



Mitochondrial dysfunction in patients with primary congenital insulin resistance

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Mitochondrial dysfunction is associated with insulin resistance and type 2 diabetes. It has thus been suggested that primary and/or genetic abnormalities in mitochondrial function may lead to accumulation of toxic lipid species in muscle and elsewhere, impairing insulin action on glucose metabolism. Alternatively, however, defects in insulin signaling may be primary events that result in mitochondrial dysfunction, or there may be a bidirectional relationship between these phenomena. To investigate this, we examined mitochondrial function in patients with genetic defects in insulin receptor (*INSR*) signaling. We found that phosphocreatine recovery after exercise, a measure of skeletal muscle mitochondrial function in vivo, was significantly slowed in patients with *INSR* mutations compared with that in healthy age-, fitness-, and BMI-matched controls. These findings suggest that defective insulin signaling may promote mitochondrial dysfunction. Furthermore, consistent with previous studies of mouse models of mitochondrial dysfunction, basal and sleeping metabolic rates were both significantly increased in genetically insulin-resistant patients, perhaps because mitochondrial dysfunction necessitates increased nutrient oxidation in order to maintain cellular energy levels.

Introduction

Insulin resistance underpins the tight association between the type 2 diabetes and obesity pandemics. Yet despite enormous scientific endeavor, understanding of the molecular pathogenesis of insulin resistance remains incomplete. One of the most characteristic and consistent metabolic features of the obese insulin-resistant state is lipid accumulation in sites other than white adipose tissue, so-called ectopic fat (1). Triglyceride accumulation in the liver and skeletal muscle is strongly associated with insulin resistance in these tissues, and while triglyceride is not itself thought to cause insulin resistance, more reactive lipid species, such as diacylglycerol and ceramide, are believed to impair insulin action (1).

Ectopic fat accumulation presumably reflects a cellular mismatch between the sum of lipid delivery and synthesis and the sum of lipid oxidation and disposal. A combination of recent human and rodent studies have convincingly documented mitochondrial abnormalities in insulin-resistant and diabetic states, highlighting the potential importance of impaired mitochondrial fat oxidation in the accumulation of ectopic fat and giving rise to the suggestion that mitochondrial dysfunction may be the primary defect in prevalent obesity-related insulin resistance (reviewed in ref. 2). These studies include ex vivo morphological and biochemical analyses of tissue samples and/

or isolated mitochondria (3–6) as well as noninvasive in vivo magnetic resonance spectroscopy (MRS) measures (7–13). However, although the associations appear robust, the direction of causality remains uncertain (2, 14).

Where human genetic variants are identified that directly produce 1 out of 2 associated phenomena, investigating whether those same genetic variants are also associated with the second phenomenon offers a powerful means of investigating causality in the association. Such Mendelian randomization is now increasingly used in large human epidemiological genetic studies (15); however, the principle is also applicable to rare monogenic disorders. A classic early example of this was the association observed between rare mutations in the *LDLR* gene, which produce high LDL cholesterol, and premature atherosclerotic disease (16), establishing that high levels of LDL cholesterol are causally linked to atherosclerosis.

Studying patients with mitochondrial genetic defects producing primary mitochondrial dysfunction seems at first sight to offer the potential to conduct a similar Mendelian randomization experiment to test whether mitochondrial dysfunction in humans can produce insulin resistance, but this is likely to be confounded by deleterious effects of generalized mitochondrial dysfunction on β cell and muscle function (17, 18). However, patients with congenital, severe insulin resistance due to mutations in the insulin receptor (*INSR*) gene are well described and, though uncommon, afford instead the reciprocal opportunity to test whether primary insulin resistance can produce mitochondrial dysfunction, potentially accounting for some but not necessarily all of their observed association in commoner forms of insulin resistance.

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**Table 1**Characteristics of the healthy volunteers and patients with severe insulin resistance due to loss-of-function *INSR* mutations

