Efficacy of Fish Oil and/or Probiotic Intervention on the Incidence of Gestational Diabetes Mellitus in an At-Risk Group of Overweight and Obese Women: A Randomized, Placebo-Controlled, Double-Blind Clinical Trial

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OBJECTIVE

To assess whether the risk of gestational diabetes mellitus (GDM) may be lowered and glucose metabolism improved by daily administration of fish oil and/or probiotic supplements in overweight and obese pregnant women.

RESEARCH DESIGN AND METHODS

We randomized in a double-blind manner 439 women (mean 13.9 ± 2.1 gestational weeks [gw]) into four intervention groups: fish oil + placebo, probiotics + placebo, fish oil + probiotics, and placebo + placebo. Fish oil (1.9 g docosahexaenoic acid and 0.22 g eicosapentaenoic acid) and probiotic supplements (*Lactobacillus rhamnosus* HN001 and *Bifidobacterium animalis ssp. lactis* 420, 10^{10} colony-forming units each) were provided for daily consumption from randomization beyond delivery. Primary outcomes were the incidence of GDM diagnosed with oral glucose tolerance test targeted at 24–28 gw and the change in fasting glucose between randomization and late pregnancy (mean 35.2 ± 0.9 gw). Insulin concentration, insulin resistance HOMA2-IR index, and pregnancy outcomes were determined, as were adverse effects related to the intervention. Analyses were by intent to treat.

RESULTS

No differences were found among the intervention groups in the maternal and neonatal pregnancy outcomes or side effects related to the intervention ($P > 0.05$). The proportion of women with GDM (94 of 377; fish oil + placebo, 23 of 96, 24.0%; probiotics + placebo, 25 of 99, 25.3%; fish oil + probiotics, 26 of 91, 28.6%; and placebo + placebo, 20 of 91, 22.0%) and the change in glucose, insulin, or HOMA2-IR ($n = 364$) did not differ among the intervention groups ($P > 0.11$ for all comparisons).

CONCLUSIONS

An intervention with fish oil and/or probiotics during pregnancy seemed to be both safe and well tolerated but conferred no benefits in lowering the risk of GDM or improving glucose metabolism in overweight and obese women.

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Gestational diabetes mellitus (GDM) is an increasingly common condition; ~14% of pregnancies are affected worldwide (1). The need to find a means to lower the risk of GDM is important, as it affects the health of mother and child both acutely and over the long term (2,3). Because GDM is clearly associated with obesity, interventions to lower the risk have typically focused on lifestyle changes. However, these interventions have yielded inconclusive results (4,5), emphasizing the need for new preventive approaches.

The pathogenesis of GDM consists of two main factors: high insulin resistance and the decreased ability of pancreatic β-cells to produce insulin (6,7). Although genetic factors predispose to GDM, often the abnormally high insulin resistance is attributable to obesity, which is amplified by pregnancy-induced hormones (8). Furthermore, pregnancy, obesity, and GDM are associated with an increase in inflammatory markers that contribute to insulin resistance, and thus a heightened inflammatory response has been proposed to play an important role in the development of GDM (9). Both probiotics and the n-3 long-chain polyunsaturated fatty acids (LC-PUFA) present in fish oil have been demonstrated to possess anti-inflammatory properties and a capability to reduce insulin resistance (10–12). Furthermore, there is previous experimental evidence indicating that a combination of these two active components might exert synergistic immunoregulatory effects (13).

In the search for novel means to lower the risk of GDM, we conducted a randomized, placebo-controlled intervention trial of a dietary supplementation with the aim to reduce insulin resistance and improve glucose metabolism. We recruited overweight and obese pregnant women, a high-risk group for developing metabolic complications, and hypothesized that fish oil and probiotic supplements, either individually or in combination, could improve blood glucose control during pregnancy and decrease the incidence of GDM (primary outcomes). As predefined secondary outcomes, we evaluated the need for medication in the management of GDM as well as several maternal and neonatal pregnancy outcomes, including infant macrosomia.

RESEARCH DESIGN AND METHODS

We conducted a double-blind, placebo-controlled randomized trial on the effects of fish oil and/or probiotic dietary supplements on maternal and child health. This single-center trial was executed in the Turku University Hospital and University of Turku in Finland with recruitment between October 2013 and July 2017 (ClinicalTrials.gov, NCT01922791). The study complies with the Declaration of Helsinki as revised in 2000. The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol, and all participants provided written informed consent. Leaflets with the study information were distributed in maternal welfare clinics. In addition, media and social media were used to inform about the study. Women interested in participating in the study contacted the project coordinator for further information and to schedule their first study visit. Eligible women were randomly assigned to one of the four parallel groups at the first study visit during early pregnancy: fish oil + placebo (i.e., placebo for probiotics), probiotics + placebo (i.e., placebo for fish oil), fish oil + probiotics, or placebo + placebo (placebo for probiotics and placebo for fish oil). Subjects were allocated into intervention groups according to mother's parity and history of GDM (primipara, multipara, or multipara with previous GDM). The stratified randomization was performed with random permuted blocks of four, and randomization lists of the three blocks were generated by a statistician who was not involved in either study recruitment or its execution. Women were assigned to the intervention groups according to the randomization list in their order of recruitment on the first study visit. The staff responsible for enrollment of participants, study visits, and assessing outcomes remained blinded to the intervention, as were the participants.

Participants

A total of 439 women were recruited from Southwest Finland. The inclusion criteria were as follows: self-reported prepregnancy BMI $\geq$25 kg/m^2, $<$18 gestational weeks (gw), and absence of chronic diseases (asthma and allergies were allowed). Exclusion criteria were as follows: diabetes before pregnancy (HbA_{1c} $\geq$6.5% [48 mmol/mol] or fasting glucose $\geq$7.0 mmol/L at randomization), multifetal pregnancy, chronic diseases impacting on metabolic and gastrointestinal health including inflammatory bowel diseases, refusal to terminate the intake of other probiotic or fish oil supplements, diagnosis or history of coagulopathy, and use of anticoagulants.

