Supporting information

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Solvents and reagents for liquid chromatography: Reagent grade ammonium acetate and HPLC grade chloroform were purchased from Sigma Aldrich (St. Louis, MO). HPLC grade methanol was purchased from Honeywell Burdick & Jackson (Morristown, NJ). All water used was distilled in house to a final measured resistance of 18 megaohms

SEM: Scanning Electron Microscopy (SEM) images were obtained as described in ref 16.

TEM: Transmission Electron Microscopy (TEM) was achieved by negative staining the lactosome sample as described in reference 17. The grid was air-dried and viewed in a Philips CM120 Biotwin Lens, FEI Company, Hillsboro, OR.,U.S.A. made in Eindhoven, The Netherlands with Gatan MegaScan, model 794/20, digital camera (2K X 2K), Pleasanton, CA.

Determining the of milk fat globules particle size: milk fat globules (MFG) sizing was conducted as described in ref 18. In brief, MFG size was calculated as the mean diameter (in microns) of the number distribution of the particles in solution obtained from two 30 sec scans. Mean diameter = $\sum (V_i d_i^2)/\sum (V_i d_i^3)$ where V = volume percent between sizes; d = size represented by the center between any two sizes. Standard deviations for the width distribution were calculated by Microtrac S3500 10.5 software as (84%-16%)/2.

Determining lactosomes size: Particle size data of lactosomes were collected with a 90Plus Particle Size Analyzer (Brookhaven, Holtsville, NY) as described in the SI text. Device internals included a 35 mW diode laser operating at a wavelength at 638 nm with a photodetector positioned orthogonal to the incident beam. Samples were diluted in 3mL of Dulbecco Phosphate Buffered Saline (PBS) at pH 7.4; typically 50 µl of sample was placed in a 3.5 mL polystyrene cuvette filled with the buffer. Actual concentration was varied to optimize photon count rates and device efficiency. The index of refraction was 1.46 for MFG fractions and 1.40 for lactosome fractions (as deemed appropriate for lipid particles of this size range) (19). All samples were analyzed with 6 three-min and 5 five-min runs at 25°C. Particle size values generated from 11 scans for each lactosome sample were treated as independent values for each sample. Mean, median, and standard deviations were generated for each lactation stage. The effect of lactation stage (early vs. mature milks) as a group and within subjects was analyzed using independent samples t-tests.

Mass spectroscopy equipment and protocol for proteomic analysis of milk fat globules and lactosomes: Analysis was performed using an Agilent 6200 Series HPLC-Chip/TOF MS system equipped with the Agilent 1200 series microwell-plate autosampler, capillary pump, nanopump and HPLC-Chip interface. The enrichment and analytical columns were composed of 5 um Zorbax C18 material. For sample

loading, the capillary pump delivered 0.1% formic acid in 3.0% acetonitrile in water (v/v) isocratically at 3 μL/min. A nanoliter pump gradient was delivered at 0.3 μL/min using A) 0.1% formic acid in 3% acetonitrile (v/v) and B) 0.1% formic acid in 90% acetonitrile (v/v). The balance of A and B was comprised of 18 MΩ nanopure water. A 60.0 min nano-LC gradient was utilized as follows: from 0%-8% B, 0-5.0 min; 8%-35% B, 5.0-40.0 min; 35%-45% B, 40.0-45.0 min; 45%-90% B, 45.0-48.0 min and held for 3.0 min at 90% B with an equilibration time of 10.0 min at 0% B. The drying gas temperature was set at 325 ^{0}C with a flow of 4.0 L/min (2.0 L nitrogen and 2.0 L dry grade compressed air). Precursor scan data were acquired in the positive ionization mode with a mass range of *m/z* 300-2000. Tandem data were acquired in the range *m/z*50-3000 , with variable fragmentation energy of *m/z* -4.8 V + 3.6 V/100.

Modifies Folch method for extracting lipid from lactosomes: An aliquot of lactosome sample was diluted five times using Folch solution and 2 mL of 18 megaohm water was added to each diluted lactosome sample. It was experimentally determined that this volume of water added to each lactosome lipid extract was sufficient to remove the KBr from the lactosome isolation step. The lactosome lipid extracts were vortexed and refrigerated for 30 min at 4 °C after which the organic phase was extracted and dried under a gentle stream of nitrogen gas. The dried lactosome lipid extracts were reconstituted in 0.5 mL of $CHCl_3$ and stored at -80 °C until analysis by mass spectrometry.

