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Associations between human milk oligosaccharides and growth in infancy and early childhood

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Short Running Head: Human milk oligosaccharides in infant / childhood growth

Abbreviations: 2'FL: 2'-fucosyllactose; HMO: human milk oligosaccharides; HPLC: high pressure liquid chromatography; LNnT: lacto-N-neo-tetraose; pre-pregnancy BMI: pre-pregenancy body mass index; The STEPS Study: Steps to healthy development of Children

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Data described in the manuscript, code book, and analytic code will be made available upon request

ABSTRACT

Background: Breastfeeding modulates infant growth and protects from the development of obesity. However, whether or not maternal variation in human milk components like human milk oligosaccharides (HMOs) is associated with programming of child growth remains unknown.

Objective: Our objective was to determine the association between maternal HMO composition and child growth during the first 5 years of life. In addition, the association between maternal pre-pregnancy body mass index (pre-pregnancy BMI) and HMO composition was assessed.

Design: Human milk samples from 802 mothers were obtained from a prospective population-based birth cohort study (STEPS) conducted in Turku, Finland. HMO composition in these milk samples was analyzed by high-pressure liquid chromatography. Child growth data from 3 months to 5 years of age were collected from municipal well-baby clinics and linked to maternal HMO composition data to test for associations.

Results: Maternal HMO composition 3 months after delivery was associated with height and weight during the first 5 years of life in children of secretor mothers. Specifically, HMO diversity and the concentration of LNnT were inversely associated and that of 2'FL was directly associated with child height and weight Z scores in a model adjusted for maternal pre-pregenancy BMI, mode of delivery, birthweight Z score, sex, and time. Maternal pre-pregnancy BMI was associated with HMO composition.

Conclusions: The association between maternal HMO composition and childhood growth may imply a causal relationship, which warrants additional testing in preclinical and clinical studies, especially since 2'FL and LNnT are among the HMOs now being added to infant formula. Furthermore, altered HMO composition may mediate the impact of maternal pre-pregnancy BMI to childhood obesity, which warrants further investigation to establish cause-and-effect relationship.

INTRODUCTION

Breastfeeding is associated with improved health both in infancy and later in life (1). Breastfeeding has been linked with long-term health benefits including reduced risk of developing obesity and type II diabetes mellitus (1-3), which has been suggested to be mediated by bioactive compounds in human milk (4). Still, the contribution of individual human milk components remains poorly understood. The matter is further complicated by the fact that maternal obesity influences the microbiological, immunologic, lipid, and metabolite composition of human milk (5-7). In addition, maternal obesity (8,9) and shorter duration of breastfeeding (1-3) are both well-established risk factors for childhood overweight. The contribution of individual breast milk components and their interindividual variation to child growth is currently unknown.

Human milk oligosaccharides (HMOs) are a structurally complex and diverse group of glycans, which are present in human milk in high quantities (5-15 g/L in mature milk) (10). HMOs carry lactose at the reducing end, which can be further elongated by galactose- and N-acetyllactosamine-containing disaccharides, sialylated in α2-3- and α2-6-linkages as well as fucosylated in α1-2-, α1-3-, and α1-4- linkages. HMO fucosylation is catalyzed by fucosyltransferase enzymes whose expression is strongly controlled by genetics. Single nucleotide polymorphisms in the gene encoding for fucosyltransferase 2 (FUT2), for example, introduce a premature stop codon, which inactivates the enzyme and leads to a near loss of α1-2-fucosylated HMOs like 2'-fucosyllactose (2'FL) or lacto-N-fucopentaose 1 (LNFP1) in the milk of some women (Non-secretors). In contrast, women with an active FUT2 enzyme (Secretors) produce and secrete high amounts of these α1-2-fucosylated HMOs in their milk (extensively reviewed in Bode 2012 (10)). In fact, the overall HMO composition profile between Secretor and Nonsecretor women is vastly different, which is why Secretor status is often used to stratify cohort data.

HMOs are not digested by the infant but instead have a number of biological functions including selectively promoting the growth of specific microbes, inhibiting the adhesion and invasion of potential pathogens in the infant's gastrointestinal tract and modulating host immune function and intestinal epithelial cell gene expression patterns (10). It is therefore conceivable that HMOs contribute to the long-term health benefits associated with breastfeeding. While the HMO composition profile appears to be unique to each individual mother (11,12), little is known about maternal factors that influence the expression of specific HMOs, or the long-term impact of maternal HMO composition on the child.

The purpose of this study was to elucidate the links between maternal pre-pregnancy BMI, HMO composition and child growth in a birth cohort. The association between maternal pre-pregnancy body mass index (pre-pregnancy BMI) and HMO concentrations in milk samples collected at 3 months of age was investigated. In addition, we addressed the question whether maternal HMO composition correlates with child growth during the first 5 years of life.

SUBJECTS AND METHODS

Study design and subjects

The present study is based on data from mothers and children participating in a longitudinal Finland cohort, Steps to healthy development of Children (the STEPS Study), which has previously been described in detail by Lagström *et al.* (13). Briefly, all Finnish- and Swedish-speaking mothers who delivered a living child between 1 January 2008 and 31 April 2010 in the Hospital District of Southwest Finland formed the cohort population (in total 9,811 mothers and their 9,936 children). Altogether 1,797 mothers (18.3 % of the total cohort) with1,827 neonates (including 30 pairs of twins) volunteered as participants for the intensive follow-up group of the STEPS study. Written informed consent was obtained from the participants. The study protocol was approved by the Ethics Committee of the Hospital District of South West Finland in February 2007.

Milk collection and infant feeding information

Mothers were asked to collect breast milk when the infant was 3 months of age. Altogether 812 of the 1,797 mothers (45%) enrolled in the STEPS study provided a breast milk sample. Information of breastfeeding was not recorded of 572 of the 985 mothers from whom milk samples were not available. Altogether 413 mothers breastfed at least for some time but did not provide a sample and only 19 mothers reported to never have initiated breastfeeding.

A total of 802 breast milk samples were analyzed while 10 samples were excluded for technical reasons including unclear labeling and insufficient sample quantity (**Figure 1**). The mean age of the infants at the time of milk collection was 11.3 weeks (SD 2.6 weeks). Written instructions for the human milk sample collection were provided to the mothers, who obtained the samples by manual expression in the morning from single breast, first milking a few drops to waste before collecting the actual sample (10 mL) into a plastic container. The mothers brought the samples to the research center, or the samples were collected from their homes on the day of sampling.

All samples were frozen and stored at -70 degrees centigrade immediately after expression until further analysis.

Background characteristics and growth data

Self-reported height and weight before pregnancy were collected from self-administered questionnaires upon recruitment for calculation of maternal pre-pregnancy BMI (kg/m^2). Information regarding maternal age and self-reported smoking habits (before and during pregnancy) were also obtained from the questionnaires during the prenatal period. Information regarding pregnancy duration, delivery as well as children's sex, birth weight, length, and possible twin brother/sisters were obtained from the Longitudinal Census Files. Birth weight Z scores were calculated using the recently published references specific to the Finnish population (14). Delivery was defined as premature if the pregnancy lasted ≤ 37 weeks. The duration of exclusive and any breastfeeding was obtained from follow-up diaries filled by the mother in real time.

Child growth data were obtained from municipal follow-up clinics, which use standardized methods for the measurement of length/height and weight provided by the Finnish Institute for Health and Welfare. The anthropometric data closest to the time points of 3, 6 and 8 months and 1, 2, 3, 4, and 5 years of age were used in the analyses. Growth charts specific for the Finnish population (15) were used to obtain population-specific Z scores for height, weight and BMI.

HMO analysis

High-performance liquid chromatography (HPLC) was used to characterize HMOs in breast milk as previously described. Human milk was spiked with raffinose (a non-HMO carbohydrate) as

an internal standard to allow for absolute quantification. Oligosaccharides were extracted by high-throughput solid phase extraction over C18 and Carbograph microcolumns and fluorescently labeled with 2-aminobenzamide (2AB). Labeled oligosaccharides were analyzed by HPLC on an amide-80 column (15 cm length, 2 mm inner diameter, 3 µm particle size; Tosoh Bioscience) with a 50 mmol/L ammonium formate–acetonitrile buffer system. Separation was performed at 25°C and monitored with a fluorescence detector at 360 nm excitation and 425 nm emission. Peak annotation was based on standard retention times and mass spectrometric analysis on a Thermo LCQ Duo Ion trap mass spectrometer equipped with a Nano-ESI-source. Absolute concentrations were calculated based on standard response curves for each of the annotated HMOs. The total concentration of HMOs was calculated as the sum of the annotated oligosaccharides. HMO-bound fucose as well as HMO-bound sialic acid was calculated on a molar basis. The proportion of each HMO making up the total HMO concentration was also calculated. HMO Simpson's diversity and evenness were calculated based on relative abundances of all annotated HMOs.

