Journal of Molecular Medicine (2026) 104:21
https://doi.org/10.1007/s00109-025-02632-y

JMolMed

ORIGINAL ARTICLE

Inverse correlation between plasma pyrophosphate hydrolysis and early growth of abdominal aortic aneurysm: a pilot study

Ricardo Villa-Bellosta[1,2,3] · Jes S. Lindholt[4] · Jose Luis Martin-Ventura[5,6]

Received: 8 May 2025 / Revised: 25 November 2025 / Accepted: 13 December 2025

Abstract

Abdominal aortic aneurysm (AAA) is often associated with vascular calcification, the calcium-phosphate crystal deposition in the aortic wall, which could impact aneurysm progression and/or stability. Tissue-nonspecific alkaline phosphatase (TNAP) hydrolyzes pyrophosphate—a natural inhibitor of calcification—thereby promoting calcium deposition. This study analyzes phosphatase activity and pyrophosphate dynamics in the plasma of AAA patients, randomly selected from the whole VIVA cohort, including controls ($n=48$, aortic diameter in <30 mm) and small AAA patients ($n=96$, aortic diameter 30–55 mm), which were stratified by growth rate (low <2 mm/year or high >2 mm/year, $n=48$ in each group), initially adjusted by aortic diameter. The phosphatase activity was assessed at physiological (7.4) and optimal (10.5) pH levels. Phosphatase activity was significantly higher in AAA patients at physiological pH, a pattern absent at optimal pH, suggesting disease-specific alterations. Small AAA patients showed notably higher phosphatase activity and pyrophosphate hydrolysis than controls at physiological pH. An inverse correlation was also observed between phosphatase activity and aortic growth rate.

Key messages

- Increased alkaline phosphatase activity was detected in heparin-plasma samples from patients with abdominal aortic aneurysm at physiological pH (7.4), but not under optimal experimental conditions (pH 10.5), suggesting a disease-related effect that may reflect processes occurring in vivo.
- Concentrations of tissue-nonspecific alkaline phosphatase in heparin-plasma samples remained unchanged, despite increased hydrolysis of pyrophosphate, a key inhibitor of vascular calcification.
- Pyrophosphate hydrolysis in heparin-plasma samples correlated inversely with aortic growth rate in abdominal aortic aneurysm patients.

Keywords Abdominal aortic aneurysm · Alkaline phosphatase · Vascular calcification · Pyrophosphate

Introduction

Abdominal aortic aneurysm (AAA) is a chronic dilatation of the aorta (>30 mm) in its infrarenal portion, accounting for 80–90% of all types of aneurysms [1]. Among cardiovascular risk factors, advanced age (over 65 years), male sex (75%), smoking (85% past/present smokers), and increased body mass index (BMI) have been associated with AAA [2]. Rupture of AAA has a mortality rate of approximately 80% [3]. The sole treatment capable of preventing rupture is surgical or endovascular repair. In the case of AAA, this is indicated if the diameter exceeds 55 mm. Thus, for those patients with an aortic diameter between 30 and 55 mm, surveillance by imaging techniques to check for aortic diameter expansion is the only guide for clinicians. However, the progression of AAA towards rupture is non-linear, with alternating periods of acceleration and stability [4].

The pathophysiology of human AAA is a complex interplay of circulating and resident cells with systemic molecules. Proteolysis of elastin and other extracellular matrix (ECM) proteins has been proposed as a main driver of aortic wall dilatation. Another pathogenic mechanism potentially involved in AAA includes immune-inflammatory responses, angiogenesis, and fibrosis [5]. More recently, the vascular smooth muscle cells (VSMCs) phenotypic switch has gained interest as a mechanism involved in AAA progression [6]. Under pathological

Extended author information available on the last page of the article

Published online: 04 January 2026

conditions, contractile VSMCs can dedifferentiate into different phenotypes, including synthetic, inflammatory, and/or osteo/chondrogenic. Phenotypic switching of contractile VSMCs into osteo/chondrogenic VSMCs is accompanied by expression of bone-specific proteins that regulate ECM mineralization, such as RUNX2 (Runt-related transcription factor 2) [7–10]. Interestingly, the levels of RUNX2 are increased in human AAA [11]. More recently, the presence of (micro)calcification in human AAA tissues was described [12]. Localized calcification within the aneurysmal wall can create a stiffened, hardened layer, potentially reinforcing the vessel and slowing AAA progression. This reinforcement may act as a biological "scaffold," helping to bear some of the mechanical load and mitigate further dilation. However, excessive or widespread calcification can reduce the vessel's natural elasticity, making the aorta more brittle and less capable of withstanding pulsatile blood flow. This reduced flexibility can increase the likelihood of rupture, particularly in areas where calcification may coexist with underlying weak spots in the arterial wall [13]. In this respect, previous studies described increased calcification in symptomatic and ruptured AAA [14], as well as a positive association with AAA progression [15]. In contrast, other studies reported negative correlations with AAA expansion and rupture [16–19]. By comparison, in occlusive arterial disease, heavy calcification is often present without vessel dilation, highlighting a different pathophysiological context compared with AAA. This distinction underscores that calcification may exert divergent effects depending on the vascular disease setting. However, potential mediators involved in the calcification process of AAA patients are unknown.

Key biochemical players in the calcification process are tissue-nonspecific alkaline phosphatase (TNAP) and pyrophosphate, a natural inhibitor of calcification [20–23]. TNAP hydrolyzes pyrophosphate, breaking it down and thereby reducing its availability [20, 24]. With less pyrophosphate present to inhibit mineralization, calcium deposition within the vessel wall is promoted, facilitating calcification [25–27]. TNAP activity thus serves as a regulatory factor in vascular calcification, with elevated TNAP activity enhancing the calcification process [23, 27]. Since human AAA tissues can be only obtained after surgery, which is performed in patients with aortic diameter > 55 mm, we focus on systemic mediators, which could shed some light on the positive or negative impact of vascular calcification during AAA progression. Specifically, this study investigates the role of phosphatase activity, specifically TNAP, in the progression of AAA. By analyzing TNAP activity under physiological conditions, we aim to gain insight into the mechanisms by which TNAP and pyrophosphate interact to influence calcification within the aneurysmal wall.

Materials and methods

Human plasmas

This study examined heparin-plasma samples randomly selected from the whole VIVA cohort [28], including controls ($n = 48$, aortic diameter < 30 mm). Small AAA patients ($n = 96$, aortic diameter 30–55 mm) were stratified according to aneurysm growth rate (low, < 2 mm/year; high, > 2 mm/year; $n = 48$ each group), with initial adjustment for baseline aortic diameter. A growth rate cut-off of 2 mm/year was used to differentiate stable from expanding AAAs, in line with previous studies reporting this value as a clinically relevant marker of progression [29, 30]. The control group represented a random subsample of a larger pool of eligible individuals from the VIVA cohort. Clinical characteristics are shown in Table 1. In brief, control subjects had a mean age of 69 ± 2.6 years, a BMI of 26.9 ± 4.2 kg/m^2, and a mean systolic blood pressure of 145.8 ± 15.3 mmHg. Regarding medication, 30.4% were on statins and 24% used aspirin. Trained project nurses performed ultrasound scans of the aorta and collected plasma samples at baseline, which were stored at –80 °C and subsequently thawed only once immediately prior to analysis. All subjects provided informed consent, and the study was approved by the local ethics committee of the Viborg Hospital (M20080025), registered at ClinicalTrials.gov (NCT00662480), and performed in accordance with the Helsinki Declaration.

