mass spectrometry. Such small samples can only be analyzed by AMS. Sample combustion was performed with the use of a Carlo Erba EA1108 Elemental Analyzer (Carlo Erba, Milan, Italy) with tin combustion capsules cleaned in cyclohexane and distol acetone to reduce combustion blanks. After combustion, the CO_2 samples were cryogenically concentrated in a helium carrier gas with the use of a specially built two-stage capillary concentrator (capillary diameters were 0.75 mm and 0.25 mm), with the resulting CO_2-helium mixture directly injected into the AMS ion source. The combustion and gas-handling blank for "modern" (ambient radiocarbon concentration) was conservatively estimated to be 2.5 ± 0.5 μg C (typically 1.7 μg C of this being from the combustion itself); this blank makes very little difference to these results because they are very close to modern levels. With the use of a linear regression with sample size, we estimated the radiocarbon-free combustion blank to be 0.18 ± 0.10 μg C. The uncertainties in these contributions to the sample have been included in the error of the AMS analysis of ^{14}C content, which was 5% at 10 μg C and decreased to 1.5% at 100 μg C.

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32. We thank M. Humm and P. Leach at the Oxford Radiocarbon Accelerator Unit for their efforts in optimizing the AMS equipment for the analysis of microgram samples; M. Garnett at the Natural Environment Research Council (NERC) Radiocarbon Laboratory for technical advice; C. Abbott and the horticultural staff at York for looking after the plants; and S. Smith, A. Smith, I. Jakobsen, and P. Young for their help in improving this paper. Funded by the Soil Biodiversity Programme of the NERC.

7 March 2003; accepted 11 April 2003

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 $^{13}C/^{31}P$ 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 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 nonsmoking, sedentary, lean [body mass index (BMI) < 25 m^2/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 determine what tissues were responsible for the insulin resistance, we performed hyperinsulinemic-euglycemic clamp studies, in combination with [6,6-$^2H_2$] glucose and [$^2H_5$] glycerol tracer infusions (*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

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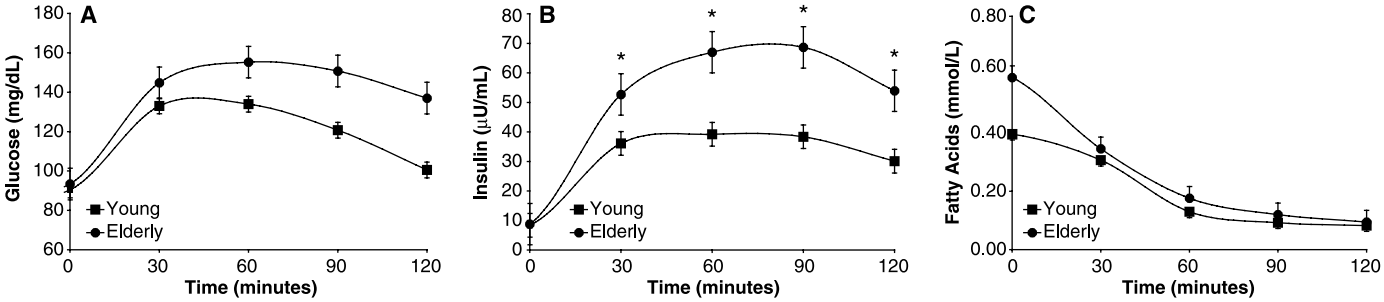

Fig. 1. Plasma concentrations of glucose, insulin, and fatty acids before and after an oral glucose tolerance test (*24*) in young and elderly participants. (**A**) Glucose [$P = 0.10$ for the area under the curve (AUC) for the elderly (16,978 ± 656) as compared with the controls (14,495 ± 1,116)]. (**B**) 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/m²)
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*).