Mass spectrometric equipment for comparison of lipid extracts: The Varian 920 TQ-FTMS consists of a modified Varian 320 triple quadrupole mass spectrometer front end that has been coupled to the FT-ICR MS by means of a hexapole accumulation cell, where ions are collected before being injected into the ICR cell by means of a radio frequency quadrupole ion guide. All mass spectrometric data collected on this instrument were acquired with the triple quadrupole front end operating in radio frequency ion guide mode only. All lipid extract samples were introduced into the mass spectrometer by direct infusion at a flow rate of 15 μL/min and the source parameters adjusted as needed to provide a consistent ion current. Typical values used for the other source parameters are 45 psi for the nebulization gas, 18 psi for the 200 °C drying gas, 5000 V for the voltage applied to the ESI capillary, and 400 V for the spray shield offset. All other instrumental parameters of the Varian 320 were controlled and optimized in the Varian MS Workstation software ver. 6.9.2 to maximize the ion current entering the FT-ICR. The FT-ICR MS was programmed in the Varian FTMS Omega software (ver. 9.1.20) with instrumental parameters adjusted as needed in order to obtain spectra with both high resolution and mass accuracies (≤ 1 ppm mass error for calibration compounds) in broadband detection mode. The high mass accuracy allowed for identification of lipid species based on accurate mass measurements with SORI-CID tandem mass spectrometry experiments performed on random ions to justify the assignments based on accurate mass. Data analysis was carried out in Varian FTMS FTDocViewer, ver. 4.1.92 (Varian Inc., Walnut Creek, CA) and Lipid Mass Spec Prediction program, ver. 1.5 (Eoin Fahy, http://www.lipidmaps.org).

Supplemental Table 1. Proteome of the MFG fraction for Sample 1000d08

HGNC Symbol	Total # of Peptides	MW
LTF	135	78.1
MFGE8	33	43.1
LALBA	19	16.2
PIGR	14	83.2
ALB	10	69.3
CLU	9	57.8
IGHM	7	53.6
XDH	7	146.3
TNC	5	240.7
LYZ	5	16.5
FABP3	9	14.8
BTN1A1	6	58.9
IGKV1-13	6	25.7
IGHA1	7	37.7
ADFP	5	48
CEL	5	79.6
CA6	8	35.3
ACTG1	3	41.8
CD36	4	53

SERPINA3	3	47.6
CSN2	7	25.4
IGKC	1	11.6
CD59	3	14.2
FN1	2	246.5
APOE	2	36.1
M6PRBP1	2	47
MUC1	2	49.2
IGL@	1	24.7
C4B	1	192.6
HLA-DRB5	1	30
TNXB	2	455.9
ACTA2	1	42
A1BG	1	54.2
PPIB	1	23.7
CD14	1	40.1
ZG16B	1	22.7
APOA1	1	30.8
MUC4	1	125
SLC2A1	2	54

GNAS	1	76.6
LPL	1	53.1
KRT24	1	55.1
KIF2C	1	81.3
RPS18P12	2	17.7

Supplemental Table 2. Proteome of the Lactosome fraction for Sample 1000d08

HGNC Symbol	Total # of Peptides	MW
LALBA	47	16.2
APOB	12	515.3
FABP3	12	14.7
PIGR	4	83.2
KRT1	4	65.8
LTF	4	78.1
MFGE8	13	43.1
SPP1	7	35.4
LYZ	2	16.5
KRT6B	1	60
ALB	4	69.3
CLU	3	57.8
CSN2	12	25.4
B2M	2	13.7
KRT2	1	65.8
CSN1S1	7	21.7
PIP	1	16.6
CST3	1	15.8
SERPINA1	1	46.7

CEL	1	79.6
PSAP	1	58.1
LRRN4	2	78.8
APOA1	1	30.8
TTR	1	20.1
KRT77	1	61.9
TSEPA	1	64.6
BTN1A1	1	58.9
YWHAZ	1	27.7
GNPDA1	1	32.6
40063	1	44.8
M6PRBP1	1	47

