Cognitive function & HDL in the oldest old

The *APOE4* allele is reproducibly associated with a greater risk for Alzheimer's disease (AD), CVD, and hypercholesterolemia [52–54]. In the last 10 years, numerous studies have investigated the possible role of dyslipidemia in the pathogenesis of AD as well as vascular dementia (VD); however, the results have been conflicting. In a prospective study, hypercholesterolemia in mid-life was associated with an increased risk of AD more than 20 years later [55]; however, others did not find any associations between TC, HDL-C, and AD [56,57]. On the other hand, the associations between low HDL-C and cognitive decline in the oldest old population seems to be more consistent. Atzmon and colleagues reported that cognitive dysfunction in centenarians was associated with a progressive decline in plasma HDL [58]. Although the pathophysiology of dementia in this population is not fully uncovered, it generally implies vascular components. However, van Exel and colleagues demonstrated that cognitive impairment in the oldest old was at least partially independent of atherosclerotic disease [59]. They reported that in the Leiden 85-plus cohort, low Mini Mental State Examination (MMSE) scores were associated with low HDL-C levels, even when subjects with CVD and stroke were excluded, suggesting that the antiaggregation and anti-inflammatory properties of HDL might explain the association between HDL and cognitive impairment. In the Tokyo Centenarians study, we demonstrated that the level of HDL-C, but not TC or LDL-C, was significantly lower in the subset with cognitive impairment [9]. Centenarians with cognitive impairment were characterized by a cluster of deteriorating traits including lower albumin, higher CRP and IL-6, lower ADL scores, and a higher rate of APOE4, so that simultaneous effects of these factors on HDL-C levels could not be excluded. Cognitive impairment in the oldest old is multifactorial, and low HDL-C may be a surrogate marker rather a determinant.

Metabolic syndrome & healthy aging: are they two sides of the same coin?

Metabolic syndrome (MS) is described as a cluster of abnormalities, including insulin resistance, high fasting glucose, central obesity, hypertriglyceridemia, and low HDL-C, on the basis of significant risk for CVD and diabetes. In some aspects, longevity phenotype can be regarded as the other side of the coin for the MS phenotype. Insulin resistance has been proposed as the prime component of the multifaceted phenotype of the MS. In contrast, preserved insulin sensitivity [60] and better adipocytokine profiles [61] were relatively common characteristics of centenarians. Atzmon and colleagues reported that the prevalence of the MS and CVD was low in centenarians and their offspring [12,62]. More recently, they investigated 66 single nucleotide polymorphisms (SNPs) in 36 candidate genes involved in lipoprotein metabolism, and found that homozygocity for the -641 C in the *APOC3* gene was associated with a lower prevalence of hypertension, greater insulin sensitivity, higher HDL-C levels and increased longevity [63], suggesting that a low frequency of MS-related traits could be associated with longevity. Caloric restriction (CR), which is the only established intervention to slow aging and extend lifespan in organisms from yeast to mammals, may provide another clue to understanding the relationship between the MS and longevity. Although the life-long effects of CR in humans remain unknown, accumulating evidence suggests that prolonged CR improves body composition, decreases levels of TC, triglyceride (TG), LDL-C, fasting insulin and blood pressure, and raised HDL-C in non-obese individuals [64,65]. Furthermore, CR in obese subjects with the MS was associated with dramatic improvement in all aspects of the syndrome [66]. We previously reported that the dietary intakes of centenarians were approximately 25 kcal/kg body weight, which was comparable to that of healthy octogenarians [67]. However, the calorie intake of centenarians does not necessarily reflect their lifelong dietary pattern. We may have to wait for several decades to obtain definite conclusions on the association between CR, the MS and longevity in humans.

Low birthweight, a marker for adverse intrauterine environment, is another issue of interest in relation to the MS and aging. In the Hertfordshire (UK) cohort born in 1911–1930, low birthweight and accelerated weight gain in early childhood was demonstrated to have large effects on the incidence of CVD and the MS in later life [68,69]. In another study of postmenopausal women aged 50–84 years, low birthweight was associated with lower HDL-C, higher waist:hip ratio in later life, and, when combined with adult obesity, the prevalence of the MS in women with low birthweight was considerably higher [70]. These findings suggested that a substantial part of the risk for CVD and MS is established during early development, presumably by developmental plasticity and

compensatory growth. Many questions on the association between intrauterine environment and healthy aging and longevity remain for future research.

Is hyperalphalipoproteinemia a longevity syndrome?

A possible association between high HDL-C levels and longevity has been proposed for more than 30 years. Familiar hyperalphalipoproteinemia, a rare form of dyslipidemia characterized by an extremely high HDL-C level and a strong genetic component, was first described as a longevity syndrome in the 1970s [4]. Thereafter, hyperalphalipoproteinemia caused by CETP deficiency was proposed as a possible longevity factor due to its antiatherogenic lipoprotein phenotype with an increased ratio of HDL_2 to HDL_3 subclass [13]. We previously studied the lipid profiles of 256 centenarians, among whom the highest value for HDL-C was 113 mg/dl, which was related to a *CETP* gene mutation at intron 14 and LPL mutation in addition to extremely high physical and cognitive function. However, CETP deficiency was not always related to good health or high HDL-C in centenarians [20]. The I405V polymorphism in the *CETP* gene was associated with longevity; however, the genotype was not associated with high levels of HDL-C, but with larger particle size of HDL and LDL [12]. Also, we should be aware that individuals with certain *CETP* mutations and normal to moderately high HDL levels are at increased risk for CVD [71]. Apart from the genetically determined hyperalphalipoproteinemia, high HDL-C is generally connected with good health. In a longitudinal follow-up study of 5888 individuals over the age of 65 years, high levels of HDL, low levels of CRP and physical activity was associated with health aging [72]. Associations between hyperalphalipoproteinemia and longevity are promising but as yet unproven. Further research should focus on a specific mechanisms underlying hyperalphalipoproteinemia.

It might be worthwhile to gain a better understanding of HDL metabolism in progeria, the premature aging syndrome, as compared with slower aging. Recently, Gordon and colleagues reported interesting results on lipoprotein profile in children with Hutchinson–Gilford Progeria Syndrome (HGPS), a rare premature aging syndrome caused by the Lamin A (*LMNA*) gene defect [73]. Although mean TC, LDL-C and HDL-C levels were similar between children with HGPS and age-matched control children, HDL-C levels decreased profoundly with age in children with HGPS, but not in controls. In addition, serum adiponectin levels also decreased with increasing age in HGPS, and levels of adiponectin were positively correlated with HDL in HGPS children. These findings were particularly informative when compared with our results demonstrating that serum adiponectin levels were significantly higher in centenarians than in healthy elderly individuals with a mean age of 75 years. Adiponectin levels were strongly correlated with HDL-C, and negatively correlated with vascular endothelial marker in centenarians [61]. The underlying mechanism of abnormalities in HDL and adiponectin metabolism in HGPS remains unknown; however, the authors speculated that loss of adipose tissue mass and function might have a key role in premature atherosclerosis in HGPS. Together with our results from the centenarian study, this suggests that adipose tissue might be essential for healthy aging.

Future perspectives

Low HDL-C is definitely a strong risk factor for CVD in the elderly; however, therapeutic targets specifically focused on raising HDL-C levels have not been established except by means of enhancing physical activity. A drug that potentially raises HDL-C levels by CETP inhibition has recently gone under trial [74]. It is an important issue to be answered whether massive elevation of HDL-C levels by pharmacological intervention could improve CVD risk as well as overall health status, including cognitive function, in the oldest old, a particularly vulnerable population for lower HDL.

Although numerous attempts have been made to describe the association between serum lipids, namely HDL-C, and longevity, we have not reached a definitive consensus. One reason for inconsistent results is that serum lipid level is affected by a number of factors such as weight change, diet and nutrition, use of medications, smoking, acute-phase response, comorbiditiy and subclinical disease, physical activities, and aging itself, particularly in the oldest old. Recently, genetic variations of microsomal triglyceride transfer protein (*MTP*) and *CETP* I405V gene polymorphism were demonstrated to be associated with longevity in two independent studies [12,75], and these findings encourage the hypothesis that lipoprotein could be a promising pathway to

Executive summary

Introduction

- Clinical evidence highlights the importance of high-density lipoprotein cholesterol (HDL-C) levels as a negative risk factor for cardiovascular disease (CVD) in the oldest old. In addition, observational studies showed that high HDL-C levels may be associated with healthy aging and longevity, suggesting that HDL-C could be used as a marker for overall health status in the oldest old.

