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ARTICLE

The metabolic syndrome in pregnancy and its association with child telomere length

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Abstract

Aims/hypothesis The aim of this study was to determine whether presence of the metabolic syndrome in pregnancy associates with child telomere length or child anthropometry (weight, BMI) and BP, measured at 10 years of age.

Methods The Screening for Pregnancy Endpoints study (SCOPE) was a multicentre, international prospective cohort of nulliparous pregnant women recruited from Australia, New Zealand, Ireland and the UK ($N = 5628$). The current analysis is a 10 year follow-up of SCOPE pregnant women and their children, from the Australian cohort. Clinical data collected at 14–16 weeks' gestation during the SCOPE study were used to diagnose the metabolic syndrome using IDF criteria. Telomere length, a biomarker of ageing, was assessed by quantitative PCR from children's saliva collected at 10 years of age.

Results In women who completed follow-up ($n = 255$), 20% had the metabolic syndrome in pregnancy. After adjusting for a range of confounders, children of mothers who had the metabolic syndrome in pregnancy had 14% shorter telomeres than children of mothers without the metabolic syndrome in pregnancy (mean difference −0.36 [95% CI −0.74, 0.01]). Height- and weight-for-age, and BMI z scores were similar in children of mothers who did and did not have the metabolic syndrome during pregnancy.

Conclusions/interpretation Children of mothers who had the metabolic syndrome in pregnancy have shorter telomeres, a biomarker of accelerated ageing. These findings warrant further studies in larger cohorts of children, as well as investigations into whether telomere length measured in cord blood associates with telomere length in childhood.

Keywords Cardiovascular · Children · Developmental programming · Maternal · Metabolic syndrome · Obesity · Offspring · Pregnancy · Telomere length

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Abbreviations

BMIz	BMI z scores
GDM	Gestational diabetes
HFAz	Height-for-age z scores
qPCR	Quantitative PCR
SCOPE	Screening for Pregnancy Endpoints study
SEI	Socioeconomic index
WFAz	Weight-for-age z scores

Introduction

Telomeres are repeating DNA sequences located at the ends of chromosomes that progressively shorten with each round of cell division [1]. Their main function is to protect the chromosomes from DNA damage thus helping to maintain genomic stability. Cells are generally unaffected by a small amount of

Research in context

What is already known about this subject?

- The metabolic syndrome is a clustering of cardiovascular risk factors, which in adults associates with an increased risk for cardiovascular diseases
- Telomere length is a biomarker of ageing and is associated with the development of future chronic diseases

What is the key question?

- Do children of mothers who had the metabolic syndrome in pregnancy have shorter telomeres than children of mothers who did not have the metabolic syndrome?

What are the new findings?

- Children of mothers who had the metabolic syndrome in pregnancy had 14% shorter telomeres than children of mothers who did not have the metabolic syndrome
- Height- and weight-for-age and BMI *z* scores were similar in children of mothers who did and did not have the metabolic syndrome in pregnancy

How might this impact on clinical practice in the foreseeable future?

- Early assessment of telomere length in children may provide insight into their potential future chronic disease risk

telomere erosion; however, once they reach a critically short length, chromosome ends become uncapped causing cell senescence, signalling the arrest of cell proliferation and subsequently leading to apoptosis [2]. Oxidative stress and inflammation shorten telomeres in vitro [3]. Consistent with this, there is human cross-sectional evidence associating shorter telomeres with oxidative stress and inflammation [4]. Shorter telomeres are a marker of the cumulative damage to which the cell has been exposed [5] and, in adults, is associated with diabetes [6], cancer [7], cardiovascular disease [8] and all-cause mortality [9].

Antenatal factors are a particularly important determinant of telomere length [10]. Adverse exposures in pregnancy, such as maternal stress, smoking, exposure to second-hand smoke and higher levels of air pollution, associate with shorter telomeres measured in cord blood [11–13], placenta [14] and fetal lung tissue [15]. For every 1 kg/m^2 increase in pre-pregnancy BMI, telomeres measured in cord blood were 0.50% shorter, while telomeres measured in the placenta were 0.66% shorter [16]. Gestational diabetes (GDM) in pregnancy was associated with shorter telomeres measured in cord blood in one study [17] but not in others [18, 19]. Importantly, exposure to maternal obesity or GDM is associated with a heightened risk for future cardiovascular disease in both the mother and the child [20, 21]. Furthermore, even before frank cardiovascular or metabolic diseases develop, children of complicated pregnancies already have markers of elevated risk for these conditions, including higher BP [22], overweight or obesity [23], elevated lipid levels [24] and type 2 diabetes [25], compared with children born after an uncomplicated pregnancy.

