Assessing maternal alcohol consumption in pregnancy: comparison of confidential postnatal maternal interview and measurement of alcohol biomarkers in meconium.

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Abstract

Objective: Knowledge of alcohol consumption in pregnancy is important for public health messaging and to help early identification of children at risk of fetal alcohol spectrum disorder. We investigated whether alcohol biomarkers fatty acid ethyl esters (FAEEs) and ethylglucuronide (EtG) in meconium are predicted by maternal or newborn demographics and/or correlate with early confidential postnatal self-report of alcohol consumption in pregnancy.

Design: Anonymised, observational population-based study.

Setting: Inner-city maternity unit in Glasgow, UK.

Patients: Singleton mother/infant dyads delivering every fourth day.

Interventions: Mother: confidential postnatal interview. Baby: meconium sample for FAEEs and EtG.

Results: 840/908 mothers consented. 370 (46.4%) reported alcohol consumption in pregnancy; for 114 (13.6%) this included drinking after 20 weeks' gestation, generally of modest amount. Alcohol consumption in later pregnancy was more commonly reported by

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women of white British ethnicity ($p<0.05$). Women who reported drinking in later pregnancy were older (31.3 *v* 29.5 years) and their babies on average 118g heavier ($p=0.032$). FAEEs were identified in all meconium samples; concentration was ≥600 ng/g in 39.6%. EtG concentration was ≥30 ng/g in 14.5%. Neither biomarker was associated with maternal age, body mass index or socioeconomic status but when EtG concentration was ≥30 ng/g, the mother was less likely to identify as white British (71.3% vs 81.8%, $p=0.028$). Sensitivity of FAEEs ≥600 ng/g and EtG ≥30 ng/g were 43.1% and 11.6% respectively at identifying postnatal self-report of alcohol use in later pregnancy (specificities 60.6% and 84.8%). *Conclusion:* Meconium biomarkers do not reliably detect modest alcohol consumption in later pregnancy.

250 words

Key Messages

What is already known on this topic

- Knowledge of alcohol consumption in pregnancy is important in targeting public health messaging and to help early identification of children at risk of fetal alcohol spectrum disorder.
- Fatty acid ethyl esters (FAEEs) and ethylglucuronide (EtG) can be measured from infant meconium but utility to document the pattern of alcohol consumption in pregnancy in an unselected UK population is not known.

What this study adds

- FAEEs and EtG measured in meconium have low sensitivity for self-reported modest alcohol consumption after 20 weeks' gestation.

How this study might affect research, practice or policy

- Measurement of alcohol biomarkers in meconium cannot currently be recommended as a reliable indicator of alcohol exposure in later pregnancy.

Contributorship statement

EMAH participated in study design, recruited all subjects, undertook some of the statistical analyses, wrote the first draft of the manuscript and participated in all subsequent revisions. DT participated in study design and critically reviewed the draft manuscript. DY advised on data analysis and reviewed the draft manuscript. DF supervised laboratory analyses and reviewed the draft manuscript. HM conceived and supervised the study, contributed to the draft manuscript and critically reviewed all manuscript revisions. All authors approved the final manuscript.

Introduction

Prenatal alcohol exposure (PAE) risks fetal damage, manifest as fetal alcohol spectrum disorder (FASD). (1) The classic triad of poor brain and somatic growth, typical facial features and learning disorder is easily recognisable as fetal alcohol syndrome (FAS) (2) but milder forms of FASD are commonly unrecognised in childhood (3-5) despite being associated with significant morbidity and financial cost to society (6). The descriptor FASD requires accurate history of PAE (2) but ascertainment of PAE is notoriously unreliable for reasons including reluctance of professionals to seek relevant information, under-reporting by expectant mothers, limited handover of information to child health services (7) and over-representation of children with FASD in the accommodated population (8). Earlier diagnosis of FASD with targeted interventions results in better outcomes and provides opportunity to protect future children of women who consume alcohol in pregnancy (9, 10).

