Inflexibility of Energy Substrate Oxidation in Type 1 Diabetic Patients

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Obese, insulin-resistant patients have been shown to have metabolic inflexibility. The goal of this study was to examine the effect of insulin administration on energy metabolism in lean, type 1 diabetic (DM1) patients. Eleven DM1 patients without vascular complications and 11 healthy controls (C) were examined. We performed a 2-step hyperinsulinemic euglycemic clamp (240 minutes; period 1: 1 mU · kg^{-1} · min^{-1} and period 2: 10 mU · kg^{-1} · min^{-1}) combined with indirect calorimetry during basal period B (B, −45 to 0 minutes), period 1, and period 2 of the clamp. The metabolic clearance rates of glucose (MCR) were lower in DM1 compared with C in period 1 (12.54 ± 3.38 *v* 17.41 ± 6.18 mL · kg^{-1} · min^{-1}; *P* < .02), as well as in period 2 (21.63 ± 6.47 *v* 26.61 ± 4.45 mL · kg^{-1} · min^{-1}; *P* < .05). Basal respiratory quotient (RQ) was lower in DM1 compared with C (0.72 ± 0.04 *v* 0.75 ± 0.04; *P* < .03). Insulin administration was accompanied by an increase in RQ in both groups, which was lower in DM1 compared with C (period 1: +0.09 ± 0.04 *v* +0.11 ± 0.07; *P* < .001; period 2: +0.13 ± 0.04 *v* +0.16 ± 0.04; *P* < .001). Glucose oxidation did not differ between the groups in period B; however, it was lower in DM1 compared with C in periods 1 (1.17 ± 0.67 *v* 3.28 ± 1.11 mg · kg^{-1} · min^{-1}; *P* < .003); and 2 (2.10 ± 0.64 *v* 3.28 ± 0.93 mg · kg^{-1} · min^{-1}; *P* < .009). Lipid oxidation was higher in DM1 in all periods compared with C; period B (3.28 ± 0.77 *v* 1.16 ± 0.55 mg · kg^{-1} · min^{-1}; *P* < .001), period 1 (1.10 ± 0.41 *v* 0.67 ± 0.54 mg · kg^{-1} · min^{-1}; *P* < .05), and period 2 (0.99 ± 0.29 *v* 0.52 ± 0.58 mg · kg^{-1} · min^{-1}; *P* < .01). The groups did not differ in protein oxidation. In conclusion, DM1 patients with secondary insulin resistance (IR) are characterized by metabolic inflexibility manifesting itself by smaller increases in RQ and glucose oxidation after insulin administration during the euglycemic clamp.

INSULIN RESISTANCE (IR) is considered to be a basic abnormality underlying type 2 diabetes mellitus.[1] Although IR is not a universal feature of type 1 diabetes mellitus (DM1), the secondary defect of insulin action has been described in both poorly and adequately controlled DM1 patients, as well as in DM1 patients with daily insulin requirements (35 ± 2 U/d), which would not clinically characterize them as being insulin resistant.[2-5] IR is characterized as a limited response of muscle to stimulate glucose metabolism. Both glucose oxidation and nonoxidative pathways are impaired in type 2 diabetes and also in DM1.[2,4,6]

In lean healthy individuals, skeletal muscle displays substantial metabolic flexibility, with an ability to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to suppression of lipid oxidation and increased glucose uptake, oxidation, and storage under insulin-stimulated conditions.[7-10] A striking physiologic characteristic of skeletal muscle is its metabolic flexibility expressed by a high capacity to modulate rates of energy production, blood flow, and substrate utilization.[8-10]

