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ORIGINAL ARTICLE

Impact of maternal diet during pregnancy and breastfeeding on infant metabolic programming: a prospective randomized controlled study

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Objectives: To evaluate the impact of maternal diet and intensive dietary counselling during pregnancy and breastfeeding on the infant's metabolic status.
Subjects/Methods: At the first trimester of pregnancy, 256 women were randomized into a control/placebo group and two dietary counselling groups (diet/probiotics and diet/placebo). The counselling, with double-blind randomization to probiotics (*Lactobacillus rhamnosus GG* and *Bifidobacterium lactis*) or placebo, targeted excessive saturated fat and low fibre consumption. Maternal diet was evaluated repeatedly during pregnancy and postpartum by means of 3 days' food diaries. Metabolic markers, serum 32–33 split and intact proinsulin, leptin/adiponectin ratio, skinfold thickness and waist circumference were measured of 194 healthy infants at the age of 6 months, and the high levels were taken to mirror adverse metabolic status.
Results: The proportion of infants with a high 32–33 split proinsulin was significantly lower in dietary counselling with probiotics ($n=6/62$, 9.7%) or placebo ($n=7/69$, 10.1%) compared with the control/placebo group ($n=17/63$, 27.0%). The high split proinsulin was associated with larger skinfold thickness, waist circumference and higher leptin/adiponectin ratio in the infants ($P<0.05$). With respect to maternal diet during pregnancy, the highest and lowest tertiles of fat intake increased the infant's risk of high split proinsulin, whereas those of butter associated correspondingly with the infant's waist circumference. Further, breastfed infants showed a reduced risk of high split proinsulin and leptin/adiponectin ratio compared with formula-fed infants.
Conclusions: Modification of maternal diet during pregnancy and breastfeeding may benefit infant metabolic health. High split proinsulin reflects adverse metabolic status in infancy, which can be improved by early dietary counselling.
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Keywords: infant; adiposity; metabolism; pregnancy; counselling

Introduction

Industrialized countries worldwide are faced with a progressive increase in metabolic conditions such as obesity, and the velocity of propagation is particularly outstanding in the paediatric population. Evidence that overweight or obesity, heightened blood pressure and impaired glucose metabolism are programmed (Barker, 2004) by early nutrition points to fetal and early postnatal life as critical periods and intervention targets.

The data implicating early nutritional influences on the cardiometabolic risks in humans derive mainly from epidemiological studies of extreme prenatal circumstances, such as exposure to famine (Ravelli *et al*., 1998; Painter *et al*., 2006). In these demonstrations, low birth weight is the main risk factor, taken to reflect a poor intrauterine nutritional environment. In well-nourished women, again, child birth weight, low or high, and a suboptimal intrauterine environment have been linked to maternal intake of single dietary factors (Godfrey *et al*., 1996; Mikkelsen *et al*., 2006; Moses *et al*., 2006; Zhang *et al*., 2006). However, a fact not previously properly addressed, but not to be ignored, is that

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the diet is always a mixture of several nutrients with possible complex interactions.

Previous demonstrations suggest that the gut microbiota composition is linked to the immunological and metabolic development of the child (Guarner and Malagelada, 2003; Rinne *et al*., 2005). The current intervention study targeted at dietary elements is associated with these developmental components, specifically at the dietary low-fibre and high saturated fat composition, together with aberrant gut microbiota development by probiotics. We have shown that the joint actions of diet and probiotics benefited the mothers' glucose metabolism (Laitinen *et al*., 2009) and weight management (Ilmonen *et al*., in press).

Here, we studied whether these benefits achieved from the mothers' diet can be extended to their children's metabolic health at the age of 6 months. The high 32–33 split proinsulin, a well-characterized predictor of insulin resistance in adults and older children (Temple *et al*., 1989; Mykkanen *et al*., 1997; Singhal *et al*., 2003), was taken as a novel marker of adverse metabolic status in infancy. To further investigate the infants' metabolic status and the usefulness of high split proinsulin in its assessment, waist circumference, skinfold thickness and adipocyte-derived cytokines—leptin and adiponectin—(Shea *et al*., 2003; Valle *et al*., 2003; Darendeliler *et al*., 2009; Corvalan *et al*., 2010) were taken as secondary outcomes.

