4-1-2011

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Recommended Citation

Bajpeyi, S., Pasarica, M., Moro, C., Conley, K., Jubrias, S., Sereda, O., Burk, D., Zhang, Z., Gupta, A., Kjems, L., & Smith, S. (2011). Skeletal muscle mitochondrial capacity and insulin resistance in type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism, 96* (4), 1160-1168. https://doi.org/10.1210/jc.2010-1621

Skeletal Muscle Mitochondrial Capacity and Insulin Resistance in Type 2 Diabetes

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Objective: The objective of this study was to determine the role of maximum mitochondrial capacity on the variation in insulin sensitivity within a population of patients with type 2 diabetes mellitus (T2DM).

Research Design and Methods: Fifty-eight participants enrolled in a cross-sectional design: eight active controls [maximum aerobic capacity (VO_{2max}) $>$ 40 ml/kg $\cdot$ min], 17 healthy sedentary controls without a family history (FH$-$) and seven with a family history (FH$+$) of diabetes, four obese participants, and 21 patients with T2DM. Mitochondrial capacity was measured noninvasively using ^{31}P magnetic resonance spectroscopy of the vastus lateralis. Maximal ATP synthetic rate (ATP_{max}) was determined from the rate of phosphocreatine (PCr) recovery after short-term isometric exercise.

Results: ATP_{max} was lower ($P < 0.001$) in T2DM and higher ($P < 0.001$) in active as compared with healthy sedentary FH$-$ (active, 1.01 $\pm$ 0.2; FH$-$, 0.7 $\pm$ 0.2; FH$+$, 0.6 $\pm$ 0.1; obese, 0.6 $\pm$ 0.1; T2DM, 0.5 $\pm$ 0.2 mM ATP/sec; ANOVA $P < 0.0001$). Insulin sensitivity, measured by euglycemic-hyperinsulinemic (80 mIU/m^2 $\cdot$ min) clamp was also reduced in T2DM ($P < 0.001$) (active, 12.0 $\pm$ 3.2; FH$-$, 7.8 $\pm$ 2.2; FH$+$, 6.8 $\pm$ 3.5; obese, 3.1 $\pm$ 1.0; T2DM, 3.4 $\pm$ 1.6; mg/kg estimated metabolic body size $\cdot$ min; ANOVA $P < 0.0001$). Unexpectedly, there was a broad range of ATP_{max} within the T2DM population where 52% of subjects with T2DM had ATP_{max} values that were within the range observed in healthy sedentary controls. In addition, 24% of the T2DM subjects overlapped with the active control group (range, 0.65–1.27 mM ATP/sec). In contrast to the positive correlation between ATP_{max} and M-value in the whole population ($r^2 = 0.35$; $P < 0.0001$), there was no correlation between ATP_{max} and M-value in the patients with T2DM ($r^2 = 0.004$; $P = 0.79$).

Conclusions: Mitochondrial capacity is not associated with insulin action in T2DM. (***J Clin Endocrinol Metab* 96: 1160–1168, 2011**)

Insulin resistance is a clear precursor to the development of β-cell failure and type 2 diabetes mellitus (T2DM). Multiple tissues are insulin resistant in T2DM, including the adipose tissue, liver, and skeletal muscle. Early studies using the euglycemic, hyperinsulinemic clamp technique emphasized the role of skeletal muscle in insulin resistance because skeletal muscle is the major site of glucose disposal under conditions commonly employed during high-dose insulin clamps.

There are multiple mechanisms for skeletal muscle insulin resistance. There is good evidence for intrinsic genetic defects in insulin action. In addition, skeletal muscle

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-1621 Received July 13, 2010. Accepted January 6, 2011.
First Published Online February 9, 2011

Abbreviations: ATP_{max}, Maximal ATP synthesis rate; BMI, body mass index; EMBS, estimated metabolic body size; FH$+$, with a family history of diabetes; FH$-$, without a family history of diabetes; GDR, glucose disposal rate; MRS, magnetic resonance spectroscopy; OXPHOS, oxidative phosphorylation; ROS, reactive oxygen species; T2DM, type 2 diabetes mellitus; VO_{2max}, maximum aerobic capacity.

becomes insulin resistant acutely when lipid supply exceeds demand. For example, Boden *et al.* (1) showed that infusions of lipid acutely lead to insulin resistance for glucose uptake, a result that has been replicated in multiple studies (2, 3). Adipose tissue dysfunction and unrestrained lipolysis have been implicated as a major source of free fatty acid (FFA) supply to muscle (4). Skeletal muscle lipid uptake is highly dependent upon the prevailing FFA concentration (5). These same cellular lipotoxic pathways can also be activated via intramyocellular sources of lipid (6), and inflammatory pathways such as nuclear factor $\kappa\beta$ may also play a role (2).