Obese subjects showed lower lipid oxidation under fasting conditions and greater lipid oxidation under insulin-stimulated conditions relative to the lean volunteers, but the absolute rates of lipid oxidation remained fixed in obese subjects and type 2 diabetes.[8-10] The failure to augment lipid oxidation during fasting conditions seems to be the key mechanism leading to lipid accumulation within skeletal muscle, whereas increased lipid stress that accumulates in muscle may, in turn, contribute to insulin-resistant glucose metabolism through substrate competition and other mechanisms.[8] This metabolic inflexibility of skeletal muscle is a clinically important feature of IR. Insulin-deficient rat models of diabetes, as well as subjects with poorly controlled DM1 patients, exhibit elevated rates of lipolysis, increased plasma free fatty acids (FFAs) and triglyceride concentrations, elevated blood ketone bodies, and decreased respiratory quotients (RQ).[8-10] While it is generally accepted that the rates of lipid oxidation are increased in obesity during insulin-stimulated conditions and, indeed, impaired suppression of lipid oxidation is a prominent manifestation of IR, much less is known about a potential link between fasting patterns of muscle FFA utilization and IR. Another important consideration is whether potential impairments within the pathways of fatty acid utilization in skeletal muscle in obesity are primary defects or arise after an individual has become obese.[8-10] Randle et al[11,12] originally proposed that the glucose/fatty acids fuel competition cycle might be responsible for IR in diabetes. Recent results suggest that the glucose/fatty acids cycle does not fully explain the interaction between glucose and fatty acids in insulin-resistant subjects.[10,11,13] Recent data indicate that lipid accumulation in skeletal muscle or in various tissues is probably more responsible for inhibiting insulin action.[14,15] Although DM1 patients express some degree of IR, there are no data dealing with metabolic flexibility in these patients. The aim of this study was to examine the effect of insulin administration on substrate metabolism in lean DM1 patients as compared with healthy subjects during a 2-step hyperinsulinemic euglycemic clamp combined with indirect calorimetry.

SUBJECTS AND METHODS

Subjects

The study groups consisted of 11 men with DM1 without specific diabetic vascular complications and 11 male healthy control subjects (C) without a family history of diabetes mellitus, dyslipidemia, and

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Table 1. Characteristics of Study Groups

	C (n = 11)	DM (n = 11)
Age (yr)	28.72 ± 2.10	32.18 ± 5.72
BMI (kg/m^2)	24.09 ± 1.0	24.36 ± 2.15
Triglycerides (mmol · L^{-1})	1.11 ± 0.76	0.77 ± 0.40
Total cholesterol (mmol · L^{-1})	4.11 ± 0.88	4.23 ± 0.59
HDL-cholesterol (mmol · L^{-1})	1.12 ± 0.42	1.67 ± 0.29
HBA_{1c} (%)	4.82 ± 0.27	7.97 ± 1.34*
Daily insulin dose (IU/d)	—	36 ± 9.8

*$P < .001$.

other metabolic diseases. All subjects gave their informed consent with the study protocol, which had been reviewed and approved by the local ethics committee. Patients were instructed to adhere to their ordinary lifestyle and avoid changes in food intake, alcohol consumption, and exercise. Characteristics of the study groups are shown in Table 1. All subjects were examined during a 3-day hospitalization while on a standard diet regimen. Dietary intakes in our study groups were: 150 to 170 mmol/24 hr of sodium, 50 to 80 mmol/24 hr of potassium, 80 g/24 hr of proteins, 275 to 325 g/24 hr of carbohydrates and daily energy intake 2,500 to 2,800 kcal/24 hr. A 2-step hyperinsulinemic euglycemic clamp combined with indirect calorimetry was performed on day 3 after hospital admission.

Procedures

Hyperinsulinemic euglycemic clamp. All studies were performed after an 8- to 10-hour overnight fast. The 2-step hyperinsulinemic euglycemic clamp, lasting 4 hours (period 1: 0 to 120 minutes and period 2: 120 to 240 minutes) was conducted as previously described.[16] Briefly, a Teflon cannula (Venflon, Viggo Helsingborg, Sweden) was inserted into left antecubital vein for infusion of all test substances. A second cannula was inserted in the retrograde fashion into a wrist vein of the same hand for blood sampling, and a hand was placed in a heated (65°C) box to achieve venous blood arterialization. A stepwise primed-continuous infusion (1 and 10 mU · kg^{-1} · min^{-1} of Actrapid HM; Novonordisk, Copenhagen, Denmark) was administered to acutely raise and maintain the plasma concentration of insulin at 75 and 1,400 μU/mL. Decreases in serum potassium concentrations during insulin infusion were prevented by coinfusion of potassium chloride with glucose (60 mmol/L KCl/L of 15% glucose). Plasma glucose concentrations during the clamp were maintained at 5 mmol · L^{-1} by continuous infusion of 15% glucose. Arterialized blood plasma glucose concentration were determined every 5 to 10 minutes. Before the clamp, only diabetics with fasting plasma glucose levels below 6 mmol/L were included into the study, and no glucose was infused until plasma glucose had decreased to the desired level.

