Endocrine and metabolic features of familial longevity : the Leiden Longevity Study

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Chapter 4: Familial longevity is marked by enhanced insulin sensitivity

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Abstract

A key question in aging research is whether the extreme effects of altered insulin signaling on lifespan observed in model organisms can be translated to humans. We aimed to compare the relation between insulin and glucose and tissue specific insulin action between subjects from long-lived families and controls. Non-fasted serum data were analyzed in 1838 subjects and a two step hyperinsulinemic-euglycemic clamp was performed in 24 subjects, comprising offspring from long-lived siblings and their partners. Clamp participants were aged 52-72 years, healthy, non-smoking, non-obese, and groups were similar with regard to sex distribution, age, exercise, BMI, waist circumference, fat mass, and fasting glucose and insulin levels. Higher glucose infusion rate was required to maintain euglycemia during high dose insulin infusion ($p = 0.036$) in offspring from long-lived siblings, reflecting higher whole body insulin sensitivity. After adjustment for sex, age and fat mass, the insulin-mediated glucose disposal rate was higher in offspring than in controls (42.5 ± 2.7 vs. 33.2 ± 2.7 μmol/kg*min, mean ± SE, $p=0.025$). The capacity of insulin to suppress endogenous glucose production and lipolysis did not differ between groups (all $p > 0.05$). Furthermore, glucose disposal rate was significantly correlated with the mean age of death of the parents. In conclusion, subjects from long-lived families are marked by enhanced insulin sensitivity and mimic the phenotype found in mammalian models with genetic disruption of IGF-1/insulin signal transduction. These observations allow for identifying biomolecular mechanisms to promote health in old age.

Introduction

A key question in aging research is whether the extreme effects of altered insulin signaling on lifespan observed in model organisms can be translated to humans. The insulin/IGF-1 system, which is highly conserved among (in)vertebrate species, adapts metabolism, growth and differentiation under various environmental conditions, including nutrient availability[1]. An ancestral gene encodes just one insulin/IGF-1 receptor tyrosine kinase in invertebrates [2], while three distinct receptors have evolved in vertebrates to mediate the metabolic and mitogenic effects of insulin, IGF-1 and IGF-2: the insulin receptor, the IGF-1 receptor and the insulin related receptor[1]. In addition, the IGF-2 receptor is thought to have evolved primarily as a clearance receptor [3]. In mammals, the production of IGF-1 is controlled by growth hormone to mediate its effects on growth and development. Extreme effects of IGF and insulin signaling on longevity were shown in invertebrates (reviewed in [4]). In mammals, enhanced insulin sensitivity is prominent in hypopituitary (GH deficient) and GH receptor deleted mice, suggesting that insulin sensitivity is a hallmark phenotype of mammalian longevity, at least in these model organisms [5].

In humans, insulin sensitivity declines progressively with age, which significantly contributes to the increased incidence of type 2 diabetes mellitus and cardiovascular disease in older people [6, 7]. A previous study suggested that glucose tolerance and insulin action are preserved in centenarians[8]. We designed the Leiden Longevity Study to examine the underlying biomolecular mechanisms of longevity in humans[9]. To this end we have recruited 421 long-lived families consisting of multiple nonagenarian siblings and their offspring (aged 33-81 years) from the Dutch population. The partners of the offspring (aged 30-80 years) were included as controls. Recently, we showed that the offspring from these families had lower mortality and lower prevalence of major age-related diseases, including diabetes[10]. Random and fasting glucose levels were also lower and we showed that glucose tolerance was better in the non-diabetic offspring when compared to controls. Offspring and controls did not differ with respect to age, sex distribution, body mass index, and lifestyle indices such as the level of physical activity [11].

To test whether the biomolecular mechanisms underlying familial longevity in humans resemble those of long-lived animal models in terms of insulin action (i.e. whether it is characterized by enhanced insulin sensitivity), here we further explored the relation between insulin and glucose in the two groups. To this end, we first compared the relationship between glucose and insulin levels as determined in random non-fasted serum samples (n=1838), which include the physiological variation in insulin levels in response to everyday challenges, such as meals. Next, we performed a double tracer, 2-step hyperinsulinemic euglycemic clamp in two subgroups comprising 12 healthy offspring from long-lived siblings and 12 partners as control subjects. This gold standard

technique allowed us to assess whole-body insulin sensitivity and distinguish between the effects of insulin on glucose disposal rate, endogenous glucose production and lipolysis.