Alcohol biomarkers explored in mother and infant include fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG) in meconium (11-14). Ethanol diffuses freely across the placenta and non-oxidative fetal metabolism produces FAEEs. These large molecules remain in the fetus including within meconium, formed in the gut from 16-20 weeks' gestation. (15) EtG crosses the placenta; its presence in meconium reflects maternal and fetal alcohol metabolism and is less prone to variation from nutritional factors and/or endogenous production than FAEEs (16). Concentration of FAEEs in meconium ≥600 ng/g has been related to regular alcohol consumption during pregnancy of >2 drinks per day (equivalent to 28 g ethanol or 3.5 UK units) (17). EtG >30 ng/g had moderate-substantial agreement with self-reported PAE after 19 weeks' gestation in a relatively heavy drinking population (12). FAEE concentration in meconium correlates inversely with educational attainment (18) (19).

In a previous anonymised study of an unselected population of infants, concentrations of FAEEs and EtG in meconium were respectively ≥600 ng/g in 42% and ≥30 ng/g in 15% although only 3% of mothers reported alcohol consumption in pregnancy (11). No association was seen between either biomarker and any maternal or infant demographic. We sought to determine if a structured, confidential early postnatal interview would be associated with more common reporting of alcohol in pregnancy, and if alcohol biomarkers in meconium would correlate with self-reported alcohol consumption in later pregnancy and/or any maternal or infant demographics.

Methods

This was an observational population-based study conducted within a large obstetric-led maternity unit in the relatively deprived city of Glasgow. All mothers delivering a live singleton infant within each fourth consecutive 24-hour period ("study days") were eligible. Women admitted to labour suite were provided with written information and a plastic bag in which to collect the baby's first meconium nappy. Written informed consent was sought as soon as possible after delivery by a single researcher; if the mother declined, retained samples were discarded. Meconium samples were transferred to a plain universal container directly from the nappy, avoiding contact with baby wipes. Containers were labelled and frozen at -40°C for thermally protected shipping to the University of Padova, Italy.

Assessment of alcohol consumption in pregnancy

Alcohol consumption in pregnancy was assessed by confidential health questionnaire administered by a single researcher immediately after consent for participation in the study. Mothers who reported alcohol consumption were asked more details including timing, type and amount of alcohol, this was recorded on an individualised calendar using a modified time

line follow back method and related to gestation. Participants were encouraged to utilise diaries and/or social media to prompt recollection. Demographic information obtained from casenotes included maternal age, gravidity, postcode of residence, ethnicity and body mass index (BMI), infant gestation, birth weight and occipitofrontal head circumference (OFC). The Scottish Index of Multiple Deprivation 2016 (SIMD16) based on postcode of the mother's address at time of delivery was used as a measure of socioeconomic deprivation (21). Scores were assigned between 1 (most deprived) and 6976 and divided into deciles.

Laboratory Analyses

Liquid chromatography/mass spectrum analysis was utilised for measurement of FAEEs (ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate) and EtG. 200 mg of meconium was sonicated for 15 minutes with 20 ng EtG-d5 and 200 ng of FAEE d-5. The supernatant was added to an aminopropyl solid-phase extraction cartridge, preconditioned with 2 ml methanol, water and acetonitrile (ACN). FAEEs were eluted with 2 ml hexane and EtG elution carried out with 2 ml water. The two mixtures were dried using nitrogen stream and recovered with 50 µl of ACN (FAEEs) and 50 µl methanol (EtG). FAEEs were detected following separation using a C8 reversed-phase column. A C18 reversed-phase column was used in isocratic mode for EtG detection. Acquisition was in multiple reactions monitoring for all the analytes in positive mode for FAEEs and negative mode for EtG. Lower limit of quantification (LOQ) values were 10-15 ng/g for summed FAEEs and 10 ng/g for EtG.

