

Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial

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Balanced glucose metabolism ensures optimal fetal growth with long-term health implications conferred on both mother and child. We examined whether supplementation of probiotics with dietary counselling affects glucose metabolism in normoglycaemic pregnant women. At the first trimester of pregnancy 256 women were randomised to receive nutrition counselling to modify dietary intake according to current recommendations or as controls; the dietary intervention group was further randomised to receive probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12; diet/probiotics) or placebo (diet/placebo) in a double-blind manner, whilst the control group received placebo (control/placebo). Blood glucose concentrations were lowest in the diet/probiotics group during pregnancy (baseline-adjusted means 4.45, 4.60 and 4.56 mmol/l in diet/probiotics, diet/placebo and control/placebo, respectively; $P=0.025$) and over the 12 months' postpartum period (baseline-adjusted means 4.87, 5.01 and 5.02 mmol/l; $P=0.025$). Better glucose tolerance in the diet/probiotics group was confirmed by a reduced risk of elevated glucose concentration compared with the control/placebo group (OR 0.31 (95% CI 0.12, 0.78); $P=0.013$) as well as by the lowest insulin concentration (adjusted means 7.55, 9.32 and 9.27 mU/l; $P=0.032$) and homeostasis model assessment (adjusted means 1.49, 1.90 and 1.88; $P=0.028$) and the highest quantitative insulin sensitivity check index (adjusted means 0.37, 0.35 and 0.35; $P=0.028$) during the last trimester of pregnancy. The effects observed extended over the 12-month postpartum period. The present study demonstrated that improved blood glucose control can be achieved by dietary counselling with probiotics even in a normoglycaemic population and thus may provide potential novel means for the prophylactic and therapeutic management of glucose disorders.

Probiotics: Dietary counselling: Glucose metabolism: Pregnancy

Early pregnancy is characterised by normal tolerance to glucose and insulin. In late pregnancy, in contrast, an increase is observed in the serum insulin concentration accompanied by insulin resistance. These metabolic adaptations aim to promote fetal growth by shunting metabolic fuels to the fetus instead of the mother, as well as preparation for breast-feeding. In some pregnant women these adaptive processes are exaggerated and lead to impaired glucose tolerance. Such individuals are predisposed to gestational diabetes mellitus and consequently to type 2 diabetes⁽¹⁾. In the case of the child, impaired maternal glycaemia predisposes toward macrosomia⁽²⁾ and impaired glucose tolerance⁽³⁾, which may develop even when maternal estimates are within normal reference ranges, i.e. not classified as gestational diabetes mellitus⁽⁴⁾. A higher-than-optimal glucose level, now acknowledged to be more common than anticipated, may involve long-term effects not only on the mother but also on the child^(5–7). Indeed, the effects on the

infant of maternal nutrition during pregnancy may initiate a cascade of metabolic and immunoinflammatory conditions manifested in later life.

We chose a combined dietary counselling and probiotic intervention to target maternal glucose metabolism, in view of the importance to maintain normoglycaemia throughout pregnancy. Previous dietary interventions with primarily reduced energy and fat intakes as well as increased fibre intakes have resulted in improved glucose tolerance test results⁽⁸⁾. Recent experimental evidence, on the other hand, points to a role for the gut microbiota composition in the harvesting and storage of nutrients^(9–11). The approach may also be justified by the demonstration that diet and microbiota may exert their effects via similar signalling pathways in regulating immune responses^(12–14). Immunoinflammatory processes and prevailing systemic low-grade inflammation may contribute to the metabolic conditions affecting glucose metabolism⁽¹⁵⁾. In a randomised clinical trial from early

Abbreviations: HOMA, homeostasis model assessment; QUICKI, quantitative insulin sensitivity check index.

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pregnancy, 256 women were allocated to three groups: modification of dietary intake according to current recommendations with probiotics or placebo and a control group receiving placebo only. The women were followed clinically and their glucose metabolism repeatedly evaluated from early pregnancy up to 12 months postpartum.

Methods

Participants

Altogether 256 pregnant women were recruited to participate in a randomised, prospective, parallel-group, combined dietary counselling and probiotics intervention study from April 2002 to November 2005 (NCT00167700; section 3, <http://www.clinicaltrials.gov>). The overall aims of the study were to optimise maternal dietary intake and metabolism to advance maternal health and thus to reduce the risk of disease in the child. The present report explores the impact of intervention on maternal nutrition with the main focus on glucose metabolism. The subjects were informed about the study by leaflets distributed during their first visit to maternal welfare clinics in

the city of Turku and neighbouring areas in South-West Finland. Interested recipients contacted the research nurse, who gave further information on the study and scheduled their first visit to the study clinic in Turku University Central Hospital. Women were eligible for participation if they were at less than 17 weeks' gestation and had no metabolic or chronic diseases such as diabetes. The study complies with the Declaration of Helsinki as revised in 2000. Written informed consent was obtained from the participants and the study protocol was approved by the Ethics Committee of the Hospital District of South-West Finland.

