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Osteoglycin Across the Adult Lifespan

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Abbreviations: BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HOMA-IR, homeostatic model assessment for insulin resistance; OGN, osteoglycin.

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Abstract

Context: Osteoglycin (OGN) is a proteoglycan released from bone and muscle which has been associated with markers of metabolic health. However, it is not clear whether the levels of circulating OGN change throughout the adult lifespan or if they are associated with clinical metabolic markers or fitness.

Objective: We aimed to identify the levels of circulating OGN across the lifespan and to further explore the relationship between OGN and aerobic capacity as well as OGN's association with glucose and HOMA-IR.

Methods: 107 individuals (46 males and 61 females) aged 21-87 years were included in the study. Serum OGN levels, aerobic capacity (VO_{2peak}), glucose, and homeostatic model assessment for insulin resistance (HOMA-IR) were assessed. T-tests were used to compare participant characteristics between sexes. Regression analyses were performed to assess the relationship between OGN and age, and OGN and fitness and metabolic markers.

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Results: OGN displayed a nonlinear, weak "U-shaped" relationship with age across both sexes. Men had higher levels of OGN than women across the lifespan ($\beta = 0.23$, $P = .03$). Age and sex explained 16% of the variance in OGN (adjusted $R^2 = 0.16$; $P < .001$). Higher OGN was associated with higher VO_{2peak} ($\beta = 0.02$, $P = .001$); however, those aged <50 showed a stronger positive relationship than those aged >50. A higher OGN level was associated with a higher circulating glucose level ($\beta = 0.17$, $P < .01$). No association was observed between OGN and HOMA-IR.

Conclusion: OGN was characterized by a U-shaped curve across the lifespan which was similar between sexes. Those with a higher aerobic capacity or higher glucose concentration had higher OGN levels. Our data suggest an association between OGN and aerobic fitness and glucose regulation. Future studies should focus on exploring the potential of OGN as a biomarker for chronic disease.

Key Words: osteoglycin, metabolic health, glycemic control, VO_{2peak}, fitness

Osteoglycin (OGN) is a proteoglycan released from various tissues, including bone and muscle, which is suggested to be involved in energy metabolism and muscle function, at least in mice (1). OGN-deficient mice have impaired glucose tolerance, and OGN treatment in wild-type mice improves insulin-stimulated glucose uptake in skeletal muscle (1). In humans, higher circulating levels of OGN are associated with lower body mass index (BMI) and greater lean body mass (1, 2). Individuals who experienced significant weight loss through gastric bypass surgery had increased levels of OGN (1). While these data suggest associations between OGN and metabolic health, the results have not always been consistent. For example, a recent study in humans reported no association between OGN and glucose, HbA1c, or body mass index (BMI) in individuals with diabetes (3). It is plausible that some of the inconsistent findings are due to the diverse demographics of the populations studied, including different ages, BMI, and disease status. To date, there are no reference ranges for OGN; furthermore, it is unclear whether the circulating levels of OGN change with age. As such, before exploring whether OGN can be used as a marker of glucose regulation and future disease development, it is essential to uncover how circulating levels of OGN change during normal aging, which itself is known to be associated with increased risk of cardiometabolic diseases and has a substantial effect on other hormones involved in bone–muscle crosstalk and glucose regulation (4-6).

In addition, it is well recognized that fitness, as measured by VO_{2peak}, is strongly associated with a reduction in cardiometabolic risk and improved glucose regulation (7, 8). As such, it will be of interest to examine whether there are associations between circulating OGN levels and VO_{2peak} as well markers of glucose regulation (glucose and homeostatic model assessment of insulin resistance [HOMA-IR]). The primary aim of this study was to identify the levels of circulating OGN across the lifespan in community-dwelling men and women. The secondary aim was to explore the relationship between OGN and VO_{2peak}, as well as its association with glucose and HOMA-IR.

Materials and Methods

Participants

The data for this study were cross-sectional and are a secondary analysis of a subset of data derived from the following studies, the Gene Smart Study (aged 18-45 years) (9, 10); the Wellderly study (aged 60 years or older) (11); and smaller exercise studies at Victoria University and Deakin University (age range 18 and older), that included a resting blood sample and fitness assessment (12-15). Patients with diabetes and/or people with a HOMA-IR of 3.5 or higher were excluded from the study. This HOMA-IR cut-off level was selected as this value represents the upper limit of the "healthy" threshold (16). Only 1 participant was being treated for osteoporosis (with antiresorptive medication), but their OGN levels were similar to participants of a similar age, so they were included in analyses. All participants were asked to avoid exercise in the 48 hours prior to the laboratory visits, and to the best of our knowledge, all volunteers either drove or used public transport to travel to testing (ie, did not use active transport).