A reduced rate of skeletal muscle lipid oxidation has been hypothesized to contribute to an increased intramyocellular lipid content and lipotoxic intramyocellular lipid derivatives such as diacylglycerol and ceramide that may cause insulin resistance. Skeletal muscle mitochondria are the major site of fatty acid oxidation and have been hypothesized to play an important role in preventing lipotoxicity. An increased glycolytic capacity relative to oxidative capacity of skeletal muscle has been shown to be associated with insulin resistance in obese women (7). Later work by Kelley *et al.* (8) demonstrated a reduction in mitochondrial volume and a strong relationship to insulin resistance. Several studies from Petersen, Shulman, and colleagues (6, 9) demonstrated reduced resting ATP turnover rates in conditions of insulin resistance. Along with microarray studies showing down-regulation of gene sets necessary for mitochondrial oxidative phosphorylation (10, 11), these results spurred on greater attention to the mitochondria as central to the development of insulin resistance.

In contrast to these studies, several investigators have shown several situations where insulin sensitivity and mitochondrial function in T2DM are dissociated. For example, Toledo *et al.* (12) showed that weight loss improved insulin action without changing the content of skeletal muscle mitochondria (12). Similarly, treatment with rosiglitazone improves insulin sensitivity without changing mitochondrial function (13). Hancock *et al.* (14) showed a high-fat diet that induced insulin resistance also increased skeletal muscle mitochondria in rats. Nair *et al.* (15) showed that diabetic Indians had similar muscle oxidative phosphorylation (OXPHOS) capacity as nondiabetic Indians. In addition, preclinical models have cast doubt on the theory that mitochondrial function is a determinant of insulin action. Animals with defective mitochondria and oxidative phosphorylation in skeletal muscle are not insulin resistant (16).

Because of the growing debate and controversy, we embarked on a clinical study to relate mitochondrial function to insulin action across a broad range of subjects with and without T2DM. Using the gold standard measure of whole-body insulin sensitivity by hyperinsulinemic-euglycemic clamp and accurate, precise, *in vivo* measures of maximal mitochondrial ATP synthesis, we explored the role of mitochondrial capacity in the insulin resistance seen in T2DM.

Patients and Methods

Study population

After signing the informed written consent approved by the Pennington Biomedical Research Center (PBRC) ethical review board, patients were enrolled in clinical trials performed in Baton Rouge, LA, at the PBRC. Volunteers qualified and were enrolled in www.Clinicaltrials.gov NCT00402012 (TAKE TIME) if they had known T2DM, were diet controlled, or were taking metformin, insulin, and/or sulfonylureas but not thiazolidinediones and were otherwise healthy. Volunteers qualified and were enrolled in NCT00401791 (ACTIV) if they were age 20–40 yr, body mass index (BMI) 20–30 kg/m^2, were nondiabetic, were taking no medications, and were otherwise healthy. Subjects in this study were recruited based on their level of habitual physical activity and a family history of T2DM. Physical activity level was calculated from a 7-d physical activity questionnaire recall and a triaxial accelerometer worn for at least 4 d. Physical activity index (total daily energy expenditure/resting metabolic rate) was calculated using both methods, and daily activity level was scrutinized from accelerometer data ensuring sedentary healthy control and active subjects have an activity index of less than 1.4 and greater than 1.6, respectively, with no long bout (over 30 min) of high physical activity/exercise in the sedentary group. Another inclusion criterion for the sedentary and active groups was a maximum aerobic capacity (VO_{2max}) lower than or above 40 ml/kg · min, respectively. A family history of T2DM was considered positive if one first-degree relative had T2DM. The obese group met the same criteria as sedentary controls but had a BMI higher than 30 kg/m^2.

Volunteers with chronic illnesses such as heart disease, hypothyroidism, and renal, lung, and liver diseases were excluded. The use of β-blockers and other drugs known to affect body weight or adrenergic tone were also exclusionary. All participants consumed a standard American diet (15% protein, 30–35% fat, and 50–55% carbohydrate) for 3 d before the test day. Also, all participants were admitted to PBRC inpatient clinic a day before the test day and fasted overnight.

Body composition

Body weight was measured in a gown after voiding and waist circumference measured using a standardized protocol. Height was measured on a calibrated stadiometer using PBRC standard protocols. Body fat mass and lean mass were measured on a Hologic dual-energy x-ray absorptiometer (QDR 4500A; Hologic, Inc., Waltham, MA).

Euglycemic-hyperinsulinemic clamp

The clamp was performed as previously described (17). Briefly, after an overnight fast, iv catheters were inserted in an antecubital vein for infusions and in a vein on the dorsum of the contralateral hand for sampling of arterialized blood. After baseline blood samples were obtained, a primed low-dose infusion of

regular insulin (5 mU/min · m^2) was then initiated and continued for 60 min, followed by a high-dose rate of 80 mU/min · m^2 for 90 min, where the dose of the insulin infusion was calculated before the study for each participant. Insulin was infused for at least 1 h after reaching a concentration of glucose of approximately 90 mg/dl. Plasma glucose was measured every 5 min and maintained by a variable 20% glucose infusion. The mean rate of exogenous glucose infusion during steady state (last 30 min) was corrected for changes in glycemia and divided by estimated metabolic body size (EMBS) (kilograms fat free mass + 17) to assess insulin sensitivity (18).