Subjects and methods

Study design

A total of 256 pregnant women were recruited from maternal welfare clinics in the area of Turku, Southwest Finland, in the first trimester of pregnancy between April 2002 and November 2004 to participate in a prospective, randomized mother–infant nutrition and probiotics study (NCT00167000; section 3, http://www.clinicaltrials.gov) (Laitinen *et al*., 2009). Pregnant women afflicted with any form of chronic disease, except allergy, were excluded from the study. The women visited the study clinic three times during pregnancy, at a median of 14 (range 7–18), 24 (20–27) and 34 weeks of gestation (30–37), respectively. At the first study visit, representing the baseline, mothers were randomized into three study groups (Figure 1), two dietary intervention and one control group, according to computer-generated block randomization of six women. The study statistician, who was not involved in the study visits, generated the randomization list. The mother–infant pairs visited the study clinic at a median infant age of 6 months (range 5–8). The study complies with the Declaration of Helsinki, as revised in 2000. Written informed consent was obtained from the women and the Ethics Committee of the Hospital District of Southwest Finland approved the study.

Maternal dietary and probiotic intervention

All pregnant women attended municipal well-women clinics. At each study visit, women in the dietary intervention groups (diet/probiotics and diet/placebo) received intensive dietary counselling in accordance with that currently recommended (Nordic Working Group on Diet and Nutrition, 1996; Becker *et al*., 2004), with this being additional to standard counselling given to all pregnant women in well-women clinics in Finland. The counselling was given in layman terms by a nutritionist who encouraged

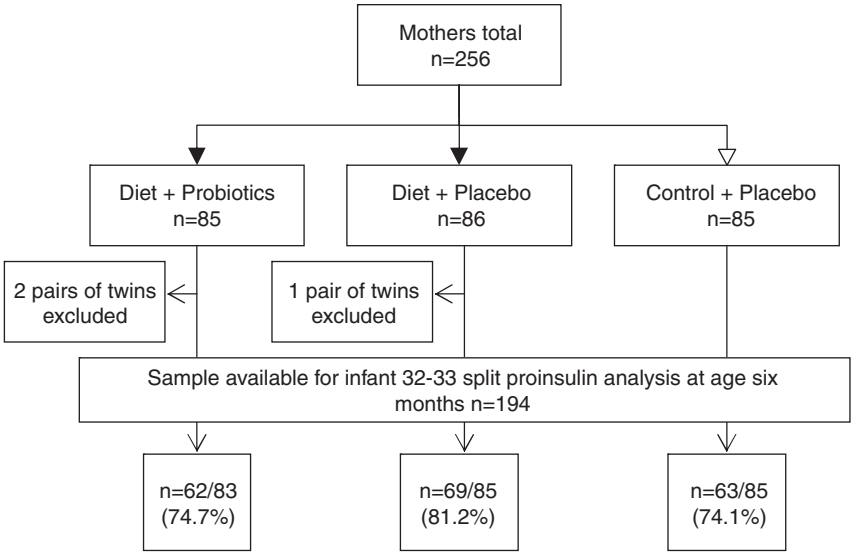

Figure 1 Subject flow. The mothers were randomized into three study groups at the first study visit. The study was open with respect to the dietary intervention, double-blinded (black arrow head) to intervention with probiotics or placebo, and single-blinded (white arrow head) to controls with placebo.

the participants to pay attention to the amount and type of fat and the amount of fibre in the diet (Piirainen *et al*., 2006). Further, to strengthen the dietary course and to demonstrate sources of favourable fat and fibre content, various food products, available on the market, were provided for use at home. The efficacy and safety of this dietary counselling during pregnancy have been reported elsewhere (Piirainen *et al*., 2006). The dietary intervention groups (diet/probiotics and diet/placebo) received capsules of probiotics (*Lactobacillus rhamnosus GG*, American Type Culture Collection 53103, Valio Ltd, Helsinki, Finland; and *Bifidobacterium lactis*, Chr. Hansen, Horsholm, Denmark; 10^{10} colony-forming units each daily) or placebo (microcrystalline cellulose and dextrose anhydrate; Chr. Hansen) in a double-blind manner, whereas the control group (control/placebo) received placebo in a single-blind manner. The maternal probiotic intervention continued from early pregnancy till the end of exclusive breastfeeding, a maximum of 6 months postpartum.

Evaluation of maternal food and nutrient intake

The maternal dietary intake was evaluated in the context of all study visits by means of 3 days' food diaries, including one weekend day, and using household measures. The daily dietary intakes of energy, foods and nutrients were calculated by the computerized program Micro-Nutrica, version 2.5 (Research Centre of the Social Insurance Institution, Turku, Finland). Maternal energy, fibre and energy-yielding nutrient intake levels were analysed. The foods consumed were combined as groups (grain, meat, fish and dairy products, fruits and berries, soft margarine and vegetable oil, sugar and sweets) in the analyses, but milk, cheese, sour milk products, vegetables and butter consumption levels were also analysed separately.

