Clinical and molecular evidence of accelerated ageing following very preterm birth

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Figure 1 Peripheral blood mononuclear cell telomere length in young adults. T-F: Term women (n=23); PT-F: Preterm women (n=21); T-M: Term men (n=29); PT-M: Preterm men (n=15); Data are mean ± s.e.m; *p<0.05, **p<0.01, ***p<0.001
Title: Clinical and molecular evidence of accelerated ageing following very preterm birth

James RC Parkinson 1,2 PhD, Robby Emsley 1 BSc, Jane L Tarry Adkins 3 PhD, Nick Longford 1 PhD, Susan E Ozanne 3 PhD, Elaine Holmes 4 PhD, Neena Modi 1 MD

Affiliations: 1 Section of Neonatal Medicine, Department of Medicine, Chelsea and Westminster campus, Imperial College London, 369 Fulham Road, London SW10 9NH, UK
2 Research Centre for Optimal Health, Department of Life Sciences, University of Westminster, London, W1W 6UW, UK
3 University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke’s Hospital, Hills Road, Cambridge, CB2 0QQ, UK
4 Department of Surgery and Cancer, South Kensington campus, Imperial College London, London SW7 2AZ, UK

Address correspondence to: Dr James Parkinson, Department of Life Sciences, 15 New Cavendish Street, London, W1W 6UW, Tel: 0203 5064609
Email: j.parkinson@westminster.ac.uk

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Category of Study: Basic science
Abstract

Background
The mechanisms responsible for the associations between very preterm birth and a higher risk of poor cardiovascular and metabolic health in adult life are unknown.

Methods
Comparison of the clinical and molecular phenotypes of healthy, normal weight young adults (18-27 years), born very preterm (<33 weeks gestational age (GA)) and at full-term (37-42 weeks GA). Outcomes included whole body magnetic resonance imaging; hepatic and muscle 1H magnetic resonance spectroscopy; blood pressure (BP) measurement, urine and blood sampling and telomere length measurement.

Results
We recruited 156 volunteers, 69 born very preterm (45 women; 24 men) and 87 born at full-term (45 women; 42 men). Preterm men had significantly more internal-abdominal adipose tissue (mean difference 0.33L (95% CI 0.04, 0.62), p<0.05), significantly fewer long telomeres (145-48.5kb: preterm men 14.1 ± 0.9%, term men 17.8 ± 1.1%, p<0.05; 48.5-8.6kb: preterm men 28.2 ± 2.6, term men 37.0 ± 2.4%, p<0.001) and a significantly higher proportion of shorter telomeres (4.2-1.3kb: preterm men 40.4 ± 3.5%, term men 29.9 ± 3.2%, p<0.01) compared to full-term men.

Conclusion
Our data indicate that healthy young adults born very preterm manifest clinical and molecular evidence of accelerated ageing.
Introduction

Rates of preterm birth and survival are rising globally \(^1\). In high income countries around 1-2% of births are at or below 33 weeks gestational age (GA) with the majority of these infants surviving to discharge from neonatal care \(^2\). In addition to the well-recognised risks to neurodevelopmental attainment, a growing volume of epidemiological data indicates that preterm birth is a risk to metabolic health in later life. Studies describe higher blood pressure \(^3\), and increased risk of stroke \(^4\), metabolic syndrome \(^5\), type-2 diabetes \(^6\), and premature death \(^7\) in comparison with birth at full-term. However, current knowledge has been insufficient for translation into preventive health care for this growing population.

We have shown previously that both preterm infants at term \(^8\) and adults born preterm \(^9\) have an altered body composition, with significantly greater internal-abdominal adipose tissue (IAAT) and intra-hepatocellular lipid (IHCL). Abdominal adipose tissue and ectopic lipid play a major role in the pathogenesis of the metabolic syndrome and correlate strongly with hypertension, insulin resistance and cardiovascular disease \(^10,11\), conditions that typically increase in prevalence with ageing.

In this proof-of-concept cohort study, we postulated that disruption of the normal pattern of third trimester development by very preterm birth would affect multiple systems that influence metabolic health. We tested the primary hypothesis that young non-obese adults born at or below 33 weeks gestation would have greater IAAT than those born at full-term. We also compared ectopic lipid in liver and muscle, biochemical parameters, blood pressure, and serum and urine metabolomes. We sought evidence of a molecular correlate of ageing by evaluating peripheral mononuclear blood cell (PMBC) telomere length.
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We obtained approval for the study from the National Research Ethics Service (London City and East Ethics Committee; REC: 12/LO/1053) and written informed consent from all participants. With the help of Bliss, the national UK preterm and sick baby charity, and the chief investigators of preterm observational cohorts 12,13, we invited the participation of healthy, young adult volunteers. Bliss advertised the study on their website, and the chief investigators sent letters of invitation to individuals from their cohorts for whom they had a current address. The cohorts were the EPICure study of children born below 26 weeks gestation and a follow-up study of extremely preterm children cared for at University College London Hospitals that has been underway since 1979 13. Inclusion criteria were age 18-27 years, with a Body Mass Index (BMI) ≤25kg/m², born very preterm (≤33 GA) or full-term (37-42 weeks GA). Exclusions were type-2 diabetes, personal or family history of dyslipidaemia, claustrophobia, moderate or severe neurodisability, presence of implanted metallic devices, possibility of pregnancy and any condition requiring medication.

Following an overnight fast, participants had a single half-day visit to the Section of Neonatal Medicine, Imperial College London, Chelsea and Westminster Hospital, London, UK. Anthropometric measurements, blood sampling, blood pressure measurements, magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS) were performed. BMI (kg/m²) was calculated from height and weight.

Physical activity was quantified using continuous scores derived from the short form of the IPAQ physical activity questionnaire 14. Standard clinical biochemistry analysis was performed on 8ml blood samples. Insulin sensitivity was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) 15 and Quantitative Insulin Sensitivity Check Index (QUICKI) 16. Participants’ post-codes were used to obtain the index of multiple deprivation (IMD) (https://www.gov.uk/government/statistics/english-indices-of-deprivation-2015) 17. An automatic
digital sphygmomanometer was used to record systolic (SBP) or diastolic (DBP) blood pressure as an average of 3 readings taken at one-minute intervals, following 5 minutes rest in a seated position. Participants were instructed on how to wear an ambulatory blood pressure monitor (Spacelabs Medical, Hertfordshire, UK) for 24 hours following their study visit. The monitor was programmed to take a reading every 30 minutes during the day (8am-10pm) and a reading every hour overnight (10pm-8am). Data upload, extraction and analysis were carried out blinded to study group. Parameters obtained were ambulatory SBP (aSBP), DBP (aDBP), mean blood pressure (aMBP), pulse pressure (aPP) and heart rate (aHR). Blood pressure monitor outcomes are presented as an overall average or “day-time” and “night-time” average readings. The point at which “night-time” readings began was identified by the expected nocturnal fall in aSBP. This was checked against prospectively reported wake/sleep cycles in the study visit questionnaire. In cases where no clear drop was observed, reported wake/sleep cycles were used. When there was a disparity between the observed blood pressure fall and reported sleep time, the blood pressure fall, which has been reported to be more accurate was used. “Non-dipping” was defined as a less than 10% fall in nocturnal aSBP. The difference between SBP or DBP readings and their corresponding day-time average ambulatory measurements (day-time average aSBP, day-time average aDBP) was calculated in order to assess a potential white coat hypertensive response.

