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BELFAST Centenarians: A Case of Optimal Cardiovascular Risk?

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Abstract: *Introduction*: Centenarians are reservoirs of genetic and environmental information to successful ageing and local centenarian groups may help us to understand some of these secrets. The current centenarian cohort in Belfast survived the 1970s epidemic of death from coronary heart disease in Northern Ireland, where cardiovascular mortality was almost highest in the world. These centenarians provided an opportunity to assess biological and genetic factors important in cardiovascular risk and ageing.

Methods: Thirty-five (27 female, 8 male) centenarians, participants of the Belfast Elderly Longitudinal Free-living Ageing STudy (**BELFAST**), were community-living and of good cognition at enrolment.

Results: Centenarians showed median Body Mass Index (BMI) at 25.7, systolic blood pressure 140mmHg and diastolic blood pressure 90mmHg respectively, and fasting glucose of 5.54mmol/l with no sex-related difference. Lipoproteins showed median cholesterol 5.3, High Density Lipoprotein (HDL) 1.10 and Low Density Lipoprotein (LDL) 3.47mmol/l respectively. Centenarian smokers showed no different blood pressure or lipid measurements compared with non-smokers. Malondialdehyde, a measure of lipid peroxidation, was low at 1.19 umol/l, and measures of antioxidant status showed variable results. Male centenarians did not carry any of the vascular risk genotypes studied ApoE4 for Apolipoprotein E (ApoE), DD for Angiotensin-Converting Enzyme (ACE), and TT for Methylene Tetrafolate-dehydrogenase reductase (MTHFR), though this is not true for female centenarians.

Conclusions: This small local study shows, that Belfast centenarians carry a reasonably favourable risk profile with respect to cardiovascular disease. There is also some evidence suggesting that vascular risk factors and genotypes may be tolerated differently between the male and female centenarians. Maintaining an optimal cardiovascular risk profile seems likely to improve the chance of becoming a centenarian, especially for males.

Keywords: Centenarians, BELFAST, ApoE, ACE, cholesterol, anti-oxidants, malondiadehyde, mortality.

INTRODUCTON

In the past century, the developed world has seen a greater increase in life expectancy than in all recorded history to the year 1900 [1]. This has generated interest in centenarians and the intriguing mystery of their successful aging. It is not understood why there are more centenarians in Okinawa, Japan than anywhere else in the world [2], why there are more male centenarians in Sardinia [3] and why females outnumber male centenarians worldwide [4]. Although the very elderly are the fastest growing sector of the United Kingdom population, there is remarkably little known about centenarians in either Britain or Ireland.

Current estimates of the Northern Ireland population are 1.7 million with a predicted estimate of around 150 centenarians [5], though no truly accurate data exists. A number of medical and geographical factors arguably make Irish centenarians of interest. Firstly, Northern Ireland has historically suffered very high cardiovascular mortality [6] and secondly, the All Ireland Cancer Registry has highlighted an excess prevalence of cancer with a significant male excess [7,8]. Many geneticists have also argued that the Irish population is more genetically homogeneous compared to mainland Europe or even Britain [9]. These arguments suggest that genetic and environmental studies in Irish centenarians may yield important information not available in more heterogeneous population groups.

METHODS AND RESULTS

BELFAST CENTENARIANS

The centenarians in this study were enrolled as part of an ongoing longitudinal study of ageing called -Belfast Elderly Longitudinal Free-living Aging Study - acronym (**BELFAST**), described previously [10]. All octo/nonagenarian subjects, including centenarians, when enrolled, were of good cognition [11], community-living and approximated 'Senieur'status [12]. Ethical permission for the study was given by the Queens University of Belfast.

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AGE AND SEX OF CENTENARIAN SUBJECTS

The centenarian group contained 8 male and 27 female centenarians (Table 1).

