The epidemiology of abdominal adiposity

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Chapter 6

Ultrasound for visceral and subcutaneous abdominal fat in infancy

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ABSTRACT:

Background: The use of direct imaging to measure internal-abdominal (visceral) and subcutaneous abdominal adipose tissue (IA-AT and SCA-AT, respectively) in infants is limited. Anthropometry and other body composition techniques do not discriminate between these depots. Therefore, we validated the use of ultrasound (US) to estimate IA-AT and SCA-AT volumes and used this technique to examine their determinants in infancy.

Methods: In 22 term newborn infants, we validated the use of US-visceral depth and US- subcutaneous abdominal depth by comparison to MRI measures of IA-AT and SCA-AT volumes. In the Cambridge Baby Growth Study (CBGS), 487 infants had US assessments at age 3 months, and 495 infants at 12 months (360 at both ages). All infants also had weight, length and skinfold thickness measurements at birth, 3 and 12 months. Linear regression was used to relate US measures to infant growth parameters adjusted for sex.

Results: In the validation study, the US measures were positively correlated with IA-AT (US-visceral depth: $r=0.48$, $p<0.05$) and SCA-AT volumes (US-subcutaneous abdominal depth: $r=0.71$, $p<0.001$). In CBGS, US-visceral depth increased by around 20% between ages 3-12 months ($P<0.0001$) and showed weak evidence of longitudinal correlations between these ages (inter-correlation: $r=0.11$, $P=0.04$, $N=360$). US-visceral depth at both 3 and 12 months was *inversely* related to skinfold thickness at birth, particularly after adjustment for current skinfold thickness ($P=0.03$ and $P=0.009$ respectively); while US-subcutaneous abdominal depth at 3 months was *positively* related to skinfold thickness at birth ($P=0.004$).

Conclusion: US is a valid method to estimate infancy abdominal adipose tissue distribution when compared to MRI measures. Both antenatal and postnatal factors may influence the quantity of IA-AT and SCA-AT in infancy.

INTRODUCTION

Childhood obesity has become a major public health issue and its prevalence is increasing worldwide (1-3). More important than BMI or overall adiposity, increased internal-abdominal (visceral) adiposity is a major risk factor for insulin resistance, dyslipidemia, hyperinsulinemia and hypertension (4-6) . This risk has also been documented in studies of obese children, where greater abdominal adipose tissue distribution appeared to be associated with an unfavourable metabolic profile (7, 8).

Several epidemiological studies have shown that early life factors, such as impaired or excess weight change, are associated with later obesity and its associated co-morbidities (9-13). Growth in fetal life as well as in infancy has been associated with subsequent abdominal adipose tissue accumulation (9, 14). However, these studies have used indirect measures of abdominal adiposity such as skinfolds and waist–hip ratios and therefore did not investigate the contribution of these early life determinants to the development of internal-abdominal and subcutaneous-abdominal adipose tissue compartments.

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are considered the gold standard for the assessment of different abdominal adipose tissue compartments. However, their use is limited in childhood research studies due to practical difficulties including: a high burden to participants and investigators (expensive equipment and need for specialised technicians), high sensitivity to movement artefacts and exposure to ionising radiation (CT only) (15, 16). MRI has been previously used to quantify different body adipose tissue compartments at birth (17). However, between the ages 3-4 months to around 5-6 years MRI is not feasible as a research tool as sedation or even general anaesthesia is usually required for clinically-indicated MRI scans at these ages.

Research studies in infants usually use standard anthropometry to evaluate adiposity. However, these measures do not differentiate between internal-abdominal and subcutaneous adipose tissue (18, 19); skinfold thicknesses exclusively measure subcutaneous adipose tissue (16).

Ultrasound (US) has been assessed as an alternative non-invasive method to estimate abdominal adipose tissue distribution. US-visceral depth and abdominal US-subcutaneous depth have been shown to be reliable and reproducible estimates of internal-abdominal (visceral) adipose tissue (IA-AT) and abdominal subcutaneous adipose tissue (SCA-AT) quantities, respectively, when compared to either CT or MRI in middle-age and older adults and in adolescents (20-24). However, its validity has yet to be studied in infants. We therefore tested the validity of US-visceral depth and US-abdominal subcutaneous depth by comparison to MRI measures of IA-AT and SCA-AT mass in newborn infants. We used this technique to identify possible early life factors, such as early growth parameters and nutrition that might influence these abdominal adipose tissue depositions in the first year of life in a prospective birth cohort.