Adipose tissue content and distribution

Whole body MRI was employed to determine total and regional adipose tissue content, as previously described. MR data were acquired on a Siemens Magnetom Avanto 1.5T scanner (Siemens Medical Systems, Erlangen, Germany). Participants were scanned using a T1 weighted fast spin echo sequence; repetition time (TR): 516ms, echo time (TE) 8.8ms, echo train length (ETL) 4, 3 signal averages and using the integrated body coil. Images were acquired with a 10mm slice thickness, 10mm gap, a field of view (FOV) of 500x500mm, matrix of 512x512. Adipose tissue images were analysed independently of the investigators, blinded to subject and group.

Intra-hepatocellular and intra-myocellular lipid

For IHCL, \(^1\)H MR Spectra were acquired from the right lobe of the liver using a phased-array surface coil and a spine array coil. Single voxel measurements were acquired using 5 STEAM sequences (TE:20, 30, 40, 50 and 60ms; TR: 1500ms acquired during breath-hold of 15 seconds; voxel size: 20x20x20mm, with 6 averages. Single voxel measurements were acquired using a PRESS sequence; TR: 3000ms, TE: 135ms, 128 averages and 15x20x20mm FOV. Water suppressed and non-water suppressed spectra were acquired for each muscle. MRS data were analysed using the Advanced Magnetic Resonance (AMARES) fitting algorithm within the Java-based MR user interface (jMRUI) software package, version 5 23. To calculate IMCL, peak areas for water and 6 lipid resonances were obtained using prior knowledge for the water suppressed and non-water suppressed spectra, and T2 corrections. IMCL was determined relative to total muscle water signal and converted to an absolute concentration presented as mmol/kg wet weight (mmol/kg ww) 24.

Telomere analysis

Telomere length was quantified, as described previously 25. PMBC were extracted from a whole blood sample by density gradient centrifugation. DNA was transferred to nylon membranes by Southern blotting and telomere length measured using Telo TAGGG telomere length assays (Roche Diagnostics, Germany). Telomere signals were analysed using Adobe Photoshop (Adobe Systems, San Jose, CA, USA) and MacBas software (Fujifilm UK, Bedford, UK). Four telomere molecular length groups (145 - 45.8kb, 48.5 - 8.6kb, 8.6 - 4.2kb, 4.2 - 1.3kb) were assessed.

Metabolic profiling of urine samples
Samples were analysed using a combination of NMR spectroscopy and global UPLC profiling methods for lipids and polar molecules using previously published methods. Data variables were normalized by a probabilistic quotient method. Multivariate data analysis was performed using the SIMCA package (v.13.0.2, Umetrics, Umeå, Sweden). Multivariate Pareto scaled data were modelled using principal components analysis and orthogonal partial least squares discriminant analysis (OPLS-DA). Orthogonal projection to latent structure discriminant analysis (O-PLS-DA) was used to optimally model class differences and evaluated using a permutation test. Comparisons were made between preterm and term (men and women combined), and separately for the two sexes. The OPLS-DA parameters were evaluated to assess the robustness each model. A two-tailed t-test was applied to the discriminatory features of each model.

**Sample size**

We approximated the sample size for key outcomes by simulations on pilot data with 10,000 permutations based on a linear model with effects for sex, BMI, preterm birth and a residual error. We determined that a total sample size of 150 participants would have over 80% power (5% significance) to detect a difference of 0.4 litre in IAAT, a difference of 4.6mmHg in SBP and 3.5mmHg in DBP.

**Statistical analysis**

We calculated the mean (SD) for each variable and each of the four groups, preterm-women, term-women, preterm-men, term-men. We used multiple linear regression to examine group (preterm-term) and sex (men-women) differences and sex-group interactions, adjusting for age, sex, BMI, IMD and IPAQ score. Unless otherwise stated, comparisons are presented as mean difference with 95% Confidence Interval (CI). S-IMCL and T-IMCL are presented as geometric mean and ratio (95% CI). To address their non-normally distributed nature, IHCL values were log transformed (0.00001 + x) and a square-root transformation was used for IMCL. Telomere data were analysed...
using 2-way ANOVA with sex and GA as the independent variables and Duncan’s post-hoc testing using Statistica 7 software (Statsoft Inc, Milton Keynes, Buckinghamshire, UK). Values provided are mean difference (95% CI) unless otherwise stated.
Results

We recruited 156 volunteers; 69 born preterm (45 women; 24 men), at a mean GA of 29 weeks (range 23 to 33) and mean birth weight of 1.26 kg (range 0.51 to 2.95), and 87 born at full-term (45 women; 42 men). Participants identified themselves predominantly as “white” ethnicity (preterm: 46; full-term: 49), with the remainder spanning a diverse range of single and mixed-race groupings. Preterm participants were significantly shorter (mean difference (95% CI) -5cm (-8 to -2), p<0.001) but there were no statistically significant differences in weight, BMI or IPAQ score. Baseline characteristics are shown in Table 1. After adjustment by regression, preterm men had significantly more IAAT than full-term men (mean difference 0.35L (0.07 to 0.63, p<0.05). There were no statistically significant differences in other AT compartments, total AT, IHCL, S-IMCL or T-IMCL (Table 2).

Compared to full-term born participants, the preterm group had higher mean SBP (6.5mmHg (4.2 to 8.9), p<0.05) and greater differences between daytime single and ambulatory systolic (7.0mmHg (4.4 to 9.6), p<0.05) and diastolic (2.1mmHg (0.0 to 4.2); p<0.05) blood pressure (Table 3). There was a statistically significant preterm-sex interaction for day, night and overall aSBP, and day aMAP and aPP, with preterm men showing higher daytime aSBP (6.0mmHg (1.9 to 10.1), p<0.05), higher overall aSBP (5.6mmHg (1.8 to 9.3); p<0.05), daytime aHR (greater by 5.4 beats per minute (0.8 to 10.1); p<0.05), overall aHR (greater by 4.8 beats per minute (0.1 to 9.5); p<0.05), daytime aPP (greater by 5.9mmHg (3.2 to 8.6); p<0.05) and overall aPP (greater by 5.9mmHg (3.3 to 8.5); p<0.05) (Table 3). In comparison with term women, preterm women showed lower overall aSBP (-3.2mmHg (-6.2 to -0.2); p<0.05) and aMAP (-3.0mmHg (-5.5 to -0.6); p<0.05). Serum leptin was significantly higher in the preterm group (0.28ng/ml (0.03 to 0.54), p<0.05) (Table 4). No significant differences in the proportion of subjects not showing nocturnal dipping were observed between term or preterm groups (p=NS) (Proportion of subjects not
showing nocturnal dipping: Preterm women: 22% (10/45), Term women: 22% (9/41), Preterm men: 21% (5/24), Term men: 21% (9/42)).