Evaluation of clinical characteristics

The weight, height, blood pressure and fasting plasma glucose concentration of the women were measured at every study visit (glucose was not measured in the second visit) (Aaltonen *et al*., 2008; Laitinen *et al*., 2009). Total gestational weight gain was calculated and the appropriate gestational weight gain evaluated according to the prepregnancy body mass index (Institute of Medicine, 1990). We recorded maternal diagnoses, including gestational diabetes mellitus, from the well-women clinic records, and determined their smoking habits. The duration of pregnancy was calculated from the date of last menstruation.

Infants' weight, length and head circumference at birth were measured in the hospital maternity ward, with measurements comparable to those used in the study visits. At the age of 6 months they underwent a physical examination, breastfeeding status was recorded and their anthropometrics were measured; weight was measured with Data Baby Scale 930 (Oriola, Espoo, Finland), length with an Infantometer (Pedihealth, Oulu, Finland), waist and head circumference with a measuring tape and the supra-iliac skinfold with a Holtain Tanner/Whitehouse Skinfold caliper (Marsden Weighing Group, Henley-on-Thames, Oxfordshire, UK).

Sampling

To evaluate the infants' metabolic status, serum 32–33 split proinsulin, intact proinsulin and adiposity-derived hormones, leptin and adiponectin were chosen as metabolic markers, as these are not sensitive to the non-fasting state (Glauber *et al*., 1986; Karlsson *et al*., 2004; Gil-Campos *et al*., 2010) unlike blood glucose and insulin concentrations. Indeed, for obvious ethical reasons, overnight fasting was not possible at the age of 6 months. Venous blood samples were collected before noon and were successfully obtained from 194 (76%) infants (Figure 1).

Analytical methods

Serum was separated immediately and the samples were initially stored at $-20\,^{\circ}C$ and then at $-70\,^{\circ}C$. The NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory (Cambridge, UK), analysed the infants' samples. Serum leptin, adiponectin, 32–33 split proinsulin and intact proinsulin concentrations were assayed on a 1235 AutoDELFIA immunoassay system (PerkinElmer Life Sciences, Boston, MA, USA). All assays were in-house, two-step time-resolved fluorometric assays as previously described (Hales *et al*., 1991; Semple *et al*., 2006; Temple *et al*., 1989), and samples were analysed in duplicate. Samples in which the coefficient of variation of the duplicates was greater than 10% were repeated. Quality control samples with concentrations spanning the working range of the assay were run each day. The between-batch imprecision for the quality control samples was less than 8% for all assays and analyte concentrations. Maternal plasma glucose concentrations were analysed on the day of sampling as previously reported (Laitinen *et al*., 2009).

Statistical analyses

The serum 32–33 split and intact proinsulin concentrations were primary outcome variables, leptin and adiponectin concentrations, waist circumference and supra-iliac skinfold were secondary outcome variables. Any 32–33 split and intact proinsulin values above the 85th percentile of the concentrations (7.9 and 6.64 pmol/l, respectively) were considered high values. These were dichotomized, as the clinically important difference in mean levels is not known, and we considered differences in proportions of higher values to be more relevant than those in overall mean levels. As the agreement between high concentrations was good (kappa-coefficient $\kappa = 0.80$), only the dichotomized 32–33 split proinsulin concentration was analysed as a final outcome variable. To evaluate high split proinsulin as a metabolic marker, logistic regression analysis was used for

dichotomized split proinsulin, which was explained by continuous or dichotomized (the 85th percentile as a cutoff point) adipocytokine ratio and anthropometrics measured at the age of 6 months.

The group comparisons in categorized and continuous outcome variables were assessed by univariate logistic regression analysis or by analysis of variance when appropriate. As most of the associations between the clinical characteristics (Table 1) and high split proinsulin were nonlinear, the characteristics of mother and child were categorized according to median, tertiles or quartiles. The effects of other possible explaining factors (Table 1) on infants' high 32–33 split proinsulin were analysed using univariate logistic regression analyses. In the final multivariate models, the intervention was forced and the explaining factors, if $P<0.10$ in univariate analysis, were introduced to the forward stepwise logistic model (criterion for entry $P<0.10$). The group comparisons are given as unadjusted and adjusted odds ratios (OR) with 95% confidence intervals using the control/placebo group as a reference group.

