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The Insulin-Sensitizing Effect of Rosiglitazone in Type 2 Diabetes Mellitus Patients Does Not Require Improved *in Vivo* Muscle Mitochondrial Function

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Aims: Our objective was to investigate whether improved *in vivo* mitochondrial function in skeletal muscle and intramyocellular lipids (IMCLs) contribute to the insulin-sensitizing effect of rosiglitazone.

Methods: Eight overweight type 2 diabetic patients (body mass index = 29.3 ± 1.1 kg/m^2) were treated with rosiglitazone for 8 wk. Before and after treatment, insulin sensitivity was determined by a hyperinsulinemic euglycemic clamp. Muscular mitochondrial function (half-time of phosphocreatine recovery after exercise) and IMCL content were measured by magnetic resonance spectroscopy.

Results: Insulin sensitivity improved after rosiglitazone (glucose infusion rate: 19.9 ± 2.8 to 24.8 ± 2.1 μmol/kg·min; $P < 0.05$). *In vivo* mitochondrial function (phosphocreatine recovery half-time: 23.8 ± 3.5 to 20.0 ± 1.7 sec; $P = 0.23$) and IMCL content (0.93 ± 0.18% to 1.37 ± 0.40%; $P = 0.34$) did not change. Interestingly, the changes in PCr half-time correlated/tended to correlate with changes in fasting insulin ($R^2 = 0.50$; $P = 0.05$) and glucose ($R^2 = 0.43$; $P = 0.08$) levels. Changes in PCr half-time did not correlate with changes in glucose infusion rate ($R^2 = 0.08$; $P = 0.49$).

Conclusion: The rosiglitazone-enhanced insulin sensitivity does not require improved muscular mitochondrial function. **(*J Clin Endocrinol Metab* 93: 2917–2921, 2008)**

In recent years, mitochondrial dysfunction has been implicated in the etiology of type 2 diabetes mellitus. The initial finding that a cluster of oxidative genes under control of peroxisome-proliferator activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) is reduced in the (pre-) diabetic state (1, 2) was rapidly followed by the demonstration of impaired skeletal muscle mitochondrial function in insulin resistance using noninvasive phosphorous magnetic resonance spectroscopy (^{31}P-MRS) (3–6).

One intervention that improves insulin sensitivity in type 2 diabetes patients is the treatment with insulin-sensitizing thiazolidinediones (TZDs), which belong to the class of PPARγ agonists. Interestingly, recent studies revealed that TZDs can cause significant improvements in mitochondrial function in adipose tissue of both animals and humans (7–9). Rosiglitazone up-regulated a set of genes encoding mitochondrial proteins, including PGC-1α, accompanied by an increase in mitochondrial mass and changes in mitochondrial structure in cultured adipocytes (8). We recently reported that rosiglitazone is also able to restore PGC-1α mRNA levels in skeletal muscle of type 2 diabetic patients (10). Together, these findings suggest that the insulin-sensitizing effect of rosiglitazone could be due to an improvement in *in vivo* mitochondrial function in skeletal muscle. Therefore, we investigated if: 1) an improvement of mitochondrial function underlies the TZD-induced improvement in insulin sensitivity, and 2) such an effect is mediated by reducing skeletal muscle lipid

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Abbreviations: FFA, Unesterified free fatty acid; GIR, glucose infusion rate; IMCL, intramyocellular lipid; MRS, magnetic resonance spectroscopy; PGC-1α, peroxisome-proliferator activated receptor-γ coactivator-1α; ^{31}P-MRS, phosphorous magnetic resonance spectroscopy; PPARγ, peroxisome-proliferator activated receptor-γ; TZD, thiazolidinedione.

content. Hence, we investigated insulin sensitivity and *in vivo* skeletal muscle mitochondrial function using ^{31}P-MRS together with intramyocellular lipid (IMCL) content using proton magnetic resonance spectroscopy in type 2 diabetic patients before and after 8-wk treatment with rosiglitazone.

Subjects and Methods

Subjects

Eight male overweight patients with type 2 diabetes mellitus participated in this study. Subjects were on oral antidiabetic medication (metformin or sulfonylureas-derivatives) and had no major health problems besides their diabetes. The protocol was approved by the institutional Medical Ethics Committee, and every subject signed an informed consent after procedures had been explained.

Study design

Fourteen days before the experimental trial, any prior antidiabetic medication was discontinued. Subjects were asked not to participate in (exhaustive) physical activity the last 3 d preceding the measurements and to consume a diet according to the Dutch guidelines for a healthy diet. After baseline measurements of *in vivo* mitochondrial function, IMCL content, insulin sensitivity, and plasma concentrations of glucose and insulin, subjects received rosiglitazone (2 × 4 mg/d) during 8 wk. All measurements were repeated after the treatment. During the treatment, subjects visited the laboratory every 2 wk for measurements of fasting glucose and to check compliance.

