

2.28) for year 2, 0.43 (0.13 to 1.41) for year 3, 0.46 (0.22 to 0.99) for year 4, 0.52 (0.18 to 1.56) for year 5, and 0.43 (0.16 to 1.16) for year 6. The P value for the trend of the effect on the risk of colorectal cancer over time is 0.34. Thus, there is no evidence that the effect of the hormone on colorectal cancer changes over time.

Our original statement that the proportion of colorectal cancers diagnosed at an advanced stage was higher in the hormone group than in the placebo group is correct. That said, we agree with Dr. Schürmann and colleagues that a major effect of estrogen and progesterin on the risk of colorectal cancer was a reduction in the absolute number of localized cancers (hazard ratio for localized cancer, 0.26; 95 percent confidence interval, 0.13 to 0.53). However, the effect on regional tumors (hazard ratio, 0.76; 95 percent confidence interval, 0.44 to 1.30) and metastatic tumors (hazard ratio, 1.54; 95 percent confidence interval, 0.50 to 4.71) is less

clear. However, as previously stated, even within the category of regional or metastatic disease, the cancers in the hormone group were associated with a greater number of positive nodes than were the cancers in the placebo group (3.6 ± 4.2 vs. 1.6 ± 2.1 nodes, $P=0.012$), and the number of deaths was very similar in the hormone and placebo groups (nine and eight, respectively). The biology underlining these findings and their clinical implications are unclear.

Rowan T. Chlebowski, M.D., Ph.D.

Harbor-UCLA Research and Education Institute
Torrance, CA 90502
rchlebowski@rei.edu

Rebecca J. Rodabough, M.S.

Fred Hutchinson Cancer Research Center
Seattle, WA 98109

Jean Wactawski-Wende, Ph.D.

University at Buffalo
Buffalo, NY 14214

Impaired Mitochondrial Activity and Insulin-Resistant Offspring of Patients with Type 2 Diabetes

TO THE EDITOR: Petersen et al. (Feb. 12 issue)¹ provide further evidence that insulin resistance is associated with a reduction in mitochondrial function in muscle² and an increase in lipid content.³ They propose that mitochondrial dysfunction causes lipid accumulation and insulin resistance, but the relation among these variables is probably more complex. For example, insulin can increase mitochondrial transcript levels, protein synthesis, and ATP production in healthy people but not in people with type 2 diabetes.^{4,5} Thus, one could argue that insulin signaling is required for maintenance of muscle mitochondria and that insulin resistance results in mitochondrial dysfunction, rather than the reverse.

Furthermore, there is growing evidence that links among mitochondria, lipids, and insulin resistance are indirect. First, increasing mitochondrial capacity by aerobic exercise is not sufficient to improve insulin sensitivity⁶; second, endurance-trained athletes have elevated muscle lipids (and mitochondria) yet excellent insulin sensitivity³; and third, in mice, an increase in muscle lipids is not sufficient to lower insulin-stimulated glucose uptake. Thus, although many interesting associations

have been revealed, there remains much to learn about the causes and effects of insulin resistance.

Kevin R. Short, Ph.D.

K. Sreekumaran Nair, M.D., Ph.D.

Mayo Clinic School of Medicine
Rochester, MN 55905
short.kevin@mayo.edu

Craig S. Stump, M.D., Ph.D.

University of Missouri-Columbia
Columbia, MO 65211

1. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med* 2004;350:664-71.
2. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 2002;51:2944-50.
3. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 2001;86:5755-61.
4. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci U S A* 2003;100:7996-8001.
5. Halvatsiotis P, Short KR, Bigelow M, Nair KS. Synthesis rates of muscle proteins, muscle functions, and amino acid kinetics in type 2 diabetes. *Diabetes* 2002;51:2395-404.
6. Short KR, Vittone JL, Bigelow ML, et al. Impact of aerobic training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes* 2003;52:1888-96.