Intervention and study conduct

Study visits took place three times during pregnancy (at 13.9 (SD 1.6), 23.8 (SD 1.4) and 33.9 (SD 1.4) weeks of gestation) and at 1, 6 and 12 months postpartum. At baseline, subjects were randomly assigned to three study groups (Fig. 1) according to computer-generated block randomisation of six women: dietary counselling with probiotic capsules (diet/probiotics); dietary counselling with placebo (diet/placebo); controls (control/placebo). The randomisation list was generated by a

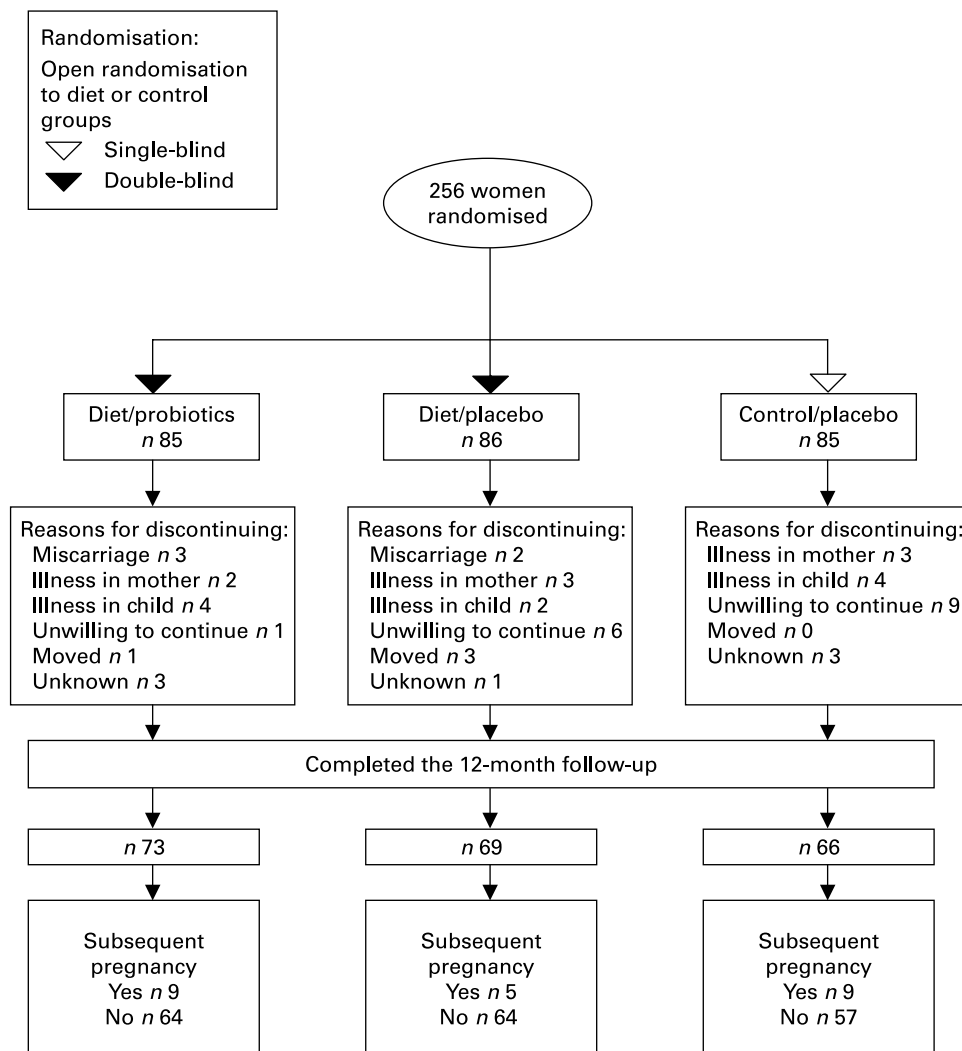


Fig. 1. Flow chart of the study.

statistician (T. P.) who was not involved in recruitment or study visits. Sealed envelopes contained subject numbers corresponding to numbered probiotics and placebo containers and information on whether the subject would receive dietary counselling. All pregnant women participating in the study also attended communal well-women clinics.

At the first study visit the envelopes were opened by the research nurse and nutritionist in the presence of each study subject in their order of recruitment. The random allocation sequence was thus concealed until interventions were assigned. Research nurses and researchers ensured that capsules with corresponding numbers were given to the subjects and that appropriate dietary counselling intervention was carried out. The capsule containers were numbered according to the randomisation list by a member of the research group not involved with the conduct or reporting of the study. The trial data were collected on printed case record forms and the members of the research group performed data entry. All data were kept confidential.

Randomisation to receive probiotics (*Lactobacillus rhamnosus* GG, ATCC 53 103, Valio Ltd, Helsinki, Finland and *Bifidobacterium lactis* Bb12, Chr. Hansen, Hoersholm, Denmark, 10^{10} colony-forming units/d each) or placebo (microcrystalline cellulose and dextrose anhydrate; Chr. Hansen, Hoersholm, Denmark) in the dietary counselling groups took place in a double-blind manner, while the control group received placebo in single-blind manner (Fig. 1). The choice of the probiotic combination was based on *in vitro* results⁽¹⁶⁾, and previous clinical intervention studies suggesting that *L. rhamnosus* GG promotes a bifidogenic microbiota and bifidobacteria dominate the microbiota of healthy breast-fed infants^(17,18), with high bifidobacteria levels linked to the risk of allergy⁽¹⁹⁾. Probiotics and placebo capsules and contents looked, smelled and tasted identical. Dosing with standard content capsules commenced at the first study visit and lasted until the end of exclusive breast-feeding. All capsules were stored at +5°C and the viability of the probiotic capsules was confirmed by regular analysis of blind in the laboratory under Professor S. Salminen. Compliance in consumption of study capsules was assessed by interview.

