SIRT1 mRNA Expression May Be Associated With Energy Expenditure and Insulin Sensitivity

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OBJECTIVE—Sirtuin 1 (SIRT1) is implicated in the regulation of mitochondrial function, energy metabolism, and insulin sensitivity in rodents. No studies are available in humans to demonstrate that SIRT1 expression in insulin-sensitive tissues is associated with energy expenditure and insulin sensitivity.

RESEARCH DESIGN AND METHODS—Energy expenditure (EE), insulin sensitivity, and SIRT1 mRNA adipose tissue expression (n = 81) were measured by indirect calorimetry, hyperinsulinemic-euglycemic clamp, and quantitative RT-PCR in 247 nondiabetic offspring of type 2 diabetic patients.

RESULTS—High EE during the clamp (r = 0.375, P = 2.8 × 10⁻⁷) and high ΔEE (EE during the clamp – EE in the fasting state) (r = 0.602, P = 2.5 × 10⁻²⁴) were associated with high insulin sensitivity. Adipose tissue SIRT1 mRNA expression was significantly associated with EE (r = 0.289, P = 0.010) and with insulin sensitivity (r = 0.334, P = 0.002) during hyperinsulinemic-euglycemic clamp. Furthermore, SIRT1 mRNA expression correlated significantly with the expression of several genes regulating mitochondrial function and energy metabolism (e.g., peroxisome proliferator–activated receptor γ coactivator-1α, estrogen-related receptor α, nuclear respiratory factor-1, and mitochondrial transcription factor A), and with several genes of the respiratory chain (e.g., including NADH dehydrogenase [ubiquinone] 1α subcomplex 2, cytochrome c, cytochrome c oxidase subunit IV, and ATP synthase).

CONCLUSIONS—Impaired stimulation of EE by insulin and low SIRT1 expression in insulin-sensitive tissues is likely to reflect impaired regulation of mitochondrial function associated with insulin resistance in humans. Diabetes 59:829–835, 2010

Compromised mitochondrial function in skeletal muscle predisposes to insulin resistance and diabetes (1,2). In contrast, physical activity and weight loss in obese and sedentary subjects stimulate mitochondrial biogenesis and improve insulin sensitivity (3). Animal and human studies have shown that mitochondrial function is associated with insulin sensitivity, but the mechanisms explaining this association are largely unknown (4).

The mammalian sirtuins (SIRT1–SIRT7) are implicated in gene silencing, mitochondrial function, energy homeostasis, insulin sensitivity, and longevity (5). We previously demonstrated that treatment with SIRT1 activator, resveratrol, enhanced mitochondrial activity and protected mice from diet-induced obesity and insulin resistance (4). The effects of resveratrol were seen in both muscle and adipose tissue and resulted in an increase in mitochondrial function, which translated into an increase in energy expenditure (EE) and insulin sensitivity. Small molecule activators of SIRT1, that are structurally unrelated to resveratrol, have also been shown to improve insulin sensitivity, lower plasma glucose, and increase mitochondrial capacity (6). In many rodent models (4,7), the upregulation of the oxidative phosphorylation (OXPHOS) pathway is coordinated by peroxisome proliferator–activated receptor γ coactivator (PGC) 1α, which is a target of SIRT1 (8). Similarly, insulin resistance in human skeletal muscle has been associated with decreased mitochondrial oxidative capacity and ATP synthesis and decreased expression of genes that control mitochondrial activity, including PGC-1α (9–11).

The offspring of type 2 diabetic subjects are known to be insulin resistant, and they have defects in mitochondrial OXPHOS associated with increased intramyocellular lipid content (9). The association of EE and insulin sensitivity with SIRT1 and PGC-1α mRNA expression has not been previously investigated. Therefore, we studied here the association of EE, insulin sensitivity, and adipose tissue SIRT1 and PGC-1α mRNA expression in 247 nondiabetic offspring of subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

