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7 Title: Breastfeeding Behaviours in Women with Obesity; Associations with Weight
8 Retention and the Serum Metabolome: A Secondary Analysis of UPBEAT.

Author Names: Kathryn V Dalrymple^{α1}, Annette L Briley^{α1,2}, Florence AS Tydeman³,
 Paul T Seed³, Claire M Singh⁴, Angela C Flynn^{1,4}, Sara L White^{3,5}, Lucilla Poston³ on
 behalf of the UPBEAT Consortium^{*}.

Affiliations: ¹ Department of Nutritional Sciences, School of Life Course and
 Population Sciences, King's College London, London, UK

² Caring Futures Institute, CHNS, Flinders University, Adelaide, Australia

³ Department of Women and Children's Health, School of Life Course and Population

16 Sciences, King's College London, London, UK

⁴ School of Population Health, Royal College of Surgeons in Ireland, Dublin, Ireland

⁵ Guy's and St Thomas' NHS Foundation Trust, London, UK

19 ^αALB and KVD equal contribution

Authors' last names: Dalrymple, Briley, Tydeman, Seed, Singh, Flynn, White,
Poston.

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Corresponding author: Kathryn Dalrymple, Department of Nutritional Sciences,
School of Life Course and Population Sciences, King's College London.

25 Kathryn.dalrymple@kcl.ac.uk

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33 **Short running head**: Breastfeeding in women with obesity

Abbreviations: Body mass index (BMI), Postpartum weight retention (PPWR) UK
 Pregnancy Better Eating and Activity Trial (UPBEAT), World Health Organisation
 (WHO)

37 **Clinical trial registry:** ISRCTN reference 89971375

Data Availability Statement: The datasets generated during and analysed during the
 current study are available from the corresponding author on reasonable request

40	pending application (via a research application form) and approval by the UPBEAT
41	Consortium.
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46	

47 Abstract

Background/Objectives: Maternal obesity is associated 48 with a decreased intention and initiation of breastfeeding 49 50 as well as a shortened duration of breastfeeding. This analysis was undertaken to identify breastfeeding 51 52 behaviours, and relationships with maternal 53 anthropometry and the serum metabolome at 6-months 54 postpartum in an ethnically diverse cohort of women with 55 obesity.

56 Subjects/Methods: A cohort analysis of 715 women from 57 the UK Pregnancies Better Eating and Activity Trial 58 (UPBEAT); a multi-centre randomised controlled trial of an antenatal lifestyle intervention in women with obesity. 59 Maternal data were collected in early pregnancy and 60 included body mass index (BMI), socio-demographic 61 62 characteristics and anthropometry. At 6-months 63 postpartum, breastfeeding behaviours, anthropometry and 158 maternal metabolic measures from blood samples 64 were recorded. Kaplan-Meier curves of breastfeeding 65 66 duration were constructed and were stratified by obesity class (I: BMI 30.0-34.9kg/m², II: 35.0-39.9kg/m², III: 67 68 ≥40.0kg/m²). Relationships breastfeeding between 69 behaviours, socio-demographic characteristics, the metabolome, and anthropometry were determined using 70 71 regression analyses.

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72 Results: 82% (591/715) of the cohort-initiated 73 breastfeeding and at the 6-month follow-up 40% (283/715) 74 were breastfeeding exclusively or partially. Duration of 75 exclusive breastfeeding decreased with increasing BMI: Compared to BMI class I (mean 90.4±64 days) the 76 77 difference in mean for classes II and III were -15.8 days 78 (95% confidence interval: -28.5, -3.1, p<0.05) and -16.7 79 (95% CI: -32.0 to -1.35, p<0.05), respectively. Compared 80 to no breastfeeding, any breastfeeding at 6-months 81 postpartum was associated with improvements in 82 metabolites towards a healthier profile, reduced weight 83 retention by -1.81kg (05% CI -0.75, -2.88) and reduced anthropometric measures, including mid-upper arm and 84 85 hip circumferences. The breastfeeding related changes in anthropometry were not evident in women of Black 86 87 ethnicity.

Conclusions: Greater emphasis on enabling
breastfeeding for women with obesity could improve
duration, women's weight management and metabolic
health. The lack of breastfeeding related anthropometric
effects in Black women requires further investigation.

93

94 **Introduction:** The World Health Organization (WHO) 95 recommends exclusive breastfeeding for the first 6-months 96 of a child's life for optimum infant development and health¹. 97 Despite this advice, exclusive breastfeeding rates in the UK at hospital discharge and 6-8 weeks postpartum 98 remain low at ~70%² and~33%, respectively³. Some 99 100 reports have shown that maternal obesity 101 (BMI≥30.0kg/m²) is associated with a lower prevalence of 102 breastfeeding compared with women of a healthy BMI (18.5-24.9kg/m²)^{4,5}, with up to 13% lower rates of initiation 103 and 20% decreased likelihood of any breastfeeding by 6-104 105 months postpartum^{4,6}, whereas others have reported no 106 differences between initiation of breastfeeding and 107 maternal BMI⁷. Despite wide cultural and international 108 variation, maternal obesity has been associated with reduced breastfeeding rates, independent of country of 109 study⁸. 110

111 Barriers to breastfeeding in BMI heterogeneous women, 112 such as embarrassment, fear of pain and concerns about insufficient milk are commonly reported^{9,10}. However, in 113 114 women with obesity a range of factors further impact on 115 breastfeeding initiation and duration, such as delayed 116 lactogenesis, low prolactin and poor body confidence 117 ^{6,8,11,12}. These additional barriers faced by women with 118 obesity are reflected in breastfeeding behaviours, including lower initiation and duration.