METHODS

Validation study population

The validation study was carried out in 22 healthy term singleton newborn infants (10 boys and 12 girls). Mean (range) age was 10.6 (6-19) days; gestational age at birth 39.9 (37.1-40.8) weeks; weight 3.3 (2.5-3.9) kg; length 53.1 (47-57) cm and waist circumference 33.8 (29-39) cm. Mothers and babies were recruited from the Neonatal Unit and postnatal wards of the Chelsea and Westminster Hospital, London UK, between 2008 and 2009 and attended the Robert Steiner MRI Unit, Hammersmith Hospital, London UK. This study was approved by the Hammersmith and Queen Charlotte's & Chelsea Hospital research ethics committee. Written parental consent was obtained prior to the participants' visit.

Cambridge Baby Growth Study (CBGS)

Details of the study have been described elsewhere (25). Briefly, mothers were recruited from the Rosie Maternity Hospital, Cambridge, UK between 2001 and 2009 at their first antenatal clinic by trained paediatric research nurses. The study comprises a total of 1655 live births. Offspring were followed up at birth, 3 and 12 months. In September 2006, abdominal US was introduced to the follow-up protocol at ages 3 and 12 months and the current analysis is based on those infants with follow-up assessments between September 2006 and June 2010. A total of 487 infants (254 boys and 233 girls) had US measures at 3 months, and 495 infants (258 boys and 237 girls) had US measures at 12 months. US measures at both 3 and 12 months were available in 360 infants (187 boys and 173 girls). Longitudinal data from birth were available on length, weight and skinfold thickness. No significant differences were observed between infants who had US only at 3 months, infants who had US only at 12 months, and those who had US at both 3 and 12 months with regard to gestational age, anthropometry at birth and at 3 months (see Supplementary Table 1). Written informed consent was obtained from the mothers and ethics approval for the study was given by the Cambridge local research ethics committee.

Anthropometry

In the validation study, weight (WT), length (LT), and waist circumference (WC) were measured by one of three trained Clinical Research fellows. WT was obtained using a Marsden Professional Baby Scale (London, UK) and recorded to the nearest 0.1 kg. Crown-heel LT was measured with a Rollametre, a recumbent infant board with a sliding footboard (Raven Equipment Ltd., Dunmow, Essex, UK). WC was measured as the circumference at the midpoint between the inferior border of the costal margin and the anterior superior iliac crests using a D-loop tape measure (Chasmors, London, UK).

In CBGS, infants were measured at birth, 3 months and 12 months by trained paediatric nurses or research assistants. WT was measured to the nearest 1 g using a SECA 757 digital scale (Chasmors Ltd, London, UK). LT was measured using a Kiddimeter (Chasmors Ltd, London, UK). WC was measured as described above. Triceps, quadricepes, flank and subscapular skinfold thicknesses were measured in triplicate on the right side of the body using Holtain Tanner skinfold calipers (Chasmors Ltd, London, UK).

Ponderal index was calculated as weight (kg)/length $(m)^3$. SD scores (SDS) were derived for weight and length by comparison to the 1990 British Growth Reference (Cole et al 1998). Separate SDS were calculated for each of the skinfold thicknesses and then an overall skinfold thickness SDS was calculated as the mean of the four skinfold SD scores in each individual.