Statistics

Between group comparisons were done using Z-tests for two proportions for categorical data and t-tests for numerical data. Pearson correlations were used to determine relationships between numerical measurements. The performance of FAEEs and EtG to predict alcohol consumption was quantified by computing sensitivity, specificity, positive and negative

predictive values. All analyses were done using Minitab (version 18) at a 5% significance level.

The study was approved by West of Scotland Research Ethics Committee 3; funding was from Yorkhill Children's Charity.

Results

1021 singleton infants were born on 71 study days (Figure 1). 840 (92.5%) eligible mothers consented and 828 completed a health questionnaire.

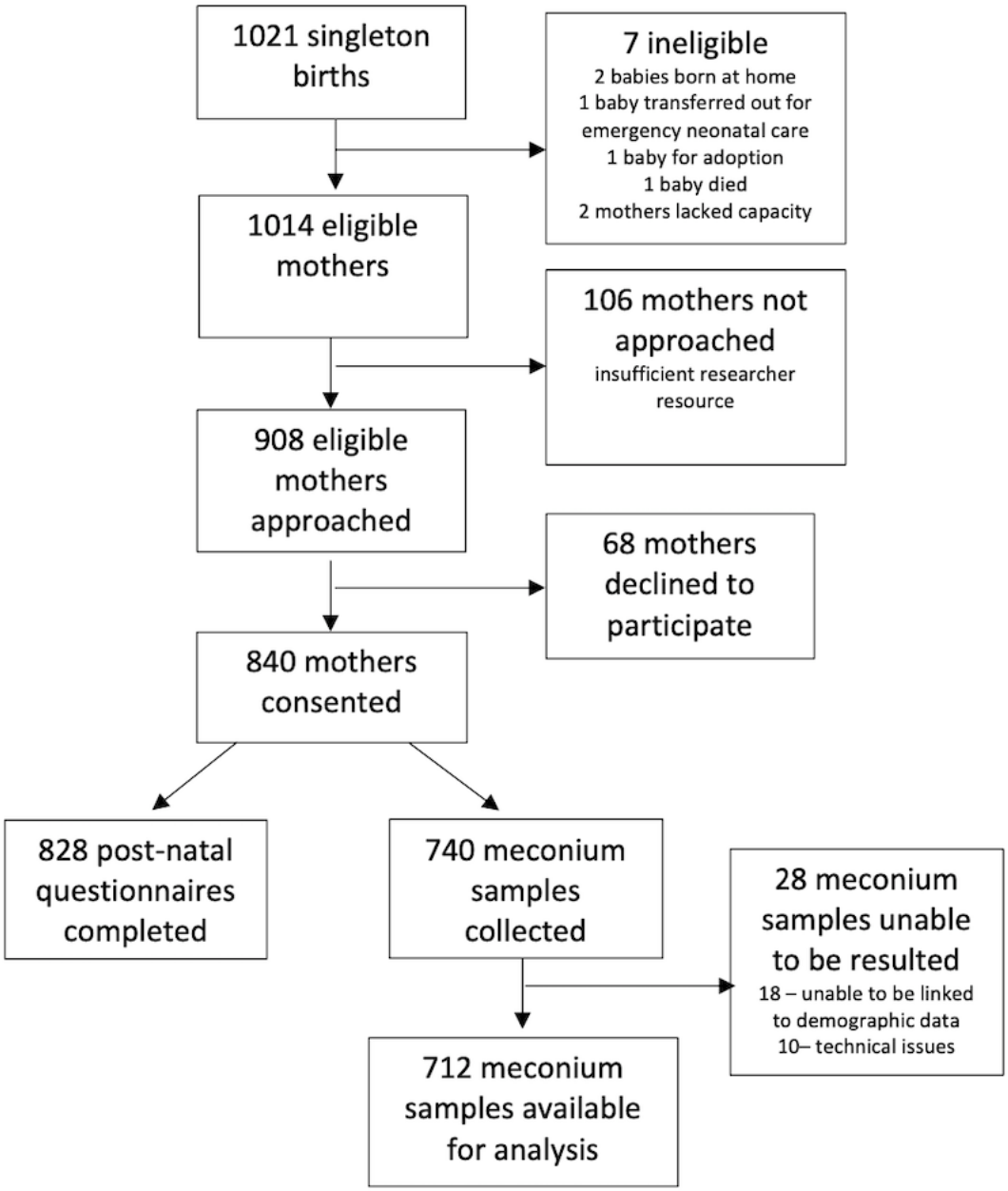

Figure 1. Study recruitment and collection of meconium samples.

Smoking and alcohol consumption

223/828 mothers (26.9%) declared smoking prior to pregnancy; 145 continued to smoke during pregnancy. A further two mothers started smoking in the current pregnancy (Table 1). 384 (46.4%) mothers reported consuming alcohol at any point in pregnancy; for the majority

(252) this was only prior to knowledge of pregnancy. 114 (13.8%) reported alcohol beyond 20 weeks' gestation; three reported one or more episodes of binge drinking (*i.e.* ≥ five UK units (40 g of ethanol) on one occasion). Alcohol consumption was less common in mid-pregnancy (5.3% of all mothers).

Any alcohol consumption in pregnancy was more common in women who identified as white British ($p<0.05$) and/or smoked in pregnancy ($p<0.05$). Self-reported alcohol beyond 20 weeks' gestation was more common in mothers aged >35 years ($p=0.0004$) and who identified as white British ($p<0.05$) (Table 1).

Babies with declared PAE after 20 weeks' gestation had a higher mean birthweight (3470 *vs* 3352 g ($p=0.032$)); this was not explained either by gestation or maternal BMI and was not accompanied by greater OFC.