Indirect calorimetry. Substrate utilization and energy expenditure (EE) measurements were made in either group by indirect calorimetry.[17] Gas exchange measurements were taken during a 45-minute basal period and during the final 45-minute periods of the 2 insulin-infusion steps. A transparent plastic ventilated hood was placed over the subject's head and made airtight around the neck. A slight negative pressure was maintained in the hood to avoid loss of expired air. A constant fraction of air flowing out of the hood was automatically collected for analysis. Air flow and O_2 and CO_2 concentrations in expired and inspired air were measured by a continuous open-circuit system (metabolic monitor VMAX; Sensor Medics, Anaheim, CA).

Urinary collections. Evaluation of urinary urea nitrogen excretion was made to calculate protein oxidation. Urinary collections during the clamp were divided into the basal period (−120 to 0 minute) and into period 1 (0 to 120 minutes) and period 2 (20 to 240 minutes). At times 0 minute and 120 minutes and at 240 minutes, blood urea nitrogen was measured.

Analytical Methods

Plasma glucose concentrations were measured on a Beckman analyzer (Beckman Instruments, Fullerton, CA) using the glucose oxidase method. Immunoreactive insulin (IRI) was determined by radioimmunoassay using an IMMUNOTECH Insulin IRMA kit (IMMUNOTECH, Prague, Czech Republic). Single measurement of glycosylated hemoglobin using the Bio-Rad heamoglobin A_{1c} column test (Bio-Rad Laboratories, Munich, Germany) was performed before the testing.

Data Analysis

Hepatic glucose production (HGP) was not measured in this study, but it is known to decrease by more than 90% at insulin levels >50 μU/mL in healthy men.[16,18] Thus, the total amount of glucose infused was a measure of the glucose metabolized by all cells of the body during clamp studies. Calculations of substrate oxidation were made using standard equations.[17] Urinary urea excretion was assessed in urine samples before and after correction for changes in urea pool size.[19] Insulin action was estimated as the metabolic clearance rate of glucose (MCR) and glucose disposal (M) calculated at minutes 80 to 120 minutes (MCR_{glu} submax and M_{glu}submax) and between 200 to 240 minutes (MCR_{glu}max and M_{glu}max). MCR was calculated by dividing the amount of glucose infused, after correction for changes in glucose pool size, by mean plasma glucose concentration.[16] Nonoxidative glucose disposal ($NEOX_{glu}$submax and $NEOX_{glu}$max) was calculated by subtracting the rate of glucose oxidation from M. Data were statistically analyzed by Mann-Whitney rank test. All data are expressed as means ± SD.

RESULTS

MCR_{glu} submax in DM1 were lower than in C in period 1 ($P < .02$), as well as MCR_{glu} max in period 2 ($P < .05$) (Fig 1). M_{glu} submax during the first period of the clamp was not statistically different, but M_{glu}max was lower in DM1 compared with C in period 2 of the clamp ($P < .01$) (Fig 2). Nonoxidative glucose disposal during the period 1 was comparable in DM1 and C (DM1: 9.87 ± 2.76 *v* C: 11.06 ± 2.3 mg · kg^{-1} · min^{-1}), while during the second period of the clamp, it was lower in DM1 (16.93 ± 5.29 *v* 19.87 ± 2.62 mg · kg^{-1} · min^{-1}; $P < .02$) (Fig 2).

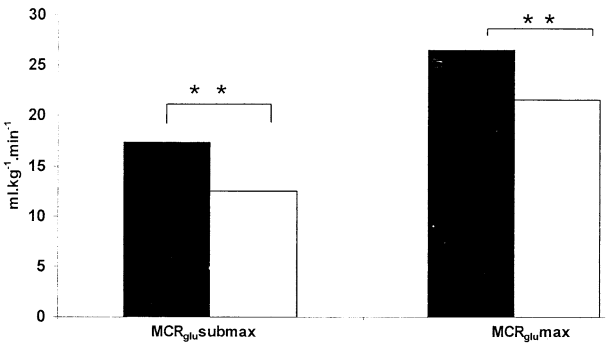

Fig 1. MCR_{glu}submax and MCR_{glu}max during the hyperinsulinemic euglycemic clamp in C (■, n = 11) and DM1 (□, n = 11). *$P < .01$.

