Status and physiological significance of circulating adiponectin in the very old and centenarians: an observational study

Takashi Sasaki 1*, Yoshinori Nishimoto 1,2, Takumi Hirata 1,3, Yukiko Abe 1, Nobuyoshi Hirose 1,4, Michiyu Takayama 1,5, Toru Takebayashi 6, Hideyuki Okano 1,7, Yasumichi Arai 1,8

1 Center for Supercentenarian Medical Research, Keio University School of Medicine, Tokyo, Japan
2 Department of Neurology, Keio University, School of Medicine, Tokyo, Japan
3 Institute for Clinical and Translational Science, Nara Medical University, Nara, Japan
4 Houtokukai Utsunomiya Hospital, Tochigi, Japan
5 Center for Preventive Medicine, Keio University School of Medicine, Tokyo, Japan
6 Department of Preventive Medicine and Public Health, Keio University School of Medicine, Tokyo, Japan
7 Department of Physiology, Keio University School of Medicine, Tokyo, Japan
8 Faculty of Nursing and Medical Care, Keio University, Tokyo, Japan
9
*Corresponding author
Center for Supercentenarian Medical Research, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, JAPAN
Telephone: 81-3-5269-2468
E-mail: sasasa@z5.keio.jp
Abstract

Background: High levels of circulating adiponectin are associated with increased insulin sensitivity, low prevalence of diabetes, and low body mass index (BMI); however, high levels of circulating adiponectin are also associated with increased mortality in the 60–70 age group. In this study, we aimed to clarify factors associated with circulating high-molecular-weight (cHMW) adiponectin levels and their association with mortality in the very old (85–89 years old) and centenarians.

Methods: The study included 812 (women: 84.4%) for centenarians and 1,498 (women: 51.7%) for the very old. The genomic DNA sequence data were obtained by whole genome sequencing or DNA microarray-imputation methods. LASSO and multivariate regression analyses were used to evaluate cHMW adiponectin characteristics and associated factors. All-cause mortality was analyzed in three quantile groups of cHMW adiponectin levels using Cox regression.

Results: The cHMW adiponectin levels were increased significantly beyond 100 years of age, were negatively associated with diabetes prevalence, and were associated with SNVs in CDH13 (p = 2.21 × 10^{-22}) and ADIPOQ (p = 5.72 × 10^{-7}). Multivariate regression analysis revealed that genetic variants, BMI, and high-density lipoprotein cholesterol (HDLC) were the main factors associated with cHMW adiponectin levels in the very old, whereas the BMI showed no association in centenarians. The hazard ratios for all-cause mortality in the intermediate and high cHMW adiponectin groups in very old men were significantly higher rather than those for all-cause mortality in the low level cHMW adiponectin group, even after adjustment with BMI. In contrast, the hazard ratios for all-cause mortality were significantly higher for high cHMW adiponectin groups in very old women, but were not significant after adjustment with BMI.

Conclusions: cHMW adiponectin levels increased with age until centenarians, and the contribution of known major factors associated with cHMW adiponectin levels, including BMI and HDLC, varies with age, suggesting that its physiological significance also varies with age in the oldest old.

Funding: This study was supported by grants from the Ministry of Health, Welfare, and Labour for the Scientific Research Projects for Longevity; a Grant-in-Aid for Scientific Research (No 21590775, 24590898, 15KT0009, 18H03055, 20K20409, 20K07792, 23H03337) from the Japan Society for the Promotion of Science; Keio University Global Research Institute (KGRI), Kanagawa Institute of Industrial Science and Technology (KISTEC), Japan Science and Technology Agency (JST) Research Complex Program "Tonomachi Research Complex" Wellbeing Research Campus: Creating new values through technological and social innovation (JP15667051), the Program for an Integrated Database of Clinical and Genomic Information from the Japan Agency for Medical Research and Development (No. 16kk0205009h001, 17jm0210051h0001, 19dk0207045h0001); the medical-welfare-food-agriculture collaborative
consortium project from the Japan Ministry of Agriculture, Forestry, and Fisheries; and the Biobank Japan Program from the Ministry of Education, Culture, Sports, and Technology.
Introduction

Adiponectin is an adipocyte-derived hormone that plays a vital role in metabolism, including lipid and glucose metabolism, and occurs in circulation at concentrations of up to 0.05% of total plasma protein\textsuperscript{1,2}. Circulating adiponectin forms three major multimer complexes, including a trimer, hexamer, and high-molecular-weight form. Among these forms, circulating high-molecular-weight (cHMW) adiponectin shows more potent biological activity than that of the other two forms\textsuperscript{3}. Previous studies in the mouse model studies have shown that cHMW adiponectin enhances insulin sensitivity and plasma lipid clearance; high levels of cHMW adiponectin improve the stability of lipid homeostasis and provided systemic tolerance to obesity under normal physiological conditions\textsuperscript{4, 5, 6}. Adiponectin knock-out mice showed mild or moderate insulin resistance, which is exacerbated by a high-fat diet\textsuperscript{7,8}. However, adiponectin knock-out mice are viable under regular physiological conditions, indicating that adiponectin is not essential for survival under regular dietary conditions\textsuperscript{7,8}. Therefore, adiponectin function is considered inconspicuous under normal conditions and should become prominent under physiological stress such as hyperglycemia.

In humans, adiponectin shows strong negative associations with body mass index (BMI), the prevalence of type 2 diabetes (T2DM), and hypertension\textsuperscript{9,10,11}. However, high levels of adiponectin have also been associated with an increased risk of cardiovascular disease (CVD) in adults in their 60s and 70s\textsuperscript{12,13,14,15}. These contradictory findings indicate that environmental and related physiological changes could alter the level and function of adiponectin; therefore, the analysis of adiponectin in adults aged 80 years and older would be essential to elucidate the significance of adiponectin in aging.

Centenarians are individuals aged 100 years and older and characterized by a low incidence of life-threatening diseases, such as CVD and T2DM. They serve as potential models for successful aging\textsuperscript{16,17}. Previous studies have reported that cHMW adiponectin levels increase with age and, specifically, that centenarians show comparatively high levels\textsuperscript{18,19}. Low BMI may contribute to high adiponectin levels and insulin resistance in older adults ages above 60 years, and transgenic mouse models have shown prolonged health span and median lifespan. However, the physiological significance of high cHMW adiponectin levels in adults aged above 80 years is still unclear\textsuperscript{20,21,22}. To provide evidence for understanding the physiological function and significance of adiponectin in the oldest old, this study aimed to determine the status and factors associated with cHMW adiponectin levels in 2,310 adults aged ≥85 years, including 812 centenarians.
Methods

Study populations

We used data from four prospective cohort studies of the oldest old in Japan: the TCS and JSS for centenarians and the TOOTH and KAWP for the very old (aged 85–99 years). Recruitment was conducted as previously described\(^{17, 23, 24, 25, 26, 27, 28}\). From the TCS and JSS, 155 participants were excluded due to a lack of cHMW adiponectin level data; thus, 812 centenarians were enrolled (127 men and 685 women with a median age of 105.3 [interquartile range (IQR): 100.9–106.8] and 106.0 years [IQR: 103.9–107.2], respectively). The TOOTH and KAWP surveys are community-based prospective cohort studies of individuals between 85 and 102 years (TOOTH) and 85 and 90 years (KAWP), respectively. Data for 542 (236 men and 306 women) and 1,026 (513 men and 513 women) individual medical examinations are included in the TOOTH and KAWP studies, respectively. Of these, 63 individuals from the TOOTH study were excluded because they were older than 90, and 7 individuals from the KAWP study were excluded due to a lack of cHMW adiponectin level data; thus, 1,498 individuals were enrolled as the very old (724 men and 774 women with a median age of 86.9 [IQR: 85.9–88.2] and 87.0 years [IQR: 86.0–88.4], respectively, Figure 1–figure supplements 1 and 2, Supplementary File 1).