Study Conduct

Women attended two study visits during gestation (mean $\pm$ SD 13.9 $\pm$ 2.1 and 35.2 $\pm$ 0.9 gw). On the first study visit, height was measured with a wall stadiometer to the nearest 0.1 cm. Prepregnancy BMI was calculated using height and self-reported prepregnancy weight obtained from the maternal welfare clinic records. Blood pressure was measured on both visits.

Supplements were provided from the first study visit, throughout the pregnancy, and until 6 months postpartum. Women were advised to take two fish oil capsules and one probiotic capsule daily. The fish oil capsules (Croda Europe Ltd., Leek, U.K.) contained a total of 2.4 g of n-3 fatty acids, of which 79% (1.9 g) was docosahexaenoic acid (22:6 n-3) (DHA) and 9.4% (0.22 g) eicosapentaenoic acid (20:5 n-3) (EPA), the rest being other n-3 fatty acids, including docosapentaenoic acid. Placebo capsules for fish oil contained an equal amount of medium-chain fatty acids (capric acid C8 54.6% and caprylic acid C10 40.3%) and were of the same size, shape, color, and lemon flavor as the fish oil capsules. The oil capsules were stored at room temperature.

Probiotic capsules contained *Lactobacillus rhamnosus* HN001 (ATCC SD5675; DuPont, Niebüll, Germany) and *Bifidobacterium animalis* ssp. *lactis* 420 (DSM 22089; DuPont), each 10^{10} colony-forming units per capsule. Placebo for the probiotics consisted of microcrystalline cellulose; the capsules were identical to the probiotic capsules in size, shape, and color. Capsules were stored at $-$20°C until provided to the subjects, who were instructed to store the capsules in a refrigerator.

The stability of the supplements was monitored by both manufacturers regularly during the trial. All capsules were identically packaged and identified by trial codes. Women were asked not to consume other probiotic and n-3

LC-PUFA products during the study. Compliance with the consumption of capsules was assessed first by a phone call at mean 28 gw, subsequently by interview at the second study visit (good compliance being defined as taking study capsules ≥5 days/week reported at both time points), and third by counting the numbers of consumed fish oil capsules, i.e., subtracting the capsules returned to the study unit from the total provided by a random sample of 62 women (14% of participants).

Women filled in questionnaires and were interviewed concerning their health, education, smoking habits, obstetric medical history, and family history of diabetes. During the intervention period, women were asked to keep a diary on a weekly basis to record possible adverse effects related to supplement consumption. Data on pregnancy and delivery were obtained from medical records.

Blood Sampling and Analysis

On the morning of the study visit, after at least 9 h of overnight fasting, blood samples were drawn from an antecubital vein. A certified laboratory (TYKSLAB, the Hospital District of Southwest Finland) was used for the sampling, with analyses of glucose and insulin conducted by an enzymatic method using hexokinase and by immunoelectrochemiluminometric assay, respectively. Insulin resistance (IR) was determined by calculating the HOMA (HOMA2-IR) (14).

GDM Diagnosis

GDM was diagnosed on the basis of a 2-h 75-g oral glucose tolerance test (OGTT) if one or more values were at or above the threshold level: 0 h ≥5.3, 1 h ≥10.0, and 2 h ≥8.6 mmol/L, according to the Finnish Current Care Guidelines (15). OGTT was offered by maternal welfare clinics to all women between 24 and 28 gw and to high-risk women also at 12–16 gw (BMI ≥35 kg/m^2, previous GDM, glucosuria, polycystic ovarian syndrome, or family risk of diabetes). We also used the diagnostic criteria from the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) with the following diagnostic thresholds: 0 h ≥5.1, 1 h ≥10.0, and 2 h ≥8.5 mmol/L. Regardless of the timing of OGTT, treatment for GDM was offered soon after diagnosis by health care services independent of the research protocol and in accordance with the national guidelines.

Outcomes

The primary outcome was the incidence of GDM based on the OGTT result targeted at 24–28 gw and the change in fasting plasma glucose between the early and late pregnancy study visits.

Prespecified secondary outcomes included the change in insulin and HOMA2-IR values, the need for medication in the management of GDM (insulin or metformin), gestational hypertensive disorders, mode of delivery, postpartum hemorrhage, birth weight, and neonatal macrosomia (birth weight >90th percentile).

Pregnancy-induced hypertension was diagnosed as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg occurring after 20 gw in a previously normotensive woman. Pre-eclampsia was defined as pregnancy-induced hypertension combined with new-onset proteinuria of ≥0.3 g/24 h or urine dipstick protein ≥2+. Superimposed pre-eclampsia was defined by the same criteria as pre-eclampsia but in women with essential hypertension (similar blood pressure levels occurring before 20 gw).

Power Calculations

The sample size was calculated on the basis of the main outcome variables (power of 80% and significance level $P < 0.05$). Based on a 20% reduction in the incidence of GDM in the fish oil or probiotic group from 50% to 30% (16,17) and a further 5% decrease in the combined intervention group (from 50% to 25%), a sample size of 93 per group was estimated. For fasting plasma glucose levels, a sample size of 50 subjects per group was calculated in order to detect a treatment effect of −0.2 mmol/L in glucose, assuming that the SD was 0.35 (18). We aimed to recruit 440 volunteers to the study (110 in each intervention group), allowing for 20% dropout.

Statistical Analyses

Our analysis was by intent to treat. The normality of the data was checked visually from histograms. The data were summarized as frequencies and percentages for categorical variables and as means and SD for normally distributed continuous variables. Postpartum hemorrhage was not normally distributed, and hence, median with interquartile range was reported and Kruskal-Wallis test applied when comparing the intervention groups.