The metabolome, a descriptor for the small-molecule 120 121 chemicals of body processes, responds to environmental 122 and genomic interactions, and is increasingly utilized as a 123 precision medicine tool with which to identify those at risk of cardiometabolic and other diseases. Whilst the benefit 124 125 of breastfeeding on maternal weight and BMI is well 126 recognized, the impact of breastfeeding on maternal metabolic health remains unclear¹³. Exploration of the 127 128 metabolome in breastfeeding women may provide further 129 insight into biological changes that occur during 130 lactogenesis.

131 Participants were from the UK Pregnancy Better Eating and Activity Trial (UPBEAT), a multicentre randomized 132 133 controlled trial of an antenatal lifestyle intervention. As the 134 UPBEAT intervention did not affect breastfeeding initiation or duration, this data was treated as a cohort. In a 135 136 secondary analysis, we have investigated breastfeeding 137 initiation after birth and behaviours (exclusivity, mixed or 138 breastfeeding), anthropometry, postnatal weight no retention (PPWR) and the impact breastfeeding had on the 139 140 maternal metabolome in a population of women with obesity up to 6-months postpartum¹⁴. As UPBEAT 141 142 participants were an ethnically diverse cohort, the 143 influences of ethnicity have also been explored.

Materials/Subjects and Methods: Between 2009-2014 144 UPBEAT recruited 1555 women (15⁺⁰-18⁺⁶ weeks' 145 146 gestation), with a BMI \geq 30kg/m² (median 35.1kg/m² (IQR 147 32.8, 38.5)) from UK inner city settings, including, London, 148 Glasgow, Newcastle, Sunderland, Bradford and 149 Manchester. Participants were randomized to standard 150 antenatal care or to a lifestyle intervention aimed at 151 reducing dietary glycaemic load and increasing physical 152 activity, superimposed on standard antenatal care. The 153 primary aim of UPBEAT was to reduce the incidence of 154 gestational diabetes (GDM) and large-for-gestational age 155 infants. Sociodemographic, pregnancy information, 156 anthropometric measurements and blood samples were 157 obtained at study entry and at two further time points 158 during pregnancy and at 6-months postpartum (2010-2015)¹⁵. All participants provided written informed consent. 159 160 The NHS research ethics committee granted approval for 161 participating centres (UK integrated all research application system, reference 09/H0802/5). Additional 162 163 approvals were obtained through local Research and 164 Development departments in each participating centre. 165 UPBEAT was also registered with the ISRCTN reference 166 88971375).

167 At birth, pregnancy outcomes and mode of feeding were168 recorded. At 6-months postpartum, data on infant feeding

intention in pregnancy, breastfeeding initiation at birth and
duration were obtained. Additional data regarding infant
weaning practices and the rationale for feeding choices for
their infant were also recorded.

For the mother, weight was measured at the 6-month visit and the following circumferences and skinfold thicknesses were measured in triplicate and a mean obtained: neck, mid-arm, waist, hip (cm) and wrist (mm) using a tape measure. Skinfold thickness of triceps, biceps, suprailiac and subscapular were measured using Harpenden callipers and the sum of skinfolds was generated.

180 The primary maternal outcomes for this analysis were 181 breastfeeding behaviours. For this study, these were 182 defined as the percentage of all women who intended to 183 breastfeed, the percentage of women who initiated 184 breastfeeding (baby put to the breast on at least one 185 occasion), the average duration of exclusive breastfeeding 186 (infant received only breast milk, directly or expressed, and no other liquids or solids) and the percentage of any 187 188 breastfeeding at 6-months postpartum.

Secondary maternal outcomes included the relationship
between breastfeeding behaviours and obesity class
(WHO obesity class I [BMI 30.0-34.9kg/m²], II [BMI 35.039.9kg/m²], III [≥40.0 kg/m²]), ethnicity, mode of birth,

diagnosis of gestational diabetes and infant birthweight
≥4kg. Maternal postnatal weight retention and
anthropometry in relation to breastfeeding initiation and
duration were also explored.

Maternal Metabolome: A total of 158 metabolites were 197 198 evaluated using serum blood samples from 6-months 199 postpartum. Targeted to multiple pathways relevant to 200 obesity and insulin resistance, we used a high throughput NMR metabolomic platform (Nightingale Health Ltd, 201 202 Finland). This platform accurately quantifies numerous lipid measures; lipoprotein particles include very low 203 204 density (VLDL) subdivided into 6 subclasses (extremely 205 large, very large, large, medium, small, very small), 206 (Intermediate) IDL, (low) LDL subdivided into 3 subclasses 207 (large, medium, small), and high (HDL) subdivided into 4 208 subclasses (very large, large, medium, small). The 209 platform also elucidates the constituents within each 210 lipoprotein particle type (triglyceride, total cholesterol, free 211 cholesterol and cholesterol ester levels, and phospholipid 212 concentrations). Fatty acids, amino acids, glycolysis related metabolites, ketone bodies and inflammatory 213 214 markers are also measured.

