Am J Physiol Endocrinol Metab 316: E347–E357, 2019.
First published November 13, 2018; doi:10.1152/ajpendo.00320.2018.

RESEARCH ARTICLE

Evidence of human milk oligosaccharides in maternal circulation already during pregnancy: a pilot study

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Submitted 3 August 2018; accepted in final form 4 November 2018

Jantscher-Krenn E, Aigner J, Reiter B, Köfeler H, Csapo B, Desoye G, Bode L, van Poppel MN. Evidence of human milk oligosaccharides in maternal circulation already during pregnancy: a pilot study. *Am J Physiol Endocrinol Metab* 316: E347–E357, 2019. First published November 13, 2018; doi:10.1152/ajpendo.00320.2018.—Human milk oligosaccharides (HMOs) are bioactive glycans linked with health benefits to both the breast-fed infant and lactating mother. We hypothesized that HMOs are present before lactation, already during pregnancy, and are influenced by maternal body composition. In a pilot study, we investigated individual and temporal variations in HMO composition and concentration in maternal serum at *gestational weeks 10–14* (*visit 1*), *20–24* (*visit 2*), and *30–35 (visit 3*) (V1, V2, and V3, respectively) and associations with maternal body composition. HMOs were quantified by HPLC and confirmed by enzymatic digest and mass spectrometry. Associations of maternal prepregnancy body mass index (BMI), subcutaneous adipose tissue (SAT) thickness, and adipokines with absolute and relative HMO concentrations were analyzed by Spearman correlation. We identified 16 HMOs and 2 oligosaccharides not common to human milk. HMO concentration and composition varied with gestational age and secretor status. HMO concentration increased with gestational age and changed from a predominantly sialylated profile at V1 to a more balanced fucosylated-to-sialylated ratio at V3, mostly due to a profound increase in 2′-fucosyllactose (2′-FL), reflecting secretor phenotype. In secretor-positive women, BMI was negatively correlated with 2′-FL at V2. SAT at V1 and V2 were strongly negatively correlated with 2′-FL concentrations. This pilot study shows that prenatal HMOs are present in maternal serum, suggesting roles for HMOs already during pregnancy. Our result that maternal body composition is associated with prenatal HMOs might indicate that maternal metabolism modulates HMO composition with unknown implications for maternal and fetal health already during pregnancy.

2′-fucosyllactose; human milk oligosaccharides; metabolic programming; pregnancy; secretor status; subcutaneous adipose tissue

INTRODUCTION

Human milk oligosaccharides (HMOs) are highly abundant, bioactive glycans in human milk that offer a variety of potential benefits to the breast-fed infant (3). The best studied HMO effects are their topical effects in the infant gut, such as prebiotic effects on beneficial bacteria and antiadhesive/antimicrobial functions against pathogens. However, recent research has also increasingly focused on a wide range of potential systemic effects of HMOs, including protection from allergies, autoimmune diseases, metabolic disorders, or respiratory and urinary tract infections (10, 13, 14, 23, 25, 27, 29–31). HMOs are taken up from the infant gut into the circulation and can be detected in blood (19, 36) and urine (11, 12) of breast-fed infants. In vitro and in vivo studies showed HMOs can modulate immune functions (13, 14), inflammation (5, 6, 23, 29), and metabolic functions (2), supporting the emerging paradigm of HMOs as signal molecules in the systemic circulation.

More than 150 different HMO structures have been described (43). They all comprise lactose, which can be elongated by galactose/*N*-acetyl-glucosamine disaccharide units and/or modified by sialic acid and/or fucose residues. As the structure of an HMO determines its function, the relative abundance of individual structures may shape the resulting effect of a complex HMO mixture (4).

In milk, HMO concentration and composition vary within individuals and over the time of lactation (7, 8, 17, 38, 45). HMO concentrations are highest in colostrum and decline as milk matures. Concentration and composition also vary greatly between mothers (37). Part of this variation is explained by genetic polymorphisms in two genes, the *Secretor* and *Lewis* genes, leading to varying activities of the encoded fucosyltransferases [fucosyltransferase-2 (FUT-2) and fucosyltransferase-3 (FUT-3)] and therefore distinct fucosylation patterns in HMO profiles (39–41). Individuals with an inactive FUT2 have a negative secretor status and lack α1–2 fucosylated structures in their HMO profiles, such as 2′-fucosyllactose (2′-FL). In turn, the presence of 2′-FL is indicative of a positive secretor status. In addition to genetic factors, an emerging body of evidence suggests that environmental factors such as geographic location and maternal nutritional status also influence HMO concentration and composition (15, 32).

Although HMO research has traditionally focused on lactation and the roles of HMOs for the breast-fed infant, several studies dating back to the 1970s reported the presence of HMOs in the urine of pregnant women as early as 8 wk of gestation (20–22, 46). These observations suggest that HMOs

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are already synthesized during early pregnancy, circulate in the pregnant woman's blood, and might already affect both the mother and the growing fetus long before the start of lactation. Circulating HMOs may contribute to the physiological maternal adaptations of metabolism and immune functions during pregnancy. However, investigation of such potential novel roles of HMOs in pregnancy requires the primary information of HMO concentration and composition in the circulation of healthy pregnant women.

Thus, the aim of this pilot study was to analyze HMO concentration and composition in maternal serum in pregnant women over the course of gestation. We hypothesized that HMO concentration and composition in serum vary with maternal metabolic characteristics, such as body mass index (BMI), body composition, maternal serum leptin, and adiponectin. Here, we used a prospective, longitudinal pregnancy cohort to address these questions.

MATERIALS AND METHODS

Study overview. Subjects were selected from a prospective, observational, longitudinal study of 51 healthy pregnant women, recruited at the Department of Obstetrics, Medical University of Graz, between February and October 2013 (ClinalTrials.gov ID NCT03277807). Women were examined at 3 time points during routine visits for neonatal care, *visit 1* (V1, 10–14 gestational weeks), *visit 2* (V2, 20–24 gestational weeks), and *visit 3* (V3, 30–35 gestational weeks). Exclusion criteria were gestational age greater than the fourteenth week of gestation, multiple pregnancy, more than three consecutive miscarriages, fetal anomalies, diabetes type 1 or 2, prepregnancy hypertension, preeclampsia/hemolysis, elevated liver enzymes, low platelets (HELLP), and self-reported smoking. The selection criteria for this pilot study were absence of gestational pathologies developed during pregnancy and availability of serum samples at all three visits, resulting in a final sample size of 25 women. For association studies, subjects providing a serum sample from V1 and V2 were included ($n = 31$). Serum samples from 14 nonpregnant healthy women in the reproductive age served as nonpregnant controls. The study complied with the Declaration of Helsinki guidelines as revised in 2000 and was approved by the ethical committee of the Medical University of Graz (no. 26–380 ex 13/14). All subjects provided written informed consent.

Anthropometrics. Height and body weight were measured using a fixed stadiometer and a digital scale, respectively, with light clothing and without shoes at all three time points. BMI was calculated as weight (kg) per height (m^2). Prepregnancy BMI was calculated based on self-reported prepregnancy weight. Maternal subcutaneous adipose tissue (SAT) thickness was assessed at each visit using a Lipometer, a patented optical device (EU Pat. no. 0516251) measuring backscattered light intensities corresponding to SAT thickness (33). Measurements were performed in 15 well-defined body sites in triplicates on the left side of the body. SAT thickness (in mm) of all sites was summed as the estimation of total SAT.

Leptin and adiponectin measurements. Serum levels of leptin and adiponectin were measured in serum samples by multiplex assay according to the manufacturer's instructions (eBioscience, San Diego, CA).

Human milk oligosaccharide standards. 2′-FL, 3-fucosyllactose (3-FL), lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), lacto-*N*-fucopentaose (LNFP)-1, LNFP-2, and LNFP-3, lacto-*N*-difucohexaose (LNDFH)-1, and lacto-*N*-hexaose (LNH) were purchased from Dextra Laboratories (Reading, UK). Lactodifucotetraose (LDFT), 3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL), 3′-sialyllactosamine (3′-SLN), 6′-sialyllactosamine (6′-SLN), sialyllacto-*N*-tetraose (LST)-a, LST-b, and LST-c, and disialyllacto-*N*-tetraose (DSLNT) were purchased from Prozyme (Hayward, CA). HMOs isolated from pooled human milk were used as a standard reference for HMOs in human milk. This HMO mixture was prepared as previously described (25) and provided by the University of California, San Diego.