HDL cholesterol & its subpopulation in the oldest old

- In general, levels of HDL-C as well as total cholesterol decreased with aging; however, in the oldest old the levels of HDL-C were variable and likely to reflect the underlying metabolic context. Larger particles in HDL subclasses may be a better indication of healthy aging and longevity.

Antiatherogenic property of HDL

Reverse cholesterol transport

- Reverse cholesterol transport is a key concept in terms of the antiatherogenic and longevity-promoting effects of HDL. Several polymorphisms in the cholesteryl ester transfer protein (*CETP*) gene, which cause reduced CETP activity and higher HDL-C levels have been demonstrated to be associated with longevity.

Antioxidative and anti-inflammatory properties of HDL

- Although data are limited, evidence suggests that antioxidative as well as anti-inflammatory effects of HDL could be related to longevity. Paraoxonase activity and gene polymorphisms have been vigorously investigated in relation to healthy aging; however, a definitive conclusion has not yet been reached.

Practical use for HDL-C as a marker of overall health status in the oldest old

HDL-C as a marker of frailty in the oldest old

- CVD and its risk factors are often associated with frailty in the oldest old. Low levels of HDL-C, especially accompanied by low albumin and/or high CRP is proposed as a predictive factor for frailty and physical disability.

Cognitive function & HDL in the oldest old

- Low HDL-C levels were consistently associated with cognitive decline in the oldest old; however, the pathophysiology of dementia in this population is multifactorial and HDL-C levels may be a surrogate marker, rather than a determinant.

Metabolic syndrome & healthy aging: two sides of the same coin?

- Accumulating evidence suggests that the low frequency of metabolic syndrome-related traits could be associated with longevity. Although the life-long effect of calorie restriction in human is still unclear, prolonged calorie restriction improved all aspects of the metabolic syndrome and may be associated with health aging.

Is hyperalphalipoproteinemia a longevity syndrome?

- In general, high HDL-C levels are associated with good health in the elderly. Familiar hyperalphalipoproteinemia has been proposed as a longevity syndrome for long time; however, detailed mechanisms remain unknown. There is a striking contrast between HDL metabolism in Hatchinson-Gilford progeria syndrome and centenarians, suggesting adipose tissue might be essential for normal aging and longevity.

Future perspective

- The question of whether massive elevation of HDL-C levels by pharmacological intervention, including a CETP inhibitor, could improve CVD risk as well as overall health status including cognitive function in the oldest old is an important issue.
- Longitudinal follow up of the offsprings of centenarians could be a powerful tool in investigating the association between lipoproteins and longevity.
- The Association between HDL-C and frailty is an issue to be vigously focused on in the future with a common platform.

Conclusion

- Low HDL-C levels in serum is definitely a strong and independent risk factor for CVD in the elderly, and those with the lowest HDL-C levels have benefitted most from pharmacological intervention. To establish the therapeutic strategy for low HDL-C among the oldest old is a priority; however, underlying mechanism of low HDL level should be carefully interpreted especially in the oldest old.

human longevity. It was reported that offspring of centenarians had favorable lipid profile and low CVD risk [76]. Longitudinal follow up of this cohort could bring further progress in this field.

The association between HDL-C and frailty is an issue to be vigorously focused on in the future, since from the point of view of health promotion, frailty among the elderly is of great concern and we have not identified reliable biomarkers for the syndrome. Diversity in the clinical phenotype makes it difficult to establish a common approach to frailty even in its definition. Recently the Interventions on Frailty Working Group developed recommendations for clinical trials aimed at the prevention of

disability [77]. These methodological developments could efficiently yield further insights into this field in the near future.

Conclusion

Low serum HDL-C levels are definitely a strong and independent risk factor for atherosclerosis in the elderly and the very old (aged 85 years and over). More importantly, in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER), the first clinical trial to specifically focus on the clinical benefits from statins in the elderly, it was demonstrated that those patients with the lowest HDL-C levels had the greatest risk reduction for CVD [51]. Therefore, establishing a therapeutic strategy for low HDL-C among the oldest old is a priority. However, we should carefully interpret the metabolic context underlying low HDL levels, especially in the oldest old, because, as mentioned above, coexisting malnutrition, age-related inflammatory activation, catabolism and weight loss (a feature of frailty) become much more common in this population. For this reason, dual measurement of serum albumin and CRP might enhance the prognostic value of HDL-C as a marker for overall health status in the oldest old.

Although research is still limited, some epidemiological evidence suggests that high HDL-C levels could be relevant to healthy aging and longevity, presumably by cardioprotective effects. Obviously, we need much more evidence, especially from interventional studies, to confirm this hypothesis. At present, enhancing or maintaining HDL-C levels by encouraging physical exercise and dietary intervention with careful observation should be recommended, even in the oldest old.

To obtain further insight into the mechanistic roles of HDL on the pathophysiology of CVD with or without geriatric syndrome, much more effort, including longitudinal follow up of cohorts and clinical intervention with proper targets will be needed.

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Functional Status of Centenarians in Tokyo, Japan: Developing Better Phenotypes of Exceptional Longevity

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Background. Centenarians are sometimes said to be representative of lifelong healthy aging. Whether they are, in fact, examples of healthy aging remains a subject of debate. The existence of heterogeneity in functional status has been reported repeatedly in previous studies of centenarians. However, there is as yet no standardized classification system with which to describe their functional phenotype.

Methods. As part of a dynamic cohort study, we studied 304 centenarians (65 men and 239 women) living in Tokyo. Their functional status (sensory, physical, and cognitive), which we used to represent their phenotype, was assessed and subsequently classified by standard assessment methods (simple questionnaire, Barthel index, Mini-Mental State Examination, and the Clinical Dementia Rating, respectively).

Results. We classified participants into 4 categories according to their functional status. Only 5 (2%) were classified as "Exceptional," with all of their functions graded as excellent, and 56 (18%) were "Normal," exhibiting maintenance of fine cognitive and physical functions. One hundred sixty-seven (55%) were "Frail," exhibiting impairment of either cognitive or physical functions, and the remaining 76 (25%) were "Fragile," exhibiting deterioration of both physical and cognitive functions.

Conclusions. The relationships between biochemical marker, mortality rates, lifestyle, and functional phenotypes demonstrated by this classification method indicate that the system is reliable to address the functional status of extremely old persons. Thus, this framework would be a useful tool for exploring the factors that contribute to exceptional longevity as well as those that help to maintain the functional status of the extremely old population.

CENTENARIANS are sometimes said to represent lifelong healthy aging (1,2), although whether they are, in fact, examples of healthy aging is a subject that is currently under discussion (3,4). The literature describes declines in sensory, cognitive, and physical functions in centenarians (4–11). Scientific studies of centenarians have focused on explorations of their environmental and genetic backgrounds. However, the recent proliferation of centenarians (12) and the heterogeneity in their phenotype has introduced confusion into the consensus that, as a whole, they are representative of healthy aging (4,13). An advisory panel on exceptional longevity, which was set up by the National Institute on Aging (14), noted that the identification of intermediate phenotypes, and hence homogenous subgroups, would increase the likelihood of finding the genes that contribute to longevity.

The majority of previous reports have noted the functional status of the centenarians that were studied, but they were separated into different domains with different definitions. If we wish to explore efficiently the factors involved in longevity, then a more parsimonious evaluation method with standardized measures is needed (13). Two centenarian studies proposed the classification method of centenarians. Using retrospective morbidity profiles, the New England study (15) categorized people into three phenotypes: the "Escapers," who could accomplish disease-free aging until they reached 100 years, the "Delayers," who developed disease only very late in life, and the "Survivors," who survived with disease. By adopting a more complicated categorizing system, the Italian study (13) categorized people into three different phenotypes: "A," who had good functional status without specific morbidity history; "B," who were in intermediate condition; and "C," who had poor functional status with a history of morbidity. In addition, they subdivided group "C" into "C1," where cognitive impairment was evident; "C2," where both physical and cognitive impairment were observed; and "C3," where physical impairment was evident.