Table 1. Age Distribution of Centenarian Subjects

Age	Male	Female	Total
100 yrs	4	7	11
101	1	7	8
102	1	6	7
103	2	2	4
104	0	2	2
106	0	3	3
	8	27	35

ANTHROPOMETRIC CHARACTERISTICS

Median weight was 55kg with no significant difference between male and female subjects (p=0.41), Table 2. As at younger ages, height was significantly greater in males compared to females (p=0.007). Median skin fold thickness, taken with calipers over the triceps skin fold, was low at 11mm with no sex difference (p=0.42). Mean body mass index (BMI) was 25.7 with females having non-

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Table 2. Anthropometric Measurements and Blood Pressure

	TOTAL (34)	MALE (7)	FEMALE (27)	Mann Whitney U Male v Female
Weight kg	55 [39-95]	58.5 [53-81]	52 [39-95]	P=0.41
Height cm	148 [138-175]	160 [150-175]	147 [138-166]	P=0.007*
Triceps Skinfold Thickness mm	11 [4-18]	10 [8-15]	12 [4-18]	P=0.42
BMI Weight kg^2/ Height m^2	25.7 [16.6-47.1]	24.4 [18.6-26.7]	25.7 [16.6-47.1]	P=0.16
Systolic mmHg	140 [95-200]	130 [110-150]	140 [95-200]	P=0.18
Diastolic mmHg	90 [60-117]	90 [80-90]	90 [60-117]	P=0.50

Body Mass Index (BMI)
Median and range []
* p<0.05

signficantly higher BMI compared to males; higher BMI in aged females being related to reduction in height due to osteoporosis viz a viz weight in the BMI calculation. Values for weight and height were lower than those for BELFAST nonagenarians [13].

BLOOD PRESSURE

Blood pressure was measured in the morning at home with the subject sitting after a 10 minute rest and used the 1st and 5th Kortakoff sounds as standard. Median systolic blood pressure was 140mmHg with no significant sex-related change (p=0.18) (Table 2). Diastolic blood pressure was 90mmHg with no sex-related change.

BLOOD GLUCOSE

Fasting blood glucose was 5.54mmol/l with no difference between male (5.84mmol/l) and female (5.46mmol/l) centenarians.

CHOLESTEROL, LIPOPROTEINS AND LIPOPROTEIN (A)

Cholesterol was collected after an overnight fast, and measured by standard laboratory methods. Median cholesterol for the group was 5.3mmol/l with a wide range 2.8-8.9mmol/l (Table 3). Higher values were noted for females compared to males, mirroring sex differences at younger ages, but with no significant difference in centenarians (p=0.20). Median High Density Cholesterol (HDL) cholesterol was 1.0mmol/l (range 0.2-1.7) with no sex-related difference (p=0.8). Similarly, mean Low Density Cholesterol (LDL) had a median value of 3.47mmol/l (range 0.7-6.8) for the whole group with no differences across the sexes (p=0.2). Triglyceride was1.3mmol/l and was not different between males and females (p=0.7). Only 20 centenarians had lipoprotein (a) Lp(a) measured, 5 males and 15 females. Lp(a) was 16.5umol/l with a wide range 0.10-81umol/l, and was non significantly higher in males (p=0.09). Several centenarians had values of Lp(a) above 30umol/l which has previously been noted to be associated with premature vascular disease.

ANTIOXIDANT STATUS AND MALONDIALDEHYDE

Serum levels for vitamins C, A and E were available for 13 centenarians (3 males and 10 females). All vitamins were measured by high performance chromatography methods in blood samples freshly collected and stored at -70°C. Vitamin C showed a wide range with median level of 14umol/l, and lower levels in 3 male centenarians (3.0umol/l) compared to females (15.6umol/l) (Table 4). The male centenarians had Vitamin C levels in either mild or severe deficiency range [14], compared to 2/10 female centenarians who showed mild deficiency. Median serum Vitamin A was 1.95umol/l with generally higher values in female (1.97umol/l) compared to male centenarians (1.51umol/l). One female centenarian had vitamin A level in the *'at risk'* range. Median serum Vitamin E was 26.2umol/l with one male centenarian having an *'at risk'* Vitamin E level. Vitamin E corrected for serum cholesterol was 5.2 umol/mmol for the whole group with no sex difference. Gluthathione peroxidase activity, a measure of anti-oxidant status, was measured in whole blood and serum by standard methods [15] and was not different between male and female centenarians but demonstrated lower values than for BELFAST nonagenarians [16].

Malondialdehyde, a measure of lipid peroxidation, was analysed in 12 centenarians (2 males and 10 females) as previously described [16]. Mean value was 1.19umol/l, with female centenarians having a value of 1.14umol/l, range 0.4-1.7umol/l (data not shown). Control subjects of 20-40 years from the same geographical area had a higher mean value (1.7umol/l) [17,10] suggesting that subjects who achieve centenarian status may have a lower malondialdehyde burden compared with younger subjects.