Human milk oligosaccharide isolation from maternal serum samples. Maternal venous blood samples were collected at each of the three visits. After centrifugation at 3,500 *g*, serum samples were stored at −80°C until later analysis. Oligosaccharides were isolated as previously reported (24). In brief, 50 μl serum were added to 350 μl H_2O containing the internal standard raffinose (Sigma Aldrich), and samples were subjected to two cycles of chloroform-methanol (2:1) extraction, followed by solid phase extraction using C18 columns (Thermo Fisher Scientific). Eluents were collected and loaded onto graphitized carbon columns (Thermo Fisher Scientific). After being washed with H_2O, HMOs were eluted with 40% acetonitrile containing 0.05% trifluoroacetic acid.

Human milk oligosaccharide analysis by HPLC. Recovered, dried HMOs from serum samples were fluorescently labeled with 2-aminobenzamide (2-AB), as previously described (25). The 2-AB-glycans were separated by HPLC with fluorescence detection on a TSKgel Amide-80 column (Tosoh Bioscience), with a linear gradient of a 50 mmol/l ammonium formate-acetonitrile solvent system. Separation was performed at 35°C and monitored by a fluorescence detector at 360 nm excitation and 425 nm emission. Standard retention times were used to annotate HPLC peaks. The amount of each individual HMO was calculated based on predetermined response factors. Relative abundance of each of the individual HMOs was determined by setting the sum of the 18 identified oligosaccharides as 100% total HMOs.

HMO peak annotation by comparison with retention times of commercially available HMO standards was confirmed by exoglycosidases and LC-MS.

Determination of oligosaccharides by LC-MS/MS. Five samples of the latest time point (35 wk of gestation) were prepared as described for the HPLC method and pooled. An Accela HPLC (Thermo Fisher Scientific) was used with a TSKgel Amide-80 (Tosoh Bioscience) and a linear gradient of a 50 mmol/l ammonium formate-acetonitrile solvent system. Oligosaccharides were determined by a TSQ Quantum Ultra (Thermo Fisher Scientific) triple quadrupole instrument in positive ESI mode. The spray voltage was set to 4,000 V, capillary voltage was set to 35 V, and vaporizer temperature was 250°C. Scan width was 1 Da with 0.1 s scan time for each transition.

Human milk oligosaccharide identification by enzymatic digest. Pooled 2-AB-labeled samples were aliquoted and either treated with α-2,3-sialidase, α-1,2-fucosidase (both New England Biolabs) or without enzyme according to the manufacturer's instructions. Enzyme-treated and untreated samples were run in parallel and analyzed by HPLC with fluorescence detection as described above.

Statistics. Concentrations of individual and total oligosaccharides were normalized to the internal standard raffinose (expressed as medians and range in nmol/ml). Relative abundances were calculated as percentage of an individual HMO of the summed unambiguously identified oligosaccharides (total HMOs). The slope of the HMO increase per week was calculated for each individual as change in HMO concentration from V1 to V2, divided by the time interval in weeks. The HMO 3-FL was excluded from all calculations because of a coelution with an unidentified peak.

Statistical analyses used SPSS (version 23; IBM SPSS, Chicago, IL) and GraphPad Prism (version 7.00; GraphPad Software, La Jolla, CA).

Differences between secretor-positive and secretor-negative women were assessed with Student's *t*-test for normally distributed variables and Mann-Whitney *U*-test for skewed variables. For analysis of differences in selected HMO variables between time points in matching samples, Friedman ANOVA followed by Dunn's multiple comparison testing was used. Bivariate correlations between HMOs and SAT thickness and other maternal parameters were assessed with the

Spearman's rank correlation coefficient because of skewed data. Adjustment of the analyses for maternal age did not change the results and are therefore not shown. Statistical significance was assumed when $P < 0.05$.

RESULTS

Human milk oligosaccharides are found in serum of pregnant women. To test whether HMOs can be detected in maternal serum during pregnancy, we used an HPLC-based method previously described for HMO analysis in rat serum (24). We first analyzed a small subset of samples from five individuals at *week 35* of gestation and five nonpregnant control samples and compared HPLC chromatograms to the HMO profile of pooled human milk. Representative chromatograms are shown in Fig. 1. Peak annotation of pregnant serum profiles using standard retention times revealed 16 HMOs also present in pooled human milk (2′-FL, 3-FL, LDFT, 3′-SL, 6′-SL, LNT, LNnT, LNFP-1, -2, and -3, LST-a, -b, and -c, LNDFH, LNH, and DSLNT). The total number of oligosaccharide peaks found in maternal serum profiles was smaller than in pooled human milk; 3′-SL and 2′-FL were the most abundant HMOs in serum. LNT, one of the major HMOs in pooled human milk, was observed in minute abundances in maternal serum. Maternal serum profiles showed two additional peaks absent in pooled human milk, which we annotated as 3′-SLN and 6′-SLN. Serum profiles of nonpregnant controls revealed 3′-SLN and 3′-SL peaks, but no other oligosaccharides.

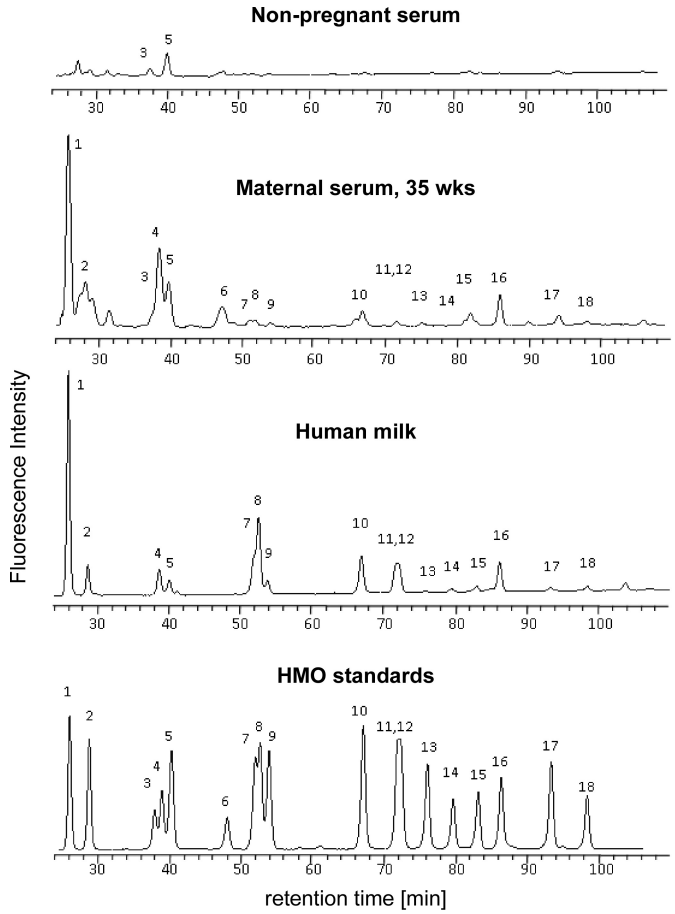

Fig. 1. Human milk oligosaccharides (HMOs) are present in maternal serum in pregnancy. Representative HPLC chromatograms of oligosaccharides in nonpregnant control serum (*first panel*) or pregnant serum at 35 wk of gestation (*second panel*) are shown. As controls, oligosaccharides from pooled human milk (*third panel*) and a mixture of commercially available human milk oligosaccharide standards (*fourth panel*) are shown. (1), 2′-fucosyllactose (2′-FL); (2), 3-fucosyllactose (3-FL); (3), 3′-siallyllactosamine (3′-SLN); (4), lactodifucotetraose (LDFT); (5), 3′-sialyllactose (3′-SL); (6) 6′-siallyllactosamine (6′-SLN); (7), 6′-sialyllactose (6′-SL); (8), lacto-*N*-tetraose (LNT); (9), lacto-*N*-neotetraose (LNnT); (10), lacto-*N*-fucopentaose-1 (LNFP-1); (11, 12), lacto-*N*-fucopentaose-2/3 (LNFP-2/3); (13), sialyllacto-*N*-tetraose-a (LST-a); (14), sialyllacto-*N*-tetraose-b (LST-b); (15), sialyllacto-*N*-tetraose-c (LST-c); (16), lacto-*N*-difucohexaose (LNDFH); (17), lacto-*N*-hexaose (LNH); (18), disialyllacto-*N*-tetraose (DSLNT).