Hyperinsulinemic euglycemic clamp

After an overnight fast, insulin sensitivity was measured with a 3-h hyperinsulinemic euglycemic clamp as reported earlier (10). The rate at which glucose was infused during the last 30 min of the clamp to maintain euglycemia [glucose infusion rate (GIR)] was used as a measure of whole body insulin sensitivity.

Magnetic resonance spectroscopy (MRS)

MRS measurements were performed at 1.5 T (Intera; Philips Medical Systems, Best, The Netherlands).

In vivo *mitochondrial function by ^{31}P-MRS*

In vivo mitochondrial function was investigated by ^{31}P-MRS (6-cm surface coil, free induction decay, repetition time = 4 sec, n = 1) by assessing phosphocreatine recovery half-time of the mono-exponentially fitted time course after knee-extension exercise in the vastus lateralis muscle as reported earlier (5).

IMCL by ^{1}H-MRS imaging

Image-guided ^{1}H-MRS imaging (20 × 20 voxels, field of view = 10 cm, point resolved spectroscopy, repetition time = 1500 msec, spin echo pulse sequences = 24 msec) was performed in the vastus lateralis muscle as reported earlier (5) and fitted with Java-based magnetic resonance user interface (11).

Because of insufficient spectral quality in one case, the IMCL values of only seven subjects are reported.

Blood analyses

Blood was collected in EDTA-containing tubes, and plasma was frozen in liquid nitrogen and stored at −80 C until assayed. Plasma unesterified free fatty acid (FFA) and glucose were measured with enzymatic assays automated on a Cobas Fara/Mira (FFA: Wako Nefa C test kit; Wako Chemicals, Neuss, Germany; glucose: hexokinase method, La-Roche, Basel, Switzerland). Insulin concentration was determined using a RIA (LINCO Research, Inc., St. Charles, MO).

Statistics

Results are reported as mean ± SEM and considered significant if $P < 0.05$. The effect of rosiglitazone on selected parameters was determined by a two-sided paired Student's *t* test. To evaluate the relationship between variables, Pearson correlation coefficients were calculated (SPSS for Windows 11.5 software; SPSS, Inc., Chicago, IL).

Results

Subject characteristics

Subject characteristics before and after 8-wk rosiglitazone treatment are shown in Table 1.

Insulin sensitivity and substrate oxidation

Whole body insulin sensitivity (GIR) significantly improved after 8-wk rosiglitazone treatment (from 19.9 ± 2.8 to 24.8 ± 2.1 μmol/kg·min; $P = 0.04$). Basal and insulin-stimulated glucose and lipid oxidation were not affected by 8-wk rosiglitazone treatment. However, the increase in respiratory quotient upon insulin stimulation was more pronounced after rosiglitazone, suggesting improved metabolic flexibility (before: from 0.82 ± 0.01 to 0.87 ± 0.02; after: from 0.79 ± 0.01 to 0.88 ± 0.01; $P = 0.06$).

IMCL content

IMCL content was unchanged after rosiglitazone (0.69 ± 0.14% before treatment and 1.01 ± 0.29% after treatment; $P = 0.35$). IMCL content did not correlate with insulin sensitivity

TABLE 1. Baseline subject characteristics

	Before	After	*P* value
Age (yr)	61.8 ± 1.6		
Body weight (kg)	90.2 ± 5.2	91.4 ± 5.2	NS
BMI (kg/m^2)	29.3 ± 1.1	29.7 ± 1.0	NS
VO_{2max} (ml/kg·min)	31.5 ± 2.3	ND	
Glucose (mmol/liter)	9.3 ± 0.7	8.8 ± 0.7	NS
Insulin (mU/liter)	16.1 ± 2.9	13.4 ± 1.8	NS
Free fatty acid (μmol/liter)	431 ± 30	398 ± 42	NS
Insulin sensitivity (GIR, μmol/kg·min)	19.9 ± 2.8	24.8 ± 2.1	0.04

Data are mean ± SE. BMI, Body mass index; ND, not determined; NS, not significant; VO_{2max}, maximum oxygen consumption.

($P = 0.87$) or *in vivo* mitochondrial function ($P = 0.27$). Changes in IMCL content did neither correlate with changes in insulin sensitivity ($P = 0.68$) nor with changes in mitochondrial function ($P = 0.49$).