Despite this evidence for associations between adverse pregnancy exposures and progeny telomere length at birth, it is not known whether these associations persist into childhood. The metabolic syndrome is a clustering of modifiable cardiovascular risk factors including abdominal obesity, hyperlipidaemia, raised BP and insulin resistance [26]. We have recently shown that the metabolic syndrome in pregnancy increases the risk for pre-eclampsia and GDM by two- to fourfold [27]. The metabolic syndrome in adults is associated with an approximately twofold higher risk for cardiovascular diseases and all-cause mortality [28]. There are clear links not only between cardiometabolic risk factors and accelerated ageing in adults [29] but also between maternal cardiovascular risk factors and cardiovascular risk factors in children [22–25]. Therefore, assessment of telomere length in children may serve as a marker of future development of cardiovascular and metabolic diseases.

The aims of this study are to determine whether presence of the metabolic syndrome in pregnant women associates with child telomere length or child anthropometry (weight, BMI) and BP, measured at 10 years of age.

Methods

Participants The Screening for Pregnancy Endpoints study (SCOPE) is a multicentre prospective cohort study that recruited nulliparous women with singleton pregnancies from Adelaide (Australia), Auckland (New Zealand), Cork (Ireland), Leeds, London and Manchester (UK) ($N = 5628$) between November 2004 and February 2011 [30]. Detailed data collection and inclusion criteria can be found in previous

publications [27, 30]. The current analysis consisted of women from the Adelaide SCOPE pregnancy cohort, who were recruited between November 2004 and September 2008. Maternal demographics, smoking status, family, medical and gynaecological history, anthropometry (height, weight, waist circumference) and BP were recorded at 14–16 weeks' gestation. Socioeconomic status was determined using the socioeconomic index (SEI), derived from the specific occupation of the woman, producing a score between 10 and 90 with a lower score reflecting greater disadvantage [31]. Ethnicity was binary coded as white/other. Smoking status was binary coded as yes/no for any cigarette smoking at the first visit. Ethical and practical constraints meant it was not appropriate to ask pregnant women to fast prior to their antenatal visit, thus a non-fasting blood sample was taken for measurement of HDL-cholesterol and triacylglycerol at 14–16 weeks' gestation. Plasma blood glucose was measured as a random blood sample by glucometer at the same time point. Details of immunoassay methodology for measuring lipids can be found in previous publications [32].

According to the IDF criteria for adults [33], a pregnant woman was considered to have the metabolic syndrome if she had a waist circumference ≥80 cm measured at the recruitment assessment (14–16 weeks' gestation), along with any two of the following: high triacylglycerol (≥1.70 mmol/l [≥150 mg/dl]); low HDL-cholesterol (<1.29 mmol/l [<50 mg/dl]); high BP (systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg); or high fasting plasma glucose (≥5.6 mmol/l). As this was a pregnancy cohort, a random plasma glucose sample was measured; however, the same concentration cut-off was used as in IDF criteria (≥5.6 mmol/l).

Information collected from the biological father included paternal age, and paternal height and weight (to calculate BMI). Infant data (birthweight and sex) were recorded by research midwives within 72 h of birth.

Follow-up participants For follow-up, women from the SCOPE Adelaide cohort were contacted by telephone 8–10 years after their first pregnancy and were asked to attend a follow-up appointment with their child. Three attempts were made to contact each woman, using each of the telephone numbers specified in the medical records. If they did not respond, an SMS text was sent to any valid mobile telephone numbers informing them of the study and asking them to contact study coordinators. Of the 1164 women in the initial Adelaide cohort, we were able to contact 1139 (the remainder did not have valid phone numbers) (Fig. 1) and 270 mothers and their children attended the follow-up visit (24% response rate). Included in the current analysis are 255 mother–child pairs for whom both a pregnancy blood sample (for assessment of the metabolic syndrome) and a follow-up child salivary DNA sample (for the primary outcome of telomere length) were available.

