Supplementary material

Methods

**Measurement of pulse wave velocity** Pulse wave velocity was measured between the neck and femoral artery (Vicorder®, SMT Medical GmbH&Co., Wurzburg, Germany) as previously described (1).

**Measurement of body composition and intracellular fat** Total and regional adipose tissue volumes were measured after imaging data was analysed using the SliceOmatic image analysis program (Tomovision, Montreal, Quebec, Canada). All spectra were analysed in the time domain using the AMARES algorithm included in the MRUI software package (2). IMCL was expressed as a ratio to the muscle creatine signal. IHCL was expressed as a % ratio to liver water content. Single slice multi-echo was performed through the pancreas during a single breath hold of fifteen seconds. Typical parameters were field-of-view 400*300mm, repetition time 150 ms, 10mm slice thickness; twenty echo times starting at 1.15ms with spacing of 1.15ms producing alternate in and out of phase images. Analysis was performed using MATLAB to produce a measurement of pancreatic fat as previously described (3).

**Laboratory protocols** ApoB from both VLDL\textsubscript{1} and VLDL\textsubscript{2} fractions were selectively precipitated by an equal volume of isopropanol, and extracted with ethanol: diethyl ether (2:1 v/v). The precipitate from the VLDL\textsubscript{1}-apoB fraction was applied to SDS polyacrylamide gel electrophoresis to isolate apoB100. The isolated VLDL\textsubscript{1} and VLDL\textsubscript{2}-apoB were acid hydrolysed (6M HCl) at 120°C for 24h, purified by ion-exchange (Resin AG® 50W-X8 Resin, Cation Form) and derivatised with trifluoroacetic acid and trifluoroacetic anhydride (4). Isotopic enrichment was measured by negative chemical ionization gas chromatography mass spectrometry (GCMS) (Agilent 5975 inert XL MSD) monitoring the ions m/z 209 and 210.

The lipid content of VLDL\textsubscript{1} and VLDL\textsubscript{2} fractions at each time point, was extracted with chloroform:methanol (2:1 v/v), isolated by thin layer chromatography, and hydrolysed in the presence of 3% HCL in methanol (v/v) at 50°C overnight. Glycerol was further purified by ion-exchange chromatography (AG® 50W-X8 Resin, Hydrogen Form and AG1-X8 Resin, Formate Form) and
derivatised to form the triacetate derivative (5). Isotopic enrichment of glycerol was measured by positive chemical ionization GCMS (Agilent 5973 network MSD) monitoring the ions m/z 159 and 164. Plasma glycerol enrichment was determined after deproteinisation of plasma using 3.5% sulphosalicylic acid and purified by ion exchange chromatography before derivatisation (5). To measure plasma α KIC enrichment, plasma was extracted with ethanol and reacted with O-phenylenediamine (2% in 4M HCL) at 90 °C for 1 hour, then extracted with ethyl acetate. The extracted residue was redissolved in acetonitrile and derivatised to form a quinoxalinol-TMS derivative of the keto acid (4). Isotopic enrichment was measured by electron ionization GCMS (Agilent 5973 network MSD) monitoring ions m/z 275 and 276.

Plasma glucose, NEFA, TG, VLDL₁ and VLDL₂-TG and cholesterol and LDL and HDL cholesterol were measured enzymatically using a Cobas MIRA (Roche, Welwyn Garden City, UK). Insulin was measured by immunoassay (Millipore corporation, Billerica, MA, USA). VLDL₁ and VLDL₂-apoB were measured by an in-house ELISA. ALT, AST and GGT were measured by an Advia 1800 Chemistry System (Siemens Healthcare Diagnostics).

**Kinetic modelling of VLDL₁ and VLDL₂-TG**

VLDL₁ and VLDL₂, TG FCR (pools/d) were determined using SAAM II software. Plasma glycerol kinetics was described by a sum of three exponentials representing a three compartment model. The incorporation of glycerol into VLDL by the liver is subject to a delay. A five-compartment chain described time delay due to synthesis and secretion of VLDL₁ and VLDL₂-TG. The model is schematically depicted in Fig. 1.
Figure 1. Schematic description of the model used to describe TTRs of VLDL₁ and VLDL₂-TG.

