

UE de M1 « Signalisation cellulaire normale et pathologique »

ED n°1 du 5 avril 2007

Module 1 : Mécanismes moléculaires de la résistance à l'insuline (Dr C. Vigouroux)

Vous lirez le document issu de l'article « Mitochondrial dysfunction in the elderly : possible role in insulin resistance » par Petersen et collaborateurs, publié dans Science en mai 2003, puis vous répondrez par écrit aux questions proposées, de façon brève et synthétique. Le devoir sera rendu en début de séance. Une épreuve écrite de 15-20 minutes portant sur l'article et son interprétation terminera la séance d'ED.

Glossaire :

Oral glucose tolerance test : en français, hyperglycémie provoquée par voie orale ; ce test consiste à administrer 75g de glucose par voie orale à des sujets le matin à jeun, et à déterminer les concentrations plasmatiques de glucose, d'insuline et d'acides gras libres aux temps 0 (c'est-à-dire juste avant l'administration de glucose), puis 30, 60, 90 et 120 minutes après la prise de glucose.

Dual-energy x-ray absorptiometry (absorptiométrie bi-photonique) : Il s'agit d'un balayage lent de l'ensemble du corps par un faisceau de rayons X à deux niveaux d'énergie. Le rapport des atténuations de ces deux rayonnements étant dépendant de la composition de la matière qu'ils traversent, cet examen permet d'évaluer la quantité de la masse osseuse, la masse grasse, et la masse maigre.

NMR spectroscopy : La spectroscopie RMN permet de mesurer par détection externe la concentration de certaines molécules dans les tissus d'un organisme vivant, en utilisant les mêmes principes que ceux de l'IRM, reposant sur le magnétisme nucléaire de certains atomes qui se comportent comme des aimants microscopiques et possèdent donc un moment magnétique nucléaire (spin). C'est le cas du proton, qui permet ici de mesurer les concentrations de triglycérides intramyocytaires et intrahépatiques ; du phosphore 31 (^{31}P), permettant dans cette étude d'évaluer la production mitochondriale d'ATP dans le muscle ; et du carbone 13 (^{13}C), utilisé par les auteurs pour mesurer l'activité oxydative mitochondriale après perfusion d'acétate marqué par le ^{13}C . Le signal est obtenu après application d'un champ magnétique, et son intensité dépend de la concentration des molécules correspondantes.

Questions :

- 1) Quels résultats permettent aux auteurs d'authentifier une résistance à l'insuline chez les sujets âgés ? Quelles précautions ont été prises pour que les sujets soient comparables ?
- 2) En vous servant de vos connaissances, expliquer pourquoi la surcharge lipidique tissulaire identifiée par les auteurs pourrait être la cause de l'insulino-résistance.
- 3) Quelles sont les deux hypothèses initiales des auteurs pour expliquer la surcharge lipidique tissulaire ? Quels résultats permettent d'écarter la première hypothèse ?
- 4) Proposer un schéma physiopathologique intégré permettant de comprendre les causes de l'insulino-résistance du sujet âgé.

Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

Kitt Falk Petersen,¹ Douglas Befroy,^{1,7} Sylvie Dufour,^{1,7}
James Dziura,¹ Charlotte Ariyan,³ Douglas L. Rothman,⁴
Loretta DiPietro,^{5,6} Gary W. Cline,¹ Gerald I. Shulman^{1,2,7*}

Insulin resistance is a major factor in the pathogenesis of type 2 diabetes in the elderly. To investigate how insulin resistance arises, we studied healthy, lean, elderly and young participants matched for lean body mass and fat mass. Elderly study participants were markedly insulin-resistant as compared with young controls

. These changes were associated with increased fat accumulation in muscle and liver tissue assessed by ¹H nuclear magnetic resonance (NMR) spectroscopy, and with a ~40% reduction in mitochondrial oxidative and phosphorylation activity, as assessed by in vivo ¹³C/³¹P NMR spectroscopy. These data support the hypothesis that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly.