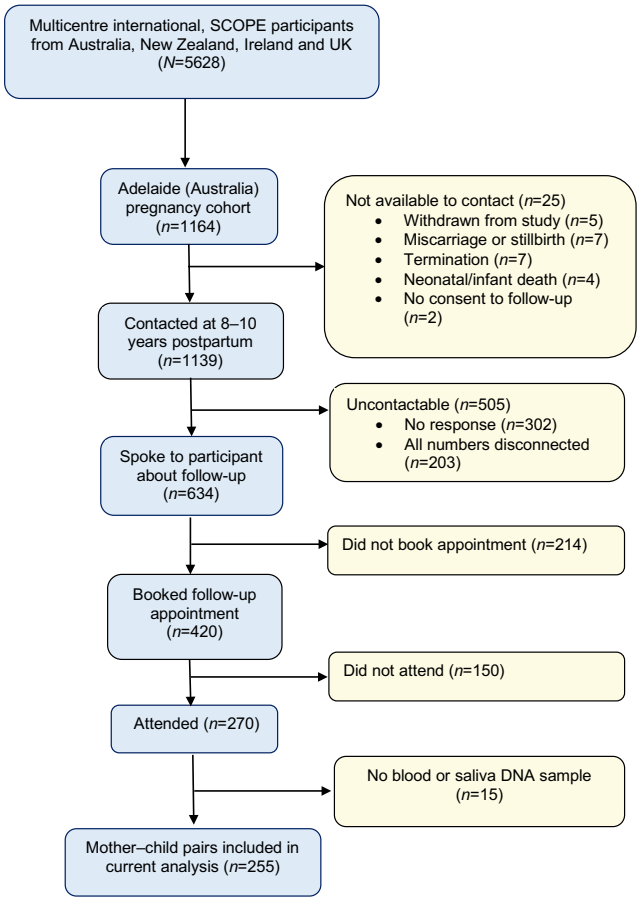

Fig. 1 Participant flow from initial SCOPE cohort through to SCOPE follow-up

Study visit During the follow-up visit, height, weight and BP were measured for the child. Height was measured with a stadiometer to the nearest 0.1 cm; weight was measured to the nearest 0.1 kg using the TANITA SC-330 (Tokyo, Japan) bio-impedance scale, which also calculates BMI. Child peripheral systolic and diastolic BP was measured using an USCOM BP+ Sphygmomanometer (Brisbane, Australia). A saliva sample (2 ml) was collected from the mother and her child, into an Oragene DNA OG-500 collection kit containing stabilisation solution (DNA Genotek, Canada). Saliva samples were stored at room temperature until DNA extraction was performed. At this visit, women and children provided written consent and assent, respectively. Ethics approvals for the SCOPE follow-up study were granted by the Human Research Ethics Committees of the University and hospitals [HREC/15/WCHN/192 and HREC/14/TQEH/277].

Outcome Child telomere length was the primary outcome. DNA was extracted from saliva samples using the prepIT L2P reagent (DNA Genotek) according to the manufacturer's instructions. DNA was allowed to gradually re-dissolve at 4°C before DNA concentration was determined by NanoDrop One (Adelaide, Australia). DNA samples were diluted and

transferred to 96-well plates at a concentration of 2.5 ng/μl. Ten-microlitre reactions were set up in each well of a 384-well white opaque PCR plate containing the following: 5 μl of 2× SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, USA); 100 nmol/l of each primer (either telomere (T) forward primer 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′ and telomere (T) reverse primer 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′ or 36B4 (S) forward primer 5′-CAGCAAGTGGGAAGGTGTAATCC-3′ and 36B4 (S) reverse primer 5′-CCCATTCTATCATCAACGGGTACAA-3′); and 5 ng of DNA [34]. Each PCR reaction was performed in triplicate. Pooled genomic DNA from unrelated samples was used as a control calibrator and was included on every plate. The quantitative PCR (qPCR) was run on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad) with the following conditions: initial denaturation step of 3 min at 98°C, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Melt curve analysis was performed at the end of 40 cycles of 65–95°C, with 0.5°C increments at 2 s per step. Any samples that failed, or where the SD of the Cq value of triplicate wells was >1, were repeated or excluded from subsequent analyses. This generated a measure of the average telomere length as a ratio (T/S) of telomere repeat length (T) to copy number of a single-copy gene (S), for each DNA sample. The mean CV was 8.04% for the telomere PCR and 6.06% for genomic DNA PCR.

Maternal telomere length, measured from the saliva sample taken at follow-up, was included as a covariate in the statistical models.