Dietary counselling given by a dietitian at each study visit aimed to modify dietary intake to conform with that currently recommended^(20,21), particular attention being paid to the quality of dietary fat. Achievement of the recommended diet was supported by providing participants with readily available food products of favourable fat composition (for example, rapeseed oil-based spreads and salad dressing) to be consumed at home. The counselling and food products provided have been described in detail elsewhere⁽²²⁾. Dietary intake was assessed at each trimester using 3 d food diaries. Energy and nutrient intakes were calculated with a Micro-Nutrica® computerised program (version 2.5; Research Centre of the Social Insurance Institution, Turku, Finland).

At baseline, background information concerning education and parity was collected by interview. Total gestational weight gain was calculated by subtracting self-reported pre-pregnancy weight from that recorded at a prenatal visit or at hospital within 1 week before delivery. Information regarding children's birth weights and heights and the course of pregnancy was obtained from hospital records. On the morning of each visit, a 10h overnight fasting blood sample was

drawn from the antecubital vein for the analysis of glucose and glycated Hb A_{1C} on the day of sampling, whilst serum was stored at -70°C for the group analysis of insulin.

Analytical methods

On the day of sampling, plasma glucose concentration was measured by an enzymic method utilising hexokinase in a Modular P800 automatic analyser (Roche Diagnostics GmbH, Mannheim, Germany) and blood glycated Hb A_{1C} was measured by ion-exchange HPLC by the Bio-Rad Variant™ II Haemoglobin A_{1C} Program (Bio-Rad Laboratories, Marnes-la-Coquette, France). Serum insulin concentration was measured by immunoelectrochemiluminometric assay in a Modular E170 automatic analyser (Roche Diagnostics GmbH). To evaluate insulin sensitivity, the quantitative insulin sensitivity check index (QUICKI) was calculated as described by Katz *et al.*⁽²³⁾. Homeostasis model assessment (HOMA) was calculated using a formula devised by Matthews *et al.*⁽²⁴⁾. All personnel who handled or analysed blood samples were blind to the intervention. Glucose challenge screening tests were taken from those performed in well-women clinics at 26 to 28 weeks of gestation according to standard procedures for women fulfilling the criteria for at-risk pregnancy: pre-pregnancy BMI over 25 kg/m²; age over 40 years; gestational diabetes mellitus in a previous pregnancy; previous delivery of a child weighing more than 4500 g; detection of glucose in the urine or suspicion of a macrosomic fetus in the present pregnancy.

Plasma glucose concentrations above 4.8 mmol/l during pregnancy and 5.6 mmol/l in the non-pregnant state, the percentage of glycated Hb in total Hb above 6.5% and a serum insulin concentration above 26 mU/l were considered raised. Results of the glucose challenge tests were considered pathological if an increased fasting glucose value (4.8 mmol/l) was combined with at least one abnormal post-glucose measurement (blood glucose >10.0 mmol/l at 1 h or >8.7 mmol/l at 2 h) according to reference values in Turku University Central Laboratories. A higher QUICKI and lower HOMA were taken to indicate better insulin sensitivity in comparison of differences amongst the groups.

Outcome measures

The primary outcome measure to explore the effects of intervention on the mother was glucose metabolism, characterised by plasma glucose concentration, blood glycated Hb A_{1C}, serum insulin and HOMA and QUICKI indices. The measurements were made at the first trimester (baseline) and third trimester of pregnancy, and at 1, 6 and 12 months postpartum, the primary time points being the third trimester of pregnancy and 12 months postpartum. Secondary outcomes were dietary energy-yielding nutrients assessed from food diaries, which were analysed to explain changes in glucose metabolism.

Statistical analyses

Data were analysed with SPSS (version 14.0; SPSS Inc., Chicago, IL, USA) by a statistician (T. P.) independent of clinical evaluations. The primary sample size calculations were based on infant sensitisation assessed by skin prick

testing at the age of 12 months. We estimated that the required sample size for analyses of glucose metabolism was sixty-six per group to detect a difference in blood glucose of 0.20 mmol/l between groups statistically significant with a 0.05 two-sided significance level and 90% power. We assumed the common SD to be 0.35. Thus the same fixed sample size ensured that the power was sufficient also for analyses of glucose metabolism.

The baseline and clinical variables were analysed using the χ^2 test, ANOVA or the Kruskal–Wallis test (5 min Apgar). Missing values for glucose metabolism (at most one during pregnancy and one during the postpartum period) were computed using the group mean or geometric mean, as linear extrapolation or interpolation methods were not appropriate due to the substantial inherent non-linear within-subject fluctuation. Serum insulin and HOMA were skewed to the right and were logarithmically transformed before analysis.