Osteoglycin Analysis

The serum samples for all cohorts were stored at –80°C until analysis. All samples were analyzed at one time point for the purposes of this study. Serum OGN was analyzed in duplicate using enzyme-linked immunosorbent assay (ELISA) (SEC688Hu, L200522411, Cloud-Clone Corp.) according to the manufacturer's protocol. This ELISA kit has been previously used to measure OGN and has

manufacturer-reported coefficients of variation of <10% (intra-assay) and <12% (interassay) (1). Serum samples were thawed on ice and then diluted 1:60 ~1:200 with a standard diluent buffer. The dilution was optimized for each sample cohort, but the final dilution for each sample was still within the optimum detection range for the ELISA kit. Standards were reconstituted 15 minutes prior to the assay, and a total of 1 blank and 6 standard wells were included in each plate. Each well was filled with 100 µL of either the standard, blank, or diluted samples and the samples were processed.

Glucose, Insulin, and HOMA-IR

Serum glucose was analyzed using an automated analysis system (YSI 2300 STAT Plus™ Glucose & Lactate Analyzer). Serum insulin was analyzed using Ultrasensitive Insulin ELISA Jumbo (Alpco, Lot 08192). HOMA-IR was calculated from both fasting insulin and fasting glucose following the equation HOMA-IR = [fasting insulin (µU/L) × fasting glucose (nmol/L)]/22.5 (17).

Aerobic fitness (VO_{2peak})

Participants in all studies completed a graded exercise test on a cycle ergometer with a gas analyzer to obtain peak aerobic capacity (VO_{2peak}). Individual protocols for the graded exercise tests were previously described within each study (9-15,18).

Statistical Analysis

Statistical analysis was performed using R version 4.1.0 (19). T-tests were used to compare age, BMI, aerobic capacity, and metabolic measures between sexes. To investigate the relationship between baseline OGN and age, a polynomial regression of degree 2 model was fitted for OGN (dependent variable) using age and sex as independent variables. To investigate the relationship between baseline OGN and metabolic markers or fitness, a linear regression model was fitted for OGN (dependent variable) using metabolic markers or fitness, age, and sex as independent variables. We also investigated whether the relationship of OGN with metabolic markers was similar between those aged 50 years or younger and those aged 50 years and older as this is the average age for onset of menopause and andropause (20, 21). To do so, we recorded age as a dummy variable ("age group") and assigned individuals to one of the following categories: "young" (<50 years) or "old" (>50 years); we then added an interaction between metabolic markers or fitness, and age group. The model was of the from OGN = age group × predictor. To meet the statistical assumptions of the regression (normally distributed residuals), HOMA-IR and OGN were log-transformed. All data are presented as mean ± SD. For all statistical analyses, $P < .05$ was considered statistically significant. The following packages were used in our analysis *lme4* (22), *lmerTest* (23), *tidyverse* (24) *MASS* (25), *data*.table (26) *OWLE*, and *lmtest* (27).

Results

Participant characteristics are shown in Table 1. Women were older and had a lower aerobic capacity than men but were otherwise similar.

OGN displayed a "U-shaped" relationship with age across the lifespan in men and women combined (Fig. 1A). While not adequately powered to explore sex differences, the trend line for men and women separately (Fig. 1B) indicated that a polynomial model (U-shaped curve) was the best fit for men and both a linear and polynomial model were equally good fits for women. For the combined data, circulating levels of OGN decreased in a nonlinear fashion, from 59.9 ng/mL at 18 years to 35.8 ng/mL at ~46 years before increasing to 73.7 ng/mL at 80 years. Overall, age and sex explained 16% of the variance in OGN (adjusted $R^2 = 0.16$; $P < .001$). In the young, but not the older cohort, men had a higher level of OGN (β = 0.54; 95% CI 0.27-0.82; $P < .001$) than women.

