

# Breast Milk from Non-Obese Women with a High Omega-6 to Omega-3 Fatty Acid Ratio, but Not from Women with Obesity, Increases Lipogenic Gene Expression in 3T3-L1 Preadipocytes, Suggesting Adipocyte Dysfunction

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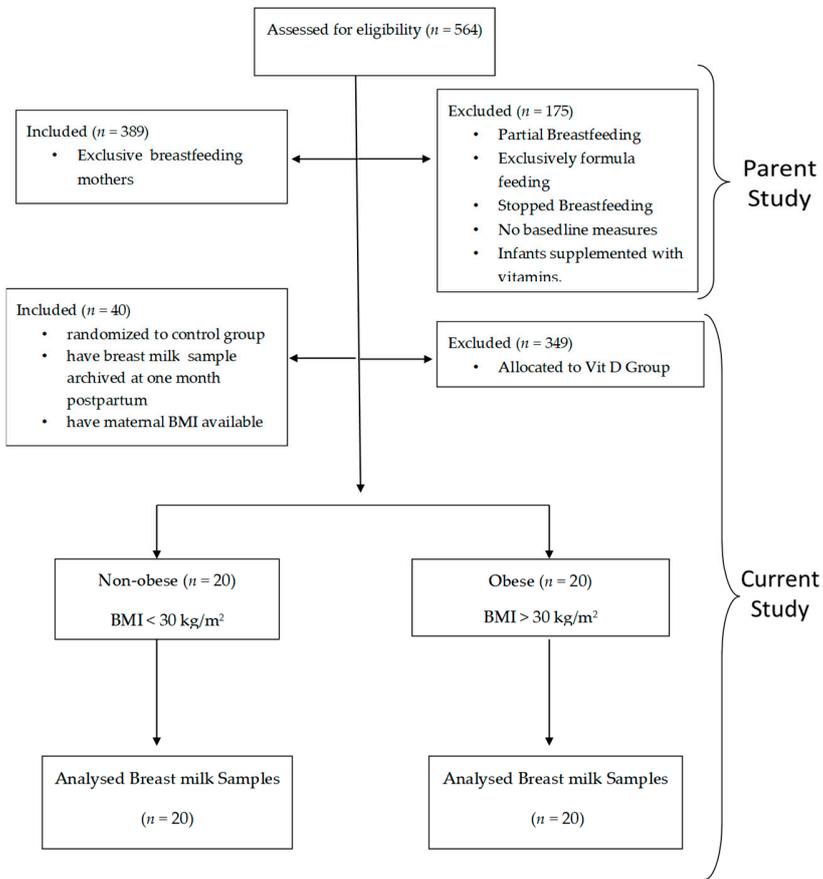
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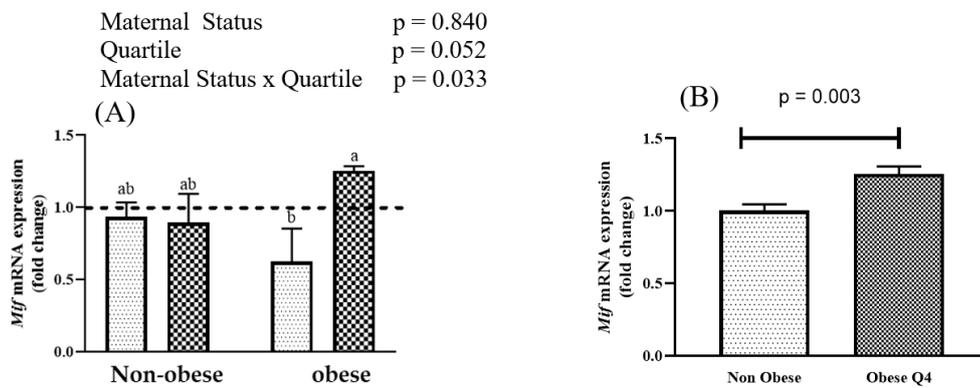
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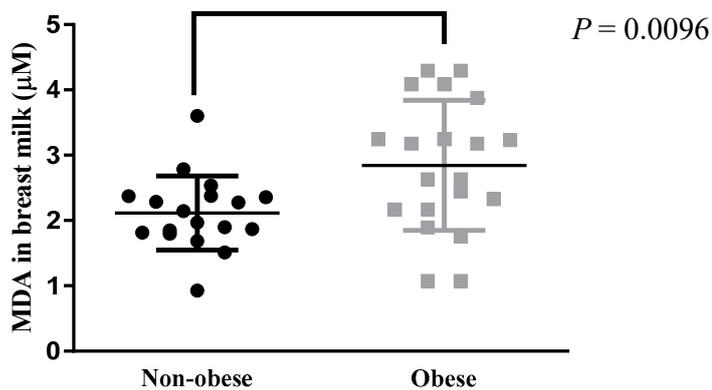
**Keywords:** adipogenesis; lipogenesis; breast milk; obesity; polyunsaturated fatty acids; 3T3-L1 preadipocytes



