meat intake and HEI-2010 score, and household income, and inversely associated with male and female BMI and male alcohol intake (Table 1). Saturated fat intake was positively associated with the male partner's red meat intake and inversely associated

Table 1. Participant characteristics by quartiles of male dietary fat intakes in PRESTO, 2015–2022 (n ¼ 697 couples).

| | Total fat | | | | Saturated fatty acids | | | | Trans-fatty acids | | | | Omega-3 fatty acids | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| Male partner | | | | | | | | | | | | | | | | |
| Age (years) | 32.0 | 32.4 | 31.2 | 32.4 | 32.7 | 31.5 | 31.9 | 31.8 | 32.5 | 32.1 | 31.6 | 31.7 | 31.8 | 31.7 | 32.2 | 32.3 |
| Education <12 years | 17% | 10% | 5% | 6% | 12% | 8% | 10% | 8% | 9% | 7% | 11% | 11% | 18% | 6% | 9% | 5% |
| Education 2:17 years | 24% | 37% | 40% | 45% | 33% | 37% | 33% | 41% | 39% | 39% | 34% | 34% | 23% | 35% | 44% | 41% |
| Total energy intake (kcal/day) | 1844 | 1742 | 1696 | 1744 | 1820 | 1762 | 1773 | 1702 | 1806 | 1775 | 1799 | 1690 | 1754 | 1764 | 1823 | 1702 |
| BMI (kg/m$^2$) | 28.9 | 28.2 | 27.6 | 27.5 | 28.1 | 28.3 | 28.1 | 28.1 | 27.6 | 27.9 | 27.8 | 29.1 | 29.0 | 29.0 | 27.4 | 27.3 |
| Physical activity (MET-hours/week) | 34.9 | 32.9 | 34.7 | 33.6 | 37.6 | 33.5 | 31.9 | 32.1 | 41.4 | 34.8 | 32.0 | 28.0 | 33.8 | 35.1 | 33.1 | 33.2 |
| Current regular smoker | 14% | 4% | 2% | 4% | 12% | 4% | 4% | 4% | 10% | 5% | 4% | 4% | 12% | 5% | 3% | 4% |
| Alcohol intake (drinks/week) | 6.9 | 4.8 | 5.4 | 4.7 | 6.7 | 6.8 | 4.6 | 3.7 | 6.5 | 6.3 | 5.3 | 3.8 | 5.2 | 5.7 | 4.8 | 6.1 |
| Caffeine intake (mg/day) | 172 | 144 | 167 | 197 | 169 | 171 | 170 | 170 | 181 | 186 | 150 | 164 | 169 | 165 | 169 | 174 |
| Sugar-sweetened beverage intake (drinks/week) | 7.7 | 3.6 | 3.0 | 1.8 | 5.4 | 3.4 | 4.6 | 2.8 | 4.5 | 3.4 | 4.4 | 3.8 | 6.9 | 3.3 | 3.6 | 2.5 |
| Past-year fish intake (oz./week) | 2.6 | 3.1 | 2.8 | 3.7 | 3.1 | 3.3 | 2.9 | 2.9 | 3.5 | 3.1 | 3.3 | 2.3 | 1.3 | 2.4 | 3.7 | 4.7 |
| Past-year red meat intake (oz./week) | 7.5 | 8.2 | 8.0 | 9.4 | 6.6 | 8.0 | 9.2 | 9.5 | 6.1 | 8.0 | 9.9 | 9.3 | 8.3 | 8.8 | 7.8 | 8.4 |
| Fish oil supplementation | 11% | 8% | 13% | 16% | 12% | 12% | 11% | 16% | 15% | 15% | 10% | 10% | 12% | 13% | 16% | 10% |
| HEI-2010 score | 58.5 | 62.4 | 63.1 | 63.8 | 63.1 | 65.8 | 61.3 | 57.4 | 65.9 | 64.7 | 60.9 | 56.7 | 56.1 | 60.8 | 65.8 | 65.3 |
| Female partner | | | | | | | | | | | | | | | | |
| Age (years) | 29.8 | 30.5 | 30.1 | 30.9 | 30.7 | 30.2 | 30.2 | 30.3 | 30.6 | 30.7 | 30.1 | 29.9 | 29.9 | 30.1 | 30.5 | 30.8 |
| Education <12 years | 4% | 2% | 2% | 2% | 3% | 2% | 1% | 2% | 2% | 0% | 3% | 3% | 5% | 1% | 3% | 1% |
| Education 2:17 years | 38% | 56% | 56% | 52% | 46% | 54% | 50% | 51% | 50% | 57% | 52% | 43% | 42% | 50% | 54% | 55% |
| BMI (kg/m$^2$) | 29.5 | 27.6 | 26.9 | 26.6 | 28.1 | 27.1 | 28.2 | 27.5 | 26.7 | 27.4 | 27.5 | 29.2 | 29.7 | 28.1 | 26.7 | 26.5 |
| Couple | | | | | | | | | | | | | | | | |
| Income <$50 000 USD | 18% | 13% | 11% | 9% | 18% | 12% | 10% | 12% | 16% | 8% | 13% | 15% | 20% | 12% | 12% | 9% |
| Income 2:$150 000 USD | 16% | 17% | 20% | 24% | 16% | 20% | 21% | 19% | 16% | 19% | 27% | 13% | 14% | 15% | 20% | 26% |
| Married | 91% | 95% | 94% | 95% | 93% | 95% | 94% | 93% | 95% | 94% | 96% | 90% | 92% | 91% | 93% | 97% |
| Doing something to improve chances of conceiving | 77% | 81% | 81% | 80% | 77% | 81% | 76% | 84% | 79% | 77% | 80% | 83% | 81% | 82% | 77% | 79% |
| Intercourse <1 time/week | 24% | 23% | 20% | 24% | 24% | 26% | 26% | 17% | 24% | 22% | 22% | 27% | 27% | 22% | 23% | 21% |
| Intercourse 2:4 times/week | 13% | 17% | 8% | 17% | 14% | 12% | 13% | 15% | 18% | 10% | 16% | 12% | 12% | 15% | 13% | 16% |
| Cycles of attempt time at study entry | 1.7 | 1.8 | 1.7 | 1.6 | 1.7 | 1.6 | 1.9 | 1.7 | 1.7 | 1.5 | 1.7 | 2.0 | 1.9 | 1.7 | 1.7 | 1.5 |