215 **Statistical analyses:** Missing data mechanism was 216 assumed missing at random therefore a complete case

11

217 analysis was used. All UPBEAT women who provided 218 infant feeding data at the 6-month postpartum visit were 219 included in the analysis. Chi-square and t-tests were used 220 investigate associations between breastfeeding to 221 behaviours and maternal variables. Descriptive statistics 222 were used to identify the maternal rationales for choice of 223 infant feeding, with percentages calculated for the 224 proportion of women opting for each given reason when 225 asked by the midwives during the follow up visits. Interval 226 regression analysis with right sided censoring as not all 227 mothers had stopped breastfeeding at the time of follow up 228 was used to assess the duration of breastfeeding between 229 obesity classes. Regression analyses were used to assess 230 the difference between maternal variables for those who 231 did and did not initiate breastfeeding.

232 For the serum metabolome, multivariable linear regression 233 was applied for each metabolite with breastfeeding as the 234 primary independent variable of interest, adjusted for the 235 following confounders: age, BMI, parity, and intervention 236 arm. The models for all women (n=485) were also adjusted 237 for ethnicity. All analytes were checked for normality and 238 transformations were made as appropriate; analytes were then scaled and centred¹⁶. Results were presented as 239 240 standard deviation (SD) differences between groups to 241 allow for comparisons across multiple measured units. We 242 present analysis for the entire cohort and, for comparison, 243 for samples from women randomised to the control arm 244 only. Metabolome analyses were also analysed by 245 ethnicity for Black and White women. Women were 246 excluded from the anthropometric and metabolomic 247 analysis if they were pregnant at the 6-month follow-up 248 visit. Statistical analyses were conducted using Stata 249 (version 18) and Rstudio version 3.5.2. *P* values ≤ 0.05 250 were considered statistically significant.

251

252 **Results:**

253 Participants: Data were available for 715/1555 (46.0%) 254 UPBEAT participants who attended the 6-month 255 postpartum follow-up visit. Of the 840 non-participants, 1 256 participant was excluded after randomisation, 19 257 pregnancies were affected by a major health problem, 258 miscarriage or sudden infant death syndrome (SIDS); 100 259 declined participation; 701 either did not respond or were 260 unable to participate as they had either, returned to full-261 time employment, living overseas or the child was not the 262 primary responsibility of the woman. 5 women had no 263 infant feeding data recorded at the 6-month follow-up. Of 264 those attending the 6-month postpartum visit with their child 354, 225 and 136 women were classified as BMI 265

obesity classes I, II and III respectively at baseline (15⁺⁰ to
18⁺⁶ weeks gestation) (for study flow diagram see Figure
1).

269 Compared to those women who did not return for the 6-270 month visit, the women who attended the 6-month follow-271 up were more likely to be of White ethnicity and less likely 272 to be of Black or Asian ethnicity. Women were more likely 273 to be nulliparous at study entry, less likely to be a smoker, 274 more likely to have had GDM in pregnancy and more likely 275 to have had an in-labour caesarean or an operative vaginal 276 birth (Table 1).

277 Effect of Intervention

There was no significant difference in breastfeeding intention, initiation or exclusive or partial breastfeeding at 6-months by UPBEAT group allocation (**Supplementary Table 1**). Therefore, data for intervention and control arms of the trial were combined and the participants treated as a cohort.

284 Breastfeeding behaviours

Table 2 summarises breastfeeding intention and practices
by maternal BMI class at trial entry for those who
completed the 6-month follow up visit. Overall, 76.3% of
women stated antenatally that they intended to breastfeed,

289 and 82.7% put their baby to the breast on at least one 290 occasion. For those women who initiated breastfeeding, 291 the percentage of any breastfeeding at 6-months 292 postpartum decreased in BMI class III compared to 293 classes I and II (class 1, 51.7%, II, 48.2% & III 30.7%, 294 p<0.05). The percentage of those using formula milk at the 295 6-month follow-up increased with BMI class, 75.0%, 80.0% 296 and 84.0%, respectively.

297 Figure 2 shows a Kaplan-Meier survival curve for 298 breastfeeding duration according to BMI category. Interval 299 regression analysis showed duration of exclusive 300 breastfeeding in women with class I obesity was 90.4 days 301 compared to 74.6 days and 73.7 days in those with class 302 II and class III obesity, respectively; mean differences 303 were: class II -15.8 (95% confidence interval (CI) -28.5 to 304 -3.1), p<0.01) and class III -16.7 (95% CI -32.0 to -1.35), 305 p<0.05), compared to class I (**Table 2**). More women with 306 obesity class I compared with class II and III were more 307 likely offering breastfeeding in combination with solids and 308 other fluids (e.g., water) at 6-months postpartum (24.8%, 309 21.6% and 14.8%) (Table 2). 92% of the cohort had 310 introduced solids by the 6-month follow-up.