Table 1. Participant characteristics

Variable	Female (n = 61)	Male (n = 46)	*P*
Age (years), mean ± SD	54.96 ± 17.26	48.09 ± 18.23	.03
BMI (kg/m^2), mean ± SD	27.15 ± 4.5	27.8 ± 4.99	.45
Glucose (mmol/L), mean ± SD	5.24 ± 0.91*	5.31 ± 0.83*	.65
OGN (ng/ul), median (IQR)	3.85 (3.42, 4.26)	4.01 (3.65, 4.51)	.06
Insulin (µIU/mL), median (IQR)	1.69 (1.41, 1.98)	1.67 (1.15, 2.08)	.44
HOMA (AU), median (IQR)	0.14 (–0.15, 0.55)*	0.26 (–0.32, 0.62)*	.34

All normally distributed data are presented as mean ± SD and not normally distributed data are presented as median (IQR).
*7 samples did not have serum glucose results and have been excluded from the glucose and HOMA analyses. Total samples for glucose, insulin and HOMA are n = 107.

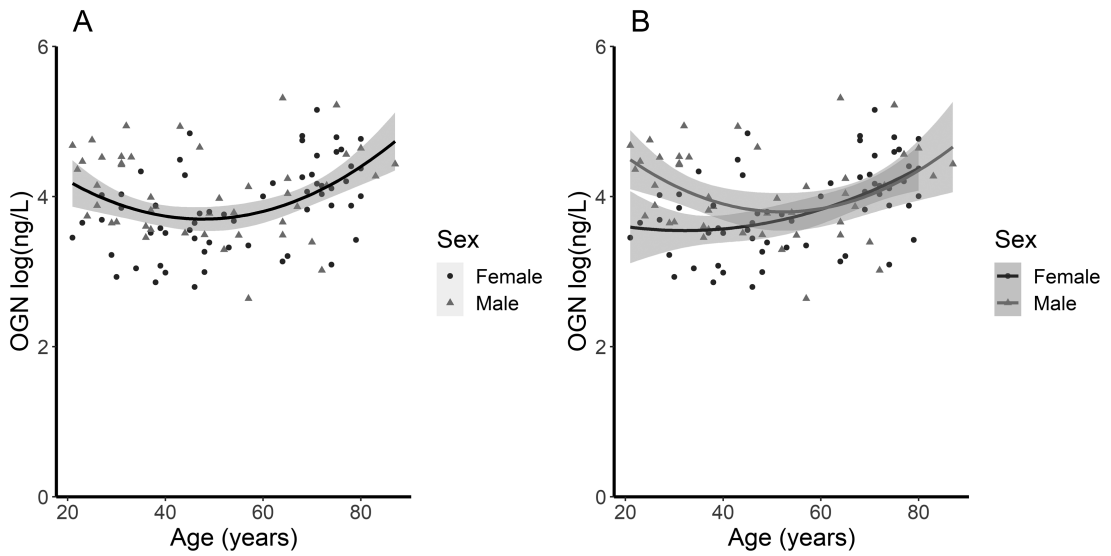

Figure 1. Relationship between age and OGN in men and women combined (A) and in men and women separately (B). Women are denoted by circles and men by triangles.