The percentage distribution of each telomere length range is shown in Figure 1. We identified a significant redistribution of telomere length in preterm men, with fewer long and more short PMBC telomeres compared to full-term men (145-48.5kb: preterm men 14.1 ± 0.9%; full-term men 17.8 ± 1.1%, p<0.05; 48.5-8.6kb: preterm men 28.2 ± 2.6%, full-term men: 37.0 ± 2.4%, p<0.001); 4.2-1.3kb: preterm men 40.4 ± 3.5%, full-term men: 29.9 ± 3.2%, p<0.01). No significant difference in the distribution of telomeres was observed between preterm and full-term women.

The metabolic profiles of full-term compared with preterm participants were not significantly different when modelled either as a whole dataset or stratified according to sex. The profiles were based on multiple metabolite groups including lipids, amines, sugars, phenols, acylcarnitines and organic acids. Similarly, when the profiles were regressed against telomere length, no significant associations were detected.
Discussion

We show that healthy, normal-weight, young adults born very preterm manifest characteristics indicative of greater risk to metabolic and cardiovascular health, compared with those born at full-term. These features, namely greater IAAT in men, and higher blood pressure, typically manifest with ageing. We also identified a molecular marker of cellular ageing, a greater proportion of shorter telomeres in preterm men compared to term men. This indicates that the early onset of age-related conditions identified in pre-term infants in epidemiological studies is more likely to be a true relationship than a reflection of confounding by factors such as socio-economic disadvantage. The lack of significant association of metabolites with term/preterm status indicates that preterm birth had no functional metabolic effect at this age in keeping with their outwardly healthy condition.

The strengths of our study lie in replicating the finding of increased IAAT and higher blood pressure we noted in an earlier, smaller study 9 and in identifying a molecular correlate in telomere shortening. Participants were young and healthy, and neither overweight nor obese, and the comparisons remained robust to adjustment for potential confounders. WE analysed telomere length by evaluating the distribution of length categories. This provides more biological insight that assessing mean length, as for a cell to become senescent the telomere length of one chromosome needs to become critically short (<5 kb). Therefore knowing the proportion of telomeres that are critically short is essential for robust inferences to be made about cellular ageing.

The principal weakness is the smaller number of preterm men in relation to women that we were able to recruit. Phenotypic characteristics indicative of risk to metabolic health differ across racial groups and a further limitation is that participants were racially heterogeneous.
Telomeres are hexameric TTAGGG repeats found at the ends of chromosomes responsible for maintenance of chromosomal integrity. They are considered to be robust markers of cellular ageing and senescence in somatic cells and are associated with longevity. A novel finding of our study is that, at the age studied, only the men experienced any effect of gestational age at birth upon telomere length with more short and fewer long telomeres in the preterm men compared with the full-term group. Oestrogens are protective against telomere shortening which may offer an explanation of why young women did not appear affected.

Epidemiological studies have long suggested increased risk of the metabolic syndrome, and type-2 diabetes in adults born preterm. Preterm boys have higher mortality than girls and are generally more susceptible to adverse health outcomes. Our data provide further evidence of greater male vulnerability. IAAT is a depot associated with type 2 diabetes, dyslipidaemia and hypertension. The magnitude of increase in IAAT in the preterm group, namely by $330 \text{ cm}^3$ on average, equivalent to about $290\text{g AT}$, is comparable to the increase of $510\text{cm}^3$ in our previous study. Kuk et al estimated that $370\text{g}$ excess IAAT is associated with an 80% increase in 5 year mortality. A novel observation is that we identified higher circulating leptin in the preterm cohort, although total AT was similar in the groups. Leptin correlates highly with total AT, so this may indicate leptin resistance, also a feature of ageing. We previously identified increased IHCL and soleus IMCL in preterm-born young men, markers of ectopic fat deposition that are predictors of peripheral insulin resistance, a key component of the metabolic syndrome, but were unable to replicate this observation in the present study.

Higher blood pressure in children and adults born preterm has been reported in several studies from around the world, as has a linear relationship with degree of prematurity. In a systematic review and meta-analysis of 27 studies, including over 300,000 participants, we previously identified significantly higher SBP, DBP and aSBP in preterm compared with full-term adults.
Ambulatory monitoring is considered a more reliable approach to assessing blood pressure, as it is less affected by the anxiety response that accompanies single measurements\(^\text{41}\). The clinical relevance of these observations is that every 2mmHg rise in SBP is associated with a 7% increase in mortality from ischaemic heart disease and a 10% increased risk of stroke\(^\text{42}\). Birth before 32 weeks GA is also associated with a near doubling in risk of cerebrovascular disease compared to birth at term\(^\text{4}\) and higher mortality from cerebrovascular disease, particularly occlusive stroke\(^\text{43}\). Blood pressure measurements in preterm women were inconclusive, with seemingly lower aSBP and aMAP than term women. Preterm women also showed greater differences between single and ambulatory SBP than term women, indicative of a greater stress response as previously reported\(^\text{44,45}\). This suggests that the higher blood pressure in preterm women reported in previous studies may have been driven by the so-called “white-coat” stress response. We recommend that future studies use ambulatory rather than single measurements of blood pressure.

Our data add to a growing recognition that preterm birth disrupts multiple organ systems and leaves a life-long legacy. Our study not only provides evidence for the direct consequences of prematurity, but also insights into the mechanisms by which this occurs. When considered in combination with other epidemiological and experimental data our study has implications for families, the growing population of young people born preterm who are now reaching adulthood, clinicians, charities, policy-makers, and researchers. Families and in due course the young people themselves, should be made aware that very preterm birth is a risk factor for early onset of features indicating susceptibility to cardio-metabolic disease, and should receive general health and lifestyle advice to mitigate against these risks, in particular the benefits of a healthy diet, exercise, and avoidance of smoking. Clinicians should recognise the relevance of asking about preterm birth when taking a general medical history. In our view, existing evidence warrants at the very least, opportunistic measurement of blood pressure in all individuals born preterm. Charities may wish to utilise the findings of this study to improve public understanding of preterm birth, strengthen...
advocacy and raise funds for research. Preterm birth is closely associated with poor maternal health and socio-economic disadvantage and growing numbers of very preterm survivors are being added to the total population pool. There is also therefore need for policy makers around the world to direct attention to the wider societal determinants of preterm birth. The challenge for researchers is to determine if the characteristics we identify reflect preterm birth or neonatal care practices, identify causal biological mechanisms, and test candidate intrauterine and new-born interventions to reduce risks to life-long health.
Acknowledgments

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Author Contributions

Dr JRC Parkinson contributed to study design, data acquisition, analysis and interpretation, wrote, and revised the final manuscript and has given final approval of this version to be published.

Mr R Emsley contributed to data acquisition, revised the article and has given final approval of this version to be published.

Dr JL Tarry Adkins contributed to data acquisition, revised the article and has given final approval of this version to be published.

Dr N Longford contributed to data analysis, revised the article and has given final approval of this version to be published.

Professor SE Ozanne contributed to study design, data analysis and interpretation, revised the article and has given final approval of this version to be published.

Professor E Holmes contributed to study design, data analysis and interpretation, revised the article and has given final approval of this version to be published.