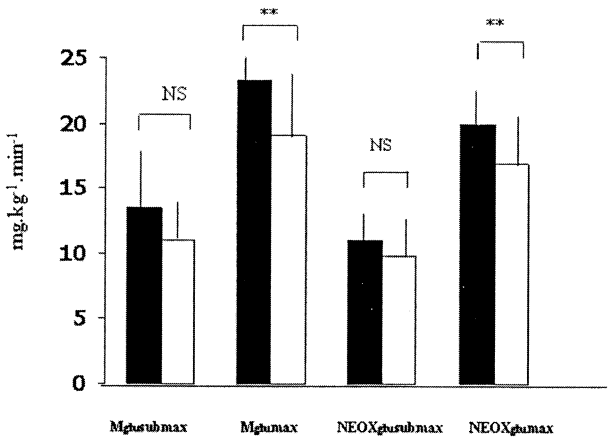

Fig 2. Glucose disposals (Mglusubmax and Mglumax) and nonoxidative glucose disposals (NEOXglusubmax and NEOXglumax) during the hyperinulinemic euglycemic clamp in C (■, n = 11) and DM1 (□, n = 11). *$P < .01$.

Basal RQ was lower in DM1 compared with C (0.72 ± 0.04 *v* 0.75 ± 0.04; $P < .03$) (Fig 3). Insulin administration was accompanied by RQ increase in both groups, which was lower in DM1 compared with C (period 1: +0.09 ± 0.04 *v* +0.11 ± 0.07; $P < .001$; period 2: +0.13 ± 0.04 *v* +0.16 ± 0.04; $P < .001$). Glucose oxidation (Fig 4A) did not differ between the groups in period B, however, it was lower in DM1 compared with C in period 1 (1.17 ± 0.67 *v* 3.28 ± 1.11 mg · kg^{-1} · min^{-1}; $P < .003$); and period 2 (2.10 ± 0.64 *v* 3.28 ± 0.93 mg · kg^{-1} · min^{-1}; $P < .009$). Overall, lipid oxidation (Fig 4B) in DM1 was significantly higher in period B (3.28 ± 0.77 *v* 1.16 ± 0.55 mg · kg^{-1} · min^{-1}; $P < .0001$), period 1 (1.10 ± 0.41 *v* 0.67 ± 0.54 mg · kg^{-1} · min^{-1}; $P < .05$) and period 2 (0.99 ± 0.29 *v* 0.52 ± 0.58 mg · kg^{-1} · min^{-1}; $P < .01$) compared with C. C and DM1 did not differ in protein oxidation (basal: 0.70 ± 0.19 *v* 0.97 ± 0.68, period 1: 0.42 ± 0.27 *v* 0.69 ± 0.39 and period 2: 0.37 ± 0.18 *v* 0.45 ± 0.32 mg · kg^{-1} · min^{-1}).

DISCUSSION

The results of this study indicate altered substrate utilization in DM1. To the best of our knowledge, this is the first report showing metabolic inflexibility in DM1 patients.

In accordance with other investigators, we have confirmed the presence of IR in DM1 patients.[2-4] The metabolic clearance rates of glucose (MCR) were significantly lower in DM1 in both periods of the clamp. Glucose disposal rates were also lower in period 2, but, in period 1, this difference was not statistically significant, as well as nonoxidative glucose disposal in period 1.

We did not measure HGP. The rate of insulin infusion (1 mU · kg^{-1} · min^{-1}) used during period 1 of the clamp study has been shown to suppress, almost completely, HGP in healthy subjects, but insufficient suppression cannot be excluded in DM1 patients. Thus, the lack of significant differences in glucose disposal and nonoxidative glucose disposal in period 1 could be due to insufficient suppression of HGP in DM1 patients.[18] It has been known for years that patients with type 1 diabetes are insulin resistant, and this has been attributed to the phenomenon of glucotoxicity rather than a factor intrinsic to the muscle. Thus, part of the glucose or IR in these patients may be caused by hyperglycemia per se.[20] Furthermore, literary data have shown that higher daily insulin (more 25 to 55 μU/mL) requirements were associated with high circulating insulin antibody titers, which could be a cause of IR in DM1. Insulin antibody titers have not been measured in our study, because we assumed that the daily doses of insulin we used were not associated with increased antibody titers.[2,20-23]

