

## Reduced expression of mitochondrial frataxin in mice exacerbates diet-induced obesity

Doreen Pomplun, Anja Voigt, Tim J. Schulz, René Thierbach, Andreas F. Pfeiffer, and Michael Ristow

*PNAS* published online Apr 2, 2007;  
doi:10.1073/pnas.0611631104

**This information is current as of April 2007.**

<b>E-mail Alerts</b>	This article has been cited by other articles: <a href="http://www.pnas.org#otherarticles">www.pnas.org#otherarticles</a>
<b>Rights &amp; Permissions</b>	Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or <a href="#">click here</a> .
<b>Reprints</b>	To reproduce this article in part (figures, tables) or in entirety, see: <a href="http://www.pnas.org/misc/rightperm.shtml">www.pnas.org/misc/rightperm.shtml</a>
	To order reprints, see: <a href="http://www.pnas.org/misc/reprints.shtml">www.pnas.org/misc/reprints.shtml</a>

Notes:

# Reduced expression of mitochondrial frataxin in mice exacerbates diet-induced obesity

Doreen Pomplun<sup>\*†</sup>, Anja Voigt<sup>‡</sup>, Tim J. Schulz<sup>\*†</sup>, René Thierbach<sup>‡</sup>, Andreas F. Pfeiffer<sup>\*</sup>, and Michael Ristow<sup>\*†§</sup>

<sup>\*</sup>Department of Clinical Nutrition, German Institute of Human Nutrition, D-14558 Potsdam-Rehbrücke, Germany; <sup>†</sup>Department of Human Nutrition, Institute of Nutrition, University of Jena, D-07743 Jena, Germany; and <sup>‡</sup>Institute of Nutrition, Department of Nutritional Toxicology, University of Potsdam, D-14558 Potsdam, Germany

Communicated by Bruce N. Ames, University of California, Berkeley, Oakland, CA, December 28, 2006 (received for review November 18, 2006)

**Published evidence suggests that adiposity in humans may be linked to impaired energy expenditure for reasons widely unresolved. We have generated mice with a systemic impairment of oxidative phosphorylation (OXPHOS) due to *aP2 cre*-mediated targeted disruption, and unexpectedly ubiquitous reduction of mitochondrial frataxin protein expression. Only when maintained on a high-calorie diet resembling Westernized eating habits, these animals accumulate additional body fat, leading to increased body mass, and develop diabetes mellitus, despite the fact that both calorie uptake and physical activity were identical to that in control animals. This phenotype is caused by a mild but significant reduction in total energy expenditure paralleled by increased expression of ATP citrate lyase, a rate-limiting step in *de novo* synthesis of fatty acids and triglycerides. Taken together, these findings indicate that a limited impairment in oxidative metabolism within the mitochondria directly predisposes mammals to excessive body weight gain.**

metabolism | mitochondria | oxidative phosphorylation

**M**itochondria are subcellular organelles where conversion of nutrient intermediates into readily available energy equivalents takes place. This conversion involves a process called oxidative phosphorylation (OXPHOS) generating adenosine triphosphate (ATP). Recent scientific evidence has implicated mitochondrial dysfunction in several human diseases, including cancer (1–4), neurodegenerative disorders (5, 6), and type 2 diabetes mellitus (7–12). Of note, the latter disease is frequently associated with increased body mass (13, 14). Obesity is a major risk factor for the development of type 2 diabetes, presumably by causing insulin resistance, i.e., impaired insulin action on peripheral tissues including adipocytes and skeletal muscle (14, 15).

Excessive accumulation of body fat in humans is the most prevalent health problem in Westernized countries. It is generally accepted that obesity is caused by an imbalance of energy uptake and energy expenditure, cumulating in storage of excess calories mainly as body fat (13). Accordingly, it has been known for many years that an unexplained decrease in energy expenditure results in a predisposition to obesity in humans (16). Because most nutritive energy is ultimately converted within the mitochondria, researchers have repeatedly addressed the question whether altered mitochondrial activity might influence body mass in mammals. For example, thyroid hormones have been shown to regulate body mass by altering mitochondrial metabolism (17). Furthermore, increased expression of mitochondrial uncoupling proteins (UCPs) or a transcriptional coactivator, PGC1, has been suggested to promote systemic energy expenditure because of thermogenic effects, i.e., generation of heat (18–21) and a concurrent decrease in OXPHOS (22).