Materials and methods

Subjects

The Leiden Longevity Study comprises 421 families, as described more extensively elsewhere[9]. Families were recruited if at least two long-lived siblings were alive and fulfilled the age-criterion of 89 years or older for males and 91 year or older for females. As no proper controls exist for this age group, for further studies the offspring of these long-lived nonagenarians were included. This generation carries on average 50% of the genetic advantage of their long-lived parent and was shown to have a 35% lower mortality rate compared with their birth cohort. Their partners, with whom most have had a relationship for decades, were included as population-based controls. Nonfasted serum samples and BMI were available for 1930 subjects. After exclusion of subjects with non-fasted glucose levels above 11 mmol/L (indicative of possible diabetes), history of diabetes or use of glucose lowering medication, non-fasted serum samples of 1838 subjects were available for the current study.

For the hyperinsulinemic euglycemic clamp study, we aimed to include twelve couples, each consisting of an offspring from long-lived siblings and his or her current partner as control subject. Subjects were selected from the database based on the following inclusion criteria: middle-age (50-75 years), residence in close proximity of the research center (less than 45 minutes by car) and normal body mass index (BMI) (22 kg/m^2 < BMI < 30 kg/m^2). Eligible subjects were screened for the following exclusion criteria: fasting plasma glucose > 6.9 mmol/L [12], presence of endocrine, renal, hepatic or other significant chronic disease, use of medication known to influence lipolysis, glucose metabolism or GH-secretion, recent weight changes or attempts to loose weight (> three kg weight change within last three months), smoking, extensive sporting activities (> 10 hours/week) and inaccessible peripheral veins for intravenous catheter insertion, as assessed by clinical examination and routine laboratory tests. During the screening interview, information on age (of death) of the parents was obtained.

In total, 87 subjects were approached, of which 17 subjects did not fulfill the inclusion criteria (19%), 44 subjects refused participation (51%) and 26 subjects agreed to participate in the study (30%). Two subjects (one offspring, one control) did not complete the study due to medical technical reasons. One of the partners of an offspring also had a long-lived parent with a long-lived sibling and was therefore included in the offspring group. In total, the group consisted of 24 subjects, comprising 8 couples and 8 unrelated subjects (4 offspring, 4 controls). The Medical

Ethical Committee of the Leiden University Medical Center approved the study and written informed consent was obtained from all subjects.

Clinical protocol.

All clamp studies started at 8:00 AM after an overnight fast. Anthropometric measurements (height, weight, waist and hip circumference) and blood pressure measurements were performed according to standard methods. Body composition was measured using bioelectrical impedance analysis (BIA). In a larger sample of the Leiden Longevity Study, body composition as measured with BIA was highly correlated with dual energy X-ray absorptiometry (DEXA) measurements. (Ling et al, unpublished) Metabolic studies were performed as described previously[13]. Subjects were requested to lie down on a bed in a semirecumbent position. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was inserted into a contralateral dorsal hand vein for blood sampling; this hand was kept in a heated box (60 ˚C) throughout the study day to obtain arterialised venous blood samples. Basal samples were taken for measurement of glucose, insulin, total cholesterol, high-density lipoprotein (HDL-) cholesterol, triglycerides, free fatty acids (FFA), glycerol, and background enrichment of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol. At 08:30 AM (t = 0 min), an adjusted primed (17.6 μmol/kg) continuous (0.22 μmol/kg per minute) infusion of [6,6-$^2H_2$]-glucose (enrichment 99.9%; Cambridge Isotopes, Cambridge, Mass) was started and continued throughout the study. At 09:00 AM (t=30 min), a primed (1.6 μmol/kg), continuous (0.11 μmol/kg per minute) infusion of [$^2H_5$]-glycerol (Cambridge Isotopes) was started and continued throughout the study. At the end of the basal period (t = 90 min), three blood samples were taken at 10 min-intervals for the determination of glucose, insulin, glycerol, triglycerides, FFA's and enrichment of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol. Subsequently, a primed continuous infusion of human recombinant insulin (Actrapid, Novo Nordisk Pharma BV, Alphen aan de Rijn, The Netherlands; 10 mU/m^2 per minute) was started (t = 120 min) for 2 hours. This low dose insulin infusion was used to determine differences in insulin sensitivity of the liver and whole-body lipolysis. Exogenous glucose 20% enriched with 3% [6,6-$^2H_2$]-glucose was infused at a variable rate to maintain the plasma glucose level at 5.0 mmol/L. From t = 210 to t = 240 minutes blood samples were taken at 10 minute intervals for the determination of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol-specific activities, glucose, insulin, glycerol, triglycerides and FFA. Next, at t=240, a primed continuous infusion of insulin was started at 40 mU/m^2 per minute. This second high dose of insulin infusion was used to determine whole-body glucose disposal. From t = 330 to t = 360 minutes, blood samples were taken at 10 minute intervals for the determination of [6,6-$^2H_2$]-glucose and [$^2H_5$]-glycerol-specific activities, glucose, insulin, glycerol, triglycerides and FFA. Plasma samples