We confirmed the identity of the most abundant HMOs in maternal serum using exoglycosidase digest. Treatment with either α-1,2-fucosidase or α-2,3-sialidase resulted in removal or major reduction of the expected peaks (2′-FL, LDFT, LNFP-1, and LNDFH for α-1–2 fucosidase and 3′-SL and 3′-SLN for α-2,3-sialidase, respectively), and an increase in respective peaks (lactose, 3-FL, LNT). Fig. 2 shows representative chromatograms with and without treatment with exoglycosidases. Using LC-MS, we confirmed the respective masses for all 18 peaks assigned by retention times of standards (Fig. 3).

Human milk oligosaccharide concentrations in maternal serum vary with gestational age. To investigate temporal variations during the pregnancy, we determined concentration and composition of HMOs in serum in a longitudinal cohort study. Table 1 shows the main characteristics of the study participants. Twenty-six (84%) of the women were normal weight and five women (16%) were overweight (mean prepregnancy BMI of 21.9 ± 2.9 kg/m^2). We found HMOs in serum samples from all pregnant women already at V1. In nonpregnant women ($n = 14$), we found traces of 3′-SLN and 3-SL, but no other HMOs were detectable, revealing a significant increase in total HMOs by virtue of pregnancy already at V1 (Fig. 4*A*). Mean total HMO concentrations were 0.24 nmol/ml [95% confidence interval (CI) of mean 0.21–0.29], 0.47 nmol/ml (95% CI of mean 0.37–0.57), and 0.49 nmol/ml (95% CI of mean 0.27–0.72) at V1, V2, and V3, respectively, revealing a significant effect of gestational age on the total HMO concentration (Friedman ANOVA $P = 0.0093$) (Fig. 4*A*). Individual and total HMO concentrations in serum at three time points during gestation are shown in Table 2. A significant increase of the secretor-active HMOs, 2′-FL and LDFT, (ANOVA $P = 0.0001$ and $P = 0.0014$, respectively) mostly accounted for the increase in total HMOs over time. This induction of 2′-FL and LDFT seemed to mostly occur between V1 and V2 (Table 2). The slope, calculated as weekly concentration change from V1 to V2, was highest for 2′-FL [9.5 pmol/ml per week; interquartile range (IQR) 2.1–23.7] and significantly higher than the slopes of LDFT ($P < 0.0001$), and of the sialylated HMOs, 3′-SL and 3′-SLN ($P = 0.0005$ and $P < 0.0001$, respectively) (Fig. 4*B*). 2′-FL and LDFT were increased by 5.5-fold (IQR 1.5–14.1) and 3.0-fold (IQR 0.86–5.4), respectively, relative to concentrations at V1, and showed the highest fold changes of individual and grouped HMOs (data not shown). 3′-SLN and 3′-SL remained relatively stable, or only modestly increased over time (Table 2, Fig. 4*B*).

Human milk oligosaccharide composition in maternal serum varies with secretor status. Having observed the appearance of secretor-active HMOs between V1 and V2, we asked to which extent the secretor phenotype, once established, accounts for

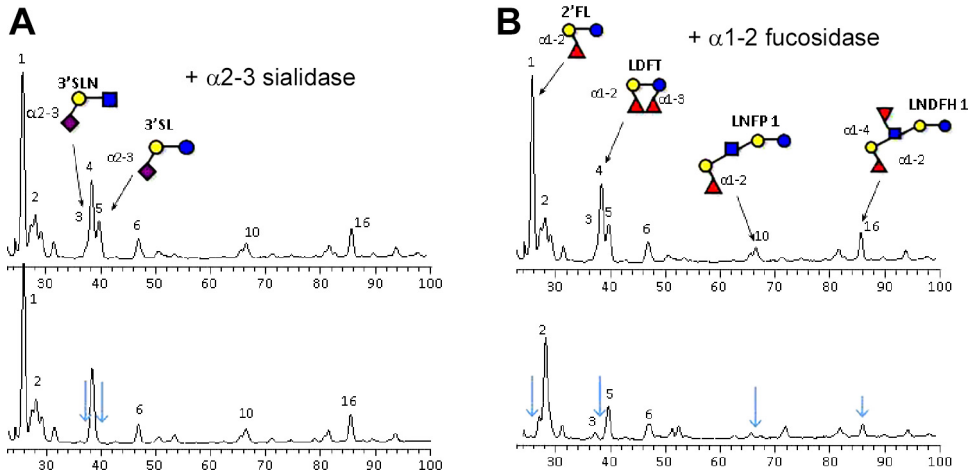

Fig. 2. Confirmation of human milk oligosaccharide (HMO) identity by enzymatic digest. 2-Aminobenzamide (2-AB)-labeled HMO isolations from 5 individuals at 35 wk of gestation were pooled and split into three aliquots for treatments without enzyme, with α-2,3-sialidase, or with α-1,2-fucosidase. Representative HPLC chromatograms show HMOs without treatment (*top*) and after enzymatic digest (*bottom*) using α-2,3-sialidase (*A*) and α1,2-fucosidase (*B*). Arrows depict removed or reduced peaks. Oligosaccharide composition of selected HMOs are shown with glucose (blue circle), galactose (yellow circle), fucose (red triangle), and sialic acid (purple diamond) as monosaccharide building blocks. (1), 2′-fucosyllactose (2′-FL); (2), 3-fucosyllactose (3-FL); (3), 3′-siallyllactosamine (3′-SLN); (4), lactodifucotetraose (LDFT); (5), 3′-sialyllactose (3′-SL); (6), 6′-siallyllactosamine (6′-SLN); (10), lacto-*N*-fucopentaose 1 (LNFP-1); (16), lacto-*N*-difucohexaose (LNDFH).

compositional variation in HMOs. We first assessed whether the secretor phenotype is consistent throughout pregnancy. At V1, 2′-FL and LDFT levels were generally very low. In 10 of 25 serum samples (40%), relative 2′-FL concentrations were below 5% of total HMOs. From V1 to V3, relative abundances of 2′-FL increased from 6.5% (IQR 3.7–10.2) at V1 to 22.7% (IQR 10.1–34.9) at V2 and 33.8% (IQR 18.1–41.6) at V3, respectively (Fig. 5*A*). In 4 of 25 women (16%) of whom we had a complete sample set from all three visits, 2′-FL remained under an arbitrarily set threshold of 5% at mid and late pregnancy and was therefore classified as secretor negative (Fig. 5*A*, open symbols). The heat map (Fig. 5*B*) shows the variation in relative 2′-FL abundances in individuals at V1, V2, and V3, and arrows depict negative secretor phenotype. All women, who at V3 expressed 2′-FL and were assigned positive secretor status accordingly, expressed 2′-FL above the threshold already at V2. For one woman who failed to provide a V3 sample but provided samples from V1 and V2 and a later sample (delivery), we also found 2′-FL below the threshold at mid and late pregnancy, and her sample at V2 was included for comparison of HMOs at V2 between the secretor-positive (n = 21) and secretor-negative group (n = 5). When absolute HMO