New England and Italian groups noted that this framework was helpful for exploring the factors underlying exceptional longevity. However, both classification systems have advantages and disadvantages. As both systems emphasize participants' medical history, they will allow exploration of the effect of disease-associated factors on longevity, under the "compression of morbidity" hypothesis (15,16), which suggests that the onset of illness is delayed among centenarians. At the same time, these systems have a disadvantage in that they cannot be used to identify those factors that either protect or delay the aging process, if indeed they exist. If a person possesses a strong protective factor against aging, he may be a "Survivor" with high functional status. The phenotype of these people should be different to that of people who classify as "Survivor" but with frailty. Likewise, as the phenotype in the latter study is affected by a

Table 1. Background Characteristics of Participants

Characteristic	Sex: Male N	Male %	Female N	Female %	Total N	Total %
No. of participants	65	21.4	239	78.6	304	100.0
Age group, y						
100	38	58.5	134	56.1	172	56.6
101–102	16	24.6	62	25.9	78	25.7
103–107	11	16.9	43	18.0	54	17.8
Mean (standard deviation)	101.0	(1.7)	101.2	(1.7)	101.1	(1.7)
Living arrangements						
Alone	2	3.1	6	2.5	8	2.6
With family	49	75.4	149	62.3	198	65.1
Institutionalized	14	21.5	84	35.1	98	32.2
Education						
No education	0	0.0	3	1.3	3	1.0
Elementary education	37	56.9	130	54.4	167	54.9
Secondary education	3	4.6	63	26.4	66	21.7
Higher education	24	36.9	36	15.1	60	19.7
Unknown	1	1.5	7	2.9	8	2.6
Occupation						
Blue collar	19	29.2	45	18.8	64	21.1
White collar	46	70.8	85	35.6	131	43.1
Housewife, or no occupation	0	0.0	100	41.8	100	32.9
Unknown	0	0.0	9	3.8	9	3.0
Birth area						
Kanto (around Tokyo)	25	38.5	121	50.6	146	48.0
Other regions	40	61.5	117	49.0	157	51.6
Unknown	0	0.0	1	0.4	1	0.3

Table 2. Distribution of Sensory Functions and Barthel Index in Centenarians by Sex

Sensory and Basic Physical Function		Male N	Male %	Female N	Female %	Total N	Total %
Visual function							
No problem		30	46.2	82	34.3	112	36.8
Incomplete		23	35.4	72	30.1	95	31.3
Big characters		8	12.3	57	23.8	65	21.4
Face outline		3	4.6	25	10.5	28	9.2
Blind		1	1.5	3	1.3	4	1.3
Hearing function							
No problem		19	29.2	64	26.8	83	27.3
Loud voice		14	21.5	65	27.2	79	26.0
Close to ear		6	9.2	29	12.1	35	11.5
Close to ear with a loud voice		25	38.5	78	32.6	103	33.9
Deaf		1	1.5	3	1.3	4	1.3
Barthel Index							
Independent	100	12	18.5	14	5.9	26	8.6
	80–99	16	24.6	32	13.4	48	15.8
Minimal help	60–79	8	12.3	32	13.4	40	13.2
Partially dependent	40–59	7	10.8	36	15.1	43	14.1
Very dependent	20–39	9	13.8	35	14.6	44	14.5
Totally dependent	<20	13	20.0	90	37.7	103	33.9
Mean (standard deviation)		59.2	34.9	40.0	33.7	44.1	34.8*†

Note: *$p < .01$.
†Main effect of sex.

mixture of causative factors (medical, biological status, environmental, and stochastic) and effects (cognitive or physical function), the role of phenotype as an independent variable in research into those persons who live an exceptional healthy long life becomes ambiguous. The purpose of the study reported here was to propose a new framework for evaluating functional characteristics in centenarians in addition to describing their functional status.

Methods

Participants

A total of 304 Japanese centenarians (65 men, 239 women) living in the 23 wards of metropolitan Tokyo participated in a survey in which they were visited by Tokyo Centenarian Study staff between July 2000 and May 2002. We randomly chose centenarians from the residential list and sent a letter inviting participation to 1194 centenarians, accounting for 68.8% of an estimated 1735 centenarians living in this area in the study period. Five hundred fourteen (43.0%) agreed to participate. Three hundred four persons, representing 25.5% of the letter recipients, participated in the visit survey.

Women outnumbered men in our sample by 1:3.6, which was not significantly different from the ratio for the total centenarian population in this area (1:3.8). Table 1 lists the background information of the participants in this study.

Procedure

After we had received a reply from the centenarian (or proxy) agreeing to participate, we sent a questionnaire that included questions about the participant's functional status. After the questionnaire had been returned, a medical doctor, a psychologist, and a nurse visited the centenarian's residence. After the group had explained the purpose of the study and obtained the permission of the centenarian (or proxy), the doctor examined the patient and took a blood sample. The psychologist conducted a cognitive assessment. The Barthel index (17), was use to assess physical function, and visual and hearing acuity was rated according to the five categories from highest ("No problem") to lowest ("Blind" or "Deaf"; see the detail in Table 2).

The, Clinical Dementia Rating scale (CDR) (18), Global Deterioration Scale (GDS) (19), two scales that were developed in Japan to assess the mental state of elderly persons (NM scales) (20), and the Mini-Mental State Examination (MMSE) (21) were used to evaluate cognitive status. The NM scales were developed for concomitant use with the N-ADL scale, which assess the basic activities of daily living of the patients (20).The MMSE was conducted on all survey participants who were visited at their residences, but 76 participants were unable to complete it for the following reasons: "Disagree to participate" (13.2%); "Bedridden and unable to give a response" (42.1%); "Frailness" (10.5%); "Inability to follow instructions" (15.8%); "Blind or deaf" (13.2%); or "Unable to speak" (5.3%). We scored those participants who were "Bedridden and unable to give a response" and "Inability to follow instructions" as MMSE 0; this test was not conducted on the others. Because of their frailty, it was not possible to perform neuropsychological tests on most of the participants. Thus, we collected data regarding the cognitive and mental status of participants by rating the questionnaires to increase the reliability of the cognitive assessment.

Table 3. Distribution of CDR, MMSE Score, and Classified Cognitive Status

Classification of Cognitive Status		Male N	Male %	Female N	Female %	Total N	Total %
CDR rating and dementia status							
No dementia	0	28	43.1	46	19.2	74	24.3*†
Probably no dementia	0.5	10	15.4	32	13.4	42	13.8
Dementia	1	11	16.9	46	19.2	57	18.8
	2	5	7.7	24	10.0	29	9.5
	3	5	7.7	45	18.8	50	16.4
	4	2	3.1	25	10.5	27	8.9
	5	4	6.2	21	8.8	25	8.2
MMSE score							
Not impaired	≥21	24	36.9	36	15.1	60	19.7
Impaired	11–20	17	26.2	76	31.8	93	30.6
Severely impaired	0–10	17	26.2	102	42.7	119	39.1
Not scored		7	10.8	25	10.5	32	10.5
Reason							
Visual problem		1	1.5	3	1.3	4	1.3
Hearing problem		1	1.5	5	2.1	6	2.0
Speech problem		1	1.5	3	1.3	4	1.3
Frailty		3	4.6	5	2.1	8	2.6
Disagreed to participate		1	1.5	9	3.8	10	3.3
MMSE mean (standard deviation)		16.1 (8.9)		11.5 (8.3)		12.5 (8.6)*†	
Cognitive status							
Excellent		24	36.9	36	15.1	60	19.7
Good		14	21.5	42	17.6	56	18.4
Moderately impaired		16	24.6	70	29.3	86	28.3
Severely impaired		11	16.9	91	38.1	102	33.6

Note: *$p < .01$.
†Main effect of sex.
CDR = Clinical Dementia Rating; MMSE = Mini-Mental State Examination.

The CDR ratings were achieved as a consensus of three expert geropsychologists at a postvisit meeting. One of the three had interviewed the centenarians by him/herself. The GDS, NM scale rating, and videotaped responses of the centenarians to the MMSE, as well as the answers given by the participant's proxy regarding their daily activity were used as a reference to obtain a CDR rating.

Written informed consent was obtained from all participants or proxy. The ethics committee of Keio University School of Medicine approved this study.

Statistical Analyses

We used chi-square tests and one-way analysis of variance (ANOVA) to compare the functional status between the men and women. We used two-way ANOVA to compare the means of serum albumin concentration, 1-year survival, habitual smoking, and alcohol drinking, with sex and functional categories as independent variables. All statistical analyses were performed using SPSS 13.0J (Chicago, IL).

RESULTS

Sensory Functions

The distributions of sensory function levels are given in Table 2. One hundred twelve (36.8%) and 83 (27.3%) participants had "No problem" with vision and hearing function, respectively. The others had moderate to severe problems with these senses, but only 1.3% ($N = 4$) were blind and only 1.3% were deaf ($N = 4$). Most of them ($N = 253$; 83.2%) had either a vision or a hearing problem, and only 51 (16.8%) had intact vision and hearing.

Physical Function

The total Barthel index score and categorized levels of basic ADL are given in Table 2. "Independent" was shown by 74 (24.3%) of the participants, 40 (13.2%) "Needed minimal help," 43 (14.1%) were "Partially dependent," 44 (14.5%) were "Very dependent," and 103 (33.9%) were "Totally dependent." Of 74 independent participants, only 26 (8.6%) were "Fully independent" (Barthel index score = 100). A one-way ANOVA for the total score revealed a significant main effect of gender ($p < .01$), indicating that the men (59.2; standard deviation [*SD*] = 34.9) were more intact than the women (40.0; *SD* = 33.7).