In vivo mitochondrial function

During the knee-extension protocol, the PCr levels decreased initially in the vastus lateralis muscle until a steady state was reached after 2–3 min. Steady-state PCr levels were similar before and after rosiglitazone treatment (at 72.7 ± 2.8% and 70.4 ± 3.2% of the resting baseline value; $P = 0.3$). None of the subjects showed substantial acidification during the exercise protocol, with similar end-exercise pH values before and after treatment (7.11 ± 0.01 and 7.03 ± 0.03; $P = 0.14$).

Eight weeks of rosiglitazone treatment did not improve mitochondrial function, as reflected by an unchanged PCr recovery half-time (from 23.8 ± 3.5 to 20.0 ± 1.7 sec; $P = 0.23$). In fact, three subjects showed an improvement in mitochondrial function, reflected by a reduced PCr recovery half-time of more than 5%, whereas five subjects showed a decrease or no change (Fig. 1). However, interestingly, the changes in PCr recovery half-time correlated positively with the changes in plasma insulin levels ($R^2 = 0.50$; $P = 0.05$) and tended to correlate with changes in plasma glucose levels ($R^2 = 0.43$; $P = 0.08$) (Fig. 2). There was no correlation between the changes in PCr recovery half-time and those in insulin sensitivity ($R^2 = 0.08$; $P = 0.49$).

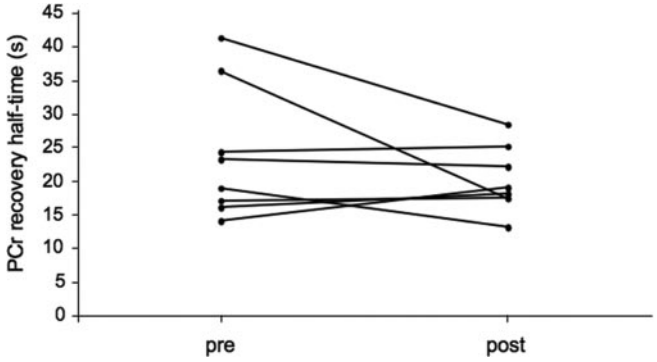

FIG. 1. Individual changes in PCr recovery half-time upon rosiglitazone treatment. post, After; pre, before; s, sec.

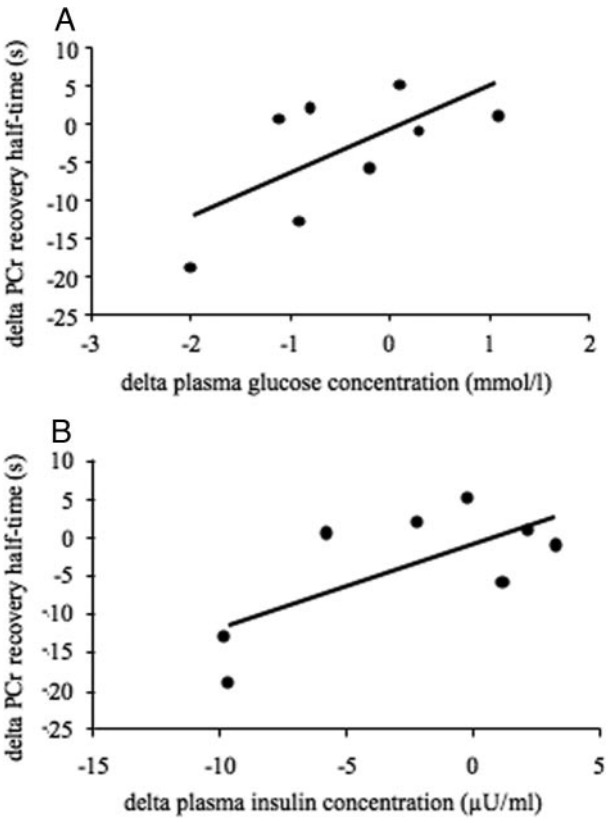

FIG. 2. A, Correlation between the change in plasma glucose levels and the change in PCr recovery half-time ($R^2 = 0.43$; $P = 0.07$). B, Correlation between the change in plasma insulin levels and the change in PCr recovery half-time ($R^2 = 0.50$; $P = 0.05$).

Discussion

A decreased mitochondrial function has been implicated in the development of insulin resistance. Based on previous results obtained in white adipose tissue showing induction of mitochondrial biogenesis (7) and in myotubes, showing increased PGC-1α expression and insulin-stimulated glycogen synthesis (12) upon rosiglitazone treatment, we predicted that treating human type 2 diabetic patients for 8 wk with the PPARγ agonist rosiglitazone would result in improved mitochondrial function that could underlie the improvement in insulin sensitivity. Although 8-wk rosiglitazone treatment significantly improved insulin sensitivity, we did, however, not observe significantly improved mitochondrial function, as determined by postexercise PCr recovery half-time, and no change in IMCL content. Moreover, changes in insulin sensitivity did not correlate with the changes in mitochondrial function or IMCL content. Thus, the present *in vivo* study in type 2 diabetic patients shows that rosiglitazone-enhanced insulin sensitivity does not require improved muscular mitochondrial function.