Ultrasound (US) abdominal depths

US-visceral depth and US-subcutaneous abdominal depth were measured with a Logic Book XP ultrasound (GE Healthcare, Bedford, UK), using the 3C MHZ -RS abdominal curved array transducer. For both measures, the transducer was positioned where the xiphoid line intercepted the waist circumference measurement position (the midpoint between the inferior border of the costal margin and the anterior superior iliac crests) and the images were taken during expiration. US-visceral depth was measured on a longitudinal plane and defined as the thickness from the peritoneal boundary to the corpus of the lumbar vertebra. US-subcutaneous abdominal depth was measured on the same location, but on a transverse plane, and was defined as the depth from the cutaneous boundary to the linea alba. For the visceral measure the probe depth was set to 9 cm and for the subcutaneous measure it was decreased to 4 cm to visualise superficial anatomical structures. The image was captured when the transducer just had contact with the skin to avoid compressing the subcutaneous adipose area. In the validation study, the US measures were performed by one of two trained operators. In the CBGS, these measures were performed by one of four trained operators. The relative intra-observer technical error of measurement (TEM) ranged between 0.6 to 1.5 % for US-visceral depth, and 1.2 to 2.4 % for US-subcutaneous abdominal depth, and the relative inter-observer TEM was 3.3% for US-visceral depth 3.8% for US-subcutaneous abdominal depth, based on repeated measurements in 6 infants. In the validation study, qualitative information on the feasibility and acceptability of the ultrasound method was also collected by asking the participants an open ended question about their views on this method. The operators were trained not to ask leading questions when asking to comment on this method.

MRI

The MRI procedure used in the validation study is described elsewhere (Harrington et al 2004). Briefly, infants were scanned in natural sleep in a 1.5 TPhillips AchiIA-AT^{M} scanner (Best, Netherlands) using a rapid T1-weighted spin-echo sequence (repetition time (TR) 600ms, echo time (TE) 16 ms, field of view =24 cm, number of signal averages=2 and a 256 x 256 matrix with phase conjugate symmetry). 5mm-thick contiguous transverse images throughout the body were obtained and analysed using the SliceOmatic (Tomovision, Montreal, Quebec, Canada), a semi automated program containing a threshold range and a contour-following algorithm with an interactive slice editor facility to distinguish between adipose tissue compartments.

The adipose tissue volumes derived by the MRI included: abdominal subcutaneous adipose tissue (SCA-AT), subcutaneous non-abdominal adipose tissue (SCNA-AT), internal-abdominal adipose tissue (visceral) (IA-AT), internal non-abdominal adipose tissue (INA-AT) which included internal adipose tissue in the head, neck, chest, pelvis, arms, and legs, and total body subcutaneous adipose tissue (total SC-AT). For the purpose of this study, only IA-AT, SCA-AT, and total SC-AT were used. IA-AT and SCA-AT volumes were calculated from the adipose tissue in the slices from the top of the sacrum to the slice containing the top of the liver or base of the lung (17). SC-AT comprised both superficial and deep subcutaneous adipose tissues.

Statistical analysis

Statistical analysis was performed using STATA version 11.0 (StataCorp). Means and standard deviations are presented separately for boys and girls and differences between them were tested using unpaired t-tests.

For the validation study: Pearson's correlation coefficients were used to describe associations between IA-AT or SCA-AT and the US and anthropometric variables. Multiple regression analysis was used to study the added value of US measures over anthropometry to explain the variance in IA-AT or SCA-AT.

For CBGS: Pearson's correlation coefficients were used to describe cross-sectional associations between US measures at 3 months and 12 months and anthropometry. The associations between early growth parameters (e.g. birth weight and skinfold at birth) and US measures were explored using linear regression models. Associations were similar in both sexes, so all analyses were performed in a pooled sample with adjustment for sex. Further adjustment for current size was included in the final models. To explore the strength of tracking of visceral and subcutaneous abdominal depths, we performed Pearson's correlations in the 360 infants with longitudinal US measures at both 3 and 12 months. Weak tracking was defined by a correlation coefficient <0.3, moderate tracking as 0.3-0.6 and strong tracking as >0.6 (26).

Table 1. Validation study: Inter-correlations between MRI IA-AT or SCA-AT and anthropometry or ultrasound measures in 22 term infants. Values are Pearson's correlation coefficients

	IA-AT (cm^3) [1]	SCA-AT (cm^3) [2]	Total SC-AT (cm^3) [3]	Ponderal Index (kg/m^3)	Length (cm)	Weight (kg)	US-sub-abdo depth (cm) [45]	US-visceral depth (cm) [4]
SCA-AT (cm^3)[2]	0.48*	1						
Total SC-AT (cm^3)[3]	0.61*	0.94**	1					
Ponderal Index (kg/m^3)	0.15	0.32	0.27	1				
Length (cm)	0.34	0.40*	0.54*	-0.40*	1			
Weight (kg)	0.39	0.6*	0.70**	0.2	0.81**	1		
US-sub-abdo depth (cm)[34]	0.52*	0.71**	0.78**	0.17	0.79**	0.92**	1	
US-visceral depth (cm)[3]	0.48*	0.22	0.31	0.14	0.31	0.40*	0.38	1
Waist (cm)	0.08	0.16	0.26	0.19	0.54*	0.72**	0.6*	0.28

