Mitochondrial Dysfunction in the Elderly: Possible Role in Insulin Resistance

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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, and this resistance was attributable to reduced insulin-stimulated muscle glucose metabolism. 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/15N 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 intramyocellular 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 habit 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 determine what tissues were responsible for the insulin resistance, we performed hyperinsulinemic-euglycemic clamp studies, in combination with [6,6-2H2] glucose and [2H5] glycero1 tracers (24). Basal rates of glucose production were similar in the young and elderly participants (Table 2) and were suppressed completely in both groups during the hyperinsulinemic-euglycemic clamp. In contrast, the rates of glucose infusion required to maintain euglycemia during the clamp and insulin-stimulated rates of peripheral glucose uptake were ~40% lower in the elderly participants (Table 2). Basal energy expenditure and respiratory quotient both tended to be lower in the elderly participants (24).

To ascertain whether lipid accumulation in muscle might be responsible for the insulin resistance in the elderly participants, we used 1H
We assessed whole-body and subcutaneous fat lipolysis, we also examined this process in vivo. 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 [14C] glycerol turnover in combination with microdialysis measurements of glycerol release from subcutaneous fat. Basal rates of whole-body glycerol turnover 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. Taken together, these data suggest that insulin resistance was confined mostly to skeletal muscle and 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 13C NMR and phosphorylation activity by 31P 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.
Regulation of Aging and Age-Related Disease by DAF-16 and Heat-Shock Factor
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The Caenorhabditis elegans transcription factor HSF-1, which regulates the heat-shock response, also influences aging. Reducing hsf-1 activity accelerates tissue aging and shortens life-span, and we show that hsf-1 overexpression extends life-span. We find that HSF-1, like the transcription factor DAF-16, is required for daf-2 overexpression to extend life-span. Our findings suggest this is because HSF-1 and DAF-16 together activate expression of specific genes, including genes encoding small heat-shock proteins, which in turn promote longevity. The small heat-shock proteins also delay the onset of polyglutamine-expansion protein aggregation, suggesting that these proteins couple the normal aging process to this type of age-related disease.

Heat-shock factor activates transcription of heat-shock genes, which encode chaperones and proteases, in response to heat and other forms of stress. Previous studies have implicated heat-shock proteins (HSPs) in aging. For example, mild heat stress can cause a period of decreased mortality rate in Drosophila, and hsp70 has been implicated in this effect (1). In addition, expression of genes encoding small heat-shock proteins (sHSPs) is increased in Drosophila lines selected for increased life-span (2), and overexpression of hsp70f increases the life-span of C. elegans (3).

Previously, we showed that reducing the activity of C. elegans HSF-1 causes a rapid-aging phenotype and shortens life-span (4). Conversely, we found that animals carrying additional hsf-1 gene copies (5), which were resistant to heat and oxidative stress (fig. S1, A and B), lived approximately 40% longer than normal (Fig. 1A). Thus HSF-1 activity promotes longevity.

The FOXO transcription factor DAF-16, which functions in the C. elegans insulin/