Maternal dietary intakes during and after pregnancy were divided into tertiles (T1 = lowest, T2 = middle and T3 = highest). The χ^2-test was used to study associations

Table 1 Clinical characteristics of mothers and their children ($n=194$) in the dietary intervention groups (diet/probiotics and diet/placebo) and in the control group (control/placebo)

	Diet/probiotics, n = *62*	*Diet/placebo,* n = *69*	*Control/placebo,* n = *63*	P-*value*
Mother				
Age, years	29.6 (4.3)	30.4 (5.2)	30.3 (4.9)	0.513
Smokers 1 year before pregnancy	20/61 (32.8%)	30/69 (43.5%)	19/62 (30.6%)	0.256 [a]
Prepregnancy BMI (kg/m^2)	22.7 (3.0)	24.2 (4.0)	24.0 (3.3)	0.045
Gestational weight gain, kg	15.2 (4.1)	15.1 (5.2)	14.7 (4.9)	0.773
Gestational diabetes mellitus	0/56 (0%)	8/67 (11.9%)	6/62 (9.7%)	0.033[a]
Plasma glucose (mmol/l)				
1st trimester	4.66 (0.40)	4.68 (0.32)	4.70 (0.29)	
3rd trimester	4.47 (0.30)	4.62 (0.48)	4.59 (0.35)	
6 months	4.88 (0.32)	4.97 (0.39)	5.01 (0.41)	0.041[b]
Child				
Sex, male	32 (52%)	33 (48%)	35 (56 %)	0.674[a]
Gestation age at delivery, weeks	40.3 (36.9–42.3)	40.4 (33.3–42.4)	40.1 (34.9–43.3)	0.985[c]
5′ Apgar	9 (6–10)	9 (3–10)	9 (4–10)	0.409[c]
Weight, (g)				
At birth	3536 (379)	3666 (363)	3621 (525)	
At 6 months	8174 (1043)	8182 (949)	8256 (973)	0.703[d]
Length, (cm)				
At birth	50.9 (1.7)	51.4 (1.6)	51.0 (2.2)	
At 6 months	68.4 (2.5)	69.0 (2.4)	69.0 (2.5)	0.310[d]
Head circumference, (cm)				
At birth	34.8 (1.2)	35.1 (1.2)	35.3 (1.4)	
At 6 months	44.1 (1.5)	44.0 (1.2)	44.2 (1.2)	0.455[d]
Waist circumference, (cm)				
At 6 months	44.2 (2.9)	43.3 (3.3)	44.4 (3.3)	0.102
Skinfold, (mm)				
At 6 months	6.7 (1.9)	6.6 (1.8)	6.7 (1.8)	0.934
Breast-fed, exclusive or partial				
At 6 months	66 (67%)	69 (75%)	44/62 (71%)	0.395[a]

Abbreviation: BMI, body mass index.
[a]χ^2-test.
[b]Analysis of covariance for repeated measurements with 1st trimester as a covariate.
[c]Kruskal–Wallis test.
[d]Analysis of variance for repeated measures.
Results are given as means (s.d.) or medians (range) or number of subjects (percentage). The statistically significant differences in mothers among the study groups have been published elsewhere (Laitinen *et al.*, 2009; Luoto *et al.*, 2010; Ilmonen *et al.*, in press).
The groups were compared using analysis of variance unless stated otherwise.

between dietary intakes and outcome variables. The effect of probiotic intervention on the association between dietary components and infant's high split proinsulin was analysed by the method of Mantel–Haenszel and by the Beslow–Day test; $P<0.20$ was used to indicate interaction. P-values <0.05 were considered statistically significant. Statistical analyses were performed with SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of mothers and infants are shown in Table 1. All pregnant women were Caucasian, and the majority had college or university education (74%). The infants were healthy and their anthropometrics were comparable in the diet/probiotics, diet/placebo and control/placebo groups. The infants' median 32–33 split proinsulin concentration was 3.3 pmol/l (range 1.2–21.9), intact proinsulin was 3.7 pmol/l (1.2–20.0), leptin was 3.7 ng/ml (0.7–14.8) and adiponectin was 13.5 μg/ml (3.6–37.3) at the age of 6 months.

The cluster of metabolic markers in infancy

By evaluating the usefulness of high split proinsulin as a marker of adverse metabolic status in infancy, we found that higher skinfold thickness (OR = 1.24, $P=0.045$), waist circumference (OR = 1.14, $P=0.035$) and leptin/adiponectin ratio (OR = 1.90, $P=0.029$), each of them included as continuous variables, were associated with increased likelihood of high 32–33 split proinsulin. Lower (<3320 g; Q1 vs Q2–Q4) birth weight infants had an increased risk for subsequent high split proinsulin (OR = 2.59, $P=0.027$), whereas the other infants' anthropometrics at birth or at the age of 6 months were not statistically significantly related to the high split proinsulin.