We here address the question whether a protein known to cause a neurodegenerative disorder named Friedreich ataxia ([www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=229300](http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=229300)) might be used as a regulator of OXPHOS capacity in mammals. This protein is called frataxin and has been shown to be encoded in the nucleus whereas its N-terminal localization signal di-

rects it posttranslationally mainly into the mitochondrial matrix compartment ([www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=229300](http://www.ncbi.nlm.nih.gov/entrez/dispmim.cgi?id=229300)). Overexpression studies have shown that frataxin increases mitochondrial membrane potential, oxygen consumption and ATP synthesis, and hence might be considered as an inducer of OXPHOS in mammalian cells *in vitro* (23, 24). The primary function of frataxin is to direct the mitochondrial synthesis of iron-sulfur clusters (Fe/S) (25, 26), which are essential parts of several mitochondrial enzymes, including aconitase, and complexes I, II and III of the respiratory chain (27). Accordingly, mammals with impaired frataxin expression exhibit tissue-specific impairments of the corresponding enzyme activities, whereas other mitochondrial enzymes remain unaffected (2).

By reducing expression of frataxin in mice we have now generated a model with limited impairment of OXPHOS in a non-tissue specific manner. These mice develop increased obesity and glucose intolerance only when maintained on a so-called Western diet because of impaired energy expenditure and concomitant increase of a rate-limiting step in *de novo* lipogenesis. These findings indicate that mildly impaired OXPHOS directly predisposes mice to obesity.

## Results

We originally aimed to disrupt expression of murine frataxin in an adipose-tissue specific manner by employing mice carrying loxP-flanked frataxin alleles (28) which were intercrossed with aP2-cre mice (29). The latter have been successfully used to disrupt expression of the glucose transporter GLUT4 (29) and the insulin receptor (30, 31) in adipose tissue only. In contrast, loxP-flanked animals intercrossed with the original aP2 cre line (29) in our hands showed an ubiquitous but incomplete disruption of the frataxin gene (Fig. 1), whereas other frataxin knockout animals generated by using an Albumin-cre line showed a complete and tissue specific disruption of the gene of interest [Fig. 1A, “control knockout” and (2)]. The reason for this inconsistency of expression patterns of this particular aP2-cre line in our hands versus previously published data (29–31) remains to be evaluated. Nevertheless, disruption of frataxin expression lead to a significantly reduced expression of frataxin protein in all tissues evaluated (Fig. 1B and additional data not shown). Of note and unlike mice with neuronal disruption of frataxin (28), our mice did not show any signs of ataxia,

Author contributions: D.P. and A.V. contributed equally to this work; A.F.P. and M.R. designed research; D.P., A.V., T.J.S., and R.T. performed research; D.P., A.V., T.J.S., and R.T. analyzed data; and R.T. and M.R. wrote the paper.

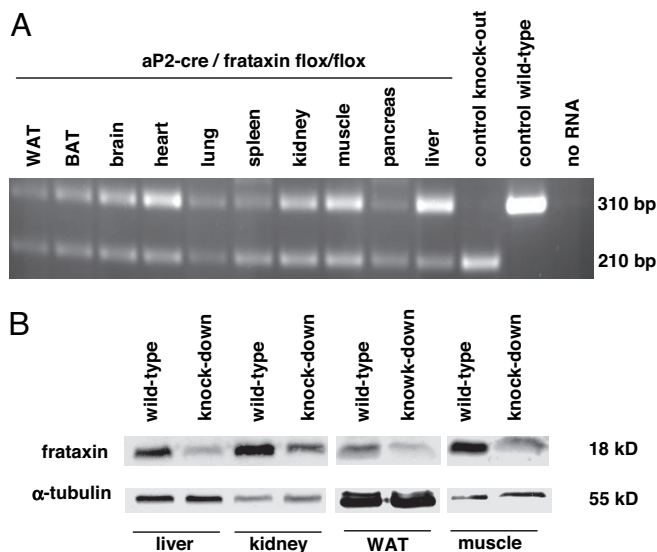
The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Abbreviations: OXPHOS, oxidative phosphorylation; UCP, uncoupling protein.

