Impact of dietary counselling and probiotic intervention on maternal anthropometric measurements during and after pregnancy: A randomized placebo-controlled trial

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Probiotics
Pregnancy
Postpartum
Anthropometric measurements

Abstract

Background & aims: To establish whether probiotic supplemented dietary counselling influences maternal anthropometric measurements during and after pregnancy.

Methods: At the first trimester of pregnancy 256 women were randomly assigned to receive nutrition counselling to modify dietary intake according to current recommendations or as controls; dietary intervention groups were further randomized to receive probiotics Lactobacillus rhamnosus GG (ATCC 53103) and Bifidobacterium lactis (diet/probiotics) or placebo (diet/placebo) capsules in a double-blind manner, whilst the controls received placebo (control/placebo). The intervention lasted until the end of exclusive breastfeeding for up to six months.

Results: The risk of central adiposity defined as waist circumference 80 cm or more was lowered in women in the diet/probiotics group compared with the control/placebo group (OR 0.30, 95%CI 0.11–0.85, p = 0.023 adjusted for baseline BMI), whilst the diet/placebo group did not differ from the controls (OR 1.00, 95% CI 0.38–2.68, p = 0.994) at 6 months postpartum. The number needed to treat (NNT) with diet/probiotics to prevent one woman from developing a waist circumference of 80 cm or more was 4. Healthy eating pattern at 12 months postpartum (p = 0.001) and BMI prior to pregnancy (p < 0.001) were strong determinants of BMI at 12 months postpartum when adjusted for dietary intervention and exercise.

Conclusion: The impact of probiotics-supplemented dietary counselling on central adiposity, may offer a novel means for the prevention and management of obesity.

This trial was registered at clinicaltrials.gov as NCT 00167700, section 3.

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1. Introduction

Balanced maternal nutrition during pregnancy ensures the physiological weight gain of the mother and thus the growth and development of the foetus. Recent evidence supports the programming theory, suggesting an impact of early nutrition on later risk of chronic life-style-related diseases: faster prenatal and postnatal growth has been associated with higher body mass index (BMI) in the child.1 Explanations for the programming effect are being sought in both restrained and excessive dietary intake.

Interestingly, it is less widely acknowledged that nutrition during pregnancy influences maternal health both short- and long-term. An excessive weight gain during pregnancy predisposes to complications in both pregnancy and labour, while also exposing the mother to a heightened risk of obesity2 and consequently obesity-related diseases in future years. Almost half of the female population are currently overweight, the problem often setting in after delivery of the first child.

Current attempts to prevent or manage obesity are based on the control of traditional life-style-related risk factors. Dietary changes such as a low-fat and fibre-rich diet may indeed be useful in weight control.3 Pregnant women are particularly receptive to dietary counselling, as we have previously demonstrated.4 An additional
means of weight control may be sought in modification of the gut microbiota. Recent advances in experimental and human studies suggest that the gut microbiota may be involved in fat accumulation, including excessive harvest and storage of nutrients. Microbiota deviations have been shown to associate with obesity, the differences identified particularly in the proportions of Firmicutes and Bacteroidetes. The normal-weight status in turn has been manifested with higher numbers of bifidobacteria further supporting their immunomodulatory role beneficial for human health. The possible mechanisms for the overweight regulating effect of microbiota arise as follows: the gut microbiota enables hydrolysis of indigestible polysaccharides to easily absorbable monosaccharides, and activation of lipoprotein lipase with consequent excessive storage of liver-derived triglycerides. These processes boost weight gain, which may be counteracted by modification of the gut microbiota by probiotics, with a potential to balance the low-grade inflammation associated with obesity.

The present study targeted maternal diet and microbiota compositions to coordinate maternal and child metabolic, microbiological and immunological programming. In the combined intervention the objective was to establish whether dietary counselling and supplementation with a probiotic combination of L. rhamnosus GG (ATCC53103) and Bifidobacterium lactis initiated in early pregnancy are effective in controlling the weight as well as the body composition of the mother during and after pregnancy.

2. Methods
2.1. Subjects

A cohort of 256 women were recruited to the study in the city of Turku and neighbouring areas in South-West Finland from April 2002 to November 2005. The women were informed of the study by leaflets outlining its aims and requirements, these being distributed during their first visit to a maternal welfare clinic. Interested recipients contacted the research nurse for information and an appointment at the study clinic in Turku University Hospital. Study eligibility required gestation at less than 17 weeks and no metabolic diseases such as diabetes. The study complied 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.