	Controls	<i>INSR</i>	<i>P</i> value
Age (yr)	27.3 ± 1.5	28.1 ± 4.8	0.889
Sex	3 M, 9 F	1 M, 6 F	0.605
Weight (kg)	71.1 ± 4.2	58.7 ± 4.5	0.072
BMI (kg/m ²)	24.2 ± 1.1	22.9 ± 1.4	0.489
Fat mass (kg)	21.2 ± 2.8	22.6 ± 3.8	0.776
FFM (kg)	48.9 ± 3.0	36.0 ± 1.4	0.002
Glucose (mmol/l)	4.43 ± 0.08	4.70 ± 0.29	0.407
Insulin (pmol/l)	34 ± 5	419 ± 102	0.009
Adiponectin (mg/l)	5.1 ± 0.6	10.5 ± 3.0	0.121
Leptin (μg/l)	12.5 ± 3.3	16.1 ± 3.3	0.482
Triglyceride (mmol/l)	0.93 ± 0.13	1.00 ± 0.12	0.739
HDL cholesterol (mmol/l)	1.46 ± 0.13	1.83 ± 0.17	0.108
NEFA (μmol/l)	238 ± 29	375 ± 61	0.033
ST			
ST V _{ATP} (mM/min)	11.1 ± 0.5	10.3 ± 0.5	0.393
[ADP] (μM)	21.2 ± 2.3	28.7 ± 1.5	0.063
T ₁ ' (s)	4.07 ± 0.07	4.33 ± 0.04	0.004
[Pi] (mM)	4.23 ± 0.21	4.05 ± 0.12	0.475
k (min ⁻¹)	2.73 ± 0.10	2.56 ± 0.18	0.391
PCr recovery			
Exercise weight (kg)	3.0 ± 0.2	2.1 ± 0.2	0.011
t _{1/2} (s)	16.6 ± 1.0	25.5 ± 2.5	0.001
Predicted VO ₂ max (ml/kg of FFM/min)	56.0 ± 1.9	51.3 ± 5.1	0.320
t _{1/2} corrected for VO ₂ max (s)	17.8 ± 1.5	25.2 ± 3.0	0.024
Resting [PCr] (mM)	30.7 ± 0.5	31.6 ± 0.8	0.356
End exercise [PCr] (mM)	24.0 ± 0.9	24.9 ± 0.9	0.520
Resting [ADP] (μM)	20.2 ± 1.5	18.1 ± 1.8	0.407
End exercise [ADP] (μM)	46.7 ± 4.4	41.9 ± 3.4	0.464
Resting pH	7.015 ± 0.010	7.020 ± 0.006	0.721
End exercise pH	7.071 ± 0.005	7.072 ± 0.009	0.939

Healthy volunteers are referred to as controls, and patients with severe insulin resistance due to loss-of-function *INSR* mutations are referred to as *INSR*. Bold *P* values are statistically significant. Data presented are mean ± SEM. M, male; F, female; FFM, fat-free mass; NEFA, nonesterified fatty acid; [ADP], concentration of adenosine diphosphate; T₁', apparent longitudinal relaxation time of Pi in the presence of saturation of the γ-ATP resonance; [Pi], concentration of inorganic phosphate; k, exchange rate constant.

Results and Discussion

Characteristics of the participants. Seven patients from 4 unrelated families with dominant-negative heterozygous missense mutations (P1236A, A1135E, M1138K, and A1121P) affecting highly conserved residues within the tyrosine kinase domain of the insulin receptor were selected for mitochondrial function studies. Such mutations are well known to produce severe insulin resistance with an autosomal dominant pattern of inheritance (19). All patients had acanthosis nigricans, a cutaneous marker of severe insulin resistance, and the typical biochemical profile of patients with primary insulin receptor defects ("insulin receptoropathies"), including severe hyperinsulinemia and normal triglyceride and HDL cholesterol levels, usually with preserved or elevated serum adiponectin levels (Supplemental Table 1; supplemental material available online with this article; doi:10.1172/JCI46405DS1). Glycated hemoglobin levels were normal in 4 *INSR* participants and only minimally elevated in the other 3 (Supplemental Table 1). Intramyocellular lipid levels were similar to those of matched controls

in 3 out of 4 *INSR* mutation carriers (Supplemental Table 1), which is in keeping with the similarly normal hepatic fat levels previously documented in this disorder (20).

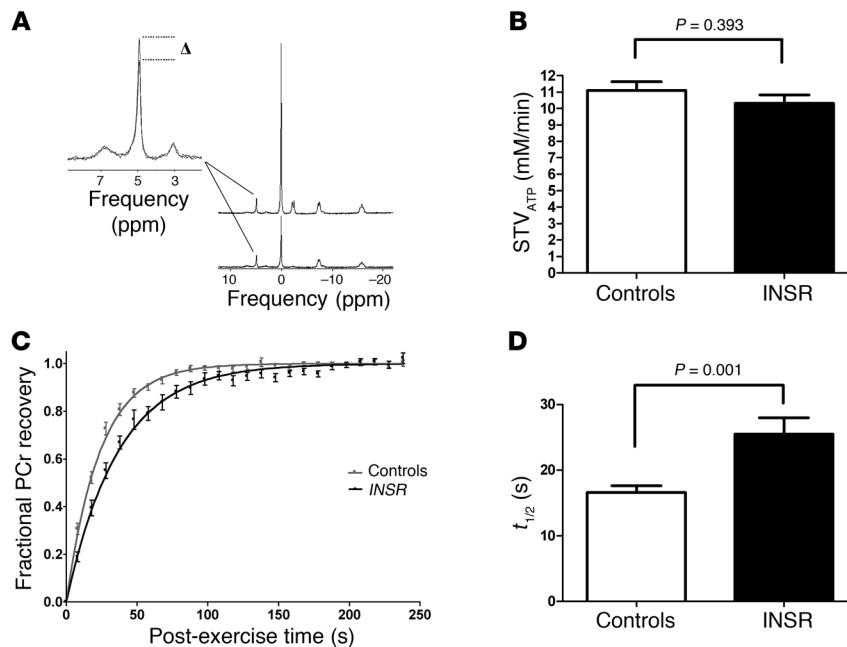
As well as being age-, gender-, and BMI-matched (Table 1), control volunteers (*n* = 12) were deliberately selected for being sedentary, as none of the patients with *INSR* mutations undertook regular exercise. Fasting biochemical parameters were all within normal limits in the healthy controls (Table 1). All patients and controls wore an Actiheart monitor (CamNtech) and underwent a standard graded exercise calibration test, from which we derived an estimate of maximal oxygen consumption (VO₂ max), measured in ml/kg of fat-free mass/min. Results from these estimates were similar in the 2 groups (Table 1).