<sup>§</sup>To whom correspondence should be addressed at: University of Jena, Institute of Nutrition, Department of Human Nutrition, 29 Dornburger Stasse, D-07743 Jena, Germany. E-mail: [mrlistow@mrlistow.org](mailto:mrlistow@mrlistow.org).

© 2007 by The National Academy of Sciences of the USA



**Fig. 1.** Ubiquitous partial deletion of exon 4 of the *frataxin* gene after *aP2 promoter* controlled expression of *cre* recombinase. (A) Depicted is an RT-PCR using primers located in exon 3 and exon 5 of the murine *frataxin* gene, while loxP sites flanked exon 4 (2, 10, 28); “control knock-out” refers to an *albumin-cre*-controlled complete *frataxin* knockout (2), and “control wild-type” refers to an animal lacking any *cre* transgene. (B) Systemic reduction of *frataxin* protein expression shown by an immunoblot using a primary antibody against murine *frataxin* (Upper lane) and a primary antibody against alpha-tubulin (Lower lane, loading control).

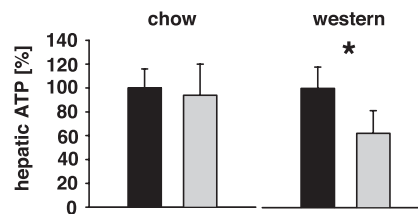
indicating that these animals should not necessarily be considered a model for Friedreich ataxia.

Based on previously published findings that *frataxin* promotes synthesis of iron-sulfur clusters (Fe/S) (25, 26) and thus induces OXPHOS (23), we first questioned whether *aP2-frataxin* knockdown mice would show impaired ATP synthesis. Whereas complete disruption of *frataxin* in, e.g., hepatic tissue causes a severe reduction of ATP content by  $\approx 80\%$  in the fed state (2), *aP2-frataxin* knockdown mice showed a significant reduction of hepatic ATP content in the fasted, i.e., overnight food-deprived state only (reduction by 38%, data not depicted). Given the fact that mitochondrial activity in yeast (32) and mammals (33) is preferably induced in states of food deprivation, it is not surprising that differences in OXPHOS capacity become unmasked preferably in the fasted state.

At an age of 4 weeks, *aP2-frataxin* knockdown mice were matched by sex and body mass, and then randomly assigned into two groups. One group was kept on fiber-rich, low-fat standard rodent chow (Table 1), whereas the other group was maintained on a so-called Western diet enriched in sugars, proteins and fat (Table 1). Based on the above-mentioned differences in e.g., hepatic ATP content in fasted state we wondered whether the dietary regimen would affect OXPHOS, i.e., ATP content. Indeed, whereas no differences in e.g., hepatic ATP within fed animals was seen between knockdown and control mice when

**Table 1. Composition of diets**

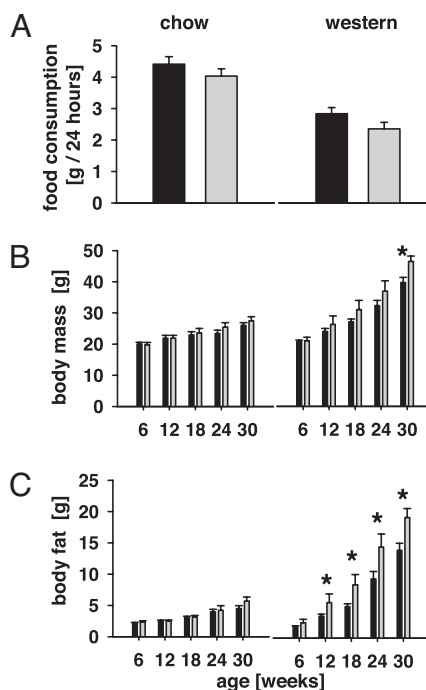
Type	Standard chow	Western diet
Proteins, g/kg	190	265
Polysaccharides, g/kg	317	177
Disaccharides, g/kg	54	108
Lipids, g/kg	40	209
Cholesterol, g/kg	<0.001	0.015
Convertible energy, kJ/g	9	17