2.2. Study design and intervention

At the first study visit, the baseline, which took place during the first trimester of pregnancy, background information concerning age, smoking, education, parity and pre-pregnancy weight was collected by interview. Subjects were randomly assigned to a prospective, parallel-group nutrition and probiotics intervention study with three groups (NCT00167700; section 3, http://www. clinicaltrials.gov). The randomization (Fig. 1) was conducted and the list generated by a statistician (TP) not involved in recruitment or study visits, according to computer-generated block randomization of six women to receive dietary counselling with probiotic capsules (diet/probiotics) or placebo (diet/placebo) and controls (control/placebo). Randomization to receive probiotics or placebo in the dietary intervention groups was conducted in double-blind manner and to receive placebo in the control group in single-blind manner. Sealed envelopes contained subject numbers corresponding to numbered probiotics and placebo containers and information on whether the subject would receive dietary counselling further to the standard counselling given in well-women clinics nationwide. The capsule containers were numbered according to the randomization list by an assistant not involved in the conduct or reporting of the study. At the first study visit the envelopes were opened by a 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 the appropriate dietary counselling intervention was carried out. The trial data were collected on printed case report forms and the members of the research group performed data entry. All data were kept confidential. The intervention code was not opened to researchers or nurses involved in the study visits. Staff in the hospital and well-women clinics was blinded to the intervention.

After baseline, the subsequent study visits took place at each trimester of pregnancy, and at one, six and 12 months postpartum. At each visit, the intervention groups received dietary counselling, supported by provision of food products for use at home and probiotics or placebo capsules. Food products and capsules were consumed from the first trimester of pregnancy until the end of exclusive breastfeeding, a maximum of 6 months postpartum. All pregnant women participating in the study also attended municipal well-women clinics.

Probiotics were administered as capsule preparations, one capsule per day, containing L. rhamnosus GG (ATCC 53103, Valio Ltd., Helsinki, Finland) and B. lactis 10^10 cfu/d each (B. lactis Bb12, Christian Hansen A/S, Hoersholm, Denmark) and placebo in capsules containing microcrystalline cellulose and dextrose anhydride (Chr. Hansen, Hoersholm, Denmark). Probiotics and placebo capsules and contents looked, smelled and tasted identical. Compliance in consumption was good, more than 95% of the subjects consuming the capsules and tolerating them well, 6% or less at the initiation of capsule consumption and 2% and 0.5% in the subsequent follow-up visits reporting adverse side-effects, mainly gastrointestinal symptoms, as previously reported.

The dietary intervention has been described in detail elsewhere. Briefly, dietary counselling given by a nutritionist aimed to modify dietary intake to conform with dietary recommendations, providing a 55–60 percentage of energy (E%) from carbohydrates,
10–15 E% from protein and 30 E% from fat. Dietary counselling focused especially on the amount and type of fat in the diet. To support the dietary group in achieving the recommendations, the mothers were provided with conventional food products with favourable fat content to be used at home, including spreads and salad dressing. Recommendations on energy intake, in other words the amount of food eaten, were made with reference to weight gain. In cases where excessive weight gain occurred, dietary advice was given on regular meal frequency, smaller portion sizes and reduction in consumption of sweet or savoury delicacies. Concomitant with dietary advice the women were encouraged to undertake regular physical activity and exercise according to their capabilities, making allowance for the stage of pregnancy. The mothers were asked to report the weekly frequency of at least 30-min sessions of exercise causing perspiration and breathlessness.

As previously described,15 on the morning of each visit, a 10-h overnight fasting blood sample was drawn. Plasma glucose concentration was measured by an enzymatic method utilizing hexokinase in a Modular P800 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany), serum insulin concentration by immuno-electrochemiluminescent assay (ECLA) in a Modular E170 automatic analyzer (Roche Diagnostics GmbH), and homeostasis model assessment (HOMA) was calculated using a formula devised by Matthews et al.16

The main hypothesis was that a combined intervention of dietary counselling and supplementation with probiotic L. rhamnosus GG and B. lactis would be effective in preventing excessive weight gain during pregnancy and in controlling the weight as well as the adiposity of the mother during the 12 months’ postpartum period.

2.3. Primary outcomes

The overall aims of the clinical follow-up study were to optimize maternal dietary intake and metabolism to advance maternal health and thus to reduce the risk of disease in the child. The primary outcome measures applied were mothers’ anthropometric measurements, most importantly BMI and adiposity defined as a waist circumference of 80 cm or more, and the proportion of body fat over the 12 months’ postpartum period. Secondary outcomes were dietary intakes of foods and nutrients and a healthy eating index during the postpartum period.

