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Inflammation and arterial stiffness

Luca Zanoli

Coordinatore Dottorato e Relatore

Chiar.mo
Prof. Pietro Castellino

Dedicated to my wife

For her patience and support during the first two years of our marriage, spent 2194 Km away.

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Background

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1.1 List of abbreviations

CRP = C-reactive protein
ENaC = Epithelial sodium channel
IBD = Inflammatory bowel disease
IMT = Intima-media thickness
IL-6 = Interleukin-6
MMP = Matrix metalloproteinases
NO = Nitric oxide
oxLDL = Oxidised low-density lipoproteins
PWV = Pulse wave velocity

1.2 Introduction

Arterial stiffness is one of the earliest detectable manifestations of adverse structural and functional changes within the vessel wall. It is well known that increased large artery stiffness independently predicts the cardiovascular risk in a variety of populations [1,2]. The identification of populations at higher risk of increased arterial stiffness and the knowledge of the mechanisms involved in arterial stiffening may help to identificate pharmacological and other treatments to reduce the arterial stiffness and improve the outcome of the patients. Recently, new advances have been proposed about the active role of inflammation and endothelial dysfunction in arterial stiffening and early atherosclerosis. The aims of this thesis were to review the literature (chapter 1) and to study for the first time the arterial stiffness in inflammatory bowel disease (chapter 2).

1.3 Arterial stiffness

Large arteries have, aside from their properties of providing a conduit for blood to reach peripheral tissues, a critical role in providing adequate vascular buffering to each ventricular contraction through arterial-ventricular coupling. This phenomenon is of key importance as diastolic flow in arteries may represent more than half of cardiac output. The histological structure of the aorta plays an important role, varying according to its site and function as a reservoir and conductive system (Windkessel principle). Stiffness can be assimilated as the resistance to deformation. The thoracic aorta and its immediate branches are rich in elastin fibers (*elastic arteries*) that allows the support of each systolic impulse and accommodates the stroke volume; more distal vessels become progressively stiffer, with a more prominent component of the muscular fibers (*muscular arteries*).

Pulse wave velocity (PWV) is a measure of regional arterial stiffness, related to the elastic modulus of the arterial wall (which represents the intrinsic stiffness of the wall), the arterial geometry (thickness and radius) and blood density. PWV can be determined by measuring the pulse transit time from the pressure waveforms at the 2 sites along a vascular segment (***Figure 1.1***). The distance (ΔL) is divided by the wave foot-to-foot time (ΔT) it takes for that forward wave to reach the end measuring point (PWV). Pulse wave velocity is inversely related to vascular compliance. Hence, the stiffer the vessel, the higher the pulse wave. Carotid-femoral (*elastic artery*) PWV is a direct measure of aortic stiffness and is used as a diagnostic test for assessment of target organ damage, as outlined in the latest 2007 European Society of

Cardiology (ESC)/European Society of Hypertension (ESH) guidelines for the management of hypertension [3]. To date, the prediction of the cardiovascular risk by the carotid-radial (*muscular artery*) PWV is not demonstrated. Moreover, whether the muscular artery stiffness is influenced by the inflammatory state is not clearly defined.

1.4 Inflammation and arterial stiffness in pathological conditions

There are good evidences that both acute and low-grade chronic inflammation are associated with arterial stiffening. In healthy individuals and in many pathological conditions, including hypertension and human immunodeficiency virus, inflammation level appears as an emerging causal factor for arterial stiffening. In autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus, psoriasis and inflammatory bowel disease, chronic and often intermittent inflammation contributes over time to the destruction of target organs that house inciting antigens or are the sites of immune-complex deposition. For some of these disorders, including rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes mellitus and psoriasis, the association of chronic inflammation and increased arterial stiffness was reported. In inflammatory bowel disease vascular remodelling in involved segments was reported [4]. However, whether the stiffness is increased in arterial districts far away from the intestinal circulation was never tested.