Results are means (continuous variables) and column percentages (categorical variables). All characteristics except for age are standardized by the age of the male partner.
BMI, body mass index; DHQ, Diet History Questionnaire; HEI-2010, Healthy Eating Index-2010; MET, metabolic equivalent of task; PRESTO, Pregnancy Study Online; USD, US Dollars.

with the male partner's alcohol and sugar-sweetened beverage intakes. For trans-fat intake, we observed positive associations with male and female BMI and male red meat intake, and inverse associations with female age, male and female educational attainment, and the male partner's physical activity, alcohol intake, fish intake and HEI-2010 score. Male omega-3 fatty acid intake was positively associated with male and female age, male and female educational attainment, male fish intake and HEI-2010 score, and household income, and inversely associated with male and female BMI, male sugar-sweetened beverage intake, and cycles of attempt time at study entry. Participant characteristics according to quartiles of monounsaturated, polyunsaturated, and omega-6 fatty acids are shown in Supplementary Tables S6, S7 and S8, respectively. Male partners in the lowest quartiles of total, trans-, omega-3, saturated, monounsaturated, and omega-6 fatty acid intakes had a higher prevalence of current regular smoking compared with quartiles 2 through 4. Fatty acid intakes were not strongly associated with doing something to improve chances of conceiving nor with intercourse frequency.

In minimally adjusted models, we generally observed positive associations of quartiles 2 through 4 of total and monounsaturated fat intakes with fecundability, whereas associations of other fat intakes with fecundability were close to null (Table 2). After adjustment for covariates, total fat and saturated fat intakes were positively associated with fecundability. Fully adjusted FRs for quartiles of total fat intake were 1.32 (95% CI 1.01–1.71), 1.16 (95% CI 0.88–1.51), and 1.43 (95% CI 1.09–1.88) for the second, third, and fourth vs first quartile, respectively. Fully adjusted FRs for quartiles of saturated fat intake were 1.21 (95% CI 0.94–1.55), 1.16 (95% CI 0.89–1.51), and 1.23 (95% CI 0.94–1.62) for the second, third, and fourth quartile, respectively. Other fat subtypes were not meaningfully associated with fecundability. Adjustment for female dietary trans- and omega-3 fat intakes made no meaningful differences to the FRs for the seven male dietary fat exposures.

The restricted cubic spline curves for total and saturated fat intakes showed positive linear associations with fecundability (Fig. 1). Monounsaturated fat intake was weakly, positively associated with fecundability, whereas polyunsaturated and omega-6 fatty acid intakes were not associated with fecundability. For trans-fat intake, the restricted cubic spline curve demonstrated a null association with fecundability for values approximately <1.6% total energy intake, and a positive association thereafter. The restricted cubic spline curve for omega-3 fat intake indicated a positive association with fecundability that plateaued around the median value (0.7% of total energy intake).

When fat exposures were modeled as continuous variables, replacing 5% of total energy intake from carbohydrates with total fat was associated with 6% higher fecundability (FR 1.06, 95% CI 0.98–1.15; Supplementary Table S9). Replacing 5% of total energy intake from carbohydrates with saturated fat was associated with 10% higher fecundability (FR 1.10, 95% CI 0.89–1.36). Other fat subtypes were not meaningfully associated with fecundability.

