Supplement

Abbreviations: AEE, activity energy expenditure; ECG, electrocardiography; FFA, free fatty acids; GCMS, gas chromatography mass spectrometry; HDL, high density lipoprotein; IHCL, intrahepatocellular lipid; IMCL, intramyocellular lipid; MCR metabolic clearance rate; Ox, oxidation rate; Ra, production rate; REE, resting energy expenditure; TCA, tricarboxylic acid; TEE, total energy expenditure; TG, triglyceride; TTR, tracer to tracee ratio; VCO₂ carbon dioxide production; VLDL, very low density lipoprotein.

Methods

Exclusion criteria. Subjects were excluded if they were not currently weight-stable, diagnosed with diabetes or suffering from either cardiovascular or endocrine disease, hepatic and renal disorders, substantial neurological or psychological illness, history of depression and previous surgical procedures for weight loss. Exclusion criteria also included the prescribed use of any medications known to alter body weight or appetite, β-blockers, fibrates and metformin, restrained eating (established with the Dutch Eating Questionnaire) and under-reporting of food intake based on a 4 day food diary.

Run-in period (Figure 1A). Participants were required to remain weight stable (less than 1 kg variation) during the run-in period in order to proceed into the intervention period. At the beginning of the run-in period REE was measured and participants were fitted with an Actiheart monitor to measure activity AEE. AEE was measured over 5 days using an Actiheart monitor (CamNtech Ltd, Cambridge, UK) worn on two standard ECG pads (Pulse Medical Limited, Woking, UK) on the upper chest. The monitors were calibrated before use by each subject using an 8 minute linear ramped step test. TEE was calculated assuming diet induced thermogenesis was 10% of REE and compared to recorded dietary intake to ensure participants were not under-reporting. Four subjects in the treatment group were studied first.
to allow the monitoring of weight loss which set the dietary intervention for the control group. After 6 weeks, 4 subjects randomised to the control group began the dietary intervention. This was then repeated in a block of 3 subjects in each group. Participants kept a continual diet diary throughout the study (including the run-in period) which was analysed using the nutritional analysis programme Windiets Professional 2005 (The Robert Gordon University). They were seen by a dietician every 2 weeks and contacted by telephone between visits. In the control group, the energy prescription was based on the estimate of the energy deficit required to achieve the same weight loss as the rimonabant group. Weight and diet targets were reviewed each week. In the rimonabant group dietary intake was closely monitored to ensure that this was maintained at baseline levels throughout the study. Medication compliance in this group was assessed by tablet counting at each visit. Participants in this group were asked to complete the Beck Depression Inventory II [1] every 2 weeks.

Study protocols 1 and 2
Body weight was measured weekly. Prior to study protocols 1 and 2 subjects were asked not to undertake any vigorous exercise or drink any alcohol for 48h. Subjects were provided with a standardised low fat, low fibre meal the evening before the study day and then fasted overnight. In study protocol 1, REE was measured using a Gas Exchange Monitor (GEM Nutrition Limited, Cheshire, UK), (Figure 1B) followed by a priming dose of 1-\(^{13}\)C sodium bicarbonate (0.085mg/kg) and a 2h infusion of [1,2-\(^{13}\)C]acetate (0.08 µmol.kg\(^{-1}\).min\(^{-1}\)) via an in-dwelling venous cannula. Measurements of \(^{13}\)CO\(_2\) production rate were made to correct palmitate oxidation for the loss of label in the TCA cycle [2]. Immediately after the acetate infusion study a 2 hour intravenous infusion of insulin (40mU/m\(^2\)/min) was given with a variable infusion of 20% dextrose to maintain blood glucose at 5 mmol/l. Study protocol 2
took place three days after the acetate infusion. An intravenous bolus of $[1,1,2,3,3-^{2}\text{H}_5]$ glycerol (75µmol/kg) was administered to measure VLDL$_1$ and VLDL$_2$ TG metabolism (Figure 1C). Blood samples were taken at intervals for 7 hours. A priming dose of $^{13}$C sodium bicarbonate (0.64mg/kg) and constant infusion of [U-$^{13}$C] palmitate bound to human albumin (5%) (0.01 µmol.kg$^{-1}$.min$^{-1}$), was administered for 2 hours to measure palmitate Ra and Ox.

**IHCL and IMCL.** On a separate day, IHCL and IMCL were measured by magnetic resonance spectroscopy at the MRC Clinical Sciences Centre Hammersmith Hospital. Subjects fasted for 6 hours before the scans. Two subjects in the rimonabant group and one subject in the control group were unable to have scans due to metal implants. IHCL and tibialis and soleus IMCL levels were acquired on an Intera 1.5T Achieva multinuclear system (Philips Medical Systems, Best, Holland) as previously reported [3]. IMCL spectra were obtained from the tibialis and soleus muscles. IMCL was expressed as a ratio to the muscle creatine signal. IHCL was expressed as a ratio to liver water content.