Secretor Status Determination

Maternal Secretor status was determined by the high abundance (Secretor) or near absence (Non-Secretor) of the HMO 2'-fucosyllactose in the respective milk samples.

Statistical analyses

The statistical analyses were performed using SAS software for Windows version 9.4 (SAS Institute Inc., Cary, NC, USA). The level of significance was set at p-value<0.05. The clinical characteristics of the mothers and the children as well as HMO concentrations are expressed as medians and interquartiles (IQR; Q1, Q3) for continuous variables and percentages for categorical variables. The comparisons of HMO concentrations between secretor and non-

secretor mothers were performed using the Wilcoxon Rank-Sum Test due to non-normal data distributions.

Analysis of covariance was used to examine associations between each HMO variable and maternal pre-pregnancy BMI; the HMO variables included HMO diversity, HMO-bound fucose and sialic acid, the sum of 19 HMOs and the concentrations of 19 individual HMOs. Natural logarithmic transformation was performed for all these HMO variables except for HMO diversity. Mode of delivery and sex of the child were selected as confounding factors. The explanatory variables (pre-pregnancy BMI and smoking during pregnancy) were treated as independent variables.

The relationships between the explanatory factors in the models were examined by one-way *analysis of variance* for continuous variables and chi-squared test for categorical variables. If assumptions for parametric models were not met, the results obtained were compared to the results of the Kruskal-Wallis and Wilcoxon signed-rank tests (within time) for categorical explanatory factors and to the Spearman's rank correlation coefficient for BMI. Nonparametric tests were used for the variable DFLNT in the models of whole data and secretors.

Hierarchical linear mixed models for repeated measurements of height and weight Z scores were used to model their associations with HMO concentrations. The models included sex of the child, mode of delivery, birth weight Z score, maternal pre-pregnancy BMI, time (years), HMO concentration and HMO*time interaction as explanatory factors. The statistical models were built separately for HMO diversity, HMO-bound fucose and sialic acid, the sum of 19 HMOs as well as the concentrations of 19 individual HMOs as explanatory variables. Time was treated as a categorical variable. The models were built separately for the ages 3 to 12 months and 1 to 5 years as well as for ages 3 months to 5 years. Interaction between HMO

concentration and time was included in the model to examine whether the mean change over time was different depending on HMO concentration. An unstructured covariance pattern was used for repeated measures. Normal distribution assumption was checked from studentized residuals.

The derived variable of logarithm of 2'FL to logarithm of LNnT ratio was also examined using a hierarchical linear mixed model for repeated measurements. In addition, categorical variables were derived from the derived variable, logarithm of 2'FL and logarithm of LNnT with quartiles (below 25q vs 25q-50q vs 50q-75q vs above 75q). Medians and quartiles of children's height z-score and weight z-score were calculated within the classes to examine height and weight.

RESULTS

Study population

Altogether 802 mother-child pairs were included in the study. Based on the abundance of the HMO 2'-fucosyllactose in the milk samples, 699 mothers (87.2%) were deemed to be secretors and 103 mothers (12.8%) non-secretors. The clinical characteristics of the mothers and their infants included in this study as well as those in the original cohort are presented in detail in **Table 1**. The participant mothers were slightly older, were more often primiparous, had lower pre-pregnancy BMI and smoked significantly less often during pregnancy. On the other hand, the participant children were less often premature, exhibited slightly higher birth weight and lower birth weight Z scores as compared to the entire cohort.

HMO composition 3 months after delivery and its association with maternal pre-pregnancy BMI

HMO concentrations in milk samples obtained 3 months after delivery are presented in **Supplementary Table 1**. As expected, significant differences in HMO composition were detected between secretor and non-secretor mothers. Secretor mothers exhibited significantly higher concentrations of fucosylated HMOs while the HMO profile in non-secretor mothers was more diverse (Supplementary Table 1).

The median maternal pre-pregnancy BMI in the entire study cohort was 23.0 (IQR 21.0-25.8). No significant differences in maternal BMI were detected between secretor and non-secretor mothers. Maternal pre-pregnancy BMI was negatively correlated with HMO diversity in secretor but not in non-secretor mothers (**Table 2**). The association was statistically significant in an analysis of covariance model adjusted for mode of delivery, child sex and maternal smoking during pregnancy. At the level of individual HMOs, maternal pre-pregnancy BMI was positively correlated with the concentration of 2'FL and negatively correlated with the concentration of

LNnT in an analysis of covariance model adjusted for mode of delivery, child sex and maternal smoking during pregnancy in secretor mothers. The negative association between pre-pregnancy BMI and LNnT concentration was also detected when both secretors and nonsecretors were analyzed together while no significant correlations between pre-pregnancy BMI and HMO concentrations were observed in non-secretor mothers, possibly due to the lower number of samples.

HMO concentrations and child height and weight during the first 5 years of life

HMO diversity as assessed 3 months after delivery was negatively correlated with height (**Table 3**) and weight (**Table 4**) Z scores during the first 12 months of life in children of secretor mothers. These associations were statistically significant in a hierarchical linear mixed model adjusted for child sex, maternal pre-pregnancy BMI, birth weight Z score and time point. The correlation remained significant throughout 1 and 5 years of age between HMO diversity and height Z scores but was no longer evident between HMO diversity and weight Z scores after 1 year of age. No correlations between HMO diversity and length or height Z scores were detected in children of non-secretor mothers.

The concentrations of several individual HMOs exhibited significant correlations with child height (Table 3, Supplementary Table 2) and weight (Table 4, Supplementary Table 3) Z scores throughout the first 5 years of life in children of secretor mothers. In the adjusted hierarchical linear mixed model, the concentration of 2'FL was positively correlated with height Z scores between 3 and 12 months and 1 and 5 years of age (Table 3) and weight Z scores between 3 and 12 months of age (Table 4). Conversely, the concentration of LNnT was negatively correlated with weight and length Z scores throughout the first 5 years of life. The 2'FL/LNnT ratio consequently exhibited significant association with height and weight Z scores from 3

228 months to 5 years of age in children of secretor mothers (**Figure 2**) but not in children of non-
229 secretor mothers (**Supplementary Figure 1**).

DISCUSSION

Breastfeeding modifies childhood growth and modestly reduces the risk of childhood overweight and obesity (3,16). In the present study, the HMO composition in human milk three months after delivery was significantly associated with childhood growth throughout the first 5 years of life. Specifically, HMO diversity and the concentration of LNnT were inversely associated and that of 2'FL was directly associated with child height and weight between the ages of 3 and 12 months in children of secretor mothers. These data are consistent with the results of a previous study according to which human milk HMO diversity 1 month after delivery is inversely correlated with infant fat mass at the same age and higher concentrations of LNnT are associated with lower body fat at the age of 6 months (17). Our current results provide the first evidence for an association between HMOs and child growth beyond infancy and breastfeeding by indicating a significant association between HMO diversity and LNnT concentration and child height and weight also between the ages of 1 and 5 years. Our results suggest that individual differences in HMO composition may modulate the impact of breastfeeding on growth.

Obtaining high-quality scientific evidence on the effects of breastfeeding on growth and long-term health is difficult since conducting randomized, double-blinded and placebo-controlled trials on breastfeeding is impossible for obvious ethical and practical reasons. Epidemiological and cohort studies are prone to confounding by factors known to affect both breastfeeding initiation and/or duration and child growth and risk of obesity. Previous studies indicate that maternal smoking (18), high pre-pregnancy BMI (19) and caesarean section delivery (20) are all associated with reduced breastfeeding rates. Given the known associations between maternal BMI and microbiological, immunologic, lipid, and metabolite composition in human milk (5-7), we wanted to investigate whether maternal HMO profiles also vary according to maternal BMI. Pre-pregnancy BMI exhibited a negative correlation with HMO diversity and the concentration of LNnT, while a positive association was detected between pre-pregnancy BMI and the

concentration of 2'FL in secretor mothers in an analysis adjusted for maternal smoking during pregnancy, mode of delivery, and child sex. This is to our knowledge the first study to demonstrate that HMO composition may be dependent on maternal pre-pregnancy BMI.