**Fig. 2.** Limited impairment of oxidative phosphorylation in *aP2-frataxin* knockdown mice. Relative hepatic ATP content in the fed state depicted for mice on standard rodent chow (Left) and Western diet (Right). Black bars, control animals (100%); gray bars, *frataxin* knockdown animals (colors apply to all subsequent panels) ( $n = 6$  per genotype). Error bars, SEM; \*,  $P < 0.05$ .

maintained on standard chow (Fig. 2), knockdown mice showed a significant reduction in ATP content when fed a Western diet (Fig. 2). Taken together, this finding indicated that knockdown mice have a limited impairment of OXPHOS irrespective of the diet in the fasted state, and reduced OXPHOS capacity in the fed state only when maintained on a Western diet.

When comparing standard chow and Western diet-fed animals, daily food uptake was similar between *aP2-frataxin* knockdown mice and control animals in each group (Fig. 3A). Nevertheless and consistent with previously published evidence, animals regardless of the genotype consumed more food when maintained on low-calorie chow in comparison with animals kept on Western diet (Fig. 3A). Accordingly, daily energy uptake in control animals versus knockdown mice was  $38.9 \pm 2.2$  versus  $36.3 \pm 2.1$  kJ/day when kept on a standard chow, whereas animals maintained on a Western diet ingested  $48.2 \pm 3.3$  versus  $40.0 \pm 3.6$  kJ per day (controls vs. knockdown). Given the findings in regards to reduced ATP content (Fig. 2), these data



**Fig. 3.** Eating behavior and body composition in *aP2-frataxin* knockdown mice. (A) Food uptake per individual mouse, depicted for mice on standard rodent chow (Left) and Western diet (Right) ( $n = 6$  per genotype). (B) Body mass in mice with impaired mitochondrial capacity on standard rodent chow (Left) and on Western diet (Right) ( $n = 8$  per genotype). (C) Body fat content in mice with impaired mitochondrial capacity on standard rodent chow (Left) and on Western diet (Right) ( $n = 8$  per genotype). \*,  $P < 0.05$ .



expression of ATP citrate lyase, a rate-limiting step in fatty acid synthesis.

In higher eukaryotic organisms, dissipation of nutritive energy mainly occurs via the mitochondria. Our findings suggest that increased long-term storage of nutritional energy as triglycerides may compensate for states of reduced OXPHOS capacity, leading to reduced amounts of short-term, energy-rich intermediates, namely ATP. These findings may explain why an unexplained decrease in energy expenditure results in a predisposition to obesity in humans (16). In this regard, impaired systemic substrate oxidation has been suggested to be a predisposing factor for obesity (35). Given the fact that in our model similar rates of food uptake and physical activity nevertheless cause significant differences in body mass and body triglyceride content, impaired OXPHOS capacity might possibly provide a biochemical basis for striking phenotypical differences in humans despite very similar eating habits. They might also provide a mechanistic basis for the well-known fact that human body mass typically increases with age, because OXPHOS capacity in humans has been shown to be significantly decreased in the elderly (36) suggesting that a similar tendency may apply to middle-aged individuals as well.

It has been shown that thyroid hormones as well as polyunsaturated fatty acids, both known to limit body mass gain, induce OXPHOS in e.g., isolated hepatocytes (37). Conversely, thyroid hormones induce expression of several components of the respiratory chain (38). In obese humans known to show impaired mitochondrial substrate oxidation (35), reduced thyroid function was proposed to be a potential cause for increased body weight (17) and reduced oxidative capacity (39, 40) potentially by induction of mitochondrial uncoupling (41). The latter, also named adaptive thermogenesis, was proposed to induce energy expenditure by dissipation of energy after a thermogenic proton leak reducing the mitochondrial membrane potential, ultimately leading to reduced long-term energy storage. Specifically, several UCPs, as well as subtypes of the transcriptional coactivator PGC1, have been shown to increase energy expenditure presumably by generation of heat (18–21). In regards to the present model, it should be noted that UCPs reduce OXPHOS capacity, i.e., limit ATP production (22). In contrast, there is sufficient evidence that frataxin rather induces OXPHOS by increasing mitochondrial membrane potential (23, 24). Accordingly the effects of thermogenic activators, including UCPs and PGC1 on body mass may be considered divergent from an induction of OXPHOS by other mitochondrial proteins, namely frataxin.