The model represents the kinetics of the tracer-to-tracee ratio (TTR) profiles which change as labelled glycerol is removed from plasma and incorporated into the TG fractions. The model assumes steady state of native (unlabelled) glycerol throughout the experimental period, i.e. a constant appearance, disappearance, and incorporation of native glycerol into the TG fractions. The model included a compartment for circulating VLDL₁-TG and a compartment for circulating VLDL₂-TG with an input into both compartments from the glycerol precursor pool in liver, a loss from each compartment and a transfer from the VLDL₁-TG compartment to the VLDL₂-TG compartment. Production rate (mg/kg/d) was calculated as the product of VLDL₁ and VLDL₂ FCR and the fraction TG pool sizes. The FCR for VLDL₁-TG transfer to VLDL₂-TG is termed VLDL₁-TG FCR transfer and the FCR for direct loss of VLDL₁-TG is termed VLDL₁-TG catabolism.

The direct input into VLDL₂-TG is termed VLDL₂-TG hepatic production rate and the transfer rate of TG from VLDL₁ to VLDL₂ is termed VLDL₁ to VLDL₂ transfer rate.

Kinetic modelling of apoB

VLDL₁ and VLDL₂, apoB FCR (pools/d) were determined using a model similar to that for VLDL₁ and VLDL₂-TG, shown in Figure 1, using SAAM II software. αKIC was used as the intracellular precursor pool for protein synthesis and the model assumes a steady state concentration of unlabelled αKIC. The incorporation of αKIC into VLDL by the liver is subject to a delay accounting for the amount of time
required for synthesis and production rate of VLDL₁ and VLDL₂-apoB. Similar to the TG model, the
delay compartment consisted of a five-compartment chain. Production rate (mg/kg/d) was calculated as
the product of VLDL₁ and VLDL₂-apoB FCR and their respective apoB pool sizes.

In both models the parameters were estimated using weighted nonlinear regression analysis. The
weights were reciprocal to the variance of the measurement error. The measurement error was assumed
uncorrelated with zero mean; a constant standard deviation of 0.005% below TTR of 0.1% and a
constant coefficient of variation of 5% above TTR of 0.1%.

References

1. Hickson SS, Butlin M, Broad J, Avolio AP, Wilkinson IB, McEniery CM. Validity and
repeatability of the Vicorder apparatus: a comparison with the SphygmoCor device. *Hypertens Res.*

2. Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD. Whole body fat: content


on low-density lipoprotein apolipoprotein B100 kinetics in adult patients with GH deficiency: a stable

triglyceride kinetics and fatty acid kinetics in obese postmenopausal women and obese men. *J Clin
Endocrinol Metab.* 2012;97(7):2475-2481.
**Supplement Table 1 Dietary intake**

<table>
<thead>
<tr>
<th></th>
<th>Pre Ex n=12*</th>
<th>Post Ex n=12*</th>
<th>Within group p value</th>
<th>Pre Control n=10*</th>
<th>Post Control n=10*</th>
<th>Within group p value</th>
<th>Between group p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (MJ/d)</strong></td>
<td>9.9±0.8</td>
<td>10.2±0.7</td>
<td>NS</td>
<td>8.7±0.5</td>
<td>8.2±0.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Protein (g/d)</strong></td>
<td>105±10</td>
<td>105±7</td>
<td>NS</td>
<td>89±8</td>
<td>83±7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Carbohydrate (g/d)</strong></td>
<td>279±23</td>
<td>272±24</td>
<td>NS</td>
<td>208±6</td>
<td>203±11</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sugars (g)</strong></td>
<td>102±13</td>
<td>111±16</td>
<td>NS</td>
<td>76±6</td>
<td>71±8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fat (g/d)</strong></td>
<td>87±9</td>
<td>96±9</td>
<td>NS</td>
<td>82±8</td>
<td>72±8</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Saturated fat (g/d)</strong></td>
<td>33±4</td>
<td>32±4</td>
<td>NS</td>
<td>30±3</td>
<td>25±3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fibre (g/d)</strong></td>
<td>29±6</td>
<td>37±13</td>
<td>NS</td>
<td>17±2</td>
<td>18±1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sodium (g/d)</strong></td>
<td>3.5±0.4</td>
<td>2.9±0.5</td>
<td>NS</td>
<td>2.9±0.3</td>
<td>2.7±0.2</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Three subjects in the exercise groups and two subjects in the control group failed to complete both diet dairies*