1 **Supplement**

2 **Abbreviations:** AEE, activity energy expenditure; ECG, electrocardiography; FFA, free fatty
3 acids; GCMS, gas chromatography mass spectrometry; HDL, high density lipoprotein; IHCL,
4 intrahepatocellular lipid; IMCL, intramyocellular lipid; MCR metabolic clearance rate; Ox,
5 oxidation rate; Ra, production rate; REE, resting energy expenditure; TCA, tricarboxylic
6 acid; TEE, total energy expenditure; TG, triglyceride; TTR, tracer to tracee ratio; VCO_2
7 carbon dioxide production; VLDL, very low density lipoprotein.

8 **Methods**

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

16

17 *Run-in period (Figure 1A).* Participants were required to remain weight stable (less than 1 kg
18 variation) during the run-in period in order to proceed into the intervention period. At the
19 beginning of the run-in period REE was measured and participants were fitted with an
20 Actiheart monitor to measure activity AEE. AEE was measured over 5 days using an
21 Actiheart monitor (CamNtech Ltd, Cambridge, UK) worn on two standard ECG pads (Pulse
22 Medical Limited, Woking, UK) on the upper chest. The monitors were calibrated before use
23 by each subject using an 8 minute linear ramped step test. TEE was calculated assuming diet
24 induced thermogenesis was 10% of REE and compared to recorded dietary intake to ensure
25 participants were not under-reporting. Four subjects in the treatment group were studied first

26 to allow the monitoring of weight loss which set the dietary intervention for the control
27 group. After 6 weeks, 4 subjects randomised to the control group began the dietary
28 intervention. This was then repeated in a block of 3 subjects in each group. Participants kept a
29 continual diet diary throughout the study (including the run-in period) which was analysed
30 using the nutritional analysis programme Windiets Professional 2005 (The Robert Gordon
31 University). They were seen by a dietician every 2 weeks and contacted by telephone
32 between visits. In the control group, the energy prescription was based on the estimate of the
33 energy deficit required to achieve the same weight loss as the rimonabant group. Weight and
34 diet targets were reviewed each week. In the rimonabant group dietary intake was closely
35 monitored to ensure that this was maintained at baseline levels throughout the study.
36 Medication compliance in this group was assessed by tablet counting at each visit.
37 Participants in this group were asked to complete the Beck Depression Inventory II [1] every
38 2 weeks.
39
40 *Study protocols 1 and 2*
41 Body weight was measured weekly. Prior to study protocols 1 and 2 subjects were asked not
42 to undertake any vigorous exercise or drink any alcohol for 48h. Subjects were provided with
43 a standardised low fat, low fibre meal the evening before the study day and then fasted
44 overnight. In study protocol 1, REE was measured using a Gas Exchange Monitor (GEM
45 Nutrition Limited, Cheshire, UK), (Figure 1B) followed by a priming dose of 1-^{13}C sodium
46 bicarbonate (0.085mg/kg) and a 2h infusion of [1,2-^{13}C]acetate (0.08 μmol.kg^{-1}.min^{-1}) via an
47 in-dwelling venous cannula. Measurements of $^{13}CO_2$ production rate were made to correct
48 palmitate oxidation for the loss of label in the TCA cycle [2]. Immediately after the acetate
49 infusion study a 2 hour intravenous infusion of insulin (40mU/m^2/min) was given with a
50 variable infusion of 20% dextrose to maintain blood glucose at 5 mmol/l. Study protocol 2

51 took place three days after the acetate infusion. An intravenous bolus of [1,1,2,3,3-$^{2}H_5$]

52 glycerol (75µmol/kg) was administered to measure $VLDL_1$ and $VLDL_2$ TG metabolism

53 (Figure 1C). Blood samples were taken at intervals for 7 hours. A priming dose of ^{13}C sodium

54 bicarbonate (0.64mg/kg) and constant infusion of [U-^{13}C] palmitate bound to human albumin

55 (5%) (0.01 µmol.kg^{-1}.min^{-1}), was administered for 2 hours to measure palmitate Ra and Ox.

56 *IHCL and IMCL.* On a separate day, IHCL and IMCL were measured by magnetic resonance

57 spectroscopy at the MRC Clinical Sciences Centre Hammersmith Hospital. Subjects fasted

58 for 6 hours before the scans. Two subjects in the rimonabant group and one subject in the

59 control group were unable to have scans due to metal implants. IHCL and tibialis and soleus

60 IMCL levels were acquired on an Intera 1.5T Achieva multinuclear system (Philips Medical

61 Systems, Best, Holland) as previously reported [3]. IMCL spectra were obtained from the

62 tibialis and soleus muscles. IMCL was expressed as a ratio to the muscle creatine signal.