Fatty acid ethyl esters (FAEEs)

FAEEs were detected in all samples; total concentration ranged from 22.2 to 7549.8 ng/g. Using ≥600 ng/g to define a positive result, 282 (39.6%) were positive. FAEE concentration >2000 ng/g was found in 21 (2.9%) of samples.

The likelihood of meconium positive for FAEEs was not related to maternal age, BMI, SIMD score, ethnicity or previously having had a baby. When meconium was positive, mothers tended to have been less likely to smoke during pregnancy (14% *vs* 20%, $p=0.071$).

Mean birthweight was greater for those infants whose meconium was positive for FAEEs (3425 *vs* 3331 g, $p=0.032$)). Mean OFC did not differ.

Self-reported alcohol consumption was linked with 702 meconium samples. When infant meconium was positive or negative for FAEEs, 41/280 (14.5%) or 54/422 (13%) mothers

respectively reported alcohol consumption after 20 weeks' gestation (NS). Of the eight mothers that reported drinking at least three units of alcohol on any occasion after 20 weeks' gestation, only three infants' meconium samples were positive for FAEEs (707, 847 and 996 ng/g respectively). When FAEE concentration was > 2000 ng/g (n=21), none of the mothers reported consuming alcohol in pregnancy.

Ethyl glucuronide (EtG)

EtG was detectable in 293/712 samples but below LOQ in 96. When EtG was quantifiable (n=197), median concentration was 32.2 ng/g, IQR 17.9 to 55.8 ng/g; results were skewed with mean concentration 162.1 ng/g (SD 871). EtG was ≥30 ng/g (positive) in 103 (14.5%) samples. Infants with meconium positive for EtG were less likely to have a mother who identified as white British (71.3% *vs* 81.8%, p=0.028). When meconium was positive for EtG, mothers tended to have been less likely to have smoked during pregnancy (13.6% *vs* 18.5%); and mean birthweight was marginally lighter (3418 *vs* 3365 g) but neither difference was significant.

