

# Nonsteroidal antiinflammatory drugs are associated with increased aortic stiffness

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**Objectives:** Nonsteroidal antiinflammatory drugs (NSAIDs) have been shown to retard aneurysm growth in animal models. In vitro studies have shown an inhibitory effect of NSAIDs on matrix metalloproteinase-9, interleukin-1 $\beta$ , and IL-6 mediated arterial wall elastolysis. The aim of this study was to investigate the effects of NSAIDs on arterial stiffness, a surrogate marker of elastolysis.

**Methods:** 447 subjects enrolled in a community-based abdominal aortic aneurysm (AAA) screening program were assessed for age, blood pressure, smoking status, and drug history. Aortic diameter and stiffness were measured by M-Mode ultrasound. The concentration of the amino-terminal propeptide of type III procollagen was used as a proxy measurement of type III collagen turnover.

**Results:** NSAID ingestion was significantly ( $p=0.006$ ) associated with increased aortic wall stiffness after adjusting for age, aortic diameter, blood pressure, and smoking status. No such effect was seen for  $\beta$ -blockers, calcium channel antagonists, nitrates, angiotensin-converting enzyme inhibitors, diuretics, or antiplatelet agents.

**Discussion:** These novel data show that NSAIDs are associated with increased aortic stiffness, possibly through the effects of cytokine mediated elastolysis. This in turn may prevent aortic expansion and the development of AAA.

**Keywords:** nonsteroidal antiinflammatory drugs, abdominal aortic aneurysm, aortic stiffness, elastolysis

## Introduction

The pathogenesis of abdominal aortic aneurysm formation is not fully understood. Inflammatory processes are clearly implicated, with the principal cellular abnormality within the wall of an aneurysm appearing to be increased proteolytic capacity resulting in loss of elastin.

This disruption of extra cellular matrix is accompanied by an inflammatory infiltrate and associated with increased expression of matrix metalloproteases (MMPs) (Thompson and Parks 1996). Nonsteroidal antiinflammatory drugs (NSAIDs) have been shown to inhibit aneurysm expansion via the inhibition of cyclooxygenase 2 pathway in an animal model. This pathway controls synthesis of prostaglandin E2 (PGE2) and thus expression of MMP-9 (Miralles et al 1999). NSAIDs have also been shown to decrease production of PGE2, interleukin (IL)-1 $\beta$ , and IL-6 in an in vitro study of the effect of indomethacin on human abdominal aortic aneurysm (AAA) tissue (Franklin et al 1999).

Type III collagen is the major type of collagen found in large arteries, muscle, and skin (Prockop et al 1979a, 1979b). In vitro studies have demonstrated that aneurysmal aortic tissue is less compliant than normal aortic tissue and that this mechanical change is related to loss of elastin from the arterial wall. This occurs as the mechanical load shifts from elastin to collagen and the artery becomes less compliant (Sumner et al 1970; He and Roach 1994).

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The aim of this study was to measure aortic wall compliance, a surrogate marker of elastolysis, and compare these values against lifestyle, proteolytic activity, and cardiovascular status.

## Methods

The subjects of this study participated in a prospective, longitudinal, population-based screening program for men over the age of 50 in Huntingdon, UK (Morris et al 1994). In December 1996, 9864 men over the age of 50 had been screened. A nested case control study was performed within this population. A case was defined as a person with an infrarenal aortic diameter exceeding 29 mm. A control was defined as a person with an infrarenal aortic diameter less than 25 mm. Controls were matched for age using frequency matching and were selected at random from the screened population with an aorta diameter less than 25 mm. Data about current smoking status, smoking history, family history of aortic aneurysm, occupational history, previous medical history, and drug history were obtained through a self-administered questionnaire. Smoking status was classified into three categories: current smokers, ex-smokers, and non-smokers. A positive history of ischemic heart disease was recorded if the patient had suffered a myocardial infarction, had been admitted to hospital for treatment of angina, or had undergone a coronary bypass operation. A positive history of diabetes was recorded if a subject was currently using insulin or oral hypoglycemic drugs. A comprehensive drug history was recorded, with a positive history for exposure to a particular drug only noted if the subject was actually taking the drug when questioned. Data about duration of exposure to the drugs was not recorded.

All participants had their height, weight, blood pressure, infrarenal aortic diameter, and aortic wall distensibility measured in the same screening visit. Blood pressure was measured automatically with a Dinamap 1846 SXP (Critikon, Kettering, UK).