Assessment of oxidative phosphorylation function in vivo. The theory of measuring chemical exchange rates using nuclear magnetic resonance magnetization transfer techniques was first introduced by Forsen and Hoffman in 1963 (21). Its application to measure ATP synthesis rates in muscle in vivo has been widely used despite concerns that the measurement will contain a nonoxidative, glycolytic component that could be as large as 80% at rest and that resting ATP turnover may not be the most relevant measure of mitochondrial function (22–27). An alternative approach is to assess the kinetics of replenishment of the phosphocreatine (PCr) pool after exercise, which relies purely on oxidative ATP synthesis (28). This is most conveniently quantified as the PCr recovery half time (t_{1/2}), defined as the time taken for PCr to recover by half the amount it was depleted, which is inversely proportional to functional "mitochondrial capacity." A recent study in which rats were treated with the mitochondrial complex 1 inhibitor diphenyleneiodonium highlighted reservations about measuring ATP turnover using magnetization (saturation) transfer techniques by documenting a significant slowing of PCr recovery but no change in the rate of ATP synthesis determined from saturation transfer

(ST) measurements (26). Given these concerns, we determined both resting ST and postexercise PCr recovery in this study.

All participants completed the MRS scans, apart from 2 *INSR* subjects who did not undertake the ST measurement due to claustrophobia. The rate of ATP synthesis measured with the ST technique (ST V_{ATP}) was similar in both groups (Figure 1, A and B, and Table 1), whereas the rate of PCr recovery after exercise was significantly slower in patients with severe insulin resistance due to *INSR* mutations than that in healthy controls (Figure 1, C and D, and Table 1). This difference remained significant after correcting for minor differences in VO₂ max (Table 1). There was no correlation between ST V_{ATP} and the postexercise PCr recovery half time (t_{1/2}) (Spearman's rho, *r* = -0.235, *P* = 0.363).

The significantly slowed rate of PCr recovery after exercise in the *INSR* patients shows that insulin resistance due to a well-defined primary defect in insulin signaling is associated with evidence of mitochondrial dysfunction in vivo. This suggests that the association between mitochondrial dysfunction and insulin resistance

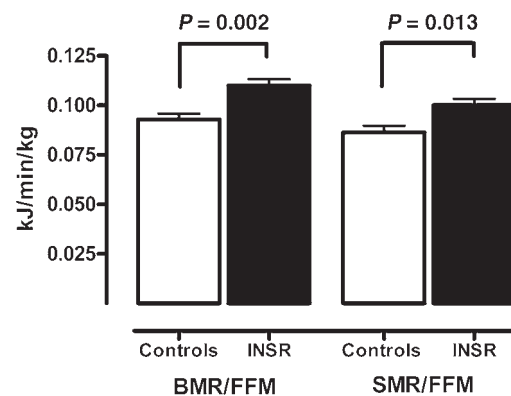
**Figure 1**

³¹P MRS measurements of mitochondrial function. (A) Representative ST spectra, with saturation of the γ -ATP resonance (right, bottom) and corresponding control spectrum (right, top). The 2 spectra are superimposed (left) to show the difference (Δ) in the Pi resonance. (B) ST V_{ATP} in both the controls (white bars; $n = 12$) and in patients with *INSR* mutations (black bars; $n = 5$). (C) Mean fractional PCr recovery curves for controls (gray squares; $n = 12$) and for patients with *INSR* mutations (black circles; $n = 7$). Five spectra were averaged to give a time resolution of 10 seconds for clarity in this figure. The monoexponential fit of the mean recovery rate constant is shown for controls (gray line) and *INSR* patients (black line). (D) Half time for PCr recovery ($t_{1/2}$) as measured from the recovery rate after exercise for both controls (white bars; $n = 12$) and for patients with *INSR* mutations (black bars; $n = 7$). In B–D, data are mean \pm SEM.

reported in prevalent forms of insulin resistance of unknown etiology cannot be assumed to imply that mitochondrial dysfunction causes insulin resistance. Our data are consistent with the mitochondrial dysfunction reported in insulin-deficient patients with type 1 diabetes (29) and several recent murine studies reporting mitochondrial dysfunction in mice with either primary genetic defects in the insulin signaling cascade (30–32) or high-fat feeding-induced insulin resistance, in which the insulin resistance was shown to precede mitochondrial dysfunction (33).