When meconium was positive for EtG, 10.7% of mothers reported alcohol consumption after 20 weeks' gestation compared to 14% of mothers when meconium was negative. Eight mothers reported drinking at least three units of alcohol on any occasion after 20 weeks' gestation; only one meconium had detectable EtG (17.3 ng/g).

Correlation between FAEEs and EtG in meconium

Overall, there was no correlation between FAEEs and EtG. There was a weak positive correlation in those meconium samples positive for both FAEEs and EtG (n=51) (Figure 2) (Pearson's coefficient= 0.283, p value=0.044).

Sensitivity and specificity of alcohol biomarkers

Using maternal self-report of alcohol after 20 weeks' gestation as gold standard, the sensitivities of FAEEs ≥600 ng/g and EtG ≥30 ng/g in meconium were 43.1% and 11.6% respectively (Table 4). Combination of FAEEs ≥600 ng/g and EtG ≥30 ng/g had high specificity for alcohol consumption in later pregnancy (92.4%), but very low sensitivity (5.3%). The positive predictive value of two positive infant biomarkers for self-reported alcohol consumption in later pregnancy was 9.8%.

Discussion

Reports have examined the utility of measuring FAEEs and/or EtG in meconium as a marker of PAE (12, 22-25). Chan *et al.* concluded a summed value of > 600 ng/g for four FAEEs was indicative of PAE using a cohort of six "confirmed heavy alcohol consumers" compared to non-drinkers (n= 207) (17, 23). Using maternal self-report of heavy drinking (3.5 units daily and/or thrice weekly binge drinking) as the gold standard, the sensitivity of FAEEs in meconium ≥600 ng/g is consistently reported at 100% with specificity varying from 13 - 98.4% (23, 26).

For EtG, reported cut off values range from 10-440 ng/g. Himes *et al.* determined that EtG >30 ng/g had the highest sensitivity (81.8%) and specificity (75%) of any other biomarker or combination of biomarkers, using a TLFB method of self-report as the gold standard (n=107) (12). EtG in meconium reflects both maternal and fetal alcohol metabolism and the higher specificity and sensitivity of EtG may also be attributable at least in part to relative stability compared to FAEE (16).

In the current study the prevalence of FAEEs ≥600 ng/g and EtG ≥30 ng/g was very similar to a pilot study of a comparable unselected Scottish maternity population, suggesting both

reproducibility of laboratory analyses and lack of behavioural change over the intervening three-year period (11). In the pilot study, no newly delivered mother reported drinking alcohol in pregnancy compared to 13.8% in this study, demonstrating that much higher rates of alcohol consumption are reported when mothers are asked detailed, confidential questions in a systematic way.

The prevalence of alcohol consumption in pregnancy in the Scottish population was examined previously via national Infant Feeding Surveys (IFS) in 2010 and 2017 and in the 2010-11 representative birth cohort (27-29). In 2017 at six weeks postnatal, 12% mothers reported alcohol consumption during pregnancy(29), comparable with 13.8% of mothers drinking after 20 weeks' gestation in the current study, conducted between 2015-16. Accuracy of reporting of alcohol consumption reduces with time from delivery (30). In the current study almost half of mothers reported some alcohol during pregnancy although this was mostly before awareness of pregnancy, suggesting that self-report underestimates drinking in the earliest stages of pregnancy, when the embryo is at risk of damage from alcohol(20). In the 2010 IFS 40% of mothers reported alcohol consumption during pregnancy, albeit generally of modest amount (<3 UK units)(28); whether this change in reporting over a seven year period including much public health messaging around the dangers of PAE reflects a true reduction or more inhibition about reporting alcohol in very early pregnancy is not clear. A similar retrospective reporting system noted a reduction in alcohol consumption during pregnancy in Scotland between 2005/6 and 2010/11(27). The 2017 IFS and the current study are consistent in that almost half of women of reproductive age in Scotland are regularly consuming alcohol prior to conception (25,26). Given that up to 40% of pregnancies are unplanned (27), this has implications for public health messaging. In our study mothers who self-reported alcohol consumption beyond 20 weeks' gestation were more likely to be older, again similar to the IFS (28).