*P-value <0.05; **P-value <0.001

[1]Internal-abdominal (visceral)l adipose tissue volume by MRI

[2] Subcutaneous abdominal adipose tissue volume by MRI

[3] Total body subcutaneous adipose tissue volume by MRI

[4] US: Ultrasound

[5]Sub-abdo depth: subcutaneous abdominal adipose tissue depth

RESULTS

Validation study

In the 22 newborn infants, mean (range) IA-AT was 18 (8 – 32) cm^3 and SCA-AT was 104 (59 - 202) cm^3. Mean (range) US-visceral depth was 2.0 (1.2 – 3.0) cm and US-subcutaneous abdominal depth was 0.30 (0.2 – 0.4) cm. IA-AT showed moderate positive correlations with US-visceral depth (r=0.48) and US-subcutaneous abdominal depth (r=0.52), and these were higher than with any anthropometric variable (Table 1). SCA-AT was most strongly positively correlated with US-subcutaneous abdominal depth (r=0.71), followed by weight (r=0.60). US-subcutaneous abdominal depth was also strongly positively correlated with total body SC-AT, weight and length. Examination of scatter plots (see Supplementary figure 1 and 2) showed no obvious heteroschedasticity (i.e. the scatter did not change with increasing IA-AT or SCA-AT).

Table 2. Validation study: Prediction models for IA-AT and SCA-AT

	Model	Constant	$B^5 \pm$ SE					Total R^2 (%)	*P-value for model change*
			Weight (kg)	Sex	Age (days)	US sub-abdo depth (cm)[34]	US visceral depth (cm)[3]		
IA-AT (cm^3)[1]	1	-1.4	5.9±3.1	-	-	-	-	15	*0.07*
	2	-1.5	5.7±3.4	0.4±3.1	-	-	-	16	*0.1*
	3	-0.7	4.8±3.4	-0.8±3.2	0.3±0.3	-	-	22	*0.2*
	4	23.7	- 12.9±8.0	-0.3±2.9	0.4±0.3	113.8±45.4	-	43	*0.1*
	5	20.9	-15.0±6.7	2.7±2.6	0.5±0.2	116.6±38.1	6.6±2.3	62	*0.02*
SCA-AT (cm^3)[2]	1	-42.6	43.6±12.9	-	-	-	-	36	*0.003*
	2	-48.6	36.2±13.2	19.8±12.2	-	-	-	44	*0.01*
	3	-49.4	37.1±14.0	21.0±13.2	-0.3±1.2	-	-	44	*0.02*
	4	66.7	-47.4±0.03	23.4±0.01	0.08±0.09	540.0±171.4	-	65	*0.1*

[1] Visceral adipose tissue volume by MRI

[2] Subcutaneous abdominal adipose tissue volume by MRI

[3] US: Ultrasound

[4] Sub-abdo: subcutaneous abdominal

[5] B = Regression coefficient

From the multiple regression analysis (Table 2), the addition of US-visceral depth to weight, sex, age and US-subcutaneous abdominal depth (used as a proxy for skinfold thickness which was unavailable) improved the explained variance in IA-AT from 43% to 62% (P-value for model change = 0.02). For the prediction of SCA-AT, the addition of US-subcutaneous abdominal depth to weight, sex and age improved the explained variance from 44% to 65% (P=0.1). Mean (range) IA-AT regenerated from predication models described in Table 2 was 21.6 (13.5 – 31.6) cm^3 and the mean (range) SCA-AT from the prediction models was 105 (39– 182) cm^3.

Around half of the mothers provided qualitative comments regarding the measurements. 9 commented favourably on the shorter duration of US compared to MRI, and four mothers commented favourably on the lack of separation from their infants using US.