Statistical analyses Frequencies and descriptive statistics for the characteristics of women and children are expressed as *n* (%) or as mean (95% CI). The impact of the metabolic syndrome on child telomere length was analysed in the following ways: (1) as a composite; (2) as each metabolic component; and (3) with an increasing number of components of the metabolic syndrome above the IDF cut-off point. These data were compared using a generalised linear model. A univariable model was first tested and then a multivariable model (model 1) adjusting for maternal age, SEI, ethnicity (white/other), smoking at first visit (yes/no), and maternal telomere length at follow-up, was run. A second multivariable model was then run, containing all factors from model 1 plus infant sex and birthweight. We then ran a third model, which further included paternal age and BMI. A supplementary analysis was investigated assessing the effect of the metabolic syndrome in the mother during pregnancy on child telomere length, by fetal sex. Causal diagrams (directed acyclic graphs) and content knowledge were used to guide selection of potential confounders. Children's BMI was converted to age- and sex-specific BMI *z* scores (BMIz) using the WHO's Child Growth Standards, with height-for-age *z* scores (HFAz) and weight-for-age *z* scores (WFAz) calculated [35] using the WHO Anthro Survey Analyser R macro package [36]. The impact of the metabolic syndrome on child anthropometry (raw and *z* scores) was then assessed using linear regression and the same models as described above. All analyses were conducted using SPSS (IBM SPSS Statistics for Windows, Version 24.0; IBM Corporation, Armonk, NY, USA).

Results

Participants Within the initial Adelaide SCOPE cohort, 22% of women attended the 10 year follow-up visit with their child and are included in the current analysis. Mothers of children who did and did not participate (unable to contact or declined) in this study are compared in electronic supplementary material (ESM) Table 1. Compared with the women who did not attend the follow-up visit, women included in the study were older and had a higher SEI and BMI, and there was a higher percentage who were white and a lower percentage who smoked. The percentage of women who had the metabolic syndrome was similar in women who attended the follow-up and those who did not (19.2% vs 16.0%). There were no differences in anthropometry of mothers who did and did not attend the follow-up (ESM Table 1).

Of the 255 women included in the current analysis, 20% ($n = 51$) had the metabolic syndrome in pregnancy. Maternal and paternal characteristics at 14–16 weeks' gestation and child characteristics at follow-up are shown in Table 1. Of women who had the metabolic syndrome, all had high waist circumference, 92% had high glucose and 80% had high triacylglycerol. In women without the metabolic syndrome ($n = 204$), 72% had a high waist circumference and 35% had high glucose. Women who had the metabolic syndrome in pregnancy had a higher mean BMI, larger waist circumference, higher systolic BP, higher random glucose, higher triacylglycerol and lower HDL-cholesterol (all $p < 0.001$) compared with women without the metabolic syndrome. The mean (SD) age of children at follow-up was 9.6 (0.6) years. There were no differences in child age, weight, BMI (raw or *z* scores) or BP in children whose mothers did and did not have the metabolic syndrome in pregnancy (Table 1).

Association between the metabolic syndrome in a mother during pregnancy and child telomere length at 10 years of age The mean (95% CI) child telomere length was 2.58 (2.43, 2.73). On univariable analysis, children of mothers who had the metabolic syndrome during pregnancy had shorter mean (95% CI) telomere length than children of mothers without the metabolic syndrome (2.27 [1.94, 2.61] vs 2.65 [2.49, 2.82], $p = 0.016$). After adjusting for maternal variables in model 1, children of mothers who had the metabolic syndrome in pregnancy had 14% shorter telomeres than children of mothers

Table 1 Maternal and paternal characteristics at 14–16 weeks' gestation and child characteristics at follow-up, comparing women who had vs did not have the metabolic syndrome in pregnancy