The lack of effect of rosiglitazone on *in vivo* mitochondrial function was not anticipated because we previously observed that 8-wk rosiglitazone treatment up-regulated the skeletal muscle expression of PGC-1α, and restored succinate dehydrogenase activity (10) and uncoupling protein-3 protein content (13) in type 2 diabetic patients. However, in these previous studies, we also noted that these improvements were not accompanied by changes in mitochondrial content, as assessed by measuring the protein content of five structural subunits of complexes I–V of the respiratory chain (10). The unchanged *in vivo* mitochondrial function of the current study suggests that the earlier reported increase in PGC-1α expression and succinate dehydrogenase activity (10) do not necessarily translate into improvements in *in vivo* mitochondrial function within the time frame of the study.

How rosiglitazone improves whole body and muscular substrate metabolism and insulin sensitivity remains to be elucidated. It has been suggested that the improvement in skeletal muscle insulin sensitivity upon rosiglitazone could be an indirect

effect, mediated by metabolic changes in adipose tissue, leading among others to increased levels of adiponectin and decreased levels of FFA, which may in turn influence muscular metabolism to result in an improved insulin sensitivity (for a recent review, see Ref. 14). Indeed, it has been shown repeatedly that rosiglitazone increases plasma adiponectin levels; in fact, we observed in a similar population and intervention a 5-fold increase in adiponectin levels after 8-wk rosiglitazone treatment (from 3.3 ± 0.4 to 11.8 ± 1.8 μg/ml, n = 11; $P < 0.001$; unpublished observations). Although this notion of an indirect mechanism is perfectly in line with the results presented here, we cannot exclude that rosiglitazone also exerts direct effects on muscular metabolism that are involved in the improvement of muscular insulin sensitivity. However, what the present study does show is that such improvement is not mediated by an increased *in vivo* mitochondrial function.

Although rosiglitazone did not have an effect on *in vivo* mitochondrial function, the change in PCr recovery half-time did correlate with the rosiglitazone-induced decline in fasting plasma insulin and glucose levels. Thus, those subjects in which rosiglitazone decreased fasting insulin and glucose levels the most showed the most prominent improvement in mitochondrial function. This can still be compatible with rosiglitazone having a stimulating effect on mitochondrial function but may indicate that the duration of our study was too short to already detect these changes at a statistically significant level. In addition, the number of subjects in our study was low. However, it should be noted that only in three of eight subjects mitochondrial function improved more than 5%, whereas it decreased or was unaltered in the other five. However, regardless of this, rosiglitazone treatment did significantly improve insulin sensitivity. Therefore, although it cannot be excluded that rosiglitazone will on the longer term also improve mitochondrial function, such improvement is not a prerequisite for the insulin-sensitizing effect of rosiglitazone.

Alternatively, the relationship between plasma glucose/insulin and *in vivo* mitochondrial function may indicate that hyperinsulinemia and/or hyperglycemia has a negative impact on *in vivo* mitochondrial function of skeletal muscle and that a reduction in glucose/insulin levels thereby improved mitochondrial function. Support for mitochondrial dysfunction being a consequence of insulin resistance comes from a recent study by Asmann *et al.* (15), in which plasma insulin and glucose levels were artificially kept similar between type 2 diabetic patients and healthy controls. Under this condition of similar postabsorptive insulin and glucose levels, *ex vivo* skeletal muscle ATP production and mitochondrial DNA copy number were not different between control subjects and diabetic patients, but type 2 diabetic subjects had a blunted increase in mitochondrial ATP production in response to insulin. The authors concluded that the mitochondrial aberrations observed in diabetic patients can be attributed to multiple factors related to insulin action rather than resulting from intrinsic mitochondrial defects.

Consistent with the lack of effect of rosiglitazone on *in vivo* mitochondrial function, also IMCL content was unchanged after rosiglitazone treatment. This confirms earlier studies by us (10) and others (16), and these results are in line with the notion that although IMCL content is generally correlated with insulin resistance, the IMCL content is not a direct determinant of insulin sensitivity.

In conclusion, we have shown that PCr recovery half-time was not affected by 8-wk rosiglitazone treatment in type 2 diabetic patients but that rosiglitazone did improve whole body substrate metabolism and insulin sensitivity. These changes occurred in the absence of rosiglitazone-induced changes in muscular fat content. Thus, neither an improved mitochondrial function nor the lowering of IMCL is a prerequisite for rosiglitazone-enhanced insulin sensitivity.

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