It is striking that HMO diversity and the concentrations of LNnT and 2'FL were associated with both maternal pre-pregnancy BMI and child growth during the first 5 years of age. Maternal obesity is a major risk factor for excessive fetal growth (21) and childhood overweight and obesity (9,16). It is therefore vitally important to rule out the possibility that the observed HMO profiles are merely an indicator of maternal pre-pregnancy BMI but have no direct association with child growth. Mode of delivery has also been reported to modulate both human milk composition (22) and childhood growth patterns (23). In our analyses adjusted for maternal pre-pregnancy BMI, birth weight Z scores, mode of delivery and child sex, significant associations between HMO composition and child growth were detected. We interpret this to suggest that while prenatal exposures such as maternal pre-pregnancy BMI affect both HMO composition and childhood growth, HMO composition may independently modulate growth patterns after birth and up to the age of 5 years. Establishing whether a causal relationship exists between HMO profiles and growth patterns is beyond the scope of this observational study and this hypothesis needs to be tested using experimental and interventional designs.

It is intriguing to speculate that the association between maternal milk HMO composition and infant growth might be mediated via the developing infant gut microbiota. HMOs are known to specifically modulate the gut microbiota by promoting the growth of specific organisms such as bifidobacteria (10,24). On the other hand, epidemiological and experimental studies indicate that early-life gut microbiota is causally associated with the development of both the growth failure associated with malnutrition (25) and the development of overweight and obesity (26,27). Unfortunately, however, fecal samples were not analyzed from the infants in the present study.

The present study is purely observational and thus provides only associations without proving causal relations. Nonetheless, the large unselected prospective cohort followed with a standardized protocol and statistical analyses adjusted for confounding factors considerably improve the reliability and relevance of the observed associations. The study has some limitations. Relatively subtle difference between the participants and the subjects in the entire birth cohort were detected, which may to some extent compromise the generalizability of the results. Relying on self-reported weight and height may lead to inaccuracies in calculation of pre-pregnancy BMI (28). Moreover, underreporting of weight and overreporting of height may result in systematic bias.This limitation must be taken into consideration when interpreting our results. Maternal BMI after delivery was unfortunately not available. On the other hand, the weights and lengths of the children were measured by healthcare professionals using standardized methods, which limits measurement error. Data on the performance of actual individual measurements were not available and variance in performance may have resulted in error. Not all factors potentially affecting HMO concentration or child growth, including maternal diet or infant and child morbidity were available. Furthermore, relying on a single human milk sample for each mother is an obvious limitation of the study since compositional changes within subjects over time are possible. It is of note, however, that all human milk samples in the study were collected 3 months after delivery from mothers living in Southwest Finland, which diminishes the potentially confounding impact of lactation stage (29) or geographical area (30) on HMO concentrations. All infants in the study were at least partially breastfed at the age of 3 months and the median duration of breastfeeding was 10 months, which implies that sufficient exposure to breast milk and HMOs was achieved to elicit physiological effects on growth.

Most of the observed associations were statistically significant in secretor mothers only, which may simply reflect the much higher number of secretor mothers (n=699) in our study compared to nonsecretors (n=103). 2'FL specifically and per definition is almost absent in the milk of

nonsecretor mothers, which is likely another reason why there was no significant association between either 2'FL or 2'FL/LNnT and maternal factors or infant/childhood outcomes.

The results of the present study suggest that (i) the HMO composition of milk varies depending on maternal pre-pregenancy BMI and (ii) HMOs may be one mediator of the programming of child growth attributed to breast milk. These notions have several clinical implications. Differences in individual HMO composition may provide one explanation for the discrepant data on the impact of breastfeeding on child growth. In particular, the association between maternal pre-pregenancy BMI and HMO profiles may contribute to the increased obesity risk in children of obese mothers. Furthermore, if a causal relationship with specific HMOs and childhood growth patterns is established, new nutritional interventions may be developed that (i) aim to modulate HMO composition in mother's milk or (ii) provide specific HMOs to infants or children to support healthy childhood growth and development. In fact, some infant formula products already contain either 2'FL alone or a combination of 2'FL and LNnT together (31). While the currently added amounts of 2'FL (0.2 or 0.8 g/L) are below the concentrations we measured in human milk samples in the current study (median 2.96 g/L), it will be important to understand how different HMOs alone and in combination affect infant short- and long-term growth and development.

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Table 1. Clinical characteristics of the mothers and children in the study presented as medians (IQR) or percentages.

Variable	Total population[1] (n=9,009)	STEPS Study participants (n=802)	P[2]	Secretors (n=699)	Non-secretors (n=103)	P[4]
Mothers						
Age, years	30 (26, 33)	31 (28, 34)	<0.001	31 (28, 34)	31 (28, 34)	0.87
Pre-pregnancy BMI, kg/m^2	23.4 (21.1, 26.5)	23.0 (21.0, 25.8)	0.033[3]	23.0 (21.0, 25.8)	23.5 (20.8, 25.8)	0.98[3]
Previous births, %	55	41	<0.001	41	40	0.82
Previous pregnancies, %	66	53	<0.001	53	52	0.87
Caesarean section, %	14	14	0.17	13	19	0.27
Smoking during pregnancy, %	18	7.8	<0.001	7.8	7.9	0.97
Children						
Sex, boys, %	51	54	0.12	53	56	0.54
Duration of gestation, weeks	$40^{0/7}$ ($39^{1/7}$, $40^{6/7}$)	$40^{0/7}$ (39, $41^{0/7}$)	0.018[3]	$40^{0/7}$ ($39^{1/7}$, $41^{0/7}$)	$40^{0/7}$ ($39^{1/7}$, $41^{0/7}$)	0.90[3]

Premature birth, %	5.7	3.4	0.013	3.3	3.9	0.76
Birth weight, g	3530 (3200, 3870)	3540 (3280, 3860)	0.033	3540 (3270, 3860)	3530 (3320, 3870)	0.43
Birth weight, z-score	-0.061 (-0.825, 0.686)	-0.014 (-0.669, 0.688)	0.039	-0.015 (-0.692, 0.695)	-0.002 (-0.634, 0.657)	0.46
Duration of any breastfeeding, months		10.0 (6.5, 12.4)		10.1 (6.5, 12.4)	9.7 (7.0 , 12.6)	0.80[3]

Two-sample t-test was used for continuous variables and Chi-squared test for categorical variables.

[1]All Finnish women with one live birth from 1st January 2008 to 31st December 2010

[2] Difference between total population and STEPS study participants

[3]*Wilcoxon Rank-Sum Test was used because of the exception of normal distribution.*

[4]Difference between secretors and non-secretors

Table 2. The association between maternal BMI and HMO diversity concentrations (nmol/mL).