In our study, the rate of basal (fasting) lipid oxidation was higher in DM1 compared with C and even higher under hyperinsulinemic euglycemic clamp conditions, just as RQ, a marker of substrate utilization, was lower during basal and hyperinsulinemic euglycemic periods. The suppression of lipid oxidation by insulin was higher in period 1 and persisted over period 2 of the clamp in DM1, but the absolute rate of lipid oxidation was higher as compared with C. These data indicate lipids were the predominant fuel under basal (fasting) conditions in both groups; however, during period 2 of the clamp, the rate of lipid oxidation remained higher in DM1 as compared with the basal period in C.

The concept that pertubated skeletal muscle fatty acid metabolism may contribute to skeletal muscle IR is not new, although the hypothesis that IR is associated with decreased fasting fatty acid oxidation is a novel reformulation, but the classic Randle glucose-fatty acid cycle is only one of a number of mechanisms by which fatty acids might influence muscle glucose metabolism and insulin action.[7,8,11-13,24]

In the presence of insulin stimulation, fatty acid utilization by skeletal muscle is normally suppressed.[25] Results obtained with rodents or humans, which more directly examined muscle fuel selection have shown that skeletal muscle in IR is accompanied by increased, rather than decreased, muscle glucose oxidation under basal conditions and decreased glucose oxidation under insulin-stimulated circumstances, producing a state of "metabolic inflexibility".[9,10] The situation in DM1 appears to be different. However, this cannot be determined from the

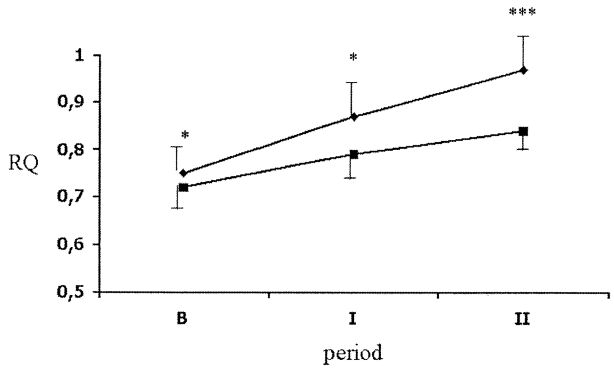

Fig 3. Changes in RQ during the hyperinsulinemic euglycemic clamp in C (◆, n = 11) and DM1 (■, n = 11). *$P < .05$, ***$P < .001$.

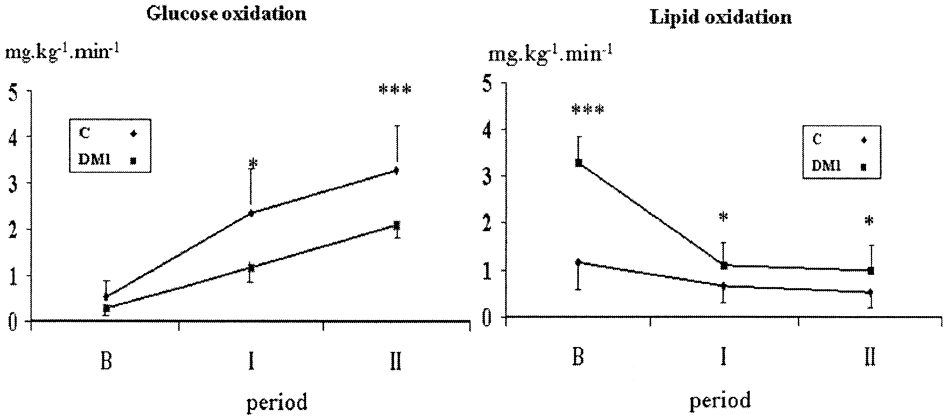

Fig 4. Changes in glucose and lipid oxidation during the hyperinsulinemic euglycemic clamp in DM1 (■, n = 11) and C (◆, n = 11). *P < .05, ***P < .001.

present study because systemic indirect calorimetry does not contain much information about fuel use in muscle, which only contributes about 15% to 20% of systemic gas exchange under the basal, resting condition.

Increased glucose oxidation negatively correlates with insulin sensitivity under fasting conditions.[9,25] Under insulin-stimulated conditions in insulin-resistant subjects, this inflexibility is characterized by a lower rate of elevation of glucose oxidation and a higher rate of lipid oxidation.