The Cambridge Baby Growth Study

Characteristics of CBGS infants with US measures at age 3 months (N=487) or 12 months (N=495) are summarised in Table 3. As expected, boys had a higher birth weights, birth lengths and lower skinfold thicknesses at birth compared to girls, despite no difference in gestational age. Boys remained heavier and taller than girls at ages 3 and 12 months, and boys had slightly greater mean US-visceral depth than girls at 12 months (P=0.04), but not at 3 months (P=0.9).

Mean US-visceral depth at age 12 months was around 20% higher at 12 months than at 3 months, with no obvious age differences in US-subcutaneous abdominal depth (or sum of skinfolds). This apparent increase in US-visceral depth was confirmed in the 360 infants with US measures at both time points (mean change: +0.4 cm; P<0.0001). In this longitudinal sample US-visceral depth showed only weak tracking between 3-12 months (r=0.11; P=0.04); in contrast the inter-correlation coefficients between 3-12 months were stronger for: mean skinfold thickness SDS (r=0.30; P<0.0001), ponderal index (r=0.30; P<0.0001), US-subcutaneous abdominal depth (r=0.40), waist circumference (r=0.50), weight (r=0.70) and length (r=0.73). Despite these marked changes during infancy, US-visceral depths at both 3 and 12 months were slightly lower in infants who were exclusively breast-fed at age 3 months compared to other infants (at 3 months: mean +SD: 2.3+0.6 vs. 2.4+0.6 cm, P=0.04; at 12 months: 2.7+0.5 vs. 2.8+0.5 cm, P=0.05). US-visceral depth was unrelated to time from last feed at 3 months (r= - 0.01, P=0.8) and 12 months (r= - 0.06, P= 0.1).

Abdominal ultrasound measures and infancy growth

In cross-sectional analyses (Table 4), US-visceral depth was positively associated with ponderal index at 3 months (P=0.02) with mean skinfold thickness SDS at 12 months (P=0.02). US-subcutaneous abdominal depth at both 3 and 12 months was positively associated with all measures of current body size (P<0.005).

Table 3. Summary of measurements in CBGS infants. Data are means (±SD)

	Boys	Girls	*P-value*[1]
Birth	n=362	n=333	
Gestational age at birth (weeks)	39.8±1.6	39.9±1.3	*0.6*
Weight (kg)	3.5±0.5	3.4±0.4	*0.006*
Length (cm)	51.5±3.5	51.0±2.6	*0.004*
Ponderal Index (kg/m^3)	26.0±3.4	26.0±3.1	*0.2*
Sum of Skinfolds (cm)	2.4±0.6	2.5±0.6	*0.04*
3 months[2]	n=254	n=233	
Weight (kg)	6.4±0.83	5.8±0.7	*<0.0001*
Length (cm)	61.8±2.5	60.2±2.5	*<0.0001*
Ponderal Index (kg/m^3)	27.0 ±2.1	27.0±2.4	*0.1*
Sum of Skinfolds (cm)	4.4±0.8	4.4±0.8	*0.6*
US-visceral depth (cm)	2.3 ±0.6	2.3±0.6	*0.9*
US-subcut abdo depth (cm)	0.4 ±0.1	0.4±0.1	*0.7*
12 months[3]	n=258	n=237	
Weight (kg)	10.2±1.1	9.6±-1.1	*<0.0001*
Length (cm)	76.4±2.7	74.9±2.6	*<0.0001*
Ponderal Index (kg/m^3)	23.0 ±1.6	23.0±1.8	*0.7*
Sum of Skinfolds (cm)	4.3±0.8	4.5± 0.8	*0.01*
US-visceral depth (cm)	2.8±0.6	2.7±0.5	*0.04*
US-subcut abdo depth (cm)	0.4±0.1	0.4±0.1	*0.6*

[1]Student's-test was used to compare boys versus girls

[2]3 months ultrasound measurements were performed in 487 infants (254 boys and 233 girls)

[3]12 months ultrasound measurements were performed in 495 infants (258 boys and 237 girls)

In models without adjustment for current body size, US-visceral depth at 3 months (P=0.06) and 12 month (P=0.03) showed *inverse* trends or associations with mean skinfold thickness SDS at birth, and these inverse associations strengthened on adjustment for current skinfolds (at 3 months: P=0.03; at 12 months: P=0.009) (Table 5). By contrast, US-subcutaneous abdominal depth at 3 months was *positively* associated with mean skinfold thickness SDS at birth (P=0.004), but not at age 12 months (P=0.1) and no associations remained on adjustment for current skinfolds (Table 5). In unadjusted models no US measure was associated with birth weight; inverse associations emerged between birth weight and US-subcutaneous abdominal depth at 3 and 12 months only emerged after adjustment for current body weight (at 3 or 12 months: both P=0.01).