The subjects were selected from an ongoing study and included healthy nondiabetic offspring of patients with type 2 diabetes, as previously described (12). The diabetic patients (proband) were randomly selected among type 2 diabetic subjects living in the region of the Kuopio University Hospital. Spouses of the probands had to have a normal glucose tolerance in an oral glucose tolerance test (OGTT). The study protocol was approved by the ethics committee of the University of Kuopio. All study subjects gave an informed consent. A total of 247 offspring (one to three from each family) were studied. Their mean ± SD age was 35.1 ± 6.3 years and BMI 26.3 ± 4.7 kg/m².
Clinical and laboratory methods. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (in kilograms) divided by square of height (in meters). On the first day, an OGTT was performed with 75 g of glucose. Subjects with normal glucose tolerance (n = 210), isolated impaired fasting glycating (n = 5) or impaired glucose tolerance (n = 1), defined on the basis of the World Health Organization criteria (13), were included in further studies. An intravenous glucose tolerance test (IVGTT) was performed to determine the first-phase insulin release after an overnight fast. After baseline blood collection, a bolus of glucose (300 mg/kg in a 50% solution) was given within 30 s into the antecubital vein. Samples for the measurement of blood glucose and plasma insulin (5 and 15 min after glucose loading) were drawn on ice and cleaned with an RNeasy Plus Micro Kit (Qiagen). RNA was transcribed to cDNA using the Advantage cDNA kit (Clontech). A housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), was used to normalize the expression of genes of interest. For Sirt1, primers, the membranes were blocked in Tris-buffered saline (TBS) with 3% milk and 0.05% Tween-20 for 1 h at room temperature, washed with TBS-0.05% Tween-20 for 3 × 5 min, and incubated overnight at +4°C with SIRT1 (no. 07-131; Millipore) primary antibodies (1:1,000). The membranes were washed with TBS-0.05% Tween-20 for 3 × 5 min before incubating them with secondary anti-rabbit horseradish peroxidase–conjugated immunoglobulin (goat, NA931V; GE Health Care, Amersham, U.K.) (1:40,000) for 2 h at room temperature. The membranes were finally washed with TBS-0.05% Tween-20 for 3 × 5 min. For GAPDHI, the membranes were blocked in TBS with 5% milk and 0.1% Tween-20 for 2 h at room temperature, washed with TBS-0.1% Tween-20 for 3 × 5 min, and incubated with GAPDHI (no. ab8245; Abcam) primary antibodies (1:5,000) overnight at +4°C. The membranes were washed with TBS-0.1% Tween-20 for 3 × 5 min before incubating them with horseradish peroxidase–conjugated immunoglobulin (goat, NA301V; GE Health Care) (1:10,000) in TBS-0.1% Tween-20 for 1 h at room temperature. The membranes were finally washed with TBS-0.1% Tween-20 for 3 × 5 min. The bands were visualized using chemiluminescence (ECL plus; GE Health Care), and images were captured in an Image Quant RT-ECL machine (version 1.0.1; GE Health Care). Quantification of the bands was done by applying Quantity One software. The band intensities were expressed as arbitrary units to calculate the ratio of the expression of interest to GAPDHI protein levels. The experiments were repeated four times.

Statistical analysis. Data analyses were carried out with SPSS 14.0 for Windows. The results for continuous variables are given as means ± SD. Variables with skewed distribution (glucose, insulin, FFAs, and subcutaneous and intra-abdominal fat) were logarithmically transformed for statistical analyses. Linear regression was used to calculate the correlations. Uni- and multivariate regression models were applied to adjust for confounding factors. For mixed-model analysis, we included the pedigree factor for the experiment as a random effect, the tertiles of the tertiles of the tertiles of the tertiles as a fixed effect, and age as a covariate.