Cognitive Status, as Assessed by CDR and MMSE

Of the 304 participants, 74 (24.3%) had a CDR score of 0 ("No dementia"), 42 (13.8%) had a score of 0.5 ("Probably no dementia"), and 188 (61.8%) were "Mildly to severely demented" (CDR score = 1–5; Table 3). A chi-square test for the frequency of dementia status indicated that women were more likely than men to have dementia ($p < .01$). Cognitive function, as assessed by MMSE, was classified into three levels (Table 3): "Not impaired" (score ≥21); "Impaired" (score 11–20); or "Severely impaired" (score 0–10) by original cutoff point (21). One-way ANOVA for the MMSE total score revealed a significant main effect of gender ($p < .01$), indicating that the men (mean score 16.1; *SD* = 8.9) were generally more cognitively intact than the women (mean score 11.5; *SD* = 8.3).

We classified the cognitive status of centenarians based on those two scales as follows: "Excellent," those who were classified as having "No dementia" by CDR and as being "Not impaired" by MMSE; "Good," those who were classified as having "No dementia" or "Probably no dementia" by CDR regardless of the MMSE score; "Moderately impaired," those who had a CDR score of 1–2; and "Severely impaired," those who had a CDR score of 3–5. As a result, of the 304 participants, 60 (19.7%) were classified as "Excellent," 56 (18.4%) as "Good," 86 (28.3%) as "Moderately impaired," and 102 (33.6%) as "Severely impaired."

Categorizing Centenarians According to Functional Status

We were able to divide the visit survey participants into 4 categories using sensory, physical, and cognitive functions. First was the category of "Exceptional," for participants who had intact visual and hearing functions ("No problem" in the questionnaire), were "Fully independent" with regard to their basic ADL (Barthel index = 100), and had "Excellent" cognitive functions (CDR = 0; MMSE ≥ 21). Second was the category of "Normal," for participants who were somewhat independent with regard to their basic ADL (Barthel index ≥ 80) and had "Good" cognitive function (CDR ≤ 0.5). Third was the category of "Frail," for participants who had impaired basic ADL (Barthel index ≤ 79) or impaired cognitive function (CDR ≥ 1). Those who

Table 4. Comparison of External Criteria (Serum Albumin Level, 1-Year Mortality Rate, and Lifestyle) Among the Four Functional Status Groups

	Male								Female								Total							
	Exceptional		Normal		Frail		Fragile		Exceptional		Normal		Frail		Fragile		Exceptional		Normal		Frail		Fragile	
	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*	Mean	*SD*
No. (%)	2	3.1	24	36.9	32	49.2	7	10.8	3	1.3	32	13.4	135	56.5	69	28.9	5	1.6	56	18.4	167	54.9	76	25.0
Age	102.5	3.5	100.7	1.4	101.2	1.8	101.6	2.1	100.3	0.6	100.5	1.0	101.1	1.6	101.6	2.1	101.2	2.2	100.6	1.2	101.1	1.6	101.6	2.1
Serum albumin (g/dl)	4.0	0.3	3.9	0.3	3.6	0.4	3.4	0.4	4.3	0.2	4.0	0.3	3.6	0.4	3.4	0.4	4.2	0.3	3.9	0.3	3.6	0.4	3.4	0.4**†
1-y mortality	0.0	0.0	0.3	0.5	0.3	0.5	0.6	0.5	0.0	0.0	0.1	0.3	0.2	0.4	0.4	0.5	0.0	0.0	0.2	0.4	0.2	0.4	0.4	0.5*†
Lifestyle																								
Drinkers ratio	1.00	0.0	0.74	0.4	0.71	0.5	0.29	0.5	0.67	0.6	0.31	0.5	0.30	0.5	0.16	0.4	0.80	0.4	0.49	0.5	0.38	0.5	0.17	0.4**†‡
Smokers ratio	0.00	0.0	0.30	0.5	0.48	0.5	0.14	0.4	0.00	0.0	0.03	0.2	0.14	0.3	0.14	0.4	0.00	0.0	0.15	0.4	0.20	0.4	0.14	0.4*†

Notes: Serum albumin concentrations were calculated only for visit survey participants ($N = 264$; men = 59, women = 205). One visit survey participant was lost to follow-up within 1 year after participation, so 1-year mortality data were collected from 303 participants. One-year mortality, and the ratios of drinkers and smokers were calculated as 0 for no (alive) and 1 for yes (dead).

*$p < .05$; **$p < .01$.

†Significant effect was observed among the four groups.

‡Significant difference was observed between male and female.

SD = standard deviation.

were "Totally dependent" (Barthel index < 20) and had "Severely impaired" cognitive function (CDR ≥ 3) were categorized as "Fragile." Table 4 gives the number of centenarians categorized in each of the 4 categories for each gender. Only 5 (1.6%) of the centenarians were categorized as "Exceptional" and 56 (18.4%) as "Normal." Of the "Normal" centenarians, 19 (33.9%) were "Fully independent" (Barthel index score = 100) and 32 (57.1%) had "Excellent" cognitive ability. Most of the centenarians (167; 54.9%) were categorized as "Frail"; of this "Frail" group, only 13 (7.8%) were physically "Independent" but had cognitive problems, while 23 (13.8%) had "Good" cognitive status but had physical problems. Seventy-six participants (25.0%) were categorized as "Fragile."

We did not evaluate the psychiatric aspects of these participants. However, five of the "Exceptional" centenarians had no adverse psychiatric symptoms: two usually go out of the house for shopping, two participate in the day service program provided by the local government for hobby activities, and one is the chairperson of the Brussels Sprout Association.

Validity of the New Categorization

To confirm the validity of the new categorization, we assessed serum albumin concentration and 1-year mortality after participation in the study as external criteria, and compared these values among the groups (Table 4). We also compared (alcohol) drinking and smoking status among the groups as examples to explore the influences of environmental factors on the functional phenotype (Table 4). Participants were defined as being drinkers or smokers if they ever had or now have a drinking or smoking habit, respectively. No significant effect of age was observed among the groups. A significant effect of group ($p < .01$) was observed for serum albumin concentration. Further multiple comparisons indicated that the "Exceptional" and "Normal" groups had higher serum albumin concentrations than the "Frail" and "Fragile" groups did ($p < .05$). The "Fragile" group had significantly lower serum albumin concentrations than the "Frail" group did ($p < .05$). The same analysis for 1-year mortality revealed a significant effect of group ($p < .05$), indicating that those categorized as "Normal" and "Frail" survived longer than those categorized as "Fragile" did. Quite remarkable is the fact that every one of the "Exceptional" centenarians survived for at least 1 year after participation in this survey. Although the differences in serum albumin concentration and 1-year mortality between the "Exceptional" and "Normal" groups were not significant (because of the small number of centenarians in the former group), our new classification method could appropriately discriminate two higher functional groups (Figure 1). This is a particularly notable characteristic of our classification method in comparison with single-domain classification methods (for example, using CDR, MMSE, and Barthel index alone; Figure 1).

With regard to the influence of lifestyle, the higher functional centenarians included fewer habitual smokers and more drinkers. Statistically, the main effect of group was observed for lifestyle ($p < .01$). Further multiple comparisons identified no group-by-group differences. Although there were few smokers among the centenarians ($n = 47$, 15.4%), three currently smoking centenarians were categorized as "Frail," and all five centenarians categorized as "Normal" among the smokers had quit smoking in their early 60s. No such characteristics were found for drinking habit.