Table 4. Cross-sectional correlations between anthropometry1 and abdominal ultrasound measures at 3 months (487 infants) and 12 months (495 infants). Data are Pearson's coefficients

	US-Visceral depth		US-Subcutaneous abdominal depth	
	3 months	12 months	3 months	12 months
Anthropometry at 3 months				
Weight SDS	0.02		0.31**	
Length SDS	-0.05		0.20**	
Ponderal Index SDS	0.11*		0.27**	
Mean of Skinfolds SDS	0.05		0.31**	
Anthropometry at 12 months				
Weight SDS		0.03		0.30**
Length SDS		0		0.11**
Ponderal Index SDS		0.04		0.26**
Mean of Skinfolds SDS		0.10*		0.30**

[1]SDS: sex and age-adjusted Standard deviation scores

*P<0.05, **P<0.005

DISCUSSION

Our results show that US provides valid estimates of IA-AT and SCA-AT volumes assessed by MRI in infants. US measures showed stronger correlations with IA-AT or SCA-AT than did the traditional anthropometric variables, and the addition of US measures to those variables substantially improved the predictions of IA-AT and SCA-AT. Furthermore, the reproducibility and reliability of the US measures was high as indicated by low inter- and intra-observer technical errors of measurement (TEM), and they were unrelated to time since last feeding.

The findings of our validation study in infants are consistent with the positive results of similar US validation studies in adults and adolescents using MRI (20, 23). In contrast, our earlier validation study in young children aged 6-7 years old showed only weak correlations between US-measures and IA-AT

Table 5. Abdominal ultrasound measures at 3 months (487 infants) and 12 months (495 infants) related to size at birth.

		Birth weight SDS		Mean skinfold thickness SDS at birth	
		B±SE[1]	*P-value*	B±SE[1]	*P-value*
Model 1					
US-Visceral Depth (cm)	3 months	-0.024±0.027	*0.4*	-0.059±0.031	*0.06*
	12 months	-0.041±0.024	*0.09*	**-0.062±0.028**	***0.03***
US-subcut abdo depth (cm)	3 months	0.005±0.005	*0.3*	**0.015±0.005**	***0.004***
	12 months	0.002±0.004	*0.6*	0.007±0.005	*0.1*
Model 2					
US-Visceral Depth (cm)	3 months	-0.041±0.031	*0.2*	**-0.073±0.033**	***0.03***
	12 months	-0.045±0.026	*0.09*	**-0.073±0.028**	***0.009***
US-subcut abdo depth (cm)	3 months	**-0.012±0.005**	***0.01***	0.005±0.005	*0.3*
	12 months	**-0.011±0.004**	***0.01***	0.002±0.005	*0.7*

Model 1: adjusted for sex

Model 2: also adjusted for current weight or skinfolds, respectively

[1]B = Regression coefficient; this represents the SD change in each parameter per 1 SDS change in birth weight or skinfold thickness at birth

by single-slice CT scan (27). Validation studies at younger ages are severely limited by the practical difficulties in performing gold-standard MRI or CT scans in infants and young children.Our previous studies have uniquely described that MRI estimates of IA-AT and SCA-AT 3-dimensional volumes are feasible in newborn infants without sedation, when they are asleep, securely swaddled and wearing protective ear muffs, and this formed the basis of our current validation study. We also collected qualitative information from mothers on the feasibility of US versus MRI. Not surprisingly, mothers invariably preferred US to MRI because no separation from their infants was required, and it was much faster to perform than MRI. The actual MRI scanning time is approximately 12 minutes, but the whole procedure including preparation time to settle the infant could take up to an hour.