These investigators also reported that the inflexibility, which includes impaired suppression of lipid oxidation under insulin-stimulated conditions, is a component of the IR phenotype of skeletal muscle.[9,10]

This metabolic flexibility is the key to the major role that skeletal muscle may play in overall fuel balance. In obesity and type 2 diabetes, there is a loss of this plasticity and, instead, there is metabolic inflexibility. Rates of lipid oxidation are not suppressed effectively in response to insulin, but neither do rates of lipid oxidation effectively increase during transition to fasting conditions. An important morphologic characteristic of skeletal muscle in obesity and type 2 diabetes is an increased content of triglycerides, which can be associated with IR.[9,10] DM1 compared with control subjects had lower insulin-stimulated whole body total, oxidative, and nonoxidative glucose disposal rates. They also exhibited higher levels of lipid oxidation both during basal and hyperinsulinemia conditions. Although DM1 was associated with muscle triglyceride content, muscle triglyceride was not associated with insulin sensitivity.[26]

The higher levels of plasma triglycerides are associated with reduced insulin sensitivity, and such a situation could contribute to triglyceride accumulation within the myocyte, as has been observed in IR and type 2 diabetes.[9,10,27] Recent knowledge of insulin receptor signaling indicates that the accumulation of lipid products in muscle may interfere with insulin signaling and produce IR.[9] This observation was in contradiction with the Randle glucose fatty acids cycle, although this cycle is a valid physiologic principle, but it may not fully explain IR in skeletal muscle in type 2 diabetes. Furthermore, there are no studies in DM1.[7,8,11,12] The data of Simonenau et al[29,30] suggest that, in obesity-related IR, the metabolic capacity of skeletal muscle appears to be organized towards fat esterification rather than oxidation.

Moreover, metabolic inflexibility is important in obese male subjects with impaired glucose tolerance (IGT). These patients have the same defect in fatty acid utilization as subjects with type 2 diabetes, suggesting that these disturbances may play an important role in the progression from IGT to type 2 diabetes.[29] The key aspect of metabolic fitness in skeletal muscle is its capacity to switch between fuels, and that this capacity may be lost in IR in obesity and type 2 diabetes; however, some studies has shown that weight loss does not correct this disposition.[26] Although there is now evidence that weight loss through reduction of caloric intake and increase in physical activity can prevent the development of diabetes, it remains an open question as to whether specific modulation of lipid metabolism will result in improvement in some, or all, of the above metabolic derangements or will prevent progression from IR to type 2 diabetes.[30]

Unless there is a significant weight loss, short- or medium-term dietary manipulation does not alter insulin sensitivity as much in humans as in rodent models, and there is a considerable interest in pharmacologic intervention. Studies using thiazolidinediones, peroxisome proliferators-activated receptorγ (PPARγ) receptor agonists, have supported the concept that reduced muscle lipid accumulation is associated with an increased insulin sensitivity. Other potent systemic lipid-lowering agents, such as PPARα receptor agonists (eg, fibrates) or antilipolytic agents (eg, nicotinic acid analogs) might improve insulin sensitivity, but further work is needed, particularly to clarify implications for muscle metabolism.[24,31,32]

Recent experimental data has shown that treatment with novel PPARα/γ agonists is highly effective in correcting the extreme metabolic inflexibility and impaired insulin action in the Zucker rat.[33] There are no data about the effect of PPARα/γ in human studies, but a possible positive effect should be expected in DM1 with high doses of insulin and type 2 diabetics as well. These interactions are fundamental to understanding the metabolic effects of new insulin "sensitizers," eg, thiazolidinediones, which alter lipid metabolism and improve muscle insulin sensitivity in IR states in both types of diabetes.

In conclusion, DM1 with secondary IR are characterized by metabolic inflexibility manifested by (1) a lower increase of RQ during the hyperinsulinemic euglycemic clamp; (2) lower fasting and insulin-stimulated oxidation of glucose; (3) higher fasting and insulin-stimulated lipid oxidation. While obesity and type 2 diabetes were mostly studied, the importance of IR in DM1 is still unknown. More data and a comparison between diabetic patients and their phenotypes are needed just as we need to elucidate IR in DM1 in connection with substrate utilization.

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