	Total (n= 481)		**Secretors (n=418)**		**Non-secretors (n=63)**	
	BMI slope estimate (95% CI)	P	BMI slope estimate (95% CI)	P	BMI slope estimate (95% CI)	P
Diversity	-0.03 (-0.06, 0.0005)	0.054	-0.04 (-0.08, -0.006)	0.022	0.02 (-0.03, 0.07)	0.40
Sum of HMOs	-0.00007 (-0.004, 0.004)	0.98	0.002 (-0.0001, 0.003)	0.064	-0.001 (-0.004, 0.001)	0.30
HMO-bound Sialic acid	-0.002 (-0.008, 0.003)	0.47	-0.003 (-0.009, 0.002)	0.24	-0.001 (-0.01, 0.009)	0.91
HMO-bound Fucose	-0.0002 (-0.008, 0.008)	0.97	0.002 (-0.0002, 0.005)	0.072	0.001 (-0.01, 0.02)	0.88
2’FL	-0.004 (-0.04, 0.03)	0.81	0.008 (0.0001, 0.02)	0.046	-0.02 (-0.07, 0.03)	0.55
3FL	0.006 (-0.006, 0.02)	0.33	0.008 (-0.002, 0.02)	0.12	0.005 (-0.02, 0.03)	0.71
LNnT	-0.01 (-0.02, -0.003)	0.006	-0.012 (-0.02, -0.004)	0.004	-0.004 (-0.03, 0.02)	0.73
3’SL	-0.003 (-0.01, 0.007)	0.60	-0.0005 (-0.01, 0.01)	0.93	-0.01 (-0.03, 0.01)	0.30
DFLac	-0.002 (-0.04, 0.03)	0.92	0.009 (-0.001, 0.02)	0.094	-0.006 (-0.08, 0.07)	0.87
6’SL	-0.004 (-0.02, 0.009)	0.57	-0.006 (-0.02, 0.006)	0.35	-0.007 (-0.04, 0.03)	0.71

LNT	0.0003 (-0.01, 0.01)	0.96	-0.001 (-0.01, 0.009)	0.83	0.01 (-0.03, 0.05)	0.50
LNFP I	-0.003 (-0.03, 0.02)	0.81	0.001 (-0.01, 0.01)	0.85	0.02 (-0.02, 0.05)	0.38
LNFP II	-0.006 (-0.02, 0.003)	0.22	-0.009 (-0.02, 0.0002)	0.054	0.001 (-0.02, 0.02)	0.91
LNFP III	-0.005 (-0.02, 0.006)	0.38	-0.008 (-0.02, 0.003)	0.16	0.006 (-0.02, 0.03)	0.61
LSTb	-0.004 (-0.01, 0.006)	0.45	-0.005 (-0.02, 0.006)	0.37	-0.005 (-0.02, 0.01)	0.54
LSTc	0.009 (-0.003, 0.02)	0.12	0.01 (-0.002, 0.02)	0.096	-0.0005 (-0.03, 0.03)	0.98
DFLNT	-0.02 (-0.03, 0.001)	0.069	-0.02 (-0.03, 0.001)	0.068	-0.002 (-0.03, 0.03)	0.91
LNH	-0.004 (-0.02, 0.009)	0.54	-0.01 (-0.02, 0.004)	0.16	0.03 (-0.004, 0.07)	0.077
DSLNT	-0.008 (-0.02, 0.001)	0.093	-0.007 (-0.02. 0.004)	0.21	-0.01 (-0.04, 0.008)	0.20
FLNH	-0.0007 (-0.02, 0.01)	0.93	-0.002 (-0.02, 0.01)	0.82	0.02 (-0.03, 0.06)	0.43
DFLNH	-0.002 (-0.01, 0.01)	0.80	0.0002 (-0.01, 0.01)	0.97	0.006 (-0.03, 0.04)	0.72
FDSLNH	-0.004 (-0.02, 0.008)	0.54	-0.009 (-0.02, 0.003)	0.15	0.01 (-0.01, 0.04)	0.25
DSLNH	-0.006 (-0.006, 0.02)	0.36	0.003 (-0.01, 0.02)	0.67	0.01 (-0.01, 0.04)	0.32

In addition to maternal pre-pregnancy BMI, the models included mode of delivery, child sex and smoking during pregnancy as explanatory factors. The estimate (95% confidence interval) is the slope indicating association between maternal pre-pregnancy BMI and HMO diversity and concentrations. Statistical analyses were performed with analysis of covariance.

Table 3. The asscociation between HMO-concentrations (nmol/mL) (ln-transformed data) and **height Z score in** children at the ages 3 to 12 months and ages 1 to 5 years separately for children of secretor and non-secretor mothers.

	Children ages 3 to 12 months				**Children ages 1 to 5 years**			
	Secretor mothers (n=674)		**Non-secretor mothers (n=100)**		**Secretor mothers (n=674)**		**Non-secretor mothers (n=100)**	
	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P
Diversity	-0.059 (-0.100, -0.017)	0.006	0.061 (-0.122, 0.243)	0.51	-0.050 (-0.094, -0.006)	0.027	0.099 (-0.089, 0.286)	0.30
Sum of HMOs	0.768 (-0.150, 1.687)	0.10	-1.093 (-3.872, 1.687)	0.44	0.665 (-0.314, 1.644)	0.18	-0.368 (-3.218, 2.482)	0.80
HMO-bound Sialic acid	-0.153 (-0.417, 0.111)	0.26	-0.573 (-1.471, 0.325)	0.21	-0.114 (-0.393, 0.166)	0.42	-0.094 (-1.044, 0.856)	0.84
HMO-bound Fucose	0.788 (0.199, 1.376)	0.009	0.123 (-0.394, 0.640)	0.64	0.707 (0.078, 1.337)	0.028	-0.268 (-0.804, 0.269)	0.33
2'FL	0.229 (0.042, 0.416)	0.016	-0.016 (-0.186, 0.154)	0.85	0.197 (-0.002, 0.397)	0.053	0.019 (-0.155, 0.192)	0.83
3FL	0.064 (-0.087, 0.215)	0.41	0.049 (-0.188, 0.287)	0.68	0.106 (-0.056, 0.268)	0.20	0.011 (-0.237, 0.258)	0.93

LNnT	-0.255 (-0.428, -0.082)	0.004	-0.053 (-0.367, 0.262)	0.74	-0.247 (-0.432, -0.062)	0.009	0.119 (-0.210, 0.448)	0.48
3’SL	0.106 (-0.034, 0.246)	0.14	-0.067 (-0.479, 0.345)	0.75	0.125 (-0.025, 0.275)	0.10	0.113 (-0.313, 0.538)	0.60
DFLac	0.028 (-0.118, 0.174)	0.71	0.047 (-0.065, 0.160)	0.40	0.026 (-0.129, 0.182)	0.74	0.032 (-0.086, 0.151)	0.59
6’SL	-0.107 (-0.238, 0.024)	0.11	-0.219 (-0.465, 0.026)	0.079	-0.072 (-0.211, 0.067)	0.31	-0.055 (-0.316, 0.206)	0.67
LNT	-0.077 (-0.224, 0.070)	0.30	0.057 (-0.156, 0.271)	0.59	-0.082 (-0.239, 0.074)	0.30	0.104 (-0.112, 0.320)	0.34
LNFP I	-0.015 (-0.126, 0.097)	0.80	0.069 (-0.088, 0.227)	0.39	-0.022 (-0.142, 0.097)	0.72	-0.025 (-0.190, 0.140)	0.77
LNFP II	-0.126 (-0.295, 0.042)	0.14	-0.029 (-0.334, 0.277)	0.85	-0.168 (-0.349, 0.013)	0.069	-0.054 (-0.369, 0.261)	0.73
LNFP III	-0.037 (-0.180, 0.104)	0.61	0.152 (-0.123, 0.427)	0.28	-0.034 (-0.186, 0.118)	0.66	-0.041 (-0.325, 0.242)	0.77
LSTb	-0.136 (-0.265, -0.008)	0.038	0.180 (-0.265, 0.625)	0.42	-0.108 (-0.247, 0.030)	0.12	0.289 (-0.173, 0.750)	0.22
LSTc	0.053 (-0.068, 0.173)	0.39	0.138 (-0.146, 0.422)	0.34	0.042 (-0.086, 0.171)	0.52	0.068 (-0.229, 0.364)	0.65

DFLNT	-0.008 (-0.090, 0.074)	0.85	0.111 (-0.184, 0.406)	0.46	-0.0001 (-0.087, 0.087)	1.00	0.090 (-0.216, 0.396)	0.56
LNH	-0.028 (-0.137, 0.081)	0.62	0.101 (-0.114, 0.316)	0.36	0.011 (-0.106, 0.127)	0.86	-0.036 (-0.259, 0.186)	0.75
DSLNT	-0.087 (-0.216, 0.042)	0.18	0.049 (-0.276, 0.373)	0.77	-0.110 (-0.248, 0.029)	0.12	0.256 (-0.078, 0.589)	0.13
FLNH	0.008 (-0.086, 0.101)	0.87	0.046 (-0.128, 0.219)	0.60	0.030 (-0.070, 0.130)	0.55	0.008 (-0.173, 0.188)	0.93
DFLNH	-0.007 (-0.140, 0.126)	0.92	0.085 (-0.129, 0.299)	0.43	-0.052 (-0.193, 0.089)	0.47	0.005 (-0.217, 0.228)	0.96
FDSLNH	-0.035 (-0.154, 0.085)	0.57	-0.010 (-0.272, 0.252)	0.94	-0.041 (-0.169, 0.086)	0.52	-0.147 (-0.418, 0.124)	0.29
DSLNH	0.033 (-0.079, 0.146)	0.56	-0.040 (-0.357, 0.278)	0.81	0.030 (-0.090, 0.150)	0.62	0.022 (-0.311, 0.354)	0.90