The comparisons of baseline characteristics, OGTT test result, GDM diagnosis, and maternal/neonatal outcomes among the intervention groups were conducted by one-way ANOVA for continuous variables and χ^2 test or Fisher exact test for categorical variables, when applicable. Differences in the change of glucose, insulin, and HOMA2-IR were also compared with one-way ANOVA. General linear models with binomial distribution and log link function were used to compare the relative risk of GDM in each intervention group with the placebo + placebo group (Supplementary Table 1). The modifying effect of potential confounding factors on the effect of the intervention (confounding factor × group interaction effect) on the incidence of new GDM diagnoses (i.e., early gestation OGTT-positive women excluded) was analyzed using the generalized linear model. Two-way ANOVA was used to analyze the modifying effect of potential confounding factors on the effect of the intervention (confounding factor × group interaction effect) on the change in fasting plasma glucose, insulin, and HOMA2-IR. Again, we used χ^2 test or Fisher exact test to compare differences among the intervention groups with respect to compliance, number of women discontinuing the study, and adverse effects. The comparison of duration of the side effects was conducted with the nonparametric Kruskal-Wallis test (Supplementary Table 2). A P value <0.05 was considered significant. All analyses were performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC).

RESULTS

A total of 988 women were screened for eligibility, with 439 women being randomized to the intervention (Fig. 1). The study was discontinued by 39 (8.9%) women before the OGTT and altogether by 59 (13.5%) women before the late pregnancy measurements of fasting glucose and insulin concentrations (both nonsignificant among the intervention groups). A few test results were unavailable because of failure to fast, interruption of the OGTT, or giving birth

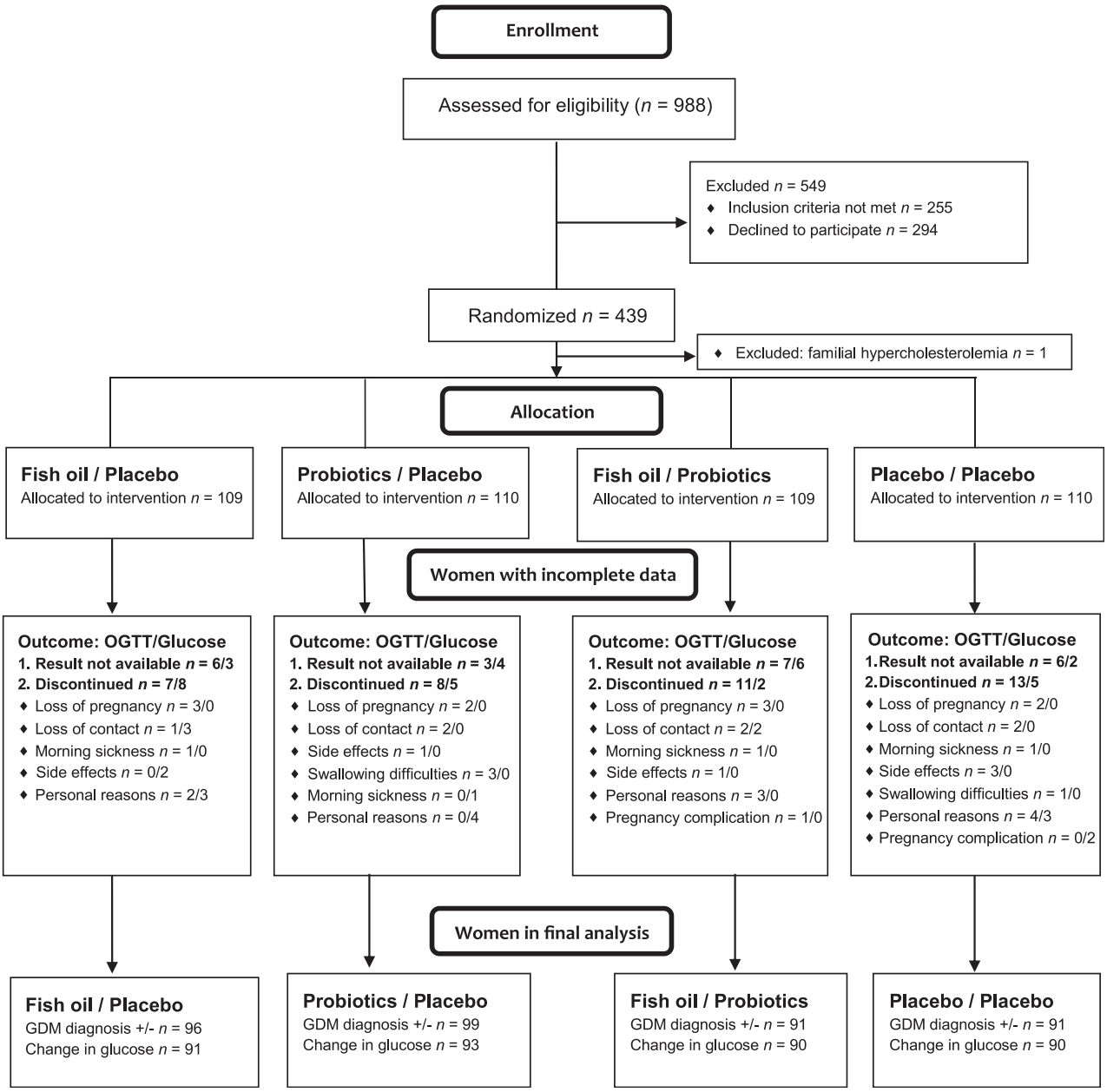

Figure 1—Flow diagram. Incomplete data are reported as either absent data before OGTT or absent data between OGTT and late gestation fasting plasma glucose measurement.

prematurely. Good compliance was reported by 88.4% of the women, with this value being similar in the four groups ($P > 0.98$, data not shown). The compliance calculated from the returned fish oil capsules indicated that a mean of 91.8% (SD 15.9) of the capsules had been consumed.