All of the KAWP, TOOTH, TCS, and JSS have been managed by the Center for Supercentenarian Medical Research, Keio University School of Medicine. Written informed consent was obtained either from the participant or from a proxy if the participant lacked the capacity to provide consent. The ethics committee approved all cohort studies of the Keio University School of Medicine (ID: 20021020, 20022020, 20070047, and 20160297). The TOOTH and KAWP studies are registered in the University Hospital Medical Information Network Clinical Trial Registry (ID: UMIN000001842 and UMIN000026053).

Baseline examination

All participants were examined by experienced geriatricians at the time of enrolment, following previously described protocols\(^{17, 23, 24, 25, 26}\). Our assessment considered medical histories, lifestyle factors, and physical and cognitive functions. A mini-mental state examination (MMSE; 0–30 points) was used to assess cognitive function. The five-item world health organization well-being index (WHO5; 0–5 points) was used to assess current mental well-being. Instrumental activities of daily living (IADLs) were assessed using the Lawton scale (0–5 points) and independent IADL was defined as 5 points on the Lawton scale. The concentration of blood biomarkers, including cHMW adiponectin, NTproBNP, cystatin C, and interleukin-6 (IL-6), was measured according to previously described protocols\(^{29}\). Blood test results for HDLC, LDLC, TCHO, TG, choline esterase (CHE), aspartate aminotransferase
AST, γ-glutamyl transpeptidase (γGTP), lactate dehydrogenase (LDH), uric acid (UA), albumin (ALB), and HbA1c content were also obtained using previously described protocols. A person with DM was defined as follows: individuals with glycated hemoglobin (HbA1c) ≥ 6.5%, those receiving antidiabetic drug therapy, or those receiving insulin injections (Supplementary File 1).

Measurement of cHMW adiponectin levels
The plasma cHMW adiponectin levels were measured using the Human HMW Adiponectin/Acrp30 Immunoassay Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) according to manufacture protocol.

Whole-genome DNA sequencing
Total genomic DNA was extracted from whole blood using a FlexGene DNA Kit (Qiagen, Hilden, Germany). The whole-genome DNA sequence of 440 centenarians was determined using whole-genome DNA sequencing with previously described protocols. The genotypes of 0.65 M SNVs of 367 centenarians were determined using an Axiom Japonica Array NEO according to the manufacturer’s protocol. The genotypes of 0.65 M SNVs of 1,015 individuals in the KAWP study were determined using an Infinium Asian Screening Array-24 v1.0 BeadChip kit according to the manufacturer’s protocol. All DNA microarray scan images were analyzed using previously described protocols.

Meta quantitative trait association analysis for cHMW adiponectin level
To identify cHMW adiponectin level-associated SNVs in the very old and centenarians, we analyzed the association among cHMW adiponectin level and genetic variants using quantitative trait association analysis with the PLINK program (version 1.90) adjusted for sex and age at entry against 440 WGS and DNA microarray-imputed data for 367 centenarians and 1,015 very old, respectively. These quantitative trait association analyses were meta-analyzed using Metal (released on 2020-05-05). Finally, we obtained meta-quantitative trait association data between 5.75 M SNVs and cHMW adiponectin levels for 1,822 individuals. A Manhattan plot was created using the qqman package (version 0.1.8) in program R. An enlarged view of a Manhattan plot with recombination rate information was generated using LocusZoom.

Genotyping and minor allele frequency of rs4783244 (CDH13) and rs11711353 (ADIPOQ)
To determine the rs4783244 and rs11711353 genotypes and minor allele frequency in the very old and centenarians, we genotyped these SNVs using the TaqMan SNP Genotyping Assay system according to the manufacturer’s protocols. Minor allele frequency of rs4783244 and rs11711353 for Japanese controls (ToMMo 38KJPN) was used in the jMorp database (https://jmorp.megabank.tohoku.ac.jp).

**LASSO and multivariate analysis**

For LASSO and further multivariate analysis, cHMW adiponectin level was used as the outcome, and LASSO was used to evaluated 32 factors by LASSO including age at entry, BMI, systolic blood pressure (SBP), years of education, smoking history, IADL score, hand grip, cognitive impairment (MMSE: ≤23), WHO5 score, self-reported disease histories (heart disease, diabetes, cancers, renal disease, fracture), biomarkers in blood (HDLC, TCHO, LDLC, TG, CHE, AST, ALT, γGTP, LDH, UA, ALB, CstC, NTproBNP, HbA1c, IL6), and genetic factors (sex, CDH13 rs4783244, ADIPOQ rs11711353) for the very old, and 26 factors including age at entry, BMI, SBP, five educational category, smoking history, activities of daily living (ADL) score, self-reported disease histories (heart disease, diabetes, cancers, renal disease, fracture), biomarkers in blood (HDLC, TCHO, LDLC, TG, CHE, γGTP, UA, ALB, CstC, NTproBNP, HbA1c, IL6), and genetic factors (sex, CDH13 rs4783244, ADIPOQ rs11711353) for centenarians (Supplementary File 1). After excluding samples with any missing values in the selected factors, 1,314 very old and 352 centenarians were selected.

**Survival analysis**

For survival analysis, all-cause, cancer-case, CVD-cause, and pneumonia-cause mortalities were used as outcome, and BMI, cHMW adiponectin level, disease history (DM), number of allele for CDH13 rs4783244 and ADIPOQ rs11711353, age at entry, HDLC, and years of education were used as potential confounder and effect modifiers based on the results of multivariate analysis. The very old and centenarians were grouped into three quantile cHMW adiponectin level groups (high, intermediate, and low) against 1,425 very old (678 men and 747 women) and 545 centenarians (90 men and 455 women) for whom survival time information was available.

**Statistical analyses**

Baseline characteristics, medical history, plasma biomarkers, and genotype data are expressed as a median or number with a percentage or interquartile range (IQR). The difference in baseline data was evaluated using Wilcoxon rank-sum, chi-square, and Fisher’s Exact tests (Supplementary File 1). Multivariate logistic regression analyses were performed using a
generalized linear model with factors selected by LASSO. All statistical analyses were performed using program R (version 4.0.3) with exactRankTests (wilcox.exact, Wilcoxon rank-sum test [version 0.8-31]), glmnet (LASSO and multivariate analyses [version 4.1]), survival (survival analysis (survfit, coxph, and cox.zph) [version 3.2-13]), powerSurvEpi (statistic power calculation [ver. 0.1.3]) and default packages.

Results

Baseline characteristics of the very old and centenarian cohorts

This study used data collected from prospective cohort studies, including the Tokyo Oldest Old Survey on Total Health (TOOTH) and Kawasaki Aging Wellbeing Project (KAWP) for the very old (aged 85–89 years) as well as the Tokyo Centenarian Study (TCS) and Japanese Semi-supercenentarian Study (JSS) for centenarians (aged 100 years and older, Figure 1a)17,23,24,25,26,27,28. The data for cHMW adiponectin levels were available for 812 centenarians (woman: 84.4%, 87.7% in Japanese census data in 2020) and 1,498 very old (woman: 51.7%, 64.4% in Japanese census data in 2020, Figure 1–figure supplement 1). Participant characteristics at enrollment are presented in Supplementary File 1. The flow chart for the analysis is shown in Figure 1–figure supplement 2.

cHMW adiponectin levels increased with age from 30–70 years old and are higher in women than those in men19. Our findings in this study were consistent in that cHMW adiponectin levels gradually increased with age (Figure 1b; also observed in the longitudinal data of the TOOTH study, Figure 1–figure supplement 3), with a similar difference observed between sexes of the very old and centenarians (Figure 1c,d).