RESULTS

Energy expenditure and insulin sensitivity. EE during the clamp positively correlated with insulin sensitivity (r = 0.375, P = 2.8 × 10−6) (Fig. 1). Even stronger correlation was found between insulin-stimulated energy expenditure (EE) (defined as EE during the clamp − EE in the fasting state) and insulin sensitivity (r = 0.602, P = 2.5 × 10−24). In contrast, fasting EE was not correlated with insulin sensitivity (r = −0.004). To further investigate the association of EE and insulin sensitivity, we analyzed the rates of WBGU during the hyperinsulinemic clamp according to the tertiles of EE (Fig. 1). We did not find differences in WBGU among the tertiles of fasting EE, glucose oxidation, or nonoxidative glucose disposal (data not shown). In contrast, subjects in the highest tertile of EE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (49.85 ± 15.43 vs. 55.02 ± 15.46 vs. 63.44 ± 18.76 mol/kg LBM/min, P = 2.2 × 10−6), which was attributable to both high-glucose oxidative (19.54 ± 5.42 vs. 20.96 ± 5.62 vs. 22.75 ± 6.11 mol/kg LBM/min, P = 0.007) and nonoxidative (30.31 ± 12.81 vs. 34.06 ± 13.24 vs. 40.68 ± 16.52 mol/kg LBM/min, P = 1.2 × 10−6) glucose disposal. These differences were even more pronounced across the tertiles of ΔEE/LBM, where subjects in the highest tertile of ΔEE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (48.85 ± 15.43 vs. 55.02 ± 15.46 vs. 63.44 ± 18.76 mol/kg LBM/min, P = 2.2 × 10−6), which was attributable to both high-glucose oxidative (19.54 ± 5.42 vs. 20.96 ± 5.62 vs. 22.75 ± 6.11 mol/kg LBM/min, P = 0.007) and nonoxidative (30.31 ± 12.81 vs. 34.06 ± 13.24 vs. 40.68 ± 16.52 mol/kg LBM/min, P = 1.2 × 10−6) glucose disposal. These differences were even more pronounced across the tertiles of ΔEE/LBM, where subjects in the highest tertile of ΔEE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (43.82 ± 13.25 vs. 55.75 ± 13.64 vs. 67.96 ± 16.31 mol/kg LBM/min, P = 2.5 × 10−8), attributable to both high-glucose oxidative (17.51 ± 4.34 vs. 20.81 ± 5.45 vs. 24.58 ± 5.31 mol/kg LBM/min, P = 5.9 × 10−5) and nonoxidative (20.31 ± 12.08 vs. 34.94 ± 12.24 vs. 43.38 ± 15.16 mol/kg LBM/min, P = 3.6 × 10−5) glucose disposal.

Subjects in the highest ΔEE tertile used more glucose for energy production than did subjects in the lower ΔEE tertiles. However, differences in glucose availability between tertiles were not significant. WBGU/LBM during the clamp positively correlated with insulin sensitivity (r = 0.375, P = 2.8 × 10−6) (Fig. 1). Even stronger correlation was found between insulin-stimulated energy expenditure EE (defined as EE during the clamp − EE in the fasting state) and insulin sensitivity (r = 0.602, P = 2.5 × 10−24). In contrast, fasting EE was not correlated with insulin sensitivity (r = −0.004). To further investigate the association of EE and insulin sensitivity, we analyzed the rates of WBGU during the hyperinsulinemic clamp according to the tertiles of EE (Fig. 1). We did not find differences in WBGU among the tertiles of fasting EE, glucose oxidation, or nonoxidative glucose disposal (data not shown). In contrast, subjects in the highest tertile of EE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (49.85 ± 15.43 vs. 55.02 ± 15.46 vs. 63.44 ± 18.76 mol/kg LBM/min, P = 2.2 × 10−6), which was attributable to both high-glucose oxidative (19.54 ± 5.42 vs. 20.96 ± 5.62 vs. 22.75 ± 6.11 mol/kg LBM/min, P = 0.007) and nonoxidative (30.31 ± 12.81 vs. 34.06 ± 13.24 vs. 40.68 ± 16.52 mol/kg LBM/min, P = 1.2 × 10−6) glucose disposal. These differences were even more pronounced across the tertiles of ΔEE/LBM, where subjects in the highest tertile of ΔEE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (48.85 ± 15.43 vs. 55.02 ± 15.46 vs. 63.44 ± 18.76 mol/kg LBM/min, P = 2.2 × 10−6), which was attributable to both high-glucose oxidative (19.54 ± 5.42 vs. 20.96 ± 5.62 vs. 22.75 ± 6.11 mol/kg LBM/min, P = 0.007) and nonoxidative (30.31 ± 12.81 vs. 34.06 ± 13.24 vs. 40.68 ± 16.52 mol/kg LBM/min, P = 1.2 × 10−6) glucose disposal. These differences were even more pronounced across the tertiles of ΔEE/LBM, where subjects in the highest tertile of ΔEE/LBM during the hyperinsulinemic clamp had highest WBGU/LBM (43.82 ± 13.25 vs. 55.75 ± 13.64 vs. 67.96 ± 16.31 mol/kg LBM/min, P = 2.5 × 10−8), attributable to both high-glucose oxidative (17.51 ± 4.34 vs. 20.81 ± 5.45 vs. 24.58 ± 5.31 mol/kg LBM/min, P = 5.9 × 10−5) and nonoxidative (20.31 ± 12.08 vs. 34.94 ± 12.24 vs. 43.38 ± 15.16 mol/kg LBM/min, P = 3.6 × 10−5) glucose disposal.