1.4.1 Acute inflammation

Endothelial dysfunction may provide a possible mechanism linking acute inflammation and arterial stiffness [5,6]. Both endothelial dysfunction and arterial stiffness are described in patients with systemic vasculitis [7,8] and in healthy individuals after vaccination [5].

A number of circulating and endothelial-derived factors, including nitric oxide (NO) and endothelin-1, influence large artery stiffness [9-11]. Endothelial dysfunction may lead to functional stiffening of the large arteries reducing NO bioavailability and increasing the activity of opposing mediators such as endothelin-1. Moreover, successful anti-inflammatory therapy in patients with acute inflammation can re-establish 'normal' endothelial function [7,8]. Induction of acute inflammation by vaccination results in endothelial dysfunction in humans [5], which can be attenuated by co-administration of aspirin [12]. In few ours after vaccination, the plasma concentration of the proinflammatory cytokine IL-6 increased. This elevation is clinically relevant because IL-6 is an important stimulus to the synthesis of C-reactive protein (CRP) [13-15] and there are evidences that elevated CRP predicts the risk of cardiovascular events [13-18]. The mechanisms by which CRP may affect vascular function are discussed in **chapter 1.6**.

1.4.2 Chronic inflammation

Recent studies reported an association between chronic low-grade inflammation and arterial stiffening [19,20]. Systemic inflammation appears as an emerging causal factor for increased arterial stiffness in chronic inflammatory disease states such as systemic vasculitis [2], systemic lupus erythematosus [19], rheumatoid arthritis [20], and human immunodeficiency virus [21]. The arterial stiffening in chronic inflammatory disorders can be independent of the presence of atherosclerosis and related to disease duration [19], a marker of chronic inflammation. The associations of disease duration and level of CRP with arterial stiffening in patients with systemic

lupus erythematosus and rheumatoid arthritis suggest that the cumulative burden of inflammation is of primary importance in this abnormality [19]. Evidences that the prevalence of carotid atherosclerosis in rheumatoid arthritis is at least as high as in diabetes mellitus [22] support the hypothesis that chronic low-grade inflammation may have an important role in the development of atherosclerotic lesions. Moreover, the finding that therapy with methotrexate [23,24] reduces the risk of cardiovascular disease confirms the causality and suggests that the inflammatory status should be considered in the management of cardiovascular risk. Finally, in opposite with augmentation index, a composite measure of systemic arterial stiffness dependent on the site and degree of wave reflection and the speed of wave travel (the PWV) [25], brachial PWV is correlated with CRP levels [26]. This finding suggests that inflammation may influence muscular artery stiffness and that an increase in arterial stiffness rather than enhanced wave reflection is associated with increasing inflammatory load.

Arterial stiffness can be caused by both structural and functional changes within the walls of the major conduit arteries [27]. This process can develops via the increased production of reactive oxygen species [28] which, in turn, triggers an inflammatory process leading to the proliferation of smooth muscle cells, the influx of leukocytes, and the production of proinflammatory substances and chemoattractants [29] resulting in an increase in pulse wave velocity. Stiffening of the large arteries may predispose to atherosclerosis, in part, by changes in mechanical stress within the arterial wall and a reduction in shear stress [30].

Chronic inflammation may induce structural changes in the arterial wall, by altering the balance between elastin breakdown and synthesis. An increase in circulating inflammatory mediators promotes white cell infiltration into arteries, and a change in vascular smooth muscle phenotype. Both these cell types release a number of inducible matrix metalloproteinases (MMP), including MMP-9, which can degrade elastin. Also, endothelial dysfunction inflammation-induced leads to several changes in the extracellular matrix including smooth muscle cell proliferation and increased synthesis of structural proteins including collagen [31].