Comparison of glucose metabolism between the three study groups at the third trimester of pregnancy or at 12 months postpartum was made by analysis of covariance (ANCOVA) and that in the postpartum period (1, 6 and 12 months) was analysed using ANCOVA for repeated measurements. In both cases the baseline was included as a continuous covariate. The results are given as baseline-adjusted means or geometric means with 95% CI or standard deviations. Paired group comparisons were Bonferroni-adjusted. The proportions of subjects with elevated glucose concentrations (≥ 4.8 mmol/l during pregnancy, ≥ 5.6 mmol/l postpartum) were compared between the study groups using the χ^2 test. Results of group comparisons are given as OR with 95% CI. For dietary intake the study groups were compared at the third trimester, during the postpartum period and at 12 months postpartum using the same methods as described for glucose metabolism.

The analyses were based on the intention-to-treat population, apart from the twenty-three women who were pregnant again by the end of the follow-up of 12 months and were excluded, a new pregnancy being considered to be a strong confounder.

Results

The participants (Table 1) were Caucasian. The majority had higher college or university education (79% in the diet/probiotics, 69% in the diet/placebo and 79% in the control/placebo groups; $P=0.210$) and were expecting their first child (65% in the diet/probiotics, 51% in the diet/placebo and 57% in the control/placebo groups; $P=0.197$). The infants were delivered at term and their mean heights and weights were within population reference ranges (Table 1). The mean duration of exclusive breast-feeding and thus the duration of the probiotics/placebo intervention did not differ amongst the study groups, nor did the groups diverge with regard to pregnancy weight gain. Of the total participating women, 99.5% (216 out of 217), 99% (209 out of 212) and 95% (195 out of 205) at the second, third and fourth study visit, respectively, reported without significant difference between groups that they consumed the capsules regularly daily. An adverse event was not the reason for non-compliance in any case. On initiation of capsule consumption 7% (five out of seventy-three) of the women in the diet/probiotics group, 8% (six out of seventy-five) in the diet/placebo group, 3% (two out of sixty-nine) in the control/placebo group and 6% (thirteen out of 217) in all three groups together reported gut-associated adverse events including flatulence, loose stools or constipation. Subsequently the prevalence of reported symptoms was reduced to 2% and 0.5% at subsequent study visits.

Of the recruited women, 81% (208 out of 256) were followed up till 12 months postpartum (Fig. 1). The reasons for discontinuing were representative of a normal population of pregnant women. Additionally, twenty-three women were pregnant again by the end of the follow-up and were excluded from the postpartum analysis.

Although energy intakes did not differ amongst the groups, dietary counselling resulted in changes in the intakes of energy-yielding nutrients compared with controls (Table 2). Particularly the intakes of MUFA and PUFA as a proportion of energy intake were highest in the diet/placebo group; thus, the intake of SFA as a proportion of energy intake was lowest

Table 1. Characteristics of the women and their infants (Mean values and standard deviations)

Group...	Diet/probiotics		Diet/placebo		Control/placebo		<i>P</i> *
	Mean	SD	Mean	SD	Mean	SD	
Women							
Subjects (<i>n</i>)	85		86		85		
Age (years)	29.7	4.1	30.1	5.2	30.2	5.0	0.813
Weight gain over pregnancy (kg)	15.0	4.3	14.8	5.1	14.8	5.1	0.946
Duration of exclusive breast-feeding (months)	3.3	1.8	3.6	1.9	3.4	1.6	0.587
Duration of total breast-feeding (months)	7.6	4.3	9.1	5.8	8.3	4.5	0.287
Infants							
Subjects (<i>n</i>)	75–81		76–79		76–78		
Birth at weeks of gestation	39.9	1.3	39.9	1.8	40.1	1.3	0.672
Birth weight (g)	3489	431	3602	439	3600	515	0.209
Birth height (cm)	51	2	51	2	51	2	0.197
Head circumference (cm)	34.8	1.3	35.1	1.3	35.1	1.4	0.257
Apgar at 5 min							
Median	9		9		9		0.280†
Range	6–10		3–10		4–10		

* ANOVA.

† Kruskal–Wallis test.

Table 2. Daily intake of energy and energy-yielding nutrients and dietary fibre at first trimester (baseline) and third trimester of pregnancy, during the postpartum period (mean of 1, 6 and 12 months) and at 12 months postpartum in the study groups* (Mean values and standard deviations or baseline-adjusted means and 95 % confidence intervals)