2.4. Anthropometric measurements

Weight was measured at each study visit using a calibrated scale, pre-pregnancy weight being self-reported. Weights and height, measured at the first study visit, were used to calculate BMI as weight (kg) divided by the square of height (m). The women were classified according to pre-pregnancy BMI as normal-weight (BMI ≤ 25.0 kg/m²), overweight (25.0 kg/m² ≤ BMI < 30.0 kg/m²), or obese (BMI ≥ 30.0 kg/m²). Total gestational weight gain was calculated by subtracting self-reported pre-pregnancy weight from the weight recorded at prenatal visit or at the hospital within one week before delivery. Assessment of weight gain in comparison to that recommended for pregnancy was made according to mothers’ pre-pregnancy BMI categories. Weight gain during pregnancy was classified as normal, excessive or low,18 the recommended gain being 12.5–18.0 kg for women with a pre-pregnancy BMI below 19.8, 11.5–16.0 kg for those with a BMI from 19.8 to 26.0, and 7–11.5 kg for those with a pre-pregnancy BMI above 26.0. The skinfold thicknesses at four sites, triceps, biceps, subscapular and suprailiac (Holtain Tanner/Whitehouse skinfold caliper; Marsden Weighing Group, Henley-on-Thames, Oxfordshire, UK) and waist, hip, upper arm and thigh circumferences were measured at each visit, suprailiac skinfold and waist circumference not, however, being measured during pregnancy. The proportion of body fat19 and mid-upper arm muscle circumference (MUAMC) were calculated (mid-upper arm circumference (cm) minus pi times triceps skinfold (cm)). Subjects were divided into two groups with a waist circumference less than 80 cm and 80 cm or more according to the postpartum measurement. Waist circumference exceeding the cutoff-off 80 cm may be taken to reflect central obesity and has been found to be related to an increased risk of Western diseases.

Information on children’s birth weights, heights and head circumferences was obtained from the hospital records.

2.5. Analysis of dietary intake

Dietary intake was assessed at each visit with three-day food diaries, from which daily energy and nutrient intakes were calculated by the Micro-Nutrica computerized program (version 2.5, Research Centre of the Social Insurance Institution, Turku, Finland), which uses the Food and Nutrient Database of the Social Insurance Institution and is continuously updated with data on commercial foods. Also, to describe the quality and health-promoting properties of the diet, a healthy eating index was calculated. This was based on guidelines given by the European Health Monitoring Program,21 in which the selected nutrition indicators for health include the intakes of vegetables, fruits and berries, fish and bread and intakes of saturated fatty acids and salt. The intakes of these foods and nutrients as recorded in the food diaries were scored and summed, a maximum index of 25 representing the best health-promoting diet.

2.6. Statistical analysis

The primary sample size calculations for the main study were based on infant atopic sensitization. At the planning stage of this sub-study of anthropometric primary variables there was no a priori information as to the possible effect of probiotics on the primary variables, or the anticipated prevalence of a waist circumference of 80 cm or more in a population such as ours. Thus, the groups receiving dietary counselling were combined in the sample size calculations, which were based on the assumption of a 2.0 kg/m² reduction in BMI at 12 months postpartum in the dietary counselling group compared to the controls. A decrease in BMI by approximately 2 kg/m² is of significance in terms of the risk of metabolic disorders.22 To detect this difference and assuming a common standard deviation of 3.77 using a two group t-test with a 0.050 two-sided significance level, we estimated that a sample size of 76 subjects in the control group and 76 subjects in the combined dietary intervention group would have 90% power. The available sample size was estimated to be sufficient.

The analysis of variance (ANOVA) and the Chi-squared test were used for baseline clinical characteristics and other factors pertaining to the women and their children. Despite random allocation of the subjects to the study groups there was a difference in weight, the women in the diet/probiotics group having the lowest weight at baseline, the first trimester of pregnancy, mean 64.7 (SD 9.2) kg in the diet/probiotics, 71.0 (13.5) kg in the diet/placebo and 68.5 (11.5) kg in the control/placebo groups; ANOVA p = 0.004. Hence, results for anthropometric measurements and other continuous outcome variables were presented as adjusted for baseline (visit 1) or pre-pregnancy value, when appropriate (ANCOVA or ANCOVA for repeated measures). A strong correlation between self-reported pre-pregnancy weight and measured weight at baseline was detected (r = 0.977, p < 0.001). The results are given as adjusted marginal means (SEM or 95% confidence interval). The pregnancy and postpartum periods were analyzed separately.
No differences were noted in dietary intakes of foods or nutrients between the diet/probiotics and diet/placebo groups, the only divergence in the intervention between the two groups being in the consumption of probiotics or placebo capsules not containing nutrients; hence the results are presented for the combined dietary intervention groups compared to the controls. The healthy eating index was considered an explaining factor for BMI and was divided into quartiles (12 or less, 13–15 and 16 or more) at 6 and 12 months. The association with BMI at 6 and 12 months was analyzed using ANOVA and ANCOVA. Logistic regression analysis was used to assess the effect of the intervention with respect to the prevalence of overweight and the prevalence of a waist circumference of 80 cm or more. For both variables BMI prior to pregnancy, exercise (≥3 times per week) and healthy eating index were included as covariates. The results are given as odds ratios (OR) with 95% confidence intervals. The possible associations between BMI and waist circumference with markers of glucose metabolism were analyzed by Pearson’s correlations. HOMA was skewed to the right and was logarithmically transformed before analysis.

The analyses were based on the intention-to-treat population, apart from the 23 women who were pregnant again and excluded from the postpartum analysis, a new pregnancy being regarded as a strong confounder. Analysis was performed using SPSS (version 15.0, SPSS Inc, Chicago, IL, USA) by a statistician (TP) independent of clinical evaluations.