Metabolic rate measurements. Changes in body weight reflect a mismatch between energy intake and energy expenditure. Although human genetic studies increasingly suggest that changes in energy intake are the primary driver of weight changes in most cases in humans, changes in metabolic rate can also lead to weight gain or weight loss. For example, thyrotoxicosis promotes mitochondrial uncoupling and ultimately weight loss (34). As well as being a major determinant of activity-associated energy expenditure, skeletal muscle is also a significant contributor (~20%–30%) to resting energy expenditure (35). In order to determine whether insulin resistance and/or mitochondrial dysfunction might alter metabolic rate, we evaluated basal metabolic rate (BMR) and sleeping metabolic rate (SMR) in our patients with *INSR* mutations and in the healthy controls. Surprisingly, we found that both the BMR and SMR were significantly increased in patients with *INSR* mutations when corrected for fat-free mass (Figure 2). While both these independent measures of metabolic rate reflect whole body rather than just skeletal muscle energy metabolism, they suggest that reduced mitochondrial function need not lead to a reduction in resting energy expenditure.

These human observations contradict the intuitive expectation that defects in mitochondrial energy metabolism will lead to reduced energy expenditure. Nevertheless, the data are consistent with observations in mice with primary defects in mitochondrial function, in which the resultant increase in cellular AMP levels activate AMP kinase, producing a lean insulin-sensitive phenotype (36). The primary determinant of the rate of oxidative phosphorylation is thought to be the homeostatic need

**Figure 2**

Energy expenditure in patients with loss-of-function *INSR* mutations. BMR and SMR in healthy controls (white bars; $n = 11$) and in patients with *INSR* mutations (black bars; $n = 7$). Results are expressed per kilogram of fat-free mass (FFM). Data are mean \pm SEM.



to defend the cellular energy charge, so one of a number of possible explanations for these observations is that mitochondrial dysfunction necessitates increased nutrient oxidation in order to maintain cellular energy levels.

Germline dominant-negative defects in the insulin receptor gene may confidently be assumed to produce congenital, severe insulin resistance, and co-inheritance of a primary mitochondrial defect in all the unrelated patients studied is vanishingly unlikely. Thus the association of genetic defects in insulin receptor function with impaired oxidative phosphorylation *in vivo* may be taken to establish that primary insulin resistance can produce secondary mitochondrial dysfunction. Such an observation in one group with a rare monogenic form of insulin resistance is not necessarily directly transposable to the situation in prevalent obesity-related insulin resistance, which most likely encompasses a heterogeneous group of postreceptor defects. Nevertheless there is currently no evidence that our findings are not generalizable to prevalent insulin resistance, and, even viewed most conservatively, they demonstrate that the association between prevalent insulin resistance and mitochondrial dysfunction must not be assumed to be solely accounted for by a unidirectional effect of primary mitochondrial dysfunction on insulin sensitivity.

Methods

Participants. Each participant provided written informed consent, and all studies were conducted in accordance with the principles of the Declaration of Helsinki. Clinical studies were approved by the National Health Service Research Ethics Committee, United Kingdom, and were conducted in the WTCRF.

Patients with severe insulin resistance due to loss-of-function mutations in the *INSR* gene were identified as part of a long-standing program of research into genetic and acquired forms of severe insulin resistance. Patients with features of Donohue syndrome or Rabson-Mendenhall syndrome were not included, as these patients tend to manifest severe

metabolic disturbances, including poorly controlled hyperglycemia, a known cause of mitochondrial dysfunction (37). Instead we recruited patients with normal or near-normal glycated hemoglobin (HbA1C) levels. Healthy age-, gender-, and BMI-matched control volunteers were recruited by advertisement. All were sedentary non-smokers without medical disorders likely to affect energy metabolism and without a family history of diabetes.

Experimental protocol and magnetic resonance studies. See the Supplemental Methods for details regarding these studies.

Biochemical assays. Insulin, leptin, and adiponectin were measured as previously described (20).

Statistics. All statistics were performed in SPSS PASW Statistics 18 (SPSS Inc.). Quantitative data are presented as mean \pm SEM. The 2-tailed independent-sample *t* test was used to compare means between groups, with significance classed as $P < 0.05$.

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