Statistical associations were tested with hierarchical linear mixed model for repeated measurements. The models include mode of delivery, sex, birth weight Z score, maternal pre-pregnancy BMI, HMO, time and HMO*time interaction as explanatory factors. [1]*Negative slope estimate indicates negative correlation between child's height SDS and ln-transformed HMO-concentrations and positive slope estimate indicates positive correlation. The 95% confidence interval of the estimate is given in parentheses. The estimate of the HMO variable is the coefficient of main effect of HMO.*

Table 4. The association between HMO-concentrations (nmol/mL) (ln-transformed data) and **weight Z-score** in children at the ages 3 to 12 months and ages 1 to 5 years separately by maternal secretors and non-secretors.

	Children ages 3 to 12 months				**Children ages 1 to 5 years**			
	Secretor mothers (n=674)		**Non-secretor mothers (n=100)**		**Secretor mothers (n=674)**		**Non-secretor mothers (n=100)**	
	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P
Diversity	-0.048 (-0.087, -0.009)	0.017	0.093 (-0.091, 0.278)	0.32	-0.038 (-0.079, 0.002)	0.063	0.103 (-0.076, 0.283)	0.25
Sum of HMOs	0.901 (0.032, 1.771)	0.042	-3.086 (-5.819, -0.353)	0.027	0.828 (-0.063, 1.718)	0.068	-1.253 (-3.961, 1.455)	0.36
HMO-bound Sialic acid	-0.018 (-0.268, 0.233)	0.89	-0.187 (-1.090, 0.717)	0.68	0.030 (-0.225, 0.284)	0.82	-0.030 (-0.930, 0.870)	0.95
HMO-bound Fucose	0.837 (0.280, 1.394)	0.003	0.113 (-0.409, 0.634)	0.67	0.610 (0.037, 1.183)	0.037	-0.095 (-0.609, 0.418)	0.71
2'FL	0.210 (0.033, 0.387)	0.020	-0.017 (-0.189, 0.156)	0.85	0.165 (-0.016, 0.347)	0.075	0.029 (-0.137, 0.195)	0.73
3FL	0.182 (0.039, 0.324)	0.012	0.141 (-0.097, 0.378)	0.24	0.162 (0.014, 0.309)	0.032	0.134 (-0.102, 0.370)	0.26

LNnT	-0.225 (-0.389, -0.061)	0.007	-0.194 (-0.508, 0.120)	0.22	-0.213 (-0.381, -0.044)	0.014	-0.062 (-0.377, 0.253)	0.70
3’SL	0.161 (0.028, 0.293)	0.017	0.151 (-0.265, 0.567)	0.47	0.153 (0.017, 0.289)	0.028	0.290 (-0.116, 0.696)	0.16
DFLac	0.154 (0.016, 0.292)	0.028	-0.013 (-0.127, 0.102)	0.83	0.115 (-0.026, 0.256)	0.11	-0.008 (-0.121, 0.106)	0.89
6’SL	-0.075 (-0.199, 0.049)	0.23	-0.375 (-0.612, -0.138)	0.002	-0.012 (-0.140, 0.115)	0.85	-0.145 (-0.392, 0.101)	0.25
LNT	-0.136 (-0.275, 0.002)	0.053	0.121 (-0.095, 0.336)	0.27	-0.091 (-0.234, 0.051)	0.21	0.103 (-0.103, 0.310)	0.32
LNFP I	-0.090 (-0.195, 0.016)	0.10	0.060 (-0.100. 0.219)	0.46	-0.061 (-0.169, 0.048)	0.27	-0.039 (-0.196, 0.118)	0.62
LNFP II	-0.031 (-0.191, 0.129)	0.71	-0.002 (-0.307, 0.303)	0.99	-0.091 (-0.256, 0.075)	0.28	-0.062 (-0.365, 0.242)	0.69
LNFP III	0.056 (-0.079, 0.191)	0.42	-0.027 (-0.304, 0.251)	0.85	0.043 (-0.096, 0.182)	0.54	0.003 (-0.270, 0.275)	0.99
LSTb	-0.149 (-0.271, -0.027)	0.017	0.082 (-0.370, 0.534)	0.72	-0.071 (-0.197, 0.055)	0.27	0.222 (-0.223, 0.666)	0.32
LSTc	0.037 (-0.077, 0.150)	0.53	-0.021 (-308, 0.265)	0.88	0.033 (-0.085, 0.149)	0.59	0.059 (-0.224, 0.342)	0.68

DFLNT	-0.049 (-0.127, 0.028)	0.21	-0.003 (-0.302, 0.296)	0.99	-0.058 (-0.137, 0.021)	0.15	-0.014 (-0.308, 0.280)	0.92
LNH	0.024 (-0.079, 0.127)	0.65	0.143 (-0.071, 0.358)	0.19	0.021 (-0.085, 0.127)	0.70	0.008 (-0.204, 0.220)	0.94
DSLNT	-0.114 (-0.236, 0.009)	0.068	0.101 (-0.226, 0.427)	0.54	-0.068 (-0.194, 0.058)	0.29	0.170 (-0.152, 0.491)	0.30
FLNH	-0.022 (-0.110, 0.066)	0.62	0.046 (-0.129, 0.221)	0.60	-0.018 (-0.109, 0.073)	0.69	0.022 (-0.151, 0.194)	0.80
DFLNH	-0.032 (-0.157, 0.094)	0.62	-0.039 (-0.254, 0.176)	0.72	-0.059 (-0.188, 0.069)	0.37	-0.025 (-0.238, 0.189)	0.82
FDSLNH	0.058 (-0.055, 0.172)	0.31	0.228 (-0.033, 0.490)	0.086	0.003 (-0.114, 0.120)	0.96	0.026 (-0.234, 0.287)	0.84
DSLNH	0.094 (-0.013, 0.200)	0.085	-0.031 (-0.352, 0.289)	0.85	0.079 (-0.031, 0.188)	0.16	-0.102 (-0.420, 0.215)	0.52

Statistical associations were tested with hierarchical linear mixed model for repeated measurements. The models include mode of delivery, sex, birth weight Z score, maternal pre-pregnancy BMI, HMO, time and HMO*time interaction as explanatory factors. [1]*Negative slope estimate indicates negative correlation between child's height SDS and ln-transformed HMO-concentrations and positive slope estimate indicates positive correlation. The 95% confidence interval of the estimate is given in parentheses. The estimate of the HMO variable is the coefficient of main effect of HMO.*

Figure Legends

Figure 1. Flowchart summarizing exclusion and inclusion criteria for present study samples from the STEPS Study.

Figure 2. Children's height and weight Z-score from 3 months to 5 year of age related to medians of the lowest (below 25) and highest (above 75) quartiles of 2'FL /LNnT ratio, 2'FL and LNnT in the group of secretor mothers (n=699). Log-transformed data.