Impact of maternal dietary intervention on the infants' metabolic status

The infants' risk of high 32–33 split proinsulin concentration was lower in dietary counselling with probiotics or placebo groups compared with the control group (Figure 2); diet/probiotics unadjusted OR = 0.29 (95% confidence intervals 0.11–0.80, $P=0.016$) and diet/placebo OR = 0.31 (0.12–0.80, $P=0.015$). To determine the difference between the diet/probiotics (9.7%) and the diet/placebo groups (10.1%) with a 0.05 two-sided significance level and 90% power, the required sample size was more than 100 000 infants per group. The independent effect of dietary intervention on the infants' high split proinsulin concentration remained statistically significant in multivariate analysis, with breastfeeding at 6 months, mother's glucose concentration at 6 months and weight gain during pregnancy. These adjusted and unadjusted logistic regression analyses are shown in Table 2. According to univariate analyses, the intervention did not affect other metabolic markers measured in the infants (data not shown); however, breastfed infants tended to have a reduced risk of high leptin/adiponectin ratio compared with formula-fed infants (OR 0.54; $P=0.054$).

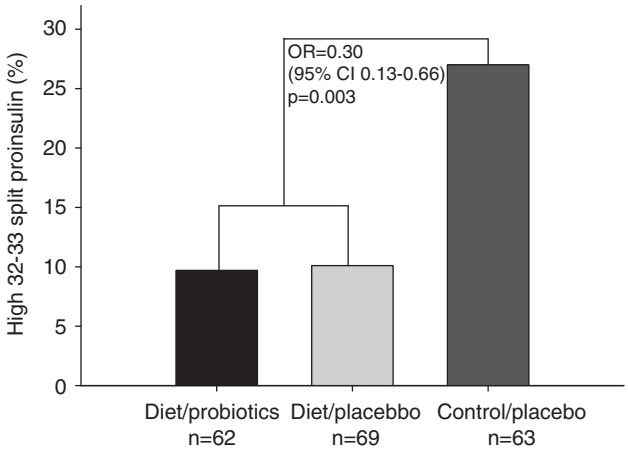

Figure 2 The impact of dietary intervention on the infants' high (above the 85th percentile cut-off point) 32–33 split proinsulin concentration. The number of infants with high 32–33 split proinsulin was 6 (9.7%) in diet/probiotics, 7 (10.1%) in diet/placebo and 17 (27.0%) in the control/placebo group. The group comparison, diet/probiotics and diet/placebo groups combined versus control/placebo group, is given as unadjusted odds ratio (OR) with 95% confidence interval (95% CI).

Impact of diet on the infants' metabolic status

To evaluate the impact of maternal nutrition during pregnancy and at 1 and 6 months postpartum on the infants' risk of adverse metabolic programming, by means of high 32–33 split proinsulin, skinfold thickness, waist circumference and leptin/adiponectin ratio, maternal dietary consumption was investigated in tertiles, as depicted in Table 3. Infants whose mothers' intake of fat, cheese or soft margarines and vegetable oil was in the highest or in the lowest tertile were more prone to high split proinsulin than were those whose mothers' intake was in the middle tertile. A corresponding nonlinear association was found between maternal butter intake and infants' waist circumference and also an association between maternal fat intake and infants' waist circumference. In contrast, extreme consumption of fruits and berries, butter and milk in the maternal diet resulted in lower infant risk of high split proinsulin compared with the middle tertile. The highest tertile of grain products intake in the maternal diet was associated with the greatest risk of high split proinsulin and high skinfold thickness in infants.

Interaction between probiotics and dietary components—exploratory analysis

To take into account the complexity of the diet, the interaction between probiotics and dietary components

Table 2 The effect of interventions and clinical characteristics on infant's high 32–33 split proinsulin (above the 85th percentile cut-off point) in univariate and multivariate analysis

Explaining factors	*Univariate analysis*		*Multivariate analysis*[a]	
	OR (95% CI)	P-*value*	*OR (95% CI)*	*Adjusted* P-*value*
Intervention[b]				
Control/placebo ($n=63$)	1.00		1.00	
Diet/probiotics ($n=62$)	0.29 (0.11–0.80)	0.016	0.24 (0.07–0.79)	0.019
Diet/placebo ($n=69$)	0.31 (0.12–0.80)	0.015	0.27 (0.09–0.79)	0.017
Breastfeeding at 6 months				
Ended ($n=57$)	1.00		1.00	
Exclusive or partial ($n=136$)	0.21 (0.09–0.47)	<0.001	0.24 (0.09–0.60)	0.002
Maternal blood glucose 6 months postpartum				
<4.90 mmol/l ($n=90$)	1.00		1.00	
$\geqslant 4.90$ mmol/l ($n=104$)	3.37 (1.37–8.28)	0.008	3.06 (1.10–8.50)	0.032
Weight gain during pregnancy[c]				
As recommended ($n=77$)	1.00		1.00	
$>$Recommended ($n=80$)	2.12 (0.81–5.58)	0.128	2.77 (0.92–8.30)	0.070
$<$Recommended ($n=32$)	3.33 (1.09–10.17)	0.034	4.04 (1.13–14.46)	0.032