Separate calculation of 18- vs 2:20-carbon omega-3 and omega-6 fatty acids demonstrated that the majority of omega-3 intake was from 18-carbon polyunsaturated fatty acids (polyunsaturated fatty acids 18:3 and 18:4; Supplementary Table S10), as

Table 2. Associations of male dietary fat intakes with fecundability in PRESTO, 2015–2022 (n ¼ 697 couples).

| | Quartile boundaries[a] | | | | | Minimally adjusted[b,c] | Fully adjusted[b,d] | Adjusted for female partner fatty acid intakes[b,e] |
|---|---|---|---|---|---|---|---|---|
| Total fat or fat subtype | DHQ II | DHQ III | Couples | Cycles | Pregnancies | FR (95% CI) | FR (95% CI) | FR (95% CI) |
| Total fat | | | | | | | | |
| Q1 | 8.1, 33.4 | 14.5, 32.1 | 173 | 777 | 95 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 33.4, 37.1 | 32.2, 34.8 | 175 | 722 | 127 | 1.40 (1.09–1.82) | 1.32 (1.01–1.71) | 1.32 (1.02–1.71) |
| Q3 | 37.1, 40.8 | 34.9, 38.0 | 175 | 786 | 114 | 1.18 (0.91–1.54) | 1.16 (0.88–1.51) | 1.15 (0.88–1.51) |
| Q4 | 40.9, 65.0 | 38.2, 56.6 | 174 | 685 | 129 | 1.51 (1.16–1.97) | 1.43 (1.09–1.88) | 1.41 (1.07–1.86) |
| Saturated | | | | | | | | |
| Q1 | 1.8, 10.2 | 4.2, 9.8 | 173 | 782 | 104 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 10.2, 11.8 | 9.9, 11.4 | 176 | 734 | 126 | 1.18 (0.92–1.50) | 1.21 (0.94–1.55) | 1.23 (0.95–1.58) |
| Q3 | 11.8, 13.3 | 11.4, 12.5 | 175 | 763 | 113 | 1.07 (0.82–1.38) | 1.16 (0.89–1.51) | 1.17 (0.89–1.53) |
| Q4 | 13.3, 20.2 | 12.6, 21.3 | 173 | 691 | 122 | 1.16 (0.89–1.52) | 1.23 (0.94–1.62) | 1.24 (0.94–1.64) |
| Monounsaturated | | | | | | | | |
| Q1 | 2.3, 12.3 | 4.5, 11.5 | 174 | 770 | 100 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 12.4, 13.8 | 11.6, 13.1 | 173 | 760 | 115 | 1.15 (0.88–1.51) | 1.04 (0.79–1.37) | 1.05 (0.79–1.38) |
| Q3 | 13.9, 15.8 | 13.1, 14.4 | 177 | 724 | 127 | 1.31 (0.99–1.75) | 1.16 (0.85–1.57) | 1.17 (0.86–1.59) |
| Q4 | 15.8, 27.8 | 14.5, 26.6 | 173 | 716 | 123 | 1.29 (0.93–1.79) | 1.08 (0.76–1.54) | 1.08 (0.76–1.53) |
| Polyunsaturated | | | | | | | | |
| Q1 | 0.8, 6.0 | 2.6, 6.5 | 174 | 747 | 100 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 6.0, 7.2 | 6.5, 7.5 | 174 | 748 | 116 | 1.08 (0.83–1.39) | 1.07 (0.83–1.39) | 1.07 (0.82–1.39) |
| Q3 | 7.2, 8.4 | 7.5, 8.6 | 174 | 746 | 130 | 1.19 (0.92–1.53) | 1.10 (0.85–1.42) | 1.08 (0.83–1.40) |
| Q4 | 8.4, 14.3 | 8.7, 14.0 | 175 | 729 | 119 | 1.09 (0.83–1.44) | 1.06 (0.80–1.41) | 1.06 (0.79–1.42) |
| *Trans* | | | | | | | | |
| Q1 | 0.2, 1.4 | 0.5, 1.2 | 172 | 699 | 116 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 1.4, 1.7 | 1.2, 1.4 | 175 | 784 | 111 | 0.83 (0.65–1.05) | 0.90 (0.70–1.15) | 0.89 (0.69–1.14) |
| Q3 | 1.7, 2.0 | 1.4, 1.7 | 174 | 770 | 119 | 0.92 (0.73–1.16) | 1.08 (0.84–1.38) | 1.09 (0.84–1.41) |
| Q4 | 2.0, 4.6 | 1.7, 2.8 | 176 | 717 | 119 | 0.98 (0.77–1.24) | 1.11 (0.87–1.42) | 1.13 (0.86–1.50) |
| Omega-3 | | | | | | | | |
| Q1 | 0.2, 0.5 | 0.2, 0.6 | 168 | 745 | 100 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 0.6, 0.7 | 0.6, 0.7 | 177 | 732 | 117 | 1.11 (0.86–1.42) | 1.09 (0.85–1.41) | 1.11 (0.86–1.43) |
| Q3 | 0.7, 0.9 | 0.7, 0.9 | 179 | 761 | 128 | 1.17 (0.91–1.50) | 1.06 (0.82–1.37) | 1.05 (0.81–1.37) |
| Q4 | 0.9, 2.3 | 0.9, 2.0 | 173 | 732 | 120 | 1.06 (0.80–1.40) | 1.07 (0.81–1.43) | 1.07 (0.79–1.45) |
| Omega-6 | | | | | | | | |
| Q1 | 0.7, 5.4 | 2.3, 5.8 | 175 | 724 | 104 | 1.00 (ref) | 1.00 (ref) | 1.00 (ref) |
| Q2 | 5.4, 6.4 | 5.8, 6.6 | 173 | 775 | 114 | 0.96 (0.75–1.24) | 1.00 (0.77–1.30) | 0.99 (0.77–1.29) |
| Q3 | 6.4, 7.5 | 6.6, 7.6 | 174 | 723 | 128 | 1.12 (0.87–1.44) | 1.01 (0.78–1.31) | 1.00 (0.77–1.29) |
| Q4 | 7.5, 12.6 | 7.6, 12.5 | 175 | 748 | 119 | 0.98 (0.74–1.29) | 0.97 (0.73–1.29) | 0.97 (0.72–1.29) |