were put on ice immediately after withdrawal, and all samples were centrifuged at 1610 x *g* at 4 ˚C for 20 minutes and stored at -80 ˚C until assay.

Assays

All serum measurements were performed with fully automated equipment. For glucose, cholesterol, HDL-cholesterol, triglycerides and FFA, the Modular P2 analyzer was used from Roche (Almere, the Netherlands). Insulin was measured using the Immulite 2500 from DPC (Los Angeles, CA, USA). CVs for these measurements were all below 9%.

[6,6-$^{2}H_2$]-glucose and [$^{2}H_5$]-glycerol were determined in a single analytical run using gas chromatography-mass spectrometry as described previously[13]. LDL-cholesterol was calculated using the Friedewald formula (11). In case insulin and glycerol values were below threshold for correct estimation of concentration we estimated the concentration to be half of the threshold value.

Calculations

An isotopic steady state was achieved during the steady-state and during the last 30 minutes of the hyperinsulinemic clamp periods. Therefore, steady-state equations were used to calculate tracer infusion rates, according to the modified Steele's steady state equations [14, 15]. The rates of appearance (Ra) and disappearance (Rd) for glucose and glycerol were calculated by dividing the tracer infusion rate by the tracer-to-tracee ratio. Glucose disposal rates were expressed in µmol/kg body weight per minute. Endogenous glucose production (EGP) during the basal steady state and during the hyperinsulinemic state was calculated as the difference between the rates of glucose appearance and glucose infusion.

Statistical analyses

A piece wise change-point model was used to model the relation between glucose and ln(insulin) for the two groups (n=1838). Within each group, the expected glucose level for a person at a certain level of ln(insulin) were modeled using the formula: predicted glucose = α1 + β1 * ln(insulin), for ln(insulin) < gamma and α2 + β2 * ln(insulin), for gamma < ln(insulin), with restrictions on α1 and α2 such that the function is continuous in the transition point gamma, i.e., (α1 + β1 * gamma) = (α2 + β2 * gamma). Because transition points were similar between groups, group differences in slopes before (difbeta1) and after (difbeta2) the transition point (gamma) were modeled using the formula: predicted glucose_ = ALPHA+ difalpha*partner + (BETA1+difbeta1*partner) * (lnInsuline-gamma)*((lnInsuline-gamma)<0) + (BETA2+difbeta2*partner) * (lnInsuline-gamma)*((lnInsuline-gamma)>0). The model was fitted using software for nonlinear regression models. Data are presented as mean with standard

deviation (baseline characteristics) or mean with standard error (SE) to assess differences between groups. Differences in outcomes between groups as well as the associations of glucose disposal rate (GDR) with age of parents were calculated using a linear regression model with correction for age, sex and fat mass. Statistical significance was set a $p < 0.05$. All analyses were performed using SPSS version 17.0.

Results

The relationship between non-fasted glucose and insulin in the baseline cohort.