The clinical characteristics of the women at baseline (Table 1) did not differ among the intervention groups, except for a family history of diabetes that was more common in women in the fish oil + placebo group as compared with the probiotics + placebo and placebo + placebo groups. With respect to the characteristics of the women, 47.9% were expecting their first child, 9.1% had previous GDM, 19.2% were ≥35 years of age, and 39.3% were obese. Women participating in the study were generally in good health, although some mild medical conditions, including allergy and/or atopy (20.5% of the women), asthma (8.8%), migraine (8.8%), and hypothyroidism (7.0%), were reported (all nonsignificant between the intervention groups).

Incidence of GDM

OGTT, scheduled for all women, was performed at mean 26.4 ± SD 2.2 gw, when the duration of the intervention was a mean of 12.5 ± 3.1 weeks. Of the women, 145 of 379 (38.3%) were diagnosed with GDM according to IADPSG criteria, and 94 of 377 (24.9%) with the Finnish criteria. We observed no significant difference in the incidence of GDM between the intervention groups (Table 2). Furthermore, no differences in the incidence of GDM or OGTT values were detected between the groups when

Table 1—Characteristics of the pregnant women in the intervention groups

	n	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	*P*
Age	110/109/109/110	30.4 ± 4.8	30.8 ± 4.8	30.8 ± 4.6	30.4 ± 4.1	0.820*
Prepregnancy weight	110/109/109/110	82.8 ± 13.4	83.6 ± 14.9	81.7 ± 12.6	83.1 ± 12.8	0.765*
Prepregnancy BMI (kg/m^2)	110/109/109/110	30.0 ± 4.2	29.9 ± 4.7	29.3 ± 3.9	29.7 ± 4.2	0.594*
Overweight		62 (56.4)	70 (64.2)	68 (62.4)	66 (60.0)	0.663†
Obese		48 (43.6)	39 (35.8)	41 (37.6)	44 (40.0)	
Primipara	110/109/109/110	53 (48.2)	52 (47.7)	52 (47.7)	53 (48.2)	1.000†
Ethnic region	110/109/109/110					0.762‡
European		109 (99.1)	107 (98.2)	106 (97.3)	108 (98.2)	
Asian		0 (0.0)	0 (0.0)	1 (0.92)	1 (0.91)	
Middle Eastern		1 (0.91)	1 (0.91)	0 (0.0)	1 (0.91)	
Other/mixed		0 (0.0)	1 (0.91)	2 (1.83)	0 (0.0)	
College or university education	100/94/99/98	66 (66.0)	59 (62.8)	56 (56.6)	58 (59.2)	0.546†
Previous GDM	110/109/109/110	10 (9.1)	10 (9.2)	10 (9.2)	10 (9.1)	1.000†
Family history of diabetes	93/89/94/86	25 (26.9)§	12 (13.5)	16 (17.0)	8 (9.3)	0.012†
Smoking during pregnancy	100/95/98/98	2 (2.0)	6 (6.3)	5 (5.1)	6 (6.1)	0.437‡
Essential hypertension	110/109/109/110	4 (3.6)	4 (3.7)	2 (1.8)	2 (1.8)	0.759‡
Systolic blood pressure in early gestation (mmHg)	110/108/109/109	116.4 ± 10.7	117.4 ± 11.3	116.0 ± 9.5	118.1 ± 9.7	0.437*
Diastolic blood pressure in early gestation (mmHg)	110/108/109/109	77.6 ± 9.0	76.2 ± 8.9	75.8 ± 7.7	76.6 ± 7.5	0.445*
Used probiotics before intervention	110/109/109/110	26 (23.6)	18 (16.5)	31 (28.4)	20 (18.2)	0.128†
Used fish oil before intervention	110/109/109/110	15 (13.6)	16 (14.7)	18 (16.5)	20 (18.2)	0.801†

Data are presented as mean ± SD or *n* (%). *One-way ANOVA. †χ^2 test. ‡Fisher exact test. §Significantly different from probiotics + placebo ($P = 0.025$) and placebo + placebo ($P = 0.002$).

only the new diagnoses were evaluated (114 of 339 [33.6%] and 83 of 360 [23.1%] according to IADPSG and Finnish criteria, respectively), i.e., early gestation OGTT-positive women being excluded from the analysis (Table 2). In early gestation, 132 women at high risk for GDM were referred to OGTT after randomization at a mean 14.7 ± 2.0 gw, and 61 (47.3%) were diagnosed with GDM according to IADPSG criteria and 36 (27.9%) according to the Finnish criteria.

We also evaluated the relative risk of GDM in each intervention group compared with the placebo + placebo group but detected no statistically significant differences ($P > 0.24$ for all comparisons) (Supplementary Table 1).

Every fifth (24 of 119, 20.2%) woman diagnosed with GDM (Finnish criteria) needed insulin or metformin for the management of GDM (nonsignificant between the intervention groups) (Table 2).

Glucose and Insulin Concentrations

Fasting plasma glucose concentrations decreased and the serum insulin concentration and HOMA2-IR increased significantly in all intervention groups from early to late pregnancy (Table 2). No significant differences were detected in the change of glucose or insulin concentrations or HOMA2-IR between the intervention groups ($P > 0.05$) (Table 2).

Role of Potential Confounding Factors

The effect of the intervention on the incidence of new GDM diagnoses at late pregnancy was not influenced by confounding factors, including compliance or duration of the intervention, maternal age <35 or ≥35 years, prepregnancy BMI, gestational weight gain between study visits, consumption of fish oil or probiotic supplements before randomization, family history of diabetes, or previous GDM ($P > 0.05$ for confounding factor × group interactions in all comparisons). Furthermore, the differences between the intervention groups in the change of fasting plasma glucose, insulin, or HOMA2-IR did not differ between women with and without GDM ($P > 0.05$ for GDM × group interactions in all comparisons).