CI, confidence interval; FR, fecundability ratio; PRESTO, Pregnancy Study Online.

[a] Quartiles represent percent of total energy intake from the respective fat subtype, calculated separately by DHQ version. Category boundaries are non-mutually exclusive due to rounding.

[b] Results were combined across 20 multiply imputed datasets using PROC MIANALYZE.

[c] Results are from models adjusted for the percent energy from total fat minus the respective fat subtype (continuous; not applicable to the model for total fat), percent energy from protein intake (continuous), percent energy from alcohol intake, and total energy intake (kcal/day, continuous) of the male partner; the age of the female partner; and DHQ version. The model for trans-fat is additionally adjusted for calendar year.

[d] Results are from models adjusted for the percent energy from total fat minus the respective fat subtype (continuous; not applicable to the model for total fat), percent energy from protein intake (continuous), percent energy from alcohol intake, total energy intake (kcal/day, continuous), age, BMI, educational attainment, physical activity, caffeine intake, and sugar-sweetened beverage intake of the male partner; the age and BMI of the female partner; household income; and DHQ version. The model for trans-fat is additionally adjusted for calendar year.

[e] Results are from models adjusted for the percent energy from trans-fat intake (continuous), percent energy from omega-3 fatty acid intake (continuous), and total energy intake of the female partner, in addition to the variables listed in footnote (d).

was the majority of omega-6 intake (polyunsaturated fatty acid 18:2). Omega-3 and omega-6 fatty acid intakes were not strongly associated with fecundability when separated into 18- vs 2:20-carbon fatty acids.

In models stratified by male BMI, we observed stronger positive associations of total fat intake in quartiles 2 through 4 with fecundability among couples with male BMI 2:30 kg/m$^2$, but not among couples with male BMI <25 or 25–29 kg/m$^2$ (Supplementary Table S11). Associations of saturated and monounsaturated fat intakes with fecundability were stronger among couples with male BMI 25–29 kg/m$^2$ (saturated fat only) and 2:30 kg/m$^2$ compared with couples with male BMI <25 kg/m$^2$, although the associations were imprecise and non-monotonic.

Restriction to nulliparous couples who never tried to conceive before with <3 cycles of attempt time at study entry yielded stronger positive FRs for total and monounsaturated fatty acid intakes, and attenuated FRs for saturated fat intake, although precision was limited (Supplementary Table S12). When we truncated follow-up to the first three cycles and limited analysis to couples with <3 cycles of attempt time at enrollment, FRs were closer to the null for total fat, whereas FRs for saturated and trans-fat were slightly stronger (Supplementary Table S13).

