Circulating visfatin level is correlated with inflammation, but not with insulin resistance

Short title: visfatin is an inflammation marker

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Summary

Objective

Recent studies, both in vitro and in vivo, have indicated that visfatin is one of the inflammatory cytokines, although the relationship between visfatin and insulin resistance remains inconclusive. Accordingly, we assessed the association between visfatin concentrations in serum and those of interleukin-6 (IL-6) and C-reactive protein (CRP) known as markers of systemic inflammation, and also investigated the relationship between these serum concentrations and insulin resistance.

Research design and method

Two-hundred ninety-five Japanese Americans living in Hawaii (126 men and 169 women, mean age, 68.7 ± 14.9 years) were enrolled. The serum levels of visfatin, IL-6 and CRP levels were measured, and HOMA-IR was calculated as a marker of insulin resistance.

Results

Significant positive correlations were found between serum levels of visfatin and IL-6 or CRP (r=0.271, $P<0.001$; r=0.118, $P<0.05$, respectively). Multiple regression analysis revealed that correlations between serum levels of visfatin and IL-6 or CRP remained significant after adjustments for the age, sex, body mass index, percent body fat, and waist girth. There was no significant trend of the HOMA-IR for the tertiles of serum concentrations of visfatin. On the other hand, a significant trend towards increase of HOMA-IR with increasing tertile of serum concentrations, from the lowest to the highest, was observed for both IL-6 and CRP. The HOMA-IR

in subjects with serum concentration of IL-6 or CRP in the highest or intermediate tertiles of IL-6 or CRP were significantly higher than that in the subjects in the lowest tertile, even after adjustment for age and sex (IL-6, $P<0.001$ and $P<0.001$, respectively; CRP, $P<0.001$ and $P<0.01$, respectively).

Conclusion

Serum visfatin levels were positively correlated with the serum levels of IL-6 and CRP, but not with HOMA-IR, in Japanese Americans. Our results indicate that circulating visfatin may reflect inflammation status.

Introduction

Visfatin, which was recently identified as a new adipocytokine,[1] has been recognized as a pre-B-cell colony enhancing factor (PBEF), which acts synergistically with interleukin-7 and stem cell factors to promote the growth B cell precursors.[2] It has been reported that the production of interleukin-6 (IL-6) in human monocytes is induced by visfatin via the p38 mitogen-activated protein kinase (MAPK) and MAPK kinase 1 (MEK1) pathways.[3] In addition, visfatin has been shown to activate nuclear factor-kappaB (NF-κB), which plays an important role in triggering and coordinating immune responses in mice.[3] Taken together, the results from both in vitro and in vivo studies suggest that visfatin activates IL-6, although there have been no reports until date on the association between serum levels of visfatin and IL-6 in humans. Furthermore, CRP, which is synthesized in the liver, is known to be mainly stimulated by IL-6.[4] It is speculated that circulating visfatin may promote the production of not only IL-6, but also CRP, however the relationship between serum visfatin and serum CRP levels in humans are also remains unclear.

Chronic inflammation has been postulated to play a role in the pathogenesis of insulin resistance.[5,6] Clinical studies have shown a relationship between serum concentrations of various markers of inflammation, such as CRP and IL-6, and the risk of development of insulin resistance.[7,8] It has been suggested that visfatin may also be involved in the development of insulin resistance, because, it also appears to be a marker of inflammation, as stated above, and it is expressed predominantly in the visceral adipose tissue and exhibits insulinomimetic effects.[1]

We hypothesized that serum visfatin concentrations may be associated with serum levels of IL-6 and CRP, which are known as reliable biomarkers of inflammation, and that visfatin may also be a marker of insulin resistance. Thus, in the present study, we investigated; 1) the presence/absence of correlations between serum visfatin and IL-6 or CRP levels, 2) the relationship of visfatin, as well as IL-6 and CRP, to insulin resistance, as assessed by homeostasis model assessment for insulin resistance (HOMA-IR) or not.

The subjects in the Japanese American population examined in this study, have been reported to be at a higher risk of developing obesity and diabetes than the Japanese living in Japan, plausibly because of their more westernized lifestyle.[9] Therefore, we opted to enroll subjects from this population as the most appropriate, to determine the associations between markers of inflammation and insulin resistance.

