Chapter 11: General discussion and synopsis of part A

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Introduction

World life expectancy has rapidly increased over the last two centuries, however not all of the gained years of life are spent in good health. There is a strong need to identify candidate targets for interventions to prevent age-related loss of function and morbidity. Substantial evidence supports the familial clustering of exceptional longevity, suggesting a genetic etiology for longevity. In search for the biology of healthy longevity we therefore set off to study the phenotypes of exceptionally long-lived families in the Leiden Longevity Study. In this chapter, we give a summary of the endocrine and metabolic characteristics that appear to be pertinent for familial healthy longevity.

Mortality and morbidity

Previous studies have shown that familial factors contribute substantially to the ability to reach very old age [1]. Indeed, families from the Leiden Longevity Study display a striking survival benefit of 30% when compared to the general population (**chapter 2**). This lower mortality risk is not only present in nonagenarian sibling pairs but extends to first-degree family members of the nonagenarian siblings as well [2;3]. Persistence of a survival advantage in siblings up to the highest age ranges suggests the involvement of genetic factors since many environmental factors shared early in life are likely to diverge as the siblings grow older [4].

Apart from a delay in mortality, another important indicator of aging retardation is a later onset of age-related diseases [5]. The incidence of diseases declines with age and is an important contributor to mortality [6]. We found that the offspring of nonagenarian siblings had a markedly reduced risk for age-related diseases as myocardial infarctions, hypertension and most notably type II diabetes (**chapter 2**) [2]. Our outcomes are in accordance with prior studies which showed lower disease prevalence in children from parents who reached an exceptionally high age when compared to control subjects whose parents died at younger ages [7;8]. However in these earlier studies significant differences in major cardiovascular risk factors were present between these groups, including years of education and smoking habits. Exact determination of the contribution of genetic, behavioural, and lifestyle factors therefore remained cumbersome. Moreover, since centenarians generally engage in healthy lifestyles, their offspring may have copied their behaviour [9]. To rule out potential confounding by differences in environmental factors, we compared offspring from long-lived cases with their partners in the Leiden Longevity Study. We hypothesized that as the offspring and their partners by and large share the same environment, it is unlikely that any observed differences between the two groups are due to differences in environmental factors. Indeed, major indicators of lifestyle, including estimates for BMI, current smoking, and associated prevalence of chronic obstructive pulmonary disease were similar

between both groups, indicating that the difference in health status is more likely due to genetic than environmental factors [2;10].

We did not find a difference in cancer prevalence nor deaths due to cancer when comparing offspring with controls. This finding is at odds with earlier research showing a relative lower risk of cancer mortality in offspring of centenarians as compared to controls [11]. The discrepancy might be explained by a difference in age: the study groups from the Leiden Longevity Study are approximately 10 years younger than those in the reported study.

IGF insulin signaling

The role of the evolutionarily conserved insulin/ insulin-like growth factor (IGF-1) signaling (IIS) pathway in the regulation of lifespan is well documented in worms [12], flies [13], and rodents [14;15]. Genetic mutations that partially blunt IIS activation prolong lifespan in these organisms, particularly in the female sex. Invertebrates have a single receptor that binds multiple ligands comparable to insulin/IGF-1, whereas in mammals distinct receptors have evolved for insulin and IGF-1, with different but overlapping functions. IGF-1 is involved in growth, while insulin primarily regulates metabolism [16].

In mammals, a hallmark phenotype shared by many of the long-lived mutants [17] including those with genetically induced IGF-1 resistance is their preserved insulin sensitivity and low fasting blood glucose levels. Preserved insulin sensitivity is also closely linked to the lower mortality observed in mammals under dietary restriction conditions. Several findings in the Leiden Longevity Study imply that preserved insulin sensitivity is at play in human longevity as well.