Although we are reasonably confident that self-reporting of frequency of alcohol consumption was accurate, those mothers who reported drinking in pregnancy may have under-estimated the amount. It is also possible that mothers who declined to participate, or who provided consent but no meconium sample, were more likely to have consumed alcohol (selection bias). Inaccurate self-report may reflect lack of recollection, underestimation of poured volume and concern about clinician judgement.(31) Similar to smoking during pregnancy, women who report alcohol use will be telling the truth as there is no advantage to reporting alcohol use when none has been consumed. Therefore, the sensitivity of meconium biomarkers in the current study will be accurate.

Any comparison with maternal self-report either through individualised or standardised risk questionnaires such as TWEAK, MAST, CAGE must be close to delivery (17, 24, 25) since patterns of alcohol consumption may change during pregnancy as evidenced in the current study.

The strengths of this large population-based study are the high rates of recruitment, the consistency of confidential, early postnatal interviewing and the prompt handling of samples. A weakness is the lack of inclusion of known heavy alcohol consuming mothers. Despite attempts to recruit high consumers of alcohol (including an amendment to protocol to enable recruitment outwith study days) none was highlighted to the research team. It is unclear whether this was due to midwives not being confident in identifying mothers or reluctance of staff to refer to the research team.

If clinicians are to make early diagnosis of FASD to allow early more effective intervention, a marker of PAE needs to be *sensitive* to allow as many affected infants as possible to receive intervention and *specific* to not subject unaffected infants to unnecessary intervention and the mother to the stigma of an alcohol related disorder. This study shows that FAEE and EtG

measurement in meconium is neither sensitive nor specific and therefore should not be used in isolation as a marker of PAE (11).

Conclusion

FAEEs and EtG measured in meconium have low sensitivity for self-reported modest alcohol consumption after 20 weeks' gestation in an unselected Scottish population. Measurement of these alcohol biomarkers in meconium cannot currently be recommended for the identification of newborns at risk of FASD.

Word count 2439

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	Entire recruited population (N=840)	Ever drank alcohol in pregnancy (N=384)	Drank alcohol after 20 weeks' gestation (N=114)	Did not drink alcohol after 20 weeks' gestation (N=713)
Maternal age (years) mean (SD)	N=840	384	114	713
	29.8 (5.7)	29.5 (5.8)	31.3 (5.7)	29.5 (5.6)
% aged > 35 years	19.5	20.6	31.6*	17.4*
Maternal BMI (kg/m^2) median (IQR)	N=822	380	112	697
	25.8 (22.9 – 30.3)	27.0 (16.0-53.0)	25.4 (23.0 – 29.8)	25.8 (22.8 – 30.3)
SMID decile median (IQR)	N=831	379	110	709
	3 (1 – 6)	4 (1-7)	5 (2- 8)	4 (1 – 6)
Ethnicity (% White British)	N=803	370	107	686
	80.3%	90.5%	91.6%*	79.7%*
Smoked during pregnancy	N=828	383	113	712
	17.8%	20.1	13.3	18.5
Previous child (%)	N=822	375	111	699
	56.3%	54.1%	54.1%	56.8%
Mode of delivery (% vaginal)	N=839	383	114	712
	61.5%	62.9%	60.5%	61.7%
Infant sex (% male)	N=840	384	114	713
	50.1%	50.8%	48.2%	50.6%
Gestation (weeks) mean (SD)	N=837	382	113	711
	38.9 (1.7)	38.9 (1.5)	39 (1.3)	38.9 (1.7)
Birthweight (g) mean (SD)	N=838	383	113	712
	3367 (526)	3369 (511)	3470 (474)*	3352 (532)*
OFC (cm) Mean (SD)	N=825	375	111	702
	34.6 (1.5)	34.7 (1.5)	34.7 (1.5)	34.6 (1.5)
APGAR score mean (SD)	N=829	381	112	705
	9 (1.3)	9 1.2	9 (1.3)	8.6 (1.5)