Table 1 Clinical characteristics of control individuals and AAA patients ($n = 48$ per group). Asterisks indicate significance levels relative to the control group: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. The symbols "##" denote significance ($P < 0.01$) comparing the high vs. the low group (HG vs. LG) in AAA patients

	Control	Small AAA Low growth	Small AAA High growth
Continuous variables			
Age (years)	69.4 ± 2.6	70.2 ± 2.8	69.6 ± 2.6
BMI (kg/m^2)	26.9 ± 4.2	27.9 ± 3.4	26.8 ± 3.4
SBP (mm Hg)	145.8 ± 15.3	157.3 ± 21.4*	157.3 ± 18.2*
DBP (mm Hg)	79.7 ± 8.6	86.3 + 10.3*	87.5 ± 10.8*
Cholesterol (mM)	4.9 ± 1.3	4.7 ± 0.9	4.8 ± 1.0
Aortic diameter (mm)	18.1 ± 2.3	**32.7 ± 2.2***	**32.6 ± 3.5***
Growth (mm/year)	-	**0.9 ± 0.4**	**3.8 ± 1.3**$^{\#\#}$
Categorical variables			
Smoking (%)	17.4	29.8	57.7$^{\#\#}$
Statins (%)	30.4	58.7	38.7$^{\#\#}$
Previous CVD	13	17	20
Aspirin	24	52	34

Phosphatase activity and inhibition assay

Alkaline phosphatase (ALP) activity was assessed using para-nitrophenyl phosphate (pNPP; N4645, Sigma-Aldrich) as the substrate. In this assay, 50 µL of human plasma was incubated with 150 µL of Tris-HCl buffer (adjusted to the specified pH) containing 10 mmol/L pNPP for 12 h. The absorbance of each sample was measured at 405 nm at 30-min intervals (37 °C). Activity and slope values were calculated through linear regression using GraphPad Prism software. To evaluate the inhibitory effect of SBI425 (SML2935, Sigma-Aldrich) on TNAP activity, 50 µL of plasma was incubated with Tris-HCl buffer containing 10 mmol/L pNPP and various concentrations of SBI425. Fold change was determined by normalizing each individual measurement to the mean value of the 48 controls, thus setting the average control activity to 1 while retaining the variability of the data.

Quantitative determination of alkaline phosphatase (ALP) activity

ALP activity was quantitatively measured using a stable p-nitrophenol phosphate substrate with the QuantiChrom™ Alkaline Phosphatase Assay Kit (DALP-250, Bioassay Systems, Hayward, CA, USA).

Quantitative determination of TNAP levels

Plasma TNAP levels were quantified using a specific ELISA kit (SEB091Hu, Wuhan USCN Business Co., Ltd., Houston, TX) according to the manufacturer's instructions and procedures from previous studies [27].

Pyrophosphate hydrolysis in plasma samples

Pyrophosphate hydrolysis was assessed by incubating plasma samples (15 µL) in 45 µL of Tris-HCl buffer (adjusted to the specified pH) containing 5 µmol/L pyrophosphate (S6422, Sigma-Aldrich, St. Louis, MO) and 10 µCi/mL [^{32}P]pyrophosphate (Perkin Elmer, Boston, MA). After 4 h, orthophosphate was separated from pyrophosphate as previously described [9, 25, 27]. Briefly, 20 µL of the sample was mixed with 400 µL of ammonium molybdate solution (to bind orthophosphate; 09913, Sigma-Aldrich) and 0.75 mol/L sulfuric acid (258,105, Sigma-Aldrich). The mixture was then extracted with 800 µL of isobutanol/petroleum ether (4:1) to isolate phosphomolybdate from unreacted pyrophosphate (Sigma-Aldrich; 77,379 for petroleum ether and 360,465 for isobutanol). A 400 µL aliquot of the organic phase containing phosphomolybdate was transferred to liquid scintillation fluid (UltimaGold™, 6013329; Perkin Elmer), and radioactivity was measured using a Tri-Carb 2810TR liquid scintillation analyzer (Perkin Elmer).

Cell culture of human aortic smooth muscle cells

Human aortic smooth muscle cells (ASMCs, Lonza, Walkersville, USA) were cultured in SmGM-2 medium (Lonza) following the manufacturer's protocol. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. To sustain culture growth, a 1:3 split ratio was applied during each trypsinization step.

Calcification assays in human aortic smooth muscle cells (ASMCs)

Human ASMCs were cultured to confluence and then subjected to a quiescence step by overnight incubation in medium containing 0.1% fetal bovine serum (FBS). For calcification assays, the cells were incubated for 5 days in minimum essential medium (MEM) supplemented with 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, 0.1% FBS, and 10 µCi/mL calcium-45 (Perkin Elmer, Boston, MA) as a radiotracer. MEM was supplemented with the specified calcium concentration and recombinant human alkaline phosphatase/ALPL protein, CF (2909-AP-010; R&D Systems) to create a controlled environment for the calcification assay.

To quantify the calcium content in ASMCs, the wells were washed five times with 9 g/L NaCl and treated with 200 µL of 0.6 M HCl and incubating overnight at 4 °C to release calcium. Then, 50 µL of the HCl solution containing calcium-45 was transferred to liquid scintillation fluid (UltimaGold™, 6013329; Perkin Elmer), and radioactivity was measured using a Tri-Carb 2810TR liquid scintillation analyzer (Perkin Elmer).

Statistical analysis

The Shapiro-Wilk test was used to assess data normality. Depending on the distribution assumptions, statistical analyses were performed using either the student's *t*-test or one-way ANOVA followed by Tukey's multiple comparison post hoc test for normally distributed data. For non-normally distributed data, the Mann-Whitney test or Kruskal-Wallis test with Dunn's post hoc test was applied. All statistical analyses were conducted using GraphPad Prism software, and the significance was determined accordingly.

Differences between AAA patients and controls or between AAA patients with low and high growth rate were assessed by the chi-square test (categorical variables) and the Kruskal-Wallis test with Dunn's post hoc test (continuous variables) to identify potential confounders. Associations

with a probability below 0.10 were considered to be potential confounders and used in the multivariate analyses. The association between pyrophosphate hydrolysis and AAA growth was studied by Pearson correlation analysis. Multivariate linear regression analysis was performed to assess the association between pyrophosphate hydrolysis and AAA growth, adjusting for the potential identified confounders.