Supplemental Table 3. Proteome of the MFG fraction for Sample 1005d08

HGNC Symbol	Total # of Peptides	MW
LTF	111	78.1
ALB	32	69.3
LALBA	26	16.2
MFGE8	38	43.1
CLU	13	57.8
PIGR	9	83.2
ACTB	9	41.7
XDH	9	146.3
PIGR	6	83.2
CEL	9	79.6
FABP3	6	14.8
ACTG1	3	41.8
LYZ	4	16.5
ADFP	9	48
CD14	4	40.1
CD36	7	53
IGHA1	7	37.7
CD59	7	14.2

BTN1A1	7	58.9
M6PRBP1	4	47
CSN2	9	25.4
MUC1	3	49.2
CSN1S1	2	21.7
A1BG	3	54.2
CA6	8	35.8
IGHM	2	53.6
APOA2	1	11.2
IGKC	3	11.6
SERPINA3	1	47.6
STOM	1	31.7
SPP1	1	35.4
HLA-DRB1	1	29.9
IGKV1-13	1	25.7
APOA1	1	30.8
IGL@	1	24.7
GNB2	1	37.3
CIC	2	163.7
GGT2	1	61.8
TNC	1	240.7

SLC16A3	2	49.4
GNAS	1	76.6
APOE	1	36.1
ANXA6	1	75.2
APOH	1	38.3
TNXB	1	455.9
B2M	1	14
FAM63A	1	51.7
SSR4	1	19

Supplemental Table 4. Proteome of the Lactosome fraction for Sample 1005d08

HGNC Symbol	Total # of Peptides	MW
LALBA	46	16.2
FABP3	7	14.7
SPP1	11	33.8
KRT1	5	65.8
CLU	6	57.8
KRT2	3	65.8
LYZ	3	16.5
MFGE8	4	43.1
LTF	3	78.1
PIGR	2	83.2
CSN2	9	25.4
ALB	2	69.3
B2M	2	14
CSN1S1	6	21.7
APOB	2	515.3
CST3	1	15.8
KRT13	1	49.6
LRRN4	1	78.8
TTR	1	20.1

SERPINA1	1	46.7
TSEPA	1	64.6
KRT77	1	61.9
M6PRBP1	1	47
MYOF	1	234.6

Supplemental Table 5. Proteome of the MFG fraction for Sample 1008d08

HGNC Symbol	Total # of Peptides	MW
LTF	96	78.1
ALB	15	69.3
MFGE8	33	43.1
LALBA	24	16.2
CLU	20	57.8
FABP3	11	14.7
PIGR	11	83.2
XDH	10	146.3
LYZ	7	16.5
CEL	7	78.3
IGKC	5	11.6
IGHA1	6	37.7
BTN1A1	9	58.9
ADFP	8	48
CD59	9	14.2
CA6	7	35.8
CD36	5	53
RAB10	2	22.5
IGHM	2	53.6

APOA1	2	30.8
MUC1	5	49.2
CSN2	6	25.4
CD14	2	40.1
M6PRBP1	2	47
STOML3	3	32.1
IGL@	3	24.7
HLA-DRB3	1	29.9
SERPINA3	1	47.6
SPP1	3	35.4
ENSP00000380341*	1	44.8
CSN1S1	1	21.7
ACTN4	2	104.8
RAP1B	1	20.8
GNB2	1	37.3
HLA-DRA	1	28.6
APOH	1	38.3
GGT2	1	61.8
TNXB	1	455.9
PABPC1L	1	68.3
SLC2A1	1	54