Single nucleotide variations in the promoter regions of CDH13 and ADIPOQ were associated with cHMW adiponectin levels in the very old and centenarians

A previous genome-wide association study (GWAS) has revealed that cHMW adiponectin levels are associated with two major loci, including CDH13 (also called T-cadherin) and ADIPOQ (gene corresponding to adiponectin), both in European and multi-ethnic cohorts35. To confirm this association in the very old and centenarians, we quantitatively assessed 5.75 M single nucleotide variants (SNVs) adjusted for age at entry and sex from the genome data for 1,822 individuals, including whole-genome DNA sequences for 440 centenarians, imputed microarray analysis data for 367 centenarians, and imputed microarray analysis data for 1,015 very old (Figure 2a, Figure 2–figure supplement 1). We found that rs12051213 T>C SNV, located near exon 1 of CDH13, was the locus most significantly associated with cHMW adiponectin levels ($p = 2.21 \times 10^{-22}$, Z score = -9.73), and rs11711353 A>G SNV, located near exon 1 of ADIPOQ, was the second-most significant locus ($p = 5.72 \times 10^{-7}$, Z score = 5.00). The
GWAS results also revealed that rs12051213, rs11711353, and other associated SNVs were mainly located around exon 1, indicating that these variants would be associated with the expression of CDH13 and ADIPOQ genes (Figure 2b,c). For the CDH13 locus, rs4783244 ($p = 5.39 \times 10^{-22}$, Z score = -9.64) was located near rs12051213, another SNV commonly used as a cHMW adiponectin level-associated SNV; therefore, we selected rs4783244 as a representative SNV among CDH13-associated SNVs. To confirm the association between these SNVs and cHMW adiponectin levels, we determined the genotype of these two SNVs against the very old and centenarian men and women using a TaqMan assay. As a result, no significant difference in minor allele frequency was found between Japanese control (ToMMo 38KJPN), the very old, and centenarian men and women using Fisher’s exact test and multiple testing (Figure 2–figure supplement 2). We compared the genotype-based distribution of cHMW adiponectin levels by genotype (Figure 2d, Figure 2–figure supplement 2). cHMW adiponectin levels were found to vary significantly between the rs4783244 reference allele homozygote and rs4783244 alternative allele heterozygote both in the very old and centenarians. However, except for very old men, no significant difference was observed between the rs4783244 alternative allele heterozygote and rs4783244 alternative allele homozygote in the very old or centenarians. Additionally, cHMW adiponectin levels varied significantly among several allele combinations of rs11711353 in very old or centenarian women but not in very old or centenarian men (Figure 2–figure supplement 3). These data indicated that both major loci (rs4783244 of CDH13 and rs11711353 of ADIPOQ) were associated with cHMW adiponectin levels in the very old and centenarians. However, the effects depended on age and sex.

The characteristics of cHMW adiponectin levels in the very old and centenarians

A previous study reported a negative association between cHMW adiponectin levels and T2DM prevalence and BMI, as well as a positive association with insulin sensitivity index, triglyceride (TG) content, and high-density lipoprotein cholesterol (HDLC) levels. To evaluate these associations in the oldest old, we analyzed the association between cHMW adiponectin levels and diabetes mellitus (DM), HDLC, and BMI (Figure 3). A person with DM was defined as follows: individuals with glycated hemoglobin (HbA1c) ≥ 6.5%, those receiving antidiabetic drug therapy, or those receiving insulin injections (Figure 3a,b). We found that cHMW adiponectin levels in the DM group were significantly lower than those in the non-DM group in both the very old and centenarians, indicating that adiponectin is associated with the DM pathway, regardless of age. Although blood-lipid contents, including total cholesterol (TCHO), HDLC, low-density lipoprotein cholesterol (LDLC), and TG gradually decreased with age from the very old to centenarians (Supplementary File 1), a positive association was observed between cHMW
adiponectin and HDLC levels (Figure 3c,d). A negative association between cHMW adiponectin levels and BMI was observed in the very old, though this association was less prominent in centenarians (Figure 3e,f). These findings suggested that the physiological factors associated with adiponectin may vary from the very old to centenarians.

The factors associated with cHMW adiponectin levels vary between the very old and centenarians.

In our multivariate regression analysis of cHMW adiponectin levels, we initially selected 32 factors for the very old, including cHMW adiponectin level-associated genetic factors (genotypes of rs4783244 in CDH13 and rs11711353 in ADIPOQ), and 26 factors for centenarians based on a previous report. To reduce the effects of multicollinearity, we used a Least Absolute Shrinkage and Selection Operator (LASSO) method with five-fold cross-validation and identified 19 factors for the very old and 7 factors for centenarians (Figure 4—figure supplement 1). According to the multivariate regression analysis for the very old, 14 significant factors for men and 10 significant factors for women were identified (Figure 4a, Supplementary Files 2–4); among centenarians, three significant factors for men and four significant factors for women were identified (Figure 4b, Supplementary Files 5–7). An analysis of deviance revealed that the total variance of known cHMW adiponectin level-associated factors was 36.8–42.0% in the very old and centenarian men and 18.4% in centenarian women (Figure 4c,d, Supplementary Files 8–11). These results suggest that the genotypes of rs4783244 in CDH13, HDLC, BMI, and lipid metabolism-associated factors, including HDLC and TG, are major factors associated with cHMW adiponectin levels in both sexes of the very old. Furthermore, the genotypes of rs4783244 in CDH13 and HDLC were also associated with cHMW adiponectin levels in centenarians. Significantly, the current known factors associated with cHMW adiponectin levels were expected to correspond to 18.4% of the total variance in centenarian women, indicating a reduced contribution of known factors associated with cHMW adiponectin levels in centenarians. Thus, major cHMW adiponectin-associated factors found in the very old would not be responsible for the age-dependent increment of cHMW adiponectin levels.

Higher cHMW adiponectin levels in very old men was positively associated with high all-cause mortality rates, independent of BMI.

Higher cHMW adiponectin levels are associated with increased all-cause mortality and CVD risk in adults in their 60s and 70s. To evaluate the effects of cHMW adiponectin levels on mortality in the very old and centenarians, hazard ratios of all-cause mortality were analyzed using Cox promotional hazards models for three quantiles of cHMW adiponectin levels (i.e.,
high, intermediate, and low) in 1,425 very old (678 men and 747 women) and 545 centenarians (90 men and 455 women) for whom both survival time information and a number of covariates were available. Prior to the analysis, the availability of sufficient samples and events for all-cause mortality were ensured for the survival analysis of the very old and centenarian women and there was no significant difference in the proportional hazards assumption of the cHMW adiponectin level and each of the covariates (Figure 5 – figure supplements 1 and 2). However, the statistical power analysis indicated that there were not sufficient events, and samples were ensured for the centenarian men even if they were divided into two groups. Within the follow-up periods, 145 (21.3%) men and 101 (13.5%) women died in the very old, whereas 89 (98.9%) men and 542 (99.4%) women died in the centenarians (Figure 5 and Supplementary File 1). As a result, the hazard ratios of all-cause mortality for intermediate and high levels of cHMW adiponectin groups in very old men were significantly higher (HR: 1.67 and 2.32) rather than those of the all-cause mortality of the low cHMW adiponectin level group (reference), even after adjustment for BMI (HR: 1.60 and 2.12). In contrast, the hazard ratio for all-cause mortality was significantly higher for the high cHMW adiponectin levels group in very old women was significantly higher (HR: 1.89), but was not significant after adjustment for BMI (HR: 1.41, (Figure 5)). This trend was also observed in the centenarian women. To further elucidate the factors associated with mortality, we also analyzed cause-specific mortality associated with cancer, CVD, and pneumonia in the very old (Figure 5 – figure supplements 3 – 5). The total number of events for each cause-specific mortality was 59 (cancer), 53 (CVD), and 40 (pneumonia), indicating that the analysis lacked sufficient statistical power. Testing populations with a 5% difference in event frequency would require approximately 440 samples for each group.