**Figure S1: Consort flow diagram for participant selection.**



**Figure S2. Effects of breast milk from women with obesity (obese) on the mRNA expression of Macrophage migration inhibitory factor (*Mif*).** Values are expressed as mean  $\pm$  SD,  $n = 3$ . Data were assessed using 2 way ANOVA in (A), and Student's *t*-test was used to analyze differences between control untreated cells (No BM) and obese Q4 (high n-6:n-3 PUFA) (B).  $P < 0.05$  was considered significant. Different superscripts (a, b) are used to denote significant differences between the treatment groups, BM = breast milk; Q = quartile.



**Figure S3. The effect of maternal obesity on malondialdehyde (MDA) levels in breast milk.** MDA levels in the breast milk whey of non-obese and women with obesity (obese) at one month postpartum were measured using a Thiobarbituric acid reactive substances (TBARS) assay kit (KGE013, R & D Systems, Minnesota, USA) as previously described (Enstad et al., 2021). The pre-optical density readings were subtracted from the optical density values after 2 hours of incubation to get the final concentration and multiplied by the dilution factor to determine the MDA concentration in the samples. Data were expressed as micromole ( $\mu\text{M}$ ), and the values are expressed as mean  $\pm$  SD,  $n = 20$ . Data were analyzed using Student's *t*-test to determine the difference between the group;  $P < 0.05$  was considered significant.

**Table S1. Primer sequences for qPCR.**

<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>Acc1</i>	GGCCAGTGCTATGCTGAGAT	AGGGTCAAGTGCTGCTCCA
<i>Atgl</i>	AACACCAGCATCCAGTTCAA	GAACTTGCCCATGTCCTTGT
<i>Fasn</i>	CTGCGGAAACTTCAGGAAATG	GGTTCGGAATGCTATCCAGG
<i>Hsl</i>	AGACACCAGCCAACGGAT	GGGCATAGTAGGCCATAGCA
<i>Mif</i>	TCAAGCGAAGGTGGAACCGTT	CGGACCGGGTCTACATCAA
<i>Pparg</i>	GAGCTGACCCAATGGTTGCTG	GCTTCAATCGGATGGTTCTTC
<i>Plin1</i>	TGCTGGATGGAGACCTC	ACCGGCTCCATGCTCCA
<i>Rplp0</i>	AATTTCAATGGTGCCTCTGG	TCACTGTGCCAGCTCAGAAC
<i>Scd1</i>	CACCTGCCTCTTCGGGATTT	CTTGACAGCCGGGTGTTTG

*Acc1*, acetyl-CoA carboxylase; *Atgl*, adipose triglyceride lipase; *Fasn*, fatty acid synthase; *Hsl*, hormone-sensitive lipase; *Mif*, macrophage migration inhibitory factor; *Plin1*, perilipin; *Pparg*, peroxisome proliferator-activated receptor-gamma; *Rplp0*, large ribosomal Protein; *Scd1*, stearyl-CoA desaturase.

Table S2. The fatty acids composition of breast milk by quartiles with low and high n-6:n-3 PUFA.