Results

Phosphatase activity is significantly elevated in the plasma of patients with AAA

To evaluate enzyme function in human plasma at different hydrogen ion concentrations (pHs) reflecting physiological and pathological states, we sought to elucidate phosphatase activity in human plasma over a physiological range of pHs. As shown in Fig. 1A, the phosphatase activity gradually increased at successive pH levels. Although direct measurements of pH at the aneurysm site are scarce, ischemic or inflammatory microenvironments could theoretically lead to localized alterations in hydrogen ion concentration. Importantly, Fig. 1A shows that below pH 7.5, phosphatase activity does not differ, supporting the use of pH 7.4 as a physiologically relevant condition for our analyses.

Clinically, alkaline phosphatase is typically assessed at a highly alkaline pH of 10.5, which maximizes its catalytic activity. However, human plasma is maintained at a physiological pH of approximately 7.4. To better understand phosphatase activity under both optimal and physiological conditions, we measured phosphate activity at an alkaline pH of 10.5 (optimal conditions) and at a physiological pH of 7.4. At a physiological pH of 7.4, AAA patients presented

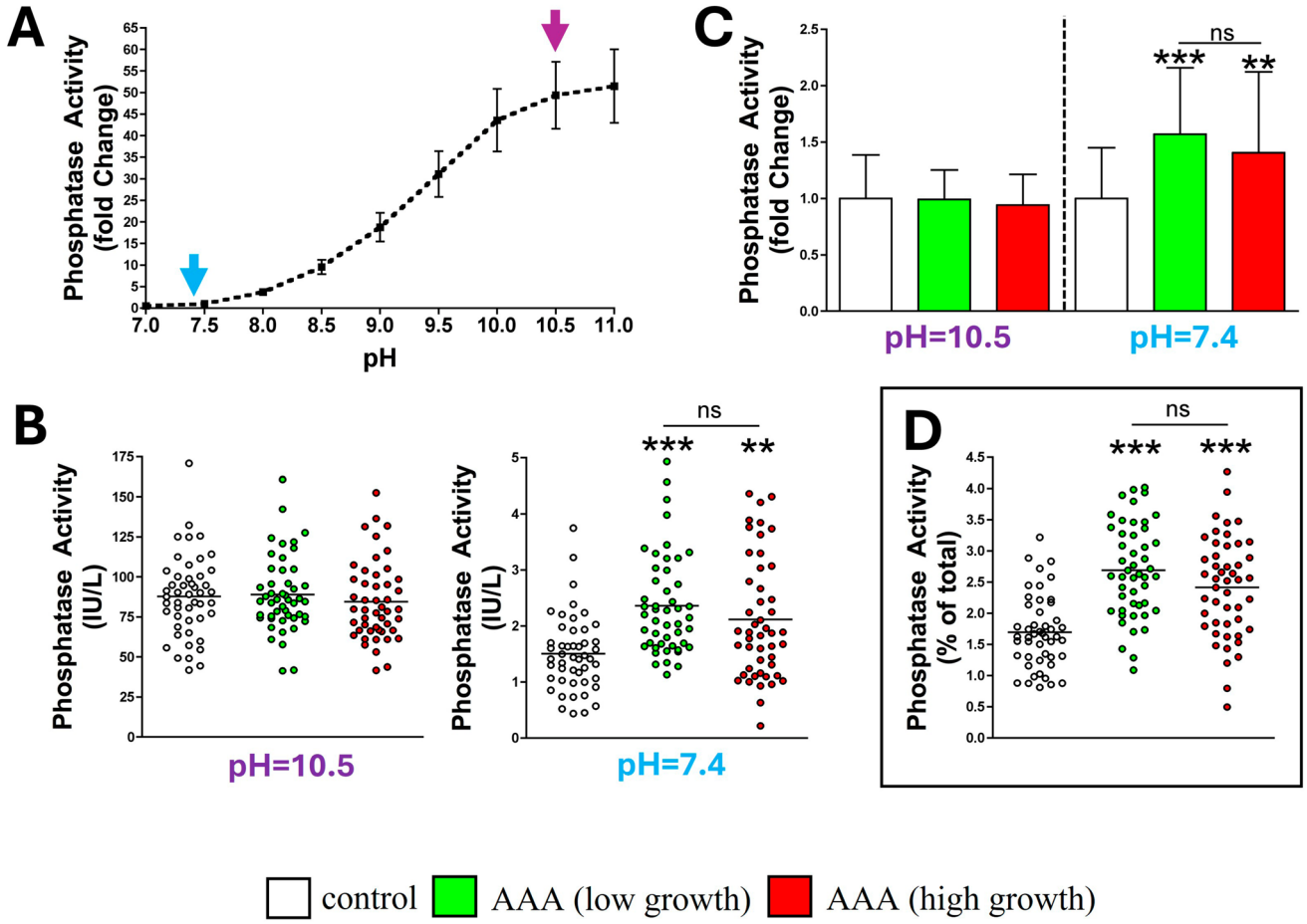

Fig. 1 Phosphatase activity in the plasma of patients with abdominal aortic aneurysm (AAA). **A** Phosphatase activity in human plasma samples across varying hydrogen ion concentrations (pH). **B** Phosphatase activity measured at an optimal pH of 10.5, and at a physiological pH of 7.4. Activity was evaluated in plasma from controls and AAA patients stratified by aortic growth rate, including low and high growth. **C** Phosphatase activity shown as fold change relative to the control group. **D** Percentage of phosphatase activity at physiological pH (7.4), relative to the optimal activity observed at pH 10.5. The data are presented as mean ± SEM (**C**) or Scatter plot (**B, D**). Statistical analyses were performed using the Kruskal-Wallis test with Dunn's post hoc test. Asterisks indicate significance levels: $^{**}P < 0.01$; $^{***}P < 0.001$

significantly greater phosphatase activity (2.36 ± 0.89 IU/L in low growth; 2.12 ± 1.08 IU/L in high growth) than controls (Fig. 1B, C; 1.51 ± 0.68 IU/L). In contrast, there were no significant differences in phosphatase activity between patients with AAA and control subjects under optimal conditions (Fig. 1B, C; 87.81 ± 25.18 IU/L in control; 88.99 ± 23.46 IU/L in low growth; 84.59 ± 24.58 IU/L in high growth). In addition, phosphatase activity as a percentage of total activity at physiological (pH 7.4) vs. optimal alkaline pH conditions (pH 10.5) was markedly elevated in AAA patients (Fig. 1D).

The elevated phosphatase activity is consistent with the presence of alkaline phosphatase

To confirm that the observed activity was due to alkaline phosphatase, a specific alkaline phosphatase inhibitor (SBI425) was used [31, 32]. First, the inhibition of phosphatase activity in the plasma of healthy individuals with increasing concentrations of the inhibitor was shown (Fig. 2A). The study found that SBI425 is a highly effective inhibitor of plasma phosphatase activity, with an IC_{50} of 698.8 nmol/L and complete inhibition achieved at 100 µmol/L. To further explore its efficacy, we assessed phosphatase inhibition at pH 7.4 using 200 µmol/L SBI425 across three experimental groups, with EDTA serving as a benchmark for maximum inhibition. SBI425 significantly suppressed phosphatase activity in all groups, with no notable differences compared to EDTA, confirming complete inhibition at physiological pH (Fig. 2). These results strongly suggest that plasma phosphatase activity at pH 7.4 primarily reflects the activity of TNAP.