However, a significant interaction was detected between previous GDM and the intervention groups ($P = 0.016$) with respect to the change in fasting plasma glucose. This was attributable to the association of previous GDM with the decrease in glucose values in the placebo + placebo group when compared with the increase in the fish oil + placebo group ($P = 0.049$). Furthermore, after exclusion of women with early-pregnancy GDM, the change in glucose levels was different among the intervention groups depending on the duration of intervention ($P = 0.039$) or prepregnancy BMI ($P = 0.043$); in the probiotics + placebo group and the probiotics + fish oil group, but not in the other groups, the longer duration of the intervention was associated with a greater decrease in glucose levels ($P = 0.026$ and $P < 0.0001$, respectively); in the fish oil + placebo group and the probiotics + placebo group, but not in the other groups, a higher prepregnancy BMI value was significantly related to a greater increase in the glucose level ($P = 0.022$ and $P = 0.043$, respectively).

Maternal and Neonatal Outcomes

There were no differences in maternal or infant pregnancy outcomes among the intervention groups, including numbers

Table 2—Impact of intervention on the incidence of GDM and concentrations of glucose, insulin, and insulin resistance in the different intervention groups

	n	Fish oil + placebo			Probiotics + placebo			Fish oil + probiotics			Placebo + placebo			*P*
GDM diagnosis by IADPSG criteria*														
GDM in early pregnancy	42/32/32/23	19 (45.2)			15 (46.9)			14 (43.8)			13 (56.5)			0.80†
GDM at approx. 24–28 gw	96/99/93/91	36 (37.5)			35 (35.4)			38 (40.9)			36 (39.6)			0.87†
GDM, new diagnosis‡	83/88/84/84	27 (32.5)			25 (28.4)			31 (36.9)			31 (36.9)			0.59†
GDM diagnosis by Finnish criteria§														
GDM in early pregnancy	42/32/32/23	11 (26.2)			9 (28.1)			7 (21.9)			9 (39.1)			0.56†
GDM at approx. 24–28 gw	96/99/91/91	23 (24.0)			25 (25.3)			26 (28.6)			20 (22.0)			0.77†
GDM, new diagnosis‡	91/94/88/87	20 (22.0)			23 (24.5)			23 (26.1)			17 (19.5)			0.74†
Insulin and/or metformin medication	30/29/28/23	7 (23.3)			3 (10.3)			7 (25.0)			7 (30.4)			0.33†
OGTT results														
gw at OGTT	97/99/94/91	26.6 ± 2.5			26.3 ± 1.7			26.5 ± 2.5			26.2 ± 2.0			0.49‖
Duration of intervention until OGTT	97/99/94/91	12.6 ± 3.5			12.6 ± 2.9			12.5 ± 3.1			12.1 ± 2.8			0.63‖
Fasting plasma glucose (mmol/L)	97/99/94/91	4.9 ± 0.38			4.9 ± 0.43			5.0 ± 0.60			4.8 ± 0.32			0.11‖
Plasma glucose at 1 h (mmol/L)	96/99/93/91	7.7 ± 1.5			7.5 ± 1.7			7.9 ± 1.9			7.7 ± 1.6			0.44‖
Plasma glucose at 2 h (mmol/L)	95/99/92/91	6.4 ± 1.1			6.5 ± 1.3			6.5 ± 1.7			6.4 ± 1.4			0.87‖
Glucose, insulin, and insulin resistance		Early gestation	Late gestation	Mean change (95% CI)	Early gestation	Late gestation	Mean change (95% CI)	Early gestation	Late gestation	Mean change (95% CI)	Early gestation	Late gestation	Mean change (95% CI)	
gw at testing	91/93/90/90	14.0 ± 2.2	35.1 ± 0.89	21.2 (20.7; 21.6)	13.6 ± 2.1	35.3 ± 0.96	21.6 (21.2; 22.1)	13.9 ± 2.1	35.2 ± 0.93	21.3 (20.8; 21.8)	13.9 ± 2.0	35.2 ± 1.0	21.3 (20.8; 21.7)	0.51‖
Fasting plasma glucose (mmol/L)	91/93/90/90	4.80 ± 0.38	4.71 ± 0.43	−0.09 (−0.2; −0.0)	4.73 ± 0.31	4.59 ± 0.37	−0.14 (−0.2; −0.1)	4.74 ± 0.37	4.64 ± 0.43	−0.10 (−0.2; −0.0)	4.73 ± 0.36	4.56 ± 0.41	−0.17 (−0.3; −0.1)	0.49‖
Insulin (mU/L)	91/93/90/90	12.1 ± 6.4	20.1 ± 10.4	8.0 (6.4; 9.6)	10.1 ± 5.7	15.6 ± 6.8	5.5 (3.9; 7.0)	10.6 ± 4.6	17.2 ± 8.8	6.6 (5.0; 8.2)	11.7 ± 6.1	18.1 ± 12.6	6.4 (4.8; 8.0)	0.16‖
HOMA2-IR	91/93/90/90	1.54 ± 0.82	2.51 ± 1.28	0.98 (0.8; 1.2)	1.29 ± 0.71	1.94 ± 0.84	0.65 (0.5; 0.8)	1.35 ± 0.59	2.15 ± 1.1	0.80 (0.6; 1.0)	1.48 ± 0.76	2.22 ± 1.45	0.75 (0.6; 0.9)	0.12‖

Data presented as mean ± SD or *n* (%) unless marked otherwise. *IADPSG criteria: 0 h ≥5.1 mmol/L, 1 h ≥10.0 mmol/L, 2 h ≥8.5 mmol/L, and one pathologic value sufficient to diagnose GDM. †χ^2 test. ‡GDM, new diagnosis = women with GDM diagnosed at early pregnancy excluded from analyses. §Finnish criteria: 0 h ≥5.3 mmol/L, 1 h ≥10.0 mmol/L, 2 h ≥8.6 mmol/L, and one pathologic value sufficient to diagnose GDM. ‖One-way ANOVA.