Table 1 Maternal and infant demographics in relation to maternal consumption of alcohol in mid-late pregnancy * (P=<0.05)

	Meconium sample resulted (N=712)	FAEE ≥600ng/ml (N=282)	FAEE <600ng/ml (N=430)
Maternal age (years) mean (SD)	N=712	282	430
	29.8 (5.6)	30.1 (5.3)	29.7 (5.8)
Maternal BMI (kg/m^2) median (IQR)	N=696	274	422
	25.7 (22.8 – 30.1)	25.6 (23 – 30.6)	26.9 (22.5 – 30)
SIMD decile median (IQR)	N=704	280	424
	3 (1 – 6)	3 (1 – 7)	3 (1 – 6)
Ethnicity N= (% White British)	N=682	276	406
	547 (80.2%)	218 (79%)	329 (81%)
Smoked during pregnancy N= (%)	N=703	279	424
	126 (17.9%)	41 (14.7%)	85 (20%)
Previous child N= (%)	N=699	280	419
	390 (55.8%)	118 (57.9%)	191 (54.4%)
Mode of delivery N= (% vaginal)	N=711	282	429
	434 (61%)	174 (61.7%)	260 (60.6%)
Infant sex N= (% male)	N=712	282	430
	365 (51.3%)	145 (51.4%)	220 (51.2%)
Gestation (weeks) mean (SD)	N=709	282	427
	38.9 (1.6)	39 (1.5)	38.8 (1.7)
Birthweight (g) mean (SD)	N=710	282	428
	3373 (526)	3425 (523)	3331 (525)
OFC (cm) Mean (SD)	N=700	276	424
	34.6 (1.4)	34.7 (1.4)	34.6 (1.5)
APGAR score mean (SD)	N=703	278	425
	8.6 (1.2)	9 (1.2)	8.6 (1.3)

Table 2. Maternal and infant demographics in relation to meconium positive (≥ 600 ng/g) or negative for FAEEs

	Meconium sample resulted (N=712)	EtG ≥30ng/ml (N=103)	EtG <30ng/ml (N=609)
Maternal age (years) mean (SD)	N=712	103	609
	29.8 (5.6)	29.4 (6.5)	29.9 (5.6)
Maternal BMI (kg/m^2) median (IQR)	N=696	100	596
	25.7 (22.8 – 30.1)	26.9 (22.8 – 30)	27.1 (22.7 – 30.2)
SIMD decile median (IQR)	N=704	101	603
	3 (1 – 6)	2 (1 – 6)	3 (1 – 6.5)
Ethnicity (% White British)	N=682	101	581
	547 (80.2%)	72 (71.3%)	475 (81.8%)
Smoked during pregnancy	N=703	103	600
	126 (17.9%)	14 (13.6%)	112 (18.5%)
Previous child (%)	N=690	101	598
	390 (55.8%)	53 (52.5%)	337 (56.4%)
Mode of delivery (% vaginal)	N=711	102	609
	434 (61%)	66 (64.7%)	368 (60.4%)
Infant sex (% male)	N=712	103	609
	365 (51.3%)	47 (45.6%)	318 (52.2%)
Gestation (weeks) mean (SD)	N=709	103	606
	38.9 (1.6)	38.8 (1.8)	38.9 (1.6)
Birthweight (g) mean (SD)	N=710	103	607
	3373 (526)	3418 (524)	3365 (526)
OFC (cm) Mean (SD)	N=700	101	599
	34.6 (1.4)	34.8 (1.5)	34.6 (1.4)
APGAR score mean (SD)	N=703	100	603
	8.6 (1.2)	8.5 (1.1)	8.6 (1.3)

Table 3 Maternal and infant demographics in relation to meconium sample positive (≥ 30 ng/g) or negative for EtG