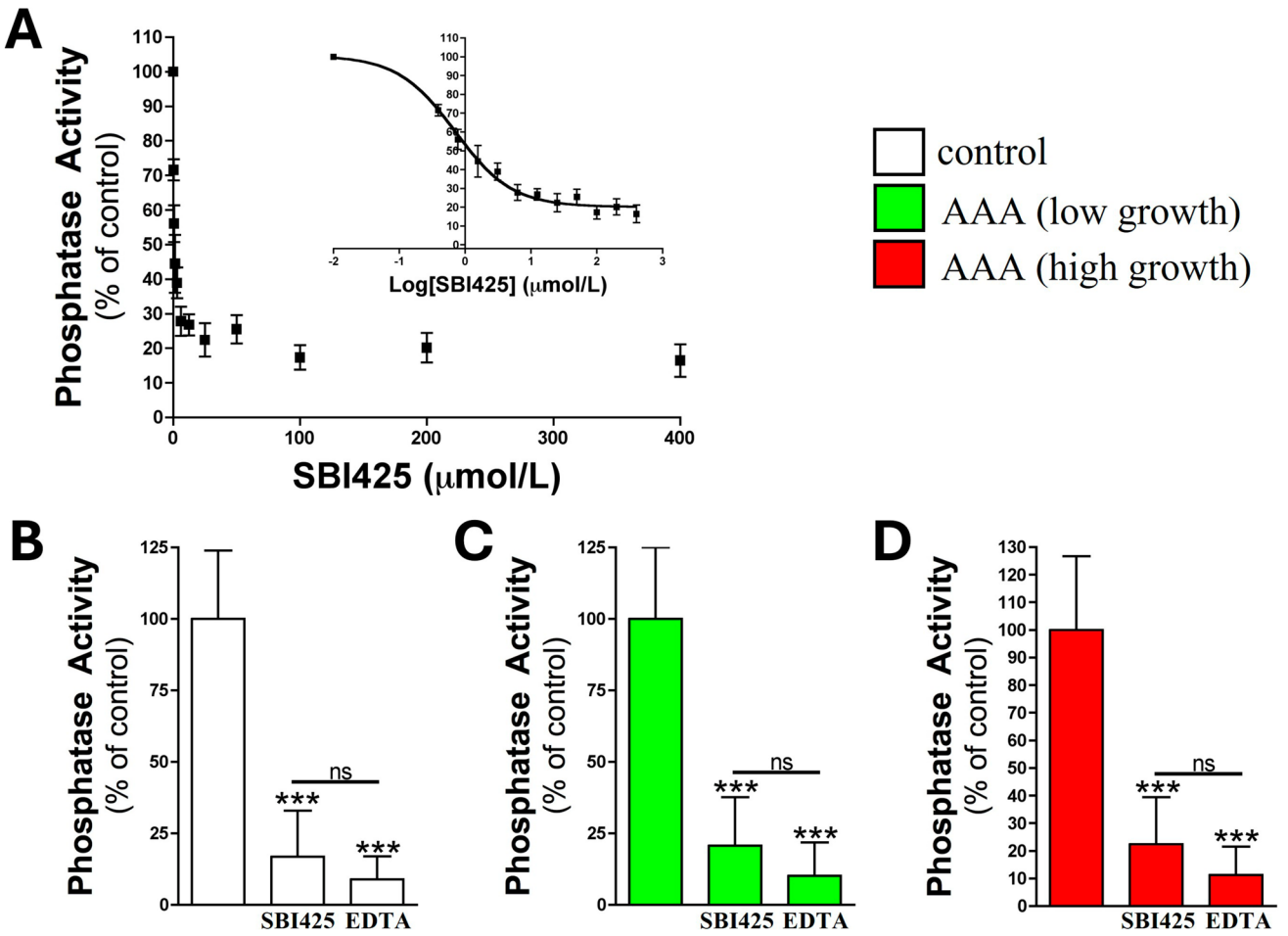

Fig. 2 Inhibition of plasma phosphatase activity by a specific alkaline phosphatase inhibitor. **A** Inhibition of phosphatase activity in human plasma samples was evaluated at various concentrations of the specific alkaline phosphatase inhibitor, SBI425. **B–D** Phosphatase activity, with or without SBI425 or EDTA, was measured in plasma from healthy controls and in groups stratified by aortic growth rate (low vs. high). The data are presented as the mean ± SD. Statistical analyses were conducted using the Kruskal-Wallis test followed by Dunn's post hoc test (**B–D**). Asterisks denote significance levels: ***$P < 0.001$

Tissue-nonspecific alkaline phosphatase is unchanged in plasma samples

To ensure that phosphatase activity remains stable under standard clinical testing conditions (optimal for alkaline phosphatase activity), three strategies were employed. First, TNAP levels were quantified using an ELISA kit. No significant differences in plasma TNAP levels were observed between AAA patients and control subjects (Fig. 3A).

Second, phosphatase activity was measured under optimal standard clinical testing conditions using a commercial alkaline phosphatase assay kit. As shown in Fig. 3B, phosphatase activity levels did not differ significantly between control subjects and AAA patients. As expected, TNAP protein levels correlated significantly with phosphatase activity under these optimal conditions, confirming that the enzymatic activity observed directly reflects the enzyme concentration (Fig. 3C).

Third, alkaline phosphatase activity was measured using another, and in fact the natural substrate, pyrophosphate, which is an inhibitor of vascular calcification [24, 25]. Pyrophosphate hydrolysis remained consistent between the control group and AAA patients at optimal pH, regardless of stratification by aortic diameter or growth rate (Fig. 4A). These findings further support that phosphatase activity is stable across all groups, even when measured with a biologically relevant substrate under optimal conditions.