Biopsy and fiber typing

After an overnight fast and local anesthesia with lidocaine/bupivicaine, skeletal muscle samples were collected using the Bergstrom technique with suction from the *vastus lateralis* (Propper Manufacturing Co., Long Island City, NY). At the bedside, samples were rapidly cleaned and blotted dry before mounting in a mixture of optimal cutting temperature (OCT) compound and tragacanth powder for immunohistochemistry/fiber typing or snap frozen in liquid nitrogen for Western immunoblotting. Fiber typing was done by immunohistochemistry performed on 12-μm sections using mouse monoclonal antibody specific for slow muscle (MAB1628; Chemicon, Temecula, CA) along with a rat monoclonal antibody to laminin (AB2500; Abcam Inc., Cambridge, MA). Images were taken using a confocal microscope (Zeiss 510 META; Carl Zeiss, Thornwood, NY) and type I fibers were counted. Western immunoblotting was performed using OXPHOS antibody (MS601; Mitosciences, Eugene, OR).

Maximum aerobic capacity (VO_{2max})

Cardiorespiratory testing was conducted in the Exercise Testing Core using a standardized graded exercise testing protocol on a stationary bicycle ergometer (Lode Excalibur, Gronig, Netherlands). VO_{2max} was not measured in T2DM subjects.

Mitochondrial capacity measured by maximal ATP synthesis rate (ATP_{max})

ATP_{max} was determined as described previously (19) on a 3T GE Signa MNS magnet (GE, Milwaukee, WI) using a 4- or 6-cm ^{31}P-tuned surface coil positioned over the distal *vastus lateralis*. After the acquisition of a fully relaxed spectrum, ^{31}P spectra were acquired every 6 sec at rest (4 NEX) and continuously during a 24-, 30-, or 36-sec ballistic exercise obtained by kicking against Velcro straps positioned tight across the leg and thigh. Exercise time and intensity were targeted to drop PCr by 33–50% of basal PCr and to avoid a pH of less than 6.8 because lower pH inhibits oxidative phosphorylation and results in an artificially low ATP_{max}. Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) contains the pH in resting muscle and the minimum achieved after exercise in each group. ATP_{max} was calculated using the PCr recovery time constant (τ) and [PCr]rest: ATP_{max} = [PCr]rest/τ (Supplemental Fig. 1). Evidence that τ is independent of the starting level of PCr, in accordance with first-order reaction kinetics, is shown in Supplemental Fig. 2. Confirmation that ATP_{max} is a good measure of phosphorylation capacity comes from animal and human studies that have found this rate to vary in direct proportion to the oxidative enzyme activity of healthy muscle (20, 21). ATP_{max} has previously been validated against mitochondrial content (22). The reproducibility of muscle ATP_{max} determinations has been published (23), and repeated measures on the same subject at PBRC agree to within ±7% (n = 19; Fig. 1A). The ATP flux in the resting muscle (ATPase) was measured during ischemia as previously described (24).

Statistical analysis

All statistical analyses were performed and graphs were made using GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA). All values are presented in figures as means ± SE and in tables and text as means ± SD. One-way ANOVA was used to compare measures across groups. Tukey *post hoc* multiple-comparison tests were performed to determine specific differences between groups. Because all variables were normally distributed, pairwise Pearson correlations were performed. Significant differences were assumed for $P < 0.05$.

Results

Subject characteristics

The characteristics of the study population are presented in Table 1. Subjects in all groups were males with the exception of the T2DM group, which had both males and females. The T2DM subjects were older and BMI matched with obese subjects. As expected, the active group had higher VO_{2peak}, type I fibers (percent), glucose disposal rate (GDR), and ATP_{max}, whereas T2DM subjects had higher fasting glucose, lower GDR, and lower ATP_{max} compared with healthy nondiabetic sedentary controls without any family history of diabetes. Obese subjects had lower VO_{2peak}, lower GDR, lower ATP_{max}, and a higher percentage of type I fibers compared with healthy nondiabetic sedentary controls.

Precision and validity of the ATP_{max} measures

Test retest reliability

ATP_{max} was measured in two different occasions (at least 7 d apart) in 19 subjects (Fig. 1A). ATP_{max} measurement was highly reproducible in two different tests ($r^2 = 0.83$; $P < 0.0001$; average coefficient of variation = 6.94%).

External validation

ATP_{max} is considered an integrated measure of the functional properties of mitochondria *in vivo* that reflects both mitochondrial content and efficiency (22). In this study, ATP_{max} was correlated with other measures of skeletal muscle mitochondrial content and function such as VO_{2max}, percentage of type I fibers, and total OXPHOS protein content (complex I–V), used as a marker of mitochondrial content (Fig. 1, B–D). Moreover, we found a higher ATP_{max} in the active group (1.0 ± 0.2 mM/sec) and a lower ATP_{max} in T2DM (0.5 ± 0.2 mM/sec) compared with healthy sedentary nondiabetic controls (0.7 ± 0.2 mM/sec) (Table 1 and Fig. 2A).