Results remained consistent with the main analysis when we additionally adjusted for the male partner's intakes of red meat, poultry, and fish (Supplementary Table S14), the male partner's intakes of vegetables and fruit (Supplementary Table S15), and the female partner's parity (Supplementary Table S16). When we limited analysis to couples in which the male partner did not use fish oil supplements, fully adjusted FRs for omega-3 fatty acid intake were 1.12 (95% CI 0.85–1.47), 1.13 (95% CI 0.86–1.48), and 1.09 (95% CI 0.80–1.49), for the second, third, and fourth vs first quartiles, respectively. The male partner's use of fish oil capsules was not meaningfully associated with fecundability (FR 0.88 (95% CI 0.68–1.14)). When we restricted analysis to participants who enrolled during 2015–2018 (n ¼ 1579 cycles, 330 couples, 213 pregnancies), before the trans-fat ban went into effect, the fully

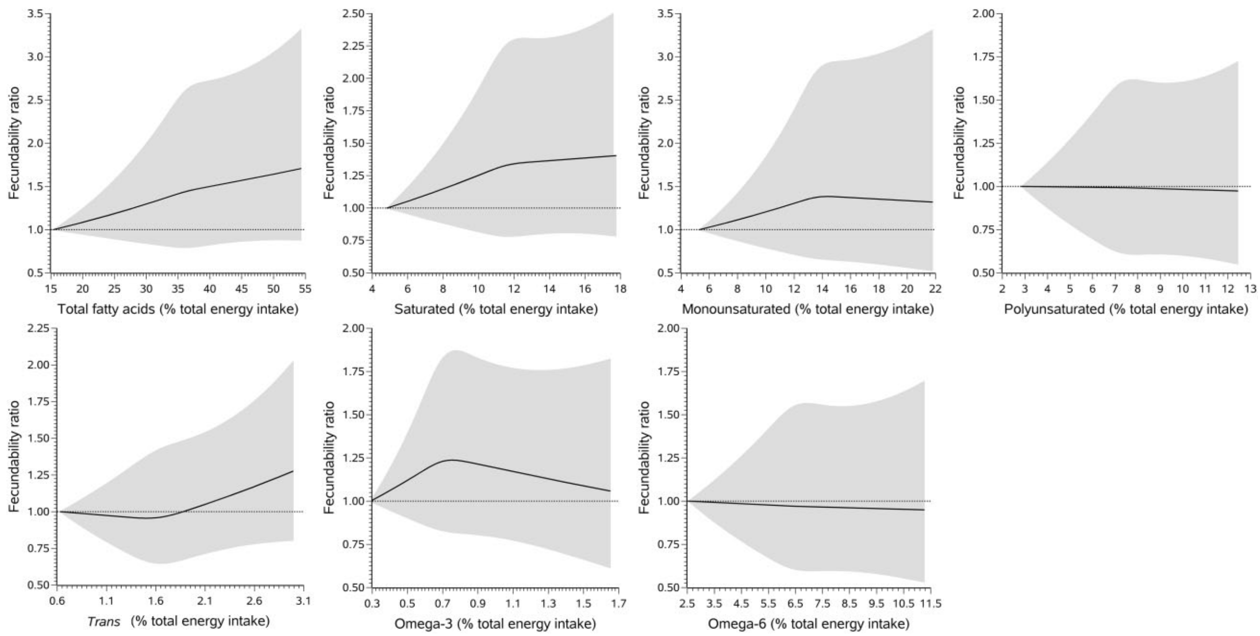


Figure 1. Restricted cubic spline curves for associations of male dietary fat intakes with fecundability in PRESTO. Results are from restricted cubic spline models with knots at 25th, 50th, and 75th percentiles, with values trimmed at the 1st and 99th percentiles, adjusted for the percent energy from total fat minus the respective fat subtype (continuous; not applicable to the model for total fat), percent energy from protein intake (continuous), percent energy from alcohol intake (continuous), total energy intake (kcal/day, continuous), age, BMI, educational attainment, physical activity, caffeine intake, and sugar-sweetened beverage intake of the male partner, as well as the age and BMI of the female partner and household income and DHQ version. The model for trans-fat is additionally adjusted for calendar year. BMI, body mass index; DHQ, Diet History Questionnaire; PRESTO, Pregnancy Study Online.

adjusted FRs for trans-fat intake were 0.93 (95% CI 0.64–1.33), 1.32 (95% CI 0.88–1.98), and 0.99 (95% CI 0.67–1.47), for the second, third, and fourth vs first quartiles, respectively.

## Discussion

In a prospective cohort study of North American pregnancy planners, we observed weak positive associations of male dietary intakes of total and saturated fatty acids with fecundability. We did not observe dose–response associations with quartiles of fat intakes; however, intake in the lowest quartile was associated with the lowest fecundability. Spline curves demonstrated weak positive associations of intakes of total and saturated fatty acids, and to a lesser extent monounsaturated and trans-fatty acids, with fecundability. Sensitivity analyses demonstrated that results were not explained by confounding by the male or female partner's diet. The associations of total, saturated and monounsaturated fatty acids with fecundability were strongest among couples with male BMI 2:30 kg/m$^2$, although these associations were imprecise and non-monotonic. Polyunsaturated fatty acid intakes were not meaningfully associated with fecundability. Overall, the results indicate a weak positive association with fecundability of replacing carbohydrate intake with total fat intake, but no individual fat subtype was strongly associated with fecundability.