Discussion

The results of this study showed that cHMW adiponectin levels increased with age up to centenarians, although the associated factors varied with sex. Therefore, we are further elucidating whether the increment of cHMW adiponectin level with age extends into very old and exceptionally old age. Meta-GWAS with cHMW adiponectin levels revealed that the SNVs of two loci containing the promoter regions of CDH13 and ADIPOQ genes were associated with cHMW adiponectin levels. The levels of HDLC were associated with those of cHMW adiponectin both in the very old and centenarians, though the association with BMI was relatively weaker in centenarians. The multivariate regression analysis with factor selection using the LASSO method revealed that genetic variants, BMI, and lipids were major factors associated with cHMW adiponectin level in the very old; here, BMI was not selected as an associated factor in centenarians. The analysis of deviance revealed that the contribution of...
known factors to cHMW adiponectin levels decreased in centenarian women, suggesting that
348 the major factors in the very old would not be responsible for the age-dependent increase in
349 cHMW adiponectin levels. The high cHMW adiponectin levels in very old men were associated
350 with all-cause mortality independently of BMI; however, no association was observed between
351 the cHMW adiponectin levels and all-cause mortality in very old and centenarian women.
352 Therefore, the contribution of known major factors associated with cHMW adiponectin levels,
353 including BMI and lipid content, varies with age, suggesting that its physiological significance
354 also varies with age in the oldest old.
355 The salutary effects of adiponectin on glucose homeostasis, insulin sensitivity, and chronic
356 low-grade inflammation, and the inverse association between the incidence of T2DM and
357 cHMW adiponectin levels are known\textsuperscript{36, 37, 38}. We have previously reported that a low incidence
358 of T2DM is a characteristic of centenarians; therefore, we deduced that the high cHMW
359 adiponectin levels in centenarians would be partially influenced by a low incidence of T2DM.
360 In the present study, the T2DM group showed significantly lower levels of cHMW adiponectin,
361 regardless of the cohort, suggesting the physiological significance of cHMW adiponectin levels
362 in the context of insulin sensitivity and T2DM incidence is consistent across ages.
363 We revealed that very old men with high cHMW adiponectin levels show high rates of
364 all-cause mortality, consistent with previous reports for adults in their 60s and 70s\textsuperscript{12, 13, 14, 15}.
365 Moreover, cHMW adiponectin levels were associated with all-cause mortality independently of
366 BMI. Excess weight loss can cause frailty in the oldest old, exacerbating mortality rates and
367 death due to pneumonia\textsuperscript{39}. Based on these results, we deduced that a combination of high
368 cHMW adiponectin levels and low BMI may exert synergistic effects in the mortality among
369 very old men. We also revealed that high cHMW adiponectin levels were not associated with
370 mortality both in very old and centenarian women. Surprisingly, strength of the association
371 between BMI and cHMW adiponectin level decreased in centenarians. Although the major
372 factors associated with cHMW adiponectin level in centenarians were unknown, these results
373 suggest that the factors associated with cHMW adiponectin levels vary with age, which would
374 also alter the physiological significance of cHMW adiponectin level as it relates to mortality.
375 Frailty is an important concept in health maintenance and the process of functional decline in
376 the oldest old. Recently, plasma adiponectin levels have been positively associated with frailty
377 in the oldest old\textsuperscript{40, 41}. In our cohort, most centenarians were classified as frail according to the
378 current frailty criteria, so it is difficult to assess frailty in centenarians. For the very old, only the
379 KAWP, one of the cohorts that included the very old, collected sufficient data to assess frailty.
380 Using these limited data for the very old, we analyzed the distribution of cHMW adiponectin
381 levels in each frailty category and analyzed their association with the revised J-CHS frailty
382 index criteria using multiple regression analysis\textsuperscript{42}. As a result, we found that cHMW
adiponectin levels were significantly associated with frailty, both in very old men and women (Figure 5–figure supplement 6). The cHMW adiponectin level was also significantly associated with frailty in very old women even after adjustment for BMI; however, no significant association was observed in very old men after adjustment by BMI. Thus, cHMW adiponectin levels would be associated with frailty in the very old, especially in women.

Although cHMW adiponectin levels increased with age, their association with BMI was comparatively lower in centenarians than that in the very old. This raises the question of which cells are responsible for the increased expression of adiponectin with aging. One hypothesis is that the clearance mechanism of adiponectin from the blood may be impaired by reduced kidney function, resulting in an accumulation of cHMW adiponectin. However, we did not observe a significant association between the levels of cHMW adiponectin and plasma cystatin C, one of the kidney function markers. Another hypothesis is that aging would cause ectopic ADIPOQ gene expression, increasing cHMW adiponectin levels. Re-analysis of in silico mouse single-cell transcriptomic data revealed that a small number of cells derived from subcutaneous adipose tissue expressed high levels of ADIPOQ, including brown, gonadal, mesenteric, and subcutaneous adipose tissues (Figure 5–figure supplement 7). Furthermore, a re-analysis of mouse whole-body single-cell transcriptomic data from 24 tissues during 1–30 months of age revealed that ADIPOQ mRNA was rarely expressed in tissues other than the fat tissue, even at advanced ages of 24, 27, and 30 months (Figure 5–figure supplement 8). These findings indicate that no universal mechanism between humans and mice would exist to induce cHMW adiponectin through ectopic expression of the ADIPOQ gene by aging.

The study had the following limitations: 1) Surveys of centenarian surveys tend to have many missing values due to their limited physical and cognitive function; therefore, multivariate analysis using a series of covariates tends to reduce the number of samples to be analyzed. 2) Although the short survival time of centenarian in this showed no association between cHMW adiponectin level and all-cause mortality in this study, strong factors associated with survival, such as N-terminal pro-brain natriuretic peptide (NTproBNP) and albumin (ALB), tend to be detectable, while weaker factors are more difficult to detect. 3) Cox regression for all-cause mortality in centenarian men and cause-specific mortality in the very old men was statistically underpowered due to the insufficient size of samples and/or events. CVD mortality in very old men showed a trend to be associated with cHMW adiponectin levels, but statistically, twice the number of events or twice the number of total samples are needed to assess this. 4) Analysis of cHMW adiponectin levels and frailty in centenarians is difficult because most centenarians would be classified as frail according to the current frailty criteria. Of the two cohort studies of very old participants, the TOOTH study did not have sufficient data adjusted for the evaluation of J-CHS frailty criteria. Therefore, the association between cHMW adiponectin levels and
frailty was analyzed in selected samples derived only from the KAWP study only. This was only a cross-sectional analysis, and further analysis would be needed to prove causality. Therefore, these are described only in the Discussion section.

In this study, we verified the association among cHMW adiponectin level, BMI, and all-cause mortality in the very old and centenarians. Due to changes in the physiological significance of BMI between young and old ages, the appropriate BMI value is expected to vary with age. While a low BMI is recommended at a young age due to the risk of diabetes and metabolic syndrome, a high (though not excessively high) BMI is recommended at a later stage of life to decrease the risk of frailty and mortality. Therefore, the biological significance of cHMW adiponectin levels would be also changed depending on the biological significance of BMI in the aging process. The reasons for the high cHMW adiponectin levels and loss of association with BMI in centenarians remain unknown; however, future research should focus on identifying cells that expressing adiponectin, which should clarify its physiological significance in the oldest old.

Data availability
The cHMW adiponectin levels and covariates data were deposited with this manuscript as source data files. The data with age for the very old and centenarians have ethical and legal restrictions to public deposition due to avoid personal identification, and will be available upon request with an appropriate research arrangement with approval of the Research Ethics Committee of Keio University School of Medicine for Clinical Research. To request, please contact Takashi Sasaki (corresponding author) via e-mail: sasasa@z5.keio.jp.
Reference


Figure legends

Figure 1 | Analysis workflow and distribution of circulating high-molecular-weight (cHMW) adiponectin levels in the very old and centenarians

(a), Sample summary and analysis workflow of cHMW adiponectin levels. (b), Distribution of cHMW adiponectin levels in older adults and centenarians. cHMW adiponectin levels gradually increased with age in the very old to centenarians. (c), Distribution of cHMW adiponectin levels in older men and women. (d), Distribution of cHMW adiponectin levels in centenarian men and women. The difference in cHMW adiponectin levels was significant between sexes in both the very old and centenarians.