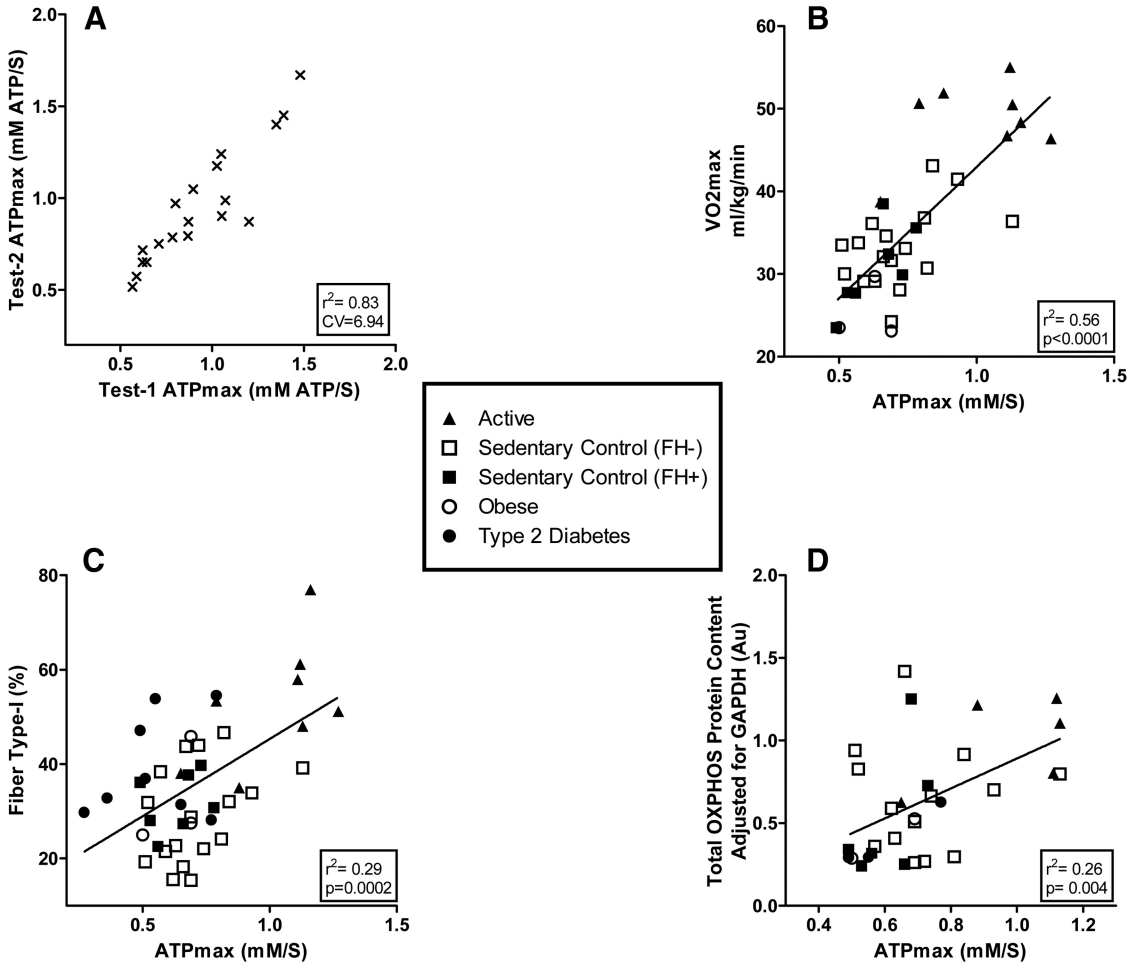

FIG. 1. Precision and validity of ATP_{max} measurement. A, Precision of ATP_{max} measurement is shown by a test-retest plot of MRS/ATP_{max} measurement on two different occasions; B–D, ATP_{max} is positively correlated to VO_{2max} (B), fiber type I (C), and total OXPHOS protein content (D).

Comparisons across groups

Insulin sensitivity measured by hyperinsulinemic-euglycemic clamp (80 mIU/m^2 · min) shows an expected high GDR in the active group and a low GDR in the obese and T2DM groups (active, 12.0 ± 3.2 mg/kg EMBS · min; obese, 3.1 ± 1.0 mg/kg EMBS · min; and T2DM, 3.4 ± 1.6 mg/kg EMBS · min; ANOVA $P < 0.0001$) (Fig. 2B). Consistent with the literature, ATP_{max} was higher in the active and lower in the T2DM group compared with healthy sedentary nondiabetics. There was no difference in ATP_{max} between sedentary men with a family history (FH+) or without a family history (FH−) of T2DM. Unexpectedly there was a broad range of ATP_{max} within the T2DM population (range, 0.23–0.79 mM ATP/sec). Then