Group...	Diet/probiotics (n 75)		Diet/placebo (n 81)		Control/placebo (n 76)		ANCOVA P†	Group comparisons P‡
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI		
Energy(kJ)								
First trimester of pregnancy								
Mean	8196		8263		8042			
sd	1774		1736		1874			
Third trimester of pregnancy	8531	8155, 8904	8255	7883, 8627	8427	8046, 8807	0.586	
Postpartum period§	7878	7523, 8234	7929	7581, 8280	8083	7715, 8452	0.718	
Postpartum at 12 months	7217	6795, 7640	7431	7012, 7845	7481	7046, 7916	0.659	
Carbohydrates (% of energy)								
First trimester of pregnancy								
Mean	50.2		50.9		49.8			
sd	4.9		5.7		6.1			
Third trimester of pregnancy	53.2	51.9, 54.5	51.3	50.0, 52.5	51.0	49.7, 52.3	0.036	0.056; 0.111; 1.000
Postpartum period§	48.8	47.8, 49.9	48.3	47.3, 49.4	48.3	47.3, 49.4	0.760	
Postpartum at 12 months	48.9	47.4, 50.4	47.7	46.2, 49.1	47.8	46.3, 49.4	0.441	
Protein (% of energy)								
First trimester of pregnancy								
Mean	16.9		16.4		16.6			
sd	2.5		2.9		2.7			
Third trimester of pregnancy	15.4	14.8, 16.0	16.9	16.3, 17.4	16.8	16.2, 17.4	0.001	0.004; 0.002; 1.000
Postpartum period§	17.4	16.9, 18.0	17.1	16.6, 17.7	17.3	16.7, 17.9	0.723	
Postpartum at 12 months	17.9	17.1, 18.7	17.7	16.9, 18.5	17.9	17.1, 18.8	0.890	
Fat (% of energy)								
First trimester of pregnancy								
Mean	31.4		31.2		32.3			
sd	4.8		5.6		5.7			
Third trimester of pregnancy	30.0	28.8, 31.3	30.3	29.1, 31.5	30.8	29.5, 32.0	0.698	
Postpartum period§	31.9	30.9, 32.9	32.4	31.5, 33.4	32.4	31.3, 33.4	0.749	
Postpartum at 12 months	31.3	29.8, 32.8	31.8	30.4, 33.3	31.5	30.0, 33.1	0.877	
MUFA (% of energy)								
First trimester of pregnancy								
Mean	10.6		10.3		10.9			
sd	2.6		2.2		2.5			
Third trimester of pregnancy	10.6	10.0, 11.1	10.9	10.3, 11.4	10.1	9.5, 10.7	0.154	
Postpartum period§	11.2	10.8, 11.7	11.6	11.2, 12.0	10.6	10.2, 11.0	0.008	0.124; 0.865; 0.006
Postpartum at 12 months	10.9	10.2, 11.6	11.1	10.4, 11.8	10.4	9.7, 11.1	0.369	
PUFA (% of energy)								
First trimester of pregnancy								
Mean	5.1		5.1		5.3			
sd	1.5		1.8		1.5			
Third trimester of pregnancy	5.7	5.4, 6.1	5.8	5.4, 6.1	5.1	4.8, 5.5	0.030	0.071; 1.000; 0.056
Postpartum period§	5.8	5.5, 6.1	6.1	5.8, 6.3	5.4	5.1, 5.6	0.001	0.081; 0.455; 0.001
Postpartum at 12 months	5.7	5.3, 6.2	5.7	5.3, 6.2	5.3	4.8, 5.7	0.267	
SFA (% of energy)								
First trimester of pregnancy								
Mean	12.9		13.0		13.2			
sd	2.5		3.0		2.9			
Third trimester of pregnancy	11.1	10.5, 11.7	11.0	10.4, 11.6	12.5	11.9, 13.1	0.001	0.003; 1.000; 0.002
Postpartum period§	11.8	11.3, 12.4	11.9	11.4, 12.4	13.2	12.6, 13.7	0.002	0.002; 1.000; 0.004
Postpartum at 12 months	11.7	10.9, 12.4	11.8	11.1, 12.6	12.6	11.9, 13.4	0.162	
Dietary fibre (g)								
First trimester of pregnancy								
Mean	20.2		20.5		18.6			
sd	6.0		6.6		6.3			
Third trimester of pregnancy	22.3	20.9, 23.7	21.7	20.3, 23.1	20.6	19.1, 22.0	0.222	
Postpartum period§	19.1	17.8, 20.3	19.9	18.6, 21.1	18.7	17.4, 20.0	0.409	
Postpartum at 12 months	18.2	16.7, 19.7	18.4	16.9, 19.9	17.6	16.0, 19.1	0.729	

ANCOVA, analysis of covariance.

* Number of subjects in analysis 232 (75 in diet/probiotics, 81 in diet/placebo and 76 in control/placebo) at first trimester of pregnancy, 209 (70, 71 and 68) at third trimester of pregnancy, 203 (68, 71 and 64) during the postpartum period and 173 (58, 60 and 55) at 12 months postpartum.

† Baseline-adjusted univariate ANOVA.

‡ The group comparisons (diet/probiotics v. control/placebo; diet/probiotics v. diet/placebo; diet/placebo v. control/placebo) are given Bonferroni-corrected.

§ Baseline-adjusted mean of measurements at 1, 6 and 12 months.