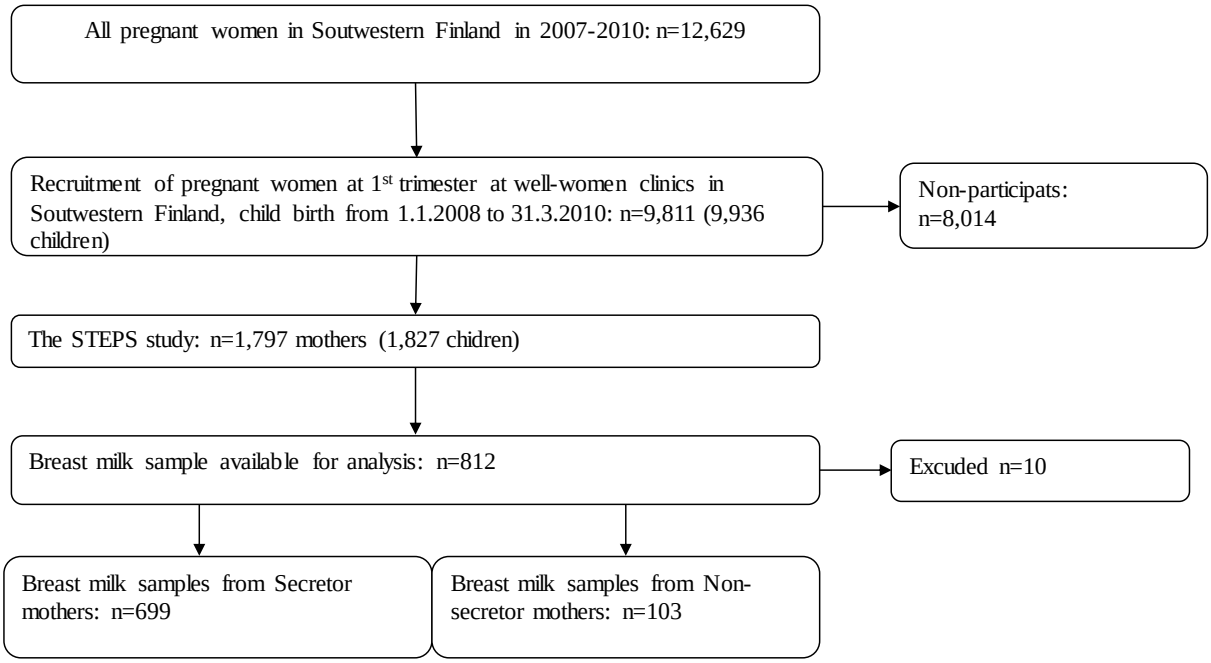

All pregnant women in Soutwestern Finland in 2007-2010: n=12,629
Recruitment of pregnant women at 1st trimester at well-women clinics in Soutwestern Finland, child birth from 1.1.2008 to 31.3.2010: n=9,811 (9,936 children)
Non-participats: n=8,014
The STEPS study: n=1,797 mothers (1,827 chidren)
Breast milk sample available for analysis: n=812
Excuded n=10
Breast milk samples from Secretor mothers: n=699
Breast milk samples from Non-secretor mothers: n=103

Figure 1.

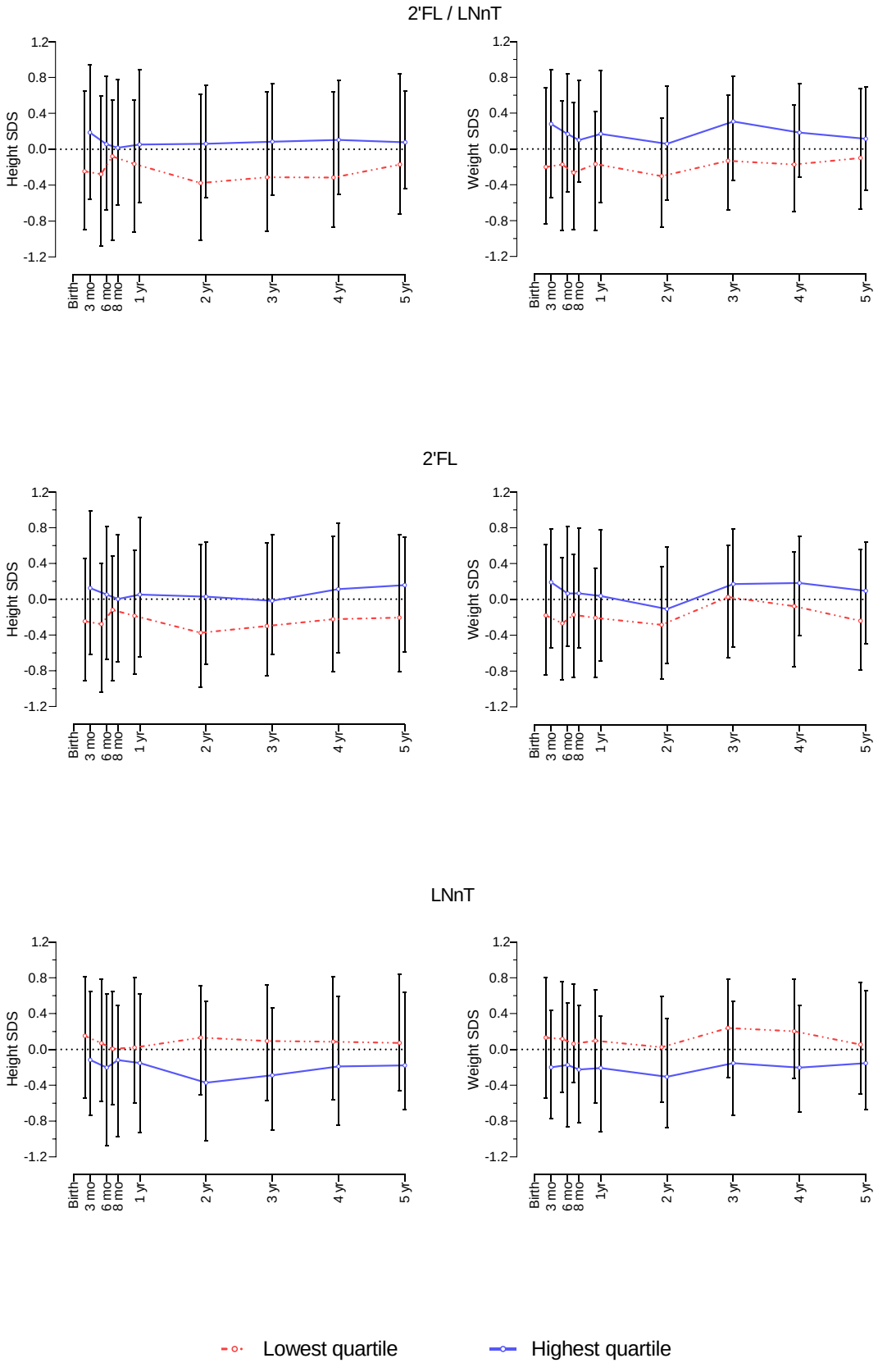

2'FL / LNnT
Height SDS
Weight SDS
2'FL
LNnT
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
Birth
3 mo
6 mo
8 mo
1 yr
2 yr
3 yr
4 yr
5 yr
Lowest quartile
Highest quartile

Figure 2.

Associations between human milk oligosaccharides and growth in infancy and early childhood

Lagström H, Rautava S* et al.

* These authors contributed equally to the manuscript.

Online Supplementary Material

Supplementary table 1. Human milk oligosaccharide (HMO) concentrations (nmol/mL) as median (Q1, Q3) of mothers with 3-month-old children (n=802). Comparison between secretors and non-secretors is based on the Wilcoxon Rank-Sum Test.

	Total (n=802)	**Secretors (n=699)**	**Non-secretors (n=103)**	P
Diversity	5.1 (4.0, 6.2)	5.0 (3.8, 6.3)	5.5 (4.8, 6.0)	**0.003**
Sum of HMOs	16180 (15250, 17070)	16370 (15650, 17220)	9203 (8882, 9536)	**<0.001**
HMO-bound Sialic acid	2823 (2340, 3397)	2710 (2290, 3187)	4140 (3631, 4611)	**<0.001**
HMO-bound Fucose	14460 (12930, 15680)	14780 (13640, 15900)	5551 (4807, 6242)	**<0.001**
2'FL	6059 (4405, 7863)	6455 (4993, 8237)	46 (30, 108)	**<0.001**
3FL	347 (240, 492)	374 (274, 524)	122 (77, 168)	**<0.001**
LNnT	983 (772, 1279)	978 (772, 1268)	1065 (767, 1380)	0.32
3'SL	507 (387, 680)	526 (406, 705)	395 (312, 525)	**<0.001**
DFLac	498 (344, 684)	542 (405, 710)	8.8 (4.0, 17)	**<0.001**
6'SL	561 (396, 877)	521 (383, 774)	1200 (786, 1802)	**<0.001**
LNT	857 (605, 1158)	882 (630, 1168)	632 (363, 1025)	**<0.001**
LNFP I	1137 (637, 1733)	1237 (844, 1833)	78 (40, 112)	**<0.001**
LNFP II	1535 (1108, 2030)	1440 (1056, 1839)	3083 (2783, 3521)	**<0.001**
LNFP III	74 (54, 105)	71 (52, 93)	144 (101, 201)	**<0.001**
LSTb	112 (80, 154)	107 (77, 146)	161 (128, 213)	**<0.001**
LSTc	74 (50, 107)	77 (53, 110)	60 (40, 81)	**<0.001**
DFLNT	1494 (953, 1846)	1578 (1237, 1890)	584 (432, 810)	**<0.001**
LNH	59 (37, 84)	58 (37, 83)	59 (38, 97)	0.23
DSLNT	322 (227, 446)	318 (223, 444)	384 (257, 480)	**0.01**
FLNH	52 (29, 83)	56 (34, 88)	23 (14, 45)	**<0.001**
DFLNH	38 (25, 52)	40 (29, 53)	13 (8.7, 19.4)	**<0.001**
FDSLNH	240 (158, 368)	223 (147, 322)	560 (391, 745)	**<0.001**
DSLNH	69 (45, 103)	67 (44, 100)	87 (58, 119)	**<0.001**

