Investigating a Liver Fat Arterial Stiffening Pathway in Adult and Childhood Obesity

Oliver J. Rider,* Rajarshi Banerjee,* Jennifer J. Rayner, Ravi Shah, Venkatesh L. Murthy, Matthew D. Robson, Stefan Neubauer

Objective—To investigate the relationship between hepatic fat content, circulating triglyceride levels and aortic stiffness in adult and childhood obesity.

Approach and Results—Seventy-seven adults and 18 children across a wide range of body mass index (18.5–52.6 kg/m² percentile 8–100) with no identifiable cardiac risk factors underwent; 1H- magnetic resonance spectroscopy to quantify hepatic fat content and magnetic resonance imaging to assess aortic pulse wave velocity (PWV) and regional distensibility. In adults, multivariable regression showed age (β=0.09; P=0.02), liver fat (β=2.5; P=0.04), and serum triglyceride (β=0.47; P=0.01) to be independent predictors of PWV. Age and blood pressure–adjusted, moderated regression showed that 43% of the total negative effect of hepatic fat on PWV is attributable to indirect effects via increased triglyceride (P=0.005). In addition, regional distensibility was positively correlated with hepatic fat (ascending; r=−0.35; descending, r=−0.23; abdominal, r=−0.41; all P<0.001). Similar to that seen in adults, PWV (r=0.72; P<0.001) and abdominal regional distensibility (r=−0.52; P<0.001) were correlated with liver fat in children.

Conclusions—Increasing age, liver fat, and triglyceride are all related to increased aortic stiffness in adults. Even when controlling for the effects of age and blood pressure, hepatic fat has a negative effect on PWV, with substantial indirect effect occurring via increased circulating triglyceride level. This relationship between hepatic fat and aortic stiffness occurs early in the obesity process and is also seen in children. As such, hepatic fat content is a potential therapeutic target to treat the elevated vascular risk in obesity. (Arterioscler Thromb Vasc Biol. 2016;36:198-203. DOI: 10.1161/ATVBAHA.115.306561.)

Key Words: liver • magnetic resonance imaging • obesity • pulse wave analysis • triglycerides

Aortic pulse wave velocity (PWV) is a useful, well-validated, clinical measure of central arterial stiffness and has been shown to be predictive of increased mortality, coronary heart disease, and stroke in multiple patient groups.1-3 Obesity, even in the absence of the metabolic syndrome, is associated with increased PWV,4 and changes in adipose levels over time are emerging as an important modifiable determinants of arterial aging, with large epidemiological studies showing 12% of body mass index–related increase in cardiovascular disease being attributable to adipose mediated increases in PWV.5

The mechanisms by which obesity causes vascular stiffness are not fully elucidated, but the fact that obesity is strongly related to non alcoholic fatty liver disease (NAFLD) and that NAFLD is an independent risk factor for arterial stiffness6-8 make it likely that this is an important process by which obesity impairs arterial elastic function. In support of this, in addition to the association with the metabolic syndrome and diabetes mellitus, NAFLD is thought to independently promote coronary vascular disease and predicts greater carotid intima-media thickness,9 as well as impaired endothelial function and lower adiponectin levels.10 Whether a link exists between liver fat deposition and vascular stiffness in children remains unknown, but given the major increase in childhood obesity and childhood NAFLD is certainly worthy of investigation (Figure 1).

One potential pathway through which NAFLD could act to impair arterial function is by increasing circulating triglyceride levels, which is known to impair aortic elastic function.11 Circulating triglyceride levels are dictated not only by dietary intake but also by hepatic triglyceride production, which is elevated in NAFLD. This suggests that NAFLD may be exerting its negative effects on vascular function indirectly via increasing blood triglyceride levels. As a result, we hypothesized that the elevated liver fat levels in obesity would be linked to aortic stiffness, and that effect would, at least in part, be mediated indirectly via increasing serum triglyceride levels. Should
there be a strong liver fat–arterial stiffening pathway, this would be an attractive potential therapeutic target to reduce obesity-related morbidity and cardiovascular mortality.

To investigate this, 77 adults and 18 children underwent magnetic resonance imaging assessment of aortic PWV, regional aortic distensibility (AD), and abdominal visceral fat, IH magnetic resonance spectroscopy assessment of liver fat content, dual-energy x-ray absorptiometry assessment of total fat mass, and fasting blood tests for serum triglyceride levels.

### Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

### Results

A summary of the anthropometric data for the adult and childhood study groups is shown in Table 1.