TO THE EDITOR: Petersen et al. demonstrate that offspring of persons with type 2 diabetes have impaired mitochondrial activity. In the light of the possibility that decreased AMP kinase activity, brought about by decreased adiponectin signaling, is a likely explanation for these findings, it is surprising that in this study plasma adiponectin concentrations did not differ between offspring and controls. We and our colleagues¹ and others² have shown that plasma adiponectin levels in insulin-resistant offspring of persons with type 2 diabetes are lower than those in control subjects. Furthermore, the expression of both isoforms of the adiponectin receptor (AdipoR1 and AdipoR2) is decreased in the skeletal muscle of these offspring.¹ We proposed that an impairment in the expression of the adiponectin receptors in combination with reduced concentrations of plasma adiponectin may reduce AMP kinase activation by adiponectin,³ thereby reducing fat oxidation within the skeletal muscle of these offspring. Although Petersen et al. studied persons who were leaner than those we examined (a factor that may have had some effect on their adiponectin concentrations), it is important to know whether these offspring had reduced expression of adiponectin receptors.

Mandeep Bajaj, M.D.

Lawrence J. Mandarino, Ph.D.

University of Texas Health Science Center
San Antonio, TX 78229
mandeepbajaj@hotmail.com

1. Civitarese AE, Jenkinson CP, Richardson D, et al. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. *Diabetologia* (in press).
2. Pellme F, Smith U, Funahashi T, et al. Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. *Diabetes* 2003;52:1182-6.
3. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;8:1288-95.

TO THE EDITOR: Petersen et al. suggest that a reduction in fatty acid oxidation in muscle that leads to intramyocellular lipid accumulation is a primary, inherited cause of insulin resistance, and they speculate about the role of peroxisome-proliferator-activated receptor γ coactivator 1 (PGC1). However, a cause-and-effect relation has not been demonstrated; only an association between insulin resistance (and hyperinsulinemia) and a reduction in mitochondrial function has been observed. No study has shown that such abnormalities precede the de-

velopment of insulin resistance; in fact, Jove et al.¹ have suggested that an improvement in insulin resistance with a reduction in hyperinsulinemia restores the reduced PGC1 transcript levels seen in Zucker diabetic fatty rats, suggesting that the process is a secondary one. Similarly, ectopic fat accumulation in liver and muscle may be a secondary process resulting from an increase in de novo lipogenesis. Hyperinsulinemia leads to up-regulation of sterol regulatory element-binding protein 1c (SREBP1c), a critical transcription factor that regulates the lipogenic genes. Knockout of SREBP1c in ob/ob mice ameliorates hepatic fat accumulation but does not alter insulin sensitivity.² Changes in mitochondrial function may be important in the progression of disease but it has yet to be shown that they are a primary insult.

Peter J. Raubenheimer, M.B., B.Ch.

University of Edinburgh
Edinburgh EH9 1AT, United Kingdom
p.raubenheimer@ed.ac.uk

1. Jove M, Salla J, Planavila A, et al. Impaired expression of NADH dehydrogenase subunit 1 and PPARgamma coactivator-1 in skeletal muscle of ZDF rats: restoration by troglitazone. *J Lipid Res* 2004;45:113-23.
2. Yahagi N, Shimano H, Hasty AH, et al. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)Lep(ob) mice. *J Biol Chem* 2002;277:19353-7.

TO THE EDITOR: Petersen et al. conclude that impairment of mitochondrial metabolism precedes type 2 diabetes. This observation suggests that diseases with defined alterations of mitochondrial metabolism must cause diabetes as well. Friedreich's ataxia is an autosomal recessive disorder that results in impaired oxidative phosphorylation in skeletal muscle¹ similar to that observed by Petersen et al. in diabetic persons. Friedreich's ataxia occurs as a result of reduced expression of a mitochondrial protein called frataxin, which has been functionally linked to cellular respiration and ATP replenishment.² Diabetes frequently develops in patients with Friedreich's ataxia,³ and even heterozygous (i.e., unaffected) carriers of mutations causing Friedreich's ataxia have insulin resistance.⁴ Furthermore, such mutations have been found in association with type 2 diabetes.³ Finally, genetic studies indicate that type 2 diabetes is linked to chromosome 9q13, the location of the frataxin gene.³ Hence, the interesting findings of Petersen et al. regarding a mitochondrial pathogenesis of type 2 diabetes are strongly supported by metabolic and genetic data

from studies of a primarily neurodegenerative disease — Friedreich's ataxia.

Matthias Möhlig, M.D.

German Institute for Human Nutrition
D-14558 Potsdam-Rehbrücke, Germany

Frank Isken, M.D.