Breastfeeding behaviours demonstrated associations witheducational attainment; a higher education attainment was

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313 associated with a likelihood of partial or exclusive 314 breastfeed at 6-months postpartum. Maternal age 315 ≥30years and cohabitation were associated with a higher 316 percentage of breastfeeding initiation and duration 317 (**Supplementary Table 2**). Whereas being of White 318 ethnicity and smoking were associated with lower rates of 319 breastfeeding initiation and duration.

320 Table 3 presents the birth outcomes stratified by BMI 321 class. There was no statistical difference in the relationship 322 between initiation of breastfeeding and maternal obesity 323 category (Table 3). There were no differences between 324 mode of birth or infant birthweight \geq 4kg when stratified by 325 maternal BMI. Diagnosis of GDM was significantly more 326 common in BMI classes II and III, compared to class I 327 (**Table 3**).

328 <u>Weight and Anthropometric measures</u>.

329 Supplementary table 3 summarises the data stratified by 330 any vs no breastfeeding, at 6-months postpartum. Women 331 who were offering any breast milk at 6-months (n=283) 332 weighed 1.12kg less than their pre-pregnancy weight. 333 Whereas women who were not breastfeeding at 6-months 334 demonstrated weight retention of 0.70kg (mean difference 335 of 1.81kg (95% CI 0.75, 2.88) p<0.01). Changes from 336 baseline to 6-months in neck, midarm, wrist and hip circumferences were also significantly lower in women
who were fully or partially breastfeeding at 6-months
compared to those women who were not breastfeeding.
There were no statistically significant associations
between maternal skinfold thicknesses and breastfeeding
behaviours (Supplementary Table 3).

343 Subgroup analysis between women of Black and White 344 ethnicity showed that changes in weight retention and 345 circumferences associated with breastfeeding apparent in 346 the White women were not evident in women of Black 347 African or Black Afro-Caribbean ethnicity (Supplementary 348 Table 4). Black women who were breastfeeding at the 6-349 month follow-up had an average postpartum weight 350 retention of 3.35kg (95% CI 1.39, 5.30) compared to White 351 women. Similar observations were apparent in mid-upper 352 arm [0.93 (0.36, 1.51)] and hip circumferences [3.59 (2.00, 353 5.19)]. However, women of Black ethnicity were more likely 354 initiate breastfeeding (95% vs 78%) and be to 355 breastfeeding at the 6-month follow-up (62% vs 32%) 356 compared to women of White ethnicity (Supplementary 357 Table 2).

358 <u>Metabolome</u>

There were no significant differences in the metabolome at6 months postpartum between women randomised to the

361 control and intervention arms following adjustment for age, 362 ethnicity and parity, and the data were therefore treated as 363 a cohort. Exclusive or partial breastfeeding vs no 364 breastfeeding at 6-months postpartum was associated 365 with marked changes in the NMR metabolome (Figures 3 366 & 4). Breastfeeding was associated with a reduction in 367 some metabolites and an increase in others. A marked 368 reduction in total triglycerides was observed (Figure 4b), 369 reflecting a reduction within multiple subclasses of VLDL, 370 LDL and HDL lipoprotein particles, and within the IDL 371 lipoprotein subclass (Figure 3b). VLDL particle 13-1 was 372 smaller (Figure 4b), and there were lower total lipids in 373 VLDL (Very large, large, medium, small and very small) 374 subclasses which was attributable to lower VLDL 375 triglycerides, cholesterol, and phospholipids (Figures 3a & 376 **b**). HDL particle size was higher and there were higher 377 total lipids in HDL (very large and large) subclasses 378 (Figure 4b) which was attributable to greater total 379 cholesterol and phospholipid content (Figures 3a). 380 Apolipoprotein A-1 concentration was higher and 381 Apolipoprotein B and the ApoA/ApoB ratio were lower 382 (Figure 4b). When expressed as proportions of total fatty 383 acids, polyunsaturated fatty acids were increased (linoleic, omega-6 and PUFA), there was a decrease in mono-384 385 unsaturated fatty acids (Figure 4a). In addition, 386 glycoprotein acetyls, an inflammatory marker, was 387 reduced, whereas acetate and the amino acids alanine 388 glycine were increased (Figure 4a) in the and 389 breastfeeding group compared to those who did not 390 breastfeed.

391 When considered according to ethnic group, a similar 392 metabolic profile to the whole group was observed in the 393 White women who were breastfeeding at 6-months 394 postpartum (Supplementary Figures 1 & 2). However, 395 although numbers were smaller there were a number of 396 notable differences seen in the metabolome of Black 397 breastfeeding mothers compared to White mothers; IDL 398 and LDL particle size, concentration and content, were 399 little impacted by breastfeeding in White women (apart 400 from triglyceride content), there was a tendency for these 401 to be lower in breastfeeding women of Black ethnicity, 402 likely as a result of lower phospholipid and total cholesterol 403 content in these particles (Supplementary Figures 1a & 1b). Reductions including 404 in total fatty acids 405 polyunsaturated, monounsaturated and saturated fatty 406 acids appeared more marked in women of Black ethnicity 407 who breastfed (Supplementary Figure 2a). 408 Supplementary Figures 3 & 4 demonstrate that the 409 metabolic profile comparing breastfeeding and non-410 breastfeeding women in the control arm only (n=253), was

similar to that in the entire cohort (n=485).