Characteristic	No MetS ($n = 204$)	MetS ($n = 51$)	p value
Maternal characteristics			
Age, years	25.5 (24.5, 26.2)	26.4 (24.9, 27.9)	0.311
BMI, kg/m^2	27.2 (26.3, 28.1)	31.4 (29.3, 33.5)	<0.001
White ethnicity, n (%)	195 (95.6)	51 (100)	0.129
SEI[a]	29.2 (27.6, 30.8)	31.0 (28.1, 33.9)	0.312
Smoking at first visit, no, n (%)	175 (85.8)	51 (100)	0.226
Metabolic variables (continuous)			
Waist circumference, cm	88.8 (86.9, 90.7)	99.1 (94.9, 103.4)	<0.001
SBP, mmHg	108.9 (107.6, 110.2)	115.7 (113.0, 118.4)	<0.001
DBP, mmHg	64.3 (63.2, 65.3)	66.6 (64.2, 69.0)	0.065
Random glucose, mmol/l	5.45 (5.36, 5.54)	6.19 (5.94, 6.43)	<0.001
Triacylglycerol, mmol/l	1.34 (1.27, 1.41)	2.24 (2.04, 2.44)	<0.001
HDL-cholesterol, mmol/l	1.82 (1.78, 1.87)	1.55 (1.44, 1.66)	<0.001
Metabolic variables (categorical), n (%)			
High waist circumference, ≥80 cm	147 (72)	51 (100)	<0.001
High SBP/DBP, ≥130 mmHg/≥85 mmHg	3 (1.5)	6 (12)	0.003
High random glucose, ≥5.6 mmol/l	71 (35)	47 (92)	<0.001
High triacylglycerol, ≥1.70 mmol/l	30 (15)	41 (80)	<0.001
Low HDL-cholesterol, <1.29 mmol/l	6 (3)	19 (37)	<0.001
Paternal characteristics			
Age, years	28.3 (27.4, 29.3)	30.0 (28.3, 31.8)	0.868
BMI, kg/m^2	27.7 (27.0, 28.4)	28.4 (26.7, 30.1)	0.277
Follow-up data			
Maternal telomere length	2.23 (2.06, 2.39)	2.08 (1.81, 2.35)	0.368
Child characteristics			
Male/female sex, n (%)	91 (46.6) / 113 (55.4)	21 (41.2) / 30 (58.8)	0.390
Age, years	9.63 (9.55, 9.70)	9.55 (9.38, 9.72)	0.321
Height, cm	137.6 (136.6, 138.6)	138.1 (136.0, 140.2)	0.731
Height, z score	0.246 (0.081, 0.410)	0.253 (−0.025, 0.531)	0.369
Weight, kg	34.4 (33.1, 35.8)	36.1 (33.3, 38.8)	0.797
Weight, z score	0.438 (0.209, 0.666)	0.710 (0.300, 1.119)	0.247
BMI, kg/m^2	17.9 (17.4, 18.4)	18.7 (17.6, 19.9)	0.401
BMI, z score	0.374 (0.140, 0.608)	0.726 (0.283, 1.169)	0.122
SBP, mmHg	111.4 (109.6, 113.1)	110.2 (107.2, 113.2)	0.171
DBP, mmHg	60.0 (58.7, 61.3)	59.7 (57.7, 61.7)	0.319

Data are presented as mean (95% CI) or n (%)

p values were calculated using Student's t test, test or χ^2 test

[a] SEI is a measure of socioeconomic status and is derived from the specific occupation of the woman, producing a score of 10–90, with a lower score reflecting greater disadvantage [31]

DBP; diastolic BP; MetS, the metabolic syndrome; SBP systolic BP

without the metabolic syndrome (Table 2). When including fetal (model 2), and paternal factors (model 3), the estimates remained similar, with 14% shorter telomeres in children of mothers with the metabolic syndrome (model 3: mean difference −0.36 [95% CI −0.74, 0.01]).

Among the 255 included women, 32 (12.5%) had no elevated components of the metabolic syndrome, 88 (34.5%) had one elevated component, 83 (32.5%) had two and 52 (20.4%) had three or more. Table 3 shows the association between the metabolic syndrome in pregnancy (its individual components and increasing number of components) and child telomere length. Few women had a low waist circumference ($n = 57$), and most had high HDL-cholesterol ($n = 230$) or low BP ($n = 246$). Of the individual components of the metabolic

Table 2 Associations between the metabolic syndrome in women during pregnancy and child telomere length[a, b]

	Model 1 ($n = 255$)	Model 2 ($n = 255$)	Model 3 ($n = 235$)
Telomere length	−0.37 (−0.73, −0.02)	−0.38 (−0.74, 0.26)	−0.36 (−0.74, 0.01)

[a] Data are mean difference (95% CI) representing presence vs absence of the metabolic syndrome in pregnancy

[b] Telomere length is reported as a ratio (T/S) of telomere repeat length (T) to copy number of a single-copy gene (S), therefore there are no units

Model 1 adjusted for maternal age, SEI, ethnicity (white/other), smoking at first visit (yes/no), maternal telomere length at follow-up; model 2 is model 1 plus infant sex and birthweight; model 3, is model 2 plus paternal age and BMI

syndrome, only waist circumference was associated with 12% shorter child telomeres, with similar mean T/S ratios after adjusting for maternal factors in model 1 (low vs high waist circumference: 2.52 [2.08, 2.96] vs 2.94 [2.47, 3.41], $p = 0.019$) and fetal and paternal factors (2.51 [2.06, 2.29] vs 2.84 [2.35, 3.32], $p = 0.082$). Compared with women who had no components of the metabolic syndrome, women who had ≥3 of any components of the metabolic syndrome had children with 23% shorter telomeres (adjusted mean [95% CI] 1.82 [1.01, 2.62] vs 2.35 [1.49, 3.20], $p = 0.037$).