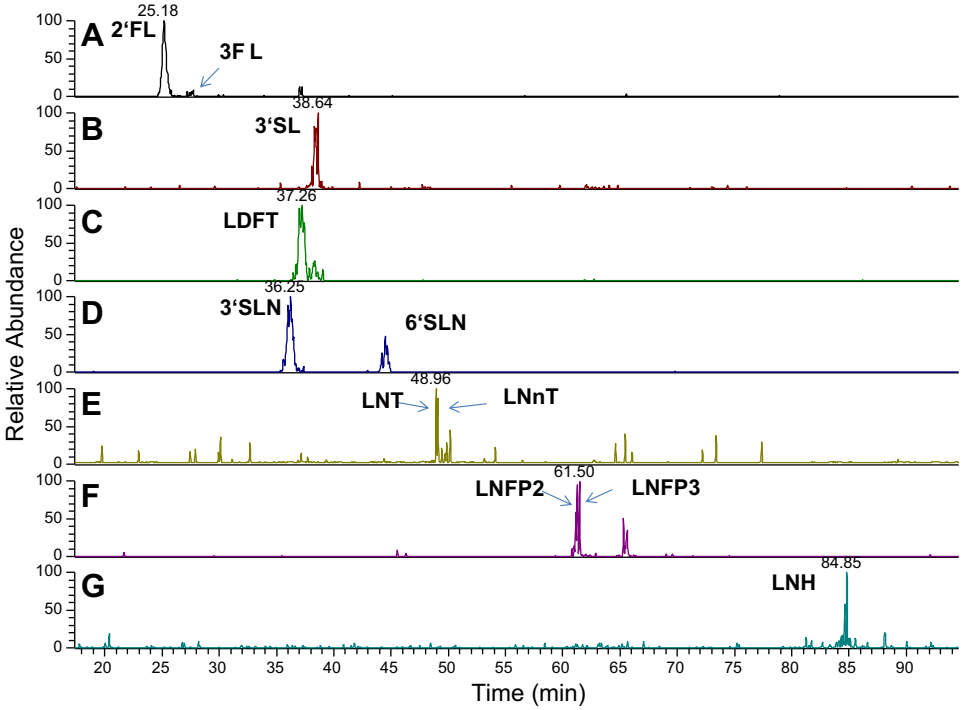

Fig. 3. Confirmation of human milk oligosaccharide (HMO) identity by mass spectrometry. Mass chromatograms of oligosaccharides isolated from pooled serum samples of 5 pregnant women at 35 wk of gestation, acquired by LC-MS/MS in multiple reaction monitoring mode are shown. Panels show the mass transitions 609/301 for 2′-fucosyllactose (2′-FL) and 3-fucosyllactose (3-FL) (*A*), 754/301 for 3′-sialyllactose (3′-SL) (*B*), 755/301 for lactodifucotetraose (LDFT) (*C*), 795/325 for 3′-siallyllactosamine (3′-SLN) and 6′-siallyllactosamine (6′-SLN) (*D*), 828/301 for lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT) (*E*), 974/301 for lacto-*N*-fucopentaose 2/3 (LNFP2/3) (*F*), and 1193/138 for lacto-*N*-hexaose (LNH) (*G*).

Table 1. *Maternal characteristics of study population*

Maternal Characteristics	Total, $n = 31$	Se Positive, $n = 26$	Se Negative, $n = 5$
Age, yr	34.2 ± 4.9	34.3 ± 4.8	33.8 ± 5.8
Height, cm	167.9 ± 5.8	167.5 ± 6.2	170.2 ± 3.2
Weight, kg (prepregnancy)	61.7 ± 8.2	61.2 ± 8.2	64.4 ± 8.1
Gestational weight gain, kg	15.3 ± 5.7	14.8 ± 5.2	18.2 ± 7.8
Weight gain V1 to V2, kg	4.1 ± 2.0	4.2 ± 1.9	3.8 ± 2.6
BMI, kg/m^2 (prepregnancy)	21.9 ± 2.6	21.8 ± 2.6	22.2 ± 2.8
BMI category			
Normal (BMI 18–25) (*n*)	26 (84%)	22 (85%)	5 (80%)
Overweight (BMI 25–30) (*n*)	5 (16%)	4 (15%)	1 (20%)
BMI			
V1, kg/m^2	22.3 ± 2.4	22.1 ± 2.3	23.3 ± 2.7
V2, kg/m^2	24.4 ± 2.4	24.3 ± 2.6	24.7 ± 2.1
V3, kg/m^2	27.0 ± 2.4	27.0 ± 2.4	26.7 ± 2.7
Total SAT			
V1, mm	148.2 ± 36.8	143.8 ± 37.8	170.1 ± 23
V2, mm	159.8 ± 34.2	155.3 ± 31.4	178.4 ± 42.9
V3, mm	149.8 ± 27.5	151.3 ± 27.4	142.4 ± 32.9

Values are means ± SD; n = number of participants. BMI, body mass index; SAT, subcutaneous adipose tissue; V1, *visit 1* (10–14 gestational weeks); V2, *visit 2* (20–24 gestational weeks); V3, *visit 3* (30–35 gestational weeks). Student's *t*-test was performed to test for differences in the characteristics between secretor (Se)-positive and Se-negative group. There were no differences in any of the parameters.

concentrations were compared in secretor-positive and secretor-negative women separately, the temporal change in total HMOs was only significant in the secretor-positive group (Fig. 6). The lack of rise in 2′-FL in secretor-negative women seemed to account for lower total HMO concentrations at V2 and V3. However, this did not reach significance. Absolute and relative 2′-FL concentrations at V2 significantly correlated with concentrations at V1 ($r = 0.42$, $P = 0.034$ and $r = 0.42$, $P = 0.039$, respectively) and with 2′-FL concentrations at V3 ($r = 0.50$, $P = 0.01$, and $r = 0.65$, $P < 0.0001$, respectively) (data not shown). Moreover, at V2, the secretor-active HMOs, 2′-FL, LDFT, and LDFH strongly positively correlated with each other ($P < 0.00001$), indicating common regulation, whereas 3′-SL was strongly positively correlated with 3′-SLN, and to a lesser extent with 6′-SL and 6′-SLN, and independent from fucosylated HMOs (data not shown).

Overall fucosylation increases with gestational age. Having found that individual HMO structures increased at different rates, we next investigated compositional changes in the overall abundance of grouped sialylated, fucosylated, and unmodified HMOs during pregnancy. Not surprisingly, composition of sialylated and fucosylated HMOs as percentage of total HMOs significantly changed with gestational age, and these changes were only seen in secretor-positive women (data not shown). The fucosylated HMOs significantly increased during the pregnancy, from 26.3% (95% CI of mean 21.7–30.9) of total fucosylation at V1 to 46.6% (95% CI of mean 39.8–56.3) at V2 and 57.9% (95% CI of mean 51.6–64.2) at V3 (repeated measures ANOVA $P < 0.0001$) in the group of secretor-positive women ($n = 21$). In turn, relative abundance of sialylated HMO peaked at 71.8% (95% CI of mean 67.5–76.1) at V1 and dropped to 51.7% (95% CI of mean 44.9–58.5%) at V2 and 40.5% (95% CI of mean 34.4–46.6) at V3 (repeated measures ANOVA $P < 0.0001$). In secretor-negative women ($n = 4$), relative fucosylation (V1: 17.4%, V2: 16.2%, V3: 15.4%) and sialylation (V1: 81.9%, V2: 79.2%, V3: 78.9%) did not significantly change over time.