412 <u>Reasons for maternal choices regarding infant feeding</u>

413 Of the 124 women who did not initiate breastfeeding, 64% 414 reported "never planned to", 13% gave reasons including 415 "difficulty in establishing lactation", "feeding issues with a 416 previous child" and "inverted nipples". A further 5% 417 reported being "advised not to breastfeed". Of those who 418 initiated breastfeeding but stopped before their child was 419 6-months old, the most common reason was "perceived 420 inadequate milk supply" (23%); others were "discomfort" 421 (7%) and "convenience" (5%). Thirty nine percent of 422 women reported "other" reasons for cessation including: 423 infant tongue tie, difficulty in 'latching on', needing to return 424 to work and partner/family members wanting to feed the 425 baby. Of the 290 women who maintained some 426 breastfeeding at 6-months, the most cited reasons for continuing were that breast milk is the "best nutrition for 427 428 baby" (74%), "convenience" (55%), "enjoyment" (47%), 429 "cheaper" (37%) and "maternal weight loss" (22%).

430 **Discussion**:

431 This study reports original observations on breastfeeding 432 behaviours and the metabolome in an ethnically diverse 433 cohort of women with obesity. The findings also strengthen 434 the case for additional support and strategies to enable 435 women with obesity to breastfeed for a longer duration. We 436 have described the relationship between severity of 437 obesity and breastfeeding and found that women with a 438 higher BMI had a shorter exclusive breastfeeding duration 439 of 17 days compared with women from a lower obesity 440 class. We have also described the previously recognised 441 relationships between breastfeeding and reduction of 442 postpartum weight and measures of anthropometry, 443 although these positive changes in anthropometry were 444 not observed amongst women of Black ethnicity, despite 445 Black women being more likely to initiate and continue to 446 breastfeed until 6-months postpartum, compared to other 447 ethnic groups.

448 Breastfeeding duration has previously been identified as 449 an important determinant of maternal weight loss 450 postpartum^{17,18}, with previous reports documenting the 451 reduction in weight largest amongst women of 452 heterogeneous BMI breastfeeding at 6-months and 453 beyond^{17,19}. Compared to no breastfeeding, we have 454 reported a -1.8kg difference in those women who were 455 giving any breastfeeding at 6months postpartum. Although 456 modest, our study confirms that breastfeeding duration is 457 equally relevant to weight loss in women with obesity; and 458 indeed, to reduce adiposity, providing further evidence for 459 healthcare professionals to support women with obesity to 460 breastfeed, and to encourage longer durations of 461 breastfeeding to aid postpartum weight loss. An ongoing 462 randomized controlled trial in Columbia is investigating the 463 impact of breastfeeding counselling on breastfeeding 464 prevalence and postpartum weight loss in women with a 465 BMI>24.9kg/m², which may contribute to an evidence 466 based intervention for encouraging women with a higher BMI to breastfeed²⁰. 467

468 In contrast to the present study, a study from the USA with 469 smaller sample size (n=37) reported that postnatal weight 470 retention in women with obesity was associated with 471 increased energy intake, independent of breastfeeding, 472 eating behaviours and metabolic biomarkers ²¹. The 473 authors investigated body composition, diet and activity 474 from early pregnancy until 12 months postpartum and 475 stratified results by PPWR vs postpartum weight loss. 476 Duration of breastfeeding was similar in both groups (30±5) 477 vs 29±6 weeks), indicating that higher energy intake could 478 override the role of breastfeeding in postpartum weight479 loss.

480 We found that intention to, and initiation of, breastfeeding 481 in the study cohort of women with obesity was higher than reported in a general UK population^{2,3}. Contributary factors 482 483 may include participation in a clinical trial, or changes in 484 local midwifery and health visitor practice to support 485 breastfeeding in line with the UK Baby Friendly Initiative²². 486 The follow up rate of 46% of the original trial participants 487 could also reflect selection bias, with findings less 488 generalisable to the whole study cohort, although those 489 choosing not to take part at 6-months postpartum had 490 similar characteristics at baseline to the participants 491 included in this analysis.

492 We found that increasing BMI class was associated with 493 decreased duration of breastfeeding. A retrospective 494 cohort study of women from the USA reported a similar 495 relationship, although with different BMI classification; 496 30.0-39.9kg/m²; 18.5-24.9kg/m²; 40.0-49.9kg/m²; 23 497 ≥50.0kg/m² The authors found that overall 498 breastfeeding rates were low (32%) with no data on 499 continued breastfeeding beyond hospital discharge, a 500 strength of the present study.

23

Previously reported reasons for decreased duration of breast feeding in women with obesity have included reduced maternal confidence to breastfeed associated with larger breasts^{8,11} and delayed lactogenesis II²⁴. Once initiated, milk supply may be impacted by hormonal imbalance^{8,25} or through consequences of the mother's perceived body image¹².