As shown in ESM Table 2, when stratified by sex, girls but not boys of mothers who had the metabolic syndrome in pregnancy had shorter telomere length than girls of mothers who did not have the metabolic syndrome in pregnancy (mean difference −0.51 [95% CI −1.00, −0.01]). However, there was no evidence of an interaction, such that the effect of the metabolic syndrome on child telomere length is not modified by sex, and is consistent for boys and girls (interaction term effect 0.18 [95% CI −0.57, 0.93], $p = 0.6$).

Metabolic syndrome in women during pregnancy and child phenotype at 10 years of age In adjusted analyses, HFAz, WFAz and BMIz were similar in children from mothers who did and did not have the metabolic syndrome in pregnancy (Table 4). Child systolic and diastolic blood pressures similarly did not differ between children of mothers who did or did not have the metabolic syndrome while pregnant.

Discussion

In this follow-up study of pregnant women and their 10-year-old children, the metabolic syndrome in early pregnancy was associated with 14% shorter telomeres in the child compared with children of mothers who did not have the metabolic syndrome. There were no differences in the children's anthropometry measures (raw or *z* scores of weight-for-age, height-for-age and BMI-for-age) or BP, when comparing offspring of mothers who did or did not have the metabolic syndrome.

Several studies have reported associations between intrauterine exposures and measurement of telomere length in placenta or cord blood leucocytes [37]. There is less information on maternal exposures in pregnancy and telomere length in children. A recent study ($n = 439$) demonstrated 14% shorter telomeres in 12-year-old children of mothers who had GDM, driven by shorter telomeres in female but not male children [38]. We demonstrated no effect modification with fetal sex, when assessing the metabolic syndrome and child telomere

Table 3 Association between individual components of the metabolic syndrome in women during pregnancy, and increasing number of components, and child telomere length[a]

Metabolic component	*n*	Crude ($n = 255$)	Model 1 ($n = 255$)	Model 2 ($n = 255$)	Model 3 ($n = 235$)
Low waist circumference, <80 cm	57	0.46 (0.10, 0.82)	0.42 (0.07, 0.76)	0.41 (0.07, 0.76)	0.33 (−0.04, 0.69)
High HDL-cholesterol, ≥1.29 mmol/l	230	0.31 (−0.19, 0.82)	0.31 (−0.18, 0.81)	0.32 (−0.17, 0.81)	0.30 (−0.24, 0.83)
Low triacylglycerol, <1.70 mmol/l	184	0.17 (−0.17, 0.50)	0.17 (−0.15, 0.49)	0.17 (−0.16, 0.49)	0.17 (−0.17, 0.51)
Low glucose, <5.6 mmol/l	137	0.07 (−0.24, 0.37)	0.05 (−0.24, 0.34)	0.05 (−0.24, 0.34)	0.04 (−0.26, 0.34)
Low systolic/diastolic BP, <130 mmHg/<85 mmHg	246	0.27 (−0.54, 0.10)	0.23 (−0.55, 1.00)	0.25 (−0.53, 1.01)	0.14 (−0.64, 0.92)
No. of components					
0	32	Reference	Reference	Reference	Reference
1	88	0.31 (−0.17, 0.79)	0.22 (−0.21, 0.66)	0.19 (−0.24, 0.63)	0.25 (−0.20, 0.70)
2	83	0.25 (−0.29, 0.78)	0.11 (−0.39, 0.62)	0.11 (−0.40, 0.62)	0.06 (−0.47, 0.58)
≥3	52	0.61 (0.13, 1.09)	0.54 (0.08, 1.09)	0.55 (0.07, 1.02)	0.53 (0.03, 1.03)

Data are mean difference (95% CI) representing presence vs absence of the metabolic syndrome in pregnancy

Model 1 adjusted for maternal age, SEI, ethnicity (white/other), smoking at first visit (yes/no), maternal telomere length at follow-up; model 2 is model 1 plus infant sex and birthweight; model 3 is model 2 plus paternal age and BMI

Table 4 Associations between the metabolic syndrome during pregnancy and child phenotype