Table 3—Pregnancy outcomes in the intervention groups

	n	Fish oil + placebo	Probiotics + placebo	Fish oil + probiotics	Placebo + placebo	*P*
Maternal						
Duration of intervention until delivery (weeks)	94/96/96/92	25.8 ± 2.6	26.1 ± 2.6	25.4 ± 3.1	25.6 ± 2.5	0.27*
Miscarriage <22 gw	110/109/109/110	3 (2.7)	1 (0.9)	3 (2.8)	2 (1.8)	0.84†
Stillbirth	95/96/96/93	0	0	0	1 (1.1)	0.24†
Pregnancy-induced hypertension	95/96/96/93	7 (7.4)	4 (4.2)	6 (6.3)	4 (4.3)	0.80
Pre-eclampsia including superimposed	95/96/96/93	1 (1.1)	4 (4.2)	3 (3.1)	2 (2.2)	
Intrahepatic cholestasis of pregnancy	95/96/96/92	2 (2.1)	3 (3.1)	1 (1.0)	4 (4.4)	0.47†
Postpartum hemorrhage (mL)‡	95/96/96/92	400 (300)	400 (300)	400 (200)	400 (200)	0.96§
Postpartum hemorrhage >1,000 mL	95/96/96/92	6 (6.3)	8 (8.3)	5 (5.2)	7 (7.6)	0.83‖
Mode of delivery	95/96/96/92					0.28‖
Vaginal unassisted		66 (69.5)	77 (80.2)	69 (71.9)	65 (70.7)	
Vacuum extraction		13 (13.7)	5 (5.2)	8 (8.3)	13 (14.1)	
Elective cesarean		5 (5.3)	4 (4.2)	10 (10.4)	6 (6.5)	
Acute or emergency cesarean		11 (11.6)	10 (10.4)	9 (9.4)	8 (8.7)	
Neonatal						
gw at delivery	95/96/96/92	39.8 ± 1.6	39.8 ± 1.4	39.4 ± 2.0	39.6 ± 1.4	0.34*
Sex: girl	95/96/95/92	46 (48.4)	51 (53.1)	52 (54.7)	43 (46.7)	0.66‖
Premature (<37 gw)	95/96/96/92	4 (4.2)	4 (4.2)	11 (11.5)	3 (3.3)	0.05‖
Post date (>42 gw)	95/96/96/92	0 (0.0)	2 (2.1)	2 (2.1)	1 (1.1)	0.67†
Birth weight (g)	95/96/96/92	3,610 ± 516	3,620 ± 539	3,530 ± 647	3,600 ± 503	0.70*
Birth weight *z* score	92/96/93/92	0.1 ± 1.0	0.1 ± 1.1	−0.0 ± 1.1	0.1 ± 1.0	0.87*
Small for gestational age (<10th percentile)	92/96/92/92	7 (7.6)	7 (7.3)	8 (8.7)	9 (9.8)	0.93‖
Macrosomia (>90th percentile)	92/96/93/92	6 (6.5)	13 (13.5)	8 (8.6)	13 (14.1)	0.26‖
Apgar points at 5 min	94/96/96/91	9.1 ± 0.7	9.0 ± 0.7	8.9 ± 0.8	9.0 ± 0.8	0.41*
Umbilical artery pH	87/86/84/89	7.25 ± 0.09	7.26 ± 0.09	7.28 ± 0.08	7.28 ± 0.08	0.13*
Hypoglycemia ≤2.4 mmol/L	93/95/93/89	20 (21.5)	20 (21.1)	19 (20.4)	12 (13.5)	0.48‖
Phototherapy	94/96/93/91	16 (17.0)	12 (12.5)	11 (11.8)	8 (8.8)	0.40‖
Admitted to neonatal intensive care unit	94/96/94/92	12 (12.8)	13 (13.5)	16 (17.9)	11 (12.0)	0.76‖
Congenital malformations¶	94/96/95/92	3 (3.2)	4 (4.2)	5 (5.3)	2 (2.2)	0.79†

Data are expressed as mean ± SD or *n* (%) unless marked otherwise. ‡Median (interquartile range). *One-way ANOVA. †Fisher exact test. §Kruskal-Wallis. ‖χ^2 test. ¶Congenital malformations consist of five hip dislocations and two cardiac, one chromosomal, two urogenital, two skin, and two bone abnormalities.

of miscarriages, hypertensive complications, mode of delivery, or macrosomia (Table 3). In addition, there were no differences in the frequencies of postpartum hemorrhage or in the number of women bleeding >1,000 mL among the intervention groups.

Adverse Effects

Adverse effects of the capsule consumption were reported by 109 of 389 (28.0%) of the women, with no significant differences among the intervention groups; half of those reporting headache or other side effects were women in the placebo + placebo group (Supplementary Table 2). Gastrointestinal symptoms were the most common adverse effects; 26.2% of the women experienced some degree of discomfort.

CONCLUSIONS

We demonstrated here that this intervention with fish oil providing 2.4 g of n-3 LC-PUFA, probiotics *L. rhamnosus* HN001 and *B. animalis ssp. lactis* 420, or their combination did not lower the incidence of GDM, fasting glucose concentration, or insulin resistance in overweight and obese pregnant women. As the frequencies of pregnancy complications were similar in all groups, our intervention appeared to be safe and, in the light of the minimal adverse effects, also well tolerated.

As far as we are aware, this is the first time that the potential synergistic benefits of combining fish oil with probiotics have been investigated in pregnant women. The published literature on this topic is scanty; one previous study demonstrated promising synergistic results on insulin sensitivity in a population of healthy overweight adults (19). Both supplements have been proposed individually to possess health-promoting metabolic effects such as an ability to reduce insulin resistance and inflammatory status. Putative mechanisms include improvements in intestinal barrier integrity and reduction in the risk of metabolic endotoxemia and subsequent low-grade inflammation (20). Our intervention targeted these metabolic disturbances, which are also known to manifest in type 2 diabetes. Along with insulin resistance, the genetic backround behind impaired insulin secretion also plays a role in the pathogenesis of GDM (7) and may be more difficult to influence by dietary means. However, it has been reported that nonpregnant individuals with impaired glucose tolerance can benefit from lifestyle interventions in preventing the development of type 2 diabetes, independent of their genetic background or familial risk of type 2 diabetes (21).