508 We report here reasons given by the study participants for non-initiation and shorter duration of breastfeeding. These 509 510 indicated that family and healthcare staff support are important in facilitating breastfeeding in women with 511 512 obesity. To achieve this would require relevant healthcare 513 staff in acute and primary care settings to acquire 514 appropriate competencies and skills, with inherent cost 515 implications. Insufficient regulations of the marketing of 516 breastmilk substitute as well as food insecurity also undermine breastfeeding prevalence^{26,27}. Also, more 517 518 research is required to explore women's perceptions of 519 inadequate milk supply and to identify why some of the 520 participants, and indeed the wider population of women, 521 do not consider breastfeeding.

522 A 2017 narrative review suggested that interventions 523 aimed at breastfeeding women will not be successful 524 unless there is protection, promotion, and support at a 525 population health level, along with increased investment in 526 health services to support women to breastfeed²⁸. 527 Furthermore, open responses from the participants 528 indicated that at 6-months postpartum many had returned 529 to paid employment, or were imminently planning to, a 530 common reason for early weaning and cessation of 531 breastfeeding. Comments from women on reasons for not 532 initiating breastfeeding, or for stopping early, highlighted 533 several areas for further research to better comprehend 534 the complexity surrounding maternal breastfeeding 535 behaviours.

536 Our study was also supported by a biological 'read out' of 537 metabolic health through the NMR metabolome at 6-538 months postpartum, until which time the WHO recommend exclusive breastfeeding. To our knowledge changes in the 539 540 metabolome in women with obesity continuing to 541 breastfeed at 6-months postpartum, either partially or 542 exclusively, have not previously been reported and may provide insight into the mechanisms of weight loss 543 associated with breastfeeding^{19,29}. This could contribute to 544 545 the protective effect of breastfeeding against progression to diabetes after gestational diabetes^{30,31}. There were 546 547 numerous indications of metabolic health, when compared with non-breast feeding women, including a reduction in 548 549 atherogenic VLDL particles and triglycerides across

550 lipoprotein particles, and an increase in anti-atherogenic 551 larger HDL particles, including constituent HDL cholesterol 552 and phospholipids. In addition, continuation of 553 breastfeeding was associated with higher polyunsaturated 554 fatty acids and lower mono-unsaturated fatty acids. The 555 majority of fats in breastmilk comprise triglycerides, 556 synthesised in the mammary glands of the breast from denovo lipogenesis of breast fat^{32,33} and intact triglycerides 557 558 are not directly transported from the circulation into breast 559 milk³⁴. The fall in maternal plasma triglycerides observed 560 in association with breastfeeding likely reflects increased 561 mobilisation of maternal fat stores and enhanced 562 metabolism to fatty acids that would contribute to 563 generation of energy to meet the demands of 564 breastfeeding. Mechanistically, it has been proposed that 565 the stimulation of prolactin during lactation would lead to a 566 fall in maternal oestrogens which, in turn, would stimulate 567 lipolysis¹⁸. An increase in the proteogenic amino acid 568 glycine could be advantageous to maternal health. Our 569 findings are consistent with a targeted mass spectrometry 570 metabolome undertaken 6–8 weeks postpartum in a cohort 571 of normoglycaemic individuals but with previous GDM, 572 where lactation intensity was associated with lower 573 triglycerides (and diglycerides) and higher phospholipids³⁵; 574 indeed they suggest that downregulation of

575 triglycerides/diglyceride lipogenesis during lactation is 576 directly associated with formation of phospho- and 577 sphingolipids through the CEPT1 gene. In addition, the 578 'healthy' metabolic profile seen here mirrors that seen 579 during pregnancy in individuals with obesity who were normoglycaemic compared to those who had GDM³⁶ and 580 581 may reflect a comparatively insulin-sensitive state in those 582 who are breastfeeding. As we had previously reported a 583 beneficial effect of the intervention on the maternal metabolic profile between 16 and 36 weeks of gestation³⁷, 584 585 exclude any residual effect of the intervention to 586 postpartum we also analysed the metabolome in women 587 in the control arm, which demonstrated similar differences 588 in the profile between the breastfeeding and non-589 breastfeeding mothers as that of the metabolome from the 590 whole cohort.

591 The lack of effect of breastfeeding on weight and 592 measures of adiposity in women of Black African or Black 593 Afro-Caribbean ethnicity compared with women of White 594 ethnicity is a novel observation. There may be fundamental 595 differences of genetic origin in fat metabolism, or 596 differences in postpartum physical activity, and diet. We have previously reported³⁸ the longitudinal dietary 597 598 trajectories in the UPBEAT women across pregnancy and 599 up to 3-years postpartum. Amongst the women who had a 600 high adherence to an African/Caribbean dietary pattern, 601 we observed a dietary rebound for those women who 602 followed a high adherence to the African/Caribbean 603 trajectory at 6-months postpartum. This may reflect food 604 insecurity, sociocultural drivers of diet such as social 605 support or family food preferences and may contribute to 606 the null findings for postpartum weight loss for women of 607 Black ethnicity. Differences in the metabolome observed 608 between White and Black women who breastfeed are 609 novel. These may be driven by a modification in their 610 diets³⁸, or implicated by the lower sample of Black women 611 in the study; these findings need to be evaluated in a larger 612 cohort.