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.
[a]The intervention was forced to the model and the following categorical variables were given to the stepwise logistic regression model (criterion for entry $P<0.10$): breast-feeding at 6 months, mothers glucose at 6 months (median = 4.90 mmol/l), weight gain during pregnancy, gestational diabetes mellitus (yes vs no), maternal smoking over 1 year before pregnancy (yes vs no) and birth weight (quartiles Q1 vs Q2–Q4; <3320 g vs $\geqslant 3320$ g).
[b]Global effect $P=0.012$ in univariate and $P=0.014$ in multivariate model.
[c]Global effect $P=0.098$ in univariate and $P=0.075$ in multivariate model.
The results are given as non-adjusted (95% CI) and adjusted odds ratios for clinical characteristics using multivariable logistic regression analysis. Only explaining factors included in the final multivariate model of infants' high 32–33 split proinsulin concentration are shown.

was studied. The effect of probiotic versus placebo administration on the percentage of infants with high 32–33 split proinsulin in tertiles of intake of dietary components is shown in Table 4. The probiotic intake contributed to the pattern of association between dietary components and infants' high split proinsulin according to the Breslow–Day test. The interaction was suggested if $P<0.200$. Interestingly, even when Bonferroni-adjusted, the maternal high milk, fruits and berries consumption combined with probiotics culminated in a more prevalent high split proinsulin concentration in this group of infants compared with children whose mothers were not receiving probiotics. Further, the probiotics tended to reduce the detrimental effect of maternal low cheese, middle milk and low or middle fruit and berries consumption, as well as high fat intake, on the infant's risk of high split proinsulin concentration.

Discussion

Our results indicate that dietary counselling and a balanced nutritional environment early in life support a beneficial metabolic development of the infant. Together with our previous demonstration that maternal nutrition during pregnancy contributes to the infant's blood pressure (Aaltonen *et al*., 2008), the present findings would further support the conception that several risk factors in the metabolic syndrome may be modifiable by diet during critical and sensitive periods of life, as previously shown in adults (Shiell *et al*., 2000).

From our prospective study, we have thus far learned that by modifying the dietary intake of fat and fibre by detailed dietary counselling and by the provision of appropriate food products combined with probiotics, we are not only improving the quality of the maternal diet (Piirainen *et al*., 2006) but also the maternal glucose metabolism up to 1 year after pregnancy (Laitinen *et al*., 2009). In the present study, the risk reduction in infants' high 32–33 split proinsulin concentration was already detected at the age of 6 months, although, the intervention did not influence the infants' adiposity measurements or leptin/adiponectin ratio. Further, maternal dietary counselling *per se*, with or without probiotics, was independently related to a lowered risk of high split proinsulin concentration in infants, irrespective of possible confounding variables such as maternal gestational diabetes mellitus, pregnancy weight gain or child birth size, previously linked to adverse metabolic outcomes in the child (Boney *et al*., 2005; Wrotniak *et al*., 2008).

As the maternal and fetal nutritional environments are closely related, an explanation for the beneficial effect of dietary counselling could be extrapolated from the current knowledge that a higher intake of unsaturated fatty acids can improve insulin sensitivity (Riserus *et al*., 2009), whereas a high saturated fat content in the diet promotes the secretion

Table 3 Tertiles for intake of foods and energy-yielding nutrients in maternal diet during pregnancy and breastfeeding, and the proportion of infants with high (above the 85th percentile cut-off point) 32–33 split proinsulin concentration, skinfold thickness, waist circumference and leptin/adiponectin ratio in each tertile of intake