Higher maternal adiposity associates with lower 2′-FL concentrations. Despite the relatively small sample size in this pilot study, we asked whether maternal body composition might also influence HMO concentration or composition in serum of pregnant women. Because HMO concentrations significantly increased from V1 to V2, mostly by the induction of two secretor-active HMOs, we focused on associations of maternal factors before and early in pregnancy (prepregnancy, V1 and V2), with absolute or relative concentrations of HMOs (individual or grouped HMOs) in midpregnancy (V2). To increase sample size, here we also included women of whom we had serum samples from V2 available, despite missing samples from V3, leading to a total sample size of $n = 31$ for further analyses. Because secretor status has a major influence on HMOs, we analyzed the group of secretor-positive women only ($n = 26$). We investigated associations of different proxy

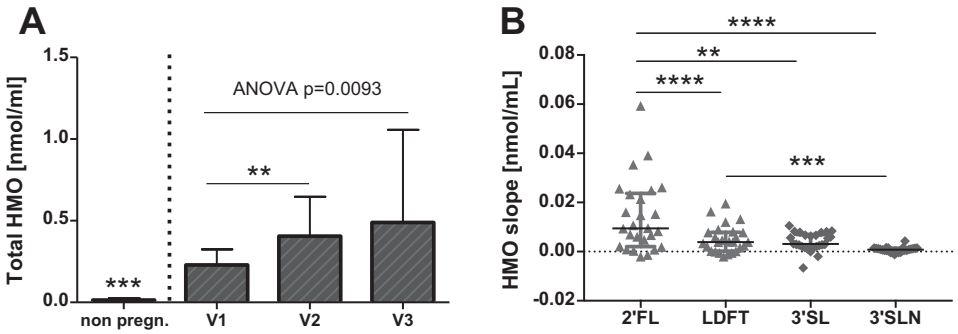

Fig. 4. Human milk oligosaccharide (HMO) concentrations increase with gestational age at different rates for individual HMOs. *A*: HMOs were measured by HPLC in serum of nonpregnant women ($n = 14$) and pregnant women ($n = 25$) at each of the 3 visits during pregnancy and expressed as the sum of individual HMO concentrations. Asterisks show significant difference between the nonpregnant controls and all time points during pregnancy (Student's *t*-test, $P < 0.0001$). ***$P < 0.001$, **$P < 0.01$. Total HMOs were significantly different over time (Friedman ANOVA, $P = 0.0093$). Data are means with SD. Total concentrations were significantly different between V1 and V2 (Dunn's test, $P = 0.0089$). *B*: Fold changes of total HMOs, grouped fucosylated or sialylated HMOs, and individual HMOs at V2 relative to V1. Groups of HMOs are shown as triangles (fucosylated) and diamonds (sialylated). Asterisks mark significant changes compared with concentration at V1 by Wilcoxon test. Data are medians and interquartile ranges. 2′-FL, 2′-fucosyllactose; LDFT, lactodifucotetraose; 3′-SL, 3′-sialyllactose; 3′-SLN, 3′-sialyllacotsamine; V1, *visit 1* (10–14 gestational weeks); V2, *visit 2* (20–24 gestational weeks); V3, *visit 3* (30–35 gestational weeks). ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$; ($n = 25$ pregnant women).

Table 2. *HMO concentration in maternal serum during pregnancy*

	V1			V2			V3			
HMO	Se pos. (n = 26)	P	Se neg. (n = 5)	Se pos. (n = 26)	P	Se neg. (n = 5)	Se pos. (n = 25)	P	Se neg. (n = 4)	ANOVA P value
2′-FL	16.4 ± 16.1[b]	0.63	11.8 ± 6.5	135.8 ± 113.5[a]	**<0.0001**	7.0 ± 5.7	181.0 ± 183.1[a]	**<0.0001**	4.5 ± 3.3	**<0.0001**
3′-SLN	24.2 ± 10.4	0.41	26.2 ± 5.5	28.7 ± 11.1[c]	0.70	31.3 ± 11.3	52.8 ± 96.7[b]	0.93	24.2 ± 28.7	**0.04**
LDFT	15.0 ± 11.5[b,c]	**<0.01**	4.8 ± 0.4	57.2 ± 44.3[a]	**<0.0001**	1.9 ± 2.6	84.5 ± 106.5[a]	**<0.0001**	1.1 ± 1.6	**<0.0001**
3′-SL	79.3[b] ± 30.5	0.85	78.2 ± 23.3	102.5 ± 33.3[a]	0.62	110.3 ± 43.9	148.7 ± 195.1	0.93	88.5 ± 103.9	**0.02**
6′-SLN	14.1 ± 9.4	0.22	15.6 ± 5.7	18.7 ± 9.3[c]	0.90	17.8 ± 8.6	29.9 ± 59.3[b]	0.97	17.9 ± 20.3	**0.02**
6′-SL	7.0 ± 6.2	0.16	3.0 ± 3.0	10.5 ± 8.2[c]	0.28	13.1 ± 7.7	7.5 ± 10.3[b]	0.20	11.9 ± 10.5	**0.007**
LNT	2.2 ± 4.5	0.25	ND	1.2 ± 1.7	0.06	5.5 ± 6.0	1.3 ± 2.9	**0.03**	8.7 ± 12.4	0.40
LNnT	3.4 ± 10.2	>0.99	1.2 ± 2.7	6.5 ± 6.5	0.69	7.3 ± 5.3	6.5 ± 10	0.64	3.6 ± 3.6	**0.002**
LNFP-1	22.7 ± 14.6	0.59	17.4 ± 8.2	20.3 ± 15	0.81	17.7 ± 6.5	21.1 ± 27.9	0.37	7.4 ± 5.3	0.09
LNFP-2/3	1.1 ± 4.7[b,c]	>0.99	ND	5.0 ± 4.7[a]	**<0.05**	11.5 ± 5.9	7.5 ± 12.5[a]	0.19	14.1 ± 19.4	**<0.0001**
LST-c	24.9[c] ± 28.6	0.72	20.4 ± 23.7	30.6 ± 16.9[c]	0.62	33.3 ± 10.0	5.6 ± 18.2[a,b]	0.16	5.2 ± 7.6	**<0.0001**
LNDFH	0.9 ± 2.5[b,c]	0.59	ND	9.2 ± 8.9[a]	**0.002**	ND	8.6 ± 14.6[a]	0.22	ND	**<0.0001**
DSLNT	0.9 ± 2.2[b,c]	0.85	0.4 ± 0.9	4.0 ± 3.2[a]	0.10	7.4 ± 4.1	6.1 ± 8.8[a]	0.94	5.0 ± 8.5	**0.0004**
Fucosylated	56.0 ± 33[b,c]	**0.03**	33.6 ± 11.9	227.5 ± 158.5[a]	**0.001**	39 ± 15.7	302.7 ± 312.9[a]	**0.001**	27.2 ± 26.7	**<0.0001**
Sialylated	151.3 ± 57.9[b]	>0.99	143.5 ± 41.8	200.3 ± 62[a,c]	0.70	221.1 ± 69.4	254.8 ± 316.4[b]	0.98	159.8 ± 176.4	**0.007**
Unmodified	5.6 ± 12.5	0.44	1.3 ± 2.9	7.7 ± 7.0	0.21	12.8 ± 6.7	7.8 ± 11.8	0.22	12.4 ± 16.0	0.09
Total HMO	213.0 ± 80.6[b]	0.49	178.4 ± 46.4	435.5 ± 203.6[a]	0.08	272.9 ± 76.1	565.2 ± 608.5	0.11	199.3 ± 218.4	**0.0021**

Values are means ± SD (in pmol/ml); n = number of participants. 2′-FL, 2′-fucosyllactose; 3′-SLN, 3′-siallyllactosamine; 3′-SL, 3′-sialyllactose; 6′-SL, 6′-sialyllactose; 6′-SLN, 6′-siallyllactosamine; DSLNT, disialyllacto-*N*-tetraose; LDFT, lactodifucotetraose; LNDFH, lacto-*N*-difucohexaose; LNFP-1, lacto-*N*-fucopentaose-1; LNFP-2/3, lacto-*N*-fucopentaose-2/3; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; LST-c, sialyllacto-*N*-tetraose-c; ND, not detectable. Mann-Whitney test was used to test for significant differences in concentrations between the secretor (Se)-positive and Se-negative group. Friedman ANOVA with Dunn's multiple comparison testing was performed in the Se-positive group to test whether time (V1, V2, V3) had a significant effect on individual, grouped fucosylated, sialylated, and unmodified or total human milk oligosaccharide (HMO) concentrations. Significant correlations are shown in bold ($P < 0.05$). Superscript letters indicate significance ($P < 0.05$) with the denoted visits ([a]V1; [b]V2; [c]V3).

measures for body composition and fat mass, such as BMI, leptin, adiponectin, and SAT thickness with the absolute and relative abundance of the most abundant HMOs, 2′-FL and 3′-SL, and grouped fucosylated and sialylated HMOs at V2. Table 3 shows the Spearman's rank coefficients; Fig. 7 shows correlations with SAT and 2′-FL and 3′-SL.