613 Strengths and Limitations: Strengths of the study 614 include the rich UPBEAT dataset which provided 615 comprehensive information breastfeeding on and 616 anthropometry outcomes at 6-months postpartum in an 617 ethnically diverse cohort. To our knowledge, this study is 618 the first to report metabolomic changes associated with 619 breastfeeding in a cohort of women with obesity. The main 620 limitation is the observational study design, which is 621 residual subject to confounding and potential overestimation of reported effects³⁹. Although the 622 623 breastfeeding outcomes are reported in detail, these 624 outcomes are self-reported by the mother which may be subject to recall bias⁴⁰. Furthermore, while the findings are
generalisable amongst women with obesity, they may not
be directly generalisable to the general population of
women with a heterogeneous BMI.

629 **Conclusion:** Given the prevalence of obesity in women of 630 reproductive age, strategies to support and enable women 631 with a BMI \geq 30.0kg/m² to initiate and continue to 632 breastfeed are required to improve long-term maternal 633 health outcomes. This study supports strategies to 634 encourage, support and enable all women to continue 635 breastfeeding for at least 6 months, according to the WHO 636 recommendation, and to overcome barriers associated 637 with breastfeeding. The initiation and duration of 638 breastfeeding requires collective societal approaches, 639 including support from family members, healthcare 640 professionals, as well as government action to enable and 641 support breastfeeding, such as the development of 642 actionable policies which promote breastfeeding and 643 reduce the misleading advertising and marketing of breastmilk substitutes⁴¹. Further exploration into the null 644 645 findings for postpartum weight loss in women of Black 646 ethnicity are required.

647

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654 **Conflict of Interest:** None

Authors' Contribution: AB, CS, PT and LP designed
research; AB, CS, AF conducted research, KD and FT
analysed data, KD, AB and LP, wrote the paper. SW
provided metabolomic expertise. KD had primary
responsibility for the final content.

660 **Details of Ethics Approval:** The NHS research ethics 661 committee granted approval for all participating centres 662 (UK integrated research application system, reference 663 09/H0802/5). Additional approvals were obtained through 664 local Research and Development departments in each 665 participating centre. UPBEAT was also registered with the 666 ISRCTN reference 88971375).

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668 **Data availability statement:** The data that support the 669 findings of this study are available from the corresponding author, upon reasonable request and approval by theUPBEAT consortium.

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- 673 UPBEAT consortium authors: Annette L Briley⁶, Paul T
 674 Seed⁶, Claire M Singh⁶, Angela C Flynn⁶ and Lucilla
 675 Poston⁶
- ⁶⁷⁶ ⁶King's College London/Guy's and St Thomas' NHS
- 677 Foundation Trust, London, UK.

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Figure legend

Figure 1: Summary diagram of recruitment process by maternal BMI at trial entry

Figure 2: Kaplan-Meier survival curve for duration of exclusive breastfeeding from birth until cessation in women in each obesity class

Figures 3a & 3b: Standard deviation difference in lipoprotein particle concentration and subclass constituents between breastfeeding and nonbreastfeeding UPBEAT women at 6 months postpartum (n=485). The right-hand side of the x-axis represents positive associations with breastfeeding (fully or mixed) at 6 months, compared to non-breastfeeding women, and negative associations to the left-hand side.

Figures 4a and 4b: Standard deviation differences for (a) fatty acids, amino acids, glycaemic and other markers and (b) lipoprotein particle groups between breastfeeding and non-breastfeeding UPBEAT women at 6 months postpartum (n=485). The right-hand side of the x-axis represents positive associations with breastfeeding (fully or mixed) at 6 months, compared to non-breastfeeding women, and negative associations to the left-hand side.

	Included in analysis Not included in		Comparison		
	n= 715	analysis n=839			
	Mean (SD)/N (%)				
			coefficient/Risk		
			ratio/Odds ratio		
			(95% CI)		
Age (years)	31.2 (5.31)	29.9 (5.60)	1.32 (0.78 to 1.87)		
BMI (kg/m ²)	36.3 (4.90)	36.3 (4.66)	0.04 (-0.43 to 0.52)		
Ethnic origin					
White	506 (71%)	467 (56%)	ref		
Black	141 (20%)	260 (31%)	0.50 (0.39 to 0.63) ***		
Asian	5 (3%)	70 (8%)	0.33 (0.20 to 0.53) ***		
Other	43 (6%)	42 (5%)	0.94 (0.61 to 1.47)		
Nulliparous	363 (51%)	311 (37%)	1.75 (1.43 to 2.14) ***		
Current smoker	27 (4%)	81 (10%)	0.37 (0.23 to 0.57) ***		
Index of multiple depr	rivation				
1 (least deprived)	35 (5%)	30 (4%)	1.51 (0.91 to 2.51)		
2	49 (7%)	54 (6%)	1.17 (0.77 to 1.78)		
3	76 (11%)	101 (12%)	0.97 (0.60 to 1.36)		
4	261 (37%)	272 (33%)	1.24 (0.98 to 1.56)		
5 (most deprived)	292 (41%)	378 (45%)	ref		
Pregnancy outcomes					
Diagnosis of GDM ¹	197/699 (28%)	141/606 (23%)	1.29 (1.00 to 1.66) *		
Gestation at birth ≤	32/715 (4.5%)	67/805 (8.3%)	0.52 (0.33 to 0.79)		
37 weeks					
Unassisted vaginal	345/715 (48%)	453/805 (56%)	ref		
birth					
Operative vaginal	93/715 (13%)	85/805 (10%)	1.43 (1.04 to 1.99) [*]		
birth					
Prelabour Caesarean	144/715 (20%)	151/805 (19%)	1.25 (0.96 to 1.63)		
Section					
In labour Caesarean	133/715 (18%)	116/805 (14%)	1.50 (1.13 to 2.00) **		
section					
Birthweight ≥ 4 kg101/715 (14%)109/805 (13%)1.05 (0.78 to 1.41)					