Taken together, our findings suggest that reduced expression of frataxin mildly decreases OXPHOS capacity to significantly promote *de novo* synthesis of triglycerides and hence to exacerbate

obesity in mammals. These findings suggest that a limited systemic reduction in oxidative capacity due to genetic or environmental reasons may predispose mammals to body mass gain.

## Materials and Methods

**Targeted Disruption of Frataxin Gene.** Frataxin was disrupted by removing exon 4 of the corresponding gene in C57BL/6 mice as described (2, 10, 28) except that systemic expression of Cre recombinase was obtained by using a subline of mice carrying an aP2 promoter-driven Cre transgene (29). Expression of aP2-driven Cre recombinase was obtained with a single founder kindly provided by BIDMC (Boston, MA) reported to be >90% C57BL/6.

**Hepatic ATP Content.** Methods have been described (2, 42), except that HPLC equipment was from Jasco (MD-1510; Gross-Umstadt, Germany) and a Jasco reverse phase column was used.

**Housing and Physiologic Measurements of Mice.** Housing conditions and methods for determination of body mass, body composition, locomotor activity, and energy expenditure have been described (10, 43). Footprint analysis to detect possible signs of ataxia has been described (44).

**Immunoblotting.** Methods have been described (2). Additional antibodies were against basal ATP citrate lyase and phosphorylated ATP citrate lyase (both from Cell Signaling Technology, Danvers, MA).

**i.p. Glucose Tolerance Tests.** Methods have been described (10) and were preferred over oral glucose tolerance tests because the i.p. application of glucose requires no anesthesia known to severely affect blood glucose levels (45).

**Determination of Serum Insulin Levels.** Methods have been described (10).

**Statistical Analyses.** Methods have been described (10).

We thank Susann Richter and Elke Thom for excellent technical assistance; Barbara B. Kahn (Beth Israel Deaconess Medical Center) for providing the aP2-cre line; and Barbara B. Kahn, Hélène Puccio, and Randy J. Seeley for comments on the manuscript. This study was funded by grants from the Deutsche Forschungsgemeinschaft (DFG), the Wilhelm-Sander-Stiftung, and the Fritz-Thyssen-Stiftung (all to M.R.).

- Ames BN (2005) *EMBO Rep* 6(Spec No):S20–S24.
- Thierbach R, Schulz TJ, Isken F, Voigt A, Mietzner B, Drewes G, von Kleist-Retzow JC, Wiesner RJ, Magnuson MA, Puccio H, et al. (2005) *Hum Mol Genet* 14:3857–3864.
- Ristow M (2006) *Curr Opin Clin Nutr Metabol* 9:339–345.
- Schulz TJ, Thierbach R, Voigt A, Drewes G, Mietzner BH, Steinberg P, Pfeiffer AF, Ristow M (2006) *J Biol Chem* 281:977–981.
- Ames BN (2004) *Ann NY Acad Sci* 1019:406–411.
- Manfredi G, Beal MF (2000) *Brain Pathol* 10:462–472.
- van den Ouweland JM, Lemkes HH, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PA, van de Kamp JJ, Maassen JA (1992) *Nat Genet* 1:368–371.
- Ristow M, Giannakidou E, Hebinck J, Busch K, Vorgerd M, Kotzka J, Knebel B, Müller-Berghaus J, Eppelen C, Pfeiffer A, et al. (1998) *Diabetes* 47:851–854.
- Hebinck J, Hardt C, Schöls L, Vorgerd M, Briedigkeit L, Kahn CR, Ristow M (2000) *Diabetes* 49:1604–1607.
- Ristow M, Mulder H, Pomplun D, Schulz TJ, Müller-Schmehl K, Krause A, Fex M, Puccio H, Müller J, Isken F, et al. (2003) *J Clin Invest* 112:527–534.
- Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI (2004) *N Engl J Med* 350:664–671.
- Ristow M (2004) *J Mol Med* 82:510–529.
- Spiegelman BM, Flier JS (2001) *Cell* 104:531–543.
- Chiasson JL, Rabasa-Lhoret R (2004) *Diabetes* 53(Suppl 3):S34–S38.
- Kahn CR (1994) *Diabetes* 43:1066–1084.
- Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WG, Boyce V, Howard BV, Bogardus C (1988) *N Engl J Med* 318:467–472.
- Kyle LH, Ball MF, Doolan PD (1966) *N Engl J Med* 275:12–17.
- Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS (1993) *Nature* 366:740–742.
- Koepcke J, Clarke G, Enerback S, Spiegelman B, Kozak LP (1995) *J Clin Invest* 96:2914–2923.
- Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell BB, Scarpulla RC, Spiegelman BM (1999) *Cell* 98:115–124.
- St Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, Spiegelman BM (2003) *J Biol Chem* 278:26597–26603.
- Cline GW, Vidal-Puig AJ, Dufour S, Cadman KS, Lowell BB, Shulman GI (2001) *J Biol Chem* 276:20240–20244.
- Ristow M, Pfister MF, Yee AJ, Schubert M, Michael L, Zhang CY, Ueki K, Michael MD, II, Lowell BB, Kahn CR (2000) *Proc Natl Acad Sci USA* 97:12239–12243.
- Gonzalez-Cabo P, Vazquez-Manrique RP, Garcia-Gimeno MA, Sanz P, Palau F (2005) *Hum Mol Genet* 14:2091–2098.