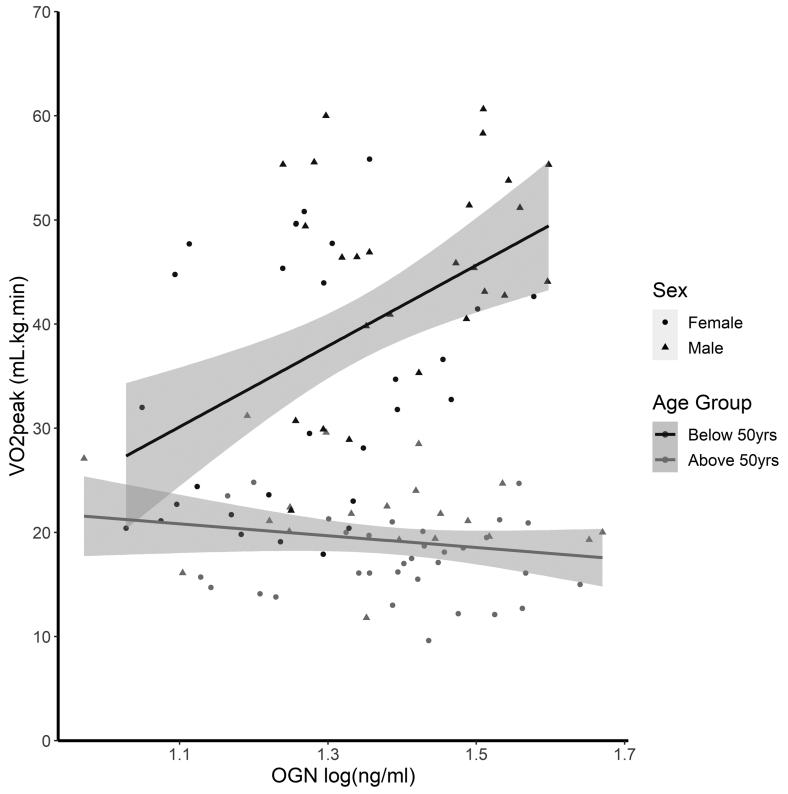

Figure 2. Relationship of OGN adjusted for age and VO_{2peak}. Women are denoted by circles and men by triangles. Those below 50 years are in red, and those above 50 years are in blue.

Higher OGN was associated with higher VO_{2peak} (β = 0.02, P = .001), which remained significant after adjusting for age and sex (Fig. 2). There was an interaction between age and VO_{2peak} (P = .02), whereby those aged below 50 showed a strong positive relationship between OGN and VO_{2peak} compared with those aged above 50 (Fig. 2). Moreover, a higher VO_{2peak} was associated with higher circulating levels of glucose (β = 0.03, SE = 0.009, P < .001) after adjusting for age and sex.

A higher OGN was associated with higher blood glucose (β = 0.17; 95% CI 0.04-0.31; P = .012) (Fig. 3A). Glucose was 0.7 mmol/L higher in the old cohort (above