Dietary component	*Study visit*	*Tertile of intake*		*Infants with high split proinsulin*		*Infants with high skinfold thickness*		*Infants with high waist circumference*		*Infants with high leptin/adiponectin*	
		Mean	*Range*	*Number (%)*	P[a]	*Number (%)*	P[a]	*Number (%)*	P[a]	*Number (%)*	P[a]
Grain products, g	*1st trim.*										
	T1 ($n=65$)	134	43–176	7 (10.8)	0.034	11 (18.6)	0.011	7 (11.7)	0.065	10 (15.4)	0.491
	T2 ($n=70$)	206	176–239	8 (11.4)		3 (4.5)		5 (7.8)		8 (11.4)	
	T3 ($n=58$)	298	239–560	15 (25.9)		12 (23.1)		12 (22.2)		11 (19.0)	
Fruits and berries, g	*2nd trim.*										
	T1 ($n=69$)	147	17–232	6 (8.7)	0.006	8 (12.3)	0.518	8 (12.5)	0.597	12 (17.4)	0.571
	T2 ($n=62$)	300	233–373	17 (27.4)		7 (12.7)		10 (16.9)		10 (16.1)	
	T3 ($n=63$)	543	373–1443	7 (11.1)		11 (19.0)		6 (10.7)		7 (11.1)	
Fat, g	*3rd trim.*										
	T1 ($n=59$)	46	23–58	11 (18.6)	0.038	8 (14.8)	0.243	7 (12.3)	0.088	8 (13.6)	0.234
	T2 ($n=66$)	67	58–76	4 (6.1)		12 (20.3)		4 (7.0)		7 (10.6)	
	T3 ($n=67$)	93	76–175	14 (20.9)		6 (9.5)		13 (20.6)		14 (20.9)	
Cheese, g	*3rd trim.*										
	T1 ($n=64$)	19	0–30	12 (18.8)	0.038	9 (15.0)	0.992	7 (12.1)	0.561	10 (15.6)	0.668
	T2 ($n=66$)	39	30–52	4 (6.1)		9 (15.0)		7 (11.3)		8 (12.1)	
	T3 ($n=62$)	77	52–177	13 (21.0)		8 (14.3)		10 (17.5)		11(17.7)	
Butter, g	*3rd trim.*										
	T1 ($n=62$)	0.0	0.0–0.3	4 (6.5)	0.029	8 (13.6)	0.876	9 (15.3)	0.011	10 (16.1)	0.495
	T2 ($n=64$)	1.5	0.3–3.8	15 (23.4)		10 (16.7)		2 (3.4)		7 (10.9)	
	T3 ($n=66$)	11.5	3.8–53.3	10 (15.2)		8 (14.0)		13 (22.0)		12 (18.2)	
Soft margarine and vegetable oil, g	*3rd trim.*										
	T1 ($n=66$)	15	1–22	15 (22.7)	0.045	8 (13.3)	0.554	7 (11.3)	0.808	10 (15.2)	0.905
	T2 ($n=59$)	28	22–34	4 (6.8)		10 (19.2)		8 (15.1)		8 (13.6)	
	T3 ($n=67$)	47	34–76	10 (14.9)		8 (12.5)		9 (14.5)		11 (16.4)	
Milk, g	*6 months*										
	T1 ($n=66$)	72	0–151	9 (13.6)	0.035	12 (19.0)	0.450	7 (12.7)	0.891	10 (15.2)	0.591
	T2 ($n=59$)	246	151–350	15 (25.4)		6 (11.1)		7 (12.5)		11 (18.6)	
	T3 ($n=59$)	549	350–1016	5 (8.5)		7 (13.2)		9 (15.3)		7 (11.9)	

Only dietary components significantly associated with infants' high 32–33 split proinsulin concentration are shown.
[a]The association between tertiled intake of dietary components and infants' high split proinsulin concentration, skinfold thickness, waist circumference and leptin/adiponectin ratio was analysed by χ^2-test in combined study groups (diet/probiotics, diet/placebo and control/placebo).

of proinflammatory cytokines (Cani *et al*., 2007), causally linked to insulin resistance (Shoelson *et al*., 2006). Further, higher fibre consumption, by reducing the risk of gestational diabetes mellitus (Zhang *et al*., 2006) or by facilitating maternal weight control (Howarth *et al*., 2001), was presupposed to benefit the infant's metabolic programming. These previous data are in agreement with our findings that maternal intake of fat and specific fat-containing food products affected metabolic markers in infancy. The effects of grain products, fruits and berries on infants' risk may be mediated by the effect of dietary fibre. Interestingly, in contrast to what has been previously shown (Godfrey *et al*., 1996), maternal energy, protein or carbohydrate intakes were not related to infants' high split proinsulin concentration in our analyses. Furthermore, a longer duration of breastfeeding reduced the infants' risk of high split proinsulin and leptin/adiponectin ratio. The advantage of breastfeeding may be related to protection against obesity (Armstrong and Reilly, 2002) or to the collective composition of the gut microbiota. Indeed, bifidobacteria, which typify the gut microbiota of the healthy breastfed infant (Fanaro *et al*., 2003), may dampen the systemic endotoxemia induced by bacterial lipopolysaccarides (Griffiths *et al*., 2004), and may thereby improve the metabolic status (Shoelson *et al*., 2006; Cani *et al*., 2007). The independent effect of maternal probiotic intervention on the infant's risk of high split proinsulin was not possible to study in this study population as the estimated sample size for such evaluation was more than 100 000 mother–infant pairs per group. However, our exploratory results suggest that