Method

Study population

The study subjects were Japanese Americans enrolled in a medical survey in 2002. The survey has been described in detail elsewhere.[9] In brief, the survey represented a long-term epidemiological study of the risk factors for diabetes, hypertension and atherosclerotic diseases. The subjects of this study were limited to a population living in Hawaii who were genetically identical to the Japanese. Subjects with symptoms of infection(s), autoimmune diseases, or any underlying acute conditions as assessed by a medical interview were excluded. The study participants comprised 126 males and 169 females (mean age, 68.7 ± 14.9 years). Written informed consent was obtained from all the subjects. This study was approved by the Ethics Committee of Hiroshima University and the Council of the Hiroshima Kenjin-Kai Association in Hawaii.

Measurements

The height, body weight, and waist girth of the subjects were measured by the standard methods, and the body mass index (BMI) was calculated. Percent body fat (%fat) was assessed by measuring the bioelectric impedance from foot to foot.[10] Venous blood samples were collected on the morning of the day of the study after the patients had fasted overnight. The blood samples were centrifuged, and the separated serum samples were immediately frozen at -80°C. The frozen samples were brought back to Japan and analyzed in Japan. The serum concentration of insulin was

measured by a double-antibody radioimmunoassay. HOMA-IR was calculated according to the following formula: fasting glucose (mg/dl) × fasting insulin (mU/l) / 405.[11] The serum visfatin level was measured with an enzyme immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). Serum IL-6 was determined by a chemiluminescent enzyme assay (Fujirebio Inc, Tokyo, Japan). CRP was measured by an enzymatic method using latex-enhanced immunonephelometric assay on a BNII analyzer (Dade Behring, Tokyo, Japan).

Statistical analysis

Quantitative data were presented as means ± SD for continuous normally distributed variables. During analysis, the values for HOMA-IR, visfatin, IL-6, and CRP were converted into their log values and expressed in medians (interquartile range), because the distribution of the data were skewed. Analyses were performed using the SPSS for Windows (release 12.0; SPSS Inc., Chicago, IL). *P* values < 0.05 were considered to be significant.

First, analyses of covariance (ANCOVA) were used to compare serum visfatin, IL-6, and CRP levels between men and women after adjustment for age. Second, we performed a simple regression analysis to determine the association between serum visfatin and IL-6 or CRP levels in entire subject population. Third, the associations between serum concentrations of IL-6 or CRP as a dependent variable, and that of visfatin as the independent variable were investigated by multiple regression analyses with age, sex, BMI, %fat, and waist girth as covariates.

We examined the association between serum concentrations of visfatin, IL-6, and CRP, and HOMA-IR. Thirty-two subjects with a history of diabetes, receiving treatment for diabetes, or serum fasting glucose levels higher than 160 mg/dl were excluded to assess HOMA-IR.[11] In the analysis of the serum CRP, nine subjects with CRP levels >10 mg/l were excluded from the analyses, because this level indicates the possibility of acute inflammatory disease.[12] Finally, ANCOVA was used to determine the relations between HOMA-IR and the tertiles of the serum visfatin, IL-6 and CRP concentrations. If they were found to be statistically significant, the Bonnferoni analysis was used to assess the relationship between the tertiles. The test for trend was perfomed with a polynominal contrast procedure.

Results

Subject characteristics

The serum levels of visfatin, IL-6, and CRP were 86.3 (63.4 - 125.7) ng/ml, 1.5 (1.1 - 2.3) pg/ml, and 0.85 (0.37 - 1.68) mg/l, respectively. There were no gender-related differences in serum concentrations of visfatin (82.4 [60.8 - 115.5] *vs*. 89.9 [65.4 - 131.8] ng/ml) or CRP (0.82 [0.32 - 1.46] *vs*. 0.89 [0.42 - 1.97] mg/l) after adjustment for age, whereas men had higher serum IL-6 levels than women (1.5 [1.1 - 2.5] *vs*. 1.4 [1.1 - 2.2] pg/ml, $P<0.05$) after adjustment for age.

Relationships between the serum visfatin and serum IL-6 or CRP levels

The association between serum visfatin levels and serum IL-6 or CRP levels as determined by simple regression analysis is shown in Figure 1. A significantly positive correlation was found between serum concentrations of visfatin and IL-6 ($r=0.271$, $P<0.001$) or CRP ($r=0.118$, $P<0.05$). The significant relationship between serum visfatin and IL-6 concentrations was still maintained after the subjects were divided into men and women ($r=0.303$, $P<0.001$ and $r=0.265$, $P<0.001$, respectively). In regard to the relationship between serum concentrations of visfatin and CRP, a significant relationship between the two was found in men ($r=0.232$, $P<0.01$), but not in women ($r=0.013$, $P<0.873$). The results of multiple regression analysis taking age, sex, and BMI, %fat, or waist girth as covariates, revealed that serum visfatin concentrations were correlated with the serum IL-6 and CRP cocentrations. Furthermore, there were no significant correlations between serum

visfatin levels and the BMI, %fat, or waist girth after adjustment for age and sex.