TABLE 1. Study subject characteristics

	Active	Sedentary control (FH−)	Sedentary control (FH+)	Obese	T2DM
n (male/female)	8 (8/0)	17 (17/0)	7 (7/0)	4 (4/0)	21 (13/8)
Height (cm)	179.2 ± 2.9[a]	177.1 ± 5.1[a]	177.5 ± 9.6[a]	181.2 ± 1.6[a]	166.9 ± 10.2[b]
Weight (kg)	76.4 ± 9.2[a]	80.2 ± 11.3[a]	85.6 ± 13.7[a,c]	115.3 ± 10.7[b]	101.5 ± 16.9[b,c]
BMI (kg/m^2)	23.8 ± 3.0[a]	25.5 ± 3.1[a]	27.1 ± 3.2[a]	35.2 ± 3.5[b]	36.4 ± 5.0[b]
Age (yr)	23.1 ± 3.6[a]	26.1 ± 4.4[a]	26.9 ± 6.1[a]	28.5 ± 4.2[a]	53.9 ± 10.1[b]
Fasting glucose (mg/dl)	89.5 ± 3.7[a]	91.4 ± 6.8[a]	87.7 ± 11.9[a]	99.0 ± 3.9[a,b]	132.4 ± 34.9[b]
GDR (mg/kg EMBS · min)	12.0 ± 3.2[a]	7.8 ± 2.2[b]	6.8 ± 3.5[b,c]	3.1 ± 1.0[c,d]	3.4 ± 1.6[d]
ATP_{max} (mM/sec)	1.0 ± 0.2[a]	0.7 ± 0.2[b]	0.6 ± 0.1[b,c]	0.6 ± 0.1[b,c]	0.5 ± 0.2[c]
VO_{2max} (ml/kg · min)	48.5 ± 4.9[a]	33.2 ± 4.7[b]	30.8 ± 5.1[b,c]	24.9 ± 3.2[c]	NA
Fiber type I (%)	53 ± 13[a]	29 ± 10[b]	32 ± 6[b,c]	34 ± 10[b,c]	36 ± 9[b,c]

Data are presented as mean ± SD. Fiber type was determined in eight subjects from the T2DM group. NA, Data not available.

[a-d] Values with different *superscript letters* in each row are significantly different ($P < 0.05$).

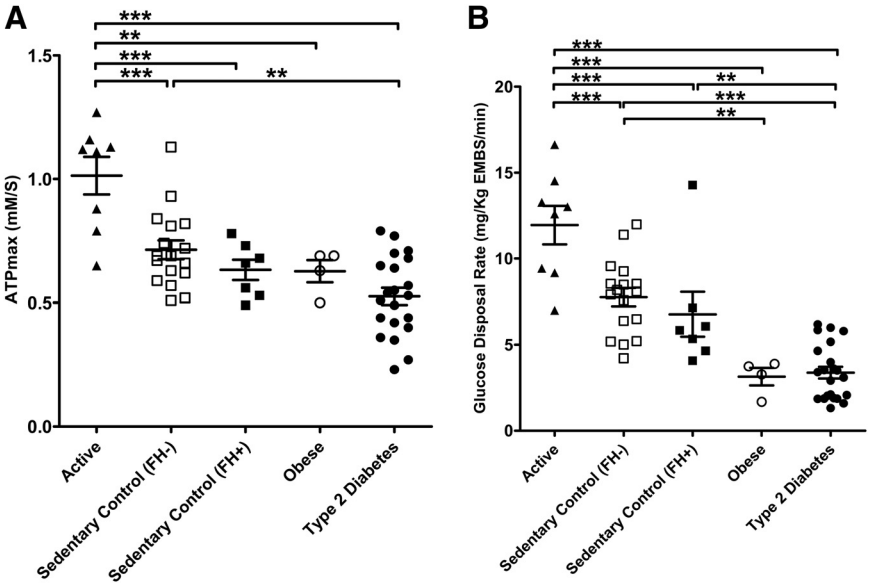

FIG. 2. Cross-sectional measurement of ATP_{max} (A) and GDR (B) in athletes, healthy young controls (FH− and FH+), and obese and T2DM subjects. **, $P < 0.01$; ***, $P < 0.001$ *vs.* healthy control FH−.

we calculated the percentage of T2DM subjects whose ATP_{max} value overlapped with healthy sedentary or active groups using the lowest ATP_{max} value as reference point in the above-mentioned groups. Fifty-two percent of the subjects with T2DM had ATP_{max} values that were within the range observed in healthy sedentary controls (range, 0.51–1.13 mM ATP/sec). In addition, 24% of the T2DM subjects overlapped with the endurance-trained active groups (range, 0.65–1.27 mM ATP/sec).