SMOKING

Some 14% of centenarians were or had been smokers. Significantly more male subjects smoked (4/8) compared to female subjects (2/27) (p=0.0095). Median and ranges for cholesterol, systolic and diastolic blood pressure, some measures of antioxidant

Table 3. Cholesterol and Lipoproteins in Subjects Achieving Centenarian Status

	Total	Male	Female	Mann-Whitney U Male v Female
Cholesterol mmol/l	5.3 [2.8-8.9]	4.2 [3.7-5.4]	5.6 [2.8-8.9]	P=0.2
HDL mmol/l	1.0 [0.2-1.7]	0.92 [0.6-1.6]	1.0 [0.2-1.7]	P=0.8
LDL mmol/l	3.47 [0.7-6.8]	2.88 [2.6-4.7]	3.61 [0.7-6.8]	P=0.2
Trigylceride mmol/l	1.30 [0.24-2.7]	1.12 [0.24-2.2]	1.3 [0.24-2.7]	P=0.7
Lp(a) umol/l	16.5 [0.01-81]	51.3 [6-81]	14 [0.01-70]	P=0.09

Median and range []
HDL-high density lipoprotein
LDL-low density lipoprotein
Lp(a)-lipoprotein a

Table 4. Antioxidant Status in Subjects Achieving Centenarian Status

	Total	Male	Female	MWU Male v Female
Vitamin C umol/l	**14.0** [1.0-231]	**3.0** [1.0-31]	**15.6** [6-231]	P=0.16
Vitamin A umol/l	**1.95** [0.89-3.2]	**1.51** [0.95-1.7]	**1.97** [0.89-3.2]	P=0.87
Vitamin E umol/l	**26.2** [11.8-38.6]	**15.3** [11.8-26.2]	**29.6** [18.0-38.6]	P=0.88
Vitamin E/ Cholesterol umol/mmol	**5.2** [3.5-9.0]	**3.5** [3.5-7.1]	**5.6** [4.0-9.0]	P=0.70
Serum Glutathione Peroxidase U/L	**245** [127-318]	**255** [236-264]	**222** [127-318]	P=0.09
Whole Blood Glutathione Peroxidase U/L	**4975** [2577-5968]	**5531** [3371-5554]	**4771** [2577-5968]	P=0.19

Median and range []
MWU Mann Whitney U

status and malondialdehyde were compared between centenarian smokers and non smokers. Although malondialdehyde, a measure of lipid peroxidation, was higher and gluthatione peroxidase activity in blood and serum was higher in smokers compared to non smokers, this did not achieve significance across the small groups (Table 5).

GENETIC STUDIES

Cardiovascular-Related Genes-ApoLipoprotein E (ApoE), Angiotensinogen Converting Enzyme (ACE) and Methylenetetrahydrofolate reductase (MTHFR)

DNA, extracted from whole blood using standardised salting out methods [18], was available in 26 of the 35 centenarian subjects for genetic typing for 3 genes associated with vascular risk-ApoE4 for apolipoprotein E (ApoE) [19], DD for Angiotensinogen Converting Enzyme (ACE) [20] and the thermolabile vanant for methylene tetrahydrofolatereductase MTHFR [21] (Table 6). ApoE alleles, E2, E3 and E4, were present in 16%, 80% and 8% in centenarians respectively. Although the number of centenarian subjects is small, there appears to be an over representation of E2 (8%) and a decrease in E4 (16%) compared with a local younger population genotyped as part of the World Health Organisations MONICA project (MONItoring of CArdiovascular Health) [22]. No centenarian male carried the E4 allele, previously associated with atherosclerosis, but 4 female centenarians carried E4, associated with E3. Of 21 centenarians genotyped for ACE and MTHFR genotypes, no male centenarians were homozygous for

Table 5. Cholesterol, Antioxidant Status and Malondialdehyde in Smokers and Non Smokers in Subjects Achieving Centenarian Status