First, the middle-aged offspring of nonagenarian siblings had relatively lower blood glucose and insulin levels (**chapter 7**) [18] as well as a more favourable glucose tolerance as assessed by oral glucose tolerance tests (**chapter 3**) [10]. Preliminary evidence suggests that this phenotype of lower blood glucose and insulin levels in families with exceptional longevity relative to the general population is present even up to the highest age ranges (**figure 1**). Remarkably, common determinants for insulin resistance such as physical activity, body composition, diet, low-grade inflammation (**chapter 5**) were similar between the middle-aged groups. Secondly, the group of offspring showed a lower prevalence of metabolic syndrome (**chapter 3**) [10], a combination of cardio-vascular risk factors for which the dominant underlying factor appears to be insulin resistance. When considering the individual components of the metabolic syndrome, the group of offspring contained fewer individuals with low HDL levels and a lower number of individuals with impaired fasting glucose. Obesity related criteria, including elevated waist circumference

and fasting triglyceride levels were equal between the two groups, centralizing the role of glucose metabolism in our findings. Finally, middle-aged offspring predisposed for healthy longevity showed higher whole-body insulin sensitivity, marked by enhanced peripheral glucose disposal as assessed by hyperinsulinaemic euglycaemic clamp (**chapter 4**)). Using this technique, which is considered the gold standard for assessment of whole-body insulin sensitivity, we found that it is insulin action on glucose metabolism, and glucose disposal in particular, that distinguishes offspring of long-lived siblings from controls, rather than insulin mediated suppression of endogenous glucose production or lipolysis. The importance of sustained insulin action on glucose disposal is in agreement with prior studies on the pathophysiology of type II diabetes mellitus. Peripheral insulin resistance is regarded as one of the earliest steps in the pathophysiology of diabetes [19;20], and is already present several decades before onset of the disease [21;22]. Insufficient suppression of hepatic glucose production, on the other hand, is a consequence of fat accumulation in the liver [23], and is considered an advanced phenomenon in the trajectory towards onset of diabetes [20].

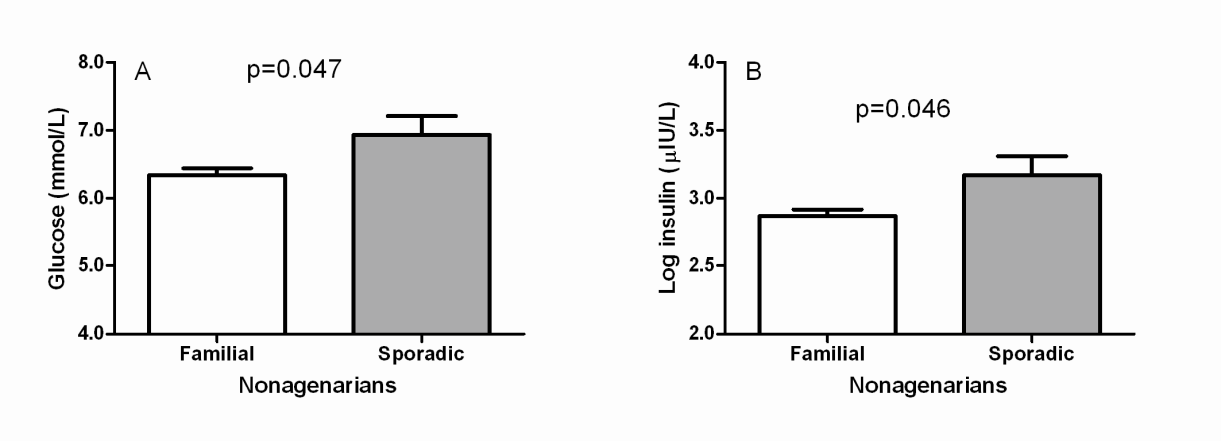

Figure 1. Non-fasted serum glucose (A) and log insulin (B) levels for familial nonagenarians (N=333) and sporadic nonagenarians (N=49) (not selected for having nonagenarian siblings). *To decrease the possible influence of differences in health status between the two groups, nonagenarians from the highest tertile of performance on Instrumental Activities of Daily Living score were included. Bars represent mean levels with standard errors of the mean adjusted for sex and age.*