Subjects in the highest ΔEE tertile used more glucose for energy production than did subjects in the lower ΔEE tertiles. However, differences in glucose availability between tertiles were not significant.
tertiles, as indicated by their higher respiratory quotient in the fasting state ($P = 0.010$) and during the hyperinsulinemic clamp ($P = 1.2 \times 10^{-12}$) (Fig. 2). Subjects with the highest ΔEE had the lowest lipid oxidation in the fasting state ($P = 0.004$) and during the hyperinsulinemic clamp ($P = 9.2 \times 10^{-12}$). In the fasting state, FFA levels were not different among the tertiles ($P = 0.417$), whereas during the hyperinsulinemic clamp, subjects with the highest ΔEE had the lowest levels of FFAs ($0.05 \pm 0.03$ vs. $0.04 \pm 0.02$ vs. $0.03 \pm 0.03$ mmol/l, $P = 8.7 \times 10^{-7}$).

To evaluate variables associated with the rates of WBGU/LBM during the hyperinsulinemic clamp, we per-

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**FIG. 1.** A: Correlation between the rates of WBGU and fasting EE (univariate linear regression). Correlation between the rates of WBGU and EE during the hyperinsulinemic clamp (B) and correlation between the rates of WBGU and ΔEE (defined as EE during the clamp − EE in the fasting state) (C). Rates of WBGU in the lowest (□), middle (○), and highest (□) EE tertiles according to fasting EE (D), EE during the hyperinsulinemic clamp (E), and the ΔEE (F). Data are means ± SD (D–F).
formed univariate linear regression analysis (Table 1). High ΔEE exhibited the strongest association with high WBGU/LBM, followed by low levels of low intra-abdominal adipose tissue mass and low total triglycerides. Low lipid oxidation during the hyperinsulinemic clamp and low subcutaneous adipose tissue mass were also associated with insulin sensitivity. In multivariate regression analyses, a model including ΔEE and intra-abdominal adipose tissue as independent variables explained a higher proportion of the variance of WBGU/LBM ($R^2 = 0.431, P = 1.7 \times 10^{-24}$) than did ΔEE alone ($R^2 = 0.362$), but adding age and sex into this model did not improve substantially the $R^2$ value ($R^2 = 0.436, P = 5.4 \times 10^{-23}$). A model that included ΔEE and subcutaneous adipose tissue mass as independent variables was not more strongly associated with WBGU/LBM ($R^2 = 0.379, P = 8.0 \times 10^{-21}$) than was ΔEE alone.

**SIRT1 mRNA expression correlation with EE, insulin sensitivity, and SIRT1 target genes.** To explore the determinants of insulin-stimulated EE and WBGU/LBM, we measured adipose tissue mRNA expression of SIRT1 and PGC-1α. SIRT1 mRNA expression correlated significantly with EE ($r = 0.289, P = 0.010$) and with WBGU/LBM ($r = 0.334, P = 0.002$) during the euglycemic clamp (Fig. 3). No statistically significant correlation was found between SIRT1 expression and EE in the fasting state ($r = 0.142$). The correlation between SIRT1 expression and PGC-1α expression was 0.448 ($P = 3.5 \times 10^{-5}$). PGC-1α expression correlated significantly only with WBGU/LBM ($r = 0.387, P = 3.9 \times 10^{-4}$) but not with EE during the clamp ($r = 0.167$).