Supplementary Table 2. The association of HMO-concentrations (nmol/mL) (ln-transformed data) to height standard deviation score (SDS) of children ages 3 months to 5 years separately for children of secretor and non-secretor mothers.

	Children ages 3 months to 5 years			
	Secretor mothers (n=674)		**Non-secretor mothers (n=100)**	
	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P
Diversity	-0.048 (-0.089, -0.006)	0.024	0.089 (-0.084, 0.261)	0.31
Sum of HMOs	0.617 (-0.297, 1.531)	0.19	-0.749 (-3.330, 1.832)	0.57
HMO-bound Sialic acid	-0.075 (-0.336, 0.186)	0.57	-0.336 (-1.197, 0.525)	0.44
HMO-bound Fucose	0.660 (0.073, 1.247)	0.028	-0.085 (-0.570, 0.400)	0.73
2'FL	0.186 (-0.0003, 0.372)	0.050	0.027 (-0.132, 0.186)	0.74
3FL	0.081 (-0.070, 0.233)	0.29	0.037 (-0.189, 0.263)	0.75
LNnT	-0.247 (-0.419, -0.074)	0.005	0.060 (-0.238, 0.358)	0.69
3'SL	0.129 (-0.011, 0.269)	0.071	0.050 (-0.342, 0.441)	0.80
DFLac	0.039 (-0.106, 0.185)	0.60	0.032 (-0.075, 0.140)	0.55
6'SL	-0.081 (-0.211, 0.049)	0.22	-0.147 (-0.381, 0.088)	0.22
LNT	-0.067 (-0.213, 0.079)	0.37	0.081 (-0.117, 0.280)	0.42
LNFP I	-0.025 (-0.137, 0.086)	0.66	0.006 (-0.145, 0.157)	0.93
LNFP II	-0.123 (-0.292, 0.045)	0.15	-0.032 (-0.322, 0.258)	0.83
LNFP III	-0.022 (-0.164, 0.120)	0.76	0.039 (-0.221, 0.299)	0.77
LSTb	-0.109 (-0.237, 0.020)	0.099	0.214 (-0.210, 0.637)	0.32
LSTc	0.050 (-0.070, 0.170)	0.42	0.087 (-0.183, 0.357)	0.52
DFLNT	-0.0004 (-0.082, 0.081)	0.99	0.109 (-0.171, 0.389)	0.44
LNH	0.003 (-0.106, 0.112)	0.95	0.014 (-0.189, 0.217)	0.89

DSLNT	-0.076 (-0.205, 0.053)	0.25	0.178 (-0.129, 0.484)	0.25
FLNH	0.018 (-0.075, 0.111)	0.71	0.006 (-0.160, 0.172)	0.94
DFLNH	-0.040 (-0.172, 0.092)	0.55	0.021 (-0.181, 0.223)	0.84
FDSLNH	-0.023 (-0.142, 0.097)	0.71	-0.086 (-0.333, 0.160)	0.49
DSLNH	0.041 (-0.071, 0.153)	0.48	-0.002 (-0.306, 0.301)	0.99

Statistical associations were tested with hierarchical linear mixed model for repeated measurements. The models include mode of delivery, sex, birth weight Z score, maternal pre-pregnancy BMI, HMO, time and HMO*time interaction as explanatory factors.

[1]*Negative slope estimate indicates negative correlation between child's height SDS and ln-transformed HMO-concentrations and positive slope estimate indicates positive correlation. The 95% confidence interval of the estimate is given in parentheses. The estimate of the HMO variable is the coefficient of main effect of HMO.*

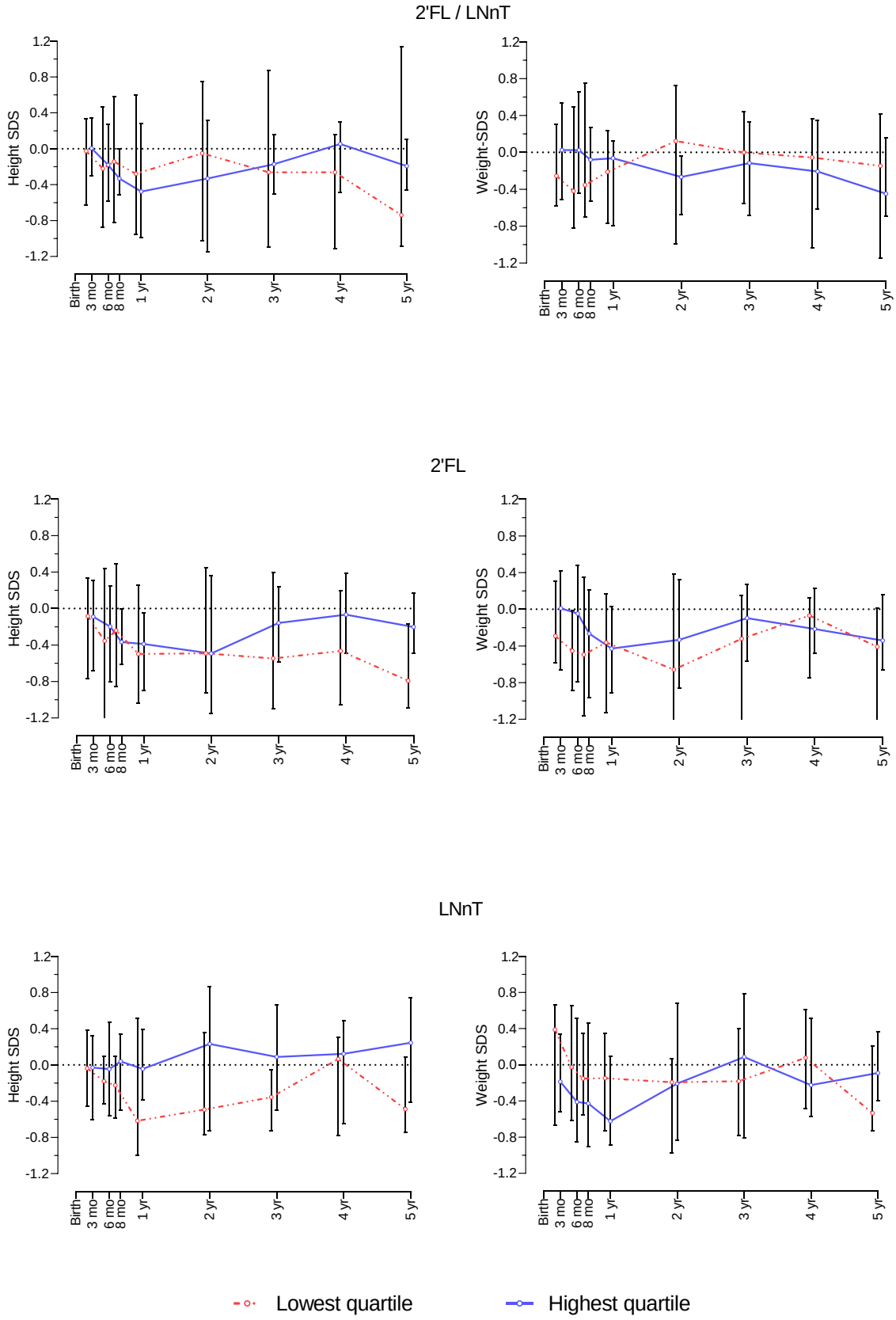
Supplementary Table 3. The association of HMO-concentrations (nmol/mL) (ln-transformed data) to weight standard deviation score (SDS) of children ages 3 months to 5 years separately by maternal secretors and non-secretors.