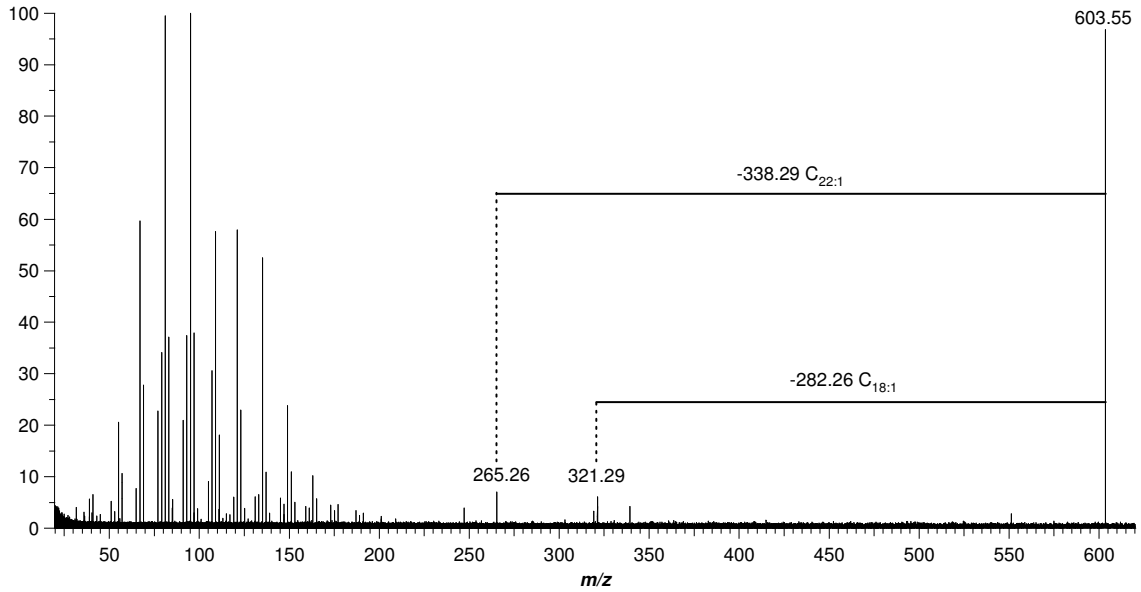
KRT13	1	49.6

*This is a human protein identifier from the Ensembl database. No HGNC Symbol was available for this uncharacterized peptide.

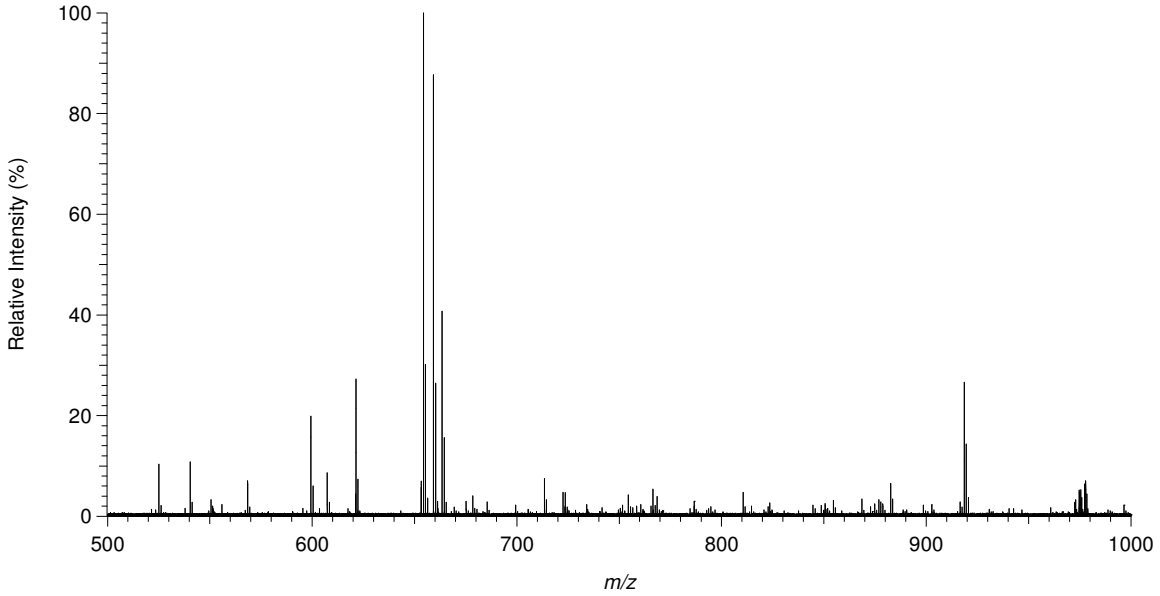
Supplemental Table 6. Proteome of the Lactosome fraction for Sample 1008d08

HGNC Symbol	Total # of Peptides	MW
KRT1	27	65.8
LTF	22	78.1
APOB	16	515.3
KRT2	10	65.8
KRT10	14	59.5
LALBA	50	16.2
MFGE8	11	43.1
FABP3	5	14.8
KRT9	5	62.1
SPP1	12	33.8
CLU	3	57.8
APOA1	3	30.8
KRT4	1	63.9
PIGR	2	83.2
CSN2	30	25.4
LYZ	3	16.5
KRT28	2	50.5
ALB	2	69.3