Under inflammatory conditions, vascular smooth muscle cells also express osteoblast markers and can take up phosphate to produce bioapatite, leading to medial calcification and reduced vessel elasticity [32]. In addition, the proteoglycan composition and state of hydration differs in the inflamed arterial wall from that of normal vessels, thus altering the biomechanical properties of large arteries. Finally, perivascular inflammation and cellular infiltration around the vasa vasorum may lead to vessel ischemia which may also promote matrix remodelling and ultimately stiffening of the vessels.

1.4.3 Hypertension

The association between low-grade inflammation, as expressed by CRP, and arterial stiffness was also reported in patients with arterial hypertension [33]. Moreover, a significant relationship between blood pressure levels and inflammation was reported in the normal range of blood pressure [34].

Several mechanisms might explain the relationship between blood pressure levels and inflammation. Hypertension may be the cause of inflammation.

The endothelial injury consequent to the high hydrostatic pressure on endothelial cells may trigger a cascade of inflammatory processes. Indeed, the expression and function of endothelial cell genes are affected, and cytokines and adhesion molecules are secreted [35]. The platelets-dependent release of substances such as P-selectin promotes inflammation [36]. Apart from the participation of the endothelium, angiotensin II also exerts an inflammatory effect, as it has been shown that a chronic increase in the plasma concentration of this hormone is associated with the upregulated expression of adhesion molecules and chemoattractant chemokines in human cells [37]. Moreover, hypertension may be, not only the cause, but also the consequence of chronic inflammation, as suggested by several studies that reported the association between serum CRP levels and the future development of hypertension [38,39].

1.4.4 Rheumatologic diseases

Many rheumatologic diseases are affected by an increase in cardiovascular diseases not completely explained by traditional atherosclerotic risk factors. In these patients, at least part of the excess of cardiovascular risk can be explained by chronic low-grade inflammation.

Rheumatoid arthritis

Patients with rheumatoid arthritis have an increased mortality risk largely due to cardiovascular disease [40–44]. Recent studies suggested that the cardiovascular risk was not completely explained by the traditional risk factors [42-45] or corticosteroid and disease-modifying therapy [41]. Taking in

mind the role of chronic inflammation in atherogenesis [46], the presence of rheumatoid arthritis may by itself explain part of the increased cardiovascular risk reported in these patients. Evidences that the extent and duration of inflammatory burden determine premature atherosclerosis [22,47-49] suggest an important role of inflammation in the pathogenesis of cardiovascular diseases in rheumatoid arthritis. Moreover, many authors recently reported that therapy with methotrexate [23,24] or TNF blockers [50] reduces the cardiovascular risk. These findings suggest that chronic inflammation is a potential target of therapy. Also, patients with rheumatoid arthritis have increased arterial stiffness [19,26,51,52], related to disease duration and inflammation [19,20] and reduced after 12 weeks of treatment with anti-TNF therapy (***Figure 1.2*** [20]) or atorvastatin [53]. Taking into account the role of arterial stiffness in the development of cardiovascular diseases [54], these findings suggest a cause-effect relationship between inflammation, arterial stiffening and increased cardiovascular risk in rheumatoid arthritis. Moreover, taking in mind that the *structural changes* of the arterial wall requires more than 12 weeks to be evident, it is reasonable that the reduction of inflammation produces, at least in the first step, *functional remodelling* of the arterial wall. To date, the effect of a long-lasting correction of inflammation is not known. Finally, to the best of my knowledge, structural and functional changes of the arterial wall are not well studied (i.e. with a high definition echotracking device) in patients with rheumatoid arthritis.

Interestingly, the pleiotropic effect of statins seems to be not significant in patients with chronic inflammation. Mäki-Petäjä et al [55] reported that cholesterol lowering per se has anti-inflammatory effects and ameliorates

aortic stiffness and endothelial dysfunction in patients with rheumatoid arthritis and that there is no difference in disease activity and inflammatory markers between patients treated with statins (cholesterol lowering and pleiotropic effect) or ezetimibe (cholesterol lowering without pleiotropic effect). Future studies are needed to establish whether the reduction of arterial stiffness and improvement of endothelial function (i.e. with antihyperlipidemic agents) translates to an improvement in cardiovascular outcome in patients with rheumatoid arthritis.

