<table>
<thead>
<tr>
<th>Citation</th>
<th>Rutanen, Jarno, Nagendra Yaluri, Shalem Modi, Jussi Pihlajamäki, Markku Vänttinen, Paula Itkonen, David A. Sinclair, et al. 2010. SIRT1 mRNA expression may be associated with energy expenditure and insulin sensitivity. Diabetes 59(4): 829-835.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td><a href="https://doi.org/10.2337/db09-1191">doi://10.2337/db09-1191</a></td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:10251496">http://nrs.harvard.edu/urn-3:HUL.InstRepos:10251496</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>

(Article begins on next page)
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 × 10⁻³) 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 type 2 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 (probands) 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.

From the 1Department of Medicine, University of Kuopio and Kuopio University Hospital, Kuopio, Finland; the 2Department of Clinical Radiology, University of Kuopio, Kuopio, Finland; the 3Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, Illkirch, France; the 4Laboratory of Integrative and Systems Physiology, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; the 5Department of Pathology and Glenn Labs for Aging Research, Harvard Medical School, Boston, Massachusetts; and 6Sirtris Pharmaceuticals, Cambridge, Massachusetts.

Corresponding author: Markku Laakso, markku.laakso@kuh.fi.


© 2010 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

diabetes.diabetesjournals.org
were centrifuged for 30 min at high speed, and supernatant was collected and used for Sirt1 protein expression studies in mice. After homogenization, tissue lysates were normalized to the copy number of GAPDH.

were purchased from Applied Biosystems. Sirt1 expression (Mm01168521_m1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Mm99999915_g1) were purchased from Applied Biosystems. Inventoried Taqman primers for Sirt1 were used to design specific primers and probes (Applied Biosystems) in a seven 500 Real-Time PCR System (Applied Biosystems) using 6 ng (RNA equivalents) of cDNA as template, gene-specific primers, and probes (Applied Biosystems) (coded as a family number) as a random factor, the tertiles as fixed factors, and sex and age as covariates.

RESULTS

Energy expenditure and insulin sensitivity. EE during the clamp positively correlated with insulin sensitivity ($r = 0.375, P = 2.8 \times 10^{-05}$) (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 ($r = 0.602, P = 2.5 \times 10^{-24}$). 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/LBM 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 $\mu$mol/kg LBM/min, $P = 2.2 \times 10^{-05}$), which was attributable to both high-glucose oxidative (19.54 ± 5.42 vs. 20.96 ± 5.62 vs. 22.75 ± 6.11 $\mu$mol/kg LBM/min, $P = 0.007$) and nonoxidative (30.31 ± 12.81 vs. 34.06 ± 13.24 vs. 40.68 ± 16.52 $\mu$mol/kg LBM/min, $P = 1.2 \times 10^{-05}$) glucose disposal. These differences were even more pronounced across the tertiles of $\Delta$EE/LBM, where subjects in the highest tertile had highest WBGU/LBM (43.82 ± 13.25 vs. 55.75 ± 13.64 vs. 67.96 ± 16.31 $\mu$mol/kg LBM/min, $P = 2.5 \times 10^{-05}$), attributable to both high-glucose oxidative (17.51 ± 4.34 vs. 20.81 ± 5.45 vs. 24.58 ± 5.31 $\mu$mol/kg LBM/min, $P = 5.9 \times 10^{-15}$) and nonoxidative (20.31 ± 12.08 vs. 34.94 ± 12.24 vs. 43.38 ± 15.16 $\mu$mol/kg LBM/min, $P = 3.6 \times 10^{-15}$) glucose disposal.

Subjects in the highest $\Delta$EE tertile used more glucose for energy production than did subjects in the lower $\Delta$EE
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 ($P = 1.6 \times 10^{-4}$) and during the hyperinsulinemic clamp ($P = 9.2 \times 10^{-35}$). 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 performed univariate linear regression. Correlation between the rates of WBGU and fasting EE (Fig. 1A), correlation between the rates of WBGU and ΔEE (defined as EE during the clamp – EE in the fasting state) (Fig. 1B), and correlation between the rates of WBGU and ΔEE (defined as EE during the clamp – EE in the fasting state) (Fig. 1C). Rates of WBGU in the lowest (□), middle (■), and highest (▲) EE tertiles according to fasting EE (Fig. 1D), EE during the hyperinsulinemic clamp (Fig. 1E), and the ΔEE (Fig. 1F). Data are means ± SD (Fig. 1D–F).
formed univariate linear regression analysis (Table 1). High ∆EE 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 ∆EE 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 ∆EE 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 ∆EE 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 ∆EE 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) 1 (complex 1) 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

---

**TABLE 1**

Variables associated with the rates of WBGU/LBM (univariate linear regression model, $n = 247$)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Standardized coefficient</th>
<th>$R^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆EE (clamp – fasting)</td>
<td>0.602</td>
<td>0.362</td>
<td>2.5 $\times 10^{-24}$</td>
</tr>
<tr>
<td>Intra-abdominal adipose tissue</td>
<td>-0.497</td>
<td>0.247</td>
<td>3.1 $\times 10^{-14}$</td>
</tr>
<tr>
<td>Total triglycerides</td>
<td>-0.480</td>
<td>0.230</td>
<td>1.3 $\times 10^{-15}$</td>
</tr>
<tr>
<td>Lipid oxidation/LBM during the clamp</td>
<td>-0.438</td>
<td>0.192</td>
<td>1.7 $\times 10^{-12}$</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue</td>
<td>-0.338</td>
<td>0.114</td>
<td>6.8 $\times 10^{-7}$</td>
</tr>
<tr>
<td>EE/LBM in the fasting state</td>
<td>-0.004</td>
<td>0.000</td>
<td>0.946</td>
</tr>
</tbody>
</table>

---

**FIG. 2.**

A: Respiratory quotient in the fasting state and during the hyperinsulinemic-euglycemic clamp in the tertiles of ∆EE. Data are means ± SD in the lowest (●), middle (■), and highest (▲) tertile. Lipid oxidation in the fasting state and during the hyperinsulinemic-euglycemic clamp in the tertiles of ∆EE (B) and FFAs in the fasting state and during the hyperinsulinemic-euglycemic clamp in the tertiles of ∆EE (C). Data are means ± SD.