Increased pyrophosphate hydrolysis in the plasma of AAA patients

Finally, pyrophosphate hydrolysis was also determined in three experimental groups to determine phosphatase activity under both optimal and physiological conditions. Pyrophosphate hydrolysis in optimal conditions (pH = 10.5) was similar in all experimental groups

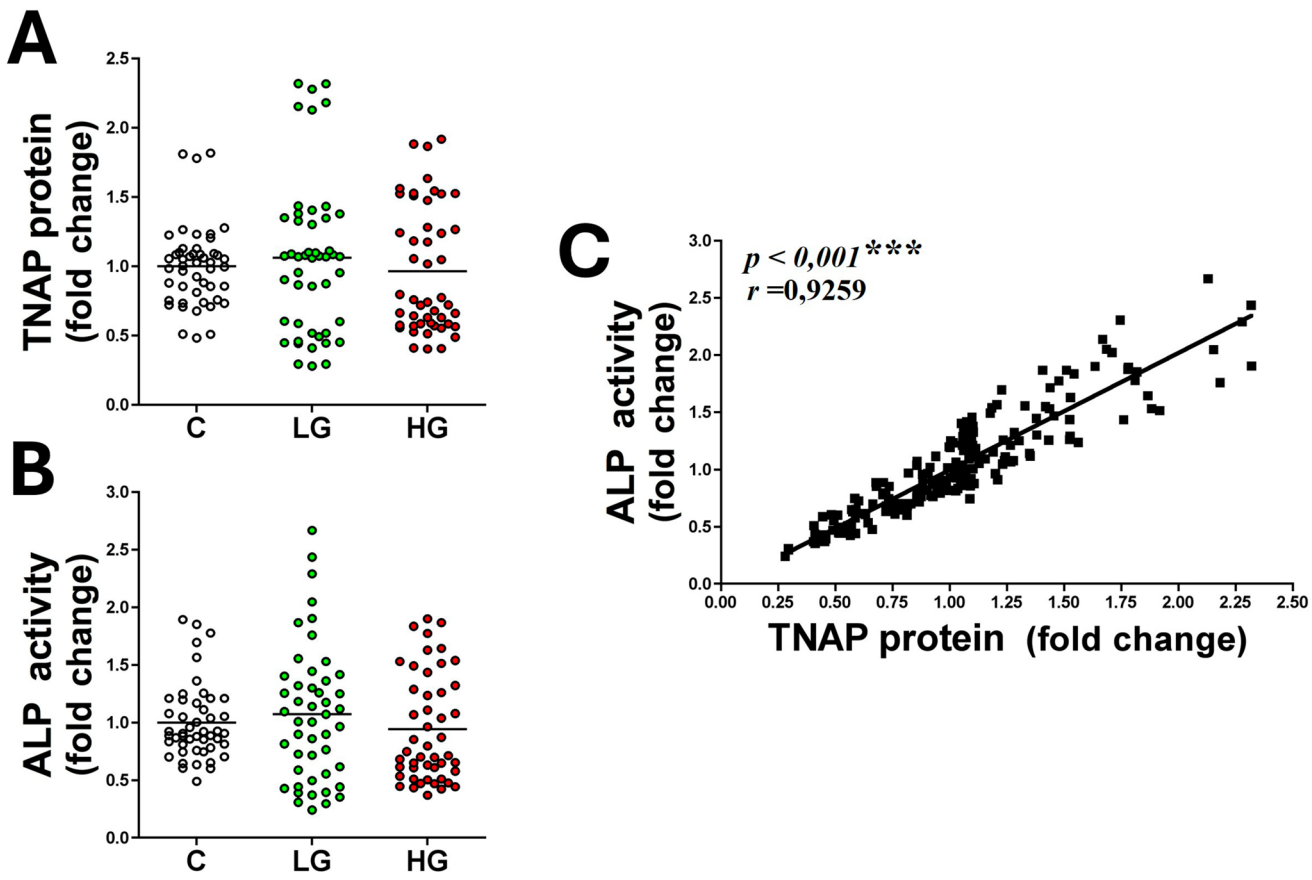

Fig. 3 Tissue-nonspecific alkaline phosphatase contents of the plasma samples were unchanged. **A** Levels of tissue-nonspecific alkaline phosphatase (TNAP) in plasma from controls (**C**) and patients with AAA, including low aortic growth (LG) and high aortic growth (HG). **B** Alkaline phosphatase (ALP) activity measured with a commercial assay kit in plasma from healthy subjects and aneurysm patients. **C** Correlation between TNAP protein levels and ALP activity, indicating a strong positive relationship. The data are presented as scatter plot (**A**, **B**). Statistical analysis was conducted using the Kruskal-Wallis test with Dunn's post hoc test. No statistically significant differences were observed between the groups assessed. Asterisks denote statistically significant differences: ***$P < 0.001$