Similar to our results, in a previous study with GDM as a primary outcome (22), fish oil exerted no impact on the incidence of GDM, even though a large number of women were examined (*n* = 2,399). However, the dose of n-3 LC-PUFA was considerably smaller, 0.8 g DHA (1.5 g n-3 LC-PUFA) (22), than in our study. Meta-analysis of randomized controlled studies, primarily

with main outcomes other than GDM, has failed to demonstrate any benefit of consuming n-3 LC-PUFA on the incidence of GDM (23). A larger number of studies have been conducted in women with GDM; a recent meta-analysis of seven randomized controlled trials did report evidence of a benefit on glucose metabolism associated with n-3 LC-PUFA consumption (24). Nevertheless, in our study, there was no difference in fasting plasma glucose or insulin levels in women with GDM receiving fish oil + placebo as compared with the placebo + placebo group. It is noteworthy that here the presence of a family history of diabetes was highest in women in the fish oil + placebo group compared with the other groups, which may have contributed to the lack of an intervention effect. Interestingly, a meta-analysis in patients with type 2 diabetes revealed that a high ratio of EPA to DHA could be beneficial in terms of glucose control (25). This may be one reason why the fish oil intervention failed to exert a glucose-regulating benefit, as DHA was the dominant fatty acid in the fish oil consumed by the women. This DHA-dominant fish oil was chosen for our study particularly for its expected benefits for the infants. Indeed, a recent meta-analysis of 70 randomized controlled trials found that n-3 LC-PUFA consumption during pregnancy was beneficial in reducing the risk of preterm birth (26). It remains to be demonstrated whether our intervention, which continued beyond delivery, exerts any long-term benefits in reducing the GDM-induced elevated risks for both mother and child.

When considering the probiotics, in contrast to our working hypothesis and previous findings (16,18), we found no impact of the intervention on the incidence of GDM or glucose metabolism in overweight and obese pregnant women. In our previous trial, we demonstrated the benefits of administration of *L. rhamnosus GG* and *B. lactis* in lean women in the regulation of glucose metabolism (18) and reducing the incidence of GDM (16). Another study with *L. rhamnosus* HN001 detected a lowered incidence of GDM in older women with a history of GDM (27). Instead, *L. salivarius* given to obese women did not affect glucose metabolism (28). Similarly to our study, these interventions took place from the first or early second trimester onwards. It is possible that different probiotic strains, their combination, or the timing and duration of the intervention may play an important role in the efficacy of probiotic interventions on glucose control. Further, the volunteering women were likely to be motivated and thus may not be representative of a general population of pregnant women.

In patients with GDM, several studies, mainly conducted on Asian populations, have reported benefits on glucose metabolism with a range of different probiotics (29–31), although there are also some trials detecting no benefits (32,33). There are recent findings for disturbances in the composition of gut microbiota in women who subsequently develop GDM or who already have GDM (34,35), although the evidence is ambiguous (36). These results suggest that manipulation of the gut microbiome may be one way to influence metabolism during pregnancy.

Based on our study, it seems that the administration of n-3 LC-PUFA and/or these two strains of probiotics was not beneficial with regard to the maternal risk of GDM and glucose regulation. In the light of the previous literature, the reasons why we failed to detect an intervention effect remain unknown. It may be that the metabolic burden of obesity in our recruited women was so severe that it could not be overcome by the potential interventional effect in regulating glucose metabolism, as also found by Callaway et al. (37). Indeed, our previous study demonstrated more pronounced changes in both microbiota and metabolic profile with increasing BMI (38). Additionally, in the previous trial, the lower threshold for fasting glucose (4.8 mmol/L) allowed a GDM diagnosis for women who would nowadays be considered healthy (16). Thus, it could be possible that probiotics are efficient in the prevention of GDM in lean women with mild glucose intolerance.

Fish oil and probiotics have been suggested to act through a range of mechanisms to exert beneficial effects on the health of the offspring (26,39,40). With regard to these potential benefits and the fact that these supplements are already widely used by pregnant women, it is worthwhile emphasizing the significance of our finding on safety related to pregnancy outcomes as well as the absence of major adverse effects in this well-conducted, randomized placebo-controlled trial. However, according to our results, these supplements do not appear to be useful in reducing the risk of GDM in overweight and obese women.

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References

1. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract 2014; 103:137–149

2. Metzger BE, Lowe LP, Dyer AR, et al.; HAPO Study Cooperative Research Group. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358:1991–2002

3. Hod M, Kapur A, Sacks DA, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on gestational diabetes mellitus: a pragmatic guide for diagnosis, management, and care. Int J Gynaecol Obstet 2015; 131(Suppl. 3):S173–S211

4. Shepherd E, Gomersall JC, Tieu J, Han S, Crowther CA, Middleton P. Combined diet and exercise interventions for preventing gestational diabetes mellitus. Cochrane Database Syst Rev 2017;11:CD010443

5. Rogozińska E, Marlin N, Jackson L, et al. Effects of antenatal diet and physical activity on maternal and fetal outcomes: individual patient data meta-analysis and health economic evaluation. Health Technol Assess 2017;21:1–158

6. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction 2010;140: 365–371
7. Buchanan TA. Pancreatic B-cell defects in gestational diabetes: implications for the pathogenesis and prevention of type 2 diabetes. J Clin Endocrinol Metab 2001;86:989–993
8. Buchanan TA, Xiang AH. Gestational diabetes mellitus. J Clin Invest 2005;115:485–491
9. Abell SK, De Courten B, Boyle JA, Teede HJ. Inflammatory and other biomarkers: role in pathophysiology and prediction of gestational diabetes mellitus. Int J Mol Sci 2015;16:13442–13473
10. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans 2017;45:1105–1115
11. Gomes AC, Bueno AA, de Souza RG, Mota JF. Gut microbiota, probiotics and diabetes. Nutr J 2014;13:60
12. Lalia AZ, Lanza IR. Insulin-sensitizing effects of omega-3 fatty acids: lost in translation? Nutrients 2016;8:pii:E329
13. Kastel R, Bomba A, Herich R, Vasko L, Svedova M. Effect on the immune system of germ-free piglets of probiotics potentiated with polyunsaturated fatty acids. Berl Munch Tierarztl Wochenschr 2007;120:221–225
14. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004; 27:1487–1495
15. Working group established by the Finnish Medical Society Duodecim, the Medical Advisory Board of the Finnish Diabetes Association and the Finnish Gynecological Association. Gestational diabetes: current care guidelines [Internet]. Helsinki, Germany, The Finnish Medical Society Duodecim, 2013. Available from www.kaypahoito.fi. Accessed 8 December 2018
16. Luoto R, Laitinen K, Nermes M, Isolauri E. Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. Br J Nutr 2010;103:1792–1799
17. Chu SY, Callaghan WM, Kim SY, et al. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care 2007;30:2070–2076
18. Laitinen K, Poussa T, Isolauri E; Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota Group. Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. Br J Nutr 2009;101:1679–1687
19. Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. Mediators Inflamm 2014;2014: 348959
20. Mokkala K, Röytiö H, Ekblad U, Laitinen K. Opportunities for probiotics and polyunsaturated fatty acids to improve metabolic health of overweight pregnant women. Benef Microbes 2017;8:3–15
21. Uusitupa MI, Stancáková A, Peltonen M, et al. Impact of positive family history and genetic risk variants on the incidence of diabetes: the Finnish Diabetes Prevention Study. Diabetes Care 2011;34:418–423
22. Zhou SJ, Yelland L, McPhee AJ, Quinlivan J, Gibson RA, Makrides M. Fish-oil supplementation in pregnancy does not reduce the risk of gestational diabetes or preeclampsia. Am J Clin Nutr 2012;95:1378–1384
23. Chen B, Ji X, Zhang L, Hou Z, Li C, Tong Y. Fish oil supplementation does not reduce risks of gestational diabetes mellitus, pregnancy-induced hypertension, or pre-eclampsia: a meta-analysis of randomized controlled trials. Med Sci Monit 2015;21:2322–2330
24. Gao L, Lin L, Shan N, et al. The impact of omega-3 fatty acid supplementation on glycemic control in patients with gestational diabetes: a systematic review and meta-analysis of randomized controlled studies. J Matern Fetal Neonatal Med 2018:1–7
25. Chen C, Yu X, Shao S. Effects of omega-3 fatty acid supplementation on glucose control and lipid levels in type 2 diabetes: a meta-analysis. PLoS One 2015;10:e0139565
26. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018;11:CD003402
27. Wickens KL, Barthow CA, Murphy R, et al. Early pregnancy probiotic supplementation with *Lactobacillus rhamnosus* HN001 may reduce the prevalence of gestational diabetes mellitus: a randomised controlled trial. Br J Nutr 2017; 117:804–813
28. Lindsay KL, Kennelly M, Culliton M, et al. Probiotics in obese pregnancy do not reduce maternal fasting glucose: a double-blind, placebo-controlled, randomized trial (Probiotics in Pregnancy Study). Am J Clin Nutr 2014;99:1432–1439
29. Kijmanawat A, Panburana P, Reutrakul S, Tangshewinsirikul C. Effects of probiotic supplements on insulin resistance in gestational diabetes mellitus: a double-blind randomized controlled trial. J Diabetes Investig 2019;10: 163–170
30. Karamali M, Dadkhah F, Sadrkhanlou M, et al. Effects of probiotic supplementation on glycaemic control and lipid profiles in gestational diabetes: a randomized, double-blind, placebo-controlled trial. Diabetes Metab 2016;42:234–241
31. Dolatkhah N, Hajifaraji M, Abbasalizadeh F, Aghamohammadzadeh N, Mehrabi Y, Abbasi MM. Is there a value for probiotic supplements in gestational diabetes mellitus? A randomized clinical trial. J Health Popul Nutr 2015; 33:25
32. Jafarnejad S, Saremi S, Jafarnejad F, Arab A. Effects of a multispecies probiotic mixture on glycemic control and inflammatory status in women with gestational diabetes: a randomized controlled clinical trial. J Nutr Metab 2016;2016: 5190846
33. Lindsay KL, Brennan L, Kennelly MA, et al. Impact of probiotics in women with gestational diabetes mellitus on metabolic health: a randomized controlled trial. Am J Obstet Gynecol 2015; 212:496.e1–496.e11
34. Mokkala K, Houttu N, Vahlberg T, Munukka E, Rönnemaa T, Laitinen K. Gut microbiota aberrations precede diagnosis of gestational diabetes mellitus. Acta Diabetol 2017;54:1147–1149
35. Crusell MKW, Hansen TH, Nielsen T, et al. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. Microbiome 2018;6:89–108
36. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470–480
37. Callaway LK, McIntyre HD, Barrett HL, et al. Probiotics for the prevention of gestational diabetes mellitus in overweight and obese women: findings from the SPRING double-blind randomized controlled trial. Diabetes Care 2019;42:364–371
38. Houttu N, Mokkala K, Laitinen K. Overweight and obesity status in pregnant women are related to intestinal microbiota and serum metabolic and inflammatory profiles. Clin Nutr 2018; 37(6 Pt A):1955–1966
39. Ostadrahimi A, Salehi-Pourmehr H, Mohammad-Alizadeh-Charandabi S, Heidarabady S, Farshbaf-Khalili A. The effect of perinatal fish oil supplementation on neurodevelopment and growth of infants: a randomized controlled trial. Eur J Nutr 2018;57:2387–2397
40. Garcia-Larsen V, Ierodiakonou D, Jarrold K, et al. Diet during pregnancy and infancy and risk of allergic or autoimmune disease: a systematic review and meta-analysis. PLoS Med 2018;15: e1002507