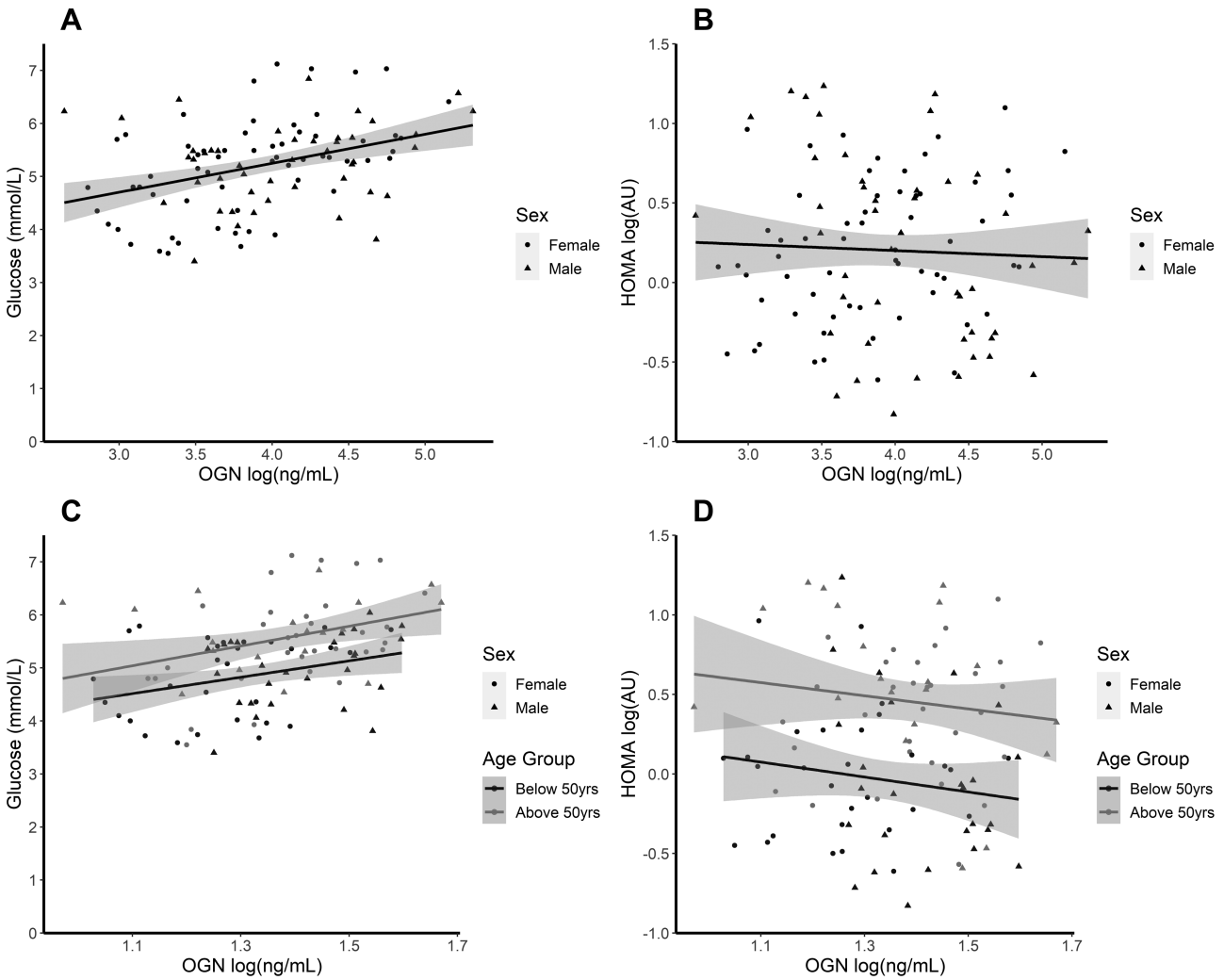

Figure 3. Relationship between glucose or HOMA-IR and OGN. Women are denoted by circles and men by triangles (A,B). Those below 50 years are in red, and those above 50 years are in blue (C,D).

50 years) when compared with the young (<50 years) cohort ($P < .001$) (Fig. 3C). We found no clear relationship between OGN and HOMA-IR in the overall sample ($P = .1$) (Fig. 3B). HOMA-IR was increased in the old cohort (above 50 years) when compared with the young (<50 years) and old cohort ($\beta = 0.49$, $P < .001$) (Fig. 3D).

Discussion

We report a nonlinear, weak "U-shape" relationship between OGN and age across the lifespan. There was an association between higher OGN levels and a higher aerobic capacity in the full cohort, and the association was even stronger in those under 50 years. Finally, those with higher glucose levels showed higher OGN levels.

The aging process has a profound impact on muscle and bone as well as circulating hormones. However, our understanding of how aging influences circulating levels of OGN, a hormone implicated not only in the function of muscle and bone but also in metabolic regulation, remains poor. We reported a nonlinear, weak "U-shaped" relationship of OGN with age, regardless of sex; older and younger adults had a higher concentration of OGN than those who are middle-aged. Interestingly, when comparing sex differences within the old and young cohorts, young men had higher OGN than women. This is a similar finding to other bone remodeling markers that are higher in younger men than in women (28). A U-shaped relationship of OGN across the lifespan for the full cohort has been shown in another study that examined the relationship between age and osteocalcin, another hormone linked with bone–muscle crosstalk, suggesting some similarities in how these bone–muscle crosstalk hormones change across the lifespan (6). The reason for the U-shaped pattern is currently unclear; however, a plausible explanation is that in young and older individuals there is an increase in the release of hormones, including OGN and osteocalcin, but the physiological reasons for the increases are very different. In younger

individuals, higher OGN could be linked to increased bone modeling and a gain of bone mineral density, but in older age, the increase in OGN may reflect an accelerated bone remodeling leading to bone loss. This hypothesis should be further explored in the future.

Interestingly, the levels of OGN in women trended toward being lower than men across the lifespan. The physiological relevance of this is unknown, but it highlights the importance of studying both sexes when examining the effects of aging on bone–muscle crosstalk hormones (29). Despite the different circulating levels of OGN throughout the lifespan, both sexes have higher OGN levels in older age. Given that some cardiometabolic diseases are more elevated in older adults, future studies should explore whether increased OGN in older adults is related to an increased risk of cardiometabolic diseases (30, 31). Indeed, in the current study, we reported that a higher OGN is associated with higher circulating glucose in our community-dwelling cohort; we also report no association between OGN and HOMA-IR. The relationship between OGN and glucose contrasts with a previous study that reported that higher OGN levels are related to improved glucose regulation in women and improved insulin action in mice (1). The results also contrast with a recent study in patients with type I and type II diabetes, which found no associations between OGN and glucose (3). The conflicting results between humans and animals as well as between healthy and clinical populations complicate our understanding of the metabolic role of OGN, and, as such, further interventional studies are needed. As our study includes participants across the lifespan, it is possible that the role of OGN in glucose regulation varies to some extent throughout the aging process. Previous studies have suggested OGN regulates energy homeostasis through alterations in glucose uptake and changes in insulin sensitivity, but we do not have the data to confirm or refute this hypothesis. Another complication that may explain the contrasting reports in the OGN literature is that it has been referred to by different names, and several different transcript and protein variants of OGN have been identified (32). It is therefore difficult to make conclusive claims about OGN or fully interpret the results with current data. This highlights the need for specifically designed studies to uncover the unique role of each OGN variant in different species.