The association with BMI at 6 and 12 months was analyzed using ANOVA and ANCOVA. Logistic regression analysis was used to assess the effect of the intervention with respect to the prevalence of overweight and the prevalence of a waist circumference of 80 cm or more.

### 3. Results

We demonstrated here that consumption of probiotics combined with dietary counselling from early pregnancy onwards lowers the risk of central adiposity over the 6 months postpartum period. Pre-pregnancy BMI and healthy eating pattern were strong determinants of maternal postpartum BMI.

#### 3.1. Clinical characteristics and dietary intake

Of the recruited women 72% (185/256) were followed up till 12 months postpartum (Fig. 1). Of these, 23 women were then pregnant again and were therefore excluded from the postpartum analysis.

### Table 1

<table>
<thead>
<tr>
<th>Diet/probiotics</th>
<th>Diet/placebo</th>
<th>Control/placebo</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>85</td>
<td>29.7 ± 4.1</td>
<td>86</td>
</tr>
<tr>
<td>Smoker prior to pregnancy</td>
<td>14</td>
<td>16%</td>
<td>18</td>
</tr>
<tr>
<td>College or university education</td>
<td>66</td>
<td>79%</td>
<td>59</td>
</tr>
<tr>
<td>Primipara</td>
<td>55</td>
<td>65%</td>
<td>44</td>
</tr>
<tr>
<td>Gestational length (weeks)</td>
<td>75</td>
<td>39.9 ± 1.3</td>
<td>77</td>
</tr>
<tr>
<td>Duration of breastfeeding (months)</td>
<td>Exclusive</td>
<td>75</td>
<td>3.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>75</td>
<td>5.4 ± 1.8</td>
</tr>
<tr>
<td>Postpartum glucose (mmol/L)</td>
<td>At 6 months</td>
<td>66</td>
<td>4.89 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>At 12 months</td>
<td>66</td>
<td>4.93 ± 0.35</td>
</tr>
<tr>
<td>Postpartum HOMA</td>
<td>At 6 months</td>
<td>45</td>
<td>1.05 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>At 12 months</td>
<td>45</td>
<td>1.16 ± 0.75</td>
</tr>
<tr>
<td>Children at birth</td>
<td>81</td>
<td>3489 ± 431</td>
<td>79</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>81</td>
<td>50.7 ± 1.8</td>
<td>79</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>77</td>
<td>34.8 ± 1.3</td>
<td>79</td>
</tr>
</tbody>
</table>

*ANOVA.

**Chi-squared test.

*Geometric mean ± SD.

At recruitment the subjects had no metabolic or chronic diseases, with the exception of allergic disease, which was not an exclusion criterion. At baseline, the first trimester of pregnancy, the characteristics of the women in each study group were comparable, the majority having college or university education (Table 1). The intervention had no influence on the outcome of pregnancy as evaluated by gestational length, birth characteristics of the children or duration of breastfeeding (Table 1).

The dietary intake data over the 12 months' postpartum period are presented for the combined dietary intervention groups (diet/probiotics and diet/placebo), compared to the controls (control/placebo), since the probiotics or placebo capsule not containing nutrients resulted in no effect in dietary intakes of foods or nutrients between the two intervention groups (data not shown). The impact of the dietary intervention was manifested in lowered intakes of butter and cheese and increased intakes of margarines and vegetable oils (Table 2). Altered food consumption was reflected in the intakes of nutrients, shown as lower intake of saturated fatty acids and higher intakes of monounsaturated and polyunsaturated fatty acids in the dietary intervention groups (Table 3). Also, the intake of vitamin E was higher and the intake of vitamin D tended to be higher in the intervention groups.

To describe the health-promoting properties of the entire diet, a healthy eating index was computed. During pregnancy dietary quality differed amongst the study groups, the healthy eating index being higher in the diet/probiotic, 15.0 (95% CI 14.4–15.6), and diet/placebo groups, 14.8 (95% CI 14.2–15.5) compared to the controls, 13.8 (95% CI 13.2–14.5; p = 0.022 amongst the groups). During the postpartum period the differences amongst the groups were no longer significant; diet/probiotics 13.9 (95% CI 13.1–14.8), diet/placebo 13.7 (95% CI 12.8–14.5) and control/placebo 13.1 (95% CI 12.2–14.0; p = 0.406).