Professor Modi contributed to study design, data analysis and interpretation, and revised the final manuscript and has given final approval of this version to be published.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Whole body MRI was employed to determine total and regional adipose tissue content, as previously described \(^{21}\). MR data were acquired on a Siemens Magnetom Avanto 1.5T scanner (Siemens Medical Systems, Erlangen, Germany). Participants were scanned using a T1 weighted fast spin echo sequence; repetition time (TR): 516ms, echo time (TE) 8.8ms, echo train length (ETL) 4, 3 signal averages and using the integrated body coil. Images were acquired with a 10mm slice thickness, 10mm gap, a field of view (FOV) of 500x500mm, matrix of 512x512. Adipose tissue images were analysed independently of the investigators, blinded to subject and group.

**Intra-hepatocellular and intra-myocellular lipid**

For IHCL, 1H MR Spectra were acquired from the right lobe of the liver using a phased-array surface coil and a spine array coil. Single voxel measurements were acquired using 5 STEAM sequences (TE:20, 30, 40, 50 and 60ms; TR: 1500ms acquired during breath-hold of 15 seconds; voxel size: 20x20x20mm, with 6 averages. Single voxel measurements were acquired using a PRESS sequence; TR: 3000ms, TE: 135ms, 128 averages and 15x20x20mm FOV. Water suppressed and non-water suppressed spectra were acquired for each muscle. MRS data were analysed using the Advanced Magnetic Resonance (AMARES) fitting algorithm within the Java-based MR user interface (jMRUI) software package, version 5 23. To calculate IMCL, peak areas for water and 6 lipid resonances were obtained using prior knowledge for the water suppressed and non-water suppressed spectra, and T2 corrections. IMCL was determined relative to total muscle water signal and converted to an absolute concentration presented as mmol/kg wet weight (mmol/kg ww) 24.

**Telomere analysis**

Telomere length was quantified, as described previously 25. PMBC were extracted from a whole blood sample by density gradient centrifugation. DNA was transferred to nylon membranes by Southern blotting and telomere length measured using Telo TAGGG telomere length assays (Roche Diagnostics, Germany). Telomere signals were analysed using Adobe Photoshop (Adobe Systems, San Jose, CA, USA) and MacBas software (Fujifilm UK, Bedford, UK). Four telomere molecular length groups (145 - 45.8kb, 48.5 - 8.6kb, 8.6 - 4.2kb, 4.2 - 1.3kb) were assessed.

**Metabolic profiling of urine samples**
Samples were analysed using a combination of NMR spectroscopy and global UPLC profiling methods for lipids and polar molecules using previously published methods \(^{26-28}\). Data variables were normalized by a probabilistic quotient method \(^{29}\). Multivariate data analysis was performed using the SIMCA package (v.13.0.2, Umetrics, Umeå, Sweden). Multivariate Pareto scaled data were modelled using principal components analysis and orthogonal partial least squares discriminant analysis (OPLS-DA). Orthogonal projection to latent structure discriminant analysis (O-PLS-DA) \(^{30}\) was used to optimally model class differences and evaluated using a permutation test \(^{31}\). Comparisons were made between preterm and term (men and women combined), and separately for the two sexes. The OPLS-DA parameters were evaluated to assess the robustness of each model. A two-tailed t-test was applied to the discriminatory features of each model.

**Sample size**

We approximated the sample size for key outcomes by simulations on pilot data with 10,000 permutations based on a linear model with effects for sex, BMI, preterm birth and a residual error. We determined that a total sample size of 150 participants would have over 80% power (5% significance) to detect a difference of 0.4 litre in IAAT, a difference of 4.6mmHg in SBP and 3.5mmHg in DBP.

**Statistical analysis**

We calculated the mean (SD) for each variable and each of the four groups, preterm-women, term-women, preterm-men, term-men. We used multiple linear regression to examine group (preterm-term) and sex (men-women) differences and sex-group interactions, adjusting for age, sex, BMI, IMD and IPAQ score. Unless otherwise stated, comparisons are presented as mean difference with 95% Confidence Interval (CI). S-IMCL and T-IMCL are presented as geometric mean and ratio (95% CI). To address their non-normally distributed nature, IHCL values were log transformed (0.00001 + x) and a square-root transformation was used for IMCL. Telomere data were analysed...
using 2-way ANOVA with sex and GA as the independent variables and Duncan’s post-hoc testing using Statistica 7 software (Statsoft Inc, Milton Keynes, Buckinghamshire, UK). Values provided are mean difference (95% CI) unless otherwise stated.
Results

We recruited 156 volunteers; 69 born preterm (45 women; 24 men), at a mean GA of 29 weeks (range 23 to 33) and mean birth weight of 1.26 kg (range 0.51 to 2.95), and 87 born at full-term (45 women; 42 men). Participants identified themselves predominantly as “white” ethnicity (preterm: 46; full-term: 49), with the remainder spanning a diverse range of single and mixed-race groupings. Preterm participants were significantly shorter (mean difference (95% CI) -5cm (-8 to -2), p<0.001) but there were no statistically significant differences in weight, BMI or IPAQ score. Baseline characteristics are shown in Table 1. After adjustment by regression, preterm men had significantly more IAAT than full-term men (mean difference 0.35L (0.07 to 0.63, p<0.05). There were no statistically significant differences in other AT compartments, total AT, IHCL, S-IMCL or T-IMCL (Table 2).

Compared to full-term born participants, the preterm group had higher mean SBP (6.5mmHg (4.2 to 8.9), p<0.05) and greater differences between daytime single and ambulatory systolic (7.0mmHg (4.4 to 9.6), p<0.05) and diastolic (2.1mmHg (0.0 to 4.2); p<0.05) blood pressure (Table 3). There was a statistically significant preterm-sex interaction for day, night and overall aSBP, and day aMAP and aPP, with preterm men showing higher daytime aSBP (6.0mmHg (1.9 to 10.1), p<0.05), higher overall aSBP (5.6mmHg (1.8 to 9.3); p<0.05), daytime aHR (greater by 5.4 beats per minute (0.8 to 10.1); p<0.05), overall aHR (greater by 4.8 beats per minute (0.1 to 9.5); p<0.05), daytime aPP (greater by 5.9mmHg (3.2 to 8.6); p<0.05) and overall aPP (greater by 5.9mmHg (3.3 to 8.5); p<0.05) (Table 3). In comparison with term women, preterm women showed lower overall aSBP (-3.2mmHg (-6.2 to -0.2); p<0.05) and aMAP (-3.0mmHg (-5.5 to -0.6); p<0.05). Serum leptin was significantly higher in the preterm group (0.28ng/ml (0.03 to 0.54), p<0.05) (Table 4). No significant differences in the proportion of subjects not showing nocturnal dipping were observed between term or preterm groups (p=NS) (Proportion of subjects not
showing nocturnal dipping: Preterm women: 22% (10/45), Term women: 22% (9/41), Preterm men: 21% (5/24), Term men: 21% (9/42)).

The percentage distribution of each telomere length range is shown in Figure 1. We identified a significant redistribution of telomere length in preterm men, with fewer long and more short PMBC telomeres compared to full-term men (145-48.5kb: preterm men 14.1 ± 0.9%; full-term men 17.8 ± 1.1%, p<0.05; 48.5-8.6kb: preterm men 28.2 ± 2.6%, full-term men: 37.0 ± 2.4%, p<0.001); 4.2-1.3kb: preterm men 40.4 ± 3.5%, full-term men: 29.9 ± 3.2%, p<0.01). No significant difference in the distribution of telomeres was observed between preterm and full-term women.