Type 2 diabetes is the most common chronic metabolic disease in the elderly, affecting ~30 million individuals 65 years of age or older in developed countries (1). The estimated economic burden of diabetes in the United States is ~\$100 billion per year, of which a substantial proportion can be attributed to persons with type 2 diabetes in the elderly age group (2). Epidemiological studies have shown that the transition from the normal state to overt type 2 diabetes in aging is typically characterized by a deterioration in glucose tolerance (3, 4) that results from impaired insulin-stimulated glucose metabolism in skeletal muscle (5, 6). Measurements of muscle triglyceride content by biopsy (7) or in-

tramyocellular lipid content (IMCL) by ¹H nuclear magnetic resonance (NMR) spectroscopy (8–10) have shown a strong relationship between increased intramuscular fat content and insulin resistance in muscle. Similar correlations have been established for hepatic insulin resistance and hepatic steatosis (11–13). Increases in the intracellular concentration of fatty acid metabolites have been postulated to activate a serine kinase cascade leading to defects in insulin signaling in muscle (14–17) and the liver (18), which results in reduced insulin-stimulated muscle glucose transport activity (14), reduced glycogen synthesis in muscle (19, 20), and impaired suppression of glucose production by insulin in the liver (11–13).

To examine whether insulin resistance in the elderly is associated with similar increases in intramyocellular and/or liver triglyceride content, we studied healthy elderly and young people that we matched for lean body mass (LBM) and fat mass. All study participants were non-smoking, sedentary, lean [body mass index (BMI) < 25 m²/kg], and taking no medications.

Sixteen elderly volunteers (ages 61 to 84 years, 8 male and 8 female) were screened with a 3-hour oral glucose (75 g) tolerance test and underwent dual-energy x-ray absorptiometry to assess LBM and fat mass (21). One elderly man was excluded from the study because of an abnormal glucose profile. Thirteen young volunteers (ages 18 to 39 years, 6 male and 7 female), who had no family history of diabetes or hypertension, were matched to the older participants for BMI and habitual physical activity, which was assessed by means of an activity index questionnaire (22). All participants underwent a complete medical history and physical examination, as well as blood tests to confirm that they were in excellent health (23).

Young and elderly participants had similar fat mass, percent fat mass, and LBM (Table 1) (24). The elderly participants had slightly higher plasma glucose concentrations (Fig. 1A) and significantly higher plasma insulin concentrations (Fig. 1B) during the oral glucose tolerance test, suggesting that they were relatively insulin-resistant as compared with the young controls. Basal plasma fatty acid concentrations (Fig. 1C) also tended to be higher in the elderly participants but were suppressed normally after glucose ingestion.

To ascertain whether lipid accumulation in muscle might be responsible for the insulin resistance in the elderly participants, we used ¹H

¹Department of Internal Medicine, ²Department of Cellular and Molecular Physiology, ³Department of Surgery, ⁴Department of Diagnostic Radiology, ⁵Department of Epidemiology and Public Health, ⁶John B. Pierce Laboratory, ⁷Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: gerald.shulman@yale.edu

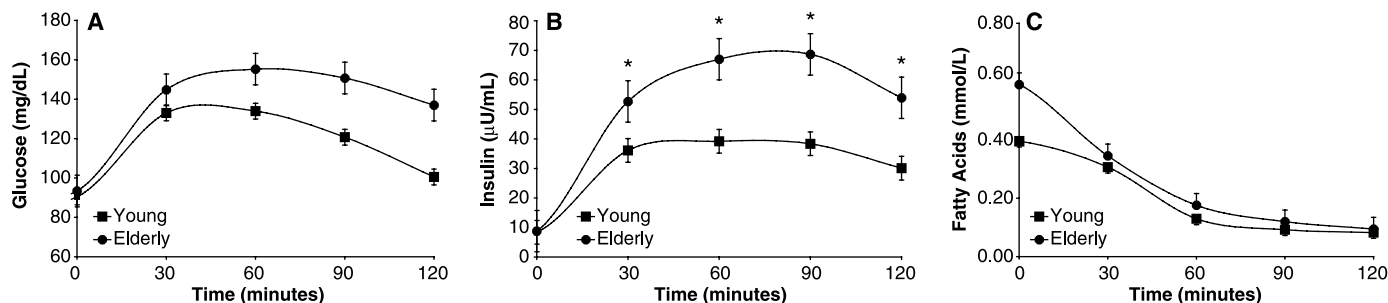


Fig. 1. Plasma concentrations of glucose, insulin, and fatty acids before and after an oral glucose tolerance test (24) in young and elderly participants. **(A)** Glucose [$P = 0.10$ for the area under the curve (AUC) for the elderly ($16,978 \pm 656$) as compared with the controls ($14,495 \pm 1,116$)]. **(B)** Insulin [asterisks indicate $P < 0.03$ for AUC for the elderly (6590 ± 853) as compared with the controls (3986 ± 519)]. **(C)** Fatty acids ($P = 0.08$ for the basal concentration of fatty acids in the elderly versus the controls).