25. Rötig A, de Lonlay P, Chretien D, Foury F, Koenig M, Sidi D, Munnich A, Rustin P (1997) *Nat Genet* 17:215–217.
26. Mühlhoff U, Richhardt N, Ristow M, Kispal G, Lill R (2002) *Hum Mol Genet* 11:2025–2036.
27. Lill R, Mühlhoff U (2005) *Trends Biochem Sci* 30:133–141.
28. Puccio H, Simon D, Cossee M, Criqui-Filipe P, Tiziano F, Melki J, Hindelang C, Matyas R, Rustin P, Koenig M (2001) *Nat Genet* 27:181–186.
29. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB (2001) *Nature* 409:729–733.
30. Blüher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR (2002) *Dev Cell* 3:25–38.
31. Blüher M, Kahn BB, Kahn CR (2003) *Science* 299:572–574.
32. Lin SJ, Kaerberlein M, Andalis AA, Sturtz LA, Defossez PA, Culotta VC, Fink GR, Guarente L (2002) *Nature* 418:344–348.
33. Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, et al. (2005) *Science* 310:314–317.
34. Castaneda TR, Jurgens H, Wiedmer P, Pfluger P, Diano S, Horvath TL, Tang-Christensen M, Tschop MH (2005) *J Nutr* 135:1314–1319.
35. Astrup A, Buemann B, Toubro S, Raben A (1996) *Proc Nutr Soc* 55:817–828.
36. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW, Shulman GI (2003) *Science* 300:1140–1142.
37. Nogueira V, Rigoulet M, Piquet MA, Devin A, Fontaine E, Leverve XM (2001) *J Biol Chem* 276:46104–46110.
38. Wiesner RJ, Kurowski TT, Zak R (1992) *Mol Endocrinol* 6:1458–1467.
39. Heald FP (1962) *J Pediatr* 61:327–330.
40. Astrup A, Buemann B, Toubro S, Ranneries C, Raben A (1996) *Am J Clin Nutr* 63:879–883.
41. Lebon V, Dufour S, Petersen KF, Ren J, Jucker BM, Slezak LA, Cline GW, Rothman DL, Shulman GI (2001) *J Clin Invest* 108:733–737.
42. Di Pierro D, Tavazzi B, Perno CF, Bartolini M, Balestra E, Calio R, Giardina B, Lazzarino G (1995) *Anal Biochem* 231:407–412.
43. Jürgens H, Haass W, Castaneda TR, Schürmann A, Koebnick C, Dombrowski F, Otto B, Nawrocki AR, Scherer PE, Spranger J, et al. (2005) *Obes Res* 13:1146–1156.
44. Simon D, Seznec H, Gansmuller A, Carelle N, Weber P, Metzger D, Rustin P, Koenig M, Puccio H (2004) *J Neurosci* 24:1987–1995.
45. Pomplun D, Mohlig M, Spranger J, Pfeiffer AF, Ristow M (2004) *Horm Metab Res* 36:67–69.