The metabolic profiles of full-term compared with preterm participants were not significantly different when modelled either as a whole dataset or stratified according to sex. The profiles were based on multiple metabolite groups including lipids, amines, sugars, phenols, acylcarnitines and organic acids. Similarly, when the profiles were regressed against telomere length, no significant associations were detected.
Discussion

We show that healthy, normal-weight, young adults born very preterm manifest characteristics indicative of greater risk to metabolic and cardiovascular health, compared with those born at full-term. These features, namely greater IAAT in men, and higher blood pressure, typically manifest with ageing. We also identified a molecular marker of cellular ageing, a greater proportion of shorter telomeres in preterm men compared to term men. This indicates that the early onset of age-related conditions identified in pre-term infants in epidemiological studies is more likely to be a true relationship than a reflection of confounding by factors such as socio-economic disadvantage. The lack of significant association of metabolites with term/preterm status indicates that preterm birth had no functional metabolic effect at this age in keeping with their outwardly healthy condition.

The strengths of our study lie in replicating the finding of increased IAAT and higher blood pressure we noted in an earlier, smaller study and in identifying a molecular correlate in telomere shortening. Participants were young and healthy, and neither overweight nor obese, and the comparisons remained robust to adjustment for potential confounders. We analysed telomere length by evaluating the distribution of length categories. This provides more biological insight that assessing mean length, as for a cell to become senescent the telomere length of one chromosome needs to become critically short (<5 kb). Therefore knowing the proportion of telomeres that are critically short is essential for robust inferences to be made about cellular ageing.

The principal weakness is the smaller number of preterm men in relation to women that we were able to recruit. Phenotypic characteristics indicative of risk to metabolic health differ across racial groups and a further limitation is that participants were racially heterogeneous.
Telomeres are hexameric TTAGGG repeats found at the ends of chromosomes responsible for maintenance of chromosomal integrity. They are considered to be robust markers of cellular ageing and senescence in somatic cells and are associated with longevity. A novel finding of our study is that, at the age studied, only the men experienced any effect of gestational age at birth upon telomere length with more short and fewer long telomeres in the preterm men compared with the full-term group. Oestrogens are protective against telomere shortening which may offer an explanation of why young women did not appear affected.

Epidemiological studies have long suggested increased risk of the metabolic syndrome, and type-2 diabetes in adults born preterm. Preterm boys have higher mortality than girls and are generally more susceptible to adverse health outcomes. Our data provide further evidence of greater male vulnerability. IAAT is a depot associated with type 2 diabetes, dyslipidaemia and hypertension. The magnitude of increase in IAAT in the preterm group, namely by 330 cm³ on average, equivalent to about 290g AT, is comparable to the increase of 510cm³ in our previous study. Kuk et al estimated that 370g excess IAAT is associated with an 80% increase in 5 year mortality. A novel observation is that we identified higher circulating leptin in the preterm cohort, although total AT was similar in the groups. Leptin correlates highly with total AT, so this may indicate leptin resistance, also a feature of ageing. We previously identified increased IHCL and soleus IMCL in preterm-born young men, markers of ectopic fat deposition that are predictors of peripheral insulin resistance, a key component of the metabolic syndrome, but were unable to replicate this observation in the present study.

Higher blood pressure in children and adults born preterm has been reported in several studies from around the world, as has a linear relationship with degree of prematurity. In a systematic review and meta-analysis of 27 studies, including over 300,000 participants, we previously identified significantly higher SBP, DBP and aSBP in preterm compared with full-term adults.
Ambulatory monitoring is considered a more reliable approach to assessing blood pressure, as it is less affected by the anxiety response that accompanies single measurements. The clinical relevance of these observations is that every 2mmHg rise in SBP is associated with a 7% increase in mortality from ischaemic heart disease and a 10% increased risk of stroke. Birth before 32 weeks GA is also associated with a near doubling in risk of cerebrovascular disease compared to birth at term and higher mortality from cerebrovascular disease, particularly occlusive stroke. Blood pressure measurements in preterm women were inconclusive, with seemingly lower aSBP and aMAP than term women. Preterm women also showed greater differences between single and ambulatory SBP than term women, indicative of a greater stress response as previously reported. This suggests that the higher blood pressure in preterm women reported in previous studies may have been driven by the so-called “white-coat” stress response. We recommend that future studies use ambulatory rather than single measurements of blood pressure.

Our data add to a growing recognition that preterm birth disrupts multiple organ systems and leaves a life-long legacy. Our study not only provides evidence for the direct consequences of prematurity, but also insights into the mechanisms by which this occurs. When considered in combination with other epidemiological and experimental data our study has implications for families, the growing population of young people born preterm who are now reaching adulthood, clinicians, charities, policy-makers, and researchers. Families and in due course the young people themselves, should be made aware that very preterm birth is a risk factor for early onset of features indicating susceptibility to cardio-metabolic disease, and should receive general health and lifestyle advice to mitigate against these risks, in particular the benefits of a healthy diet, exercise, and avoidance of smoking. Clinicians should recognise the relevance of asking about preterm birth when taking a general medical history. In our view, existing evidence warrants at the very least, opportunistic measurement of blood pressure in all individuals born preterm. Charities may wish to utilise the findings of this study to improve public understanding of preterm birth, strengthen
advocacy and raise funds for research. Preterm birth is closely associated with poor maternal health and socio-economic disadvantage and growing numbers of very preterm survivors are being added to the total population pool. There is also therefore need for policy makers around the world to direct attention to the wider societal determinants of preterm birth. The challenge for researchers is to determine if the characteristics we identify reflect preterm birth or neonatal care practices, identify causal biological mechanisms, and test candidate intrauterine and new-born interventions to reduce risks to life-long health.
Acknowledgments

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Author Contributions

Dr JRC Parkinson contributed to study design, data acquisition, analysis and interpretation, wrote, and revised the final manuscript and has given final approval of this version to be published.

Mr R Emsley contributed to data acquisition, revised the article and has given final approval of this version to be published.

Dr JL Tarry Adkins contributed to data acquisition, revised the article and has given final approval of this version to be published.

Dr N Longford contributed to data analysis, revised the article and has given final approval of this version to be published.

Professor SE Ozanne contributed to study design, data analysis and interpretation, revised the article and has given final approval of this version to be published.

Professor E Holmes contributed to study design, data analysis and interpretation, revised the article and has given final approval of this version to be published.