Our data agree with earlier studies which show that the offspring of exceptionally long-lived individuals are protected against development of cardio-vascular disease [11;24;25]. Earlier, offspring from long-lived parents were shown to have favorable lipid profiles [26;27]. However, while it was shown that offspring of exceptionally long-lived individuals are healthier in many respects [28], this has not been reported before for glucose regulation. While insulin sensitivity generally declines with age in humans, sporadic long-lived centenarians exhibit an extraordinary insulin sensitivity, comparable to that of young adults [29]. Our findings add to these previous observations by

demonstrating that a beneficial glucose metabolism is already present at middle-age in offspring of familial nonagenarians.

Apart from the preserved insulin sensitivity, exceptional human longevity is possibly associated with tempering of the IGF-1 signaling pathway. Recently, it was shown that centenarians exhibited a relative enrichment for rare genetic variants in the IGF-1 receptor which resulted in high levels of IGF-1/IGFBP3 coexisting with low levels of IGF-1 signaling [30]. We and others demonstrated that common genetic variations affecting IGF-1 signaling might contribute to differences in mortality in the general population [30;31]. In line with these studies and other studies in model organisms, we found preliminary evidence for the involvement of reduced IGF-1 signaling in familial longevity. Most recently, we observed lower serum levels of IGF-1 in female nonagenarians whose parents reached an exceptionally high age when compared to nonagenarian subjects whose parents died at younger ages (**chapter 8**). Another important feature of (lifelong) blunted IGF-signalling in both humans and model organism is a relatively small stature. In agreement with this observation, nonagenarians from parents who reached an exceptionally high age tended to be shorter than nonagenarian subjects whose parents died younger.

The findings in the group nonagenarians contrast with those in their middle-aged children. We did not observe differences in IGF-1 serum levels nor height between the offspring and their partners (**chapter 7**) [18]. Moreover a single time-point measurement after overnight fast showed similar levels of serum growth hormone levels between the two generations (**table 1**). These disparate results may be explained by differences in age. The estimated contribution of genetic factors to longevity is modest (20-30%) but was shown to become more important and specific at higher ages. Therefore it is possible that the effects of genetic variation in the IIS pathway only become detectable at advanced ages. In line, the association between genetic variation in *FOXO3A* and longevity was found to be stronger in centenarians than in nonagenarians [32]. Another possible explanation for these contrasting observations could be differences in imprinting of the IGF-1 gene, reflecting historical differences in maternal nutrition between the two generations [30;33].

Thyroid function

The hypothalamo–pituitary–thyroid axis is widely implicated in modulating the aging process [34]. Life prolonging effects related to decreased thyroid hormone levels have been reported in multiple animal models. In neonatal rats induction of hypothyroidism results in a moderate extension of lifespan [35]. Similarly, low thyroid hormone levels are characteristic of murine pituitary mutants with postponed aging: long-lived Ames and Snell dwarf mice show features that are likely related to thyroid hormone deficiency [36]. In humans a relation between low thyroid

function and extended lifespan has been observed as well. In the oldest old, higher concentrations of thyrotropin are associated with a survival advantage without apparent detrimental effects on ability or mood [37;38].

Table 1. Serum levels of endocrine parameters under fasted conditions for offspring and partners

	Offspring	Partners	P-value
Participants (N)	121	113	
Females (N, %)	62 (51.2%)	59 (48.8%)	0.90
TSH (mU/L)	2.41 (1.93 - 3.06)	1.69 (1.33 – 2.15)	0.029
free T4(pmol/L)	16.2 (15.8 – 16.6)	16.4 (16.0 – 16.9)	0.49
free T3 (pmol/L)	5.03 (4.87 – 5.20)	5.26 (5.09 – 5.44)	0.045
Growth hormone (mU/L)	1.90 (1.50 – 2.40)	2.02 (1.58 – 2.57)	0.72
IGF-1 (nmol/L)	15.3 (14.3 – 16.2)	15.0 (14.1 – 16.0)	0.71
IGFBP3 (mg/L)	4.03 (3.85 – 4.21)	3.98 (3.79 – 4.16)	0.63
Cortisol (μmol/L)	0.49 (0.47 – 0.52)	0.52 (0.49 – 0.55)	0.22
Prolactin (U/L)	10.1 (9.28 – 10.9)	10.3 (9.51 – 11.2)	0.64
HsCRP (mg/dL)	1.29 (1.17 – 1.60)	1.17 (0.93 – 1.46)	0.45