### Adult Cohort

**Hepatic Fat Content in Adults**

As expected, liver fat level was strongly positively correlated with abdominal visceral fat (r=0.81; P<0.001), total fat mass (r=0.60; P<0.001), and serum triglyceride levels (r=0.60; P<0.001) and also moderately correlated with markers of insulin resistance (glucose mmol/L: r=0.30, P=0.01; insulin mmol/L: r=0.45, P<0.001; and homeostasis model assessment for insulin resistance: r=0.49, P<0.001) and circulating fatty acid levels (r=0.48; P<0.001).

**Aortic Stiffness in Adults**

Aortic PWV was correlated positively with fat mass (r=0.39; P<0.001), liver fat (r=0.66; P<0.001; Figure 3), abdominal visceral fat (r=0.48; P<0.001), systolic blood pressure (r=0.24; P=0.04), age (r=0.40; P<0.001), fasting serum triglyceride (r=0.53; P<0.001), and homeostasis model assessment for insulin resistance (r=0.25; P=0.04; Table 2). Using Steiger Z statistic, the correlation between PWV and liver fat was stronger than that between PWV and visceral fat (Z=2.42; P<0.05), total fat (Z=2.81; P<0.01), and body mass index (Z=3.21; P<0.01; Table 3).

Stepwise multivariable regression of these variables revealed that triglyceride (β=3.6; P=0.009) and liver fat (β=2.5; P=0.008) to be independent predictors of increased PWV in this model (overall R² of the model 0.42; P<0.001; Table 4).

To further explore the relationship between PWV, liver fat, and triglyceride, age and systolic blood pressure–adjusted moderated multiple regression was performed (dependent variable PWV, independent variable hepatic fat, moderator triglyceride; Figure 2; Table 5). In this model, liver fat was positively related to PWV (c pathway; β=2.96; P<0.001), liver fat was positively related to serum triglyceride level (a pathway; β=0.49; P<0.001), and that the mediator triglyceride was positively related to PWV (b pathway; β=2.52; P=0.0045). As the a and b pathways were significant, mediation analysis was tested using 10000 bootstrap resamples to generate a 95% confidence interval (bias corrected) of the indirect effect. This confirmed the mediating role of triglyceride in the relationship between liver fat and PWV (β=0.12; 95% confidence interval, 0.15–3.3). As the direct effect of liver fat remained significant (β=1.7; P=0.03), this suggests partial mediation. Overall, 42% of the total negative effect of liver fat on PWV are attributable to the indirect effects through increasing serum triglyceride in this model (Figure 2; Table 4).

**Figure 1.** Images from (top) a normal weight child with low levels of abdominal visceral, subcutaneous fat, and hepatic triglyceride, with a normal aortic pulse wave velocity (PWV) and (bottom) an obese child with higher levels of abdominal visceral, subcutaneous fat, and hepatic triglyceride, with a higher PWV.
Abdominal distensibility showed a similar pattern, with AD in all areas being negatively correlated with liver fat (ascending thoracic aorta [Ao]: $r = -0.35$; proximal descending thoracic aorta [PDA]: $r = -0.50$; all $P < 0.001$), age (Ao: $r = -0.61$; PDA: $r = -0.55$; DDA: $r = -0.50$; all $P < 0.001$), systolic blood pressure (Ao: $r = -0.50$; PDA: $r = -0.59$; DDA: $r = -0.51$; all $P < 0.001$), serum triglyceride (Ao: $r = -0.29$; PDA: $r = -0.27$; DDA: $r = -0.21$; all $P < 0.05$), and abdominal visceral fat (Ao: $r = -0.38$; PDA: $r = -0.31$; DDA: $r = -0.45$; all $P < 0.006$). Total fat mass was correlated only with abdominal AD ($r = -0.30$; $P = 0.001$), liver fat ($r = 0.77$; $P < 0.001$), with a strong trend toward correlation with systolic blood pressure ($r = 0.46$; $P = 0.055$). Only liver fat ($r = -0.52$; $P < 0.001$) and abdominal visceral fat ($r = -0.60$; $P = 0.001$) correlated with AD, and again only in the DDA.