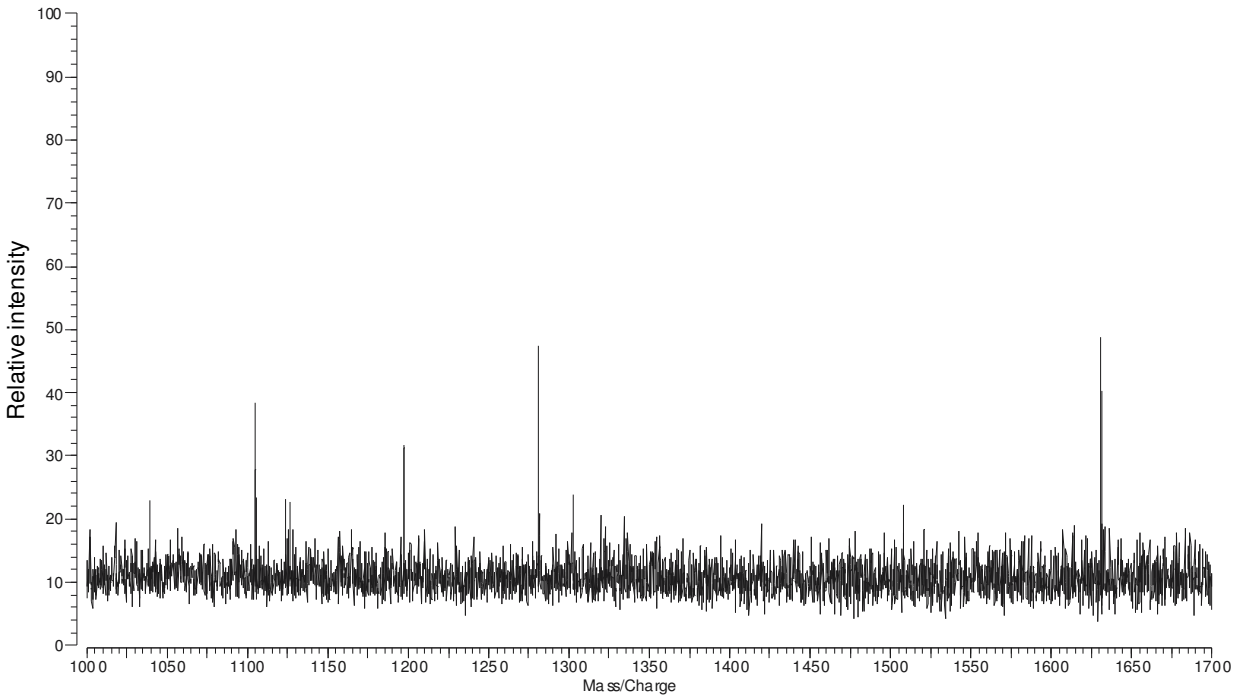
B2M	3	14
BTN1A1	2	58.9
CSN1S1	7	21.7
CST3	3	15.8
CFL1	1	18.5
KRT5	1	62.3
IGHA2	1	42.3
ARID1B	1	237.5
CD59	1	14.2
C4B	3	192.6
APOE	1	36.1
TFG	2	43.4
FABP5L2	1	15.2
PSAP	1	61.7
PKM2	1	58
TSEPA	4	64.6
ACTG1	1	41.8
H3F3B	1	15.3
LIMS1	1	20
ADFP	1	48



Supplemental Figure 1. Tandem mass spectrometry spectrum of an isolated fatty acid cluster from the MFG for sample 1008 day 8 obtained by SORI-CID on an ESI FT-ICR MS. This spectrum shows the dissociation of the cluster at *m/z* 603.55 into the two constituent fatty acids. The collection of peaks between *m/z* 50-200 are the result of the dissociation of the constituent fatty acids.



Supplemental Figure 2. ESI FT-ICR MS spectra of the lactosome fraction in the positive mode from sample 1008 day 8.



Supplemental Figure 3. Negative mode spectrum of the fraction from human milk lactosome sample 1005 day 28.