Table 4 The effect of probiotic intervention on the association between dietary components and infant's high 32–33 split proinsulin (above the 85th percentile cut-off point)

Dietary component	*Study visit*	*Tertile of intake*	*Infants with high 32–33 split proinsulin*		
			Probiotics	*Placebo*	
		(Total number)	*Number (%)*	*Number (%)*	P[a]
Fruits and berries, g	2nd trim.	T1 ($n=69$)	0/23 (0.0)	6/46 (13.0)	0.003
		T2 ($n=62$)	2/21 (9.5)	15/41 (36.6)	
		T3 ($n=63$)	4/18 (22.2)	3/45 (6.7)	
Fat, g	3rd trim.	T1 ($n=59$)	2/18 (11.1)	9/41 (22.0)	0.115
		T2 ($n=66$)	2/19 (10.5)	2/47 (4.3)	
		T3 ($n=67$)	2/25 (8.0)	12/42 (28.6)	
Cheese, g	3rd trim.	T1 ($n=64$)	1/20 (5.0)	11/44 (25.0)	0.197
		T2 ($n=66$)	2/23 (8.7)	2/43 (4.7)	
		T3 ($n=62$)	3/19 (15.8)	10/43 (23.3)	
Milk, g	6 months	T1 ($n=66$)	2/18 (11.1)	7/48 (14.6)	0.002
		T2 ($n=59$)	1/24 (4.2)	14/35 (40.0)	
		T3 ($n=59$)	3/16 (18.8)	2/43 (4.7)	

Only dietary components indicating interaction in Breslow–Day test ($P<0.200$) with infants' high 32–33 split proinsulin concentration are shown.
The results are given as number of children with high split proinsulin/as total number of children in the tertile group of intake (% of children with high 32–33 split proinsulin).
[a]The interaction was analysed by the method of Mantel–Haenszel and the Beslow–Day test.

probiotics may interact with dietary components and enhance the association between maternal dietary components and infants' high split proinsulin concentration. This is not surprising in the light of previous studies. For example, dietary fatty acids and gut microbiota share similar signalling pathways in immune responses (Laitinen *et al.*, 2006), possibly controlling the low-grade inflammation frequently detected in metabolic disorders (Fantuzzi, 2005). Thus, our results, together with those of recent human and animal studies, point to the relevance of late gestation and early postnatal life (Jones and Ozanne, 2009) and to the importance of dietary quality (Cani *et al.*, 2007) in metabolic development.

A large body of evidence supports the role of 32–33 split proinsulin as a marker of insulin resistance. Raised concentrations of split proinsulin in adults have been interpreted as evidence of β-cell dysfunction (Temple *et al.*, 1989) and a risk of impaired glucose tolerance (Hales *et al.*, 1991), but its role as a metabolic marker in infancy has remained poorly understood (Hawdon *et al.*, 1993; Singhal *et al.*, 2003). We found a positive association between the high split proinsulin concentration and the ratio of adipocyte-derived cytokines—leptin and adiponectin—which have been found to correlate with adiposity (Schubring *et al.*, 1999; Mantzoros *et al.*, 2009), and metabolic disorders (Valle *et al.*, 2003; Darendeliler *et al.*, 2009) in newborns and children, and, further, to efficaciously reflect cardio-metabolic risks (Steinberger *et al.*, 2003; Norata *et al.*, 2007). On the other hand, weight status at the age of 6 months predicts obesity in childhood (Taveras *et al.*, 2009), and here abdominal obesity, known to impair β-cell function (Hanley *et al.*, 2002), was linked to the high split proinsulin concentration. A limitation of this study was that infants were not fasted before blood sampling. As fasting infants is unethical, we selected metabolic markers that were not very sensitive to the non-fasting state (Glauber *et al.*,1986; Karlsson *et al.*, 2004; Gil-Campos *et al.*, 2010). Taken together, a high level of split proinsulin at the age of 6 months likely indicates susceptibility to adverse metabolic programming in these infants.

In conclusion, we have shown for the first time in humans that favourable metabolic programming, measured especially by a lower incidence of high 32–33 split proinsulin, can be achieved by balancing the diet of the mother during pregnancy and by breastfeeding the infant. Thus, the intrauterine and immediate postnatal period comprises a window of opportunity for interventions aiming to reduce the risk of metabolic disorders in both mother and infant and implies a prospect of achieving health benefits for two generations.

Conflict of interest

The authors declare no conflict of interest.

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