**Discussion**

NAFLD is the most common cause of chronic liver, and its prevalence is increasing worldwide. NAFLD is considered the hepatic manifestation of metabolic syndrome and is associated commonly with metabolic risk factors, including obesity, insulin resistance, and dyslipidemia. Considering the rising prevalence of obesity and type 2 diabetes mellitus worldwide, NAFLD is becoming a major global health problem. It is now becoming clear that NAFLD is an independent risk factor for arterial stiffness, but the mechanisms by which increased hepatic fat act to impair aortic elastic function remain unclear. We have shown here that aortic stiffness, measured with both PWV and AD, are strongly related to hepatic fat content in adults and, for the first time in children. We have also shown that a substantial proportion of the negative effects of liver fat on PWV are likely to be mediated via increased circulating triglyceride levels, suggesting a significant liver fat–arterial stiffening pathway exists.

**Hepatic Fat Content, Triglycerides, and Aortic Stiffness**

Triglyceride are primarily synthesized in the liver and transferred to adipose tissue for storage. However, once triglyceride homeostasis is disrupted, this eventually results in ectopic fat accumulation in the liver. The amount of liver fat is dictated

### Table 1. Anthropomorphic Data for the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult</strong> (n=77; male n = 40, female n=37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>38.3</td>
<td>11.0</td>
<td>22–61</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7</td>
<td>7.0</td>
<td>18.4–46.8</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118.5</td>
<td>10.9</td>
<td>88–139</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73.4</td>
<td>8.4</td>
<td>55–94</td>
</tr>
<tr>
<td>Abdominal visceral fat, cm²</td>
<td>67.8</td>
<td>49.3</td>
<td>13–187</td>
</tr>
<tr>
<td>Hepatic fat content (% water)</td>
<td>3.0</td>
<td>5.0</td>
<td>0.12–27.3</td>
</tr>
<tr>
<td>Total fat mass, kg</td>
<td>24.9</td>
<td>15.2</td>
<td>7.6–67.6</td>
</tr>
<tr>
<td>Insulin, μU/L</td>
<td>13.4</td>
<td>4.9</td>
<td>3–27.5</td>
</tr>
<tr>
<td>Glucose, mmol/L (n=12)</td>
<td>5.1</td>
<td>0.5</td>
<td>4–6.5</td>
</tr>
<tr>
<td>Cholesterol total, mmol/L</td>
<td>0.3</td>
<td>0.9</td>
<td>0.37–2.37</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>0.9</td>
<td>0.5</td>
<td>0.37–2.37</td>
</tr>
<tr>
<td>Pulse wave velocity, m/s</td>
<td>7.4</td>
<td>3.4</td>
<td>2.8–24.0</td>
</tr>
</tbody>
</table>

**HDL indicates high-density lipoprotein.**

### Table 2. Simple Linear Regression for the Adult and Adolescent Study Groups

<table>
<thead>
<tr>
<th></th>
<th>$r$</th>
<th>$β$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.34</td>
<td>0.17</td>
<td>0.002</td>
</tr>
<tr>
<td>Total fat, kg</td>
<td>0.39</td>
<td>0.09</td>
<td>0.001</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>0.48</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log liver fat, %</td>
<td>0.52</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.4</td>
<td>0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>0.24</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>0.17</td>
<td>0.63</td>
<td>0.15</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>0.19</td>
<td>1.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Insulin, mmol/L</td>
<td>0.21</td>
<td>0.15</td>
<td>0.09</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.25</td>
<td>0.77</td>
<td>0.04</td>
</tr>
</tbody>
</table>

**Children**

<table>
<thead>
<tr>
<th></th>
<th>$r$</th>
<th>$β$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.46</td>
<td>0.09</td>
<td>0.053</td>
</tr>
<tr>
<td>Subcutaneous fat, cm²</td>
<td>0.57</td>
<td>0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>Visceral fat, cm²</td>
<td>0.77</td>
<td>0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log liver fat, %</td>
<td>0.72</td>
<td>1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>0.42</td>
<td>–0.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>0.46</td>
<td>0.05</td>
<td>0.055</td>
</tr>
</tbody>
</table>

$P=0.01$), liver fat ($r=0.72$; $P<0.001$; Figure 3), abdominal visceral fat ($r=0.77$; $P<0.001$), with a strong trend toward correlation with systolic blood pressure ($r=0.46$; $P=0.055$). Only liver fat ($r = -0.52$; $P < 0.001$) and abdominal visceral fat ($r = -0.60$; $P = 0.001$) correlated with AD, and again only in the DDA.