Prepregnancy BMI was significantly associated with absolute concentrations of 2′-FL ($r = -0.42$, $P = 0.03$) and fucosylated HMOs at V2 ($r = -0.39$, $P < 0.05$).

Total SAT thickness, measured at V1 as the sum of all 15 assessment sites, negatively correlated with 2′-FL ($r = -0.41$, $P = 0.04$) concentration. The strongest associations were calculated for total SAT measured at V2 with absolute concentrations of total HMOs ($r = -0.51$ $P = 0.02$), fucosylated HMOs ($r = -0.63$, $P = 0.002$), and 2′-FL ($r = -0.71$, $P < 0.001$) at V2. Maternal serum leptin concentrations at V2 were positively correlated with 3′-SL concentration ($r = 0.40$, $P = 0.04$), and this association was even stronger with the 3′SL slope between V1 and V2 ($r = 0.59$, $P = 0.004$; data not shown). Weight gain from V1 to V2, or other tested maternal factors such as maternal age and height, did not significantly correlate with the concentrations of the HMOs tested (Table 3, Fig. 7).

Maternal adiposity is associated with relative human milk oligosaccharide composition. To test whether maternal pregestational factors or factors early in pregnancy might influence HMO composition, we analyzed associations with relative HMO concentrations. Spearman's rank coefficients are given in Table 3; Fig. 7 shows correlations of SAT with relative concentrations of 2′-FL or 3′-SL in serum.

Prepregnancy BMI and BMI at V2 were significantly negatively correlated with 2′-FL percentage ($r = -0.46$, $P = 0.02$

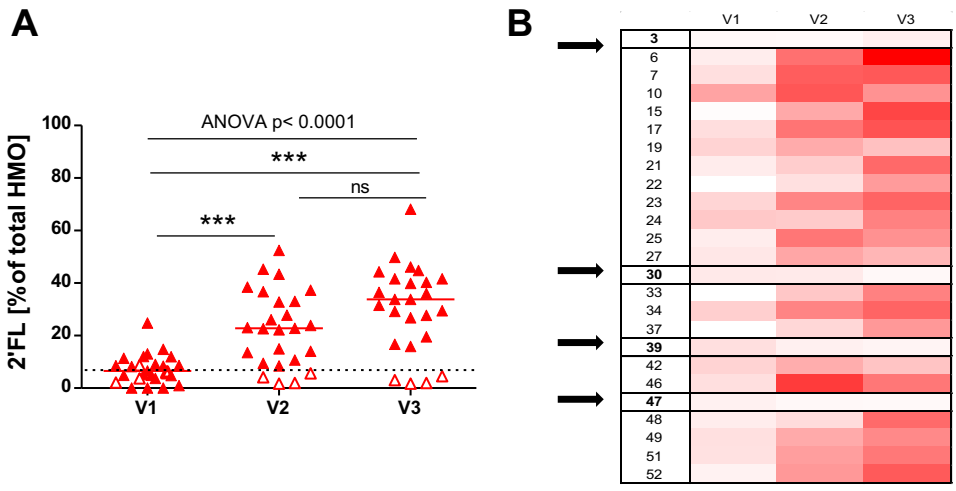

Fig. 5. Relative 2′-fucosyllactose (2′-FL) concentration in maternal serum at midpregnancy allows for assignment of secretor status. *A*: scatter plot showing median 2′-FL percentage of total human milk oligosaccharides (HMOs) increased significantly with gestational age in repeated measurements in serum during pregnancy (Friedman ANOVA $P < 0.0001$). At 20 and 33 wk of gestation, 2′-FL percentage clustered above (solid triangles) and below (open triangles) 5%. *B*: heat map showing 2′-FL percentage at 3 visits of 25 individuals. Depicted are 4 secretor-negative individuals (nos. 3, 30, 39, and 47). Intensity of red color represents 2′-FL percentage, white bars indicate no relative 2′-FL concentration (n = 25 pregnant women).

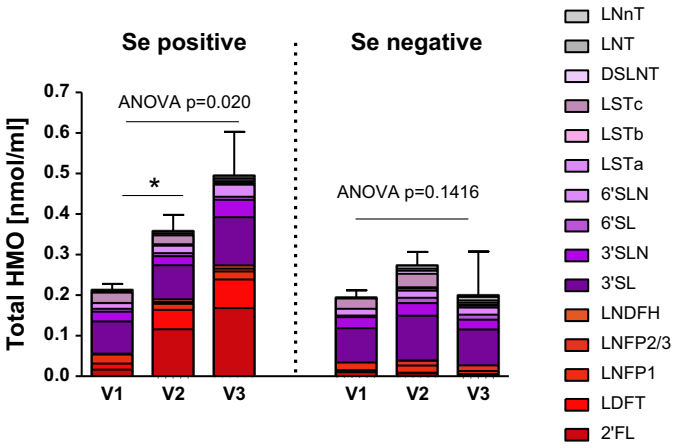

Fig. 6. Total and individual human milk oligosaccharide (HMO) concentrations in maternal serum during pregnancy in secretor-positive and secretor-negative women. Stacked bar plots show total HMO concentrations (means ± SE) in maternal serum at 3 visits (V1, V2, V3) in secretor-positive (*left*, $n = 21$) and secretor-negative (*right*, $n = 4$) women with individual HMOs colored according to their type of modification in shades of red (fucosylated), shades of purple (sialylated), and gray (unmodified). Within the secretor-positive group, we found differences in total HMO concentrations over time (Friedman ANOVA with Dunn's multiple comparison testing), whereas no difference was found within the secretor-negative group. 2′-FL, 2′-fucosyllactose; 3′-SLN, 3′-siallyllactosamine; LDFT, lactodifucotetraose; 3′-SL, 3′-sialyllactose; 6′-SLN, 6′siallyllactosamine; 6′-SL, 6′-sialyllactose; LNT, lacto-*N*-tetraose; LNnT, lacto-*N*-neotetraose; LNFP-1, lacto-*N*-fucopentaose-1; LNFP-2/3, lacto-*N*-fucopentaose-2/3; LST-a, sialyllacto-*N*-tetraose-a; LST-b, sialyllacto-*N*-tetraose-b; LST-c, sialyllacto-*N*-tetraose-c; LNDFH, lacto-*N*-difucohexaose; LNH, lacto-*N*-hexaose; DSLNT, disialyllacto-*N*-tetraose; V1, *visit 1* (10–14 gestational weeks); V2, *visit 2* (20–24 gestational weeks); V3, *visit 3* (30–35 gestational weeks).

and $r = -0.63$, $P = 0.003$, respectively) and positively correlated with percentage of 3′-SL ($r = 0.39$, $P = 0.04$ and $r = 0.49$, $P = 0.03$, respectively) and with sialylated HMOs ($r = 0.42$, $P = 0.03$ and $r = 0.52$, $P = 0.02$, respectively). Total SAT at V1 was negatively correlated with percentage of 2′-FL ($r = -0.54$, $P = 0.005$) and fucosylated HMOs ($r = -0.42$, $P = 0.03$) at V2. In turn, total SAT at V1 was positively associated with percentage of 3′-SL ($r = 0.43$, $P = 0.03$) and sialylated HMOs ($r = 0.38$, $P = 0.053$). Total SAT at V2 was highly negatively correlated with percentage of 2′-FL ($r = -0.66$, $P = 0.001$) and fucosylated HMOs ($r = -0.63$, $P = 0.002$) and positively with percentage of 3′-SL ($r = 0.66$, $P = 0.001$) and sialylated HMOs ($r = 0.61$, $P = 0.003$) (Table 3, Fig. 7). Maternal serum leptin at V1 or V2 was not significantly correlated with HMO composition. However, the leptin-to-adiponectin ratio at V2 was negatively correlated with percentage of 2′FL at V2 ($r = -0.44$, $P = 0.02$) and positively correlated with percentage of 3′-SL ($r = 0.43$, $P = 0.03$) and sialylated HMOs ($r = 0.35$, $P = 0.073$), the latter without reaching significance (Table 3).