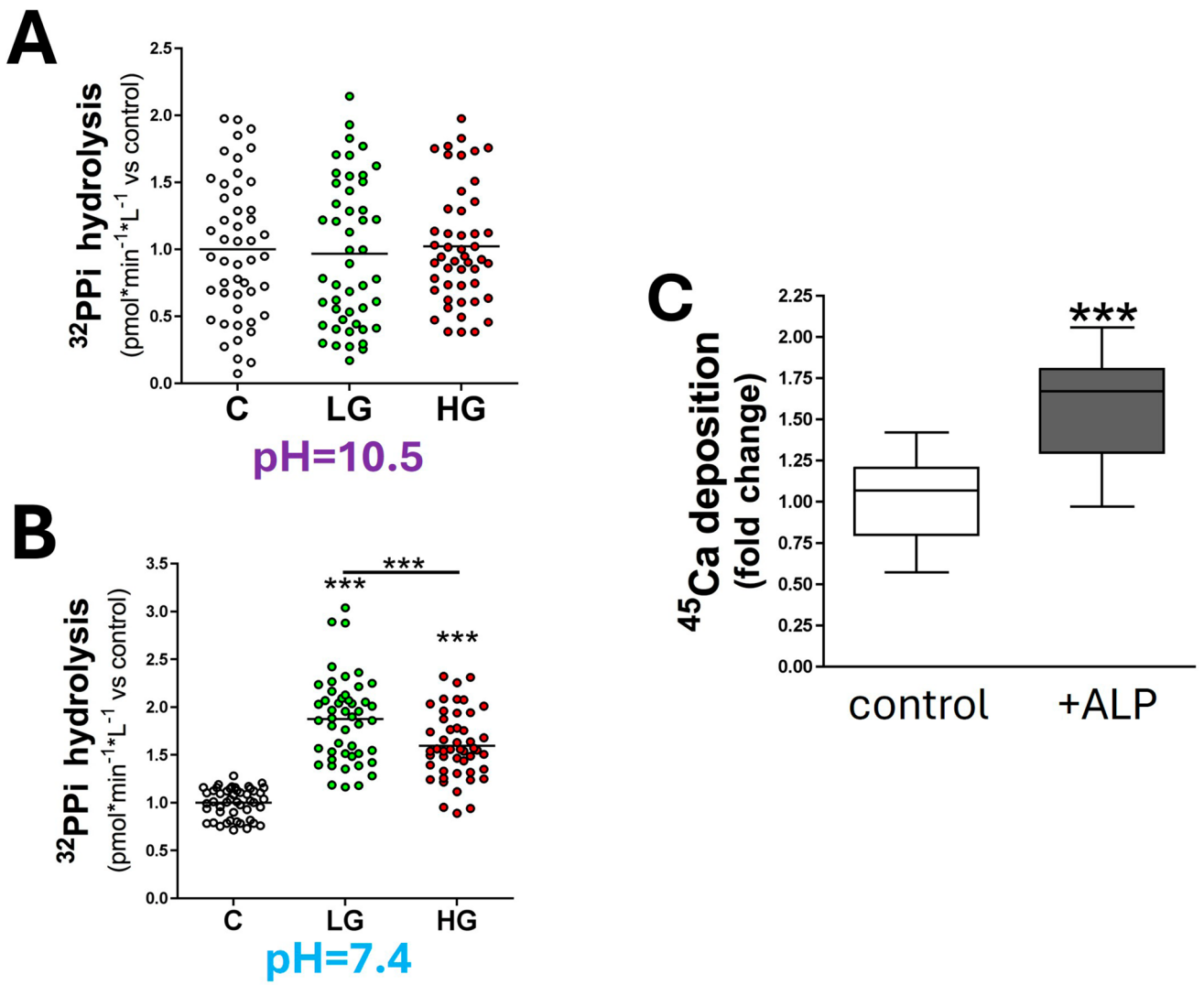

Fig. 4 Elevated pyrophosphate hydrolysis in the plasma of AAA patients. Hydrolysis of 5 µmol/L pyrophosphate, using 10 µCi/mL pyrophosphate-32 (^{32}PPi) as a radiotracer, was measured in plasma from both control subjects (**C**) and AAA patients, including low aortic growth (LG) and high aortic growth (HG). Hydrolysis of ^{32}PPi was further analyzed at two pH values: pH 10.5 (**A**) and physiological pH 7.4 (**B**). The data are presented as a scatter plot. Statistical analysis was performed using the Kruskal-Wallis test with Dunn's post hoc test. **C** Calcium deposition in human aortic smooth muscle cells incubated in MEM for 5 days with 2.5 mmol/L calcium, 0.9 mmol/L magnesium, 1 mmol/L phosphate, and 10 µCi/mL.^{45}Ca as a radiotracer, in the absence or presence of recombinant human tissue-nonspecific alkaline phosphatase (+ALP). The results are presented as box-and-whisker plots (E). Statistical analyses were conducted using *t*-tests. Asterisks denote statistically significant differences: ***$P < 0.001$

(Fig. 4A). Moreover, pyrophosphate hydrolysis in physiological conditions (pH = 7.4) was significantly higher in AAA patients compared to the control group (Fig. 4B). At the same time, different aortic growth rates also differed significantly in pyrophosphate hydrolysis. Notably, as the aortic growth rate increased, pyrophosphate hydrolysis—and consequently phosphatase activity—tended to decrease, suggesting a potential inverse relationship between the aortic growth rate and plasma pyrophosphate hydrolysis.

The impact of increased alkaline phosphatase activity on aortic calcification, which is linked to increased pyrophosphate hydrolysis and its subsequent reduction in availability, was further investigated. Calcium accumulation in human smooth aortic muscle cells was significantly greater in the presence, than in the absence, of TNAP (Fig. 4C). These findings suggest that elevated TNAP activity may promote vascular calcification by reducing pyrophosphate levels, thereby facilitating calcium deposition in aortic smooth muscle cells.

Inverse correlation between plasma pyrophosphate hydrolysis and aortic growth of AAA

The correlation between aortic growth rate of AAA and phosphatase activity and pyrophosphate hydrolysis in both optimal and physiological pH was also assessed. A significant inverse correlation was observed between aortic growth and pyrophosphate hydrolysis under physiological conditions (pH 7.4). In contrast, no significant differences were found in the other comparisons (Fig. 5). Notably, the significant inverse correlation observed between aortic growth and pyrophosphate hydrolysis under physiological conditions remained independent of potential confounding factors (Table 2).

Table 2 Multivariate linear regression analysis in AAA patients (LG vs HG)

	Beta	*t*	*P*-value
Pyrophosphate hydrolysis	−0.211	−2.073	0.041
AAA diameter	0.040	0.392	0.696
Smoking	0.240	2.236	0.028
Previous CVD	0.047	0.423	0.674
Aspirin	−0.215	−1.801	0.075
Statins	−0.067	−0.560	0.577

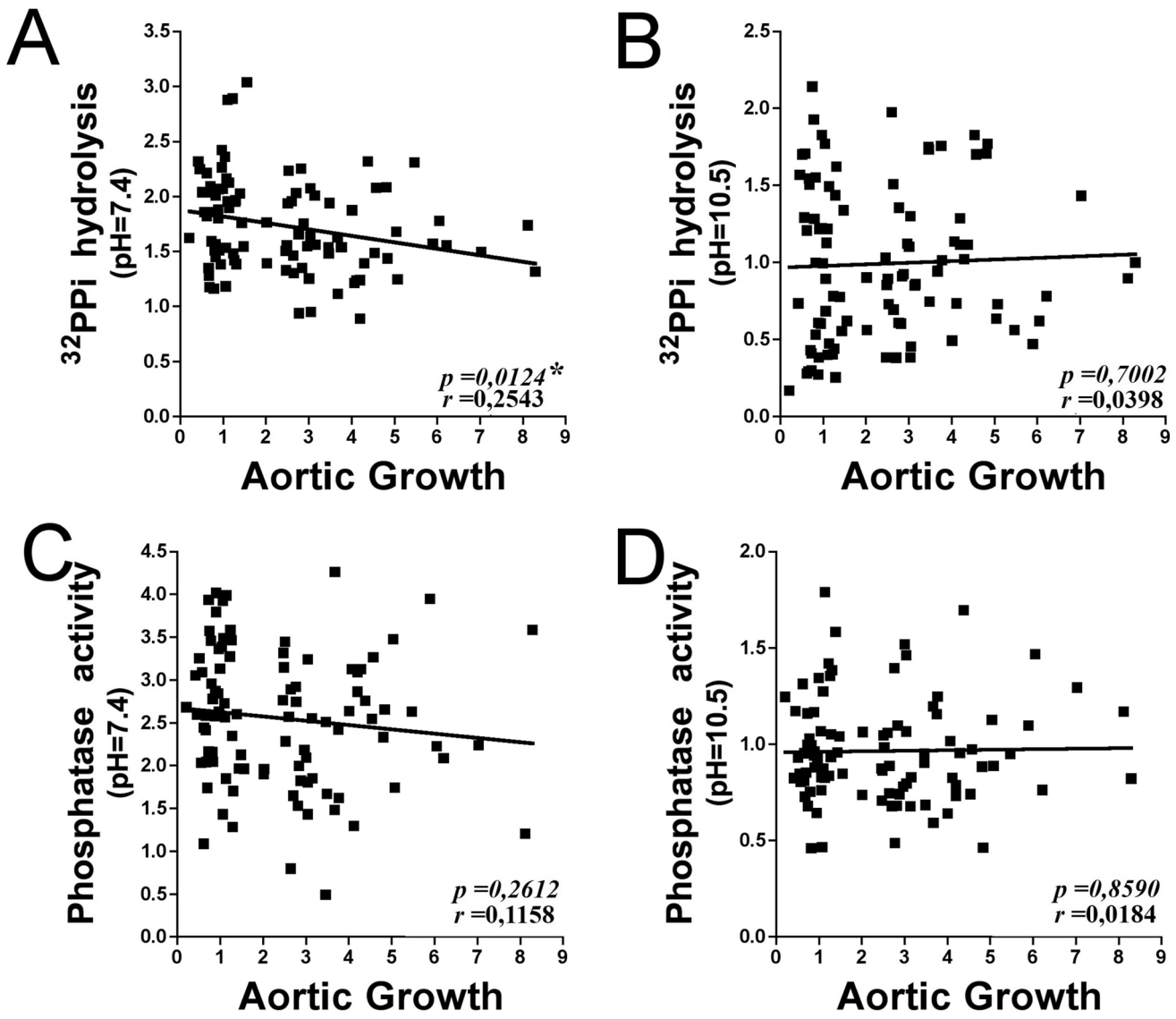

Fig. 5 Pyrophosphate hydrolysis in plasma under physiological conditions inversely correlates with AAA growth. This figure presents the correlations between aortic growth rate and **A** pyrophosphate hydrolysis at pH 7.4, **B** pyrophosphate hydrolysis at pH 10.5, **C** phosphatase activity at pH 7.4, and **D** phosphatase activity at pH 10.5. Asterisks denote statistically significant differences: $^{*}P < 0.05$