Table 1. Body composition of study participants.

	Age (years)	Body weight (kg)	Fat mass (kg)	% Fat mass (% body weight)	LBM (kg)	BMI (kg/m^2)
Young ($n = 13$)	27 ± 2	71 ± 4	19.9 ± 2.5	28 ± 3	54 ± 5	23.8 ± 1.1
Elderly ($n = 15$)	70 ± 2	70 ± 3	20.1 ± 1.7	29 ± 2	49 ± 3	25.1 ± 0.5
P value	<0.0001	0.69	0.93	0.77	0.28	0.28

Table 2. Metabolic rates and tissue lipid content of participants (24).

	Clamp peripheral glucose metabolism rate (mg/kg of LBM/min)	Intramyocellular lipid content (%)	Intrahepatic lipid content (%)	Mitochondrial TCA flux rate (nmol/g of muscle/min)	Mitochondrial ATP synthesis rate ($\mu\text{mol}/\text{g}$ of muscle/min)
Young	6.2 ± 0.6	0.96 ± 0.08	0.49 ± 0.10	96 ± 10	7.50 ± 0.77
Elderly	4.0 ± 0.4	1.39 ± 0.15	1.61 ± 0.38	62 ± 5	4.06 ± 0.65
P value	<0.002	0.035	0.036	<0.006	<0.004

NMR spectroscopy to assess IMCL and hepatic triglyceride content (24). The IMCL content in the soleus muscle was increased by ~45% in the elderly participants as compared with controls (Table 2). Intrahepatic triglyceride content was also increased by 225% in the elderly participants as compared with controls

and insulin suppression of glycerol turnover during the clamp were similar in the elderly and control participants. Consistent with this finding, the interstitial glycerol concentrations, assessed by microdialysis, decreased by a similar degree during the clamp in both groups. These data suggest

Because increases in intramyocellular and intrahepatic triglyceride content could occur secondarily to increased fatty acid delivery from lipolysis, we also examined this process in vivo. We assessed whole-body and subcutaneous fat lipolysis by measuring the rates of [$^2\text{H}_5$] glycerol turnover in combination with microdialysis measurements of glycerol release from subcutaneous fat. Basal rates of whole-body glycerol turnover

that increased basal rates of peripheral lipolysis, and/or defects in insulin suppression of lipolysis, do not play a major role in causing the increased intramyocellular and intrahepatic triglyceride content in the elderly.

We and others (25) have previously hypothesized that defects in mitochondrial oxidative and phosphorylation capacity might be a contributing factor to the increased triglyceride content in muscle and the liver (26). To test this hypothesis, we assessed in vivo rates of mitochondrial oxidative activity in skeletal muscle by ^{13}C NMR and phosphorylation activity by ^{31}P NMR (24, 27). Using this approach, we found that rates of mitochondrial oxidative and phosphorylation activity were both reduced by

~40% in the elderly participants as compared with the young controls. These in vivo results are consistent with those of a previous in vitro study, which found decreased state III (activated) mitochondrial respiration in isolated mitochondria from elderly participants (28). However, the latter study was performed with muscle strips, from orthopedic and chronic fatigue syndrome patients, under artificial substrate concentrations that do not reflect in vivo conditions.

Our results suggest that insulin resistance in the elderly is related to increases in intramyocellular fatty acid metabolites that may be a result of an age-associated reduction in mitochondrial oxidative and phosphorylation activity (fig. S2). The similarity in mitochondrial energy coupling, assessed by the ratio between adenosine triphosphate (ATP) synthase flux and tricarboxylic acid (TCA) cycle oxidation, suggests an age-associated reduction in mitochondrial number and/or function, as opposed to an acquired defect in mitochondrial energy coupling. These possibilities are consistent with a recent study demonstrating an age-associated accumulation of mutations in control sites for mitochondrial DNA replication (29). Because mitochondrial oxidative and phosphorylation activity is the major source of energy in most organs, including the brain, our data add support to the hypothesis that a decline in mitochondrial oxidative and phosphorylation energy production may also have an important role in aging (30, 31). Furthermore, because mitochondrial energy metabolism plays a critical role in glucose-induced insulin secretion (32), similar age-associated reductions in pancreatic beta cell mitochondrial function, in the setting of peripheral insulin resistance, might help explain the high prevalence of diabetes in the elderly.