**Hepatic Fat Content, Triglycerides, and Aortic Stiffness**

Triglyceride are primarily synthesized in the liver and transferred to adipose tissue for storage. However, once triglyceride homeostasis is disrupted, this eventually results in ectopic fat accumulation in the liver. The amount of liver fat is dictated
by the balance between input processes (de novo lipogenesis and fatty acid uptake) and output processes (β-oxidation of fatty acid and triglyceride export). Dysregulation of this homeostasis leads to abnormal triglyceride accumulation and increased hepatic triglyceride output. Although we have shown here that hepatic fat content predicts arterial stiffness, a direct negative effect of hepatic fat on aortic elastic function seems unlikely given the lack of spatial colocalization. As a result, we hypothesize that a major component of the observed negative effect is mediated via increased hepatic triglyceride output. This seems plausible given the fact that artificially increasing triglyceride levels not only impairs aortic function through the induction of endothelial dysfunction but also results in elevated PWV and reduced distensibility in a pattern similar to that seen in obesity. The relative contributions of liver triglyceride output and dietary intake of triglyceride to arterial stiffness are less clear, but using moderate multivariable regression, we have shown in this study that ≈43% of the increase in PWV that is attributable to elevated liver fat occurs via indirect effects through elevated triglyceride levels.

**Hepatic Fat Content, Inflammation, and Aortic Stiffness**

Although the remaining indirect effects of elevated liver fat are unmeasured in this study, the link between NAFLD and low-grade inflammation makes it likely that this also underpins some of the observed relationship between liver fat and aortic stiffness. In line with this, C-reactive protein, interleukin-6 and tumor necrosis factor-α levels have all been found to correlate with both NAFLD and aortic stiffness.

**NAFLD and Aortic Stiffness in Children**

Although it is becoming established that NAFLD is predictive of central arterial stiffness in adults, the data in childhood are conflicted. Several studies show increased carotid intima-media thickness in those with liver steatosis, but others have showed that no relation was found with carotid intima-media thickness or arterial wall stiffness. Using magnetic resonance imaging to measure central aortic elastic function, we have shown here that hepatic fat is strongly associated with aortic PWV and with abdominal AD, both suggesting that liver fat plays a role in reducing aortic elastic function in children. The reasons behind the discrepancy in the literature may be that the majority of previous studies have recorded peripheral rather than central arterial elastic function. Another explanation may well lie in the pattern of aortic stiffness observed here in children.

**Pattern of Aortic Stiffness**

In this study, we showed reduced distensibility only in the DDA in children, which confirms previous studies. This suggests that the DDA is the first area to become affected by obesity and could explain why studies of peripheral arterial

<table>
<thead>
<tr>
<th>Table 4. Stepwise Multivariable Regression for Pulse Wave Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Wave Velocity, m/s</td>
</tr>
<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Model 1</td>
</tr>
<tr>
<td>Log liver fat, %</td>
</tr>
<tr>
<td>Model 2</td>
</tr>
<tr>
<td>Log liver fat, %</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
</tr>
</tbody>
</table>

**Table 3. Comparison of Correlations of Different Metrics of Fat With PWV**

<table>
<thead>
<tr>
<th>Metric</th>
<th>PWV, m/s</th>
<th>Z Statistic vs Liver Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver fat</td>
<td>r=0.66</td>
<td>...</td>
</tr>
<tr>
<td>Visceral fat</td>
<td>r=0.49</td>
<td>2.42</td>
</tr>
<tr>
<td>Total fat</td>
<td>r=0.39</td>
<td>2.81</td>
</tr>
<tr>
<td>BMI</td>
<td>r=0.34</td>
<td>3.21</td>
</tr>
</tbody>
</table>

Two-tailed Z-critical 1.96 for P<0.05 and 2.58 for P<0.01. BMI indicates body mass index.

**Figure 2.** The proposed liver fat–arterial stiffening pathway, showing the indirect effect of hepatic fat acting via increasing circulating triglycerides (TG) to influence aortic pulse wave velocity (PWV). The indirect effect of hepatic fat on PWV is shown as a + b, a (hepatic fat to TG pathway and b, TG to PWV pathway), the direct effect is shown as the c pathway. Total effects are depicted as the c pathway. (P<0.05, ***P<0.001).