Discussion

Vascular calcification within the context of AAA has a complex pathophysiological role, as it may both contribute to and/or inhibit aneurysm progression [13]. Calcification was traditionally associated with weakened structural integrity of the aortic wall, promoting AAA expansion and increasing the risk of rupture [14]. However, emerging evidence suggests that calcification may possess a stabilizing property in the form of biological "scaffolding," which could strengthen the aneurysmal wall to decrease potential dilatation [16–19]. This dual role highlights the influence of mineralization in AAAs, where localized calcification may act as a protective factor, slowing AAA progression in some cases by reducing wall stress and preventing further degradation of the aortic tissue. These opposing effects must be elucidated to develop specific pathways potentially amenable for targeted interventions; the modulation of calcification could represent a potential strategy to either disrupt pathological calcification processes or take advantage of its stabilizing properties in the management of AAA.

In this retrospective study, we found that both plasma alkaline phosphatase activity and pyrophosphate hydrolysis significantly increased at physiological pH (7.4) in AAA as compared to controls, but not at an alkaline pH (10.5). This underscores the value of examining enzymes in physiological milieu to identify changes that occur in disease states, which may be masked during traditional clinical assays. Although measuring TNAP activity at physiological pH rather than at its optimal catalytic pH may be viewed as a limitation, since it does not reflect maximal enzyme capacity, we also consider it a strength, as it allows detection of pathophysiologically relevant differences that remain masked under optimal assay conditions. Moreover, the observed increase in phosphatase activity in patients with AAA in physiological conditions raises critical questions about the underlying regulatory mechanisms involved. These could involve deficiencies or disruptions in natural alkaline phosphatase inhibitors, structural alterations of the enzyme, or post-translational modifications that increase its activity in AAA patients.

More importantly, our study revealed an inverse correlation between pyrophosphate hydrolysis and aortic growth rate in patients with small AAAs, pointing out the possible beneficial effect of calcification contributing to the stabilization of the aneurysmal wall [16, 17]. Pyrophosphate, a potent vascular calcification inhibitor, can be hydrolyzed by alkaline phosphatase that decreases the availability of this compound and thus promotes aortic calcium deposition [24]. A previous study [33] suggests that systemic pyrophosphate, present in plasma and the extracellular milieu, may play a more decisive role in inhibiting vascular calcification than locally synthesized pyrophosphate. Nevertheless, direct measurements of pyrophosphate at the AAA site are scarce, and future studies assessing tissue-specific levels will be valuable to clarify its local contribution to aneurysm pathophysiology.

Finally, the authors acknowledge some significant limitations that deserve mention. First, there was a lack of imaging data on AAA morphology or calcification in our patients, which could have been correlated with the serological analysis to further support the conclusions of the study. Second, because of the observational nature, it would not be possible to make causal inferences between increased phosphatase activity and AAA progression. Third, the sample is not representative of the full spectrum of AAA pathophysiology, as genetic predisposition (and environmental effects on phosphatase activity and calcification) may vary between patients, but these events are likely to be missed from our cohort. In addition, the lack of longitudinal data to follow calcification over time means that we are limited in understanding how phosphatase activity relates to calcification and AAA growth or stability across various stages of disease. Furthermore, tissue-specific analyses would be highly valuable to clarify whether local TNAP activity is directly linked to AAA growth; however, obtaining both healthy vascular tissue and non-operated AAA samples remains a considerable challenge. These limitations are best addressed by larger, longitudinal cohorts in future research, as they will provide a more detailed characterization of the role of phosphatase activity and calcification in AAA development and/or progression.

Author contribution Study conception and design: Ricardo Villa-Bellosta and Jose Luis Martín-Ventura. Experimental procedures were performed and materials were provided by Ricardo Villa-Bellosta. Plasma samples were provided by Jes S. Lindholt. All authors contributed to the data collection, analysis, and interpretation of data. The first draft of the manuscript was written by Ricardo Villa-Bellosta, Jes S. Lindholt, and Jose Luis Martín-Ventura. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by grants from the Spanish Ministry of Education and Science (SAF-2014-60699-JIN and PID2020-113603RD-100, to RVB; PID2022-136979OB-I00 to JLMV), the Spanish Society of Nephrology (SEN21-3315, to RVB), the Xunta de Galicia (ED431F 2022/03, to RVB), and the Caixa Foundation (HR22/00253) and the Community of Madrid (S2022/BMD-7333) to JLMV. Additional support was provided through a Senior Research contract under the Ramón y Cajal program from the Spanish Government (RYC2019-027920-I to RVB).

Data availability The data that supports the findings of this study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This study was approved by the local ethics committee of the Viborg Hospital (M20080025) and performed in accordance with the Helsinki Declaration. Samples were randomly selected from individuals enrolled in the VIVA trial (ClinicalTrials.gov NCT00662480).

Consent to participate All subjects provided informed consent.

Competing interests The authors declare no competing interests.