#### 3.2. Impact of the intervention on anthropometric measures

**3.2.1. During pregnancy**

The mean total weight gain over pregnancy was 14.8 (SD 4.8) kg, with no significant differences amongst the study groups; diet/probiotics 14.8 (SD 4.4) kg, diet/placebo 14.7 (SD 5.0) kg and control/placebo 14.8 (SD 5.2) kg; p = 0.981. The proportions of
women falling within the recommended range of weight gain were 49.3% in the diet/probiotics group, 39.7% in the diet/placebo group and 31.4% in the control/placebo group; p = 0.100, with a tendency towards a greater proportion (44.4%) in the combined intervention groups compared to the women in the control/placebo group (31.4%); p = 0.071. The proportion showing a pregnancy weight gain exceeding that recommended was lowest in the diet/probiotics group and highest in the control/placebo group, although the difference was not statistically significant; 31.9% in the diet/probiotics, 45.7% in the diet/placebo and 49.3% in the combined intervention (diet/probiotics and diet/placebo, p = 0.246. Overall, 39.2% of the women showed a weight gain exceeding that recommended during pregnancy. The greatest

**Table 2**

<table>
<thead>
<tr>
<th>Dietary intervention</th>
<th>Control</th>
<th>Dietary intervention vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td>Grain products</td>
<td>231 (222, 241)</td>
<td>236 (221, 250)</td>
</tr>
<tr>
<td>Milk products</td>
<td>494 (463, 526)</td>
<td>485 (438, 531)</td>
</tr>
<tr>
<td>Meat products</td>
<td>104 (96, 112)</td>
<td>104 (93, 115)</td>
</tr>
<tr>
<td>Fish products</td>
<td>27.0 (22.7, 31.2)</td>
<td>26.2 (19.8, 32.6)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>261 (247, 276)</td>
<td>251 (229, 273)</td>
</tr>
<tr>
<td>Fruits</td>
<td>231 (209, 253)</td>
<td>218 (185, 250)</td>
</tr>
<tr>
<td>Butter</td>
<td>2.8 (2.0, 3.6)</td>
<td>5.4 (4.2, 6.6)</td>
</tr>
<tr>
<td>Soft margarine</td>
<td>21.5 (20.0, 23.1)</td>
<td>17.8 (15.5, 20.1)</td>
</tr>
<tr>
<td>Vegetable oils</td>
<td>12.2 (10.9, 13.6)</td>
<td>9.3 (7.4, 11.3)</td>
</tr>
<tr>
<td>Cheese</td>
<td>43 (39, 47)</td>
<td>51 (45, 57)</td>
</tr>
</tbody>
</table>

* ANCOVA, where the baseline intake was included as a continuous covariate.

**Table 3**

<table>
<thead>
<tr>
<th>Energy-yielding nutrients</th>
<th>Dietary intervention Mean (95% CI)</th>
<th>Control Mean (95% CI)</th>
<th>Dietary intervention vs. Control Mean (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates g</td>
<td>230 (222, 237)</td>
<td>234 (222, 246)</td>
<td>5 (19, 10)</td>
<td>0.531</td>
</tr>
<tr>
<td>Protein g</td>
<td>80.6 (77.8, 83.4)</td>
<td>82.3 (78.2, 86.6)</td>
<td>−1.7 (−6.7, 3.3)</td>
<td>0.499</td>
</tr>
<tr>
<td>Fat g</td>
<td>17.3 (16.9, 17.7)</td>
<td>17.3 (16.7, 17.9)</td>
<td>0.0 (−0.7, 0.7)</td>
<td>0.982</td>
</tr>
<tr>
<td>Saturated fatty acids g</td>
<td>68.0 (65.3, 70.8)</td>
<td>69.8 (65.7, 73.8)</td>
<td>−1.7 (−6.6, 3.2)</td>
<td>0.484</td>
</tr>
<tr>
<td>Monounsaturated fatty acids g</td>
<td>25.1 (23.9, 26.3)</td>
<td>26.8 (26.8, 30.4)</td>
<td>−3.4 (−5.6, −1.3)</td>
<td>0.002</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids g</td>
<td>11.5 (11.5, 12.3)</td>
<td>12.6 (12.6, 13.7)</td>
<td>1.2 (0−0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin A (RE) μg</td>
<td>9.2 (8.6, 9.9)</td>
<td>8.2 (7.2, 9.2)</td>
<td>1.0 (−0.2, 2.2)</td>
<td>0.096</td>
</tr>
<tr>
<td>Vitamin D μg</td>
<td>10.3 (9.9, 10.7)</td>
<td>9.0 (8.3, 9.6)</td>
<td>1.3 (0.6, 2.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>Thiamine mg</td>
<td>13.2 (12.5, 14.0)</td>
<td>12.7 (11.6, 13.8)</td>
<td>0.5 (−0.8, 1.8)</td>
<td>0.448</td>
</tr>
<tr>
<td>Riboflavin mg</td>
<td>1.41 (1.35, 1.47)</td>
<td>1.42 (1.33, 1.50)</td>
<td>−0.0 (−0.1, 0.1)</td>
<td>0.953</td>
</tr>
<tr>
<td>Nicotinic acid mg</td>
<td>3.14 (2.83, 3.46)</td>
<td>2.95 (2.48, 3.41)</td>
<td>0.20 (−0.36, 0.76)</td>
<td>0.484</td>
</tr>
<tr>
<td>Folate μg</td>
<td>17.4 (16.6, 18.2)</td>
<td>17.7 (16.5, 18.8)</td>
<td>−0.3 (−1.7, 1.1)</td>
<td>0.658</td>
</tr>
<tr>
<td>Vitamin C mg</td>
<td>24.4 (22.6, 26.1)</td>
<td>23.6 (21.0, 26.2)</td>
<td>0.8 (−2.4, 3.9)</td>
<td>0.623</td>
</tr>
<tr>
<td>Vitamin B₂₁₂ μg</td>
<td>6.43 (5.74, 7.12)</td>
<td>6.42 (5.40, 7.44)</td>
<td>0.01 (−0.12, 2.24)</td>
<td>0.989</td>
</tr>
<tr>
<td>Folic acid μg</td>
<td>8.69 (6.69, 10.46)</td>
<td>7.28 (4.67, 9.89)</td>
<td>1.41 (−1.75, 4.57)</td>
<td>0.379</td>
</tr>
<tr>
<td>Calcium mg</td>
<td>110 (101, 118)</td>
<td>108 (95, 121)</td>
<td>1.6 (−13.9, 17.1)</td>
<td>0.841</td>
</tr>
<tr>
<td>Magnesium mg</td>
<td>143 (132, 155)</td>
<td>132 (116, 149)</td>
<td>11 (−9, 31)</td>
<td>0.266</td>
</tr>
<tr>
<td>Iron mg</td>
<td>1116 (1064, 1168)</td>
<td>1169 (1092, 1245)</td>
<td>−53 (−145, 40)</td>
<td>0.265</td>
</tr>
<tr>
<td>Zinc mg</td>
<td>12.2 (11.7, 12.6)</td>
<td>12.0 (11.3, 12.7)</td>
<td>0.2 (−0.6, 1.0)</td>
<td>0.647</td>
</tr>
<tr>
<td>Total</td>
<td>329 (324, 360)</td>
<td>347 (320, 373)</td>
<td>−4 (−36, 27)</td>
<td>0.784</td>
</tr>
</tbody>
</table>