Infrarenal aortic diameter was measured by ultrasound (Wilmink et al 1997). All measurements of the infrarenal aortic diameter (IAD) were made by the same ultrasonographer. A longitudinal scan of the abdominal aorta was made. The maximum external anteroposterior diameter was measured at the widest part or the most distal 1 cm of the abdominal aorta, with the patient in supine position.

Aortic distensibility was measured with M mode ultrasound by a single ultrasonographer using a Toshiba Capasee with a curvilinear 3.5 MHz probe (Toshiba Medical Systems Ltd, Crawley, UK) (MacSweeney 1999). The aortic

diameter in systole and in diastole was determined by freezing the M mode picture, at the point of diameter measurement, if a clear trace could be obtained. The minimal and maximal aortic diameters were determined visually. Diameters were measured as the distance between two cursors, which were positioned on the upper demarcation line of the echogenic zone. In each patient, three measurements were taken within 5 minutes of each other. The average of three measurements was used for the strain calculations.

Strain was defined as:

$$\frac{\text{maximal systolic diameter} - \text{maximal diastolic diameter}}{\text{maximal diastolic diameter}} \quad (1)$$

Stiffness ( $\beta$ ) was defined as:

$$\frac{\ln(\text{systolic pressure}/\text{diastolic pressure})}{\text{strain}} \quad (2)$$

The elasticity of the aorta can be described as the pressure strain elastic modulus or stiffness ( $\beta$ ) (Wilson et al 1998). The stiffness index is used here because it is less dependent on pulse pressure (Länne et al 1992). Both of these variables are inversely related to aortic distensibility.

Measurement of the ankle brachial pressure index (ABPI) was performed in the supine position with a hand-held Doppler (Mini Dopplex D900, Huntleigh Diagnostics, Cardiff, UK) with an 8-MHz probe, and a standard sphygmomanometer. The systolic brachial artery pressure was recorded in both right and left arms. The higher of the two pressures was used for calculation of the ABPI. The systolic ankle pressure was recorded at the posterior tibial artery with the cuff of the sphygmomanometer immediately above the malleoli. The ABPI was calculated for both legs and the lowest value was used to determine the presence of peripheral vascular disease. Peripheral vascular disease was deemed present if the ABPI was lower than 0.9.

Serum was taken to measure lipid levels, cotinine levels, (Cozart Biosciences, Abingdon, UK), and collagen turnover. Samples were taken at the time of the aortic diameter and stiffness measurement. The samples were spun down and the serum frozen at  $-20^{\circ}\text{C}$  on the same day for analysis at a later date. Batches of 80 serum samples were analyzed by a single biochemist at the department of chemical pathology at Hinchingsbrooke hospital. The concentration of the amino-terminal propeptide of type III procollagen (PIIINP) was used as a proxy measurement of type III collagen turnover since the aminoterminal propeptide is split off the procollagen molecule during type III collagen synthesis (Prockop et al 1979a, 1979b). The concentration of PIIINP

**Table 1** Main characteristics of the study population

Variable	Mean	SD
N	447	
Age in years	71	7.7
Height in cm	173	6.6
Weight in kg	80	12.0
Systolic BP in mmHg	156	24.0
Diastolic BP in mmHg	85	15.1
Initial AAA diameter in mm	26.5	8.1

**Abbreviations:** BP, blood pressure; AAA, abdominal aortic aneurysm.

was measured using an equilibrium type serum radio-immunoassay of the amino-terminal propeptide of type III procollagen based on a highly purified specific human antigen (Pharmacia & Upjohn Ltd, Diagnostics Division, Milton Keynes, UK). This was done according to the instructions of the manufacturer with the use of duplicate 200 µL aliquots of serum (Risteli et al 1988; Satta et al 1995). The intra-assay and inter-assay coefficients of variation of this assay are less than 5% and the sensitivity is 0.2 µg/L. The reference range for adults based on data of Finnish blood donors was 1.7–4.2 µg/L, with no differences between males and females (Risteli et al 1988).

## Statistical analysis

Statistical analysis was performed using analysis of variance and linear regression methods. The association of several factors with arterial wall stiffness was investigated by multivariate linear regression analysis. Statistical significance of each individual variable in the multivariate model was tested using a t-test. All statistical analyses were performed using STATA 5.0 for Macintosh (Stata Corporation, College Station, Texas, USA) (Stata Corporation 1995; Hamilton 1998).