---

832 DIABETES, VOL. 59, APRIL 2010
diabetes.diabetesjournals.org
We observed a strong correlation between Sirt1 and used for quantitative RT-PCR and Western blot analyses of 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 ($r = 0.582, P < 0.001$) (Fig. 4).

**DISCUSSION**

Our study demonstrated that insulin-stimulated increase in EE was strongly associated with insulin sensitivity in offspring of type 2 diabetic patients. 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 ∆EE, 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 stimulates 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 non-diabetic 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 production.
PGC-1β expression, which has several overlapping functions with PGC-1α in inducing genes related to OXPHOS (24). Significant correlations of SIRT1 mRNA expression were also observed with ERRα, which mediates many of the downstream effects of activated PGC-1α on mitochondrial function (25), with NRF-1, which is an ERRα/PGC-1α target, and with TFAM, which is a target of NRF-1 (26). Furthermore, SIRT1 mRNA levels correlated with expression of several genes regulating the respiratory chain. SIRT1 mRNA expression level also correlated with the expression of soluble superoxide dismutase 1, which protects the cell from superoxide toxicity, and with catalase, which catalyzes the decomposition of hydrogen peroxide to water and oxygen (27). Because the accumulation of reactive oxygen species can induce insulin resistance (28), the role of SIRT1 in governing superoxide dismutase 1 and catalase expression could cooperate with the effects of SIRT1 on the control of OXPHOS to improve insulin sensitivity. Our findings are also 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 a small molecule activator of SIRT1 (6), were both leaner, more hypermetabolic, and showed favorable effects on glucose and lipid metabolism. Mice treated with SIRT1 agonists, a potent synthetic activator of SIRT1, enhanced insulin sensitivity (31). Furthermore, in human studies a SIRT1 activator (SRT501) has been shown to improve glucose control (32). Adipose tissue SIRT1 mRNA expression was negatively associated with hs-CRP level, which is in agreement with the anti-inflammatory effect of SIRT1 (33). The correlation of adipose tissue SIRT1 mRNA with other inflammatory markers was modest. This may reflect a poorer correlation of SIRT1 mRNA with plasma levels of cytokines compared with cytokine expression in adipose tissue.

SIRT1 mRNA expression in adipose tissue had a high correlation with skeletal muscle SIRT1 mRNA (r = 0.655). Therefore, we believe that our results obtained in adipose tissue reflect metabolic changes in skeletal muscle, which is the main tissue for EE and insulin sensitivity during insulin stimulation. Skeletal muscle mitochondrial DNA correlated closely with SIRT1 mRNA expression in skeletal muscle and adipose tissue, giving evidence that upregulation of the genes regulating mitochondrial biogenesis in adipose tissue and likely to reflect corresponding changes in skeletal muscle (Table 2). Finally, we demonstrated in mice that Sirt mRNA expression and Sirt1 protein levels were highly correlated (Fig. 4), demonstrating that our results are likely to be valid also at protein level. However, the limitation of our study is that we could not determine SIRT1 protein level from adipose tissue biopsies due to a small amount of tissue that we can obtain using needle biopsy techniques.

In summary, we demonstrated that insulin-stimulated EE is strongly associated with insulin-stimulated glucose uptake in offspring of subjects with type 2 diabetes. Impaired stimulation of EE by insulin is likely to reflect impaired regulation of mitochondrial function in insulin-resistant states. This could be at least partially explained by low expression of SIRT1 and PGC-1α, two important master regulators of mitochondrial activity. Even though it is not possible to determine the primary defect from our cross-sectional data, disturbance in mitochondrial function and low EE were strongly associated with impaired insulin-stimulated glucose uptake. Our results give evidence that activating SIRT1 could be one of the potential mechanisms to treat insulin resistance and patients with type 2 diabetes.

ACKNOWLEDGMENTS

Work in the Laakso laboratory was supported by the Academy of Finland and the EU FP6 (EUGENE; LSHM-CT-2004-512013). Work in the Auwerx laboratory was supported by grants from the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Université Louis Pasteur, the Ecole Polytechnique Fédérale de Lausanne, the National Institutes of Health (DK069966), and the EU FP6 (EUGENE2; LSHM-CT-2004-512013). H.Y. was supported by the Fondation Recherche Médicale.

D.A.S. is a consultant to Sirtris Pharmaceuticals (a GlaxoSmithKline company developing sirtuin-based drugs) and an inventor on Harvard patents licensed to GlaxoSmithKline. P.E. was and C.W. is employed by 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.

REFERENCES

3. Toledo FG, Menshikova EV, Ritov VB, Azuma K, Radikova Z, Delany J, Kelley DE. Effects of physical activity and weight loss on skeletal muscle...
mitochondria and relationship to glucose control in type 2 diabetes mellitus. Diabetes 2007;56:2142–2147