Table 1. Baseline characteristics of baseline cohort

	Offspring (n=1273)	Controls (n=565)
Female gender, n (%)	692 (54.4)	329 (58.2)
Age (yr)	59.4 (6.4)	58.7 (7.4)
BMI (kg/m^2)	25.3 (3.4)	25.5 (3.6)
Glucose (mmol/L)	5.7 (1.1)	5.9 (1.2)
Ln Insulin (mU/L)	2.7 (0.8)	2.8 (0.8)

Continuous data are presented as means with S.D

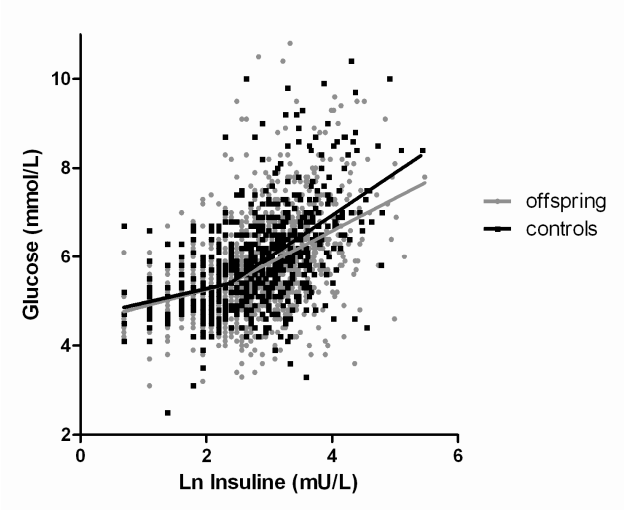

Figure 1. Relation between non-fasted insulin and glucose values in baseline cohort of the Leiden Longevity Study (n=1838).

Table 1 shows the baseline characteristic of the baseline cohort (n=1838). Baseline characteristics were similar between the offspring and controls. **Figure 1** shows the non-fasted serum insulin versus glucose levels for the two groups . For both groups, a biphasic positive association was observed between levels of glucose and ln(insulin). Initially slopes were similar between groups (0.38 in offspring vs. 0.32 in partners, p=0.73); diverged from ln(insulin)= 2.37 (corresponding to 10.7 mU/L insulin) onwards, after which slopes were significantly steeper in the partners (0.73 in offspring vs. 0.95 in partners, p=0.02).

Baseline characteristics of the hyperinsulinemic-euglycemic clamp study.

Next, we performed a hyperinsulinemic euglycemic clamp in 24 subjects. **Table 2** shows the baseline characteristics of the study groups. The group of offspring from long-lived siblings did not differ from the control group with respect to any of the baseline characteristics, although the offspring group showed a tendency towards a slightly higher age and fat mass.

Table 2. Baseline characteristics of clamp group

	Offspring (n=12)	Controls (n=12)
Female gender (%)	50.0	50.0
Age (yr)	62.7 (2.4)	61.2 (5.5)
Systolic blood pressure (mmHg)	142.5 (20.6)	143.2 (25.0)
Diastolic blood pressure (mmHg)	86.8 (10.7)	86.2 (11.1)
Weight (kg)	79.3 (10.3)	80.1 (9.7)
BMI (kg/m^2)	26.0 (2.0)	26.1 (2.3)
Fat mass (%)	33.0 (7.3)	30.9 (9.7)
Lean mass (kg)	53.6 (11.5)	53.8 (12.2)
Waist circumference (cm)	93.0 (10.8)	93.6 (7.7)
Waist/Hip ratio	0.90 (0.1)	0.89 (0.1)
Total cholesterol (mmol/L)	6.1 (1.0)	5.9 (0.8)
HDL-cholesterol (mmol/L)	1.7 (0.4)	1.7 (0.4)
LDL-cholesterol (mmol/L)	3.9 (0.9)	3.8 (0.7)
Mean age parents (yr)	88.3 (4.0)	76.6 (8.2)
Age oldest parent (yr)	97.0 (3.8)	82.3 (10.5)