Discussion

First, we confirmed the previously reported finding that there is a deterioration of functional status among centenarians in comparison with their younger cohorts (3–11,22–27). We also confirmed that male centenarians outperform female centenarians in both cognitive (6,7,9,22,24) and physical function (4,6–8,27). In addition, we adopted visual, hearing, physical, and cognitive functions as key variables and categorized the centenarians into four phenotypes. Compared with single-domain categorizations of each cognitive and physical function, this functional phenotyping method seems to have distinct advantages. The number of "Exceptional" centenarians was small in this study, and so some of the comparative data did not reach statistical significance, yet 1-year mortality and serum albumin concentrations among the "Exceptional" centenarians were

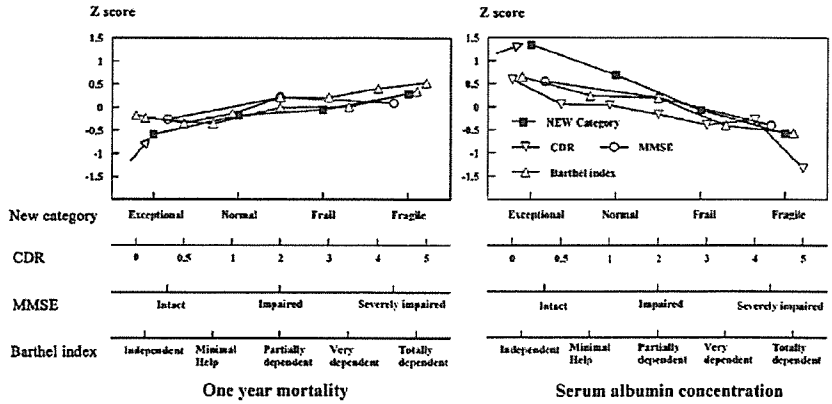

Figure 1. One-year mortality rate (*left*) and serum albumin concentration (*right*) as compared with different categorization systems. Clear differences were observed between the higher functional group (*bold arrow*) among the different classification methods. Data are presented as standardized scores.

higher than among the "Normal" centenarians (who were also categorized as being healthy) and, needless to say, among the "Frail" and "Fragile" centenarians.

Furthermore, we found that retrospective lifestyle also has an influence on the functional status of centenarians. There was a negative relationship between smoking habits and functional status. There were no smokers among the "Exceptional" participants, whereas more than 10% of each of the other groups included habitual smokers. In addition, 29% of physically "Independent" and 17% of cognitively "Excellent" centenarians were smokers. At the same time, there was a positive relationship between drinking habits and functional status. Eighty percent of "Exceptional" and 49% of "Normal" participants reported having a drinking habit, whereas less than 40% of "Frail" and "Fragile" centenarians did so. The causal relationship between drinking and functional status seems to be indistinct compared with smoking. The constitutional differences that allow drinking might influence functional status rather than the positive effect of the drinking habit itself (28). Genetic factors are thought to be more important than environmental factors for survival to an extremely old age (29); however, the results presented here indicate that lifestyles are important to the functional status of oldest old, even though they succeeded in surviving to be 100 years old.

With regard to single-domain functional status, we have confirmed the evident deterioration in both physical and cognitive function among centenarians. The New England study, which used the same scale, would be a good reference. Regarding dementia prevalence, 76% Tokyo centenarians suffer from dementia; meanwhile, this rate was 80% in the New England study (CDR $\geq$ 0.5). Regarding physical function, the New England study showed a higher independence ratio (44%) than did our study (20%). Physical frailty might be a significant characteristic of Japanese centenarians.

We adopted MMSE $\geq$ 21 as a cutoff point for a cognitive ability of "Not impaired." This criterion was based on the original MMSE article (21), was used to screen the cognitively intact centenarians in the Georgia centenarian study (11), and is 1 point higher than the criterion used in the Italian centenarian study, which used the term "absence of severe cognitive impairment" (13). This cutoff point is lower than that used in other recent studies for younger elderly persons (23,24). Previous studies did not evaluate suitable cutoff points for MMSE scores in centenarians in conjunction with external criteria. Many studies have reported a declining trend of MMSE scores with increasing age (30–32). The average MMSE score of the healthy oldest-old population in Tokyo was 25 (range 14–30) for men and 24 (range 8–30) for women (33). Moreover, MMSE scores tend to underestimate the cognitive ability of centenarians, because of sensory deterioration (22,25). Thus, we combined the MMSE and CDR to define cognitive status. We believe that this combination of assessment methods is suitable for evaluating the cognitive status of the oldest-old population. Comparative studies using the same method are needed to confirm the suitability of the MMSE cutoff-point criterion in other populations.

Compared with the available oldest-old (85+ years) data in Tokyo (34), the number of people who have impairments in cognitive or physical functions was twice as high among our centenarians. This finding indicates that the increasing number of oldest-old persons will be accompanied by a great deal of dependent people. At the same time, among 167 "Frail" centenarians, 55 (32.9%) were cognitively labeled as "Good," but had a deteriorated basic ADL score. This indicates that the psychological adaptation to functional deterioration is important in extremely old people (35–37). We need to focus more on the psychological and emotional aspects than on functional status in centenarians.

We should bear in mind the importance of having a standardized method with which to evaluate the functional status of centenarians. In Okinawa, a decline in the physical function of centenarians was reported to have occurred between 1976 and 1994 (38). This evidence led us to investigate the relationship between the increasing number of extremely old persons, the proliferation of centenarians (12), and their functional status. There have been some reports of positive generational improvements in physical and cognitive function (39,40); however, this may not be true for centenarians. The frequency of frail centenarians, who would be assumed to have low genetic advantages for longevity, might increase in the future.

We introduced a new categorization framework for classifying centenarians according to their functional

phenotype by using commonly used measures. We did not examine the influences of medical history (13,15) or other lifestyle factors, and further study is required to determine the factors that differentiate between "Exceptional" and "Normal" as well as between healthiness and frailty. The phenotypes revealed by this study will be helpful to explore factors that contribute to exceptional longevity (14) and to the discrimination between the influences of genetic and environmental factors on healthy aging.

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ORIGINAL ARTICLE

High adiponectin concentration and its role for longevity in female centenarians

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Background: Evidence from experimental models of longevity indicates that maintenance of energy homeostasis could be indispensable for longevity across various species. In humans, it has been reported that maintenance of glucose homeostasis and vascular stability is one biomedical feature of centenarians, who have reached the maximum lifespan. We hypothesized that adiponectin, a novel anti-inflammatory adipocytokine, could be a protective factor against age-related metabolic alteration and atherogeneity in centenarians.

Methods: We measured plasma adiponectin concentration in 66 female centenarians and body mass index (BMI)-matched female controls (mean age 28.3 ± 6.3 years), followed by a genetic analysis of adiponectin locus.

Results: As compared to BMI-matched female controls, female centenarians had significantly higher plasma adiponectin concentrations. In addition, high concentrations of plasma adiponectin in centenarians was associated with favorable metabolic indicators, and with lower levels of C-reactive protein and E-selectin. In contrast, genetic analysis of 10 single nucleotide polymorphism (SNP) at adiponectin locus did not show significant association between the adiponectin gene variation and longevity.

Conclusions: Our results suggested that hyperadiponectinemia in centenarians could play a role in maintenance of energy homeostasis and vascular stability, and may contribute to longevity.

Keywords: adiponectin, centenarians, inflammation marker, leptin, single nucleotide polymorphism (SNP).

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Introduction

Advancing age is frequently associated with diabetes, predominantly type 2 diabetes, and impaired glucose tolerance (IGT). Therefore, the prevalence of diabetes is substantially increasing with the explosion of the elderly population in most developed countries.[1,2] It is widely

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accepted that age-related increase in insulin resistance and changing body fat distributions, in particular visceral fat obesity, are prominent risk factors for type 2 diabetes in the elderly as well as other age-related disease such as atherosclerotic cardiovascular disease (CVD).[3,4] To date, there has been much effort to understand the pathophysiology of age-related insulin resistance, and several plausible mechanisms responsible for this phenomenon have been proposed.[5,6] Above all, roles of visceral fat depots and dysregulation in adipocyte-derived peptides, such as tumor necrosis factor-α (TNF-α),[7] leptin[8,9] and plasminogen activator inhibitor-1 (PAI-1),[10] on the metabolic alterations associated with aging have been extensively studied.

Although the number of centenarians is dramatically increasing all over the world, they are still exceptional and examples of successful aging. Accumulations of findings from centenarian studies in the last decade suggest that despite the inevitability of senescent change usually described as 'complex remodeling',[11] centenarians are examples of the 'survival of the fittest' and the physiological elite in some aspects. For example, centenarians have exceptionally preserved insulin sensitivity[12] and have an extremely low frequency of diabetes mellitus.[13] Evidence from experimental models of longevity, such as daf-2 mutant in *Caenorhabditis elegans*[14] and a caloric restriction in non-human primates,[15] indicate that maintenance of energy homeostasis, especially of insulin/insulin-like growth factor (IGF)-1 pathway, could be indispensable for longevity across various species. Aging is also a potent risk factor for CVD; nevertheless, most centenarians have escaped those fatal diseases. A recent prospective study has demonstrated that even in an apparently healthy middle-aged population, most insulin-sensitive individuals had the lowest risk for age-related disease such as hypertension, CVD and cancer.[16] Although the molecular basis of characteristics in centenarians remains poorly understood, preserved insulin sensitivity could be a fundamental factor for longevity.