Figure 2 | Meta-genome wide association study (GWAS) for cHMW adiponectin levels in the very old and centenarians

(a), Meta-GWAS analysis for cHMW adiponectin levels in the very old and centenarians. Number of samples for the very old and centenarian were 1,015 and 807, respectively. Loci for CDH13 (rs12051213, C: reference allele, T: alternative allele, p = 2.45 × 10^{-22}) and ADIPOQ (rs11711353, G: reference allele, A: alternative allele, p = 6.68 × 10^{-7}) were detected using meta-GWAS for cHMW adiponectin levels in older adults and centenarians. (b), A GWAS enlarged view of the CDH13 region. (c), A GWAS enlarged view of the ADIPOQ region. (d), Distribution of cHMW adiponectin levels in rs4783244 (CDH13) genotypes of the very old and centenarians. cHMW adiponectin levels varied significantly between the rs4783244 reference allele homozygote and rs4783244 alternative allele heterozygote in the very old and centenarians. Except in very old men, no significant difference was observed between the rs4783244 alternative allele heterozygote and rs4783244 alternative allele homozygote in the very old or centenarians.

Figure 3 | Association between cHMW adiponectin level, high-density lipoprotein cholesterol (HDLC), body mass index (BMI), and glycated hemoglobin (HbA1c)

(a, b), Distribution of cHMW adiponectin levels in the diabetes mellitus (DM) and non-DM groups. A person with DM was defined as follows: individuals with glycated hemoglobin (HbA1c) ≥ 6.5%, those receiving antidiabetic drug therapy, or those receiving insulin injections. cHMW adiponectin levels in the DM group were significantly lower than those in the non-DM group in the very old and centenarians. (c, d), Association between cHMW adiponectin levels and HDLC content. A positive association was observed between cHMW adiponectin levels and HDLC content in the very old and centenarians. (e, f), Association between cHMW adiponectin levels and BMI. A strong negative association was observed between cHMW adiponectin levels and BMI in the very old, though this association was rarely observed in
centenarians.

Figure 4 | Multivariate analysis for cHMW adiponectin levels in the very old and centenarians
(a), Multivariate analysis for cHMW adiponectin levels in very old men and women; 14 significant factors for very old men and 10 significant factors for very old women were identified. (b), Multivariate analysis for cHMW adiponectin levels in centenarian men and women; 3 significant factors for centenarian men and 4 significant factors for centenarian women were identified. (c), The contribution rate for each factor in very old men and women was estimated by analysis of variance. (d), The contribution rate for each factor in centenarian men and women was estimated using analysis of variance. The total variance of known cHMW adiponectin level associated factors corresponded to 36.8–42.0% in very old and centenarian men and 18.4% in centenarian women.

Figure 5 | Survival analysis using Cox promotional hazards model for three quantile cHMW adiponectin level groups
(a, b), Survival analysis of very old men and women using the Cox promotional hazards model for three quantile cHMW adiponectin level groups. Seven covariates (model1) and seven covariates with BMI were used for calculating the multiple regression analysis of Cox promotional hazards model. Hazard ratio for low concentration adiponectin group was calculated as the reference. The statistics power analysis using powerSurvEpi (ver. 0.1.3) indicated that survival analyses for both very old men and women have sufficient number of samples and events. (c, d), Survival analysis of the centenarian men and women using Cox promotional hazards model for three quantile cHMW adiponectin level groups. Three covariates (model2) and three covariates with BMI were used for calculation of multiple regression analysis of Cox promotional hazards model. Hazard ratio for low concentration adiponectin group was calculated as the reference. The statistics power analysis using powerSurvEpi (ver. 0.1.3) indicated that survival analysis for centenarian women has sufficient number of samples and events, however, survival analysis for centenarian men was underpowered due to insufficient number of events.
Figure 1—figure supplement 1 | Description of the cohorts in this study
(a) Description of the cohorts for centenarian studies. (b) Description of the cohorts for the very old studies.

Figure 1—figure supplement 2 | Flow chart for analysis in this study
The numbers on the flowchart indicate the number of samples used in each analysis.

Figure 1—figure supplement 3 | Transition of cHMW adiponectin level in the longitudinal data of TOOTH study
(a) Transition of cHMW adiponectin level at baseline and 3-year follow-up studies. (b) Difference of cHMW adiponectin level between baseline and 3-year follow-up studies. The cHMW adiponectin level is gradually increasing during very old.

Figure 2—figure supplement 1 | GWAS for cHMW adiponectin level
(a) GWAS for cHMW adiponectin level in centenarians (n=440) determined by whole genome sequencing (WGS). (b) GWAS for cHMW adiponectin level in centenarians (n=367) determined by genotyping by DNA microarray (Japonica V3 array) and DNA sequence imputation. (c) GWAS result for cHMW adiponectin level in the very old (n=1,015) determined by genotyping using DNA microarray (Asian screening array, Illumina) and DNA sequence imputation.

Figure 2—figure supplement 2 | Minor allele frequency comparison of rs4783244 (CDH13) and rs11711353 (ADIPOQ)
Allele frequency differences were statistically tested using the Fisher Exact test (fisher.test in R stats package (version4.2.2)). (a) rs4783244 men (CDH13). (b) rs4783244 women (CDH13). (c) rs11711353 men (ADIPOQ). (d) rs11711353 women (ADIPOQ).

Figure 2—figure supplement 3 | Distribution of cHMW adiponectin level in rs11711353 (ADIPOQ) genotypes of the very old and centenarians

Figure 4—figure supplement 1 | LASSO with five-fold cross-validation against 1,326 very old and 352 centenarians
(a) LASSO with five-fold validation analysis against 1,326 very old. As a result of five-fold cross-validation, 19 factors shown in black in the bottom column were selected for further multivariate regression analysis. (b) LASSO with five-fold validation analysis against 352 centenarians. As a result of five-fold cross-validation, 7 factors shown in black in the bottom column were selected for further multivariate regression analysis.
Figure 5—figure supplement 1 | The proportional hazards assumption test for a Cox regression model fit
(a) Very old men. (b) Very old women. For calculation of proportional hazards assumption test, cox.zph in survival package [version 3.2-13] was used.

Figure 5—figure supplement 2 | The proportional hazards assumption test for a Cox regression model fit
(a) Centenarian men. (b) Centenarian women. For calculation of proportional hazards assumption test, cox.zph in survival package [version 3.2-13] was used.

Figure 5—figure supplement 3 | Survival time analysis for cancer-cause mortality against the three quantile groups of cHMW adiponectin levels in very old men and women
Survival analysis for cancer-cause mortality of very old men and women grouped by cHMW adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi (ver. 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of events.

Figure 5—figure supplement 4 | Survival time analysis for cardiovascular disease-cause mortality against the three quantile groups of cHMW adiponectin levels in very old men and women
Survival analysis for cardiovascular disease-cause mortality of very old men and women grouped by cHMW adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi (ver. 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of events.

Figure 5—figure supplement 5 | Survival time analysis for pneumonia-cause mortality against the three quantile groups of cHMW adiponectin levels in very old men and women
Survival analysis for pneumonia-cause mortality of very old men and women grouped by cHMW adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi (ver. 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of events.

Figure 5—figure supplement 6 | J-CHS frailty index distribution against the three quantile groups of cHMW adiponectin levels and multiple regression analysis in very old men and women (KAWP)
(a) J-CHS frailty index distribution against the three quantile groups of cHMW adiponectin levels in very old men and women. (b) Multiple regression analysis between cHMW adiponectin level and J-CHS frailty index.

Figure 5—figure supplement 7 | Adiponectin mRNA expression analysis of single-cell RNA-seq for four kinds of mouse adipose tissue

We re-analyzed single-cell RNA-seq results (https://tabula-muris-senis ds.czbiohub.org/fat/droplet/) for four kinds of mouse adipose tissues including brown adipose tissue (Bat), gonadal adipose tissue (Gat), mesenteric adipose tissue (Mat), and subcutaneous adipose tissue (Scat). Dots with the blue color indicated adiponectin-expressed cells. These single-cell RNA-seq results suggested that Scat is one of the major adiponectin-expressed cells in adipose tissues.