The validation study had some limitations that must be considered when interpreting our results. Firstly, we were unable to test absolute validity using Bland-Altman analysis as the MRI and US measurements have different units and no existing prediction equations are available for this age. Secondly, we did not include skinfold thickness measurements in this protocol to reduce additional burden. A further limitation is the small sample size of infants (n = 22). Further studies are required to validate our prediction equations in other infant populations. However, we consider that our ultrasound method is sufficient for ranking of infants with higher or lower abdominal adipose tissue volumes.

In CBGS, we found that infants with lower skinfold thickness at birth tended to have lower subcutaneous abdominal depth at age 3 months, but had *greater* visceral depths at ages 3 and 12 months, suggesting a differential regulation of these adipose tissue compartments. In support of this notion, previous studies using MRI have reported that growth restricted or extremely preterm infants have reduced SCA-AT but preserved IA-AT mass at birth (17, 28).

We previously reported an inverse association between birth weight and US-visceral depth in adults after correction for adult BMI (29). The absence of this association in the current study is therefore surprising and could reflect a lack of power as similar regression coefficients are seen in both studies, but our current study had a smaller sample size and infants have a much lower range of visceral depth measurements than adults. Other studies have reported inverse associations between birth weight and subsequent abdominal adipose tissue after adjustment for current body size in both children and adults (10, 14, 30-32). However, most of those studies used outcome measures that do not distinguish between abdominal adipose tissue compartments (e.g. DEXA, waist circumference and ratio of subscapular to triceps skinfold thickness). To our knowledge, only two other studies used ultrasound measures in infancy (16, 33) and only one of these investigated the relationship with birth weight (33). That study reported an inverse association between birth weight and abdominal adipose tissue index (the ratio between the preperitoneal and subcutaneous adipose tissue). However, the technique used in that study has only been validated in adults and older children (34, 35) and does not estimate visceral adipose tissue. In summary, the stronger infant visceral depth associations that we observed with lower skinfold thickness at birth rather than lower birth weight suggest that these birth measures may be proxies for fetal growth restraint during the later antenatal period.

We also observed that the associations between lower skinfold thickness at birth and greater subsequent visceral depth strengthened with further adjustment for current skinfold thickness. Lucas and colleagues have argued that if birth size is related to later health outcomes only after adjustment for current body size, it is probably the change in body size after birth that accounts for the relationship between birth weight (36). Therefore, postnatal factors associated with the postnatal transition from lower to higher skinfolds could confer an increased propensity to visceral adipose tissue at 3 and 12 months. Visceral depth showed only weak evidence of tracking during infancy indicating poor stability of this measure during infancy (37). Within-individual variation, measurement error and imprecision will contribute to the low tracking coefficient for visceral depth and repeated measurements might help improve estimates of the tracking of body adipose tissue (38). From our experience, the US-visceral depth measurement appears to be more susceptible to bowel peristalsis and movement artifacts in infants than in older age groups. However, we found that US-visceral depth was unrelated to time from last feed. Biological factors might also affect how these compartments track overtime and our observed association between exclusive breastfeeding and lower visceral depth supports the interpretation that postnatal factors also contribute to infant visceral adipose tissue.

In contrast to the *inverse* associations with visceral depth, subcutaneous abdominal depth was *positively* associated with skinfold thickness at birth. Other MRI studies in infants have also suggested a differential regulation of these abdominal adipose tissue compartments. Both extremely preterm and growth-restricted term infants appeared to have preserved IA-AT despite marked reductions in SCA-AT (17, 28).

In conclusion, we have shown that US measures could provide valid estimates of abdominal adiposity in infancy, with particular application to large epidemiological studies, when MRI and CT imaging are not feasible for research studies. Our US findings suggest that both antenatal and postnatal factors may influence the quantity of internal-abdominal and subcutaneous abdominal adipose tissue in the first year of life.

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The authors' responsibilities were as follows – EDLR: data collection, statistical analysis and drafted the manuscript under the guidance of KKO and RPS. EDLR, NM, SU, IAH, DBD, CA, RPS and KKO: study concept and design. SU and NM supervised the data collection in London; KKO, DBD, IAH and CA supervised the data collection in Cambridge. All authors provided interpretation of the results and approved the final version of the manuscript. None of the authors had any conflicts of interest.