Given the apparently normal ATP_{max} in some patients with T2DM, we explored the relationship between ATP_{max} and insulin sensitivity measured by GDR within the T2DM population. Figure 3 shows a positive correlation between ATP_{max} and GDR in the population as a whole ($r^2 = 0.35$; $P < 0.0001$; Fig. 3A) and in the nondiabetic population ($r^2 = 0.26$; $P = 0.001$; Fig. 3C), but there was no correlation between ATP_{max} and GDR in the patients with T2DM ($r^2 = 0.004$; $P = 0.79$; Fig. 3B). We then divided patients with T2DM into two groups, those with normal and those with low ATP_{max} (*i.e.* ATP_{max} value greater than or lesser than the lowest value in healthy FH− sedentary control group, 0.52 mM ATP/sec) and found no differences in GDR ($P = 0.24$) between the two groups. Similarly, high *vs.* low GDR patients showed no differences in ATP_{max} ($P = 0.52$). We also found the same relationships between GDR and mitochondrial ATP flux in resting muscle (ATPase) (Fig. 3, D–F) as we found for ATP_{max} (Fig. 3, A–C). There was no evidence of a relationship between GDR and either maximal (ATP_{max}) or the mitochondrial flux in resting muscle (ATPase) in the T2DM population as is found in healthy, nondiabetic subjects.

Discussion

Several studies have reported lower mitochondrial content and function in T2DM. Mitochondrial oxidative phosphorylation genes and oxidative enzyme activity such as citrate synthase and succinate dehydrogenase are lower in the skeletal muscle of T2DM (25, 26). Moreover, Kelley *et al.* (27) reported smaller mitochondria with reduced activity of complex I in patients with T2DM. Our results are in agreement with the current literature that maximal ATP synthetic capacity in skeletal muscle, measured with ^{31}P magnetic resonance spectroscopy (MRS), is lower in T2DM. When a broad range of subjects, including healthy nondiabetic sedentary controls with or without family history of T2DM, obese subjects, and subjects with T2DM, are compared, ATP_{max} is correlated with insulin resistance. However, when we examined the data from the T2DM population closely, we found a 2-fold range of mitochondrial capacity; 52% of T2DM subjects had ATP_{max} values that were within the range observed in young healthy sedentary subjects, and 24% showed overlap with the active control group. Contrary to our hypothesis, within the T2DM population, we found no correlation between ATP_{max} and insulin sensitivity measured by euglycemic-hyperinsulinemic clamp. Given the apparently normal ATP_{max} in some patients with T2DM we then explored the relationship between insulin sensitivity in the T2DM population with normal *vs.* low ATP_{max} and found no difference. Our population is typical of most T2DM patients because they were insulin resistant as expected and obese. Taken together, these data suggest that the hypothesis that low mitochondrial function leads to insulin resistance in T2DM should be reexamined.

Our results should not be interpreted in isolation. Several other investigators have uncoupled insulin sensitivity and mitochondrial content in patients with T2DM. For example, Toledo *et al.* (12) showed that weight loss was able to improve insulin action without changing skeletal muscle mitochondrial content. Similarly, treatment with the antidiabetic thiazolidinedione rosiglitazone improves insulin sensitivity without changing mitochondrial function as measured by ^{31}P MRS (13). Furthermore, Boushel *et al.* (28) were unable to find defects in mitochondrial respiration in isolated skeletal muscle mitochondria even though mitochondrial mass was slightly reduced.