Continuous data are presented as means with S.D

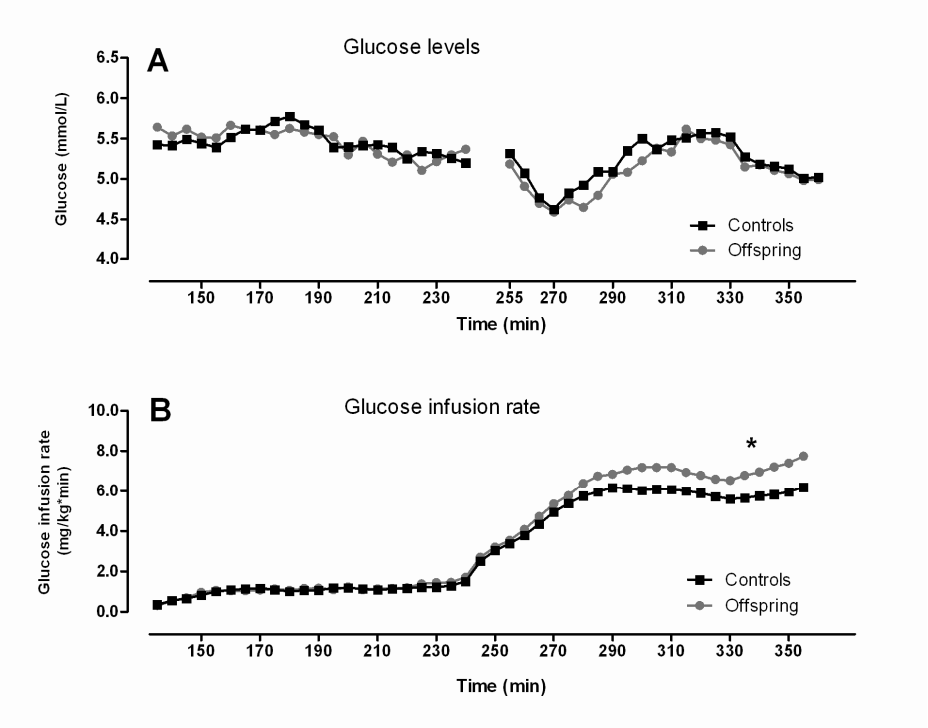

Figure 2. A. Glucose levels and B. glucose infusion rates during the clamp study.

** p <0.05 for the difference in M-value (whole-body-glucose meta-bolism) between groups during the last 30 minutes of high dose insulin infusion (corrected for sex, age and fat mass (%))*

Familial longevity associates with higher whole-body glucose metabolism

A two-step hyperinsulinemic clamp was performed using a low (10 mU/m^2/min) and a high (40 mU/m^2/min) insulin dose in the first and second clamp step, respectively. Mean insulin levels during the last 30 minutes of the clamp periods were similar between groups, both during low dose insulin infusion (11.0 ± 1.0 mU/L in offspring vs. 11.2 ± 1.0 mU/L in controls, p = 0.89) and during high dose insulin infusion (42.5 ± 2.2 mU/L in offspring vs 38.9 ± 2.2 mU/L in controls p = 0.25). Throughout the entire clamp glucose levels remained stable and were similar between groups (**figure 2a**). **Figure 2b** shows the glucose infusion rates during the clamp. During high dose insulin infusion, offspring had significantly higher glucose infusion rates (p = 0.036) compared to controls, despite a slightly higher age and fat mass in the offspring.

Familial longevity is characterized by enhanced peripheral insulin sensitivity, but not hepatic insulin sensitivity

Next, we assessed whether the higher glucose infusion rate required to maintain euglycemia in offspring was accounted for by increased glucose disposal or by enhanced insulin-mediated suppression of endogenous glucose production (**table 3**, **figure 3**). At low dose insulin infusion

(10 mU/m^2/min), the groups did not differ with respect to endogenous glucose production. At high dose insulin infusion (40 mU/m^2/min), the mean glucose disposal rate was higher in offspring than in controls (42.5 ± 2.7 vs. 33.2 ± 2.7 μmol/kg/min, p=0.025). To determine the insulin sensitivity of adipose tissue, we assessed the capacity of insulin to suppress the rate of glycerol appearance (Ra). At baseline and during both clamp conditions, the Ra of glycerol was similar in offspring and controls (**table 3**, **figure 3**), (all p> 0.05).