**Laboratory methods.** VLDL$_1$ (Sf 60-400) and VLDL$_2$ (Sf 20-60) were separated by sequential ultracentrifugation [4]. VLDL1 and VLDL2 TG were extracted with chloroform-methanol (2:1), isolated by thin layer chromatography, hydrolysed in a solution of 2%HCL in methanol and the liberated glycerol purified by ion exchange chromatography and derivatised to form the tri-acetate derivative [5]. Isotopic enrichment was measured by positive chemical ionisation gas chromatography mass spectrometry (GCMS, Agilent 5973 network MSD) monitoring the ions 159 and 164. FFA were extracted from plasma, and derivatised to their methyl esters. The isotopic enrichment of the palmitic acid methyl ester was measured by GCMS monitoring the ions 270 and 286. CO$_2$ enrichment was measured by isotope ratio MS (Delta XP, coupled with a Gas Bench II (ThermoElectron, Bremen, Germany). Plasma palmitate concentration was measured by GCMS after addition of an internal standard, heptadecanoic acid. Enzymatic assays were used to measure plasma FFA, total cholesterol,
triglyceride, VLDL1 and VLDL2 TG (ABX, Chicksands, Shefford, Bedfordshire, UK),
HDL-cholesterol (HDL-C L-Type, Alpha Labs, Eastleigh, Hampshire, UK) and glycerol
(Randox Laboratories, Antrim, N Ireland) using a Cobas MIRA (Roche, Welwyn Garden
City, UK). Insulin, adiponectin, and leptin, were measured by immunoassay using
commercially available kits (Millipore corporation, Billerica, MA, USA)

Data analysis
Tracer enrichment of palmitate, glycerol and CO$_2$ was expressed as TTR corrected for
baseline enrichment. Palmitate Ra (=Rd) was calculated as infusion rate of tracer/TTR.
Palmitate MCR was calculated by dividing palmitate Rd by palmitate concentration. The
production rate of $^{13}$CO$_2$ ($^{13}$CO$_2$ Ra) from the infused palmitate tracer was calculated as CO$_2$
TTRxVCO$_2$/k.Ac recovery where VCO$_2$ is carbon dioxide production (l/min), k is the volume
of 1 mol of CO$_2$ (22.4 l), and Ac recovery is the fractional $^{13}$C label recovery in breath CO$_2$,
obscured after infusion of labeled acetate [2] and calculated as CO$_2$ TTRxVCO$_2$/k.2F where F
is the infusion rate of [1,2-$^{13}$C]acetate. Plasma palmitate oxidation was calculated as palmitate
Rd x ($^{13}$CO$_2$ Ra/Fx16) where F is the palmitate infusion rate and 16 is the number of carbon
atoms in palmitate. VLDL$_1$ and VLDL$_2$ TG secretion rate and fractional catabolic rate were
calculated using a model of VLDL$_1$ and VLDL$_2$ TG kinetics [6], which included a
compartment for tracer recycling [7], using the SAAM II program (SAAM Institute, Seattle,
WA). The dextrose infusion rate corrected for insulin concentration between 150-180 minutes
of the clamp gave a measure of insulin sensitivity [8].
References


**Table 1. Body composition and energy expenditure (mean±SEM)**

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group</th>
<th>Control Group</th>
<th>Δ rimonabant vs Δ control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>58.1±1.9</td>
<td>57.4±1.9</td>
<td>-</td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
<td>89.0±2.2</td>
<td>84.2±3.0</td>
<td>81.1±3.1†</td>
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<tr>
<td><strong>BMI</strong></td>
<td>32.9±0.7</td>
<td>33.0±0.8</td>
<td>31.8±0.7†</td>
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<td><strong>Waist (cm)</strong></td>
<td>108±3</td>
<td>107±5</td>
<td>103±4</td>
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<tr>
<td><strong>Fat mass (kg)</strong></td>
<td>40.2±1.4</td>
<td>36.9±2.2</td>
<td>34.2±2.8†</td>
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<tr>
<td><strong>IHCL (%)</strong></td>
<td>12.3±6.4</td>
<td>19.7±11.2</td>
<td>8.7±4.4</td>
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<tr>
<td><strong>SIMCL</strong></td>
<td>10.4±2.1</td>
<td>11.1±2.2</td>
<td>9.6±2.1</td>
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<tr>
<td><strong>TIMCL</strong></td>
<td>6.2±1.0</td>
<td>8.1±0.9</td>
<td>11.0±2.0</td>
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<tr>
<td><strong>Beck Depression</strong></td>
<td>1.86±0.46</td>
<td>_</td>
<td>_</td>
</tr>
</tbody>
</table>

IHCL, intrahepatocellular lipid; SIMCL, soleus intramyocellular lipid; TIMCL, tibialis intramyocellular lipid. Significantly different from 0 weeks, †p< 0.05. * p< 0.005, ‡p=0.055.
Table 2. Food intake during the 4 week run-in period and the intervention period (mean±SEM)

<table>
<thead>
<tr>
<th></th>
<th>Rimonabant Group</th>
<th>Control Group</th>
<th>∆ rimonabant vs ∆ control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run-In</td>
<td>Intervention</td>
<td>Run-In</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>87±5</td>
<td>84±3</td>
<td>82±3</td>
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<td>SFA (g)</td>
<td>31±2</td>
<td>29±1</td>
<td>28±2</td>
</tr>
<tr>
<td>PUFA (g)</td>
<td>14±1</td>
<td>13±1</td>
<td>15±1</td>
</tr>
<tr>
<td>MUFA (g)</td>
<td>28±3</td>
<td>26±2</td>
<td>24±1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>215±21</td>
<td>213±16</td>
<td>215±10</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>78±2</td>
<td>81±4</td>
<td>78±4</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; significantly different from run-in period, †p<0.02, * p=0.001.
Figure legends

Figure 1. A) Study design, B) Study protocol 1, and C) Study protocol 2

Figure 2. Energy intake during the 4 week run-in and 12 week intervention period in the rimonabant (solid diamonds) and control group (open squares).