* ANCOVA, where the baseline intake was included as a continuous covariate.

b Percentage of energy intake.

c RE, retinol equivalent.
proportion with excess weight gain was detected among those with the highest pre-pregnancy BMI class, 26 kg/m² or more, and the proportion being 60.5% compared to 37.4% in the women with a pre-pregnancy BMI from 20 to 25.9 kg/m² and 16.7% in those with a pre-pregnancy BMI less than 20 kg/m². No differences in skinfold thicknesses or circumferences during pregnancy were seen amongst the study groups (Table 4).

3.2.2. Over 12 months postpartum

The weights of the women gradually decreased during the 12 months’ postpartum period in all groups alike (weight means 70.8 kg at 1 mo, 69.0 kg at 6 mo and 67.9 kg at 12 mo, ANOVA time effect \( p < 0.001 \), group effect \( p = 0.191 \), interaction \( p = 0.404 \)). Assessed by the mean BMI at 12 months postpartum the women in all groups were of normal-weight; baseline-adjusted means in diet/probiotics 24.3 (95% CI 23.9–24.7), diet/placebo 24.4 (24.0–24.8), control/placebo 24.5 (24.1–24.8), ANCOVA \( p = 0.843 \). Compared to the situation prior to pregnancy the women’s weights were 2.44 (95% CI 1.76–3.11) kg and BMIs 0.89 (0.65–1.13) units (kg/m²) higher at 12 months postpartum, with no statistical differences amongst the groups (ANOVA group effect \( p = 0.256 \) and \( p = 0.318 \), respectively).

In searching for factors explaining mothers’ weight, the tertiles of the healthy eating index (12 or less, 13–15 and 16 or more) at 6 and 12 months postpartum were shown to be associated with BMI at 12 months postpartum: one unit increase in the index resulted in 0.114 unit (kg/m²) decrease in BMI (Table 5). Thus, the 12 months postpartum BMI was verified by univariate regression analysis the highest index being associated with the lowest BMI (Fig. 2). Taking into account also the intervention and exercise in multivariate analysis, the BMI prior to pregnancy, and the healthy eating index at 12 months postpartum were shown to be strong determinants of the BMI at 12 months postpartum: one unit increase in the index resulted in a 0.114 unit (kg/m²) decrease in BMI (Table 5). Thus, the 12 months postpartum BMI may be explained by the following regression equation: BMI = 1.054 + 0.037 (if dietary intervention) – 0.295 (if exercise 3 or more times per week) – 0.114 × Healthy eating index at 12mo + 1.051 × BMI prior to pregnancy. The plasma glucose concentration and insulin resistance evaluated by the HOMA index (Table 1) correlated with BMI (\( r = 0.191, p = 0.008 \) and \( r = 0.421, p = 0.001 \), respectively) and waist circumference (\( r = 0.204, p = 0.004 \) and \( r = 0.454, p < 0.001 \), respectively) at 6 months postpartum. At 12 months postpartum the correlations were similar in magnitude and significance (data not shown).