**Table 5. Model Summary of Adjusted Moderated Multiple Regression Investigating the Indirect Effects of Liver Fat on PWV**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>T</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic fat to TG effect (a pathway)</td>
<td>0.48</td>
<td>0.08</td>
<td>5.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Direct effect of TG on PWV (b pathway)</td>
<td>2.54</td>
<td>0.9</td>
<td>2.98</td>
<td>0.004</td>
</tr>
<tr>
<td>Total effect of liver fat on PWV (c pathway)</td>
<td>2.84</td>
<td>0.64</td>
<td>4.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>Direct effect of liver fat on PWV (c pathway)</td>
<td>1.63</td>
<td>0.74</td>
<td>2.20</td>
<td>0.03</td>
</tr>
<tr>
<td>Partial effect of control variable age on PWV</td>
<td>0.09</td>
<td>0.03</td>
<td>2.98</td>
<td>0.04</td>
</tr>
<tr>
<td>Partial effect of control variable SBP on PWV</td>
<td>−0.01</td>
<td>0.03</td>
<td>−0.43</td>
<td>0.66</td>
</tr>
</tbody>
</table>

 PWV indicates pulse wave velocity; SBP, systolic blood pressure; and TG, triglycerides.
beds have shown conflicting data. The reasons for this pattern are unclear but may be related to an inherent susceptibility of the abdominal section of the aorta to the increases in abdominal visceral and liver fat. Given the fact that the aorta is not a heterogeneous structure, with the elastin:collagen ratio decreasing along its length, and the fact that vascular wall compliance depends on the relative ratios of collagen and elastin, the 2 major wall structural proteins, it is not surprising that the processes in obesity that are causing increased stiffness would have the earliest and greatest impact on the distal aorta, which has been shown in adult obesity studies.

Significance
Aortic stiffness is an independent risk factor for incident hypertension, cardiovascular disease, and stroke, and it is associated with age-related physical and cognitive decline. It has also recently been shown that changes in adipose levels are an important determinant of arterial aging with body mass index–related increased cardiovascular disease attributable to arterial stiffness. Given the fact that we have shown a strong relationship between liver fat, triglyceride, and arterial stiffening, this liver fat–arterial stiffening pathway may be a potential target to reduce morbidity and cardiovascular mortality in obesity. The presence of this relationship in children suggests that this negative pathway exists early in obesity process. Given the fact that liver fat is a modifiable substrate that can be quickly normalized with dietary intervention, this may well become a target to ameliorate, or even prevent, vascular risk in obesity.

Limitations
This study is a relatively small cross-sectional study and does not examine the changes over time related to liver fat, triglyceride, and PWV and does not investigate the effect of changes in liver fat or triglyceride. Insulin resistance was measured using fasting glucose and insulin levels. Although likely to be important, this study did not measure NAFLD-secreted inflammatory biomarkers, only fat content. A larger study is need to confirm these findings and investigate additional mechanisms.

Conclusions
Although obesity is known to be associated with aortic stiffness, the mechanism that underlie this are not well understood. Here, we have shown that, in obesity, it is the distribution of fat deposition that is more important than the absolute volume of excess adipose tissue in determining aortic elastic function. In this study, liver fat content, rather than total fat or abdominal visceral fat, emerges as the most powerful predictor of aortic stiffness. In addition, we have shown that a significant proportion of the negative effects of hepatic fat on arterial stiffness occurs via increasing circulating triglyceride levels, suggesting a liver fat–arterial stiffening pathway. Furthermore, we have shown that aortic stiffness occurs early, in childhood obesity, and is also related to increased hepatic fat. This not only highlights the fact that excess hepatic fat deposition is important in determining vascular function but also implies that hepatic fat is a potential therapeutic target to treat the elevated vascular risk in obesity.

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We acknowledge Professor Bernard Fingleton, CStat, for his assistance and guidance with the statistical methods and analysis in this article.

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Disclosures
None.

References


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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 (1H-MRS)
All 1H-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.\(^{(4)}\) Aortic Distensibility was calculated as \((A_{\text{max}} - A_{\text{min}})/A_{\text{min}}/(P_{\text{max}} - P_{\text{min}}),\) where \(A_{\text{max}} = \) maximal (systolic) area (mm\(^2\)), \(A_{\text{min}} = \) minimal (diastolic) area (mm\(^2\)), \(P_{\text{max}} = \) systolic blood pressure (mm Hg), and \(P_{\text{min}} = \) 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:\(^{(3)}\) 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 1.3 mm, slice thickness 5 mm, temporal resolution 10 ms. Oblique sagittal images were used to calculate the distance between the two imaging levels as previously described.\(^{(5)}\) Flow images were analysed in custom, in house software within Matlab \(^{(6)}\) and aortic PWV was determined as \(\Delta x/\Delta t \text{ (m/s)},\) 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.\(^{(8)}\) 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 5 mm slice at the level of the 4\(^{th}/5\(^{th}\) lumbar intervertebral disc (turbo factor 5, echo time TE 12 ms, TR 200 ms, slice thickness 10 mm) 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.\(^{(9)}\) 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.