FA (nmol%)	Non-obese				Obese			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
C14	7.14±1.74	6.13±1.18	6.28±1.65	5.38±1.03	6.75±0.97	5.27±0.97	7.59±3.30	6.78±3.20
C16	21.33±2.44	20.08±3.11	21.05±2.85	18.76±1.12	20.11±1.09	21.25±1.09	17.82±2.53	18.53±1.49
C18	6.96±1.06	6.38±1.26	5.46±0.51	5.57±0.98	5.38±0.83	4.20±0.83	4.62±0.48	5.42±1.25
C16:1	2.19±0.16	2.16±1.40	2.52±0.50	2.31±0.64	2.35±0.42	2.45±0.42	2.10±0.72	1.81±0.80
C18:1n9	27.07±2.44	27.58±0.99	25.83±2.65	27.81±4.63	24.38±2.00	21.81±2.00	23.50±3.86	26.73±3.37
C18:1n7	2.68±0.28	2.55±0.73	2.73±0.39	2.57±0.34	2.68±0.54	3.00±0.54	2.62±0.28	2.26±0.40
C20:1n9	0.33±0.06	0.31±0.04	0.31±0.07	0.33±0.09	0.27±0.03	0.27±0.03	0.29±0.08	0.33±0.08
C18:2n6	17.27±2.88	20.06±6.24	21.97±3.21	23.39±4.41	22.57±5.03	22.78±5.03	25.49±2.75	24.60±5.03
C18:3n6	0.23±0.06	0.25±0.04	0.22±0.04	0.22±0.02	0.26±0.06	0.20±0.06	0.25±0.05	0.26±0.07
C20:3n6	0.60±0.13	0.64±0.09	0.64±0.10	0.70±0.04	0.66±0.07	0.70±0.07	0.86±0.09	0.71±0.06
C20:4n6	0.66±0.08	0.69±0.11	0.68±0.14	0.67±0.13	0.75±0.13	0.61±0.13	0.71±0.10	0.70±0.10
C18:3n3	1.39±0.21	1.77±0.89	1.52±0.26	1.46±0.64	1.80±0.51	1.75±0.51	1.57±0.38	1.18±0.28
C20:3n3	0.17±0.04	0.17±0.01	0.21±0.05	0.15±0.03	0.18±0.03	0.14±0.03	0.21±0.06	0.18±0.02
C20:4n3	0.16±0.02	0.15±0.03	0.15±0.02	0.12±0.02	0.14±0.02	0.13±0.02	0.17±0.01	0.13±0.01
C20:5n3	0.24±0.08	0.20±0.04	0.23±0.05	0.17±0.03	0.23±0.06	0.14±0.06	0.21±0.03	0.19±0.04
C22:5n3	0.33±0.07	0.30±0.06	0.33±0.04	0.28±0.05	0.38±0.08	0.28±0.08	0.32±0.05	0.29±0.05
C22:6n3	0.47±0.09	0.40±0.17	0.42±0.09	0.38±0.12	0.45±0.17	0.30±0.17	0.33±0.04	0.34±0.04
n6-PUFA	18.77±2.88	21.63±6.28	23.52±3.15	24.99±4.50	24.23±4.91	24.29±4.91	27.32±2.80	26.27±5.03
n3-PUFA	2.77±0.44	2.99±0.90	2.86±0.35	2.56±0.69	3.18±0.34	2.73±0.34	2.81±0.36	2.31±0.39
n6/n3-PUFA	6.77±0.07 <sup>d</sup>	7.25±0.32 <sup>c</sup>	8.23±0.38 <sup>c</sup>	9.76±1.31 <sup>b</sup>	7.60±1.06 <sup>cd</sup>	8.91±1.06 <sup>b</sup>	9.77±0.58 <sup>b</sup>	11.35±0.50 <sup>a</sup>

Data were expressed as nmol% of the total extracted fatty acids; values are expressed as mean ± SD, n = 4-5. Data were analyzed by two-way ANOVA, and superscripts (a,b) were used to denote significant differences between the treatment group. Q= quartile, FA = fatty acids, ΣSFA = sum of saturated fatty acids, ΣMUFA= sum of monounsaturated fatty acids, ΣPUFA = sum of polyunsaturated fatty acids, Σn-6 sum of omega-6 polyunsaturated fatty acids, Σn-3 sum of omega-3 polyunsaturated fatty acids P<0.05 was considered significant.