Materials and Methods
The study was approved by the National Research Ethics Service (09/H0505/97). All adult participants in this study were recruited on a voluntary basis by poster advertisement from the Oxfordshire area. The poster wording was explicit that normal “healthy” volunteers were needed with no history of hypertension, diabetes or hypercholesterolaemia, and no existing heart condition. Potential participants were screened briefly on the telephone and then fully assessed in the department for exclusion criteria. All volunteered recruits were Caucasian in ethnic origin. All paediatric patients were recruited from community based paediatric outpatient clinics.

Inclusion Criteria
To limit confounding effects from obesity related co-morbidities that are known to increase aortic stiffness, all adult participants were excluded if; they were taking cardiovascular medication; had a current or past smoking habit, were diabetic (fasting serum glucose >7.0mmol/l), hyperlipidaemic (cholesterol >6.9mmol/l), hypertensive (>140/90 mmHg), or had an abnormal electrocardiograph. Participants with; history or clinical evidence of heart failure, obstructive sleep apnoea, valvular or congenital heart disease, contraindication to MR scanning, previous weight reduction surgery or recent participation in weight loss programmes, were also excluded. Written informed consent was obtained from all participants. Participants were studied after an overnight fast.

Proton Magnetic Resonance Spectroscopy (\textsuperscript{1}H-MRS)
All \textsuperscript{1}H-MR spectra were performed on a 3T MR system (Siemens, Germany) as previously described. (1, 2) A calibration pulse sequence was used to determine the optimum water suppression pulse scaling factor to better characterise the lipid peak at 1.3ppm. Five acquisitions were obtained per breath-hold with ECG-triggering. A TR of 2 seconds allowed for complete relaxation of the lipid 3 signal between successive RF pulses. This required subjects to hold their breath and lie still for 12 - 14 seconds. Six breathholds were taken – 5 with water suppression ‘on’ for lipid data, and one with water suppression ‘off’ to determine the water signal. Spectroscopy parameters were (TE 10ms; mixing time 7ms; 1024 points acquired at a bandwidth of 2000Hz; scan frequency 1.3ppm for water-suppressed spectra and 4.7ppm for water-unsuppressed spectra; TR 2s for water-suppressed data and 4s for water-unsuppressed data). Signals from different coil elements in each breath-hold were combined, and individual spectra phase- and frequency- corrected prior to summation. Spectra were analyzed using in house software (Matlab, AMARES algorithm in JMUI). Liver fat content was is presented as a percentage (signal amplitude of lipid/signal amplitude of water)×100. Example spectra are shown in Figure 1.

Aortic Distensibility
Based on sagittal-oblique pilot images aligned with the aortic arch, aortic cine images were acquired in transverse planes at 3 levels as previously described: (3) the crossing of the pulmonary arch through 1) the ascending thoracic aorta (Ao), 2) descending thoracic aorta (PDA) and 3) 12 cm below the right hemi-diaphragm piloted perpendicular to the orientation
of the abdominal aorta (DDA). A brachial artery blood pressure was recorded during image acquisition to provide pulse pressure. Aortic Distensibility was calculated as (Amax - Amin)/Amin/(Pmax − Pmin), where Amax = maximal (systolic) area (mm2), Amin = minimal (diastolic) area (mm2), Pmax = systolic blood pressure (mm Hg), and Pmin = diastolic blood pressure (mmHg).

**Aortic Pulse Wave Velocity**

To measure aortic PWV, images were acquired using a free-breathing, retrospectively ECG-gated, spoiled gradient echo sequence. Velocity-encoding gradient for phase contrast was applied to measure through-plane flow in the ascending aorta at 2 levels as previously described: the crossing of the pulmonary arch through 1) the ascending thoracic aorta, and 2) 12 cm below the right hemi-diaphragm piloted perpendicular to the orientation of the abdominal aorta. Scan parameters were: effective TR 1 RR-interval, TE 2.8 ms, in-plane resolution 1.3mm, slice thickness 5mm, temporal resolution 10ms. Oblique sagittal images were used to calculate the distance between the two imaging levels as previously described. Flow images were analysed in custom, in house software within Matlab (6) and aortic PWV was determined as \( \frac{\Delta x}{\Delta t} \) (m/s), where \( \Delta x \) is the aortic distance between two imaging levels and \( \Delta t \) is time delay between the arrival of the pulse wave between these imaging levels, as previously described.(3, 7)

**Anthropometric measurements**

Fasting venous blood was drawn for total cholesterol, triglyceride, insulin and glucose. To calculate the homeostasis model assessment for insulin resistance (HOMA-IR) the following formula was used: fasting insulin (mmol/l) x fasting glucose (mmol/l)/22.5 and used as a measure of insulin resistance. Blood pressure was recorded by an automated brachial cuff sphygmomanometer (Model, DINAMAP 1846-SX, Critikon Corp), with the average of 3 supine reading measured over 10 minutes used in analysis. Height and weight were measured using a digital station (Seca, UK) and used to calculate body mass index: as weight (kg)/ height (m)^2.

**Body Composition**

In adults, total body fat content was assessed using dual X-ray absorbitometry (DEXA, GE Lunar system). In all subjects, abdominal visceral fat mass was quantified using a water-suppressed turbo-spin-echo (TSE) transverse axial 5mm slice at the level of the 4th/5th lumbar intervertebral disc (turbo factor 5, echo time TE 12ms, TR 200ms, slice thickness 10mm) was modified so that the sequence served to suppress predominantly the water signal. As a result the images acquired contain practically only the fat signal as previously described. DEXA was not performed in adolescents due to radiation exposure, but subcutaneous fat mass was calculated by contouring the abdominal TSE images (example shown in Figure 1).

**Statistics**

All statistics were analysed using SPSS 22 (SPSS Inc, USA). All data were subjected to Kolmogorov–Smirnov tests to establish normal distribution and are presented as the mean ±
standard deviation. To assess the major determinants of PWV, a stepwise multiple linear regression model was performed. This multivariate model consisted of PWV as the dependent variable and of independent variables that had a significant relation with PWV in the simple linear regression analysis (age, liver fat, total fat, visceral fat, TG, BP and HOMA-IR). Steiger’s Z Statistic was used to compare correlation coefficients (two tailed Z-critical 1.96 for p<0.05, 2.58 for p<0.01)

To further explore the potential for an indirect effect of liver fat on PWV via TG, age and BP adjusted mediator multiple regression was performed according to the method of Preacher and Hayes 2008 (10), with 10,000 sample bootstrapping of indirect effects (dependent variable PWV, independent variable hepatic fat, moderator TG, Figure 2). A probability value of p<0.05 was considered significant. Liver fat was log transformed to achieve a linear relationship with PWV.