Circulating OGN levels were associated with aerobic fitness (VO_{2peak}) in both sexes and a higher aerobic fitness was related to increased glucose. To our knowledge, no previous studies have reported a correlation between OGN and aerobic fitness, in either humans or animals. This link between OGN and aerobic fitness is intriguing and lends further credence to the important mediating role that the bone–muscle unit contributes to fitness. Similarly, osteocalcin is also associated with higher physical activity levels (33). The clinical relevance of the association between VO_{2peak} and glucose is not clear as all participants in this analysis had normal blood glucose levels. While our understanding of the role of these bone–muscle crosstalk–related hormones and proteoglycans is in its infancy, the association between OGN and aerobic fitness is an intriguing finding, and future studies should seek to further understand the mechanisms behind this association.

The study has some potential limitations. First, we have included data from several previous studies and, as such, there is a potential that samples were handled slightly differently; however, all samples were taken in the morning, following an overnight fasting. In addition, the relatively small sample size may affect some of the correlations and the findings. The current study focused on community-dwelling individuals and future studies should include diverse clinical populations, including individuals with cardiometabolic disorders, to clarify the role of OGN in conditions where there is glucose dysregulation. There is also the possibility that there are sex-specific differences in the levels of circulating OGN across the lifespan, though this study was not powered to explore this. It is further possible that sex- and aging-related hormonal fluctuations (eg, menopause) could contribute to increased OGN in the later years of life, but hormonal changes were not the focus of any of the substudies. Future studies should consider exploring these sex-specific differences in OGN.

While our understanding of OGN is in its infancy, emerging data are encouraging regarding its potential clinical utility as a health biomarker. While our data focused on its association with metabolic function and fitness throughout the lifespan, others have further suggested that OGN could be a biomarker of future cardiac dysfunction and kidney impairment (34, 35). The variations in OGN across the lifespan further illustrate its potential as a predictive biomarker for chronic disease, particularly later in life. Robust studies are needed to advance our understanding of OGN and its clinical relevance.

In conclusion, there is a "U shape" relationship between age and OGN. Those with a higher aerobic capacity or baseline glucose concentrations had higher OGN levels. Whether OGN plays a direct role in glucose regulation in humans is unclear and should be examined in future studies.