Systemic lupus erythematosus

Patients with systemic lupus erythematosus suffer of premature development of coronary artery disease [56-59]. Similarly to patients with rheumatoid arthritis, in these patients the cardiovascular risk is not completely explained by traditional risk factors [60]. Chronic inflammation may help to explain part of the excess risk, as suggested by the onset and progression of subclinical atherosclerosis [61-63] and the burden of coronary artery disease [59,64] according with disease duration. Moreover, the increase of arterial stiffness in patients with systemic lupus erythematosus [19] and its relationship with disease duration and circulating levels of interleukin-6 and C-reactive protein [19] support the hypothesis that, also in these patients, chronic low-grade inflammation is associated with arterial stiffening. Whether anti-inflammatory and immunosuppressive therapy or of statin therapy impacts on arterial stiffening has not been clearly defined in systemic lupus erythematosus. New, well designed, prospective studies are needed to solve these questions.

1.4.5 Inflammatory bowel disease

Over the last few years, endothelium dysfunction has been recognized as the first step in the development of atherosclerosis. Several reports suggested that IBD is affected by intima-media thickening (***Figure 1.3*** [65,66]) and endothelial dysfunction (***Figure 1.4 Panel A*** [67,68,69]) which, in patients with Crohn's disease, improve after administration of TNF-alpha antagonist (***Figure 1.4 Panel B*** [69]). Interestingly, Hatoum et al. [68] observed endothelial dysfunction in chronically inflamed areas of the intestine but not in vessels isolated from segments affected by acute inflammatory processes, thereby suggesting that a prolonged inflammatory stimulus is required to determine endothelial damage. Also, serum C-reactive protein levels at diagnosis are related to the extent of disease in patients with ulcerative colitis and predict surgery in patients with either ulcerative colitis or Crohn's disease [70]. To date, no data are available about arterial stiffness in patients with IBD.

Many studies reported that the prevalence of classical cardiovascular risk factors is lower in patients with IBD than in the general population [71-73]. Interestingly, the role of non-traditional risk factors (i.e. serum white blood cell count) is stronger in IBD patients (***Figure 1.5*** [71]). Low body mass index and lipid levels are commonly seen in IBD patients [71,73-75]. IBD patients had also significantly lower rates of hypertension, diabetes, and obesity [71]. Therefore, given the risk profile of patients with IBD, cardiovascular morbidity and mortality should be lower in these patients than in the general population. However, a meta-analysis reported that the standardized

mortality ratio, which compares the mortality of patients with IBD with the mortality of the general population not considering the different prevalence of classical cardiovascular risk factors in the two groups, is not reduced in IBD patients (***Figure 1.6*** [76]). Moreover, recent studies reported an increased risk of coronary artery disease in IBD patients [71,77]. Taken together, these findings suggests that in patients with IBD the chronic inflammation may have a key role in the determining of cardiovascular risk and that the low cardiovascular risk associated with the low prevalence of cardiovascular risk factors may offset the increased cardiovascular risk associated with chronic inflammation. Further studies are needed to solve these questions.