We also measured adipose tissue mRNA levels of several target genes of SIRT1 and PGC-1α (Table 2). SIRT1 mRNA expression correlated significantly with PGC-1β expression, estrogen-related receptor (ERR) α, nuclear respiratory factor-1 (NRF-1), mitochondrial transcription factor A (TFAM), catalase (CAT), and with several genes of the respiratory chain, including NADH dehydrogenase (ubiquinone) 1α subcomplex 2, cytochrome c, cytochrome c oxidase subunit IV, and ATP synthase. SIRT1 mRNA expression also correlated with the expression of soluble superoxide dismutase 1. The correlations of mRNA expression of these genes with PGC-1α expression were quite similar but somewhat weaker. Neither SIRT1 mRNA expression nor PGC-1α mRNA expression correlated with superoxide dismutase 2.

**SIRT1 mRNA expression correlation in adipose tissue and skeletal muscle.** In 11 subjects who had both adipose tissue and skeletal muscle biopsy, the correlation of SIRT1 mRNA expression in these tissues was 0.655 ($P = 0.029$). Mitochondrial DNA in skeletal muscle also correlated positively with SIRT mRNA expression in adipose tissue ($r = 0.519$) and skeletal muscle ($r = 0.533$), although the correlations were not statistically significant due to a small sample size (supplemental Table 2 in the online appendix [available at http://diabetes.diabetesjournals.org/cgi/content/full/db09-1191/DC1]).

**SIRT1 mRNA expression correlation with cytokines and adhesion molecules.** SIRT mRNA expression negatively correlated with hs-CRP ($r = -0.241, P = 0.039$), but...
Our study demonstrated that insulin-stimulated increase in EE was strongly associated with insulin sensitivity in offspring of patients with type 2 diabetes. Furthermore, we showed for the first time that adipose tissue SIRT1 mRNA expression correlated with EE and insulin sensitivity during hyperinsulinemia. Moreover, SIRT1 expression correlated with the expression of several genes regulating the mitochondrial function.

In our study, high EE during hyperinsulinemia, and particularly high ΔEE, were strongly associated with insulin-stimulated WBGU/LBM. These results agree with previous studies (16,17) including relatively small samples of lean and obese subjects. Hyperinsulinemic clamp simulates the postprandial state with high insulin levels that promote the glucose flux from circulating blood into insulin sensitive tissues. An 8-h insulin infusion in humans has been shown to increase mitochondrial mRNA transcript levels, mitochondrial protein synthesis, and ATP production (18). This response was, however, blunted in type 2 diabetic patients. In another study (19), diabetic patients exhibited lower ATP production rate in response to high-dose insulin infusion compared with that in nondiabetic individuals. Thus, impaired mitochondrial fitness could be a consequence of impaired insulin action as supported by a study in mice fed a high-fat, high-sucrose diet showing that mitochondrial alterations do not precede the onset of insulin resistance (20). In agreement with this notion, a recent study (21) in mice demonstrated a direct effect of SIRT1 on insulin sensitivity by repressing PTP1B. Whether this mechanism is working also in humans needs to be shown.

Alternatively, primary mitochondrial dysfunction could lead to insulin resistance. An attractive possibility to explain a causal link between impaired mitochondrial function and insulin resistance is the hypothesis that impaired OXPHOS capacity leads to intramyocellular lipid accumulation (9) and thus impaired insulin signaling and insulin resistance (22). High lipid levels in the circulating blood impair insulin-stimulated ATP production in humans (23). We observed that subjects with low insulin-stimulated EE also had higher levels of FFAs, higher lipid oxidation, and lower respiratory quotient during the hyperinsulinemic clamp, reflecting changes in fuel selection in these subjects, which often lead to insulin resistance.

Further evidence supporting the hypothesis that mitochondrial activity stimulated by SIRT1 might be important for energy metabolism and insulin action are high correlations of adipose tissue SIRT1 mRNA expression with expression of genes regulating mitochondrial function. SIRT1 mRNA expression correlated significantly with otherwise the correlations with cytokines and adhesion molecules were almost entirely nonsignificant (supplementary Table 1).