Relationship between HOMA-IR and the tertiles of the serum visfatin, IL-6, or CRP levels

Subjects were divided into tertiles based on the serum visfatin, IL-6, or CRP levels. We compared HOMA-IR among the tertiles for visfatin, IL-6, or CRP by ANCOVA and a polynominal contrast procedure adjusted for age and sex. The HOMA-IR for increasing tertiles of visfatin was 1.78 [1.19 - 2.58], 1.65 [0.92 - 2.91], and 1.77 [0.98 - 2.92], revealing the absence of any significant difference or trend of the HOMA-IR among the terteiles (Fig. 2A). For IL-6, the HOMA-IR with increasing tertile of IL-6 was 1.38 [0.76 - 1.97], 1.79 [1.14 - 2.67], and 1.96 [1.08 - 3.46] (P for trend <0.001) (Fig. 2B). The value in the higher or intermediate tertile was significantly higher than that in the lowest tertile (P<0.001 and P<0.001, respectively) (Fig. 2B). The associations remained significant even after adjustment for BMI, %fat, or waist girth (data not shown). For the case of CRP, the HOMA-IR for increasing tertiles of CRP was 1.16 [0.76 - 1.78], 1.74 [0.93 - 2.32], and 1.84 [1.19 - 3.02] (P for trend <0.001) (Fig. 2C). The HOMA-IR values in the highest or intermediate tertile were significantly higher than that in the lowest tertile (P <0.001 and P <0.01, respectively) (Fig. 2C). The association remained significant, even after adjustment for %fat or waist girth, but not BMI (data not shown).

Discussion

In this study, we clarified that serum visfatin levels were positively correlated with serum IL-6 and serum CRP levels. Although serum levels of IL-6 and CRP have been reported to be correlated with HOMA-IR, no such correlation was observed between the serum visfatin concentration and the HOMA-IR. These results suggest that while visfatin may be an important inflammatory cytokine, it may not affect the insulin resistance.

Our results corroborate previous evidence suggesting the existence of positive correlations between serum visfatin levels and serum levels of IL-6 or CRP in humans. These results lend support to be recently reported results of experimental investigations in which visfatin has been shown to induce the production of IL-6 in monocytes,[3] increase circulating IL-6 in mice,[3] and stimulate the production of CRP in the liver.[4] On the other hand, IL-6 treatment of an amniotic epithelial cell line induced the visfatin/PBEF gene expression.[13] Thus, visfatin and IL-6 might have an interactive effect on each other. In our study, it appeared that the relationship between visfatin and IL-6 was stronger than that between visfatin and CRP. Therefore, it is considered that visfatin may induce IL-6, which, in turn, might induce CRP.

In our study, the circulating levels of visfatin did not reflect the insulin resistance levels as assessed by HOMA-IR. Berndt, *et al*. reported that the expression levels of visfatin mRNA in the visceral and subcutaneous fat were not correlated with glucose metabolism.[14] Two studies, one each reported by Smith, *et al*. and Varma, *et al*., have suggested that the serum visfatin levels are not

associated with parameters of body composition or the insulin resistance.[15,16] Taken together, it would appear that there is no relation between visfatin and insulin resistance. However, one study did report the existence of a correlation between the delta circulating levels of visfatin and the delata HOMA-IR in patients who performed gastric banding.[17] Therefore, further investigations are necessary to clarify the role of visfatin in glucose metabolism, including in the development of insulin resistance.

Our results indicating that the elevated serum concentrations of IL-6 or CRP might be markers of insulin resistance are consistent with previous reports.[7,8] The precise mechanisms involved in the interactions of the inflammatory cytokines with the glucose metabolic processes in humans are still unclear. Some possibilities have been suggested by the results of experimental studies, e.g., IL-6 downregulated PPAR-γ expression,[18] IL-6 affected insulin signaling in adipocytes, hepatocytes, and skeletal muscle.[19-21] Moreover, it has been speculated that while visfatin itself may not increase the insulin resistance, the effect of IL-6 or CRP on insulin resistance may be involved in the actions of visfatin.