Adiponectin, an adipocyte-derived anti-inflammatory protein, has been proposed to improve insulin sensitivity in animal models of insulin resistance caused by both obesity and lipoatrophy.[17] Plasma adiponectin concentrations are reduced in individuals with obesity,[18] type 2 diabetes[19] and CVD,[20] being inversely associated with body adiposity and with insulin resistance. This novel adipocytokine is also proposed to have protective role against vascular injury.[21] Based on these evidences, we focused on this unique adipocytokine as a protective factor of centenarians against age-related metabolic alterations and vascular injury. In the present study, we measured plasma adiponectin concentration followed by a genetic analysis of adiponectin locus to investigate whether hyperadiponectinemia could be associated with longevity.

Subjects and methods

Plasma adiponectin concentration across various ages

The details of the Second Wave of Tokyo Centenarians Study have been described elsewhere.[22] Briefly, a total of 256 Japanese centenarians (190 females and 66 males) living in the Tokyo metropolitan area, were recruited between April 1999 and January 2001 by using a national registry of centenarians published by the Ministry of Health, Welfare and Labor. For the present study, a random sample of 66 females and 28 males was additionally examined for plasma concentrations of adiponectin and vascular endothelial markers. There was no difference in age, sex or body mass index (BMI) distributions between our sample and other participants of the Tokyo Centenarians Study. Seventy-four centenarians were living at home, and 20 were institutionalized, but were in neither acute settings nor receiving tube feeding. Fifteen centenarians who received antihypertensive treatment, and one female centenarian followed by a general physician as having diabetes without antidiabetic medication were included in the present study.

We selected two categorized controls for the comparison of plasma adiponectin concentration in centenarians. For the first, BMI-matched controls (mean age 28.6 ± 6.3 years old) were recruited from hospital workers and medical and nursing school students, who were clinically well and did not have any chronic diseases including anorexia nervosa. Owing to difficulties in recruiting BMI-matched male controls (mean BMI of male centenarians was 19.1 ± 3.1, range 15.1–27.5) and gender differences in plasma adiponectin concentrations, we restricted main statistics to female participants only. For the other controls, 38 female subjects with mean age of 76.3 ± 7.9 years old were recruited from healthy elderly subjects, who underwent a medical examination at our hospital.

Plasma obtained from each participants was immediately separated by centrifugation at 4°C and stored at −80°C until subsequent assay. Blood chemistry analyzes included screening for liver and renal dysfunction. The concentrations of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined by standardized automated procedures. Glycosylated hemoglobin (HbA1c) levels were determined by an isoelectric focusing method with the normal range of 4.3–5.8%. Serum C-reactive protein (CRP) was measured with a latex-enhanced turbidimetric immunoassay. Plasma adiponectin concentrations were determined with an enzyme-linked immunosorbent assay (ELISA) as described previously (Otsuka Assay Laboratory, Tokyo, Japan).[19] Plasma leptin concentrations were determined using a commercial radioimmunoassay. For measurement of thrombomodulin and E-selectin, blood was collected separately in a tube

containing 3.8% sodium citrate. The plasma thrombomodulin concentrations were determined by enzyme-immunoassay (EIA) sandwich methods, and E-selectin were determined by using a commercially available ELISA kit (R&D Systems, Minneapolis, MA, USA)

Genetic study at adiponectin locus

Several single nucleotide polymorphism (SNP) at the adiponectin locus were reported to be associated with risk of type 2 diabetes.[23] To examine the possible association between this locus and longevity, 10 polymorphisms at the adiponectin locus including eight SNP described by Hara *et al.*,[23] were genotyped with 233 DNA from centenarians (188 female and 45 male) and 151 DNA from healthy volunteers (90 female and 61 male, mean age 37.7 ± 11.5 years, range 20–65 years) by direct sequencing. A written informed consent was obtained from every participant and the ethical committees of Keio University School of Medicine and RIKEN Yokohama Institute approved this protocol.

Statistical analysis

Data are expressed as mean (SD). Concentrations of TG, CRP, leptin and adiponectin were logarithmically transformed for statistical analysis in order to reduce skew. Comparisons between centenarians and controls were calculated by a Student's unpaired *t*-test. Pearson's simple correlation coefficients were used to assess the potential associations of BMI, lipid parameters, serum albumin, HbA1c and systemic and vascular inflammation markers with adipocytokines. Multiple regression analysis was calculated with HbA1c levels as dependent, and plasma adiponectin and other modifiable factors as independent variables to determine the quantitative effects of covariates. Because non-fasting blood sample was utilized, TG was not included for correlation analysis. Plasma adiponectin levels were reported not to be affected by food intake.[20] The χ^2 test was performed between centenarians and control subjects for each allelic frequency. $P < 0.05$ was considered as statistically significant.

Results

Clinical and biochemical characteristics of the study participants are shown in Table 1. The mean HbA1c level was significantly higher in centenarians than in BMI-matched younger controls ($P < 0.001$) but was, however, comparable with that in elderly controls. Five centenarians (6.4%) had HbA1c > 6.0% and the rest had normal values. In regard to adipocytokines, there was a striking difference between centenarians and two controls. Mean concentration of plasma adiponectin in female centenarians was almost twice as high as those in BMI-matched female controls (20.3 ± 7.4, 10.8 ± 3.9, $P < 0.001$, respectively), and also higher than those in elderly controls ($P < 0.001$). In contrast, serum leptin levels in female centenarians was significantly lower as compared to both controls. Among vascular endothelial markers, plasma thrombomodulin concentration was

Table 1 Clinical and biochemical characteristics of centenarians and controls

	BMI-matched controls ($n = 66$)	Elderly controls ($n = 38$)	Female centenarians ($n = 66$)	*P* (1) BMI-matched vs centenarians	*P* (2) Elderly vs centenarians
Age (years)	28.3 (6.3)	76.3 (7.9)	100.7 (1.0)	< 0.001	< 0.001
BMI (kg/m^2)	19.5 (2.3)	22.6 (3.6)	19.5 (3.1)	Matching factor	< 0.001
SBP (mmHg)	115 (11)	139 (18)	146 (24)	< 0.001	< 0.248
DBP (mmHg)	72 (9)	74 (10)	76 (17)	0.299	0.431
Adiponectin† (μg/mL)	10.8 (3.9)	14.9 (6.4)	20.3 (7.4)	< 0.001	< 0.001
Leptin† (ng/mL)	8.2 (5.8)	10.5 (6.4)	4.7 (3.8)	< 0.001	< 0.001
Total cholesterol (mg/dL)	165 (25)	213 (25)	175 (31)	0.057	< 0.001
Triglyceride† (mg/dL)	55 (24)	99 (25)	91 (36)	< 0.001	0.225
HDL cholesterol (mg/dL)	70 (11)	60 (13)	58 (13)	< 0.001	0.526
Albumin (g/dL)	4.7 (0.2)	4.1 (0.3)	3.8 (0.4)	< 0.001	0.013
Creatinine (mg/dL)	0.6 (0.1)	0.7 (0.1)	0.9 (0.4)	< 0.001	0.091
HbA1c (%)	4.7 (0.3)	5.2 (0.3)	5.6 (0.7)	< 0.001	0.065
CRP† (mg/dL)	0.03 (0.03)	0.17 (0.14)	0.22 (0.36)	< 0.001	0.231
Thrombomodulin (U/mL)	2.0 (0.8)	2.3 (0.5)	4.2 (1.5)	< 0.001	< 0.001
E-selectin (ng/mL)	37 (15)	ND	40 (20)	0.538	–

†Logarithmically transformed values were used for statistical analysis, but pretransformed values are expressed. Data were mean (SD) unless otherwise indicated. BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ND, not determined; HDL-C, high-density cholesterol; CRP, C-reactive protein.

significantly higher in centenarians ($P < 0.001$), while E-selectin concentration in centenarians was comparable with that in controls ($P = 0.538$).

Figure 1 shows a simple correlation of logarithmically transformed adiponectin with biomedical variables in female centenarians. In female centenarians, the logarithmically transformed adiponectin concentration was negatively correlated with BMI ($r = -0.245$, $P = 0.048$) as well as the logarithmically transformed leptin concentration. Furthermore, it showed strong negative correlations with HbA1c and CRP ($r = -0.311$; $P = 0.01$, $r = -0.316$; $P = 0.009$, respectively) and positive correlation with HDL-C ($r = 0.270$, $P = 0.029$). Adiponectin concentration was also negatively correlated with E-selectin ($r = -0.261$, $P = 0.03$). Plasma adiponectin concentration was not associated with serum albumin or with serum creatinine (Cr). In line with a previous report of consistent associations between serum leptin concentration and body adiposity,[24] plasma leptin concentration in female centenarians was highly correlated with their BMI ($r = 0.555$, $P < 0.001$).