Child characteristic	Model 1			Model 2			Model 3		
	n	Mean difference (95% CI)	*p* value	*n*	Mean difference (95% CI)	*p* value	*n*	Mean difference (95% CI)	*p* value
Height, cm	252	−0.09 (−2.33, 2.16)	0.940	252	−0.10 (−2.12, −1.93)	0.926	232	−0.12 (−2.12, 1.88)	0.905
HFAz	252	−0.11 (−0.43, 0.21)	0.491	252	−0.12 (−0.40, −0.17)	0.434	232	−0.10 (−0.39, 0.19)	0.491
Weight, kg	191	−1.43 (−4.38, 1.52)	0.342	191	−1.44 (−4.19, 1.32)	0.307	191	−1.87 (−4.47, 0.74)	0.160
WFAz[a]	191	−0.28 (−0.77, 0.21)	0.264	191	−0.28 (−0.73, 0.16)	0.214	191	−0.19 (−0.63, 0.26)	0.407
BMI, kg/m^2	252	−0.82 (−1.95, 0.31)	0.155	252	−0.82 (−1.90, 0.27)	0.139	232	−0.96 (−2.00, 0.08)	0.071
BMIz[a]	252	−0.34 (−0.78, 0.11)	0.138	252	−0.34 (−0.76, 0.08)	0.115	232	−0.37 (−0.79, 0.05)	0.085
SBP, mmHg	252	1.32 (−2.45, 5.08)	0.493	252	1.33 (−2.41, 5.06)	0.487	232	1.92 (−1.80, 5.63)	0.312
DBP, mmHg	252	0.53 (−2.21, 3.26)	0.705	252	0.52 (−2.21, 3.25)	0.709	232	0.17 (−2.35, 2.70)	0.893

Model 1 adjusted for maternal age, SEI, ethnicity (white/other), smoking at first visit (yes/no), maternal telomere length at follow-up; model 2 is model 1 plus infant sex and birthweight; model 3, is model 2 plus paternal age and BMI

[a] BMIz is a height-based calculation (WHO child growth standards, 2017) and does not depend on the weight-for-age scores, which are only valid for children up to 10 years of age, hence smaller sample size with WFAz does not impact BMIz

DBP, diastolic BP; SBP, systolic BP

length, though our sample size was small. In a study of 1396 mother–child pairs, for each 1 kg/m^2 increase in maternal pre-pregnancy BMI, the 8-year-old child's telomere length was 0.23% shorter [39]. However, that study did not adjust for maternal telomere length or paternal age, both of which may influence offspring telomere length [40, 41]. We have recently shown that women with the metabolic syndrome in early pregnancy had a near fourfold increased risk for GDM, independent of obesity [27]. The current study is unique in that the metabolic syndrome, a cluster of metabolic markers, was assessed at 14–16 weeks' gestation, far earlier than a diagnosis of GDM at 24–28 weeks' gestation. Identifying and potentially improving markers of the metabolic syndrome in early pregnancy may have a positive effect on telomere length in offspring, as well as their future disease risk.

Interestingly, when we assessed individual components of the metabolic syndrome, only high waist circumference (>80 cm) was associated with shorter child telomeres. This is somewhat surprising given that 78% of women had a high waist circumference, but supports the association described above with maternal pre-pregnancy BMI [39]. However, it is not so surprising that even shorter telomere lengths were seen when comparing children of women who had three or more markers of the metabolic syndrome with children of women who had no adverse metabolic components. While it has been shown that maternal metabolic markers change across gestation, and changes are more prominent in women who are obese or who have pre-existing hypertension of diabetes [42], it is unclear whether levels of metabolic components in our sample of low-risk women might be comparable to other study populations. As we are the first to report on individual and increasing number of components of the metabolic syndrome on child telomere length, further investigation into this area is warranted.

Mechanisms relating to the potential shortening of telomeres in children of mothers who had the metabolic syndrome in pregnancy are likely to be multifactorial. The metabolic syndrome encompasses a range of cardiovascular markers including raised lipids and BP, insulin resistance and abdominal obesity, which are associated with increased oxidative stress and inflammation [43]. Chronic inflammation in adults leads to persistent damage to telomeres and increases the rate of telomere shortening, demonstrated in cross-sectional and longitudinal studies [4]. However, whether the association between the metabolic syndrome in women during pregnancy and shorter child telomeres observed in the present study is a consequence of adverse maternal intrauterine exposures, shared genetics or environmental factors occurring after birth is unknown. Additionally, even when adjusting for factors previously shown to be associated with telomere length, such as birthweight [44–46], the impact of the metabolic syndrome on child telomeres was still significant. Importantly, child telomere length has clinical relevance. Two longitudinal studies have shown that shorter telomeres measured in children at 3.6 years of age were associated with greater intima media thickness, a non-invasive measure of subclinical atherosclerosis, at both 8 years [47] and 14 years of age [48]. Early assessment of telomere length in children may therefore provide insight into their potential future disease risk.