Although visfatin also appears to be a marker of inflammation like IL-6 or CRP, visfatin was not related to insulin resistance, unlike IL-6 and CRP. Visfatin was originally identified as a pre-B-cell colony enhancing factor (PBEF).[2] Expression of visfatin/PBEF has been shown to be upregulated in activated neutrophils and to inhibit the apoptosis of neutrophils.[22] Furthermore, the concentrations of visfatin/PBEF have been shown to be increased in the bronchoalveolar lavage

fluid in animal models of acute lung injury and in patients with inflammatory bowel disease.[4,23] Interestingly, the presence of specific single nucleotide polymorphisms in the visfatin/PBEF gene, which decrease the gene transcription rate, significantly increased the risk of development of acute lung injury in septic patients.[4] Taken together, inflammatory cells, including neutrophils, macrophages and monocytes may be the major sources of visfatin/PBEF, which might explain its involvement in several acute inflammatory diseases, but not insulin resistance or obesity.

In summary, while visfatin is associated with IL-6 and CRP, it dose not appear to be related to insulin resistance in humans. The serum visfatin concentration may reflect inflammation status.

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Figure Legends

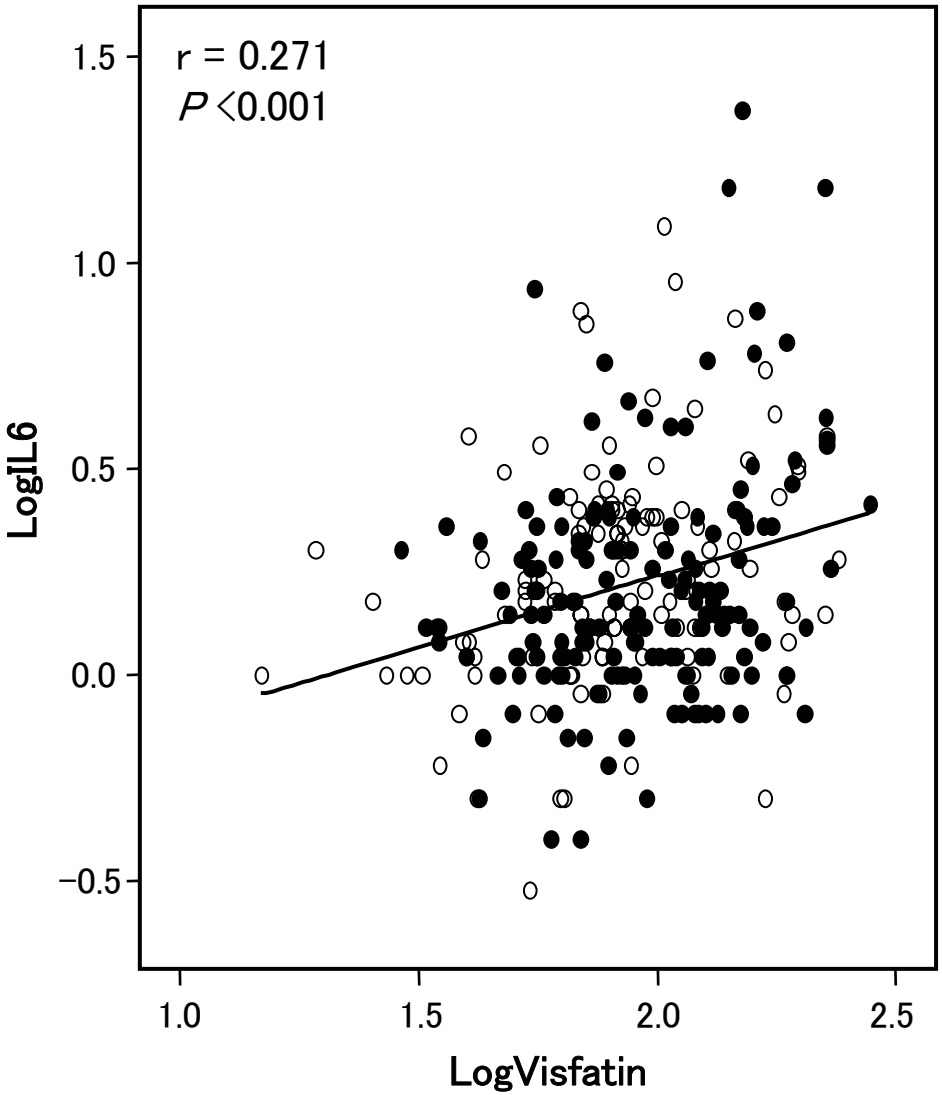
Figure 1.