	Children ages 3 months to 5 years			
	Secretor mothers (n=674)		**Non-secretor mothers (n=100)**	
	Estimate (95% CI)[1]	P	Estimate (95% CI)[1]	P
Diversity	-0.041 (-0.078, -0.004)	0.032	0.105 (-0.064, 0.273)	0.22
Sum of HMOs	0.840 (0.016, 1.664)	0.046	-2.444 (-4.919, 0.031)	0.053
HMO-bound Sialic acid	0.028 (-0.208, 0.265)	0.81	-0.141 (-0.985, 0.704)	0.74
HMO-bound Fucose	0.674 (0.144, 1.204)	0.013	0.061 (-0.414, 0.536)	0.80
2'FL	0.178 (0.010, 0.346)	0.038	0.037 (-0.118, 0.192)	0.64
3FL	0.163 (0.027, 0.299)	0.019	0.173 (-0.046, 0.392)	0.12
LNnT	-0.215 (-0.371, -0.059)	0.007	-0.139 (-0.429, 0.151)	0.35
3'SL	0.160 (0.033, 0.286)	0.013	0.255 (-0.125, 0.636)	0.19
DFLac	0.133 (0.002, 0.264)	0.046	-0.016 (-0.122, 0.089)	0.76
6'SL	-0.040 (-0.158, 0.077)	0.50	-0.300 (-0.523, -0.077)	0.009
LNT	-0.097 (-0.229, 0.035)	0.15	0.086 (-0.108, 0.281)	0.38
LNFP I	-0.074 (-0.174, 0.027)	0.15	-0.012 (-0.160, 0.135)	0.87
LNFP II	-0.058 (-0.211, 0.095)	0.46	-0.012 (-0.297, 0.273)	0.93
LNFP III	0.051 (-0.077, 0.179)	0.44	0.007 (-0.250, 0.263)	0.96
LSTb	-0.104 (-0.220, 0.012)	0.080	0.116 (-0.301, 0.532)	0.58
LSTc	0.035 (-0.073, 0.143)	0.52	0.024 (-0.241, 0.290)	0.86
DFLNT	-0.053 (-0.126, 0.020)	0.15	0.004 (-0.271, 0.279)	0.98

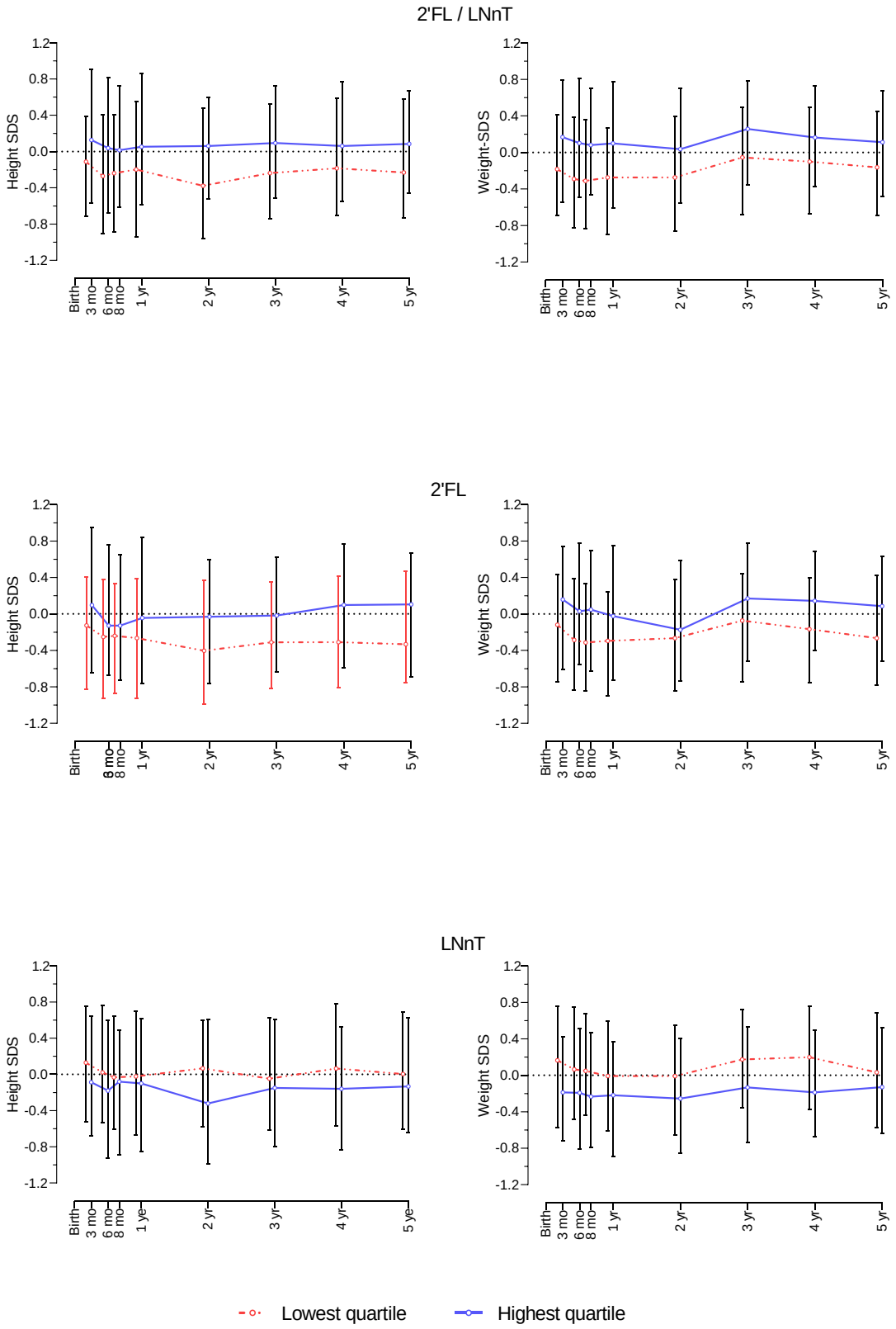
LNH	0.027 (-0.071, 0.125)	0.59	0.077 (-0.121, 0.275)	0.44
DSLNT	-0.078 (-0.195, 0.039)	0.19	0.148 (-0.153, 0.450)	0.33
FLNH	-0.019 (-0.103, 0.066)	0.67	0.017 (-0.145, 0.179)	0.83
DFLNH	-0.050 (-0.170, 0.069)	0.41	-0.041 (-0.239, 0.158)	0.69
FDSLNH	0.036 (-0.072, 0.144)	0.51	0.139 (-0.101, 0.378)	0.25
DSLNH	0.092 (-0.009, 0.193)	0.076	-0.075 (-0.372, 0.221)	0.62

Statistical associations were tested with hierarchical linear mixed model for repeated measurements. The models include mode of delivery, sex, birth weight Z score, maternal pre-pregnancy BMI, HMO, time and HMO*time interaction as explanatory factors.

[1]*Negative slope estimate indicates negative correlation between child's height SDS and ln-transformed HMO-concentrations and positive slope estimate indicates positive correlation. The 95% confidence interval of the estimate is given in parentheses. The estimate of the HMO variable is the coefficient of main effect of HMO.*



Supplementary Figure 1. Children's height and weight standard deviation score (SDS) from 3 months to 5 year of age related to medians of the lowest (below 25) and highest (above 75) quartiles of 2'FL /LNnT ratio, 2'FL and LNnT in the group of non-secretor mothers (n=103). 5Log-transformed data.



Supplementary Figure 2. Children's height and weight as standard deviation score (SDS) from 3 months to 5 year of age related to medians of the lowest (below 25) and highest (above 75) quartiles of 2'FL /LNnT ratio, 2'FL and LNnT in the group of secretor and non-secretor mothers together (n=802). Log-transformed data.