1.5 Inflammation and atherosclerosis

Atherosclerosis, the leading cause of death worldwide, is increasingly viewed as an inflammatory condition [78]. Some of the inflammatory responses elicited by activated immune cells in target organs of patients with chronic low-grade inflammation seem to share many similarities with those observed within atherosclerotic vessels [79], thereby suggesting that common cellular and molecular mechanisms are effective in both conditions. Several mechanisms by which a systemic inflammatory state can accelerate the atherosclerotic process have been suggested. The most important were the Cytokine-mediated damaging of the endothelium, immune cell activation and activation of the coagulation cascade have all been implicated. Levels of circulating white cells and inflammatory mediators, such as CRP and interleukin-6 (IL-6), are raised in patients with cardiovascular risk factors [80] and are involved in the pathogenesis of atherosclerosis and prognosis. In vitro studies and animal models of atherosclerosis reported direct evidence concerning the role of inflammation in atheroma formation [78]. Oxidised low-density lipoproteins (oxLDL) are involved in the development of atherosclerosis in systemic autoimmune diseases and are considered a pro-inflammatory stimulus which sustains chronic inflammation [81]. Once retained in the intima of the arteries, oxLDL activate endothelial cells and up-regulate the expression of adhesion molecules and the secretion of chemokines which contribute to the recruitment of circulating leukocytes [82]. When monocytes/macrophages infiltrate atherosclerotic plaques, they uptake oxLDL and form the “foam cells” that play a key role in the secretion of

inflammatory mediators [83]. A recent study also showed an association between autoantibodies against oxLDL and CV disease in rheumatoid arthritis [84]. The interaction between oxLDL and C-reactive protein (CRP) forms pro-atherogenic oxLDL/CRP complexes which, beyond perpetuating the vascular inflammation, also trigger an autoimmune response that accelerates the development of atherosclerosis [85]. In addition, CRP has direct pro-inflammatory effects on human endothelial cells in vitro [86] and can induce endothelial dysfunction [87]. Loss of the normal vasodilator, antiplatelet, and antithrombotic properties of the vascular endothelium may contribute to the development of atheromatous plaques via the vasospasm, thrombosis, and inflammation [88].

1.6 C-reactive protein and vascular function

Several studies have been designed with the aim of unravelling the possible mechanisms by which CRP, a protein rising during an inflammatory process, may affect vascular function. Levels of CRP correlate with endothelial function in patients with coronary artery disease [89] and with aortic and brachial PWV [26], peripheral and central pulse pressure in healthy individuals [26,90,91]. Finally, CRP levels independently predict outcome in patients with cardiovascular disease [16,17] and in healthy individuals [13,18].

CRP acts on vascular smooth muscle cells by up-regulating the angiotensin type I receptor [92] and stimulating the migration and proliferation of smooth muscle cells, in addition to the production of reactive oxygen species. Moreover, CRP may participate directly in the inflammatory process [93]. CRP has a direct effect on the endothelial cells, inducing the secretion of specific chemokines, particularly monocyte chemoattractant protein-1, adhesion molecules and E-selectin [86], and decreasing the expression of NO synthase [87] via inhibition of the phosphoinositide 3-kinase/Akt signalling pathway [94].

A recent study suggested a fascinating mechanism by which CRP may affect vascular function [95]. In the presence of aldosterone, CRP induces the insertion of epithelial sodium channel (ENaC) into the plasma membrane, stiffens endothelial cells, and decreases endothelial permeability (***Fig. 1.7*** [95]). This may be a compensatory mechanism to prevent a severe decrease

in blood pressure during acute inflammatory processes, in which the production of NO is increased [96,97].

Within the first minutes after exposure to CRP, a transient softening of the cells is observed. Successively, cells clearly stiffen. Administration of spironolactone or the functional ENaC blocker amiloride prevents the CRP-induced cell stiffening (***Fig. 1.8*** [95]), suggesting that the membrane insertion of ENaC is a prerequisite for CRP-induced cell stiffening.

The ENaC surface expression is increased by aldosterone. CRP enhances the aldosterone response and does not change the biomechanical properties of an endothelial cell as long as aldosterone is absent. These findings indicate that aldosterone is a prerequisite for CRP action and CRP-driven sodium channel expression (***Fig. 1.9*** [95]).