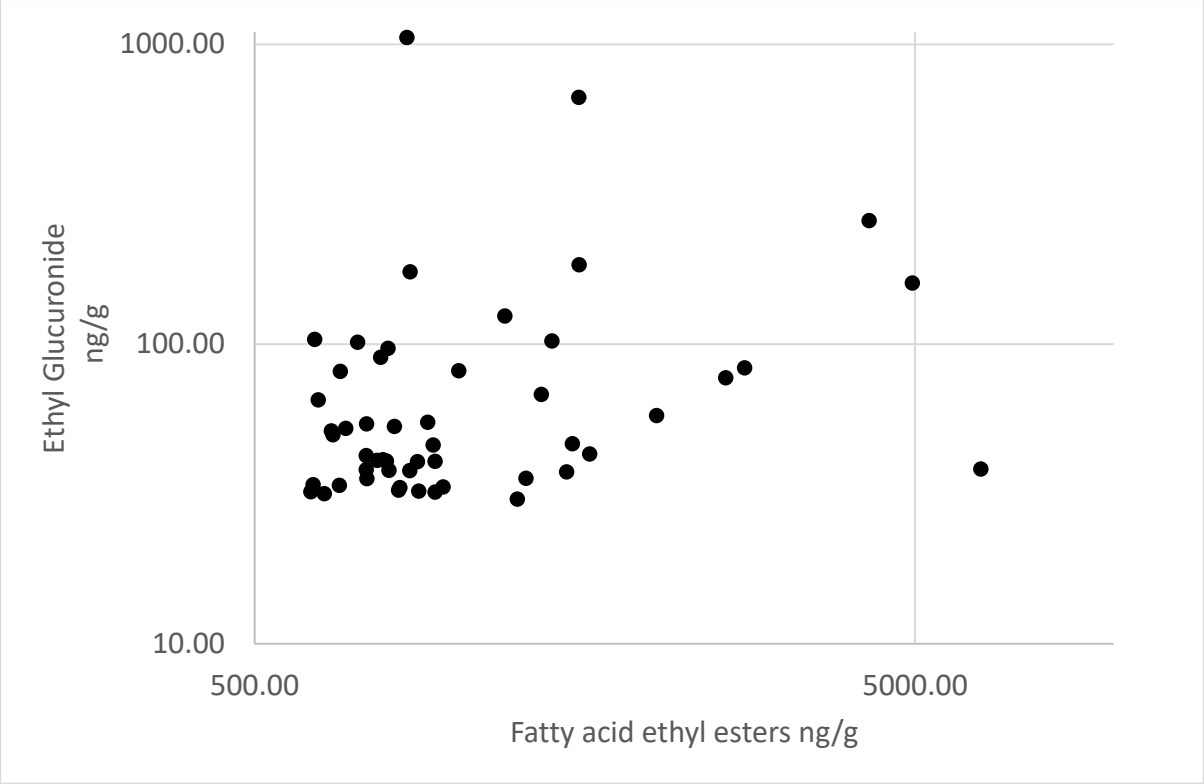

Figure 2. EtG concentration in relation to total FAEEs for meconium samples with EtG ≥30ng/g and FAEEs ≥600ng/g (N=50)

	Self reported alcohol consumption in pregnancy at any time (n=328)				Self-reported alcohol consumption in pregnancy after 20 weeks gestation (n=95)			
	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
FAEE >600ng/g	41.2	61.2	48.2	54.3	43.1	60.6	14.6	87.2
EtG >30ng/g	13.1	84	41.7	52.4	11.6	84.8	10.7	86
FAEEs >600ng/g and EtG >30ng/g	6.7	92.2	43.1	53	5.3	92.4	9.8	86.2

Table 4. Sensitivity, specificity, positive predictive value, and negative predictive value of infant biomarkers for FAEEs and EtG as ascertained by confidential postpartum maternal interview.