Figure 5—figure supplement 8 | Ectopic expression of adiponectin with aging in mouse

We re-analyzed single-cell RNA-seq results of 24 kinds of tissue in 1, 3, 18, 21, 24, 30 months (https://tabula-muris-senis ds.czbiohub.org/fat/droplet/). Major adiponectin-expressed cells were adipose tissue and no obvious ectopic adiponectin expression was observed in the time series of mouse cells.
Supplementary File 1 | Participants' characteristics at enrollment

1: Wilcoxon ranking test, 2: Fisher's exact test, 3: Chi-square test. Abbreviations: IQR, inter-quartile range; BMI, body mass index; SBP, systolic blood pressure; IADL, instrumental activities of daily living; ADL, activities of daily living; MMSE, mini mental state examination; HMW, high molecular weight; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; TCHO, total cholesterol; TG, triglyceride; CHE, choline esterase; AST, aspartate aminotransferase; γGTP, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; UA, uric acid; ALB, albumin; Alt, alternative; MAF, minor allele frequency.

Supplementary File 2 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in very old men (n=643)

Supplementary File 3 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in very old women (n=683)

Supplementary File 4 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in the very old (n=1,326)

Supplementary File 5 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in centenarian men (n=63)

Supplementary File 6 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in centenarian women (n=289)

Supplementary File 7 | Coefficients for generalized linear model analysis of plasma HMW adiponectin level in centenarian (n=352)

Supplementary File 8 | Analysis of variance of plasma HMW adiponectin level by ANOVA in very old men (n=643)

Supplementary File 9 | Analysis of variance of plasma HMW adiponectin level by ANOVA in very old women (n=683)

Supplementary File 10 | Analysis of variance of plasma HMW adiponectin level by ANOVA in centenarian men (n=63)
Supplementary File 11 | Analysis of variance of plasma HMW adiponectin level by ANOVA in centenarian women (n=289)
**Figure 1 source data**
Source data for figure 1 including 812 centenarians and 1,498 very old data.

**Figure 2 source data**
Source data for figure 1 including 812 centenarians and 1,498 very old data.

**Figure 3 source data**
Source data for figure 1 including 812 centenarians and 1,498 very old data.

**Source Code File 1**
R script code file for Figure 1c.

**Source Code File 2**
R script code file for Figure 1d.

**Source Code File 3**
R script code file for Figure 2d.

**Source Code File 4**
R script code file for Figures 3ab.

**Source Code File 5**
R script code file for Figures 3c-f.
### Table:

<table>
<thead>
<tr>
<th>Group</th>
<th>Cohort</th>
<th>Age at entry</th>
<th>n (women%)</th>
<th>Survey</th>
<th>Genetic</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centenarians</td>
<td>KAWP</td>
<td>100- yo</td>
<td>812 (84.4%)</td>
<td>Baseline</td>
<td>6M SNVs</td>
<td>~ 10 years later</td>
</tr>
<tr>
<td>n = 812</td>
<td>TOOTH</td>
<td>85-89 yo</td>
<td>1019 (49.7%)</td>
<td>Baseline</td>
<td>6M SNVs</td>
<td>~ 3.5 years later</td>
</tr>
<tr>
<td>The very old</td>
<td></td>
<td>n = 1498</td>
<td></td>
<td></td>
<td></td>
<td>~ 6 years later</td>
</tr>
</tbody>
</table>

### Adiponectin Analysis

- **GWAS**
- **Association analysis**
- **Multivariate analysis w/ genetic factors**
- **Survival analysis**

### Figure 1

**b** The very old (85-89yo) vs Centenarians (100-100yo)

- **men** (n=851, r=0.45, p < 0.001)
- **women** (n=1,459, r=0.39, p < 0.001)

**c** The very old (85-89yo)

- **men** (n=724)
- **women** (n=774)
  - p < 0.001

**d** Centenarians (100-100yo)

- **men** (n=127)
- **women** (n=685)
  - p = 0.0163

*Sasaki et al.*
**Centenarians (n=967)**

- Study: Tokyo Centenarian Study (TCS)  
  - Target age: ≥100yo.  
  - Follow up period: ~ July 2018  
  - Number of Entry: 238  

- Study: Japan Semi-supercentenarian Study (JSS)  
  - Target age: ≥105yo.  
  - Entry period: Sept. 2002 ~ March 2019  
  - Follow up period: ~ March. 2019  
  - Number of Entry: 729

**The very old (n=1,568)**

- Study: The Tokyo Oldest Old Survey on Total Health (TOOTH)  
  - Target age: ≥85yo.  
  - Follow up period: ~ Jan. 2016  
  - Number of Entry: 542  

- Study: Kawasaki Aging and Wellbeing Project (KAWP)  
  - Target age: 85-89yo.  
  - Entry period: March, 2017 ~ Dec. 2019  
  - Follow up period: ~ Sep 2022  
  - Number of Entry: 1026

---

**Data:**

- No cHMW Adiponectin data: 155

812 (127 men (median age: 105.3 yo [IQR: 100.9–106.8]), 685 women (median age: 106.0 yo [IQR: 103.9–107.2]))

- No cHMW Adiponectin data: 7

1,498 (724 men (median age: 86.9 [IQR: 85.9–88.2]), 774 women (median age: 87.0 years [IQR: 86.0–88.4]))

---

**References:**


Centenarians (n=812)  

Association analysis (Fig.1,3)  

GWAS (Fig.2)  

LASSO-multiple regression analysis (Fig.4)  

Survival analysis (Fig.5)  

The very old (n=1,498)  

1,015 Microarray  

440 WGS  
367 Microarray  

1,326 (no missing value for 32 factors)  

352 (no missing value for 26 factors)  

545 (no missing value for 4 factors)  

1,425 (no missing value for 6 factors)
[Figure a] cHMW adiponectin (µg/ml) vs age (years old) for men (n=144) and women (n=178).

[Figure b] Difference of cHMW adiponectin (µg/ml) between men (n=144) and women (n=178), p = 0.512.
Fig. 2 Sasaki et al.

CDH13 rs12051213 C>T
p = 2.21 x 10^{-22}
Z score: -9.64

CDH13 rs4783244 G>T
p = 5.39 x 10^{-22}
Z score: -9.73

ADIPQGQ 82.5 82.6 82.7 82.8
P Position on chr3 (Mb)

Very old men

Very old women

Centenarians men

Centenarians women

CDH13 (T-cadherin) rs4783244
a  Centenarians (WGS, n=440)

b  Centenarians (Japonica V3 array, n=367)

c  The very old (Asian screening array, n=1,015)
a, rs4783244 men (CDH13)

- $P = 0.858$
- $P = 0.268$
- $P = 0.335$

- $n = 127$
- $n = 722$
- $n = 11,261$

b, rs4783244 women (CDH13)

- $P = 0.703$
- $P = 0.335$
- $P = 0.213$

- $n = 682$
- $n = 774$
- $n = 27,441$

c, rs11711353 men (ADIPOQ)

- $P = 0.234$
- $P = 0.895$
- $P = 0.775$

- $n = 11,261$
- $n = 722$
- $n = 127$

c, rs11711353 women (ADIPOQ)

- $P = 0.554$
- $P = 0.022$
- $P = 0.206$

- $n = 27,441$
- $n = 774$
- $n = 682$
ADIPOQ rs11711353

Very older men

<table>
<thead>
<tr>
<th>Allele</th>
<th>Very older men</th>
<th>Very older women</th>
<th>Centenarians men</th>
<th>Centenarians women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=313)</td>
<td>(n=329)</td>
<td>(n=51)</td>
<td>(n=248)</td>
</tr>
<tr>
<td>G/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cytokine adiponectin (µg/ml)

* *: p < 0.001

p = 0.529
p = 0.052
p = 0.100
p = 0.034
p = 0.019
p = 0.423
p = 0.125
p = 0.291
p = 0.048
p = 0.486

n=313
n=329
n=82
n=313
n=357
n=104
n=51
n=63
n=13
n=248
n=339
n=95

Very older women

Centenarians men

Centenarians women
The very old

DM men
p = 0.014

DM women
p < 0.001

non DM
(n=588)
DM
(n=135)

non DM
(n=655)
DM
(n=119)