## Results

Detailed data of current medication, past medical history, lipid levels serum cotinine levels, and serum PIIINP as a proxy for collagen type III turnover were available for 447 subjects: 210 cases with an aorta larger than 3 cm, and 237 controls with a normal aortic diameter (Table 1). The type of NSAID taken and the number of subjects taking each agent is listed in Table 2. Aortic stiffness was weakly associated with age (coefficient 0.14,  $p=0.049$ ) but was strongly associated with aortic diameter (coefficient 4.6,  $p<0.0001$ ). Subjects with an aortic diameter larger than 3 cm had a significantly stiffer aorta than controls. Only 2% of these subjects used NSAIDs compared with 8% of subjects

**Table 2** Type of nonsteroidal antiinflammatory drugs and number of subjects taking each agent

Type of NSAID	N
Ibuprofen	7
Diclofenac	6
Indomethacin	4
Ketoprofen	1
Axapropazone	1
Tiaprofenic acid	1
Sulindac	1
Piroxicam	1
Mefenamic acid	1
Total	23

**Abbreviations:** NSAID, nonsteroidal antiinflammatory drug.

with an aortic diameter less than 2.5 cm ( $\chi^2=8.5$ ,  $p=0.004$ ). The association of stiffness with age disappeared in a multivariate model, which adjusted for both aortic diameter and blood pressure. Since age is known to be associated with stiffness, it was not removed from the multivariate model. Smoking status, a history of ischemic heart disease, diabetes, or the presence of peripheral vascular disease were not associated with aortic wall distensibility in a multivariate model, which was adjusted for age, aortic diameter, and systolic and diastolic blood pressure. Similarly serum lipid, cotinine, and PIIINP levels were not associated with aortic wall distensibility (Table 3).

None of the major classes of cardiovascular drugs were significantly associated with aortic wall distensibility (Table 4). However, use of NSAIDs was significantly associated with increased aortic wall stiffness (coefficient 6.5), and remained significantly associated with aortic wall

**Table 3** Relationship between aortic wall stiffness, medical history, lipid, and cotinine and PIIINP levels in a multivariate linear regression model adjusted for age initial aortic diameter and systolic and diastolic blood pressure

Variable	<sup>1</sup> N/ <sup>2</sup> Mean	Coefficient	p-value
<sup>1</sup> Current smokers	58	1.6 (1.4)	0.25
<sup>1</sup> Ex-smokers	270	-0.7 (1.2)	0.56
<sup>1</sup> History of ischemic heart disease	75	-0.2 (1.5)	0.90
<sup>1</sup> Diabetes	17	-1.6 (2.8)	0.56
<sup>1</sup> Peripheral vascular disease	95	-0.4 (1.3)	0.75
<sup>2</sup> Total cholesterol	5.85	0.35 (0.49)	0.71
<sup>2</sup> LDL cholesterol	1.15	-0.06 (0.57)	0.92
<sup>2</sup> HDL cholesterol	3.86	2.57 (1.76)	0.15
<sup>2</sup> Cotinine	82.1	0.00 (0.00)	0.81
<sup>2</sup> PIIINP	3.68	-0.20 (0.39)	0.60

NOTE: The number of subjects are classified positive for that variable. Mean values of total cholesterol, LDL-, and HDL-cholesterol in the study population are in mmol/L, and serum cotinine and PIIINP in µg/L.

**Abbreviations:** PIIINP, amino-terminal propeptide of type III procollagen; N, number; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

**Table 4** The association between aortic wall stiffness and cardiovascular drugs in a multivariate linear regression model which adjusted for age, initial aortic diameter and systolic and diastolic blood pressure

Drug	Number not exposed	Number exposed	Coefficient	p-value
Beta blockers	362	85	0.7 (1.4)	0.60
Ca channel blockers	398	49	3.0 (1.75)	0.09
ACE inhibitors	410	37	-2.0 (2.0)	0.32
Nitrates	414	33	-3.6 (2.0)	0.07
Diuretics	377	70	0.9 (1.5)	0.55
Aspirin	365	82	0.8 (1.4)	0.55
NSAIDs	424	23	6.5 (2.4)	0.006

**Abbreviations:** ACE, angiotensin-converting enzyme; NSAIDs, nonsteroidal antiinflammatory drugs.

stiffness if only controls with a normal aortic diameter were analyzed (coefficient 4.1, SE 2.0,  $p=0.03$ ) (Table 5).