DISCUSSION

This is the first study to show the presence of HMOs in maternal serum during pregnancy. Furthermore, this pilot study describes dynamic changes in concentration and composition of and individual variation in prenatal HMOs with gestational age and secretor status. Despite the relatively small sample size, we found strong negative associations of maternal fat mass in early and midpregnancy with fucosylated HMOs, i.e., 2′-FL, suggesting a potential influence of maternal metabolic status on concentration or composition of prenatal HMOs.

Human milk oligosaccharides in maternal serum versus milk. Concentrations of total HMOs in serum during pregnancy were found in the range of 0.1–1.8 nmol/ml, which is several orders of magnitude lower than mean concentrations reported in human milk (7, 8, 32, 38, 45) and within the range of HMO concentrations found in breast-fed infant serum (36). High proportions of sialylated HMOs (43–64%) in pregnant serum contrast reported HMO composition in milk, where fucosylated HMOs are most abundant (50 to 80% of total HMOs) (7, 8, 34, 35, 42). Because we did not have matching postpartum maternal serum samples or breast milk samples available, we cannot conclude whether this difference in sialylation/fucosylation reflects a temporal factor (prepartum versus postpartum), a matrix factor (milk versus blood), or both. Two studies reporting on HMOs in blood from breast-fed infants found different HMOs with little overlap between the studies (19, 36). One of these studies reported sialylated HMOs in high relative abundance (36). Despite obvious differences in the routes of HMOs into the circulation in breast-fed infants versus pregnant women, we also found high relative abundance of sialylated HMOs in maternal serum. This might lead to speculations about a generally higher sialylation of HMOs in blood

Table 3. *Spearman's rank correlations between maternal factors and serum HMOs at visit 2 in secretor-positive group*

	2′-FL				3′-SL				Fucosylated HMO				Sialylated HMO			
	Abs		%		Abs		%		Abs		%		Abs		%	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Maternal age	−0.29	0.15	−0.28	0.16	−0.09	0.65	0.37	0.06	−0.27	0.18	−0.29	0.14	−0.31	0.12	0.26	0.18
Height	0.19	0.34	0.06	0.75	−0.27	0.18	−0.16	0.43	0.25	0.23	0.19	0.33	−0.05	0.79	−0.15	0.45
BMI_{pp}	**−0.42**	**0.03**	**−0.46**	**0.02**	−0.15	0.47	**0.39**	**0.04**	**−0.39**	**<0.05**	**−0.46**	**0.02**	−0.16	0.43	**0.42**	**0.03**
BMI V2	**−0.6**	**0.006**	**−0.63**	**0.003**	−0.19	0.42	**0.49**	**0.03**	**−0.49**	**0.04**	**−0.53**	**0.02**	−0.36	0.12	**0.52**	**0.02**
Total SAT V1	**−0.41**	**0.04**	**−0.54**	**0.005**	0.21	0.31	**0.43**	**0.03**	−0.33	0.11	**−0.42**	**0.03**	0.04	0.84	0.38	0.05
Total SAT V2	**−0.71**	**<0.001**	**−0.66**	**0.001**	0.16	0.48	**0.66**	**0.001**	**−0.63**	**0.002**	**−0.63**	**0.002**	−0.09	0.7	**0.61**	**0.003**
Leptin V1	−0.18	0.37	−0.19	0.34	0.13	0.52	0.22	0.27	−0.21	0.31	−0.21	0.29	0.08	0.69	0.22	0.28
Leptin V2	−0.18	0.38	−0.29	0.15	**0.4**	**0.04**	0.31	0.11	−0.1	0.61	−0.2	0.31	0.22	0.28	0.24	0.23
Lept/Adip V1	−0.25	0.22	−0.26	0.19	0.23	0.27	0.37	0.06	−0.25	0.22	−0.29	0.15	−0.05	0.8	0.3	0.13
Lept/Adip V2	−0.33	0.1	**−0.44**	**0.02**	0.37	0.06	**0.43**	**0.03**	−0.22	0.28	−0.29	0.14	0.1	0.62	0.35	0.07

Significant correlations are shown in bold ($P < 0.05$). 2′-FL, 2′-fucosyllactose; 3′-SL, 3′-siallyllactose; abs, absolute; BMI_{pp}, body mass index prepregnancy; HMO, human milk oligosaccharide; Lept/Adip, leptin-to-adiponectin ratio; SAT, subcutaneous adipose tissue; V1, *visit 1* (10–14 gestational weeks); V2, *visit 2* (20–24 gestational weeks); V3, *visit 3* (30–35 gestational weeks). $n = 26$ secretor-positive women.

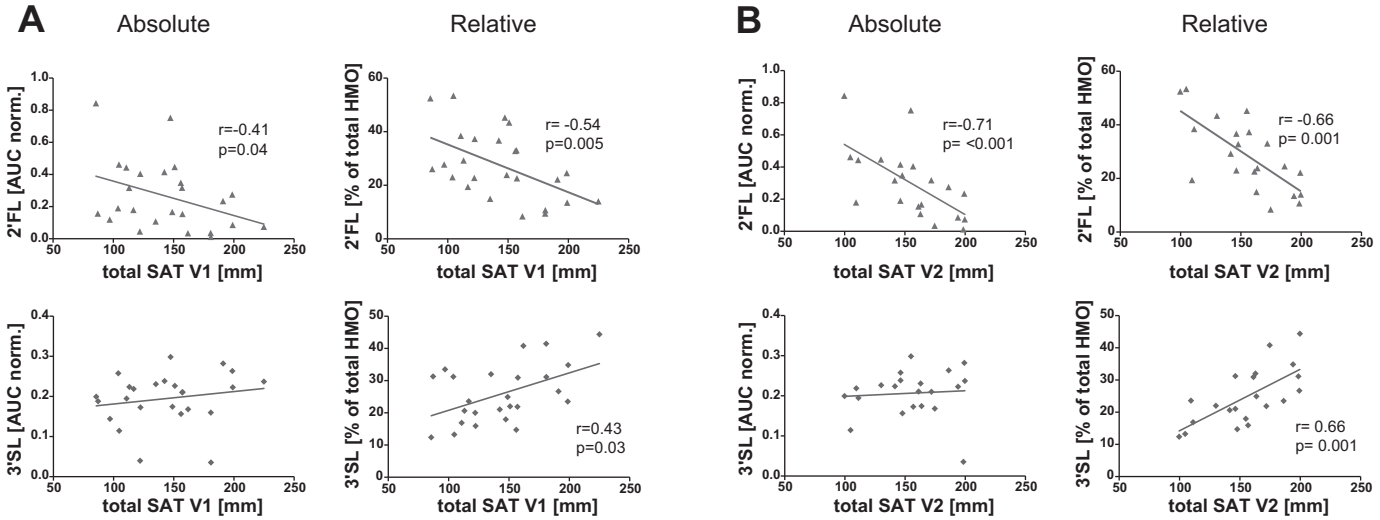

Fig. 7. Correlation of human milk oligosaccharides (HMOs) with subcutaneous adipose tissue (SAT). Spearman's rank correlations of SAT thickness with 2′-fucosyllactose (2′-FL, triangles) and 3′-sialyllactose (3′-SL, diamonds) in serum at midpregnancy (V2) in the secretor-positive group of pregnant women (n = 26). *A*: total SAT thickness (summed 15 individual sites measured by Lipometer) at *visit 1* (V1, 10–14 gestational weeks) was significantly correlated with 2′-FL absolute (*top left*) and relative (*top right*) concentrations and with relative concentration of 3′-SL (*bottom right*) at visit 2. *B*: total SAT thickness at visit 2 (V2, 20–24 gestational weeks) was significantly correlated with 2′-FL absolute (*top left*) and relative (*top right*) concentrations and with relative concentration of 3′-SL (*bottom right*).

than in milk, with potentially specific roles for circulating sialylated HMOs in pregnant women and breast-fed infants. However, because we are currently lacking evidence for specific roles of sialylated HMOs in the circulation, this remains speculative. Two lactosamines identified in maternal serum, 3′-SLN and 6′-SLN, are usually not found in human milk, but are also described in infant plasma and urine (9, 19, 36). 3′-SLN was determined in all maternal serum samples investigated, without significant changes in concentration during gestation. 3′-SLN was also found in nonpregnant women, which might speak more for a breakdown product of glycan-conjugates than a pregnancy-specific product of de novo synthesis.