	Total	Smokers (6)	Non-Smokers (23)	MWU
Cholesterol mmol/l	**5.3** [2.8-8.9]	**3.9** [3.7-8.9]	**5.5** [2.8-8.5]	P=0.41
HDL mmol/l	**1.0** [0.21-1.7]	**0.92** [0.8-1.7]	**1.02** [0.21-1.6]	P=0.45
Systolic BP mmHg	**140** [95-200]	**140** [110-200]	**140** [95-140]	P=0.77
Diastolic BP mmHg	**90** [60-117]	**90** [80-90]	**90** [60-117]	P=0.68
Vitamin C umol/l	**14.0** [1.0-231]	**17.8** [1.0-51]	**14.0** [1.0-2315]	P=0.99
Vitamin A umol/l	**1.95** [0.9-3.2]	**1.61** [0.95-2.0]	**1.96** [0.90-3.2]	P=0.49
Vitamin E umol/l	**26.2** [11.8-38.6]	**19.9** [11.8-37]	**28.7** [17-38.6]	P=0.35
Vitamin E/ Cholesterol umol/mmol	**5.2** [3.5-9.0]	**4.8** [3.5-7.1]	**5.6** [3.5-9.0]	P=0.70
Serum Glutathione PeroxidaseU/L	**245** [127-318]	**254** [127-264]	**231** [134-318]	P=0.71
Whole Blood Glutathione PeroxidaseU/L	**4975** [2577-5968]	**5531** [4212-5554]	**4771** [2577-5968]	P=0.94
MDA umol/l	**1.2** (0.4-1.7)	**1.6** (1.5-1.7)	**1.0** (0.4-1.7)	P=0.10

Median and Range [ ]
MWU Mann Whitney U
MDA Malondialdehyde
HDL- high density Lipoprotein

either DD for ACE or the thermolabile variant TT for MTHFR gene, both genotypes which have been associated with increased vascular/ atherosclerotic-related risk in younger population groups.

CAUSES OF DEATH

Currently there is a preponderance of circulatory-related death causes in over 65+ year olds in the Northern Ireland population. Approximately 55% of the local population die from circulatory diseases, 20% from neoplastic disease, 15% from respiratory disease, and 10% from other causes [5]. In the **BELFAST** follow-up study, causes of death in centenarians were identified from death certificates available from the Register General Office, Belfast and described in Table 7. In centenarians, the major cause of death was respiratory, (63%, mainly recorded as bronchopneumonia) compared with 15% in the total local over 65+ population. Similarly circulatory disease accounted for death in only 15% of centenarians compared with 55% in the over 65+ population.

DISCUSSION

This is the first study to have provided any basic clinical and genotypic data on centenarians in Northern Ireland, although interestingly, the first documented notification of a super centenarian (centenarians who live beyond 110 years), arose in Northern Ireland in 1930 and was of a woman aged 112 years [23]. Although the number of Belfast centenarians studied is small, they represent about 25% of the expected local centenarian group. The **BELFAST** centenarians are likely to represent a somewhat more *'elite'* group of centenarians, since admitting criteria included good cognition and community-living characteristics, and they therefore are unlikely to be entirely typical and this, and the small sample number are a limitations of the study.

As in most other studies in most western countries, there was an increased number of female to male centenarians, in the ratio of 4:1. The reason for the sex difference is not known but higher rates of smoking, occupational illness, war/accident and cardiovascular-

Table 6. ApoE, ACE and MTHFR Genotypes in Centenarians

		Total	Male	Female
ApoE	E2/3	8	2	6
	E3/3	14	2	12
	E3/4	4	0	4
		26		
	E2 allele	8	2(25%)	6(75%)
	E4 allele	4	0	4(100%)
ACE	II	5	1	4
	ID	11	3	8
	DD	5	0	5
		21		
	I allele	21	5(24%)	16(76%)
	D allele	21	3(14%)	18(86%)
MTHFR	CC	6	0	6
	CT	14	4	10
	TT	1	0	1
		21		
	C allele	26	4(15%)	22(85%)
	T allele	16	4(25%)	12(75%)

ApoE-apolipoproteinE
ACE-Angiotensin-Converting Enzyme
MTHFR-Methylenetetrahydrofolate reductase

Table 7. Causes of Death in Centenarians

	Circulatory	Respiratory	Neoplastic	Other
Male	1	4	-	2
Female	4	17	3	2
	5	21	3	4

related disease have been suggested as possible reasons, with variable amounts of evidence to support these suggestions [24,25].