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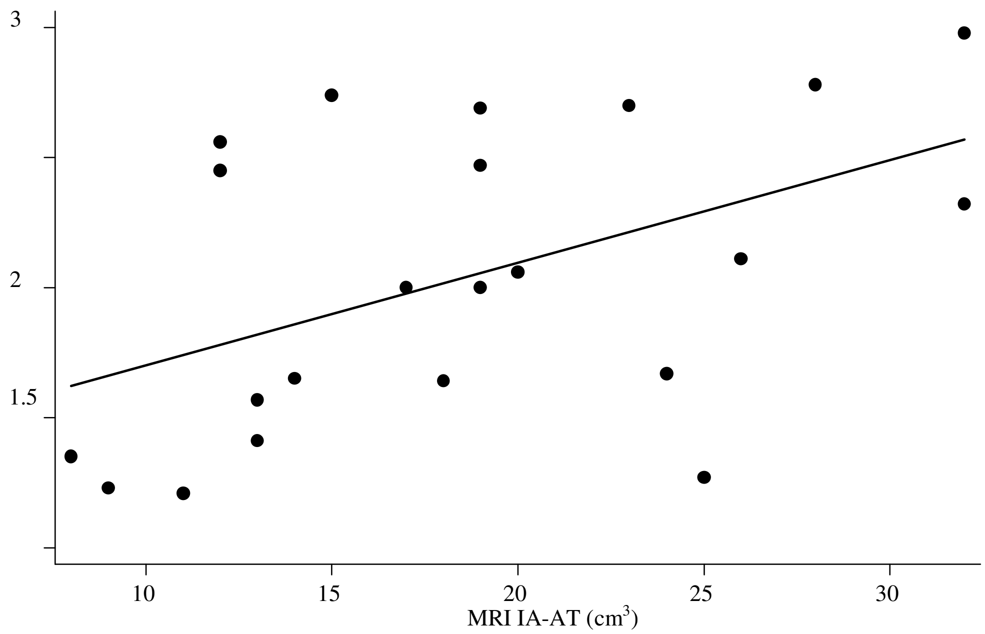
APPENDIX

Supplementary Table 1. Infants characteristics with ultrasound measures at 3 months, 12 months and both at 3 and 12 months

	US at 3 months only		US at 12 months only		US at 3 & 12 months	
	Boys n= 67	Girls n=60	Boys n= 108	Girls n=100	Boys n= 187	Girls n=173
Birth						
Gestational age at birth (weeks)	39.6±1.4	39.9±1.2	39.8±1.8	39.9±1.1	39.8±1.5	39.7±1.6
Weight (kg)	3.5±0.5	3.4±0.5	3.5±0.6	3.4±0.4	3.5±0.5	3.4±0.5
Length (cm)	51.7±2.4	51.0±2.8	51.5±2.2	51.0±2.1	51.7±2.8	51.7±2.8
Ponderal Index (kg/m^3)	25.3±3.2	26.0±2.7	26.0±3.6	26.0±3.2	25.6±3.2	25.9±3.1
Sum of Skinfolds (cm)	2.5±0.6	2.5±0.6	2.4±0.5	2.5±0.6	2.4±0.6	2.5±0.6
3 months						
Weight (kg)	6.4±0.8	5.9±0.7	6.3±0.8	5.8±0.6	6.3±0.8	5.8±0.7
Length (cm)	61.6±2.4	60.2±2.4	61.7±2.4	60.2±2.2	61.8±2.5	60.1±2.6
Ponderal Index (kg/m^3)	27.0 ±2.3	27.0±2.2	27.0±2.1	26.4±2.5	27.0±2.0	26.7±2.4
Sum of Skinfolds (cm)	4.5±0.9	4.5±0.8	4.4±0.8	4.4±0.8	4.4±0.8	4.4±0.8

[1]Data are means (±SD)

Supplementary Figure 1. SCA-ATter plot of US visceral depth against MRI IA-AT in 22 infants



Supplementary Figure 2. SCA-ATter plot of US subcutaneous depth against MRI Abdominal SCA-AT in 22 infants