Charité University Medicine
D-12200 Berlin, Germany

Michael Ristow, M.D.

German Institute for Human Nutrition
D-14558 Potsdam-Rehbrücke, Germany
mristow@mristow.org

1. Vorgerd M, Schols L, Hardt C, Ristow M, Epplen JT, Zange J. Mitochondrial impairment of human muscle in Friedreich ataxia in vivo. *Neuromuscul Disord* 2000;10:430-5.
2. Ristow M, Pfister MF, Yee AJ, et al. Frataxin activates mitochondrial energy conversion and oxidative phosphorylation. *Proc Natl Acad Sci U S A* 2000;97:12239-43.
3. Ristow M, Mulder H, Pomplun D, et al. Frataxin-deficiency in pancreatic islets causes diabetes due to loss of beta cell mass. *J Clin Invest* 2003;112:527-34.
4. Hebinck J, Hardt C, Schols L, et al. Heterozygous expansion of the GAA tract of the X25/frataxin gene is associated with insulin resistance in humans. *Diabetes* 2000;49:1604-7.

THE AUTHORS REPLY: We agree with Short et al. and Raubenheimer that our study does not prove a cause-and-effect relationship between defects in mitochondrial oxidative-phosphorylation activity and insulin resistance. However, in contrast to previous in vitro studies that showed altered expression of mitochondrial gene expression or mitochondrial enzyme activity,¹⁻³ our study revealed reduced in vivo mitochondrial phosphorylation activity in young, healthy, insulin-resistant offspring of persons with type 2 diabetes who were all lean and free of any potential confounding factors such as obesity, diabetes, or use of medications that might affect mitochondrial gene expression or mitochondrial activity. Furthermore, in contrast to the subjects in the previous studies, the subjects in our study were normoglycemic and the groups were carefully matched for body weight, body-mass index, and activity. The importance of eliminating confounding factors that might affect mitochondrial activity or biogenesis is reflected by a recent oligonucleotide microarray study that showed downregulation of mitochondrial electron-transport genes in streptozotocin-treated mice and a reversal of that process with insulin treatment.⁴

In contrast to the hypothesis proposed by Bajaj

and Mandarino, we do not believe that alterations in plasma adiponectin concentrations can explain our findings, since we did not observe any differences in plasma adiponectin concentrations between the insulin-resistant offspring and the insulin-sensitive controls. We believe that the differences between our results and those of Pellme et al.⁵ can be attributed to the fact that our subjects were lean and were matched for body-mass index. In contrast, the insulin-resistant first-degree relatives studied by Pellme et al. were significantly heavier than their age-matched control subjects (body-mass index, 25.8 ± 2.6 vs. 24.6 ± 2.6 ; $P < 0.05$) and had significantly more body fat (19.5 ± 6.1 kg vs. 16.5 ± 6.6 kg, $P < 0.03$). We are currently in the process of performing gene-chip microarray studies to assess the expression of adiponectin receptors and other hormone receptors in muscle from lean, insulin-resistant subjects.

Finally, we thank Möhlig et al. for their comments regarding the relation between inherited defects in mitochondrial dysfunction and insulin resistance in patients with Friedreich's ataxia. These studies offer further support for our hypothesis regarding a potential causative role of mitochondrial dysfunction in predisposing persons to intracellular lipid accumulation and insulin resistance.⁶

Kitt Falk Petersen, M.D.

Yale University School of Medicine
New Haven, CT 06512

Gerald I. Shulman, M.D., Ph.D.

Howard Hughes Medical Institute
New Haven, CT 06512
gerald.shulman@yale.edu

1. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 2002;51:2944-50.
2. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003;34:267-73.
3. Patti ME, Butte AJ, Crunkhorn S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci U S A* 2003;100:8466-71.
4. Yechoor VK, Patti ME, Saccone R, Kahn CR. Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. *Proc Natl Acad Sci U S A* 2002;99:10587-92.
5. Pellme F, Smith U, Funahashi T, et al. Circulating adiponectin levels are reduced in nonobese but insulin-resistant first-degree relatives of type 2 diabetic patients. *Diabetes* 2003;52:1182-6.
6. Shulman GI. Cellular mechanisms of insulin resistance. *J Clin Invest* 2000;106:171-6.