Table 3. Glucose and fat metabolism in offspring enriched for longevity and controls under different clamp conditions

	Basal steady state			Insulin (10mU/m^2/min)			Insulin (40 mU/m^2/min)		
	Offspring	Controls	p-value	Offspring	Controls	p-value	Offspring	Controls	p-value
Plasma Glucose (mmol/L)	6.1 (0.1)	5.9 (0.1)	0.27	5.6 (0.1)	5.6 (0.1)	0.69	5.5 (0.1)	5.4 (0.1)	0.64
Plasma Insulin (mU/L)	5.4 (1.1)	4.7 (1.1)	0.68	10.8 (1.1)	11.5 (1.1)	0.66	41.9 (2.2)	39.5 (2.2)	0.45
Glucose Rd (µmol/kg/min)	12.9 (0.3)	12.6 (0.3)	0.43	15.5 (1.0)	14.3 (1.0)	0.57	42.5 (2.7)	33.2 (2.7)	**0.025**
Clamp EGP (µmol/kg/min)	12.7 (0.3)	12.4 (0.3)	0.57	7.8 (0.3)	7.9 (0.3)	0.85	1.8 (0.3)	2.1 (0.3)	0.36
Glycerol Ra (µmol/kg/min)	2.1 (0.2)	2.4 (0.2)	0.34	0.9 (0.1)	1.0 (0.1)	0.36	0.6 (0.1)	0.7 (0.1)	0.52
FFA (mmol/L)	0.60 (0.1)	0.76 (0.1)	0.07	0.14 (0.03)	0.21 (0.03)	0.08	<0.05 (0.005)	<0.05 (0.005)	0.94
Triglycerides (mmol/L)	1.17 (0.2)	1.0 (0.2)	0.49	1.0 (0.2)	0.9 (1.2)	0.52	0.91 (0.2)	0.74 (0.2)	0.49

Glucose Rd = Rate of disappearance of glucose EGP = Endogenous Glucose Production Glycerol Ra = Rate of appearance of glycerol FFA = free fatty acids

During basal steady state, glucose Rd is composed of endogenous glucose production and tracer infusion.

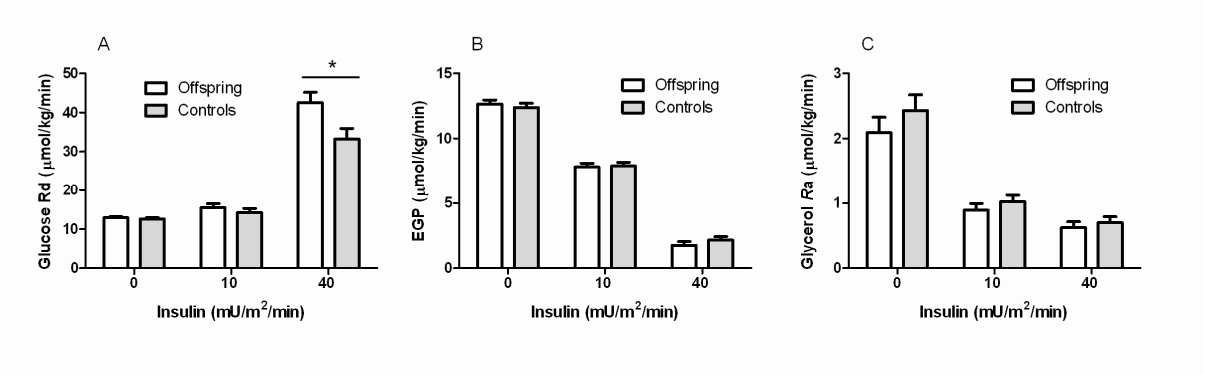

Figure 3. Glucose and fat metabolism in offspring and controls under different clamp conditions; A. glucose disposal rate (Rd), B. endogenous glucose production (EGP), C. rate of appearance of glycerol (Ra). *Results are means with standard error, after adjustment for age, sex and fat mass (%).*

Insulin sensitivity correlates positively with the age at death of the subjects' parents

The presented results suggest a relation between familial longevity and glucose disposal rate. To explore if this association was specific for offspring of long-lived siblings only or of a more general nature, we assessed the relationship between parental age (at death or censorship) with the glucose disposal rate under high dose insulin infusion in all subjects (**figure 4**, **table 4**).

After adjustment for sex, age and fat mass, we found a positive correlation between the mean age of the parents and glucose disposal rate (p = 0.007), and between the age of the oldest parent and the glucose disposal rate (p = 0.034). To exclude the possibility that these results were driven by the high age of parental death in the offspring group, we repeated the analyses for the control group only, and results did not change materially (**table 4**). Also, excluding subjects with parents who were still alive at date of censorship did not change results (data not shown).

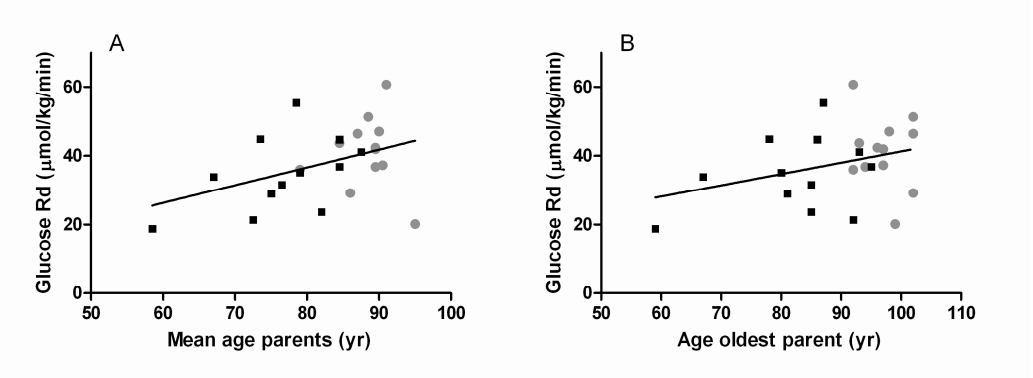

Figure 4. A Relation between mean age of both parents (at death or censorship) and glucose disposal rate; B. Relation between age of oldest parent only (at death or censorship) and glucose disposal rate. *Grey circles represent the offspring, black squares represent controls.*

Table 4. Relation between parental age and glucose disposal rate

	all subjects (n=24)			controls only (n=12)		
	β	S.E.	p-value	**β**	S.E.	p-value
1. mean age of both parents						
Crude	0.52	0.2	**0.045**	0.65	0.4	0.11
Multivariate	0.65	0.2	**0.007**	0.69	0.2	**0.022**
2. age of oldest parent only						
Crude	0.33	0.2	0.12	0.33	0.3	0.30
Multivariate	0.43	0.2	**0.034**	0.37	0.3	0.19

β represents increase in glucose disposal rate (μmol/kg/min) per year increase in age of death of the parent(s).

S.E. = standard error. When parents were still alive, current age was used.

multivariate: adjusted for age, sex and fat mass. p-value obtained using linear regression analysis.

Discussion

Here, we show that familial longevity in humans is characterized by enhanced peripheral insulin sensitivity, i.e. compared to a control group with similar distribution of age, sex and body composition, healthy offspring of long-lived siblings had a higher insulin-mediated glucose disposal rate. In contrast, the capacity of insulin to suppress endogenous glucose production or lipolysis did not differ between the groups. Interestingly, the glucose disposal rate during hyperinsulinemia was positively correlated with the age at death of the parents of the entire group, suggesting that longevity genes are involved in the control of insulin action in the general population.

This is the first study to show that subjects genetically predisposed for healthy longevity have higher whole-body insulin sensitivity when compared to a control group similar in age, sex and body composition and lifestyle indices such as smoking, socio-economic status and physical activity. A previous study showed preserved whole-body insulin sensitivity in healthy centenarians, but different to our study, these data could not be compared to a control group of similar age and body mass index[8]. Moreover, here we document that it is insulin action on glucose metabolism, and glucose disposal in particular, that distinguishes offspring of long-lived siblings from controls. Insulin action on lipolysis did not differ between the groups. These data

suggest that glucose metabolism is involved in the control of aging in humans, as has recently been demonstrated for *Caenorhabditis elegans* [16].