Simple regression analyses to determine the relation between the serum concentrations of visfatin with those of (A) IL-6 and (B) CRP. Men and women were indicated by open circles and closed circles, respectively. The serum concentrations of visfatin, IL-6, and CRP were converted to their log values.

Figure 2

Relationship between HOMA-IR and the tertile of the serum concentrations of (A) visfatin, (B) IL-6, and (C) CRP, adjusted for age and sex.

Figure 1


r = 0.271
P <0.001
LogIL6
LogVisfatin
1.5
1.0
0.5
0.0
−0.5
1.0
1.5
2.0
2.5

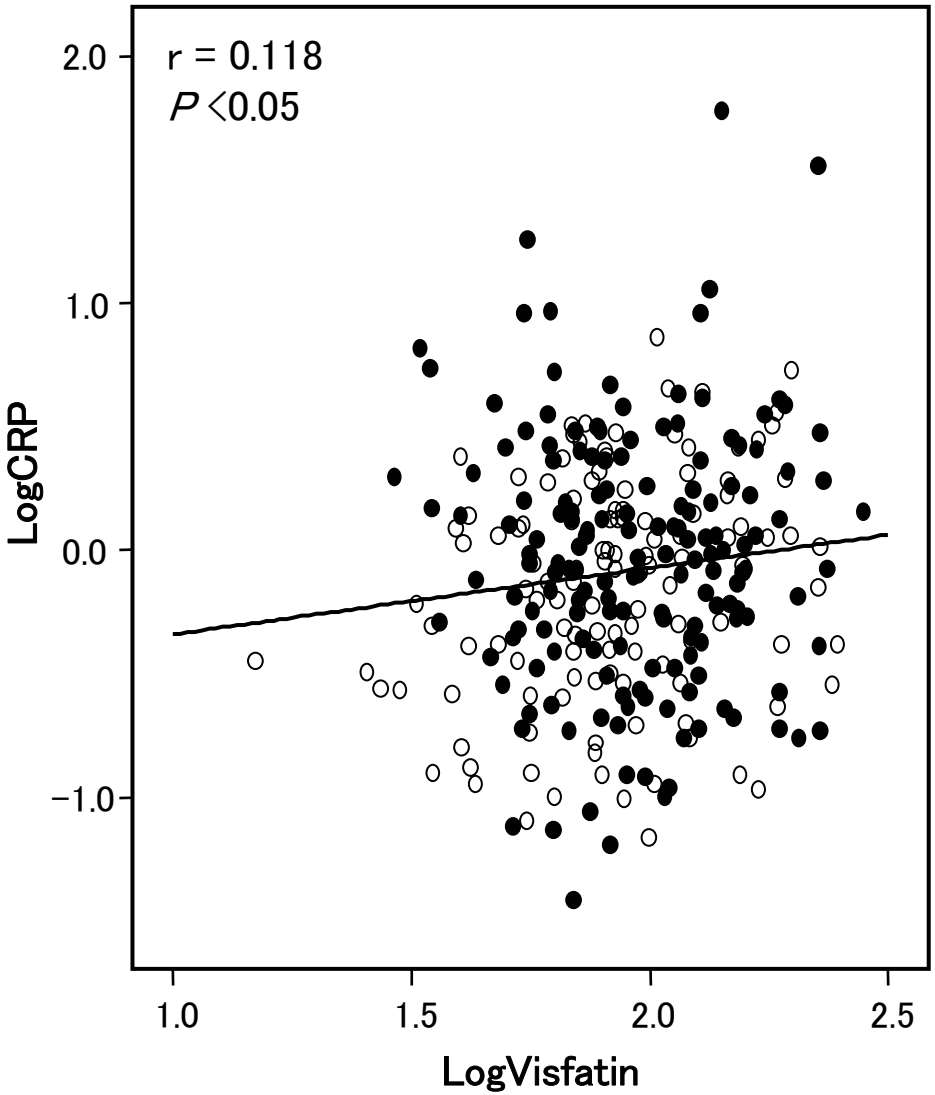
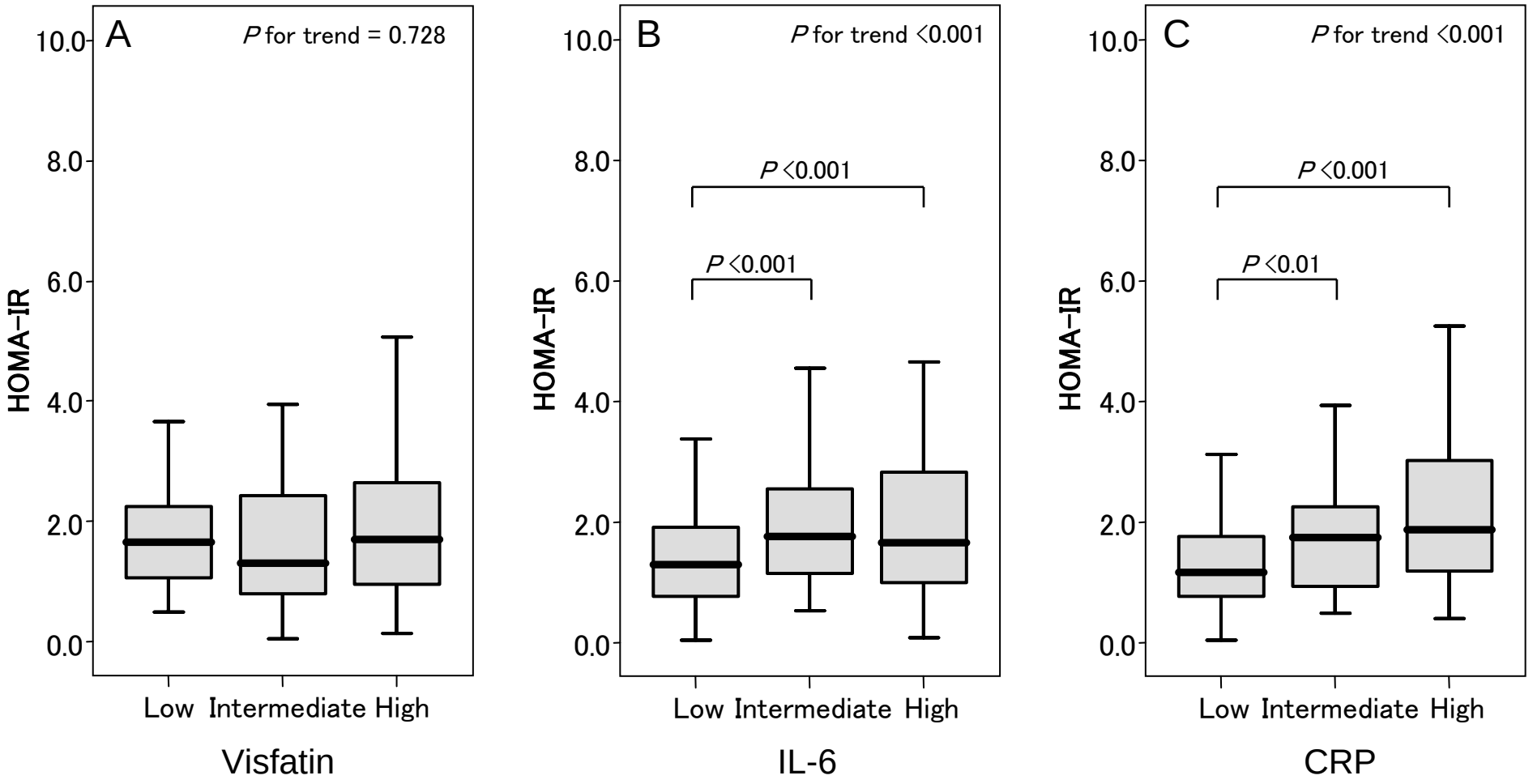

r = 0.118
P <0.05
LogCRP
LogVisfatin
2.0
1.0
0.0
−1.0
1.0
1.5
2.0
2.5

Figure 2


A
P for trend = 0.728
HOMA-IR
10.0
8.0
6.0
4.0
2.0
0.0
Low Intermediate High
Visfatin
B
P for trend <0.001
P <0.001
P <0.001
HOMA-IR
10.0
8.0
6.0
4.0
2.0
0.0
Low Intermediate High
IL-6
C
P for trend <0.001
P <0.001
P <0.01
HOMA-IR
10.0
8.0
6.0
4.0
2.0
0.0
Low Intermediate High
CRP