**Sirt1 mRNA and protein correlation.** Subcutaneous adipose tissue from 5-month-old female mice was obtained and used for quantitative RT-PCR and Western blot analyses. We observed a strong correlation between Sirt1 mRNA and Sirt1 protein expression levels \( (r = 0.882, P < 0.001) \) (Fig. 4).

**DISCUSSION**

**TABLE 2**

<table>
<thead>
<tr>
<th>Gene</th>
<th>SIRT1</th>
<th>PGC-1α</th>
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<tbody>
<tr>
<td>PGC-1β</td>
<td>( r = 0.358 )</td>
<td>( r = 0.152 )</td>
</tr>
<tr>
<td>NRF1</td>
<td>( r = 0.286 )</td>
<td>( r = 0.235 )</td>
</tr>
<tr>
<td>ERRα</td>
<td>( r = 0.339 )</td>
<td>( r = 0.260 )</td>
</tr>
<tr>
<td>TFAM</td>
<td>( r = 0.379 )</td>
<td>( r = 0.213 )</td>
</tr>
<tr>
<td>NDUFA2</td>
<td>( r = 0.392 )</td>
<td>( r = 0.273 )</td>
</tr>
<tr>
<td>CYCS</td>
<td>( r = 0.263 )</td>
<td>( r = 0.159 )</td>
</tr>
<tr>
<td>COX41</td>
<td>( r = 0.332 )</td>
<td>( r = 0.262 )</td>
</tr>
<tr>
<td>ATP5G1</td>
<td>( r = 0.248 )</td>
<td>( r = 0.196 )</td>
</tr>
<tr>
<td>SOD1</td>
<td>( r = 0.460 )</td>
<td>( r = 0.348 )</td>
</tr>
<tr>
<td>SOD2</td>
<td>( r = -0.046 )</td>
<td>( r = -0.009 )</td>
</tr>
<tr>
<td>CAT</td>
<td>( r = 0.350 )</td>
<td>( r = 0.422 )</td>
</tr>
</tbody>
</table>

Expression of all genes was normalized to RPL0 expression. ATP5G1, ATP synthase, H + transporting, mitochondrial F0 complex, subunit C1; CAT, catalase; COX41, cytochrome c oxidase subunit IV isoform 1; CYCS, cytochrome c, somatic; NDUFA2, NADH dehydrogenase (ubiquinone) 1 α subcomplex 2; SOD1, superoxide dismutase 1, soluble; SOD2, superoxide dismutase 2, mitochondrial.

FIG. 3. A: Correlation of adipose tissue SIRT1 mRNA expression level with EE during the hyperinsulinemic clamp. B: Correlation of adipose tissue SIRT1 mRNA expression level with the rates of whole-body glucose uptake in offspring of type 2 diabetic patients.
poorer correlation of SIRT1 mRNA with plasma levels of inflammatory markers was modest. This may reflect a correlation of adipose tissue SIRT1 mRNA with other negatively associated with hs-CRP level, which is in agreement with previous studies in mice (4,29). Adipose tissue SIRT1 mRNA expression was negatively associated with hs-CRP level, which is in agreement with recent studies in mice (4,29). Transgenic mice overexpressing SIRT1 (30) or mice having SIRT1 activity enhanced by the administration of the SIRT1 agonist, resveratrol (4,29), or drugs (32) and an inventor on Harvard patents licensed to Sirtris Pharmaceuticals. No other potential conflicts of interest relevant to this article were reported. The authors thank the members of the Laakso and Auwerx laboratories for discussions and technical assistance.

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4. Laakso M, Auwerx J. SIRT1, ENERGY EXPENDITURE, INSULIN SENSITIVITY

FIG. 4. A: Sirt1 protein expression Western blots in subcutaneous adipose tissue from six 5-month-old female mice (numbered from 1 to 6). GAPDH was used as a loading control. B: Correlation of Sirt1 mRNA expression level with Sirt1 protein expression level (r = 0.882, P = 0.020). A mean value of triplicates was used for Sirt1 mRNA level.
mitochondria and relationship to glucose control in type 2 diabetes mellitus. Diabetes 2007;56:2142–2147