We conducted several sensitivity analyses to reduce the potential for reverse causation and other related biases. Subfertile couples may change their diet as a result of prolonged attempt time, resulting in reverse causation and differential information bias. Likewise, a history of demonstrated fertility may result in reduced adherence to dietary recommendations if these couples are confident they will be able to conceive. Therefore, we conducted a sensitivity analysis limited to couples with the least amount of prior information about their fertility: nulliparous couples who never tried to conceive previously, with <3 cycles of attempt time at study entry. This sensitivity analysis yielded stronger results for total and monounsaturated fatty acids, but attenuated results for saturated fatty acids. Furthermore, the presence of children in the home may influence parents' dietary patterns, resulting in confounding by parity. We addressed this potential bias by conducting a sensitivity analysis adjusted for parity, which yielded similar results to our main analyses. Additionally, we conducted a sensitivity analysis truncating follow-up to the first three cycles and restricting to couples with <3 cycles of attempt time at enrollment, to reduce the potential for differential measurement error. The positive associations of saturated and trans-fat with fecundability were slightly stronger in this sensitivity analysis, which is consistent with downward bias in the main analysis due to subfertile couples changing their diets to align with dietary recommendations as a longer pregnancy attempt time elapses.

The restricted cubic spline curve showed a weak positive association of higher trans- fat intake with fecundability. The measurement of trans-fat intake in our study merits discussion, given that the study includes data collected both before and after the removal of artificial trans-fats from the USA and Canadian food supplies in 2018. To account for secular trends in trans-fat intake, we controlled for calendar year in all analyses of trans-fat. Furthermore, the removal of artificial trans-fat from the food supply is not fully accounted for by the nutrient databases used by the DHQ. Trans-fat content of foods in the DHQ III database is derived from the 2017 Nutrient Database for Research, which predates legislation to remove trans-fat from foods. As such, we may have over-estimated participants' trans-fat intake in 2018 onward. We endeavored to reduce differential measurement error over calendar time by conducting a sensitivity analysis for trans-fat restricted to participants who enrolled in 2015–2018. This analysis yielded a stronger positive FR in the third quartile, but an attenuated FR in the fourth quartile, compared with the main analysis. The interpretation of these results is limited by the small sample size, which increases the potential for chance findings. Finally, we note that although the legislation came into effect in 2018, manufacturers began to voluntarily remove artificial trans-fat from food products as early as 2003, when the US Food and Drug Administration issued a final ruling requiring the reporting of trans-fat contents in food package labeling (Albers *et al.*, 2008). Therefore, trans-fat intake might not be starkly overestimated among participants who enrolled after 2018.

Our findings for total, saturated and trans-fat disagree with observational studies of semen quality, which have generally found inverse associations between total, saturated and trans-fat intakes and semen quality parameters (Mendiola *et al.*, 2010; Attaman *et al.*, 2012; Jensen *et al.*, 2013; Chavarro *et al.*, 2014; Eslamian *et al.*, 2015; Ricci *et al.*, 2020). On the other hand, Chavarro *et al.* observed that the highest quartile of saturated fat intake was associated with the highest percent motile sperm among healthy young men (Chavarro *et al.*, 2014).

We are not aware of mechanistic research that supports a beneficial effect of saturated or trans-fats on male fertility. Intake of trans-fat, in particular, has adverse effects on male reproductive function in rodent studies (Jensen, 1976; Hanis *et al.*, 1989; Veaute *et al.*, 2007). Instead, positive associations between fat intakes and fecundability may be due to replacement of carbohydrates with fat. Dietary patterns characterized by lower intakes of refined grains, sweets and sugar-sweetened beverages are favorably associated with semen quality (Oostingh *et al.*, 2017; Danielewicz *et al.*, 2018; Efrat *et al.*, 2018; Jurewicz *et al.*, 2018). Previous research in PRESTO demonstrated inverse associations of male sugar-sweetened beverage intake with fecundability (Hatch *et al.*, 2018). Furthermore, among female PRESTO participants, higher glycemic load and higher intake of added sugar were associated with lower fecundability (Willis *et al.*, 2020). Fructose consumption may alter fatty acid synthesis (Hochuli *et al.*, 2014), which could influence the fatty acid composition of sperm and seminal fluid. Intake of added sugars can increase insulin resistance (Softic *et al.*, 2020), which may in turn adversely affect male reproductive function through oxidative damage to sperm (Agarwal *et al.*, 2014; Salas-Huetos *et al.*, 2017; Luc *et al.*, 2019) and disruption of the hypothalamic–pituitary–gonadal axis (He *et al.*, 2021).