References

1. US Preventive Services Task Force et al (2019) Screening for abdominal aortic aneurysm: US Preventive Services Task Force recommendation statement. JAMA 322, 2211–2218. https://doi.org/10.1001/jama.2019.18928
2. Golledge J, Thanigaimani S, Powell JT, Tsao PS (2023) Pathogenesis and management of abdominal aortic aneurysm. Eur Heart J 44:2682–2697
3. Reimerink JJ, van der Laan MJ, Koelemay MJ, Balm R, Legemate DA (2013) Systematic review and meta-analysis of population-based mortality from ruptured abdominal aortic aneurysm. Br J Surg 100:1405–1413. https://doi.org/10.1002/bjs.9235
4. Michel JB et al (2011) Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. Cardiovasc Res 90:18–27. https://doi.org/10.1093/cvr/cvq337
5. Torres-Fonseca M et al (2019) Pathophisiology of abdominal aortic aneurysm: biomarkers and novel therapeutic targets. Clin Investig Arterioscler 31:166–177
6. Petsophonsakul P et al (2019) Role of vascular smooth muscle cell phenotypic switching and calcification in aortic aneurysm formation. Arterioscler Thromb Vasc Biol 39:1351–1368. https://doi.org/10.1161/ATVBAHA.119.312787
7. Jono S et al (2000) Phosphate regulation of vascular smooth muscle cell calcification. Circ Res 87:E10-17. https://doi.org/10.1161/01.res.87.7.e10
8. Neven E et al (2010) Chondrocyte rather than osteoblast conversion of vascular cells underlies medial calcification in uremic rats. Arterioscler Thromb Vasc Biol 30:1741–1750. https://doi.org/10.1161/ATVBAHA.110.204834
9. Villa-Bellosta R (2018) Synthesis of extracellular pyrophosphate increases in vascular smooth muscle cells during phosphate-induced calcification. Arterioscler Thromb Vasc Biol 38:2137–2147
10. Villa-Bellosta R, Millan A, Sorribas V (2011) Role of calcium-phosphate deposition in vascular smooth muscle cell calcification. Am J Physiol Cell Physiol 300:C210-220
11. DubisJ et al (2016) Elevated expression of runt-related transcription factors in human abdominal aortic aneurysm. J Biol Regul Homeost Agents 30:497–504
12. Li Z et al (2020) Runx2 (runt-related transcription factor 2)-mediated microcalcification is a novel pathological characteristic and potential mediator of abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 40:1352–1369. https://doi.org/10.1161/ATVBAHA.119.314113
13. Vermeulen JJM et al (2023) A systematic review summarizing local vascular characteristics of aneurysm wall to predict for progression and rupture risk of abdominal aortic aneurysms. J Vasc Surg 77:288-298.e2. https://doi.org/10.1016/j.jvs.2022.07.008
14. Buijs RVC et al (2013) Calcification as a risk factor for rupture of abdominal aortic aneurysm. Eur J Vasc Endovasc Surg 46:542–548. https://doi.org/10.1016/j.ejvs.2013.09.006
15. Forsythe RO et al (2018) 18F-sodium fluoride uptake in abdominal aortic aneurysms: the SoFIA3 study. J Am Coll Cardiol 71:513–523
16. Klopf J et al (2022) The prognostic impact of vascular calcification on abdominal aortic aneurysm progression. J Vasc Surg 75:1926–1934. https://doi.org/10.1016/j.jvs.2021.11.062
17. Nakayama A et al (2016) Inverse correlation between calcium accumulation and the expansion rate of abdominal aortic aneurysms. Circ J 80:332–339
18. Lindholt JS (2008) Aneurysmal wall calcification predicts natural history of small abdominal aortic aneurysms. Atherosclerosis 197:673–678
19. Hendy K, Gunnarsson R, Cronin O, Golledge J (2015) Infrarenal abdominal aortic calcification volume does not predict small abdominal aortic aneurysm growth. Atherosclerosis 243:334–338
20. Lomashvili KA, Garg P, Narisawa S, Millan JL, O'Neill WC (2008) Upregulation of alkaline phosphatase and pyrophosphate hydrolysis: potential mechanism for uremic vascular calcification. Kidney Int 73:1024–1030
21. Villa-Bellosta R, Wang X, Millán JL, Dubyak GR, O'Neill WC (2011) Extracellular pyrophosphate metabolism and calcification in vascular smooth muscle. Am J Physiol Heart Circ Physiol 301:H61-68
22. Addison WN, Azari F, Sørensen ES, Kaartinen MT, McKee MD (2007) Pyrophosphate inhibits mineralization of osteoblast cultures by binding to mineral, up-regulating osteopontin, and inhibiting alkaline phosphatase activity. J Biol Chem 282:15872–15883
23. O'Neill WC (2006) Pyrophosphate, alkaline phosphatase, and vascular calcification. Circ Res 99:e2. https://doi.org/10.1161/01.RES.0000234909.24367.a9
24. Villa-Bellosta R, O'Neill WC (2018) Pyrophosphate deficiency in vascular calcification. Kidney Int 93:1293–1297
25. Villa-Bellosta R et al (2013) Defective extracellular pyrophosphate metabolism promotes vascular calcification in a mouse model of Hutchinson-Gilford progeria syndrome that is ameliorated on pyrophosphate treatment. Circulation 127:2442–2451
26. Lomashvili KA, Khawandi W, O'Neill WC (2005) Reduced plasma pyrophosphate levels in hemodialysis patients. J Am Soc Nephrol 16:2495–2500

27. Azpiazu D, González-Parra E, Egido J, Villa-Bellosta R (2018) Hydrolysis of extracellular pyrophosphate increases in post-hemodialysis plasma. Sci Rep 8:11089
28. Lindholt JS, Søgaard R (2017) Population screening and intervention for vascular disease in Danish men (VIVA): a randomised controlled trial. Lancet Lond Engl 390:2256–2265
29. Martín-Ventura JL et al (2011) Soluble TWEAK plasma levels predict expansion of human abdominal aortic aneurysms. Atherosclerosis 214(2):486–489
30. Vega De Ceniga M, Esteban M, Barba A, Estallo L, Blanco-Colio LM, Martin-Ventura JL (2014) Assessment of biomarkers and predictive model for short-term prospective abdominal aortic aneurysm growth—a pilot study. Ann Vasc Surg 28(7):1642–1648
31. Sheen CR et al (2015) Pathophysiological role of vascular smooth muscle alkaline phosphatase in medial artery calcification. J. Bone Miner. Res. 30:824–836. https://doi.org/10.1002/jbmr.2420
32. Pinkerton AB et al (2018) Discovery of 5-((5-chloro-2-methoxyphenyl)sulfonamido)nicotinamide (SBI-425), a potent and orally bioavailable tissue-nonspecific alkaline phosphatase (TNAP) inhibitor. Bioorg. Med. Chem. Lett. 28:31–34. https://doi.org/10.1016/j.bmcl.2017.11.024
33. Lomashvili KA, Narisawa S, Millán JL, O'Neill WC (2014) Vascular calcification is dependent on plasma levels of pyrophosphate. Kidney Int 85(6):1351–1356

Authors and Affiliations

Ricardo Villa-Bellosta[1,2,3] · Jes S. Lindholt[4] · Jose Luis Martin-Ventura[5,6]

✉ Ricardo Villa-Bellosta
metabol@hotmail.com

[1] Center for Research in Molecular Medicine and Chronic Diseases (CiMUS), University of Santiago de Compostela, Avenida de Barcelona S/N, Campus Vida, 15782 Santiago de Compostela, Spain

[2] Health Research Institute of Santiago de Compostela (IDIS), Travesia da Choupana S/N, 15706 Santiago de Compostela, Spain

[3] Department of Biochemistry and Molecular Biology, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

[4] Department of Cardiothoracic and Vascular Surgery, Center for Individualized Medicine in Arterial Diseases, Odense University Hospital, Odense, Denmark

[5] Vascular Lab, Instituto de Investigacion Sanitaria Fundacion Jimenez-Diaz-Autonoma University of Madrid (IIS-FJD, UAM), Madrid, Spain

[6] CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain