Chapter 3: Favorable glucose tolerance and lower prevalence of metabolic syndrome in non-diabetic offspring of nonagenarian siblings: the Leiden Longevity Study

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Abstract

The involvement of the insulin/IGF-1 signaling pathway in the regulation of lifespan has been demonstrated in numerous model organisms. It has been suggested that insulin sensitivity is at play in human longevity as well. The aim of this study was to explore measures of glucose metabolism in families with exceptional longevity. Therefore, we performed an oral glucose tolerance test in a group of 121 offspring of nonagenarian siblings, who were enriched for familial factors promoting longevity, in comparison to a group of 113 of their partners. All subjects were non-diabetics and body composition was similar between the two groups. The group of offspring had a lower prevalence of metabolic syndrome (p=0.031), similar body composition and lower mean fasting blood glucose levels (4.99 vs. 5.16 mmol/L; P = 0.010), lower mean fasting insulin levels (5.81 vs. 6.75 mU/L; P = 0.039), a higher mean homeostasis model assessment of insulin sensitivity (HOMA of 0.78 vs. 0.65, P = 0.018) and a more favorable glucose tolerance (mean area under the curve for glucose (13.2 vs. 14.3; P = 0.007) when compared to the group of their partners. No significant differences were observed between the group of offspring and their partners in beta cell function (insulinogenic index of 13.6 vs. 12.5; P = 0.38). Our findings imply that a preserved glucose tolerance and insulin action is already present at middle-age in offspring of familial nonagenarians.

Introduction

Healthy longevity is determined by a mix of genetic, environmental and chance elements. An increasing effort is currently being put in identifying the genetically determined pathways and mechanisms of healthy longevity in humans, as these might provide targets for specific interventions aimed at preservation of disease-free longevity. Of the genetically determined pathways that have been implicated in longevity in model organisms, the evolutionary conserved insulin/insulin-like growth factor-1 signaling (IIS) pathway clearly stands out in current literature. Mutations in the insulin/IGF-1 signaling pathway have been associated with longevity in a variety of model organisms, including nematodes, flies, and rodents [1-9]. In mammals, a hallmark phenotype shared by many of the long-lived mutants [10], including those with genetically induced insulin-like growth factor-1 (IGF-1) resistance is their preserved insulin sensitivity and/or their low fasting blood glucose concentrations. Strikingly, preserved insulin sensitivity/glucoregulation is also intimately associated with the dietary restriction mediated decreased mortality recently observed in non-human primates [11].

Recently, we found that the offspring of familial nonagenarians showed a lower prevalence of myocardial infarction, hypertension and diabetes, suggesting that they are protected against the combination of cardio-vascular risk factors that constitute the metabolic syndrome[12]. Current estimates suggest that the population-attributable fraction for the metabolic syndrome is approximately 6-7% for all-cause mortality, 12-17% for cardiovascular disease, and 30-52% for diabetes [13]. It is unclear which of the risk factors that constitute the metabolic syndrome contributes most strongly to these effects, although it had been suggested that either body mass index (BMI) or insulin sensitivity might play such a major role [13, 14].

Previous reports have shown that the offspring of centenarians had a moderately lower prevalence of metabolic syndrome [15]. Moreover, it has been reported that centenarians showed a preserved insulin sensitivity, comparable to that of healthy young subjects [16].

However, comparative cross-sectional studies involving long-lived subjects are hampered by the lack of proper controls, making it difficult to disentangle the precise contribution of genetic and lifestyle factors to the observed phenotype. We designed the Leiden Longevity Study in order to identify genetic determinants of healthy longevity in nonagenarian siblings and their offspring, which are enriched for heritable influences on morbidity and mortality [17]. In the Leiden Longevity Study, we included 420 families based on proband siblings that both exhibit exceptional longevity. We also included the middle-aged offspring of the nonagenarian siblings and the partners thereof. Recently, we found that compared to their partners, the offspring of

nonagenarian siblings had a lower prevalence of myocardial infarction, hypertension and diabetes [18] as well as lower non-fasting serum glucose levels [19]. As the offspring and their partners by and large share the same environment, it is unlikely that the observed differences between offspring and partners were confounded by environmental factors. For example, the prevalence of Chronic Obstructive Pulmonary Disease (COPD), which is almost entirely caused by behavioral factors, was similar among both groups.

The purpose of this study is twofold. First, to compare the prevalence of metabolic syndrome and its individual risk components between offspring of nonagenarian siblings and their partners. Secondly, to further explore the differences in glucose metabolism between offspring of nonagenarian siblings and their partners. For the latter, oral glucose tolerance was compared between a group of offspring of nonagenarian siblings and their partners, after exclusion of diabetes patients.

Materials and methods

The Leiden Longevity Study

The recruitment of 420 families in the Leiden Longevity Study has been described before [17]. 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. There were no selection criteria on health or demographic characteristics. For 2465 of the offspring of long-lived siblings and their partners, non-fasting serum samples were taken at baseline for the determination of endocrine and metabolic parameters. Additional information was collected from the generation of offspring and partners, including self-reported information on life style, information on medical history from the participants' treating physicians and information on medication use from the participants' pharmacists.

For the present study, a subgroup of 190 middle-age couples, living in close proximity to the Research Center (traveling distance less than 45 minutes by car) were invited to come fasted to the research Center. Of these, 137 middle-aged couples, each consisting of an offspring of a nonagenarian sibling and the partner thereof, agreed to participate. Of the 137 offspring, two participants were excluded because of current use of glucose lowering agents, nine participants because of a previous history of diabetes mellitus and five because of unreliable oral glucose tolerance test results. Of the 137 partners, six participants were excluded because of current use of glucose lowering agents, seven because of a previous history of diabetes mellitus, ten because of unreliable oral glucose tolerance test results and one because of non-compliance to the fasting

state. The Medical Ethical Committee of the Leiden University Medical Centre approved the study and informed consent was obtained from all subjects.

Anthropometric measurements

Waist circumference was measured halfway between the lower costal margin and the iliac crest with subjects in standing position. Hip circumference was measured at the level of the great trochanters. Body composition was determined by a bioelectrical impedance analysis. Measures of blood pressure, heart rate and temperature were taken at two occasions and averaged for analysis. Glucose tolerance was assessed by a two hour oral glucose tolerance test, conducted with a standard loading dose of 75g glucose/300 ml water, and venous blood samples drawn at time points of zero, 30, 60 and 120 minutes after glucose loading. Data on frequency, intensity and duration of exercise were obtained using the International Physical Activity Questionnaire (Ipaq).[20] Data were available for only 85 offspring (70.2%) and 80 partners (70.8%).

Biochemical analysis

All serum measurements were performed with fully automated equipment. For insulin the Immulite 2500 from DPC (Los Angeles, CA, USA) was applied. The coefficient of variation (CV) for this measurement was below 8%. For glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, the Hitachi Modular P 800 from Roche, Almere, the Netherlands was applied. CV's of these measurements were below 5 %. For low-density lipoprotein (LDL)-cholesterol the Friedewald formula was applied.

Definitions

Metabolic syndrome was defined according to the criteria of the Third Report of the National Cholesterol Education Program:[21] Waist > 102 cm (males), waist > 88 cm (females), Triglyceride ≥ 1.69 mmol/L, HDL cholesterol <1.04 mmol/L (men) or < 1.29 mmol/L (women), Fasting glucose ≥ 6.1 mmol, Systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥ 85 or treated hypertensive.

Areas under the curves obtained in the oral glucose tolerance test were calculated by the trapezoid rule; the homeostasis model assessment (HOMA) of insulin sensitivity was calculated by dividing 22.5 by the product of the fasting plasma insulin level (in mU/L) and the fasting plasma glucose level (in mmol/L) [22]. Insulinogenic index was calculated as the $\Delta_{30,\,0\ \text{minutes}}$ insulin (mU/L) divided by the $\Delta_{30,\,0\ \text{minutes}}$ glucose (mmol/L).

Statistical analyses

Distributions of continuous variables were examined for normality and logarithmically transformed when appropriate and used in all calculations. Geometric means (with 95% confidence intervals (CI)) are reported for transformed variables (serum insulin levels, area under the curve for insulin and insulinogenic index). Differences between offspring and partner categories were assessed with the use of linear mixed models or with logistic regression, adjusted for age and body mass index and correlation of sibling relationship. Differences in age between the group of offspring and partners were tested using a Mann-Whitney rank sum test. Differences in smoking behavior and sex distribution between the group of offspring and partners were calculated using a Chi-square test. The Statistical Package for the Social Sciences (SPSS) program for Windows, version 16.0 or STATA, version 10.1 were used for data analysis.

Results

Table 1 displays the baseline characteristics of the study populations after exclusion of diabetic participants (see methods section). In total 121 offspring and 113 partners were included in the study. The offspring group was slightly yet non-significantly older than the group of partners (median age of 63.9 years and 62.2 respectively; $p = 0.33$). Current smoking status was not different between the two groups: 11 current smokers (9.2%) in the offspring group versus 12 current smokers (10.6%) in the partners group ($p = 0.83$). Body mass index and the percentage of body fat were similar between the offspring group and partner group. In the group of offspring we observed a lower proportion of subjects using lipid lowering agents than in the group of partners. Estimated mean fasting total cholesterol and fasting LDL cholesterol levels were higher in the group of offspring than in the group of partners. However, exclusion of subjects using lipid lowering agents, diminished the difference in mean fasting total cholesterol and fasting LDL levels between offspring and partners. Furthermore, we found that the group of offspring had lower levels of fasting glucose, fasting insulin, a lower proportion of subjects using antihypertensive agents and lower systolic blood pressure.

Table 1. Baseline Characteristics of Offspring and Partners

	Offspring	Partners	P-value
Number participants (N, %)	121 (51.7%)	113 (48.3%)	
Females (N, %)	62 (51.2%)	59 (52.2%)	
Age (year)	63.9 (58.9 – 67.9)	62.2 (58.9 – 67.6)	0.33
Physical activity (Met-S/ week)	712.6 (569.9 – 891.1)	768.4 (610.5 – 967.2)	0.64
Smoking	11 (9.2%)	12 (10.6%)	0.83
Fat percentage	31.0 (29.7 – 32.4)	30.5 (29.1 – 31.9)	0.49
Body Mass Index (kg/m^2)	26.2 (25.5 – 26.9)	26.4 (25.7 – 27.2)	0.62
Waist (cm.)	97.7 (95.8 – 99.6)	99.2 (97.3 – 101.2)	0.18
Lipid lowering agents (N, %)	7 (5.8%)	20 (17.7%)	0.004
Total cholesterol (mmol/L)	5.54 (5.37 – 5.72)	5.14 (4.96 – 5.32)	0.001
Total cholesterol (mmol/L)*	5.58 (5.41 – 5.75)	5.35 (5.16 – 5.56)	0.067
Triglycerides (mmol/L)	1.25 (1.15 – 1.36)	1.28 (1.17 – 1.39)	0.74
Triglycerides (mmol/L)*	1.25 (1.14 – 1.36)	1.27 (1.16 – 1.39)	0.77
HDL cholesterol (mmol/L)	1.55 (1.48 – 1.63)	1.48 (1.40 – 1.56)	0.17
HDL cholesterol (mmol/L)*	1.56 (1.48 – 1.64)	1.49 (1.40 – 1.56)	0.19
LDL cholesterol (mmol/L)	3.37 (3.21 – 3.54)	3.03 (2.86 – 3.20)	0.002
LDL cholesterol (mmol/L)*	3.40 (3.25 – 3.56)	3.24 (3.06 – 3.41)	0.14
Fasting glucose (mmol/L)	4.99 (4.89 – 5.08)	5.17 (5.08 – 5.27)	0.006
Fasting insulin (U/L)	5.61 (4.93 – 6.37)	6.65 (5.84 – 7.59)	0.034
Antihypertensive medication (N, %)	26 (21.5%)	38 (33.6%)	0.016
Systolic blood pressure (mm Hg)	138.9 (135.4 – 142.5)	144.5 (140.9 – 148.2)	0.030
Diastolic blood pressure (mm Hg)	82.9 (81.1 – 84.7)	83.6 (81.7 – 85.5)	0.57

Data are presented as estimated mean value with 95% confidence interval. Results were adjusted for age and sex (except age). * Analyses after exclusion of subjects using lipid-lowering agents. P values for antihypertensive medication and lipid lowering agents were calculated using a logistic regression model adjusted for age and sex. Data on physical activity were available for 85 offspring and 80 partners. HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Table 2. Number of non-diabetic participants who fulfill metabolic syndrome criteria for offspring and partners

	Offspring (N=121)	Partners (N=113)	p-value
Metabolic syndrome	25 (20.7%)	36 (31.9%)	0.031
Waist*	68 (56.2%)	70 (61.9%)	0.40
Triglyceride†	29 (24.0%)	29 (25.7%)	0.73
HDL cholesterol‡	16 (13.2%)	27 (23.9%)	0.017
Fasting glucose§	1 (0.8%)	10 (8.8%)	0.019
Blood pressure¶	83 (68.6%)	86 (76.1%)	0.050

* Waist > 102 cm (males), waist > 88 cm (females), † Triglyceride ≥ 1.69 mmol/L, ‡ HDL cholesterol <1.04 mmol/L (men) or < 1.29 mmol/L (women), § Fasting glucose ≥ 6.1 mmol/L,
¶ Systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥ 85 or treated hypertensive. HDL: high-density lipoprotein.

Table 2 shows the prevalence of metabolic syndrome and its individual components for the group of offspring and the group of partners. The group of offspring showed a lower prevalence of metabolic syndrome than the group of partners (p=0.031). Moreover, in the group of offspring a lower proportion of subjects fulfilled the criteria for the glucose component (p=0.019) and the HDL component (p=0.017) when compared to the group of partners. In contrast, no differences were observed between offspring and partners for obesity related criteria, including waist and triglycerides. **Figure 1** displays the number of metabolic syndrome components for offspring and partners.

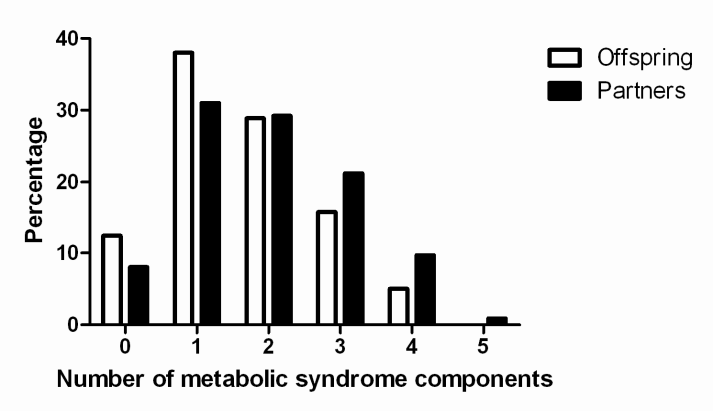

Figure 1. Distribution of number of metabolic syndrome components for offspring and partners.

To determine possible differences in peripheral glucose metabolism and insulin sensitivity between the groups of offspring and partners, participants underwent an oral glucose tolerance

test. Results of the oral glucose tolerance test are depicted in **figure 2**, in which all analyses were adjusted for BMI.

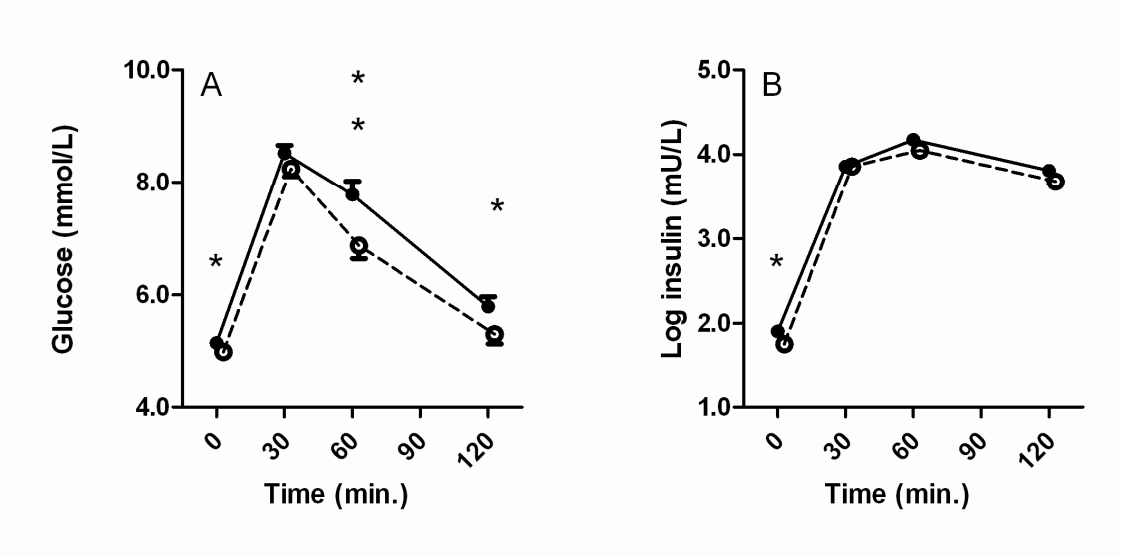

Figure 2. Results of oral glucose tolerance test for offspring and partners. *Figure 2A depicts serum glucose concentrations (mmol/L) for offspring (open circles) and partners (closed circles) for both sexes combined at 0. 30. 60 and 120 minutes (min.). Figure 2B depicts log serum insulin concentrations (mU/L) for offspring (open circles) and partners (closed circles) for both sexes combined at 0. 30. 60 and 120 minutes (min.). Data were adjusted for sex, age and body mass index. * denotes P value < 0.05. ** denotes P value < 0.01.*

Results of the oral glucose tolerance test are presented in **table 3** for the group of offspring and the group of partners. In the group of offspring as compared to the group of partners, fasting glucose levels were lower (4.99 mmol/L versus 5.16 mmol/L, P = 0.010) and the area under the curve for glucose was comparatively smaller (13.2 vs. 14.3; P = 0.007). Likewise, fasting insulin levels were lower in the group of offspring compared to the group of partners (5.81 mU/L vs. 6.75 mU/L; P = 0.039). The area under curve for insulin was non-significantly lower among the offspring group versus the partner group (92.1 vs.100.7; P = 0.18). Insulin sensitivity as assessed by the homeostasis model was higher among the group of offspring in comparison to the group of partners (0.78 vs. 0.65; P = 0.018). No differences were observed between the two groups for the insulinogenic index, an approximate measure for the pancreatic β-cell function: 13.6 in the offspring group versus 12.5 in the partner group (P = 0.38). These differences between the offspring and partner groups were most pronounced in females, while for males a trend towards these differences was observed (**table 3**). All analyses above were adjusted for age and body mass index (and in case of all, for sex). Results were not materially different when analyses were further adjusted for waist hip ratio, percentage of fat mass, current smoking and physical exercise (data not shown).

Table 3. Results of Oral Glucose Tolerance Test for Offspring and Partners.

	Offspring	Partners	P-value
All (n)	121 (100%)	113 (100%)	
Fasting glucose (mmol/L)	4.99 (4.90 – 5.08)	5.16 (5.07 – 5.26)	0.010
Area under the curve glucose	13.2 (12.6 – 13.8)	14.3 (13.7 – 14.9)	0.007
Fasting insulin levels (mU/L)	5.81 (5.20 - 6.51)	6.75 (6.02 - 7.57)	0.039
Area under the curve insulin	92.1 (83.2 - 102.0)	100.7 (90.6 - 111.8)	0.18
HOMA-insulin sensitivity	0.78 (0.69 – 0.88)	0.65 (0.58 – 0.74)	0.018
Insulinogenic index	13.6 (11.8 – 15.7)	12.5 (10.8 – 14.5)	0.38
Females (n)	62 (51.2%)	59 (52.2%)	
Fasting glucose (mmol/L)	4.88 (4.76 - 5.01)	5.13 (5.00 - 5.25)	0.007
Area under the curve glucose	13.2 (12.4 – 14.0)	14.2 (13.4 – 15.1)	0.069
Fasting insulin levels (mU/L)	5.34 (4.55 - 6.28)	7.27 (6.18 - 8.55)	0.007
Area under the curve insulin	92.7 (81.1 - 106.1)	107.0 (93.3 - 122.5)	0.13
HOMA-insulin sensitivity	0.87 (0.73 – 1.03)	0.61 (0.51 – 0.72)	0.003
Insulinogenic index	13.6 (11.5 – 16.2)	13.0 (10.9 – 15.5)	0.73
Males (n)	59 (48.8%)	54 (47.8%)	
Fasting glucose (mmol/L)	5.09 (4.95 - 5.24)	5.19 (5.04 - 5.34)	0.34
Area under the curve glucose	13.3 (12.4 – 14.2)	14.3 (13.4 – 15.3)	0.10
Fasting insulin levels (mU/L)	6.42 (5.56 - 7.41)	6.32 (5.44 - 7.34)	0.89
Area under the curve insulin	92.4 (79.6 - 107.3)	94.7 (80.8 - 111.0)	0.82
HOMA-insulin sensitivity	0.69 (0.59 – 0.81)	0.69 (0.59 – 0.81)	0.97
Insulinogenic index	14.4 (11.2 – 18.3)	12.8 (9.84 – 16.6)	0.46

Data are presented as means with 95% confidence intervals. Results were adjusted for age and body mass index, and in the case of all for age and sex. HOMA: homeostasis model assessment.

Discussion

The purpose of this study was to explore measures metabolic syndrome and differences in glucose metabolism among the middle-aged offspring of nonagenarian siblings which are enriched for heritable influences on longevity, as compared to the control group of their middle-aged partners. We found that the group of offspring had a lower prevalence of metabolic syndrome as compared to the group of partners. When considering the individual components of the metabolic syndrome, the group of offspring showed a lower fraction of subjects fulfilling the

criteria for the HDL component and the glucose component but not of obesity related criteria, including waist and triglycerides, centralizing the role of glucose metabolism in our findings.
With respect to glucose metabolism, we found that the group of offspring had lower fasting blood glucose concentrations and higher HOMA insulin sensitivity when compared to the group of partners thereof. In addition, offspring had a more favorable glucose tolerance than their partners. However, beta cell function as measured by the insulinogenic index was similar between the two groups.

These data are in accordance with earlier studies showing that the offspring of exceptionally long-lived individuals are protected against the combination of cardio-vascular risk factors that constitute the metabolic syndrome [15]. However, while it was shown that offspring of exceptionally long-lived individuals are healthier in many parameters, this has not previously been shown for glucose tolerance. Data from mammalian models show an association in diverse mutants (including those with mutations causing growth hormone/IGF-1 resistance) between enhanced lifespan and preserved insulin sensitivity i.e enhanced insulin action. Taken together, these findings suggest that in humans as in mammals decreased insulin signaling is not associated with exceptional longevity as it is in non-mammalian models.

These findings are a crucial extension of our initial observations of lower non-fasted blood glucose levels and the lower prevalence of diabetes in offspring of nonagenarian siblings compared to their partners [18, 19]. Moreover our findings add to the previous observations of a preserved glucose tolerance and insulin action in healthy centenarians [16] by demonstrating that a beneficial glucose metabolism is already present at middle-age in offspring of familial nonagenarians.

The lower prevalence of metabolic syndrome and better glucose handling in the offspring of nonagenarian siblings which we observed in the current study might have contributed to the lower prevalence of cardiovascular disease which we reported in an earlier study [18]. Prior research has demonstrated advantageous cardiovascular risk profiles in middle-aged individuals with long-lived parents compared with those whose parents died younger [23, 24], although in this study significant differences in lifestyle existed between the groups that were compared, including years of education and current smoking, which complicates disentangling the precise contribution of genetic and lifestyle factors to the observed longevity phenotype. As a strategy to minimize the potential confounding effects of differences in environment, we and others have deliberately chosen to compare offspring from long-lived cases to their partners [23, 25].

In line with previous findings [26, 27], our data suggest that differences in metabolic syndrome and glucose tolerance may result not only from environmental factors but also from genetic factors, which are transmitted in families. The lack of difference in the insulinogenic index between the offspring and their partners makes pancreatic β-cell function unlikely to account for the beneficial glucose tolerance in the offspring. As the offspring and their partners by and large share the same environment, it is unlikely that the observed differences between offspring and partners are fully explained by environmental conditions. For example, current smoking behavior and levels of physical activity were similar in both groups. Likewise, body mass index, an important risk factor for the development of insulin resistance was similar among the two groups. In order to identify mechanisms which may be involved in the better glucose handling we are planning to perform clamp studies in a representative subset of offspring and the partners thereof.

In conclusion, we observed a lower prevalence of metabolic syndrome and a favorable glucose tolerance among the offspring of nonagenarian siblings when compared to their partners. The favorable glucose tolerance could not be explained for by differences in body mass index and pancreatic β-cell function. Our findings imply that the preserved glucose tolerance and insulin action is already present at middle-age in offspring from familial nonagenarians.

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