We observed a weak positive association of monounsaturated fatty acid intake with fecundability. While some studies have found null (Chavarro *et al.*, 2014) or inverse associations (Jensen *et al.*, 2013) between monounsaturated fat intake and measures of semen quality, one study found that a higher ratio of monounsaturated to saturated fat intakes was inversely associated with odds of low sperm concentration (<15 million/ml) or low total sperm count (<39 million) (Ricci *et al.*, 2020). Intakes of polyunsaturated fat, especially omega-3 fatty acids, are generally positively associated with semen quality (Eslamian *et al.*, 2015; Oostingh *et al.*, 2017; Falsig *et al.*, 2019), although some studies have found null (Jensen *et al.*, 2013; Chavarro *et al.*, 2014) or inverse associations (Ricci *et al.*, 2020). In our study, associations of omega-3 fat intake with fecundability were close to null. In contrast to randomized controlled trials of supplementation with omega-3 supplements or nuts that observed beneficial effects on semen quality (Robbins *et al.*, 2012; Salas-Huetos *et al.*, 2018), our findings do not support a large effect size nor a dose–response association of male omega-3 fatty acid intake with fecundability.

Many studies have investigated intakes of specific foods, food groups, and dietary patterns in relation to semen quality. A 2017 systematic review of observational studies (which included 31 studies of diet and semen quality) concluded that higher intakes of fruits and vegetables, antioxidants, fiber, seafood, poultry, and low-fat dairy were positively associated with semen quality, whereas higher intakes of sugar-sweetened beverages, processed meat, soy, potatoes, cheese and full-fat dairy were associated with lower semen quality (Salas-Huetos *et al.*, 2017). Recent work on dietary patterns has supported those conclusions (Oostingh *et al.*, 2017; Danielewicz *et al.*, 2018; Efrat *et al.*, 2018; Jurewicz *et al.*, 2018; Cutillas-Tol'ın *et al.*, 2019; Ricci *et al.*, 2019; Salas-Huetos *et al.*, 2019). In PRESTO, top dietary contributors to saturated fat intake included dairy products, eggs and beef, and top contributors to trans-fat intake included fried potatoes, beef and macaroni and cheese. The positive associations of saturated and trans-fat intake with fecundability are unexpected, given the generally inverse associations of their major food sources with measures of semen quality. However, two studies identified positive associations of dietary patterns high in meat and potatoes with measures of semen quality (Vujkovic *et al.*, 2009; Cutillas-Tol'ın *et al.*, 2015), and one study observed lower semen quality among men who follow a vegetarian diet compared to men without dietary restrictions (Orzylowska *et al.*, 2016). Vujkovic *et al.* (2009) attributed the association of a diet rich in meat and potato intake with higher sperm concentration to higher intakes of folate, zinc, and vitamin B-12. Furthermore, the strong contribution of beef to trans-fat intake in our cohort may reflect naturally occurring trans-fat intake, which is considered less harmful for health than artificial trans-fat intake; this explanation is consistent with the removal of artificial trans-fats from the US and Canadian food supplies during the study period (Franco-Arellano *et al.*, 2020). Top contributors to monounsaturated fatty acid intake included some foods positively associated with semen quality (e.g. nuts and seeds, olive oil, poultry), and some foods inversely associated with semen quality (e.g. beef, cheese).

In contrast to most research about male diet and fertility, we estimated fecundability rather than relying on semen quality as a measure of male fertility. As discussed above, semen quality is an imperfect proxy for male reproductive potential (Bonde *et al.*, 1998; Buck Louis *et al.*, 2014). Furthermore, our study population differed from those of semen quality studies, which generally enrolled either healthy young men (age ~18–22) or men presenting to infertility clinics. In contrast, we enrolled male participants (average age 32 years) ranging in underlying fecundity, attempting to conceive spontaneously through intercourse. These reasons may contribute to discrepancies between our findings and previous studies of male diet and semen quality.

Few studies have evaluated male diet in relation to fertility, and they have mostly used infertility clinic populations. Braga *et al.* (2012) observed an inverse association of male meat intake with implantation rate and pregnancy among couples undergoing intracytoplasmic sperm injection. In contrast, Xia *et al.* (2015) observed no association of male meat intake (overall or specific types) with implantation, clinical pregnancy, or live birth among couples undergoing IVF. Three recent articles from the Environment and Reproductive Health prospective cohort study analyzed male diet in relation to ART outcomes among couples undergoing IVF. Mitsunami *et al.* (2021) derived a dietary score in relation to semen quality parameters, and then evaluated that dietary score as a predictor of IVF outcomes. Although the score was positively associated with total sperm count, sperm concentration, total sperm motility, progressive sperm motility, and normal sperm morphology, it was not associated with probability of implantation, clinical pregnancy, or live birth. Salas-Huetos *et al.*