Correlations of adiponectin with variables in BMI-matched female controls are shown in Figure 2. The logarithmically transformed adiponectin concentration was associated with neither BMI nor logarithmically transformed leptin in BMI-matched controls, however, was positively correlated with HDL-C ($r = 0.385$, $P = 0.0014$). Adiponectin concentrations seemed to be negatively associated with HbA1c, though the associations did not reach statistical significance ($r = -0.239$; $P = 0.053$).

We also performed stepwise multiple regression analysis to evaluate independent contributions of adipocytokines to the variance in HbA1c levels in centenarians. Plasma adiponectin and leptin concentrations, TC and E-selectin, which had significant association with HbA1c by a simple correlation analysis, were included as independent variables. Multiple regression analysis showed that plasma adiponectin concentration was the most powerful predictor of HbA1c in centenarians ($P = 0.0080$). In controls, BMI was the only parameter, which was correlated with their HbA1c levels ($r = 0.270$, $P = 0.028$).

In the genetic study, 233 Japanese centenarians and 151 healthy younger controls were genotyped for an association analysis using 10 SNP at the adiponectin

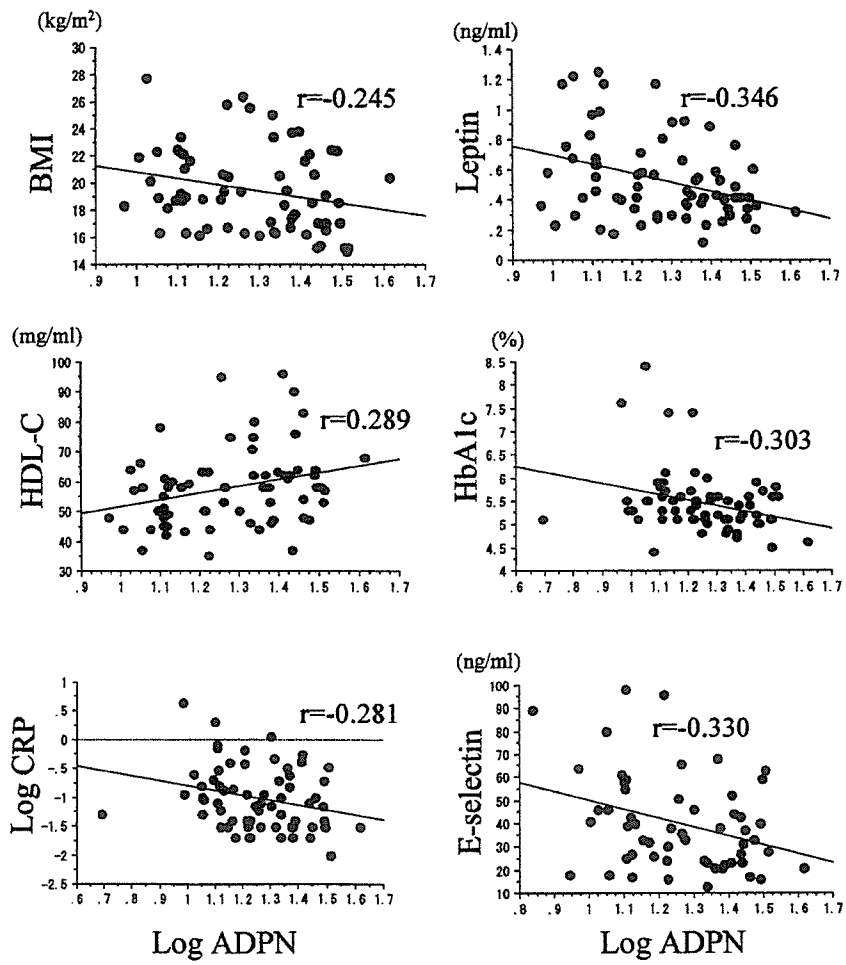

Figure 1 Simple correlations of logarithmically transformed adiponectin with clinical parameters in female centenarians. Correlation coefficient was expressed when the association was regarded as significant ($P < 0.05$). ADPN, adiponectin; BMI, body mass index; CRP, C-reactive protein.

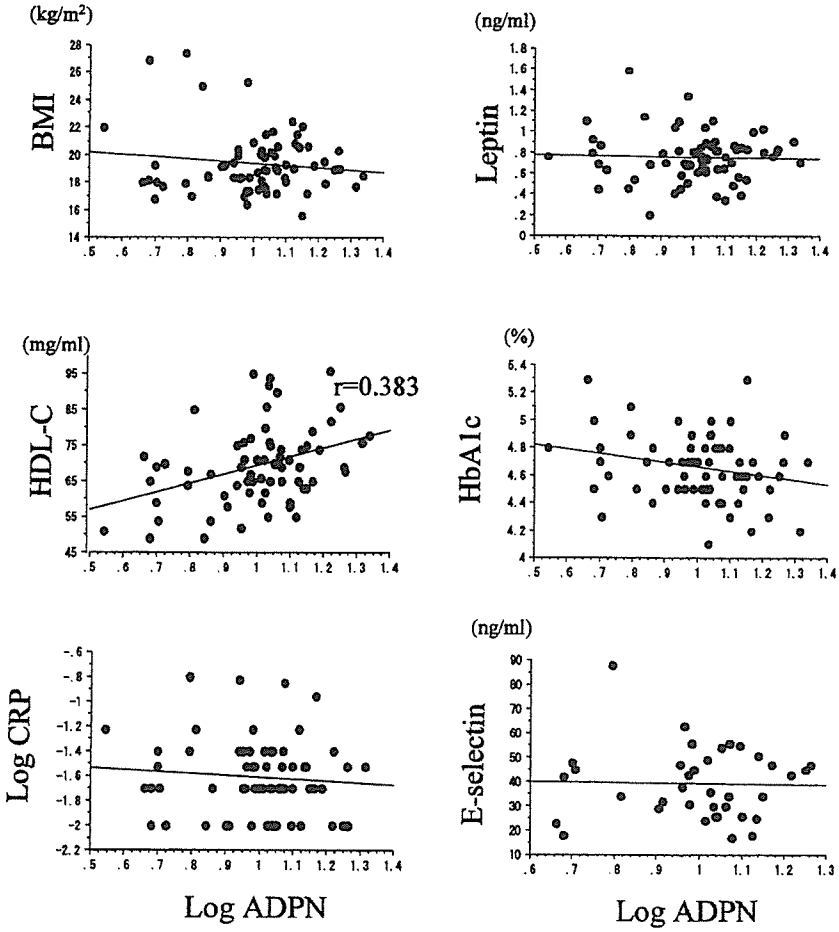

Figure 2 Simple correlations of logarithmically transformed adiponectin with clinical parameters in BMI-matched younger controls. Correlation coefficient was expressed when the association was regarded as significant ($P < 0.05$). ADPN, adiponectin; BMI, body mass index; CRP, C-reactive protein.

locus. These polymorphisms showed no significant difference between centenarians and controls (Table 2). This result was reproduced when female and male participants were examined independently (data not shown). The genotype frequencies in our sample fitted Hardy–Weinberg equilibrium expectations with remarkable fidelity (data not shown). We further studied associations between each genotype and plasma adiponectin concentration in 66 female centenarians (Table 3). There was a similar trend as in a previous report,[23] which showed a gradual increase in adiponectin concentration with T allele of SNP 276, but without significance. No other significant association was found.

Discussion

In the present study, we demonstrated a substantially high concentration of plasma adiponectin in female centenarians as compared to both BMI-matched younger controls and elderly subjects with mean age of 76 years old. We also found that plasma adiponectin in centenarians showed a strong and negative correlation with the levels of HbA1c, and positive association with HDL-C. Furthermore, plasma adiponectin concentrations in centenarians were negatively associated with systemic and vascular-specific inflammation indicated by CRP and E-selectin, respectively. Similar associations of adiponectin concentrations with HDL-C and HbA1c were observed in BMI-matched controls, however, those with inflammation markers seemed to be unique in centenarians. There is ample evidence from experimental studies that support antidiabetic and antiatherogenic properties of adiponectin. In rodents, administration of adiponectin has been demonstrated to improve insulin sensitivity through phosphorylation and activation of 5′-adenosine monophosphate (AMP)-activated protein kinase in muscle.[25] Adiponectin has also been demonstrated to suppress lipid accumulation in monocyte-derived macrophages through the suppression of macrophage scavenger receptor expression.[26] Our data on hyperadiponectinemia in centenarians sympathizes with recent lines of evidence which demonstrated metabolically favorable effects of adiponectin, suggesting possible roles of adiponectin in maintenance of energy homeostasis, especially glucose metabolism and vascular stability even in the extremely old aged.