Belfast centenarians are interesting because they are survivors of the high incidence of cardiovascular-related disease and death, which peaked in the 1970s when Northern Ireland was near the top of the world league for cardiovascular disease [6]. Conventional risk factors for cardiovascular disease such as blood pressure, lipoproteins, smoking, and diabetes predict almost 50% of cardiovascular risk [26-28]. Looking at the centenarian risk factors, apart from their age, mean blood pressure was at an acceptable level of 140/90mmHg and no centenarian took anti-hypertensive medication. Although the cholesterol values were generally above the new UK suggested lower range for cholesterol (</= 4.8mmol/l), HDL was well maintained and the HDL/LDL ratio would be considered satisfactory [29, 27]. The higher lipoprotein (a) values in centenarians have been noted previously and may track with reduced glomerular filtration rate which decreases 10% with each decade of age [2]. No Belfast centenarian was a diabetic and although centenarian smokers had higher malondialdehyde, a measure of lipid peroxidation, there were no apparent changes in cardiovascular risk factors or anti-oxidant status.

Genetic risk factors associated with vascular or atherosclerotic-related disease were conspicuously absent from centenarian males but were found in female centenarians in our study area. No male centenarian carried any of the alleles previously associated with a cardiovascular risk profile, ie ApoE4 [19], DD allele of Angiotensinogen Converting Enzyme (ACE) gene [20] and TT thermolabile allele for methylenetetrahydrofolate reductase enzyme (MTHFR) [21] but up to 20% of female centenarians, for whom DNA was available, carried the ApoE4 allele (albeit alongside ApoE3) or were homozygous for the DD allele for ACE or the 'tt' allele for MTHFR. In early work in French centenarians, Schachter [30], found an increased carriage of ApoE2 alleles with attrition of ApoE4 and an excess of DD alleles of ACE genotype. Other worldwide studies would appear to consolidate the finding that ApoE 4 alleles tend to be reduced in centenarian and nonagenarian subjects with an increase in ApoE2 carriage [22, 31-34], though the situation has remained a good deal less clear for the ACE DD and the 'tt' MTHFR alleles [35-38]. Recent work mostly supports a more atherogenic lipoprotein profile with ApoE4 compared to ApoE2 allele carriage [39,40], though whether ApoE4 can prospectively predict early cardiovascular mortality is less clear [41].

Good maintained anti-oxidant status may protect against the free-radical damage associated with ageing [42]. This study made an estimate of the antioxidant and free radical status in our centenarians. Malondialdehyde, a measure of lipid peroxidation and membrane damage, was lower in centenarians than younger local contols, suggesting that subjects who achieve centenarian status may have a lower lipid peroxidation burden [10]. In terms of their anti-oxidant capacity, Belfast centenarians showed a wide range of values, which are harder to interpret. Serum and blood glutathione peroxidase, highly important measures of anti-oxidant buffering, were lower in centenarians compared to BELFAST nonagenarians [16], though it is unclear whether these represent suboptimal glutathione peroxidase activity or perhaps reduced anti-oxidant stress/requirement. Vitamin C and E usually considered separate anti-oxidants, may better be considered together and symbiotic in their actions, with in/adequacy of one or the other, making for damaging pro-oxidant activity rather than enhanced pro-oxidant status. Vitamin C was very low in the 2 centenarian males, with a better range for females. Fat soluble vitamins A and E were generally well maintained across the centenarian group, perhaps in keeping with the increased fat body mass seen in ageing.

In this small group of centenarians, current or previous smokers had somewhat higher malondialdehyde, a measure of lipid peroxidation, but cholesterol fractions, systolic and diastolic blood pressure were apparently unchanged as was the serum and enzymatic antioxidant profile.

Belfast male centenarians seemed to show relatively low conventional vascular risk factors in life and a reduced percentage had a vascular-related death, a pattern described previously in centenarians [2, 43]. By contrast, female centenarians seem to tolerate less favourable blood pressure and lipoproteins profile. It is interesting to ask why should this be so and whether it suggests that vascular disease continues to be modulated by sex difference even in centenarians? Increasingly there is evidence that hormonal milieu can determine the degree of gene activation [44-46].

This small local study shows, apart from age, that Belfast centenarians carry a reasonably optimized risk profile with respect to cardiovascular disease. There is also some evidence to suggest that vascular risk factors and genotypes may be tolerated differently between the male and female centenarians. Maintaining an optimal cardiovascular risk profile seems likely to improve the chance of becoming a centenarian. What is not yet clear is whether this means that males can catch up with female centenarians in the longevity stakes, or whether there are other unknown factors which remain to be discovered to explain the sex-related differences in longevity.

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