3.2.3. Adiposity

The effect of the intervention during the postpartum period was manifested in lowest biceps skinfold thickness, this reflecting subcutaneous adiposity, in the diet/probiotics group (Table 4). There was a trend for percentage of body fat to differ amongst the three study groups both at 6 months (\( p = 0.076 \)) and at 12 months (0.082) postpartum when analyzed as adjusted for baseline BMI. Taking these two postpartum measurements together, the lowest body fat was seen in the diet/probiotics group 28.1% (95% CI 27.3–29.0) and in the diet/placebo group 28.2% (95%CI 27.3–29.1) compared to the control/placebo group 29.5% (95% CI 28.6–30.4; \( p = 0.052 \); adjusted for baseline BMI). The treatment effects were correspondingly −1.3% (95% CI −2.9%–0.1%) and −1.3% (−2.9%–0.2%) for diet/probiotics and diet/placebo.

For the evaluation of central adiposity the waist circumference was measured and found to be lowest in the women in the diet/probiotics group over the postpartum period (Table 4). At 6 months postpartum, the proportions of women with a waist circumference of 80 cm or more differed between the groups, being lowest in women receiving probiotics (26.2% in the diet/probiotics, 48.5% in the diet/placebo and 51.7% in the control group; \( p = 0.032 \)) adjusted for baseline BMI). The risk of large waist circumference was lowered in the diet/probiotics group compared to the control/placebo group (OR 0.30, 95% CI 0.11–0.85, \( p = 0.023 \)) adjusted for baseline BMI). Whilst the diet/placebo group did not differ from the control (OR 1.00, 95% CI 0.38–2.68, \( p = 0.994 \)). The number needed to treat (NNT) with diet/probiotics to prevent one woman from developing a waist circumference of 80 cm or more was 4. The difference remained at 12 months postpartum, albeit not statistically significant \( (p = 0.489 \) adjusted for baseline BMI), the proportions of the women with high waist circumference being 25.4%, 42.9% and 41.1%, respectively.

At 12 months after delivery, 31.3% of the women in the diet/probiotic, 23.8% in the diet/placebo and 26.3% in the control/
Further, the highest quartile (16 or more) of the healthy eating index at 12 months postpartum. Thus excessive weight prior to pregnancy predicted 25% of the cases of obesity. Of the women who were overweight prior to pregnancy (BMI more than 30), neither portion of overweight nor obesity being statistically significantly different amongst the groups (adjusted for pre-pregnancy BMI). Of the women who were overweight prior to pregnancy (BMI 25 or more), 97.9% were overweight at 12 months postpartum. On the other hand, of those who were normal-weight (BMI less than 25) prior to pregnancy, 16.2% had become overweight 12 months after delivery. Thus excessive weight prior to pregnancy predicted overweight at 12 months postpartum (OR 267.3, 95% CI 34.3–2081, \( p < 0.001 \)), with no effect from the intervention (\( p = 0.688 \)). Further, the highest quartile (16 or more) of the healthy eating index (OR 0.26, 95% CI 0.05–1.26, \( p = 0.095 \)) tended to reduce the risk of overweight.

**4. Discussion**

The present results corroborate the conception of the importance for maternal health of nutrition during pregnancy. Specifically, we showed here that the major risk factor for metabolic disorders, adiposity, was lowered by combined dietary counselling and probiotics intervention. The current findings thus extend previous demonstrations in this mother-child project; the intakes of foods and nutrients during pregnancy were modified by dietary counselling, and by concomitant probiotic intervention maternal glucose metabolism was improved. The impact on adiposity, documented here by the approximation of measured proportion of body fat and waist circumference, may be seen in the improved health and well-being of the mother in the long term.

Dietary counselling improved habitual food consumption and dietary intake for up to one year after delivery. The enduring changes in diet as well as the good compliance in attendance at study visits were most likely attributable to recurring contacts with the same study personnel and immediate feedback to the subjects, this furnishing one method of intervention applicable in health care. Indeed, life-style changes are difficult to put into practice unless repeatedly supported but on the other hand even simple advice in the form of leaflets may be productive. The strengths of the present study are further demonstrated in the conduct of anthropometric measurements by the same researchers throughout the study and in the adjustment of the measurements to baseline values, this obviating any influence of baseline group differences in interpretation of postpartum results. The adjustment for weight is particularly important for the interpretation of post-partum circumferences, considering the fact that baseline measurement could not be conducted in pregnant women. Waist circumference measurement is taken to reflect central adiposity and to show correlations with metabolic risk factors. This also being substantiated here, since associations with markers of glucose metabolism (plasma glucose and insulin resistance index HOMA) were detected. Waist circumference has also been shown to be associated with more sophisticated analytical methods to measure central obesity such as dual-energy X-ray absorptiometry. Nevertheless, it remains for future studies to confirm the effect of probiotics in reducing central adiposity, particularly in at-risk populations. In addition to the measurement techniques, one further potential source of bias in measuring waist circumference is the effect of probiotics in reducing gastrointestinal distension shown in patients with irritable bowel syndrome. However, this is an unlikely explanation here, since the subjects were healthy, with a low prevalence of gut-associated symptoms occurring only at the initiation of the intervention in all study groups alike.

