SUPPLEMENTARY MATERIAL

Supplementary Table. Body composition and biochemical parameters in a sub group of patients with severe insulin resistance due to loss-of-function *INSR* mutations.

	P. 1	P. 2	P. 3	P. 4	P. 5	P.6	P. 7	Reference values
Mutation	*INSR P1236A*	*INSR P1236A*	*INSR A1135E*	*INSR A1135E*	*INSR A1121P*	*INSR A1121P*	*INSR M1138K*	
Age (years)	15	40	20	48	20	32	18	
Gender	Female	Female	Female	Male	Female	Female	Female	
BMI (kg/m^2)	20.5	24.3	22.0	24.6	27.5	26.5	17.3	
Weight (kg)	52.0	61.0	53.8	56.0	77.5	70.8	45.2	
Fat mass (kg)	15.5	22.0	20.7	14.5	40.8	31.0	14.0	
Fat free mass (kg)	33.4	35.9	29.9	38.1	35.2	38.2	28.5	
Glucose (mmol/L)	4.5	5.7	3.8	4.9	4.3	5.7	4.0	<6.1
Insulin (pmol/L)	711	163	636	154	378	160	732	0-60
HBA1C (%)	6.7	7.1	5.0	5.9	5.6	5.9	6.6	4.9-6.3
Adiponectin (mg/L)	13.1	8.1	9.8	2.4	11.6	2.8	25.8	*
Leptin (µg/L)	21.1	15.0	22.6	3.0	ND	23.9	10.9	**
Cholesterol (mmol/L)	3.9	4.4	4.7	6.6	6.4	5.4	6.1	5.33-5.91
Triglycerides (mmol/L)	0.7	0.7	1.1	1.6	1.0	0.8	1.1	1.15-1.63
HDL-cholesterol (mmol/L)	1.8	1.5	1.7	1.1	2.4	2.1	2.2	1.37-1.71
IMCL/Cr-Soleus	ND	ND	4.9	7.8	ND	16.2 †	6.2	***

*Adiponectin sex and BMI-adjusted reference ranges 4.5-17.7 (Patients 1-3, 7), 3.5-15.5 (Patients 5,6), 2.6-12.6 (Patient 4); **Leptin sex and BMI-adjusted reference ranges 2.4-24.4 (Patients 1-3, 7), 8.6-38.9 (Patients 5,6), 0.4-8.3 (Patient 4); ***IMCL/Cr Soleus reference mean±SD 6.33±3.7 (n=20, age and BMI matched lean healthy controls). † This value should be treated with caution due to considerable extramyocellular lipid that caused a severe overlap with the IMCL resonance.

P., Patient; BMI, body mass index; HBA1C, glycated haemoglobin; HDL, high density lipoprotein; IMCL, intramyocellular lipid; ND, not determined.

Supplementary Methods

Experimental protocol

Participants were requested to refrain from strenuous exercise for at least 24 hours prior to the study and to avoid caffeinated drinks and nicotine during their research stay. Participants were admitted to the WTCRF on day 1, and, after consent and medical examination, body composition was measured using dual-energy X-ray absorptiometry (DXA) (GE Lunar, Madison, WI, USA, software version 12.2). The evening meal was of standard macronutrient composition, formulated to maintain energy balance and was provided at identical times for all participants. Sleeping metabolic rate (SMR) was measured during the overnight period from 00:00 to 07:00, in a chamber calorimeter. Instrumentation and calibration procedures have been described previously (1). O_2 consumption and CO_2 production were calculated by the method of Brown et al (2). Basal metabolic rate (BMR) was measured using a ventilated canopy indirect calorimeter (GEM, GEMNutrition, Daresbury, UK). Subject measurements were recorded every 30s over a 20 minute interval starting at 07:30 on day 2. The composition of room air was measured before and after and results corrected for any change over the interval. During BMR measurement, participants were asked to remain awake, but relaxed and motionless. Tympanic temperature was recorded after SMR and BMR measurements to rule out the possibility of subclinical pyrexia in participants. After this, volunteers provided a fasting blood sample at 08:00 before being transferred by wheelchair to the Wolfson Brain Imaging Centre for the magnetic resonance scan.

In order to calibrate individual heart rate responses to exercise and to derive an estimate of physical fitness (VO_2 max), all participants undertook an 8 minute standardised graded step test with a 2 minute recovery period (3).

Magnetic Resonance Studies

Measures of ATP synthesis rates at rest (using the saturation transfer technique) and post-exercise (PCr recovery rate) were acquired using ^{31}P magnetic resonance spectroscopy (MRS) on a 3T Siemens MAGNETOM Verio scanner.

Saturation Transfer (ST) measurement

A 12 cm diameter RAPID surface coil was placed under the calf muscle. The steady-state inorganic phosphate (Pi) magnetisation was measured in the presence of selective saturation of the γ-ATP resonance, and compared with a control (irradiation frequency symmetrical to the Pi peak), with a repetition time (TR) =25s and 48 averages each (Figure 1A). The intrinsic longitudinal relaxation time (T_1) of Pi under conditions of γ-ATP resonance saturation was measured (7 inversion times between 9-9000ms and an additional reference with inversion flip angle = 0, 16 averages and an effective TR =15s). A fully relaxed spectrum (16 averages) was used for measurements of metabolite concentrations, with [ATP] assumed to be 8.2 mM (4). The concentration of ADP was calculated using established methods (5), with the assumption that the total creatine pool (Cr + PCr) is 42.5mM.

PCr recovery kinetics post exercise

The volunteers were placed supine and a 9 cm diameter surface coil attached to their right quadriceps (1/3 distal). A weight was attached to their right ankle corresponding to 30 % of their maximal voluntary contraction, which was determined the previous day using a dynamometer chair set to the same angles of exercise as in the scanner. The exercise paradigm consisted of 1 min rest, 1 min knee extensions (0.5 Hz), then 4 min rest. The measurement was then repeated to enable two PCr recovery half time

measurements, which were then averaged. The TR = 2 s, receiver bandwidth = 5 kHz, and 360 complex spectra were acquired. The resting and depleted concentrations of PCr (and ADP) were similar between groups, and the pH decrease was minimal (<0.1) in all studies. The PCr recovery half time, $t_{1/2}$, was found using a 2 parameter monoexponential fit. The $t_{1/2}$ corrected for fitness ($t_{1/2}$ corrected) was then calculated assuming a linear relationship (6) between the rate constant and VO_2 max, and compensating individual $t_{1/2}$ values accordingly. All spectra were analysed in jMRUI (7) and fitted using the AMARES (8) algorithm with prior knowledge.

References

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