Table 2 Genotypic and allelic distributions of single nucleotide polymorphism (SNP) in adiponectin locus between centenarians and younger controls

APM1 SNP	*n*	Genotype			Allele		*P*
-11414		AA	AG	GG	A	G	
Centenarians	233	137 (58.8)	87 (37.3)	9 (3.9)	361 (77.5)	105 (2.5)	0.91
Controls	151	87 (57.6)	59 (39.19	5 (3.3)	233 (77.2)	69 (22.8)	
-11379		CC	CG	GG	C	G	
Centenarians	233	127 (54.5)	98 (42.1)	8 (3.4)	352 (75.5)	114 (24.5)	0.528
Controls	151	82 (54.3)	58 (38.4)	11 (7.3)	222 (73.5)	80 (26.5)	
-4036		AA	AC	CC	A	C	
Centenarians	232	202 (87.1)	27 (11.6)	3 (1.3)	431 (92.9)	33 (7.19	0.953
Controls	150	129 (86.0)	21 (14.0)	0 (0.0)	279 (93.0)	21 (3.0)	
-3964		AA	AG	GG	A	G	
Centenarians	232	204 (87.9)	25 (10.8)	3 (1.3)	433 (93.3)	31 (6.7)	0.993
Controls	150	130 (86.7)	20 (13.3)	0 (0.0)	280 (93.3)	20 (6.7)	
45		TT	TG	GG	T	G	
Centenarians	230	113 (49.1)	98 (42.6)	19 (8.2)	324 (70.4)	136 (29.6)	0.898
Controls	150	71 (47.0)	68 (45.0)	11 (7.3)	210 (70.0)	90 (30.0)	
276		GG	GT	TT	G	T	
Centenarians	230	118 (51.3)	96 (41.7)	16 (7.0)	332 (72.2)	128 (27.8)	0.799
Controls	151	81 (53.6)	62 (41.2)	8 (5.3)	224 (74.2)	78 (25.8)	
349		AA	AG	GG	A	G	
Centenarians	233	114 (48.9)	100 (42.9)	19 (8.2)	328 (70.4)	138 (29.6)	0.51
Controls	151	68 (45.0)	71 (47.0)	12 (7.9)	207 (68.5)	95 (31.5)	
639		TT	TC	CC	T	C	
Centenarians	233	79 (33.9)	112 (48.1)	42 (18.0)	270 (57.9)	196 (42.1)	0.411
Controls	151	61 (40.3)	62 (41.0)	28 (18.5)	184 (60.9)	118 (39.1)	
712		AA	AG	GG	A	G	
Centenarians	233	76 (32.6)	113 (48.5)	44 (18.9)	265 (56.9)	201 (43.1)	0.981
Controls	151	52 (34.4)	68 (45.0)	31 (20.5)	172 (56.9)	130 (43.0)	
967		GG	GA	AA	G	A	
Centenarians	233	111 (47.6)	104 (44.6)	18 (7.7)	326 (70.0)	140 (30.0)	0.423
Controls	151	67 (44.4)	69 (45.7)	15 (9.9)	203 (67.2)	99 (32.8)	

Numbers in parentheses indicate the values in percentages.

Numerous factors are known to affect plasma adiponectin concentrations. Adiponectin is exclusively expressed in and secreted from adipose tissue, however, plasma concentrations of this adipocytokine are inversely correlated with body fat mass and reduced in individuals with obesity,[18] type 2 diabetes[19] and CVD.[20] Adiponectin concentration is also decreased in lipodystrophic patients in proportion to the degree of fat loss and insulin resistance.[27] Given the low BMI and leptin levels, centenarians examined here are expected to have adipose tissue depletion to some extent. Nevertheless, exceptional individuals have markedly high concentrations of adiponectin compared with BMI-matched younger controls. Plasma leptin concentration is consistently correlated with total fat mass, and is demonstrated to be more closely associated with adipose cell size than with adipose tissue hyperplasia.[28] In addition, upregulated adiponectin mRNA levels in small size adipocytes induced by both heterozygous PPAR-γ deficiency and administration of peroxisome proliferators-activated receptor (PPAR)-γ agonist were demonstrated.[29] Based on these experimental evidences and our observations, we speculate that small sized adipocytes could be dominant and play some roles as an antidiabetic, and presumably thereby as an anti-aging tissue in centenarians. To support this, further evidence, especially concerning the regulation mechanism of adipocyte function/differentiation among subjects with exceptional health and longevity, seems to be essential.

Alternatively, high adiponectin concentrations in female centenarians could be explained by age-related renal dysfunction. Serum adiponectin concentration was reported to be associated with renal dysfunction in diabetic patients.[30] To clarify this point, we examined the correlation between Cr and adiponectin concentration in centenarians. Although Cr is not necessarily a reliable indicator for renal function in the elderly, we found no association between adiponectin and Cr, suggesting

Table 3 Serum adiponectin concentrations stratified by its genotypes in 66 female centenarians

SNP		Genotype		*P*
-11414	A/A	A/G	G/G	
	17.5 (7.3)	21.2 (8.2)	16.8*	0.284
-11379	G/G	G/A	A/A	
	20.6 (8.4)	18.3 (7.3)	13.0 (0.2)	0.331
-4036	A/A	A/C	C/C	
	18.8 (7.6)	23.0 (9.4)	–	0.301
-3964	A/A	A/G	G/G	
	18.8 (7.6)	23.0 (9.4)	–	0.301
45	T/T	T/G	G/G	
	19.5 (8.0)	18.6 (6.5)	23.4 (12.7)	0.537
276	G/G	G/T	T/T	
	17.8 (8.0)	20.7 (7.0)	23.1 (9.8)	0.38
349	A/A	A/G	G/G	
	20.1 (8.1)	18.3 (6.3)	18.9 (12.6)	0.756
639	T/T	T/C	C/C	
	20.4 (8.9)	18.8 (6.5)	16.9 (8.0)	0.594
712	A/A	A/G	G/G	
	20.4 (8.9)	18.3 (6.5)	18.9 (9.1)	0.685
967	G/G	G/A	A/A	
	19.7 (8.1)	17.8 (6.1)	21.6 (17)	0.594

*Only one centenarian was genotyped as G/G of SNP-11414. Log-transformed values are used in analysis but arithmetic means are presented.

renal dysfunction may not be a major determinant of adiponectin concentration in centenarians.

Genetic variation of adiponectin may have significant impacts on its concentration in plasma. Hara *et al.* reported that SNP at positions 45 and 276 were associated with risk for type 2 diabetes and, in addition, SNP at 276 contributed to adiponectin concentration in the Japanese population.[23] To investigate possible mechanisms underlying hyperadiponectinemia in centenarians as well as association of adiponectin gene variations with longevity, we genotyped 10 SNP at the adiponectin locus in centenarians. However, our data do not support a significant contribution of genetic variation to both adiponectin concentration and longevity. Although the statistical power may not be enough to detect the associations, we cannot conclude that adiponectin gene is a major locus to affect longevity in the Japanese population. Low BMI in centenarians may affect the association between SNP at 276 and adiponectin concentration. Hara *et al.*[23] also demonstrated that the effect of SNP at 276 on adiponectin concentration was BMI dependent. The association between SNP 276 and adiponectin concentration was significant in the upper tertile of BMI, but not observed in the lower tertile group. This issue should be investigated in a future study with a large sample size.

Our study has several limitations. First, stepwise elevation of plasma adiponectin concentrations across various age groups raised the possibility that high concentrations of adiponectin in centenarians could be coincidental with aging itself. This issue should be confirmed by a longitudinal prospective study. Second, the non-fasting state of our samples could restrict further investigation on mechanism(s) responsible for antidiabetic effects of adiponectin in centenarians. For example, we could not determine homeostasis model assessment for insulin resistance (HOMA-IR). Third, dysregulation of adipocytokines other than adiponectin and leptin might have significant roles in age-related metabolic alteration. Secretion of TNF-α in adipose tissue was significantly correlated with percentage of body fat and hyperinsulinemia in elderly subjects with non-insulin dependent diabetes.[31] In the future, we should conduct a comprehensive study that investigates the interplay of those adipocyte-derived peptides and integration of their roles in metabolic alteration.

Conclusions

In this cross-sectional study, we demonstrated high adiponectin concentrations in female centenarians, which were associated with better metabolic and inflammatory markers. Our results suggest that hyperadiponectinemia in centenarians could play a role in maintenance of energy homeostasis and vascular stability and may contribute to longevity.

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