63 IHCL was expressed as a ratio to liver water content.

64 *Laboratory methods.* $VLDL_1$ (Sf 60-400) and $VLDL_2$ (Sf 20-60) were separated by

65 sequential ultracentrifugation [4]. VLDL1 and VLDL2 TG were extracted with chloroform-

66 methanol (2:1), isolated by thin layer chromatography, hydrolysed in a solution of 2%HCL in

67 methanol and the liberated glycerol purified by ion exchange chromatography and derivatised

68 to form the tri-acetate derivative [5]. Isotopic enrichment was measured by positive chemical

69 ionisation gas chromatography mass spectrometry (GCMS, Agilent 5973 network MSD)

70 monitoring the ions 159 and 164. FFA were extracted from plasma, and derivatised to their

71 methyl esters. The isotopic enrichment of the palmitic acid methyl ester was measured by

72 GCMS monitoring the ions 270 and 286. CO_2 enrichment was measured by isotope ratio MS

73 (Delta XP, coupled with a Gas Bench II (ThermoElectron, Bremen, Germany). Plasma

74 palmitate concentration was measured by GCMS after addition of an internal standard,

75 heptadecanoic acid. Enzymatic assays were used to measure plasma FFA, total cholesterol,

76 triglyceride, VLDL1 and VLDL2 TG (ABX, Chicksands, Shefford, Bedfordshire, UK),
77 HDL-cholesterol (HDL-C L-Type, Alpha Labs, Eastleigh, Hampshire, UK) and glycerol
78 (Randox Laboratories, Antrim, N Ireland) using a Cobas MIRA (Roche, Welwyn Garden
79 City, UK). Insulin, adiponectin, and leptin, were measured by immunoassay using
80 commercially available kits (Millipore corporation, Billerica, MA, USA)
81
82
83 *Data analysis*
84 Tracer enrichment of palmitate, glycerol and CO_2 was expressed as TTR corrected for
85 baseline enrichment. Palmitate Ra (=Rd) was calculated as infusion rate of tracer/TTR.
86 Palmitate MCR was calculated by dividing palmitate Rd by palmitate concentration. The
87 production rate of $^{13}CO_2$ ($^{13}CO_2$ Ra) from the infused palmitate tracer was calculated as CO_2
88 TTRxVCO_2/*k*.Ac recovery where VCO_2 is carbon dioxide production (l/min), *k* is the volume
89 of 1 mol of CO_2 (22.4 l), and Ac recovery is the fractional ^{13}C label recovery in breath CO_2,
90 observed after infusion of labeled acetate [2] and calculated as CO_2 TTRxVCO_2/k.2F where F
91 is the infusion rate of [1,2-^{13}C]acetate. Plasma palmitate oxidation was calculated as palmitate
92 Rd x ($^{13}CO_2$ Ra/Fx16) where F is the palmitate infusion rate and 16 is the number of carbon
93 atoms in palmitate. $VLDL_1$ and $VLDL_2$ TG secretion rate and fractional catabolic rate were
94 calculated using a model of $VLDL_1$ and $VLDL_2$ TG kinetics [6], which included a
95 compartment for tracer recycling [7], using the SAAM II program (SAAM Institute, Seattle,
96 WA). The dextrose infusion rate corrected for insulin concentration between 150-180 minutes
97 of the clamp gave a measure of insulin sensitivity [8].
98

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100 **References**

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131

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

	Rimonabant Group		Control Group		Δ rimonabant vs Δ control
	Week 0	Week 12	Week 0	Week 12	
Age (years)	58.1±1.9	-	57.4±1.9	-	
Body weight (kg)	89.0±2.2	86.4±2.1*	84.2±3.0	81.1±3.1†	NS
BMI	32.9±0.7	31.9±0.7*	33.0±0.8	31.8±0.7†	NS
Waist (cm)	108±3	105±2†	107±5	103±4	NS
Fat mass (kg)	40.2±1.4	39.2±1.4†	36.9±2.2	34.2±2.8†	NS
IHCL (%)	12.3±6.4	14.9±8.0	19.7±11.2	8.7±4.4	NS
SIMCL	10.4± 2.1	11.9± 2.6	11.1± 2.2	9.6± 2.1	NS
TIMCL	6.2±1.0	6.8±1.2	8.1±0.9	11.0±2.0	NS
Beck Depression Score	1.86±0.46	3.43±1.02	_	_	_

133 IHCL, intrahepatocellular lipid; SIMCL, soleus intramyocellular lipid; TIMCL, tibialis

134 intramyocellular lipid. Significantly different from 0 weeks, †$p < 0.05$. * $p < 0.005$, ‡ $p = 0.055$.

135

Table 2. Food intake during the 4 week run-in period and the intervention period (mean±SEM)

	Rimonabant Group		**Control Group**		Δ rimonabant vs Δ control
	Run-In	Intervention	Run-In	Intervention	
Fat (g)	87±5	84±3	82±3	61±4†	P=0.001
SFA (g)	31±2	29±1	28±2	21±1†	P=0.046
PUFA (g)	14±1	13±1	15±1	12±1	NS
MUFA (g)	28±3	26±2	24±1	19±1†	NS
Carbohydrate (g)	215±21	213±16	215±10	185±10	P=0.03
Protein (g)	78±2	81±4	78±4	73±2	NS

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

143

Figure legends

145

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

147

148 Figure 2. Energy intake during the 4 week run-in and 12 week intervention period in the

149 rimonabant (solid diamonds) and control group (open squares).

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