	Basal rates of glucose production (mg/kg of LBM/min)	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 (μmol/g of muscle/min)
Young	2.3 ± 0.1	6.2 ± 0.6	0.96 ± 0.08	0.49 ± 0.10	96 ± 10	7.50 ± 0.77
Elderly	2.4 ± 0.1	4.0 ± 0.4	1.39 ± 0.15	1.61 ± 0.38	62 ± 5	4.06 ± 0.65
P value	0.34	<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 and fig. S1). Intrahepatic triglyceride content was also increased by 225% in the elderly participants as compared with controls, even though there was no detectable hepatic insulin resistance in the elderly participants during the clamp. It is possible that hepatic insulin resistance was not detected in the elderly participants because of the relatively high plasma insulin concentrations obtained during the clamp studies, which completely suppressed hepatic glucose production in both groups.

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 [$^2H_5$] 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 ^{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.

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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

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Materials and Methods
SOM Text
Figs. S1 and S2
References

29 January 2003; accepted 14 April 2003

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*–insulin/IGF-1 receptor mutations 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/

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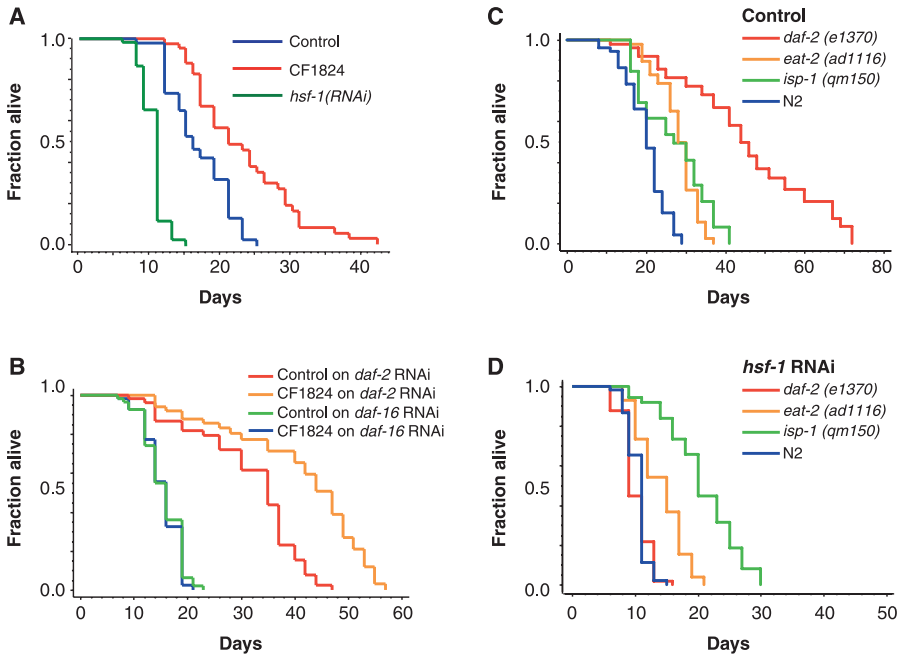

Fig. 1. HSF-1 promotes longevity. (**A**) Blue, survival of wild-type animals grown on control bacteria containing vector alone; green, animals grown on bacteria expressing *hsf-1* dsRNA; red, animals carrying additional copies of *hsf-1* (CF1824). Two additional HSF-1 overexpressing lines were obtained and were found to extend life-span (table S1). (**B**) *hsf-1* overexpression extends life-span in a *daf-16*–dependent manner. Adult life-spans of wild-type and *hsf-1*–overexpressing (CF1824) animals grown on *daf-16* RNAi bacteria (green and blue lines). In addition, *hsf-1* overexpression extends the life-span of animals treated with *daf-2* RNAi (red and orange lines). (**C** and **D**) RNAi of *hsf-1* completely prevents the *daf-2(e1370)* mutation, but not the *isp-1(qm150)* or *eat-2(ad1116)* mutations, from extending life-span. Animals were grown on *hsf-1* RNAi bacteria from the time of hatching. Adult life-spans of wild-type (N2) (blue), *daf-2(e1370)* (red), *eat-2(ad1116)* (orange), and *isp-1(qm150)* (green) animals grown on (C) control bacteria or (D) *hsf-1* RNAi bacteria. All experiments have been repeated more than once with similar effects. For statistical data, see table S1.