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Additional Information

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Data Availability: Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Lee NJ, Ali N, Zhang L, et al. Osteoglycin, a novel coordinator of bone and glucose homeostasis. *Mol Metab*. 2018;**13**:30-44.
2. Starup-Linde J, Viggers R, Handberg A. Osteoglycin and bone-a systematic review. *Curr Osteoporos Rep*. 2019;**17**(5):250-255.
3. Starup-Linde JK, Viggers R, Langdahl B, et al. Associations of circulating osteoglycin with bone parameters and metabolic markers in patients with diabetes. *Front Endocrinol (Lausanne)*. 2021;**12**:649718.
4. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part II: the aging heart in health: links to heart disease. *Circulation*. 2003;**107**(2):346-354.
5. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. *Circ Res*. 2012;**110**(8):1097-1108.
6. Smith C, Voisin S, Al Saedi A, et al. Osteocalcin and its forms across the lifespan in adult men. *Bone*. 2020;**130**:115085.
7. Aspenes ST, Nilsen TI, Skaug EA, et al. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci Sports Exerc*. 2011;**43**(8):1465-1473.
8. Solomon TP, Malin SK, Karstoft K, et al. Association between cardiorespiratory fitness and the determinants of glycemic control across the entire glucose tolerance continuum. *Diabetes Care*. 2015;**38**(5):921-929.
9. Pitsiladis YP, Tanaka M, Eynon N, et al. Athlome Project Consortium: a concerted effort to discover genomic and other "omic" markers of athletic performance. *Physiol. Genom*. 2015;**48**(3):183-190.
10. Eynon N, Voisin S, Lucia A, Wang G, Pitsiladis Y. Preface: genomics and biology of exercise is undergoing a paradigm shift. *BMC Genom*. 2017;**18**(Suppl 8):825.
11. Smith C, Lin X, Scott D, et al. Uncovering the bone-muscle interaction and its implications for the health and function of older adults (the Wellderly Project): protocol for a randomized controlled crossover trial. *JMIR Res Protoc*. 2021;**10**(4):e18777.
12. Levinger I, Jerums G, Stepto NK, et al. The effect of acute exercise on undercarboxylated osteocalcin and insulin sensitivity in obese men. *J Bone Miner Res*. 2014;**29**(12):2571-2576.
13. Parker L, Lin X, Garnham A, et al. Glucocorticoid-induced insulin resistance in men is associated with suppressed undercarboxylated osteocalcin. *J Bone Miner Res*. 2019;**34**(1):49-58.
14. Levinger I, Zebaze R, Jerums G, Hare DL, Selig S, Seeman E. The effect of acute exercise on undercarboxylated osteocalcin in obese men. *Osteoporos Int*. 2011;**22**(5):1621-1626.
15. Levinger I, Seeman E, Jerums G, et al. Glucose-loading reduces bone remodeling in women and osteoblast function in vitro. *Physiol Rep*. 2016;**4**(3):1-10.
16. Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord*. 2013;**13**:47.
17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;**28**(7):412-419.
18. Yan X, Eynon N, Papadimitriou ID, et al. The gene SMART study: method, study design, and preliminary findings. *BMC Genom*. 2017;**18**(Suppl 8):821.
19. Team. RCD. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2011.
20. Hill K. The demography of menopause. *Maturitas*. 1996;**23**(2):113-127.
21. Papierska L. Adrenopause—does it really exist? *Prz Menopauzalny*. 2017;**16**(2):57-60.
22. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;**67**(1):1-48.
23. Kuznetsova A, Brockhoff PB, Christensen RHB. lmerTest package: tests in linear mixed effects models. *J Stat Softw*. 2017;**82**(13):26.
24. Wickham H, Averick M, Bryan J, et al. Welcome to the tidyverse. *J Open Source Softw*. 2019;**4**(43):1686.
25. Venables WN, Ripley RB. *Modern Applied Statistics with S*. Springer; 2002.
26. Dowle M, Srinivasan A. data.table: Extension of 'data.frame'. R package version 1.14.0. 2021. Accessed. November 9, 2021 https://CRAN.R-project.org/package=data.table
27. Zeileis A, Kleiber C, Jackman S. Regression models for count data in R. *J Stat Softw*. 2008;**27**(8):1-25.
28. Jenkins N, Black M, Paul E, Pasco JA, Kotowicz MA, Schneider HG. Age-related reference intervals for bone turnover markers from an Australian reference population. *Bone*. 2013;**55**(2):271-276.
29. Dušková M, Kolátorová L, Jandíková H, Pospíšilová H, Stárka L. Are there sex differences in the reaction of undercarboxylated osteocalcin to hypoglycemia? *Physiol Res*. 2020;**69**(Suppl 2):S315-S320.
30. Deckx S, Heggermont W, Carai P, et al. Osteoglycin prevents the development of age-related diastolic dysfunction during pressure overload by reducing cardiac fibrosis and inflammation. *Matrix Biol*. 2018;**66**:110-124.
31. Cheng JM, Akkerhuis KM, Meilhac O, et al. Circulating osteoglycin and NGAL/MMP9 complex concentrations predict 1-year major adverse cardiovascular events after coronary angiography. *Arterioscler Thromb Vasc Biol*. 2014;**34**(5):1078-1084.

32. Deckx S, Heymans S, Papageorgiou AP. The diverse functions of osteoglycin: a deceitful dwarf, or a master regulator of disease? *FASEB J*. 2016;**30**(8):2651-2661.
33. Chahla SE, Frohnert BI, Thomas W, Kelly AS, Nathan BM, Polgreen LE. Higher daily physical activity is associated with higher osteocalcin levels in adolescents. *Prev Med Rep*. 2015;**2**:568-571.
34. Van Aelst LN, Voss S, Carai P, et al. Osteoglycin prevents cardiac dilatation and dysfunction after myocardial infarction through infarct collagen strengthening. *Circ Res*. 2015;**116**(3):425-436.
35. González-Salvatierra S, García-Fontana C, Andújar-Vera F, et al. Osteoglycin as a potential biomarker of mild kidney function impairment in type 2 diabetes patients. *J Clin. Med*. 2021;**10**(10):2209.