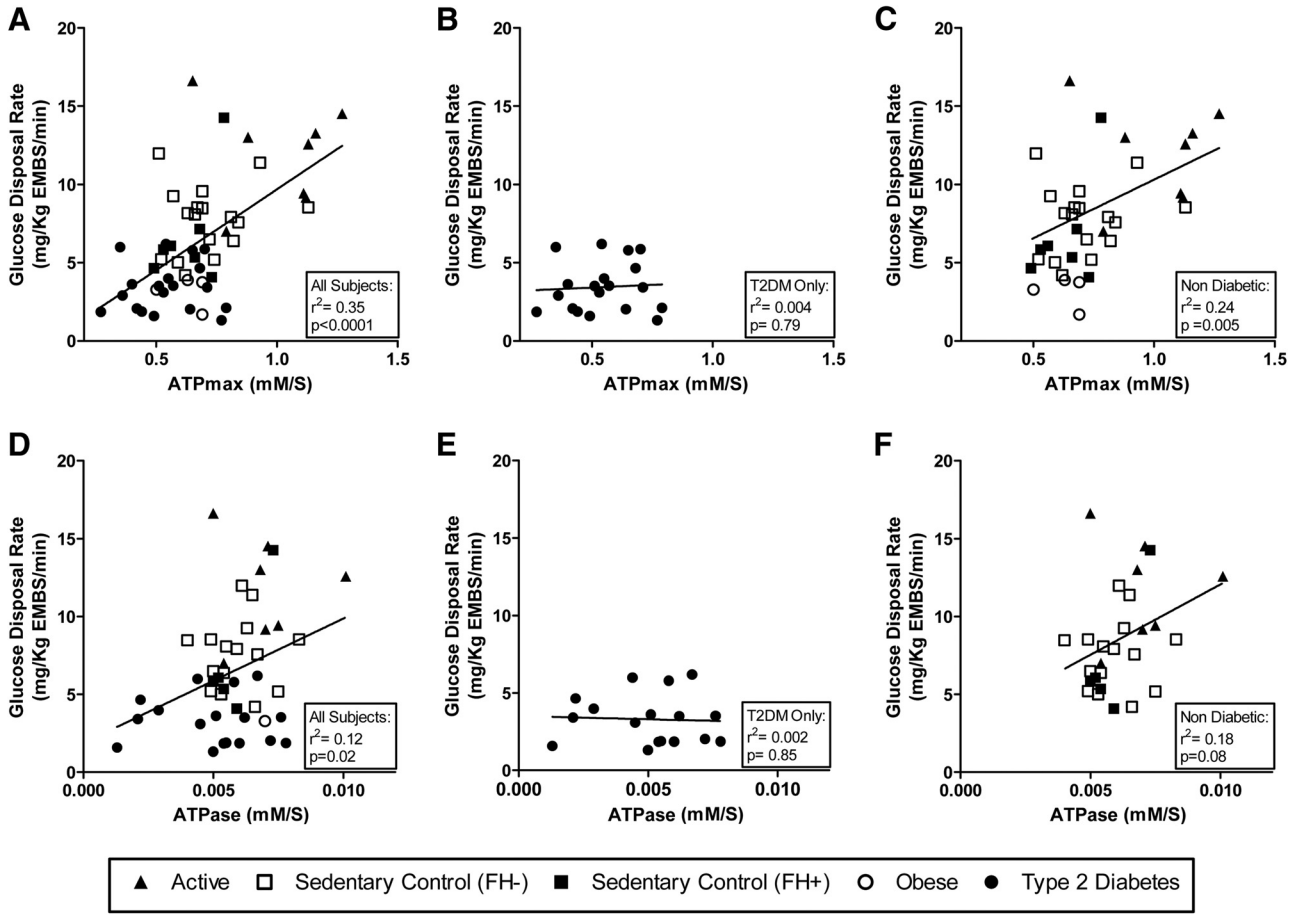

FIG. 3. Correlation of maximal ATP synthesis rate (ATP_{max}) with glucose disposal rate measured during a euglycemic hyperinsulinemic clamp in the whole population (A), in patients with T2DM only (B), and in the nondiabetic population (C). Correlation of resting ATP turnover (ATPase) with glucose disposal rate in the whole population (D), in patients with T2DM only (E), and in the nondiabetic population (F).

A link between mitochondrial function and insulin resistance in resting muscle has recently been proposed (29). Two independent approaches have found evidence for reduced ATP supply in subjects with T2DM and in the elderly using noninvasive MRS (6, 9, 24). Petersen *et al.* (6, 9) measured lower mitochondrial ATP supply in both insulin-resistant populations, whereas Amara *et al.* (24) found a corresponding decline in cellular ATP demand with age. These complementary findings demonstrate that the source of the lower mitochondrial ATP synthesis may be reduced ATP demand. Because cell ATP demand is strongly affected by the cost of protein synthesis and ion pumping, the depressed protein synthesis found in elderly muscle (30) is a possible cause of the lower ATP demand in both of these insulin-resistant populations. Thus, the lower ATP supply found in T2DM and with age is a reflection of reduced demand for ATP and not necessarily the result of mitochondrial dysfunction that limits ATP supply. Both groups also show similarly low mitochondrial capacities on average and a wide range of individual values (Supplemental Fig. 3) that overlap those in other groups with higher insulin sensitivities (Fig. 3). A factor common to both T2DM and age groups that could tie together the reduced mitochondrial content, low resting ATPase and insulin resistance is their greatly reduced physical activity levels relative to healthy adults. Inactivity, rather than age *per se* has been proposed to underlie the reduced mitochondrial capacity in the elderly (31). Perhaps inactivity is a factor common to T2DM and aging that underlies the insulin resistance in these two groups as well. In any case, neither mitochondrial capacity nor oxidative ATP supply in resting muscle appears to be an important factor in insulin resistance in T2DM.

Anderson *et al.* (32) provide evidence that mitochondrial reactive oxygen species (ROS) act as a cellular fuel gauge that regulates insulin signaling and sensitivity. ROS generation was found to be a response to excess fat intake that elevated peroxide emission from mitochondria in permeabilized fibers of both rats and humans. The shift of cellular redox to a more oxidized state triggered signaling pathways to reduce insulin sensitivity. Innovative antioxidants targeted to the mitochondria were shown to reduce this peroxide emission and to prevent development of insulin resistance in high-fat-fed animals.

This manipulation of insulin resistance occurred without changes in mitochondrial respiration, which rejects the hypothesis that mitochondrial dysfunction or mitochondrial content are important players in development of insulin resistance. Instead, insulin resistance may be regulated by mitochondrial ROS generation in response to excess fat intake to reduce energy intake and restore energy balance in the cell (33).

Given the reported reduction in insulin action (34) and ATP turnover (9) between young subjects with *vs.* without a family history of T2DM, we explored the hypothesis that a family history of T2DM might be associated with reduced mitochondrial function as defined by ATP_{max}. We did not find a significant relationship between ATP_{max} in FH+ and FH−. Our study is significantly methodologically different from earlier studies (9) where ATP turnover, not ATP_{max}, was measured. Further work is needed to resolve this apparent discrepancy.