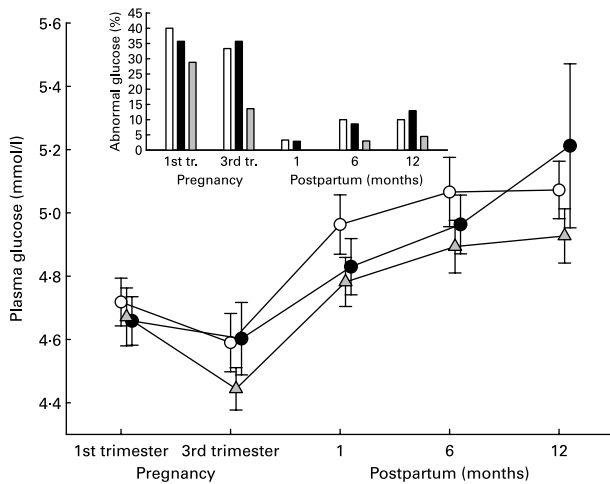


Fig. 2. Plasma glucose concentrations (mmol/l) during and after pregnancy in diet/probiotics (n 66; Δ), diet/placebo (n 70; \bullet) and control/placebo (n 60; \square) groups. Values are means, with 95% CI represented by vertical bars. There were significant differences amongst the groups at the third trimester of pregnancy ($P=0.025$), 12 months postpartum ($P=0.060$) and over the postpartum period ($P=0.025$) by analysis of covariance, where the baseline was taken as the continuous covariate. Only women with values for each time-point were included in the analysis. Bonferroni-corrected group comparisons for diet/probiotics *v.* diet/placebo and diet/probiotics *v.* control/placebo were $P=0.026$ and $P=0.165$ for the third trimester, $P=0.054$ and $P=0.878$ for 12 months postpartum, and $P=0.066$ and $P=0.048$ for over the postpartum period, respectively. The inset shows the proportion (%) of abnormal glucose concentrations in the diet/probiotics (\square), diet/placebo (\blacksquare) and control/placebo (\square) study groups. tr., Trimester. There were differences amongst the groups in the third trimester of pregnancy ($P=0.013$), 12 months postpartum ($P=0.189$) and over the postpartum period ($P=0.096$) by logistic regression, where the baseline was taken as a covariate. Only women with values for each time-point are included.

in both intervention groups compared with controls. At the same time the intakes of energy-yielding nutrients among women receiving probiotics (diet/probiotics) and placebo (diet/placebo) did not differ. This allowed comparisons of the effect of the probiotics according to the study design, except for that of the intake of protein as a proportion of energy intake, this being lowest in the diet/probiotics group at the third trimester of pregnancy, but not during the postpartum period.

Impact of the intervention on glucose metabolism

Glucose concentrations decreased from the first trimester to the third and increased during the 12 months' postpartum period in all study groups alike (Fig. 2). The levels were lowest in the diet/probiotics group throughout the follow-up period and thus results are presented adjusted for baseline, i.e. any differences before randomisation cannot explain the outcome. The difference between the study groups was significant during pregnancy, when the baseline-adjusted means were 4.45, 4.60 and 4.56 mmol/l in the diet/probiotics, diet/placebo and control/placebo groups, respectively ($P=0.025$). The same was noted at 12 months after delivery (adjusted means 4.93, 5.22 and 5.06 mmol/l; $P=0.060$) and over the 12-month postpartum period (adjusted means 4.87, 5.01 and 5.02 mmol/l; $P=0.025$). The diet/probiotics group was distinguishable from the diet/placebo group at the third trimester of pregnancy ($P=0.026$), at 12-months postpartum ($P=0.054$) and over the entire postpartum

period ($P=0.066$), and further, from the control/placebo group over the postpartum period ($P=0.048$).

Although in these healthy pregnant women, the mean plasma glucose concentrations were within normal reference ranges in all study groups, the risk of elevated concentrations was reduced in the diet/probiotics group throughout the study period (Fig. 2, inset). During the third trimester, the diet/probiotics intervention (OR 0.31 (95% CI 0.12, 0.78); $P=0.013$), unlike the diet/placebo intervention (OR 1.26 (95% CI 0.59, 2.69); $P=0.553$), had the capacity to reduce the risk of elevated plasma glucose concentrations compared with the control/placebo treatment. In sequence, over the postpartum period the risk of elevated plasma glucose concentrations remained lower in the diet/probiotics group, albeit not statistically significantly (OR 0.46 (95% CI 0.14, 1.50); $P=0.197$), but not in the diet/placebo group (OR 1.55 (95% CI 0.61, 3.95); $P=0.360$), both compared with the control/placebo group.

Altogether 45% of the subjects underwent a glucose challenge test in well-women clinics during pregnancy. The prevalence of pathological test results was lowest in the diet/probiotics group (37% of subjects) compared with the diet/placebo (58%) and control/placebo (57%) groups. However, the relative risk was not statistically significantly lowered (OR 0.44 (95% CI 0.14, 1.38) in the diet/probiotics group and OR 1.03 (95% CI 0.41, 2.61) in the diet/placebo group compared with the control/placebo group).

Glycated Hb A_{1c} remained within normal ranges throughout the study and was comparable amongst the study groups at the third trimester of pregnancy and at 12 months postpartum, but there was a tendency towards lowered glycated Hb A_{1c} in the diet/probiotics group compared with the diet/placebo group over the postpartum period (Table 3).