Professor Modi contributed to study design, data analysis and interpretation, and revised the final manuscript and has given final approval of this version to be published.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figure 1 Peripheral blood mononuclear cell telomere length in young adults. T-F: Term women (n=23); PT-F: Preterm women (n=21); T-M: Term men (n=29); PT-M: Preterm men (n=15); Data are mean ± s.e.m; *=p<0.05, **=p<0.01, ***=p<0.001
### Table 1

**Baseline characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Preterm Women</th>
<th>Term Women</th>
<th>Preterm Men</th>
<th>Term Men</th>
<th>Preterm – Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>45</td>
<td>45</td>
<td>24</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>28·2 ± 3·1</td>
<td>39·4 ± 1·6</td>
<td>27·7 ± 3·1</td>
<td>39·3 ± 1·2</td>
<td>-11·4 (-12·2, -10·6)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1·26 ± 0·57</td>
<td>3·22 ± 0·55</td>
<td>1·26 ± 0·57</td>
<td>3·38 ± 0·53</td>
<td>-2·03 (-2·21, -1·85)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22·8 ± 2·7</td>
<td>3·1 ± 2·8</td>
<td>21·9 ± 2·0</td>
<td>21·9 ± 2·0</td>
<td>0·0 (-0·8, 0·8)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58·7 ± 9·1</td>
<td>59·3 ± 8·8</td>
<td>67·4 ± 7·7</td>
<td>70·5 ± 9·1</td>
<td>-3·0 (-6·2, 0·2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 ± 7</td>
<td>165 ± 8</td>
<td>175 ± 7</td>
<td>178 ± 8</td>
<td>-5 (-8, -2)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>22·4 ± 2·4</td>
<td>21·8 ± 2·5</td>
<td>22·1 ± 2·1</td>
<td>22·3 ± 2·2</td>
<td>0·3 (-0·5, 1·0)</td>
</tr>
<tr>
<td>IPAQ</td>
<td>1727 ± 787</td>
<td>1783 ± 718</td>
<td>2438 ± 744</td>
<td>2222 ± 684</td>
<td>20 (-235, 276)</td>
</tr>
</tbody>
</table>

*Values are mean ± standard deviation; entries for Preterm-Term Difference are pooled estimates (95% confidence limits); the p value tests the hypothesis that the four groups have identical means (ANOVA).*
Table 2
Adipose tissue compartments and ectopic lipid