NSAID use was associated with significantly increased PIIINP levels. Mean levels were 5.161 (95% confidence interval [CI] 3.717–6.606) versus 3.588 (95% CI 3.476–3.700),  $p$ -value 0.0067 (Mann-Whitney test).

## Discussion

NSAID use was found to be an independent predictor of arterial wall compliance in our model that adjusted for age, blood pressure, and arterial diameter. No relationship was found with cardiovascular drugs, medical history, or serum lipid, cotinine, and PIIINP levels. Significantly increased PIIINP levels were found in patients taking NSAID therapy. This is likely to be a class effect as different types of NSAIDs were taken by the participating subjects.

Neither the duration for which a patient had been taking NSAIDs, nor the underlying diagnoses which lead to NSAID therapy were recorded. Thus we cannot exclude the possibility that an underlying disease state caused the change in aortic stiffness as opposed to NSAID therapy. However, NSAID therapy is prescribed for a wide range of disorders and this theory is unlikely to be correct. The group studied has a small total population, and the possibility of a sampling error cannot be excluded.

This method of stiffness measurement is operator dependant and difficult to verify or reproduce. Nevertheless, the results compare favorably with a Dutch study employing the same method to investigate carotid arteries. Their intra-observer coefficient of measurement variation of a single carotid artery was 30.8%. Our estimate of the coefficient of variation is 20.2% (95% CI 19.7–20.7%) if we use the same method of calculation of coefficient of variation; ie, calculate the standard error of the measurements as a percentage of

**Table 5** Unadjusted and adjusted mean stiffness and 95% confidence intervals in subjects exposed to NSAID compared to those not exposed

Exposure	Mean	95% CI	Adjusted	
			mean	95% CI
No NSAID	19.6	18.4–20.8	19.5	18.4–20.6
NSAID	24.4	19.4–29.3	26.0	21.5–30.5

NOTE: Adjusted means were calculated using a multivariate linear regression model adjusting for age, initial aortic diameter, and systolic and diastolic blood pressure.

**Abbreviations:** CI, confidence interval; NSAID, nonsteroidal antiinflammatory drug.

the mean, instead of calculating the standard deviation of the measurements as percentage of the mean (Kanters et al 1998).

Cyclooxygenase, in either isoform *cox1* or *cox2* control synthesis of PGE<sub>2</sub>, which regulates expression of MMP-9. PGE<sub>2</sub> and MMP-9 are elevated in aortic aneurysms. Miralles et al (1999) have shown that indomethacin attenuates aneurysm growth and its effects are mediated via inhibition of the *cox2* isoform, which decreases PGE<sub>2</sub> and MMP-9 synthesis. A large amount of research has implicated increased MMP activity as an important factor in increased extracellular degradation (Lassila 1993; Gronholdt et al 1998; Herman et al 2001). However, it is important to also consider increased collagen synthesis and other extracellular matrix proteins.

Increased PIIINP levels suggest up-regulation of type III collagen synthesis. We found that PIIINP levels are significantly higher in NSAID users, this suggests an up-regulation of type III collagen synthesis. This finding may partly explain the decreased aortic compliance found in patients taking NSAID therapy. However, the association between stiffness, collagen turnover, and aortic aneurysms is far less clear. It is likely that the increased stiffness is determined to a greater extent by other extra-cellular matrix proteins, in particular elastin. This is reflected by the lack of an association between PIIINP and aortic stiffness in this study population as shown in Table 3 (coefficient -0.2). We have reported previously that no association exists between PIIINP turnover and probability of having an aneurysm or an expanding aneurysm (Wilmlink et al 2000). The lack of an association between aortic stiffness and PIIINP is consistent with this previous study. The pathogenesis of aortic aneurysms is multifactorial and poorly understood. It has previously been suggested that decreased compliance in aneurysmal aortas may be protective for risk of rupture. A final increase in compliance just before rupture is thought to reflect failure of arterial wall collagen to carry the wall

load (Wilson et al 1998). Our study group consisted almost entirely of small aneurysms. Elastin may be more important in the initial stages of aneurysm formation. Our study suggests that NSAID use is associated with increased aortic wall stiffness and increased collagen turnover. However, the aortic wall stiffness is probably to a very limited extent determined by collagen in small aneurysms. Therefore, the risk and benefit in terms of taking NSAIDs as protection against aneurysm formation and rupture has not been established by this study and cannot at this point be recommended.

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