Impact of the intervention on serum insulin and insulin sensitivity indices

Insulin concentrations as well as insulin resistance, evaluated by the HOMA index, were increased and insulin sensitivity, evaluated by the QUICKI index, was reduced towards the third trimester of pregnancy in all groups. The opposite was noted after delivery, insulin concentration and HOMA index being reduced and QUICKI index increased. Mean serum insulin concentrations, insulin resistance and insulin sensitivity were found to differ amongst the groups throughout the study period (Table 3). This difference, at the third trimester of pregnancy and over the postpartum period, was explained by the lowering effect on serum insulin of the combined dietary and probiotics intervention (diet/probiotics group), which was especially pronounced when compared with controls (control/placebo group). The HOMA index was lowest and QUICKI index highest, indicating improved insulin sensitivity in the diet/probiotics group.

Discussion

Balanced glucose metabolism during pregnancy reduces the risk of pregnancy-related complications⁽²⁵⁾ and confers long-term health benefits on both the mother and the child^(3,26). In the present study, throughout the study period, combined dietary counselling and probiotics intervention yielded consistently improved glucose metabolism and

Table 3. Blood glycated Hb A_{1c} (percentage of total Hb), serum insulin (mU/l) and insulin sensitivity indices homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI) at the first trimester (baseline) and third trimester of pregnancy, during the postpartum period (mean of 1, 6 and 12 months) and at 12 months postpartum in the study groups*
(Mean values and standard deviations or baseline-adjusted means and 95% confidence intervals)

Group...	Diet/probiotics (n 76)		Diet/placebo (n 81)		Control/placebo (n 75)		P†	Group comparisons P‡
	Mean	95% CI	Mean	95% CI	Mean	95% CI		
Glycated Hb A_{1c}								
First trimester of pregnancy								
Mean	5.01		5.03		5.08			
SD	0.24		0.25		0.30			
Third trimester of pregnancy	5.19	5.13, 5.25	5.21	5.16, 5.27	5.21	5.16, 5.27	0.805	
Postpartum period§	5.29	5.24, 5.34	5.36	5.32, 5.41	5.32	5.27, 5.37	0.082	1.000; 0.078
Postpartum at 12 months	5.35	5.29, 5.41	5.41	5.35, 5.46	5.36	5.30, 5.42	0.293	
Insulin 								
First trimester of pregnancy								
Geometric mean	6.27		5.11		5.19			
SD	13.46		4.37		3.47			
Third trimester of pregnancy	7.55	6.68, 8.54	9.32	8.24, 10.54	9.27	8.06, 10.66	0.032	0.095; 0.057
Postpartum period§	4.32	3.81, 4.91	5.10	4.49, 5.79	5.51	4.77, 6.37	0.038	0.042; 0.220
Postpartum at 12 months	5.01	4.31, 5.83	6.37	5.47, 7.41	6.20	5.22, 7.37	0.06	0.211; 0.091
HOMA 								
First trimester of pregnancy								
Geometric mean	1.30		1.05		1.08			
SD	3.93		0.90		0.79			
Third trimester of pregnancy	1.49	1.31, 1.71	1.90	1.66, 2.17	1.88	1.61, 2.18	0.028	0.083; 0.040
Postpartum period§	0.94	0.83, 1.07	1.14	1.00, 1.30	1.22	1.05, 1.41	0.023	0.030; 0.119
Postpartum at 12 months	1.10	0.94, 1.30	1.46	1.24, 1.73	1.39	1.15, 1.67	0.047	0.219; 0.057
QUICKI¶								
First trimester of pregnancy								
Mean	0.38		0.39		0.39			
SD	0.03		0.03		0.03			
Third trimester of pregnancy	0.37	0.36, 0.37	0.35	0.35, 0.36	0.35	0.35, 0.36	0.028	0.103; 0.042
Postpartum period§	0.40	0.39, 0.41	0.39	0.38, 0.40	0.38	0.37, 0.39	0.070	0.067; 0.529
Postpartum at 12 months	0.39	0.38, 0.40	0.37	0.36, 0.38	0.37	0.36, 0.39	0.096	0.291; 0.133

* Number of subjects in analysis 232 (76 in diet/probiotics, 81 in diet/placebo and 75 in control/placebo) at first and third trimesters of pregnancy and 196 (66, 70 and 60) during the postpartum period and at 12 months.

† Baseline-adjusted univariate ANOVA or ANOVA for repeated measurements.

‡ The group comparisons (diet/probiotics v. control/placebo; diet/probiotics v. diet/placebo) are given Bonferroni-corrected.

§ Baseline-adjusted marginal mean or geometric mean, based on repeated measurements at 1, 6 and 12 months.

|| Data are presented as geometric mean and standard deviation or baseline-adjusted geometric mean and 95% CI for insulin and HOMA.

¶ Number of subjects 45 in diet/probiotics group, 44 in diet/placebo group and 34 in control/placebo group.

insulin sensitivity in healthy women, providing the first clinical evidence of an active dialogue between host and microbiota in glucose metabolism. These results in a general normoglycaemic population call for further research in at-risk populations.