Data are given as mean values with 95% confidence intervals. TSH, growth hormone and high-sensitivity C-reactive protein (hsCRP) are given as geometric means with 95% confidence intervals. Samples were taken between 9:00 – 9:30 a.m. data were adjusted for sex and age.

The outcomes of the Leiden Longevity Study seem to support the relation between low thyroid function and extended life span. Nonagenarians from parents who reached an exceptionally high age had higher thyrotropin levels, lower free thyroxine and lower free triiodothyronine levels when compared to nonagenarian subjects whose parents died at younger ages (**chapter 9**) [39]. The lower thyroid function in the nonagenarians was reflected in their middle-aged children, who showed lower peripheral thyroid hormone levels and a tendency towards elevated thyrotropin levels as compared to their partners during both non-fasted (**chapter 8**) [40] and fasted conditions (table 1). These observations imply that lower activity of the thyroid hormone axis is a heritable phenotype which contributes to exceptional longevity.

Lower activity of the thyroid hormone axis possibly acts as a mechanism to reallocate energy expenditure from growth and proliferation to protective maintenance. Thyroid hormones primarily regulate the basal metabolic rate of cells, thereby inducing thermogenesis and free radical production [41]. Data from model organisms show that low triiodothyronine is associated with lower production of reactive oxygen species (ROS) and ROS inflicted genomic damage [42]. The more efficient transport of electrons through the respiratory chain under conditions of low thyroid hormone might decrease the production of ROS and postpone aging.

Our study demonstrated lower thyroid function in subjects from extremely long-lived families. The prevalence of subclinical hypothyroidism and subclinical hyperthyroidism increase steeply with age [43] and the exact definition of subclinical thyroid dysfunction and the requirement for age-specific thyrotropin reference limits in clinical practice is currently a matter of intense debate. Thyrotropin levels are known to gradually increase with age, a shift that was recently shown to extend to advanced age [44]. The higher thyrotropin levels observed at old age possibly result from selective survival of subjects with constitutionally low thyroid function [37]. Furthermore, some of the changes in thyroid function that occur upon aging may be part of the age related pathology that is caused by accumulated damage, while others may actually occur in response to the accumulation of damage and may instead represent adaptive mechanisms aimed at delaying age-related pathology. Although controversial, the prevailing recommendation is to treat elderly with subclinical hypothyroidism with thyroid hormone supplementation. In view of these considerations however, the issue of reversing the endocrine changes that occur during human aging by treatment of (subclinical) hypothyroidism, remains highly controversial. While pathological changes might benefit from treatment, constitutively low thyroid function or changes in thyroid function that are part of an adaptive response might not.

Upon aging, other changes may occur in the thyroid axis beside an elevation of thyrotropin levels. In **chapter 10**, we demonstrate the existence of a mutual relationship between levels of free triiodothyronine and inflammatory cytokines. High levels of inflammatory cytokines are associated with reduced levels of free triiodothyronine, suggesting that under conditions of inflammation, the activity of the thyroid axis is dampened, possibly via reduced conversion of thyroxine to triiodothyronine.

Conclusion

As average life span continues to increase, so does the number of years spent in ill health. There is an urgent need to identify candidate targets for interventions to prevent age-related loss of function and morbidity. Studies into the phenotype of humans predisposed for an exceptional long life may delineate the determinants for healthy life span extension. Presuming that the characteristics conducive to longevity are transmitted in long-lived families, the offspring from exceptionally long-lived parents may reveal the key to successful aging.

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