<table>
<thead>
<tr>
<th></th>
<th>Preterm Women</th>
<th>Term Women</th>
<th>Preterm Men</th>
<th>Term Men</th>
<th>Preterm - Term All</th>
<th>Interaction Sex × Preterm</th>
<th>Preterm - Term Women</th>
<th>Preterm - Term Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size°</td>
<td>45</td>
<td>44</td>
<td>23</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal abdominal AT</td>
<td>1·17 ± 0·52</td>
<td>1·06 ± 0·57</td>
<td>1·54 ± 1·19</td>
<td>1·07 ± 0·58</td>
<td>0·08 (-0·01,0·16)</td>
<td>-0·30 (-0·66,0·07)</td>
<td>0·05 (-0·18,0·28)</td>
<td>0·35* (0·07,0·63)</td>
</tr>
<tr>
<td>Internal non-abdominal AT</td>
<td>1·39 ± 0·39</td>
<td>1·33 ± 0·32</td>
<td>1·48 ± 0·55</td>
<td>1·40 ± 0·38</td>
<td>0·01 (-0·02,0·05)</td>
<td>-0·01 (-0·22,0·21)</td>
<td>0·04 (-0·09,0·17)</td>
<td>0·05 (-0·11,0·21)</td>
</tr>
<tr>
<td>Subcutaneous abdominal AT</td>
<td>4·69 ± 1·62</td>
<td>4·56 ± 2·10</td>
<td>2·86 ± 1·93</td>
<td>2·36 ± 1·40</td>
<td>0·03 (-0·06,0·13)</td>
<td>-0·72 (-1·54,0·10)</td>
<td>-0·08 (-0·60,0·44)</td>
<td>0·65 (-0·02,1·27)</td>
</tr>
<tr>
<td>Subcutaneous non-abdominal AT</td>
<td>12·66 ± 3·20</td>
<td>12·62 ± 3·85</td>
<td>7·72 ± 3·39</td>
<td>7·41 ± 2·63</td>
<td>-0·03 (-0·14,0·09)</td>
<td>-0·53 (-1·93,0·87)</td>
<td>-0·28 (-1·17,0·61)</td>
<td>0·25 (-0·83,1·33)</td>
</tr>
<tr>
<td>Total AT</td>
<td>19·92 ± 5·16</td>
<td>19·57 ± 6·62</td>
<td>13·61 ± 6·73</td>
<td>12·27 ± 4·55</td>
<td>0·03 (-0·12,0·18)</td>
<td>-1·36 (-3·84,1·12)</td>
<td>-0·23 (-1·78,1·32)</td>
<td>1·13 (-0·79,3·06)</td>
</tr>
<tr>
<td>IHCL</td>
<td>-6·59 ± 1·43</td>
<td>-7·16 ± 1·73</td>
<td>-6·52 ± 2·30</td>
<td>-6·62 ± 1·69</td>
<td>0·23 (-0·20,0·65)</td>
<td>-0·14 (-1·03,0·75)</td>
<td>0·17 (-0·36,0·71)</td>
<td>0·31 (-0·39,1·02)</td>
</tr>
<tr>
<td>S-IMCL</td>
<td>1·65 ± 0·35</td>
<td>1·64 ± 0·41</td>
<td>1·73 ± 0·33</td>
<td>1·65 ± 0·38</td>
<td>-0·02 (-0·13,0·0)</td>
<td>-0·08 (-0·33,0·16)</td>
<td>-0·03 (-0·18,0·12)</td>
<td>-0·06 (-0·13,0·25)</td>
</tr>
<tr>
<td>T-IMCL</td>
<td>0·89 ± 0·36</td>
<td>1·01 ± 0·42</td>
<td>0·99 ± 0·36</td>
<td>0·97 ± 0·33</td>
<td>-0·06 (-0·17,0·06)</td>
<td>-0·19 (-0·42,0·04)</td>
<td>-0·14 (-0·29,0·01)</td>
<td>0·05 (-0·13,0·23)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation; ° Participants with missing values are not counted in the sample size; values are mean and standard deviation in litres for adipose tissue volumes and mmol/kg/ww for IHCL and IMCL; Adjusted differences (Preterm - Term): estimates and (95% confidence limits); All: based on the model without sex-preterm interaction; women and men: based on the model with sex-preterm interaction; Log transformation with offset of 0·00001, log(x + 0·00001), is used in the regression models for IHCL and IMCL; * significant at 5% level (p < 0·05)
<table>
<thead>
<tr>
<th></th>
<th>Preterm Women</th>
<th>Term Women</th>
<th>Preterm Men</th>
<th>Term Men</th>
<th>Preterm-Term All</th>
<th>Interaction</th>
<th>Preterm-Term Women</th>
<th>Preterm-Term Men</th>
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<tbody>
<tr>
<td>Sample size</td>
<td>45</td>
<td>41</td>
<td>24</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>124·3 ± 7·1</td>
<td>118·4 ± 8·0</td>
<td>133·4 ± 10·2</td>
<td>123·0 ± 6·9</td>
<td>6·5 (4·2,8·9)*</td>
<td>-4·0 (-9·1,1·1)</td>
<td>5·5 (2·3,8·7)*</td>
<td>9·5 (5·5,13·6)*</td>
</tr>
<tr>
<td>DBP</td>
<td>75·7 ± 6·4</td>
<td>73·3 ± 7·1</td>
<td>74·1 ± 9·3</td>
<td>74·4 ± 7·4</td>
<td>0·7 (-1·6,3·0)</td>
<td>4·4 (-0·2,9·1)</td>
<td>2·5 (0·3,5·4)</td>
<td>-1·9 (-5·5,1·7)</td>
</tr>
<tr>
<td>Day-time average aSBP</td>
<td>114·6 ± 9·7</td>
<td>116·6 ± 7·2</td>
<td>126·2 ± 8·3</td>
<td>119·3 ± 8·4</td>
<td>1·4 (-1·2,3·9)</td>
<td>-8·0 (-13·3,-2·8)*</td>
<td>-2·1 (-5·3,1·1)</td>
<td>6·0 (1·9,10·1)*</td>
</tr>
<tr>
<td>Night-time average aSBP</td>
<td>98·5 ± 8·0</td>
<td>100·2 ± 8·5</td>
<td>107·4 ± 8·2</td>
<td>104·4 ± 8·9</td>
<td>-0·4 (-2·9,2·1)</td>
<td>-5·4 (-10·3,0·5)*</td>
<td>-2·3 (-5·3,0·7)</td>
<td>3·1 (-0·8,7·1)</td>
</tr>
<tr>
<td>Overall average aSBP</td>
<td>109·8 ± 9·2</td>
<td>111·5 ± 6·6</td>
<td>119·7 ± 6·3</td>
<td>114·6 ± 7·0</td>
<td>0·8 (-1·8,3·3)</td>
<td>-8·8 (-13·6,4·0)*</td>
<td>-3·2 (-6·2,-0·2)*</td>
<td>5·6 (1·8,9·3)*</td>
</tr>
<tr>
<td>Difference between SBP and day-time average aSBP</td>
<td>9·8 ± 7·6</td>
<td>1·8 ± 6·1</td>
<td>6·3 ± 7·8</td>
<td>3·5 ± 7·7</td>
<td>7·0 (4·4,9·6)*</td>
<td>6·6 (2·2,11·0)*</td>
<td>8·3 (5·5,11·1)*</td>
<td>1·6 (-1·8,5·1)</td>
</tr>
<tr>
<td>Day-time average aDBP</td>
<td>71·8 ± 7·3</td>
<td>72·8 ± 5·1</td>
<td>71·8 ± 8·5</td>
<td>70·5 ± 6·3</td>
<td>-0·7 (-2·7,1·3)</td>
<td>-1·1 (-5·2,1·3)</td>
<td>-1·2 (-3·7,1·4)</td>
<td>0·1 (-3·3,3·1)</td>
</tr>
<tr>
<td>Night-time average aDBP</td>
<td>56·8 ± 5·3</td>
<td>58·5 ± 6·5</td>
<td>57·4 ± 6·9</td>
<td>57·1 ± 7·0</td>
<td>-0·6 (-2·4,1·2)</td>
<td>-2·0 (-5·7,1·7)</td>
<td>-1·4 (-3·6,0·9)</td>
<td>0·6 (-2·3,3·6)</td>
</tr>
<tr>
<td>Overall average aDBP</td>
<td>67·2 ± 6·7</td>
<td>68·4 ± 4·7</td>
<td>66·8 ± 7·1</td>
<td>66·9 ± 6·3</td>
<td>-0·9 (-2·8,1·0)</td>
<td>-2·3 (-6·2,1·6)</td>
<td>-1·9 (-4·3,0·5)</td>
<td>0·4 (-2·7,3·4)</td>
</tr>
<tr>
<td>Difference between DBP and day-time average aDBP</td>
<td>4·5 ± 7·2</td>
<td>0·7 ± 5·9</td>
<td>1·7 ± 6·1</td>
<td>3·6 ± 7·2</td>
<td>2·1 (0·4,2·9)*</td>
<td>5·4 (1·2,9·5)*</td>
<td>3·4 (0·8,6·0)*</td>
<td>-2·0 (-5·2,1·3)</td>
</tr>
<tr>
<td>Day-time average aMAP</td>
<td>83·9 ± 8·0</td>
<td>86·2 ± 5·9</td>
<td>89·1 ± 7·4</td>
<td>85·5 ± 5·8</td>
<td>-0·6 (-2·7,1·4)</td>
<td>-5·7 (-9·8,1·7)*</td>
<td>-3·5 (-6·1,-1·0)*</td>
<td>2·2 (-0·9,5·3)</td>
</tr>
<tr>
<td>Night-time average aMAP</td>
<td>72·0 ± 5·4</td>
<td>73·5 ± 6·4</td>
<td>75·4 ± 5·6</td>
<td>75·5 ± 7·6</td>
<td>-0·6 (-2·4,1·2)</td>
<td>-2·2 (-5·9,1·5)</td>
<td>-1·4 (-3·7,0·8)</td>
<td>0·8 (-2·2,3·7)</td>
</tr>
<tr>
<td>Overall average aMAP</td>
<td>80·5 ± 7·1</td>
<td>82·3 ± 5·4</td>
<td>84·5 ± 6·1</td>
<td>82·8 ± 6·0</td>
<td>-1·2 (-3·1,0·7)</td>
<td>-4·9 (-8·9,-0·9)*</td>
<td>-3·0 (-5·5,-0·6)*</td>
<td>1·8 (-1·3,4·9)</td>
</tr>
<tr>
<td>Day-time average aHR</td>
<td>79·3 ± 10·6</td>
<td>79·0 ± 10·7</td>
<td>79·0 ± 10·1</td>
<td>72·3 ± 10·4</td>
<td>3·0 (0·2,5·8)*</td>
<td>-4·5 (-9·4,1·4)</td>
<td>1·0 (-2·7,4·6)</td>
<td>5·4 (0·8,10·1)*</td>
</tr>
<tr>
<td>Night-time average aHR</td>
<td>67·2 ± 9·9</td>
<td>65·9 ± 11·3</td>
<td>63·4 ± 13·8</td>
<td>57·9 ± 8·8</td>
<td>2·3 (-0·6,5·1)</td>
<td>-2·2 (-7·8,3·4)</td>
<td>1·1 (-2·3,4·6)</td>
<td>3·4 (-1·0,7·8)</td>
</tr>
<tr>
<td>Overall average aHR</td>
<td>75·1 ± 9·3</td>
<td>74·8 ± 10·3</td>
<td>73·0 ± 9·6</td>
<td>68·3 ± 9·9</td>
<td>2·2 (-0·7,5·0)</td>
<td>-4·5 (-10·5,1·6)</td>
<td>0·4 (-3·4,4·1)</td>
<td>4·8 (0·1,9·5)*</td>
</tr>
<tr>
<td>Day-time average aPP</td>
<td>42·8 ± 5·2</td>
<td>43·4 ± 4·9</td>
<td>54·5 ± 6·1</td>
<td>48·7 ± 6·4</td>
<td>1·5 (-0·3,3·2)</td>
<td>-6·5 (-9·9,3·1)*</td>
<td>-0·6 (-2·7,1·5)</td>
<td>5·9 (3·2,8·6)*</td>
</tr>
<tr>
<td>Night-time average aPP</td>
<td>41·5 ± 5·6</td>
<td>41·7 ± 6·8</td>
<td>50·1 ± 6·9</td>
<td>47·9 ± 7·9</td>
<td>0·0 (-1·9,1·9)</td>
<td>-3·6 (-7·4,0·1)</td>
<td>-0·8 (-3·3,1·5)</td>
<td>2·8 (-0·1,5·8)</td>
</tr>
<tr>
<td>Overall average aPP</td>
<td>42·7 ± 5·1</td>
<td>42·9 ± 4·7</td>
<td>53·0 ± 4·9</td>
<td>47·9 ± 6·5</td>
<td>1·8 (0·2,3·4)*</td>
<td>-6·4 (-9·8,-3·1)*</td>
<td>-0·5 (-2·6,1·5)</td>
<td>5·9 (3·3,8·5)*</td>
</tr>
</tbody>
</table>
Values are mean ± standard deviation; * Participants with missing values are not counted in the sample size; a: ambulatory; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; HR: heart rate; PP: pulse pressure; the measurement unit for blood pressure is mmHg; heart rate is beats per minute. * Significance at 5% level.
Table 4