Several alternative mechanisms/hypotheses are also important to consider. A number of studies have shown that T2DM is characterized by lower adiponectin. Adiponectin is a potential insulin sensitizer (35, 36). Intramyocellular lipid derivatives such as long-chain fatty acyl-coenzyme A, diacylglycerol, and ceramide are often elevated in T2DM (37) and have been linked to insulin resistance (27, 33). We have recently shown that dysregulation of skeletal muscle lipases activity parallels increased intramyocellular diacylglycerol concentrations and insulin resistance *in vivo* in sedentary humans (38). Other mechanisms exist and could account for the present observations.

We are limited in our ability (small number of nondiabetic obese subjects) to perform a comparison between nondiabetic obese *vs.* T2DM subjects. When we compared T2DM subjects receiving various treatment modalities (metformin *vs.* nonmetformin: insulin and/or sulfonylureas), the apparently normal ATP_{max} values in our T2DM populations were identical. Therefore, our interpretation of a lack of association between ATP_{max} and insulin sensitivity is limited to only an uncomplicated controlled T2DM population who were not on thiazolidinediones. Also, the T2DM population was older compared with the sedentary healthy group and, therefore, may raise a question whether a decrease in ATP_{max} was due to age. When a covariate analysis was performed, age was not a significant factor in the model.

Our study has several strengths, including the ability to characterize the mitochondrial capacity for ATP synthesis. A potential criticism of the approach is that it considers all mitochondria to be uniform in properties. Skeletal muscle mitochondria can be divided into two compartments: the intermyofibrillar and subsarcolemmal mitochondria that are hypothesized to be functionally and structurally distinct. However, an electron microscopic study by Ritov *et al.* (39) found a reduction in subsarcolemmal mitochondria in patients with T2DM, which further reduced the small percentage represented by this population (20%) in healthy subjects (40). In addition, the subcellular fractionation needed to isolate these distinct populations may well be highly disruptive (41) and responsible for the striking reduction in the proportion of mitochondria electron transport chain activity in the subsarcolemmal mitochondria. Thus, it is unlikely that the purported difference in the properties of subsarcolemmal *vs.* interfibrillar mitochondria could explain the lack of relationship between mitochondrial capacity and insulin resistance that was found in our study.

The most important finding of this study is that within a T2DM population ATP_{max} is not correlated with insulin sensitivity, suggesting mitochondrial capacity may not have a causal relationship with decreased insulin sensitivity in the T2DM population. It is important to consider that in this study we measured maximum mitochondrial phosphorylation capacity, whereas the above-mentioned studies measured resting mitochondrial function. This is the first large scale *in vivo* test of the hypothesis that ATP_{max} is associated with insulin resistance in T2DM population. Our results cast doubt on the hypothesis that mitochondrial dysfunction is causatively associated with insulin resistance (12, 14).

In summary, we present data in alignment with the existent literature showing that mitochondrial capacity, measured by ATP_{max}, is significantly lower in T2DM. ATP_{max} is a valid *in vivo* measure of mitochondrial ATP synthesis capacity based on the high correlation with mitochondrial content and oxidative type I fibers. Although ATP_{max} is correlated to insulin resistance in our population as a whole, there is no correlation of ATP_{max} and insulin sensitivity within a large population of T2DM. It is clear that there is not one common underlying factor in the etiology of insulin resistance. In T2DM, there is a large range of ATP synthetic capacity with a substantial overlap with healthy young sedentary controls. In T2DM, insulin sensitivity is consistently low and independent of ATP synthetic capacity. We conclude that mitochondrial capacity is not correlated with insulin action in T2DM.

Acknowledgments

We acknowledge the expert technical assistance of Mr. Randy Neiderhofer and Mr. Randall Dean for their focus and precision in the conduct of the MRS studies and Mrs. Kori Murray in the PBRC Imaging Core. We thank Dr. Conrad Earnest and Stephanie Anaya for their help with VO_2max testing. We are thankful to Dr. Jose Galgani for insightful discussion and Jason Gramil-

lion, Benjamin Tuminello, Laura Roan, and Courtney Cain for their help with fiber typing. We also acknowledge the support and commitment of our research volunteers who made this work possible.

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This work was supported by an unrestricted research grant from Novartis, Novartis Clinical Innovation Fund (to S.R.S.); an unrestricted research grant from Takeda Pharmaceuticals North America; NIH 1R01AG030226-01A2 (to S.R.S.); and the PBRC Clinical Nutrition Research Unit (CNRU) (NIH P-30-DK072476). This work used the facilities of the Cell Biology and Bioimaging Core facilities that are supported in part by COBRE (NIH P20-RR021945) and CNRU (NIH 1P30-DK072476) center grants from the National Institutes of Health.

Clinical trial registration numbers are NCT00402012 and NCT00401791.

Disclosure Summary: S.B., M.P., C.M., K.C., S.J., O.S., D.H.B., Z.Z., A.G., and L.K. have nothing to disclose. S.R.S. received funding from Novartis.

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