A key finding of this study is that despite having shorter telomeres, there was no difference in anthropometry or BP in children of mothers who had the metabolic syndrome. This may suggest that biological/molecular ageing could occur before phenotypical changes are observed. We also found no association between child telomere length and child anthropometry at 10 years of age. Similar to our findings, Zannolli et al [49] found no difference in telomere length, measured in

blood leucocytes, when comparing obese and normal-weight white children. In contrast, Al-Attas et al and Buxton et al reported shorter telomere length, also measured in blood leucocytes, in obese vs non-obese children aged 5–12 years [50] and 2–17 years [51]. Consistent with our findings, there was no association between BMI *z* score and telomere length in the latter study [51]. Measurement of other characteristics of obesity such as elevated circulating lipids or inflammatory markers, which are observed in children who are obese [52], may assist in identifying childhood traits that are associated with biological/molecular ageing.

The study has several strengths. SCOPE was a population-based prospective cohort design that included a large number of nulliparous women across six centres in four countries. Participants comprised a clearly defined population of nulliparous low-risk women with no pre-existing disease, utilising rigorous data collection techniques. The follow-up study captured 255 mother–child pairs for whom complete information on maternal metabolic variables and telomere length were available. Assessment of telomere length isolated from saliva DNA, a non-invasive source of DNA for biomarker assays, potentially allowed us to recruit larger numbers of a vulnerable population (i.e. children) than if a blood sample was required. The follow-up study also addressed limitations of previous studies that examined intrauterine exposures and child telomere length through inclusion of paternal and infant factors in the statistical models. Potentially, the greatest weakness of this study was that 23% of women in the initial Adelaide SCOPE cohort attended the 10 year follow-up with their respective child. It is unlikely that the reasons for dropout are related to the metabolic syndrome and telomere length, thus reasons for missing data cannot be sufficiently explained by the data we hold. To account for this in statistical analyses is difficult. From the initial 1139 women who consented to follow-up, we had a high rate of failure to contact these women (44%) despite documentation of three different phone numbers, home address and maiden name information. Thirty four per cent of women were unwilling to cooperate; they were contacted but did not wish to participate ($n = 214$). While the rate of follow-up may appear low, compared with the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) follow-up study in 10- to 14-year-old children, our rate of failure to locate participants in the current study (44%) was similar to that study (41%), we had a higher rate of women attending follow-up (64% vs 52%) and, as a proportion of eligible children, the rate of attendance was similar (23% vs 26%) [53]. Pending funding, we also plan to follow-up these children again at age 16 years when they are likely to have completed puberty. Regarding the telomere length assay, the qPCR CVs in the current study were <11%, indicating reproducibility within the assay. CVs can be influenced by a number of variables, including technical variation, biological variation and sample size. However, our study was performed within a single population, all DNA was collected, stored and extracted the same way, and by the same research team. Finally, although we cannot establish causation, our results provide supporting evidence on how maternal exposures might associate with offspring telomere length.

In conclusion, the metabolic syndrome in women during pregnancy is associated with shorter telomeres in their 8- to 10-year-old children, but not with differences in the children's anthropometry (BMI, *z* scores of weight-for-age, height-for-age) or BP. Further studies in larger cohorts of children are warranted, as well as investigation of whether telomere length measured in cord blood also associates with telomere length in childhood. Such studies would provide insight into the mechanism of telomere shortening and allow possible intervention strategies aimed at attenuating telomere shortening.

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Data availability The SCOPE study, which commenced recruitment in 2004, did not seek specific consent from participants for sharing their data publicly. However, the SCOPE Consortium Scientific Advisory Board invites applications to use the collected data via e-mail to the chairperson, via A. Aherne at ei.ccu@enreha.yma. Applicants will be asked to complete a Research Application Form specifying details for their planned study which will then be reviewed by the SCOPE Scientific Advisory Board. The SCOPE Consortium is keen to promote collaboration among researchers and to see our unique SCOPE database and pregnancy biobank used in studies which meet our ethics and consenting process. The SCOPE Consortium is a member of the International Pregnancy Collaboration (https://pregnancycolab.tghn.org/) and has participated in several studies involving shared data. The SCOPE database is provided and maintained by MedSciNet AB (http://medscinet.com).

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Contribution statement GAD, CTR and JAG made substantial contributions to the conception and design of the study. Substantial contribution was made by AG, MDP and PHA to acquisition of data, by DM, JAG and SYL to data analysis and by DM, JAG, SYL, LGS, TBM and KLG to interpretation of data. All authors were involved in drafting the article or revising it critically for important intellectual content. Final approval of the version to be published was given by all authors. JAG is the guarantor of this work.

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