(2022b) calculated scores for adherence to eight dietary patterns among male partners. Adherence to any of eight dietary patterns was not associated with semen quality parameters or IVF outcomes. Finally, Salas-Huetos *et al.* (2022a) found that male partners' intakes of total omega-3, DHA þ EPA, alpha-linolenic acid, total nuts, and total fish were associated with semen quality, but not with probability of implantation, clinical pregnancy, or live birth. The authors noted that male dietary associations with semen quality may not influence the success of ART because ART procedures select for normal sperm, and that their results may not generalize to couples attempting to conceive spontaneously (Mitsunami *et al.*, 2021).

To our knowledge, only one previous study (Gaskins *et al.*, 2018) has investigated male diet (outside of caffeine or alcohol intakes) in relation to fecundability among couples attempting to conceive without fertility treatment. Gaskins *et al.* found that seafood intakes of both members of the couple were positively associated with fecundability, and that this association was partially mediated by intercourse frequency. This study was limited by a lack of data on other dietary intakes. Building upon this previous work, we used the DHQ to estimate dietary fat intakes and did not observe a meaningful association of male omega-3 fatty acid intake with fecundability. We did not observe differences in intercourse frequency according to fat intakes nor fish intake.

Major strengths of our study include the prospective study design and preconception enrollment, which reduce the potential for selection bias, reverse causation and differential measurement error. We further reduced the potential for these biases through extensive sensitivity analyses. The similarity of couples included and excluded from the analysis, in terms of lifestyle characteristics and conception over the course of study participation, provides additional evidence against selection bias. Additional strengths of the study include collection of detailed dietary and lifestyle data directly from male participants rather than relying on their partners as proxies, reducing the potential for information bias. Although we relied on self-report for dates of menstruation and conception, previous research in PRESTO has demonstrated high reliability of menstruation data (Wise *et al.*, 2015) and early detection of pregnancies using home pregnancy tests (median 4 weeks' gestation) (Wise *et al.*, 2020). We collected extensive data on male and female characteristics and adjusted for many potential confounders, including the female partner's dietary fat intakes and the male partner's food intakes, reducing the potential for residual confounding.

The study has several limitations. While the DHQ is one of the most valid and widely used FFQs used in epidemiologic research (Subar *et al.*, 2001), the assessment of dietary exposures through FFQs is notoriously prone to measurement error, most notably under-reporting (Subar *et al.*, 2003). Measurement error in the exposure is likely non-differential and independent with respect to fecundability. When exposures are modeled as quartiles, non-differential exposure misclassification can lead to bias away from the null in at least one intermediate exposure category and could obscure a true dose–response association (Yland *et al.*, 2022). Although we adjusted for many potential confounders, residual confounding by unmeasured socioeconomic, lifestyle, environmental or dietary variables is possible, given the observational study design. Chance findings are possible, and sample size was limited, particularly in stratified analyses. Finally, generalizability is limited because the study enrolled couples who identified as pregnancy planners, who may not be demographically or socioeconomically representative of the general population, and who may represent less-fertile couples than those who conceive before having the opportunity to enroll in preconception research (Savitz *et al.*, 2002).

Our results do not support a strong causal effect of male fatty acid intakes on fecundability among couples attempting to conceive spontaneously. The weak positive associations we observed between some male fatty acid intakes and fecundability may reflect a combination of causal associations (specifically, replacement of carbohydrates with fat), measurement error, chance and residual confounding. Our findings do not provide sufficient justification for clinical recommendations, nor for trials of male omega-3 supplementation or male dietary intake of fatty acids to improve fecundability among couples trying to conceive spontaneously.

## Supplementary data

Supplementary data are available at *Human Reproduction* online.

## Data availability

The data underlying this article cannot be shared publicly because the participants did not provide informed consent to share these data in this manner.

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## Authors' roles

L.A.W., A.K.W., and E.E.H. were responsible for study design and development and implementation of the cohort study. R.J.G., A.K.W., K.L.T., and L.A.W. designed the present study. R.J.G. drafted the manuscript and performed statistical analyses. A.K.W. and M.R.K. contributed to data analysis. All authors were responsible for critical interpretation of the results and revision of the manuscript. All authors approved the final version of the manuscript.

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## Conflict of interest

In the last 3 years, PRESTO has received in-kind donations from Swiss Precision Diagnostics (home pregnancy tests) and Kindara.com (fertility app). Dr L.A.W. is a fibroid consultant for AbbVie, Inc. Dr M.L.E. is an advisor to Sandstone, Ro, Underdog, Dadi, Hannah, Doveras, and VSeat. The other authors have no conflicts of interest to report.

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