Centenarians

DM men
p = 0.032

non DM
(n=121)
DM
(n=5)

non DM
(n=638)
DM
(n=38)

The very old

HDLC (mg/dL)

men
(n=724, r=0.37, p<0.001)

women
(n=774, r=0.44, p<0.001)

BMI (kg/m²)

men
(n=722, r=-0.29, p<0.001)

women
(n=744, r=-0.34, p<0.001)

Centenarians

HDLC (mg/dL)

men
(n=127, r=0.19, p=0.0289)

women
(n=684, r=0.29, p<0.001)

BMI (kg/m²)

men
(n=94, r=-0.12, p=0.261)

women
(n=462, r=-0.12, p=0.0073)
The very old (85-89yo, n=1,326)

Centenarians (100-yr, n=352)

Basic information (5)
1 Age
2 BMI
3 SBP
4 Years of education
5 Smoking history

Physical and Cognitive function (4)
6 IADL score
7 Hand grip
8 Cognitive impairment
9 WHOS score

Disease histories (5)
10 Heart disease
11 Diabetes
12 Cancers
13 Renal disease
14 Fracture

Genetic factors (3)
30 SEX
31 CDH13 rs4783244
32 ADIPOQ rs11711353

Biomarkers in blood (15)
15 HDLC
16 TCHO
17 LDLCL
18 TG
19 CHE
20 AST
21 ALT
22 γGTP
23 LDH
24 UA
25 ALB
26 CstC
27 NTproBNP
28 HBA1c
29 IL6

Biomarkers in blood (12)
12 HDLC
13 TCHO
14 LDLCL
15 TG
16 CHE
17 γGTP
18 UA
19 ALB
20 CstC
21 NTproBNP
22 HBA1c
23 IL6

Disease histories (5)
7 Heart disease
8 Diabetes
9 Cancers
10 Renal disease
11 Fracture

Genetic factors (3)
24 SEX
25 CDH13 rs4783244
26 ADIPOQ rs11711353
<table>
<thead>
<tr>
<th>Group</th>
<th>event/N</th>
<th>cHMW Adiponectin conc. (ug/ml median (range))</th>
<th>BMI (kg/m² median (25, 75%tile))</th>
<th>Hazard ratio (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Old Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>35/226</td>
<td>3.66 (0.44 – 5.11)</td>
<td>23.8 (22.3 - 25.8)</td>
<td></td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>50/226</td>
<td>9.52 (5.13 - 9.52)</td>
<td>23.3 (21.7 - 25.0)</td>
<td>1.67 (1.06 - 2.62)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>60/226</td>
<td>13.6 (9.53 - 50.20)</td>
<td>21.8 (19.7 - 23.6)</td>
<td>2.32 (1.46 - 3.70)</td>
</tr>
<tr>
<td><strong>Very Old Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>33/249</td>
<td>5.80 (0.97 - 7.96)</td>
<td>23.6 (21.3 - 25.5)</td>
<td></td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>24/249</td>
<td>10.5 (7.96 - 14.00)</td>
<td>22.7 (20.6 - 24.7)</td>
<td>0.94 (0.54 - 1.65)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>44/249</td>
<td>19.7 (14.00 - 58.9)</td>
<td>20.6 (18.8 - 22.8)</td>
<td>1.89 (1.05 - 3.40)</td>
</tr>
<tr>
<td><strong>Centenarian Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>30/30</td>
<td>10.2 (2.10 - 13.1)</td>
<td>20.4 (18.8 - 22.2)</td>
<td></td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>30/30</td>
<td>16.3 (13.2 - 19.9)</td>
<td>18.9 (17.2 - 20.7)</td>
<td>1.09 (0.69 - 1.72)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>29/30</td>
<td>26.8 (20.0 - 45.5)</td>
<td>19.6 (17.2 - 21.5)</td>
<td>0.81 (0.50 - 1.33)</td>
</tr>
<tr>
<td><strong>Centenarian Women</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>150/152</td>
<td>12.0 (1.55 - 15.3)</td>
<td>19.2 (17.4 - 21.9)</td>
<td></td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>152/152</td>
<td>18.5 (15.3 - 23.2)</td>
<td>19.1 (17.3 - 21.3)</td>
<td>1.18 (0.93 - 1.50)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>150/151</td>
<td>28.3 (23.2 - 94.4)</td>
<td>18.4 (16.8 - 20.4)</td>
<td>1.37 (1.06 - 1.76)</td>
</tr>
</tbody>
</table>

Model1 covariates: Age, cohort, HDLC, disease history (Diabetes), CDH13 rs11711353, ADIPOQ rs4783244, Years of education
Model2 covariates: Age, HDLC, CDH13 rs11711353
a. Very old men

<table>
<thead>
<tr>
<th>Variable</th>
<th>chisq</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin.3tile</td>
<td>5.6258</td>
<td>2</td>
<td>0.060</td>
</tr>
<tr>
<td>Age</td>
<td>0.4014</td>
<td>1</td>
<td>0.526</td>
</tr>
<tr>
<td>cohort</td>
<td>3.3586</td>
<td>1</td>
<td>0.067</td>
</tr>
<tr>
<td>HDLC</td>
<td>0.2860</td>
<td>1</td>
<td>0.593</td>
</tr>
<tr>
<td>DM</td>
<td>0.0184</td>
<td>1</td>
<td>0.892</td>
</tr>
<tr>
<td>rs11711353</td>
<td>0.1363</td>
<td>1</td>
<td>0.712</td>
</tr>
<tr>
<td>rs4783244</td>
<td>0.5392</td>
<td>1</td>
<td>0.463</td>
</tr>
<tr>
<td>years of education</td>
<td>2.4766</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td>BMI</td>
<td>0.9791</td>
<td>1</td>
<td>0.322</td>
</tr>
<tr>
<td>GLOBAL</td>
<td>14.6985</td>
<td>10</td>
<td>0.143</td>
</tr>
</tbody>
</table>

b. Very old women

<table>
<thead>
<tr>
<th>Variable</th>
<th>chisq</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin.3tile</td>
<td>0.2104</td>
<td>2</td>
<td>0.90</td>
</tr>
<tr>
<td>Age</td>
<td>0.3300</td>
<td>1</td>
<td>0.57</td>
</tr>
<tr>
<td>cohort</td>
<td>1.9689</td>
<td>1</td>
<td>0.16</td>
</tr>
<tr>
<td>HDLC</td>
<td>0.0023</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td>DM</td>
<td>0.8050</td>
<td>1</td>
<td>0.37</td>
</tr>
<tr>
<td>rs11711353_num.taQman</td>
<td>1.5193</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>rs4783244_num.taQman</td>
<td>0.0197</td>
<td>1</td>
<td>0.89</td>
</tr>
<tr>
<td>years_of_education</td>
<td>0.0678</td>
<td>1</td>
<td>0.79</td>
</tr>
<tr>
<td>BMI</td>
<td>0.7459</td>
<td>1</td>
<td>0.39</td>
</tr>
<tr>
<td>GLOBAL</td>
<td>6.9302</td>
<td>10</td>
<td>0.73</td>
</tr>
</tbody>
</table>
a. Centenarian men