Further efforts were made to describe the health-promoting properties of the diet in its entirety by applying a healthy eating index, another approach applicable in clinical practice. Indeed, the healthy eating index proved to be associated with BMI in that the highest quartile of the index resulted in a decrease in BMI by about 2 kg/m². This is of significant magnitude considering the risk of metabolic disorders, an example being to hand in hypertension, for which an increase in BMI per 1 kg/m² is related to a 13% increase in the risk of 5-year hypertension incidence in apparently healthy adults. An improvement in dietary quality most likely also corrects energy intake. In our intervention, focusing on a whole-some diet, women tended to show pregnancy weight gain within the recommended range. The challenge of preventing excessive pregnancy weight gain even by focused counselling on diet and physical activity has been previously demonstrated. For post-partum weight, no effect of the intervention was detected, but instead a strong determinant of weight proved to be pre-pregnancy overweight. This thus involves the women who will most likely benefit from dietary counselling, for which further support may be sought in the beneficial effects of probiotics.

In the light of the limited achievement by control of traditional risk factors, diet and physical activity, and the poor compliance in lifestyle interventions, new solutions are indeed urgently called for. In the present study such an approach was taken in the administration of probiotics. Importantly, probiotics administered during pregnancy and lactation are safe for both mother and child, as normal growth and development with no side-effects have been
demonstrated in healthy individuals. Of particular note, dietary counselling when combined with probiotics *L. rhamnosus* GG and *B. lactis* reduced central abdominal adiposity, the key factor predisposing to metabolic disorders, including diabetes and cardiovascular disease, in women during the postpartum period. To the best of our knowledge, this study represents the first trial undertaken of a probiotic intervention impacting central adiposity in a group of women during and after pregnancy. The mechanisms involved may be sought in the metabolic effects accredited to specific probiotic strains in the gut microbiota.

The instrumental role of the gut microbiota in energy homeostasis has been constructed by a series of experimental studies firstly reporting distinctive compositional differences in obesity both in mice and in humans. Faecal microbial counts have also been shown to alter with weight gain and again with weight loss. The faecal microbiota composition at an early age, during a critical period of maturation, may even predict the development of overweight. Secondarily, adiposity control by the gut microbiota may be attributed to the particular bacterial composition which contributes to energy harvest from the diet by enabling hydrolysis of indigestible polysaccharides, this influencing fat absorption and use and accumulation of the harvested energy by suppression of fasting-induced adipocyte factor with consequent activation of lipoprotein lipase. A recent study in mice has demonstrated that changes in the gut microbiota induced by antibiotic treatment result in a loss of visceral and subcutaneous adipose tissue weights but in no change in body weight itself. Alternatively, it has also been proposed that an effect on weight control is attained due to dietary composition, whereby high-fat feeding results in a rise in the proportion of lipopolysaccharide-containing microbiota in the gut and consequently in increased plasma lipopolysaccharides, the condition defined as metabolic endotoxemia. One mechanism involved here may be via increased intestinal permeability and its regulation by the administration of probiotics. The best documented probiotic effects are indeed normalization of gut barrier function and increased intestinal permeability, also linked to systemic endotoxemia in obesity. An integrative link may arise from immune regulation, given that probiotics produce anti-inflammatory cytokines and have the capacity to dampen local and systemic inflammation associated with obesity or a high-fat diet. Moreover, dietary fatty acids possess immunomodulatory properties and have been demonstrated to utilize the same signalling pathways as microbiota.

In conclusion, the results of this study demonstrate for the first time long-term health benefits in central adiposity control achieved by administration of the probiotics *L. rhamnosus* GG and *B. lactis* laying a basis for studies in at-risk populations. Overweight and its most deleterious form, central adiposity, may be taken as a source of global concern and its prevention as an integral part in reducing the risk of metabolic disorders. Means to this end are being sought in a range of life-style factors, probiotics as a component in a balanced diet offering a safe, reasonably economical, practical and potentially effective approach to a condition proved in practice to be one of the most difficult challenges to health care.

**Statement of authorship**

The authors’ responsibilities were as follows—KL and EI were responsible for the design of the study and organization of data collection. JJ contributed to data collection. KL and JJ analyzed the data and wrote the first draft of the paper. TP conducted the statistical analysis. All authors contributed to writing and revising the manuscript and approved the final draft. None of the authors had a conflict of interest.

**Conflict of interest statement**

No conflict of interest was declared.

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**References**