Table 1: Demography of women included in analysis of breastfeeding behaviour

 compared to UPBEAT women who did not attend 6 months postnatal follow up visit

Abbreviations: BMI: body mass index, CI: confidence interval, GDM: gestational diabetes, SD: standard deviation.

¹OGTT results not available for all participants therefore denominator noted here. * p<0.05, ** p<0.01, ***p<0.001

	BMI Class I	BMI Class II	BMI Class III				
	(30.0-	(35.0-	(≥40.0kg/m²)				
	34.9kg/m ²)	39.9kg/m ²)					
	N (%) / Mean (standard deviation)						
Intended to breastfeed	267/354	178/225	101/136 (74.3%)				
	(75.4%)	(79.1%)					
Initiated breastfeeding at	294/354	189/225	108/136 (79.4%)				
birth	(83.1%)	(84.0%)					
6-month breastfeeding stat	us of those who	initiated					
breastfeeding							
Age of infant at 6-month	5.9 (0.86)	5.8 (0.87)	6.0 (1.01)				
follow-up							
Exclusive breastfeeding	11/294 (3.7%)	7/189 (3.7%)	1/108 (0.9%)				
Formula milk only	8/294 (2.7%)	6/189 (3.2%)	0/108 (0%)				
Breastfeeding + other fluids	4/294 (1.3%)	4/189 (2.1%)	5/108 (4.6%)				
Breastfeeding + solids ±	73/294 (24.8%)	41/189 (21.6%)	16/108 (14.8%) [*]				
other fluids							
Formula + solids ± other	134/294	92/189 (48.7%)	68/108 (63.0%) *				
fluids	(45.6%)						
Breastfeeding + Formula	64/294 (21.7%)	39/189 (20.6%)	18/108 (16.7%) [*]				
milk + solids ± other fluids							
Any breastfeeding at 6-	152/294	91/189 (48.2%)	40/108 (30.7%) [*]				
months	(51.7%)						
All women							
Formula feeding at 6	266/354	203/255	114/136 (84.0%)				
months	(75.0%)	(80.0%)					
Introduced solids at 6	329/354	201/225	128/136 (94.1%)				
months	(92.9%)	(89.3%)					
Mean duration of exclusive	90.4 (64.0)	74.6 (66.3)	73.7 (65.1)				
breastfeeding (days)							
Difference in mean (95%CI)		-15.8 (-28.5, -	-16.7 (-32.0, -				
vs class l		3.1) *	1.35) *				
Never breastfed	60 (17.0%)	36 (16.0%)	28 (20.7%)				

Table 2: Infant feeding outcomes recorded at the 6-month follow-up, stratified by

 maternal BMI class at trial entry

* p<0.05, ** p<0.01, ***p<0.001. other fluids defined as drinks such as water or juice.

Table 3: Initiation of breastfeeding and pregnancy outcomes according to BMI class. Data presented as number of women/total (%)

	BMI Class I (30.0-34.9kg/m ²)	BMI Class II (35.0-39.9kg/m²)	BMI Class III (≥40.0kg/m²)	Class 2 compared to class 1	Class 3 compared to class 1			
		N (%)			o (95% CI)			
Initiation of	294/354 (83.1%)	189/225 (84.0%)	108/136 (79.4%)	1.07 (0.68 to 1.68)	0.79 (0.48 to 1.30)			
breastfeeding								
Parity	176/354 (50%)	120/354 (53%)	67 (49%)	1.15 (0.83, 1.62)	0.98 (0.66, 1.46)			
Unassisted vaginal	176/354 (50%)	112/225 (50%)	57/136 (42%)	ref	ref			
Operative vaginal	44/354 (12%)	33/225 (15%)	16/136 (11%)	1.18 (0.71 to 1.96)	1.12 (0.59 to 2.14)			
Pre-labour Caesarean	75/354 (21%)	37/225 (16%)	32/136 (23%)	0.78 (0.49 to 1.23)	1.32 (0.79 to 2.19)			
Caesarean in labour	59/354 (17%)	43/225 (19%)	31/136 (23%)	1.14 (0.72 to 1.81)	1.62 (0.96 to 2.75)			
GDM diagnosis	77/345 (22%)	75/218 (34%)	45/136 (33%)	1.82 (1.25 to 2.66)	1.11 (1.11 to 2.66)			
				**	*			
Infant birthweight ≥4kg	54/354 (15%)	30/221 (13%)	17/136 (13%)	0.85 (0.53 to 1.38)	0.79 (0.44 to 1.43)			
Abbreviations: BMI: body mass index, CI: confidence interval, GDM: gestational diabetes * p<0.05, ** p<0.01, ***p<0.001								









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