Modulators of human milk oligosaccharides in maternal serum. Gestational age significantly altered relative fucosylation and total HMO concentration, with the greatest changes observed from V1 to V2, indicating an induction of secretor-active HMOs. In human milk, HMO concentration and composition change with lactation stages (7, 8, 38, 45). Thus, it is tempting to speculate that fucosylated and sialylated HMOs have specific spatiotemporal roles, both during pregnancy (in the maternal circulation) and postpartum, as they are delivered to the infant via breastfeeding (in the infant's gut, and after passing the intestinal barrier, in the infant's circulation). As all pregnant women had detectable HMOs already at V1, as early as 10 wk of gestation, future studies should monitor the physiological rise in HMOs during the first trimester, also in the context of pregnancy outcome.

In addition to gestational age, secretor status significantly affected HMO composition in maternal serum. In human milk, secretor status accounts for the most pronounced HMO variations changing total concentration and composition by an interdependency of different HMOs (32, 37, 38). Many studies have used 2′-FL concentrations in milk to assign secretor status or discriminate between women with high and low 2′FL concentrations (28, 32, 38). Here, low relative 2′-FL abundance clustered below 5% at midpregnancy (V2) and late pregnancy (V3), and we used this cutoff to identify secretor-negative mothers. Milk or tissue samples were not available from mothers in this study to unambiguously confirm their secretor phenotype in milk or to compare their phenotype in pregnancy with the FUT2 genotype. However, the correlation of 2′-FL and LDFT with each other across all time points and the interdependency with other secretor active HMOs strongly support the assigned status. LNFP1, also a secretor-active HMO, did not increase in the observed period of time and was not found to be significantly different between secretor and nonsecretor women. Although the latter could be due to the high variation and small sample size, we cannot explain this observation.

Remarkably, considering the rather homogeneous sample collective with regards to secretor status (only secretor-positive women were included) and BMI (majority was healthy weight, none were obese) and despite the relatively small sample size, we found significant associations between HMOs and BMI, leptin, and most strongly with SAT. Although these associations have to be confirmed in larger studies, they suggest that maternal metabolic factors can modulate HMO biosynthesis and/or bioavailability. Our findings might indicate a delay in 2′-FL production, leading to a relatively higher 3′-SL concentration at midpregnancy in women with higher fat mass. Leptin, a commonly used surrogate marker for fat mass, was positively correlated with 3′-SL. This finding contrasts a recent study, reporting positive associations of maternal BMI and weight with 2′-FL concentrations in human milk (32). Differences in HMO production and/or bioavailability in blood and milk because of the distinctive metabolism of a pregnant versus a lactating mother may account for the opposing associations with body composition, although differences in cohorts cannot be excluded as sources of the serum-milk discrepancies.

Origin of maternal serum human milk oligosaccharides. The origin of HMOs in pregnancy remains to be elucidated, al-

though there are some indications that HMOs derive from an already synthetically active mammary gland. An earlier study found lactose in maternal plasma in the first trimester, and concentration increased over the course of gestation, peaking shortly after birth (1). The authors explained the presence of lactose in the circulation by increased permeability of the epithelial barrier during pregnancy. HMOs are known to reach the systemic circulation of the breast-fed infant, indicating transport across the intestinal barrier (19, 36). In vitro studies showed both active transport and paracellular mechanisms in intestinal epithelial cells (18). Similarly, in pregnancy, HMOs could be transported across epithelial cells of the mammary gland by active and/or passive mechanisms. Although this remains hypothetical, future studies are warranted to investigate origin and transport of HMOs. The finding of circulating HMOs in pregnant mothers also raises the intriguing question of whether HMOs can cross the placental barrier and be found in the fetal circulation. This is currently studied in our laboratory.

Strengths and limitations of the study. Strengths of this study are the novelty and high potential significance of the finding, the longitudinal design, and the measurement of total SAT thickness as surrogate for fat mass. SAT allows for a more accurate approximation of body fat mass; thus, it is a better metabolic health indicator than BMI (16, 44). A recent study showed that SAT measured at midpregnancy is an independent risk factor for obesity-related adverse pregnancy outcomes (26). Our finding that SAT was associated with HMOs in pregnancy might suggest a potential role for HMOs in pregnancy outcomes and will set the stage for future studies on the health consequences of altered HMOs during pregnancy. Obvious limitations that will be overcome in future study designs are the small sample size and observational time period limited to pregnancy, resulting in a lack of samples from birth and thereafter. The small sample size of the study disallowed investigations of associations between HMOs and pregnancy outcomes. Although birth weight, placental weight, or gestational age at birth were not associated with maternal HMOs at any time during pregnancy in this cohort (data not shown), we cannot rule out consequences of altered HMOs. Our observation that secretor status affected HMOs in serum already before midpregnancy will encourage studies on the impact of maternal secretor status on pregnancy outcomes, such as maternal infections, and common pregnancy disorders associated with aberrant immune tolerance or metabolism. Larger studies that include perinatal samples will allow us to study associations of HMOs and pregnancy and neonatal outcomes with confounders.

Potential roles of prenatal human milk oligosaccharides. Based on the diverse proposed functions of individual HMOs, it seems likely that changes in HMOs in the maternal circulation can affect pregnancy outcomes. At the moment, we can only speculate about the roles of prenatal HMOs. They could potentially reach from balancing pregnancy–induced, low-level inflammation and increased insulin and leptin resistance to adjusting the immune system to the specific condition of pregnancy and tolerance toward the fetus. One possible role of HMOs is a protective effect in the urinary tract via their anti-adhesive or antimicrobial function against pathogens (30, 31). Clearly, more studies also including maternal serum at birth and cord blood are needed to help aid our understanding of potential biological functions.

In conclusion, this work may fundamentally change our view of the role of human "milk" oligosaccharides, strengthening previous speculations that HMOs could indeed already be protective in pregnancy (31). In this context, it will be interesting to investigate how secretor-negative mothers (and their infants) are affected by the significantly different HMO signature in pregnancy (and beyond). The finding that HMOs are present early in pregnancy leads us to speculate that some HMOs might be crucial for maintenance of pregnancy, potentially contributing to the many immunological and metabolic adaptations of the maternal physiology during gestation. Monitoring HMOs by HPLC could lead to the discovery of new biomarkers for pregnancy pathologies. The novel concept of circulating HMOs in pregnancy, potentially influenced by maternal metabolic factors and with unknown implications on the health of mother and fetus, will set the stage for future research.

ACKNOWLEDGMENTS

We acknowledge Bettina Amtmann, Petra Winkler, Carina Kremslehner, Charlotte Hartwig, Karolin Tatrai, and Martina Hipfl for collecting study data and samples.

GRANTS

The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013 under grant agreement number 627056 (to E. Jantscher-Krenn) and was supported by an anniversary fund of the Austrian National Bank (OeNB) under grant number 16927 (to E. Jantscher-Krenn). The support of the Family Larsson-Rosenquist Foundation is gratefully acknowledged (L. Bode).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

E.J.-K., B.C., G.D., L.B., and M.N.V.P. conceived and designed research; E.J.-K., J.A., B.R., and B.C. performed experiments; E.J.-K., J.A., B.R., H.K., and M.N.V.P. analyzed data; E.J.-K., H.K., L.B., and M.N.V.P. interpreted results of experiments; E.J.-K., B.R., and H.K. prepared figures; E.J.-K. drafted manuscript; E.J.-K., G.D., L.B., and M.N.V.P. edited and revised manuscript; E.J.-K., J.A., B.R., H.K., B.C., G.D., L.B., and M.N.V.P. approved final version of manuscript.

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