Blood assays

<table>
<thead>
<tr>
<th>Sample size°</th>
<th>Preterm Women</th>
<th>Term Women</th>
<th>Preterm Men</th>
<th>Term Men</th>
<th>Preterm - Term All</th>
<th>Interaction</th>
<th>Preterm - Term Women</th>
<th>Preterm - Term Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38-44</td>
<td>44-45</td>
<td>22-24</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol)</td>
<td>5.03 ± 0.42</td>
<td>5.04 ± 0.36</td>
<td>5.15 ± 0.30</td>
<td>5.29 ± 0.39</td>
<td>-0.11 (-0.22, 0.00)*</td>
<td>0.00 (-0.22, 0.22)</td>
<td>-0.11 (-0.26, 0.03)</td>
<td>-0.11 (-0.28, 0.05)</td>
</tr>
<tr>
<td>Total insulin (&gt;12mU/L)</td>
<td>1.98 ± 0.55</td>
<td>1.88 ± 0.52</td>
<td>1.72 ± 0.70</td>
<td>1.58 ± 0.46</td>
<td>0.02 (-0.15, 0.18)</td>
<td>-0.11 (-0.45, 0.23)</td>
<td>-0.02 (-0.24, 0.20)</td>
<td>0.09 (-0.17, 0.35)</td>
</tr>
<tr>
<td>Proinsulin (pmol/L)</td>
<td>1.30 ± 0.32</td>
<td>1.36 ± 0.38</td>
<td>1.38 ± 0.51</td>
<td>1.33 ± 0.36</td>
<td>-0.02 (-0.11, 0.07)</td>
<td>-0.03 (-0.22, 0.15)</td>
<td>-0.03 (-0.15, 0.09)</td>
<td>0.00 (-0.14, 0.14)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol)</td>
<td>1.64 ± 0.36</td>
<td>1.67 ± 0.38</td>
<td>1.42 ± 0.34</td>
<td>1.48 ± 0.43</td>
<td>0.02 (-0.10, 0.13)</td>
<td>0.00 (-0.23, 0.23)</td>
<td>0.01 (-0.14, 0.15)</td>
<td>0.01 (-0.17, 0.19)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol)</td>
<td>2.34 ± 0.63</td>
<td>2.59 ± 0.76</td>
<td>2.34 ± 0.96</td>
<td>2.06 ± 0.61</td>
<td>-0.10 (-0.32, 0.13)</td>
<td>-0.43 (-0.89, 0.03)</td>
<td>-0.26 (-0.55, 0.02)</td>
<td>0.17 (-0.18, 0.52)</td>
</tr>
<tr>
<td>Triglycerides (mmol)</td>
<td>0.60 ± 0.17</td>
<td>0.57 ± 0.18</td>
<td>0.64 ± 0.20</td>
<td>0.56 ± 0.19</td>
<td>0.04 (-0.02, 0.10)</td>
<td>0.03 (-0.02, 0.10)</td>
<td>0.03 (-0.15, 0.09)</td>
<td>0.06 (-0.03, 0.15)</td>
</tr>
<tr>
<td>NEFA (µmol/L)</td>
<td>5.85 ± 0.54</td>
<td>5.86 ± 0.56</td>
<td>5.60 ± 0.66</td>
<td>5.45 ± 0.67</td>
<td>0.07 (-0.12, 0.26)</td>
<td>-0.17 (-0.55, 0.22)</td>
<td>-0.02 (-0.26, 0.23)</td>
<td>0.15 (-0.14, 0.45)</td>
</tr>
<tr>
<td>HS CRP (mg/L)</td>
<td>-0.26 ± 1.27</td>
<td>-0.15 ± 1.13</td>
<td>-0.23 ± 1.26</td>
<td>-0.24 ± 1.47</td>
<td>-0.10 (-0.50, 0.29)</td>
<td>-0.52 (-1.31, 0.28)</td>
<td>-0.31 (-0.83, 0.20)</td>
<td>0.21 (-0.40, 0.81)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.36 ± 0.67</td>
<td>4.62 ± 0.91</td>
<td>4.20 ± 0.99</td>
<td>3.90 ± 0.64</td>
<td>-0.08 (-0.32, 0.16)</td>
<td>-0.46 (-0.98, 0.06)</td>
<td>-0.28 (-0.60, 0.05)</td>
<td>0.18 (-0.21, 0.58)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>18.1 ± 12.3</td>
<td>13.2 ± 8.6</td>
<td>2.75 ± 3.36</td>
<td>2.07 ± 2.53</td>
<td>0.28 (0.03, 0.54)*</td>
<td>0.05 (-0.60, 0.50)</td>
<td>0.27 (-0.09, 0.63)</td>
<td>0.23 (-0.19, 0.64)</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>10.4 ± 4.2</td>
<td>10.8 ± 4.0</td>
<td>8.20 ± 3.73</td>
<td>6.86 ± 3.42</td>
<td>-0.08 (-1.26, 1.10)</td>
<td>-1.25 (-3.71, 1.21)</td>
<td>-0.43 (-2.02, 1.17)</td>
<td>0.82 (-1.05, 2.70)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.29 ± 0.24</td>
<td>0.24 ± 0.19</td>
<td>0.35 ± 0.55</td>
<td>0.17 ± 0.22</td>
<td>0.07 (-0.09, 0.23)</td>
<td>0.05 (-0.39, 0.29)</td>
<td>0.08 (-0.14, 0.29)</td>
<td>0.13 (-0.14, 0.39)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.37 ± 3.19</td>
<td>1.71 ± 7.62</td>
<td>1.49 ± 2.66</td>
<td>1.86 ± 5.22</td>
<td>0.09 (-0.17, 0.36)</td>
<td>0.06 (-0.64, 0.77)</td>
<td>0.05 (-0.39, 0.50)</td>
<td>0.01 (-0.55, 0.53)</td>
</tr>
<tr>
<td>TRG/CHOL</td>
<td>0.32 ± 0.14</td>
<td>0.28 ± 0.09</td>
<td>0.30 ± 0.11</td>
<td>0.28 ± 0.10</td>
<td>0.09 (-0.03, 0.21)</td>
<td>0.06 (-0.19, 0.31)</td>
<td>0.11 (-0.05, 0.26)</td>
<td>0.04 (-0.15, 0.23)</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation; ° Participants with missing values are not counted in the sample size; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA: non-esterified fatty acids; HS CRP: high-sensitivity C-reactive protein. * Significance at 5% level