References and Notes

1. H. King, R. E. Aubert, W. H. Herman, *Diabetes Care* **21**, 1414 (1998).
2. D. M. Huse, G. Oster, A. R. Killen, M. J. Lacey, G. A. Colditz, *JAMA* **262**, 2708 (1989).
3. A. Sasaki, T. Suzuki, N. Horiuchi, *Diabetologia* **22**, 154 (1982).
4. M. F. Saad et al., *Lancet* **1**, 1356 (1989).
5. J. W. Rowe, K. L. Minaker, J. A. Pallotta, J. S. Flier, *J. Clin. Invest.* **71**, 1581 (1983).
6. G. M. Reaven, *Physiol. Rev.* **75**, 473 (1995).
7. A. B. Rowe et al., *Clin. Sci. (London)* **82**, 219 (1992).

REPORTS

8. M. Krssak et al., *Diabetologia* **42**, 113 (1999).
9. G. Perseghin et al., *Diabetes* **48**, 1600 (1999).
10. L. S. Szczepaniak et al., *Am. J. Physiol.* **276**, E977 (1999).
11. K. F. Petersen et al., *J. Clin. Invest.* **109**, 1345 (2002).
12. E. W. Kraegen et al., *Diabetes* **40**, 1397 (1991).
13. A. Seppala-Lindroos et al., *J. Clin. Endocrinol. Metab.* **87**, 3023 (2002).
14. M. E. Griffin et al., *Diabetes* **48**, 1270 (1999).
15. A. Dresner et al., *J. Clin. Invest.* **103**, 253 (1999).
16. C. Yu et al., *J. Biol. Chem.* **277**, 50230 (2002).
17. S. I. Itani, N. B. Ruderman, F. Schmieder, G. Boden, *Diabetes* **51**, 2005 (2002).
18. J. K. Kim et al., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7522 (2001).
19. G. Boden, X. Chen, J. Ruiz, J. V. White, L. Rossetti, *J. Clin. Invest.* **93**, 2438 (1994).
20. M. Roden et al., *J. Clin. Invest.* **97**, 2859 (1996).
21. K. F. Petersen et al., *Diabetes* **47**, 381 (1998).
22. J. A. Baecke, J. Burema, J. E. Frijters, *Am. J. Clin. Nutr.* **36**, 936 (1982).
23. Written consent was obtained from each participant after the purpose, nature, and potential complications of the studies were explained. The protocol was approved by the Yale University Human Investigation Committee.
24. Materials and methods are available as supporting material on *Science Online*.
25. D. E. Kelley, J. He, E. V. Menshikova, V. B. Ritov, *Diabetes* **51**, 2944 (2002).
26. G. I. Shulman, *J. Clin. Invest.* **106**, 171 (2000).
27. V. Lebon et al., *J. Clin. Invest.* **108**, 733 (2001).
28. I. Trounce, E. Byrne, S. Marzuki, *Lancet* **1**, 637 (1989).
29. Y. Michikawa, F. Mazzucchelli, N. Bresolin, G. Scarlato, G. Attardi, *Science* **286**, 774 (1999).
30. D. Harman, *J. Am. Geriatr. Soc.* **20**, 145 (1972).
31. A. W. Linnane, S. Marzuki, T. Ozawa, M. Tanaka, *Lancet* **1**, 642 (1989).
32. R. Luft, H. Luthman, *Lakartidningen* **90**, 2770 (1993).
33. We thank Y. Kossover, M. Smolgovsky, A. Romanelli, and the staff of the Yale/New Haven Hospital General Clinical Research Center for expert technical assistance and the volunteers for participating in this study. Supported by grants from the U.S. Public Health Service (K-23 DK-02347, R01 AG-09872, P60 AG-10469, P30 DK-45735, M01 RR-00125, and R01 DK-49230).

Supporting Online Material

www.sciencemag.org/cgi/content/full/300/5622/1140/DC1

Materials and Methods

SOM Text

Figs. S1 and S2

References

29 January 2003; accepted 14 April 2003

SFig. 2