<table>
<thead>
<tr>
<th>Variable</th>
<th>chisq</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin.male.3tile</td>
<td>3.513</td>
<td>2</td>
<td>0.173</td>
</tr>
<tr>
<td>Age</td>
<td>3.243</td>
<td>1</td>
<td>0.072</td>
</tr>
<tr>
<td>HDLC</td>
<td>0.957</td>
<td>1</td>
<td>0.328</td>
</tr>
<tr>
<td>rs4783244</td>
<td>0.152</td>
<td>1</td>
<td>0.697</td>
</tr>
<tr>
<td>BMI</td>
<td>0.711</td>
<td>1</td>
<td>0.399</td>
</tr>
<tr>
<td>GLOBAL</td>
<td>12.810</td>
<td>6</td>
<td>0.046</td>
</tr>
</tbody>
</table>

b. Centenarian women

<table>
<thead>
<tr>
<th>Variable</th>
<th>chisq</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin.female.3tile</td>
<td>1.35e+00</td>
<td>2</td>
<td>0.51</td>
</tr>
<tr>
<td>Age</td>
<td>4.25e-01</td>
<td>1</td>
<td>0.51</td>
</tr>
<tr>
<td>HDLC</td>
<td>5.03e-02</td>
<td>1</td>
<td>0.82</td>
</tr>
<tr>
<td>rs4783244</td>
<td>1.63e-08</td>
<td>1</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI</td>
<td>5.93e-01</td>
<td>1</td>
<td>0.44</td>
</tr>
<tr>
<td>GLOBAL</td>
<td>2.19e+00</td>
<td>6</td>
<td>0.90</td>
</tr>
<tr>
<td>Group</td>
<td>event/N</td>
<td>cHMW Adiponectin conc. (ug/ml median (range))</td>
<td>BMI (kg/m² median (25,75%tile))</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------</td>
<td>---------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very Old Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>13/226</td>
<td>3.66 (0.44 - 3.64)</td>
<td>23.8 (22.4 - 25.8)</td>
</tr>
<tr>
<td>Intermedante conc. Adiponectin</td>
<td>13/226</td>
<td>9.52 (5.11 - 9.52)</td>
<td>23.3 (21.8 - 25.0)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>13/226</td>
<td>13.5 (9.52 - 50.2)</td>
<td>21.8 (19.7 - 23.6)</td>
</tr>
<tr>
<td>Very Old Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>3/249</td>
<td>5.80 (0.97 - 7.96)</td>
<td>23.6 (21.3 - 25.5)</td>
</tr>
<tr>
<td>Intermedante conc. Adiponectin</td>
<td>8/249</td>
<td>10.5 (7.96 - 14.00)</td>
<td>22.7 (20.6 - 24.7)</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>9/249</td>
<td>19.7 (14.00 - 58.9)</td>
<td>20.6 (18.8 - 22.8)</td>
</tr>
</tbody>
</table>

Model1 covariates: Age, HDLC, CDH13 rs11711353
## Cardiovascular disease-cause mortality

<table>
<thead>
<tr>
<th>Group</th>
<th>event/N</th>
<th>cHMW Adiponectin conc. (ug/ml median (range))</th>
<th>BMI (kg/m² median (25, 75%tile))</th>
<th>Hazard ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Model1</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Model1+BMI</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Model1 covariates: Age, HDLC, CDH13 rs11711353</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Very Old Men</td>
<td></td>
<td></td>
<td>Model1</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>6/226</td>
<td>3.66 (0.44 - 3.64)</td>
<td>23.8 (22.4 - 25.8)</td>
<td>1.58 (0.52 - 4.79)</td>
<td>0.418</td>
</tr>
<tr>
<td>Intermediate conc.</td>
<td>8/226</td>
<td>9.52 (5.11 - 9.52)</td>
<td>23.3 (21.8 - 25.0)</td>
<td>1.50 (0.49 - 4.55)</td>
<td>0.475</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>12/226</td>
<td>13.5 (9.52 - 50.2)</td>
<td>21.8 (19.7 - 23.6)</td>
<td>3.16 (1.06 - 9.41)</td>
<td>0.039</td>
</tr>
<tr>
<td>Very Old Women</td>
<td></td>
<td></td>
<td>Model1</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>12/249</td>
<td>5.80 (0.97 - 7.96)</td>
<td>23.6 (21.3 - 25.5)</td>
<td>0.30 (0.08 - 1.11)</td>
<td>0.105</td>
</tr>
<tr>
<td>Intermediate conc.</td>
<td>3/249</td>
<td>10.5 (7.96 - 14.00)</td>
<td>22.7 (20.6 - 24.7)</td>
<td>0.27 (0.07 - 1.03)</td>
<td>0.056</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>12/249</td>
<td>19.7 (14.00 - 58.9)</td>
<td>20.6 (18.8 - 22.8)</td>
<td>1.04 (0.35 - 3.09)</td>
<td>0.946</td>
</tr>
</tbody>
</table>

Model1 covariates: Age, HDLC, CDH13 rs11711353
## Pneumonia disease-cause mortality

<table>
<thead>
<tr>
<th>Group</th>
<th>event/N</th>
<th>cHMW Adiponectin conc. (ug/ml median (range))</th>
<th>BMI (kg/m^2 median (25, 75%tile))</th>
<th>Hazard ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Old Men</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>6/226</td>
<td>3.66 (0.44 - 3.64)</td>
<td>23.8 (22.4 - 25.8)</td>
<td>Model1</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model1+BMI</td>
<td>0.80</td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>11/226</td>
<td>9.52 (5.11 - 9.52)</td>
<td>23.3 (21.8 - 25.0)</td>
<td>Model1</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model1+BMI</td>
<td>0.64</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>11/226</td>
<td>13.5 (9.52 - 50.2)</td>
<td>21.8 (19.7 - 23.6)</td>
<td>Model1</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model1+BMI</td>
<td>0.74</td>
</tr>
</tbody>
</table>

| **Very Old Women**           |         |                                               |                                   |              |       |
| Low conc. Adiponectin        | 4/249   | 5.80 (0.97 - 7.96)                            | 23.6 (21.3 - 25.5)                | Model1       | 1.31  |
|                             |         |                                               |                                   | Model1+BMI   | 0.21  |
| Intermediate conc. Adiponectin | 2/249 | 10.5 (7.96 - 14.00)                           | 22.7 (20.6 - 24.7)                | Model1       | 1.13  |
|                             |         |                                               |                                   | Model1+BMI   | 0.18  |
| High conc. Adiponectin       | 6/249   | 19.7 (14.00 - 58.9)                           | 20.6 (18.8 - 22.8)                | Model1       | 5.87  |
|                             |         |                                               |                                   | Model1+BMI   | 1.01  |

Model1 covariates: Age, HDLC, CDH13 rs11711353
### Multiple regression analysis between cHMW adiponectin level and Frailty

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>cHMW Adiponectin level (ug/ml median (range))</th>
<th>OR (95%CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Model1</td>
<td></td>
</tr>
<tr>
<td>Very Old Men</td>
<td></td>
<td></td>
<td>Model1+BMI</td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>165</td>
<td>3.58 (1.23 - 5.11)</td>
<td>0.99 (0.79 - 1.24)</td>
<td>0.929</td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>178</td>
<td>6.98 (5.13 - 9.52)</td>
<td>0.98 (0.79 - 1.22)</td>
<td>0.868</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>148</td>
<td>13.2 (9.58 - 42.20)</td>
<td>1.28 (1.00 - 1.63)</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.22 (0.95 - 1.56)</td>
<td>0.126</td>
</tr>
<tr>
<td>Very Old Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low conc. Adiponectin</td>
<td>164</td>
<td>6.08 (1.60 - 7.92)</td>
<td>1.12 (0.90 - 1.39)</td>
<td>0.329</td>
</tr>
<tr>
<td>Intermediate conc. Adiponectin</td>
<td>170</td>
<td>10.5 (7.96 - 13.90)</td>
<td>1.12 (0.90 - 1.39)</td>
<td>0.319</td>
</tr>
<tr>
<td>High conc. Adiponectin</td>
<td>150</td>
<td>19.4 (14.0 - 58.9)</td>
<td>1.35 (1.04 - 1.74)</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.36 (1.04 - 1.78)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Model 1 covariates: Age, cohort, HDLC, disease history (Diabetes), CDH13 rs11711353, ADIPOQ rs4783244, Years of education

---

![Graphs showing frequency and median cHMW adiponectin levels for Very Old Men and Women by frailty status.](image)
BAT+GAT+MAT  1375 cells
Gat 731 cells
Mat 1023 cells
Scat 2999 cells
SCAT 640 cells
non fat tissue

adipose tissue