The importance of preservation of insulin action on glucose disposal is in line with previous studies on the pathophysiology of type 2 diabetes mellitus. Muscle insulin resistance is being regarded as one of the earliest steps in the pathophysiology of diabetes mellitus[17, 18], and can be found already several decades before onset of the disease [19, 20]. Insufficient suppression of hepatic glucose production, on the other hand, is seen as a consequence of fat accumulation in the liver [21], and is regarded a later phenomenon in the trajectory towards onset of diabetes[18]. Virtually all of this is mediated by increased fat mass, overweight and reduced physical exercise explaining for the epidemic of diabetes associated with increased disabilities and decreased life expectancy. It should be emphasized that the phenomena that we describe here are independent of fat mass and exercise and are likely to reflect a different and evolutionary conserved biomolecular mechanism of longevity.

Several possible explanations exist for the link between preserved insulin sensitivity and familial longevity. Insulin resistance and compensatory hyperinsulinemia are risk factors for a variety of (age related) diseases, including obesity, type 2 diabetes [22] and cardiovascular disease [23]. Furthermore, insulin resistance shows familial clustering [24, 25] and is more prominent in non-diabetic offspring of patients with diabetes type 2 [26]. Thus, the present data are in keeping with our previous observation of a reduced prevalence of type 2 diabetes and myocardial infarction among offspring of long-lived siblings comprising the entire Leiden Longevity Study cohort (4). Less age related morbidity and associated mortality could readily explain why the offspring of long-lived siblings have a propensity to live longer[9].

Alternatively or in addition, in middle-aged individuals, insulin sensitivity may be a hallmark of a different physiological state associated with increased life expectancy. Interestingly, enhanced insulin sensitivity in the offspring of long-lived siblings co-occurs with other phenotypic features, including lower levels of active thyroid hormone [27], a different spectrum of cellular responses to oxidative stress *in vitro* [28] and larger LDL particle sizes [29]. The co-occurrence of multiple beneficial features is reminiscent of the phenotype seen in genetically modified long-lived mammals as well as in calorie-restricted mammals [30]. In long-lived mammalian models, including hypopituitary (GH deficient) dwarf- and GH receptor knock out mice, enhanced insulin sensitivity often co-occurs with enhanced protection against oxidative damage [31, 32]. Conversely, it has recently been shown that mitochondrial superoxide production is a common feature of many different *in vitro* and *in vivo* models of insulin resistance [33]. Pathways implicated in mediating longevity phenotypes in genetically modified long-lived mammals as well as in

calorie-restricted mammals include modulation of FOXO, AMPK, Sirtuins and mTOR [34]. Interestingly, genetic variants of FOXO3A have been been linked to human longevity in seven different cohorts, including Hawaiians of Japanese descent, Italians, Ashkenazi Jews, Californians, New Englanders, Germans and Chinese (reviewed in [4]). Given the complexity of pathways and the generally small but possibly additive effects observed for individual genetic variants [35], stronger effects will possibly be observed when entire genetic pathways will be analysed [36].

The strict selection criteria for the clamp study participants may have diminished the experimental contrast between the groups and masked even greater differences in insulin action. The groups were comparable for age, gender, environmental conditions and lifestyle indices, and type 2 diabetes and/or any other chronic disease were reasons to exclude individuals from participation (whether it concerned offspring or control). Since the prevalence of age-related pathology, including diabetes and cardiovascular disease associated with insulin resistance, is higher in controls[10], inclusion of all cohort members (irrespective of the presence of chronic disease) would probably have revealed an even more explicit difference in insulin action between offspring and controls but would have hampered causal inference.

The insulin levels during the hyperinsulinemic clamp study were comparable to insulin levels in the non-fasted, randomly obtained serum samples in the larger baseline cohort of the Leiden Longevity Study. Likewise, the different response to insulin in offspring under experimental high insulin clamp conditions was reflected by a comparable difference in the relationship between randomly taken non-fasted insulin and glucose levels in the higher range of insulin levels. This suggests that the differences in insulin sensitivity found between offspring and controls under controlled, experimental conditions may reflect everyday physiological conditions.

In conclusion, familial longevity in humans is marked by an increased capacity of insulin to stimulate glucose disposal, which confirms observations in mammalian models of longevity. Moreover, the age at death of the parents predicts the glucose disposal rate in response to insulin infusion in their offspring, suggesting that longevity genes are involved in the control of insulin action in man. Taken together, our data suggest that genetic predisposition for longevity is associated with altered insulin sensitivity in man as it is in model organisms. Our future research will focus on identifying the underlying biomolecular mechanisms and pathways.

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