Combined dietary counselling and probiotic intervention with *L. rhamnosus* GG and *B. lactis* Bb12 moderated plasma glucose concentrations and afforded glycaemic control in healthy young females during and after pregnancy. Previous evidence regarding the effects of probiotics on glucose metabolism has been limited to experimental studies, specifically in mice with existing alterations in glucose metabolism. A diet enriched with *L. rhamnosus* GG has resulted in improved glucose tolerance test results as well as in reduced blood glycated Hb⁽²⁷⁾ and *L. casei* administration in reduced glucose levels⁽²⁸⁾ in diabetic mice. Likewise the Indian fermented milk product dahi supplemented with *L. acidophilus* and *L. casei*⁽²⁹⁾ or *Lactococcus lactis*⁽³⁰⁾ delayed the disturbance of glucose metabolism in diabetic rats. The present study, indeed, provided the first evidence of consistently improved glucose metabolism in humans. The impact of the intervention extended several months beyond the period of probiotic

consumption, which has also been shown in other clinical studies, for example, the effect of probiotic intervention lasting for up to 7 years in reducing the risk of atopic eczema⁽³¹⁾. This is probably due to a relatively long duration of probiotic consumption, from early pregnancy until the end of exclusive breast-feeding, and occurring in the critical period of the maturing infant, thus inducing an enduring small change in intestinal microbiota composition, sufficient to stimulate the metabolic change observed in blood glucose metabolism.

The microbes may impact on the glucose metabolism by processing dietary polysaccharides, indigestible by human enzymes, adding to the pool of gastrointestinal absorbable glucose⁽⁹⁾. The gut microbiota may also enhance glucose storage in adipose tissue by suppressing the fasting-induced adipocyte factor gene transcription with ensuing enhanced lipoprotein lipase activity⁽⁹⁾. Thus, as a consequence of probiotic consumption, a less saccharolytic microbiota in the gastrointestinal tract may diminish both fermentation of polysaccharides and induction of fasting-induced adipocyte factor gene transcription. In addition to the effects seen as lowered blood glucose levels in our study, it has been recently reported

that differences in the gut microbiota content may predict overweight in children, bifidobacterial content and composition being determinants of normal weight⁽³²⁾. Fermentation of dietary fibre in the gastrointestinal tract is known to be associated with improved glucose metabolism⁽³³⁾, but is not a likely explanatory factor here since dietary intake of fibre did not differ in women having received probiotics or not. Alternatively, the mechanism may be independent of energy harvest and storage as shown by resistance to diet-induced obesity and glucose intolerance^(11,34).

We suggest that the observed pronounced effect of probiotics on glucose metabolism is most probably attributable to their immunoregulatory properties. Probiotics elicit powerful anti-inflammatory capabilities by inhibiting the NF- κ B pathway, which mediates microbial activation of the immune system through toll-like receptors⁽³⁵⁾. Regulation of inflammatory pathways by probiotics may be of particular importance due to the fundamental involvement that inflammation plays in insulin resistance⁽¹⁵⁾. The concomitance of elevated blood glucose concentrations, insulin resistance and dyslipidaemia with activation of inflammation pathways⁽³⁵⁾ is related to an enhanced risk of a range of metabolic disorders, including obesity and CVD^(36,37). Indeed, alterations in gut microbiota composition have recently been documented in obesity, providing a target for probiotic intervention^(10,32,38). Here we need to acknowledge that specific probiotic strains may influence the microbiota composition in a manner favouring lower circulating lipopolysaccharide levels, possibly ensuing via the CD14 receptor⁽³⁹⁾, that associate with lower insulin resistance and blood glucose levels^(40,41). Thus a universal presence of microbes or microbiota *per se*, may not be crucial in determining glucose-regulating effects but rather specific compositions may form the key factors.

Intriguingly, probiotics were shown here to provide a more profound glucose-lowering effect than dietary counselling alone. This notwithstanding, the impact of dietary counselling, focused on fat composition⁽²²⁾, is most probably also afforded by the specific regulatory properties of fats, beyond their nutritional value⁽⁴²⁾. In fact, the microbes and fatty acids engage the same signalling channels through toll-like receptor 4⁽³⁵⁾ and soluble CD14⁽¹⁴⁾ of innate immunity. The innate immune system, again, apart from microbial recognition, has been demonstrated to participate in the regulation of glucose metabolism and insulin resistance⁽³⁵⁾. Furthermore the composition of the gut microbiota has recently been proven instrumental in energy metabolism. High-fat feeding is associated with lower intestinal *Bifidobacterium* content in mice, and increase in *Bifidobacterium* content positively correlated with improved glucose tolerance⁽³⁹⁾. It may be projected that the clinical effects are under the same regulatory mechanisms. Thus the present study calls for the precise characterisation of the mechanisms involved in the combined regulatory properties of probiotics and specific dietary compounds.

Modification of gut microbiota composition by probiotics, thereby altering the intestinal immunological milieu, may be seen as a novel means of attaining regulation of glucose metabolism. This dietary approach would offer a cost-effective tool for both prophylaxis and therapy in the metabolic disorders that constitute the metabolic syndrome. The benefit is expected to be most pronounced during the critical period of human development in view of the programming of later diseases by events in the uterus⁽⁵⁾.

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