Moreover, the knockdown of the α-ENaC subunit leads to a significant softening of the cortical zone of endothelial cells [99]. It was suggested that the disturbed Na^+ influx may be responsible for the altered biomechanical properties of the cells. Another possibility is that ENaC in the plasma membrane interacts with proteins of the cytoskeleton (i.e., F-actin [100,101]) in the submembranous layer of endothelial cells. It has been suggested that during inflammatory processes, when the levels of both aldosterone and CRP are in the high range, endothelial cells are supposed to be mechanically stiff. This most likely influences NO metabolism. Indeed, stiff endothelial cells release reduced amounts of NO, which leads to increased vascular resistance [102]. Soft cells are more sensitive (i.e., cells are more deformable) to shear stress and thus have the ability to release more NO. In contrast, stiff cells better resist shear stress (i.e., cells are less deformable)

and therefore release less NO [103]. During acute inflammatory processes, a decrease in blood pressure caused by an increased NO production is described [96,97]. In such situation, when the levels of CRP and aldosterone are high, the CRP-induced insertion of ENaC molecules into the plasma membrane and subsequent stiffening of the endothelial cells may therefore lead to reduced NO production and vasoconstriction. The CRP-dependent reduction in endothelial permeability may help to retain fluid in the vascular system, thereby stabilizing the arterial blood pressure.

1.7 Perspectives

As inflammation is related to adverse cardiovascular outcome, impairment of arterial function by inflammation may convey these harmful effects. This may also have important therapeutic implications. Identification of population at risk of increased arterial stiffness and early atherosclerosis before the onset of cardiovascular complications will help to develop more accurate secondary prevention programs. Moreover, identification of a pharmacological or other treatment that could improve arterial stiffness reducing the inflammation will have clinical implications. A major issue would be to determine whether a reduction of arterial stiffness is associated with a concomitant reduction in cardiovascular events of patients with chronic low-grade inflammation, independently of the normalization of classical cardiovascular risk factors.

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1.9 Figures of Chapter 1

Figure 1.1

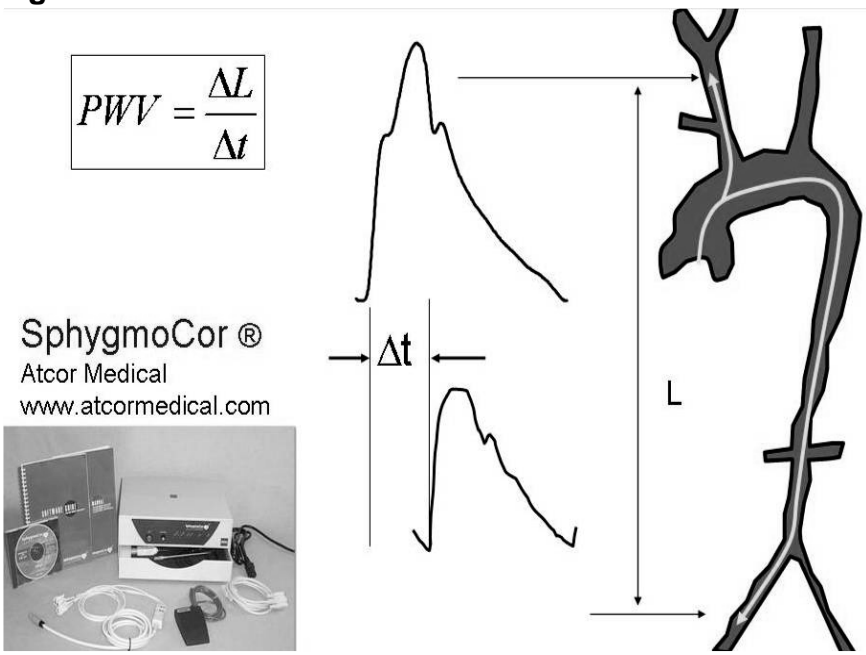

Figure 1.1 legend. Pulse wave velocity (PWV) measurement.
PWV = subtracted distance (ΔL, metres) / delay (Δt, seconds) [54].

Figure 1.2

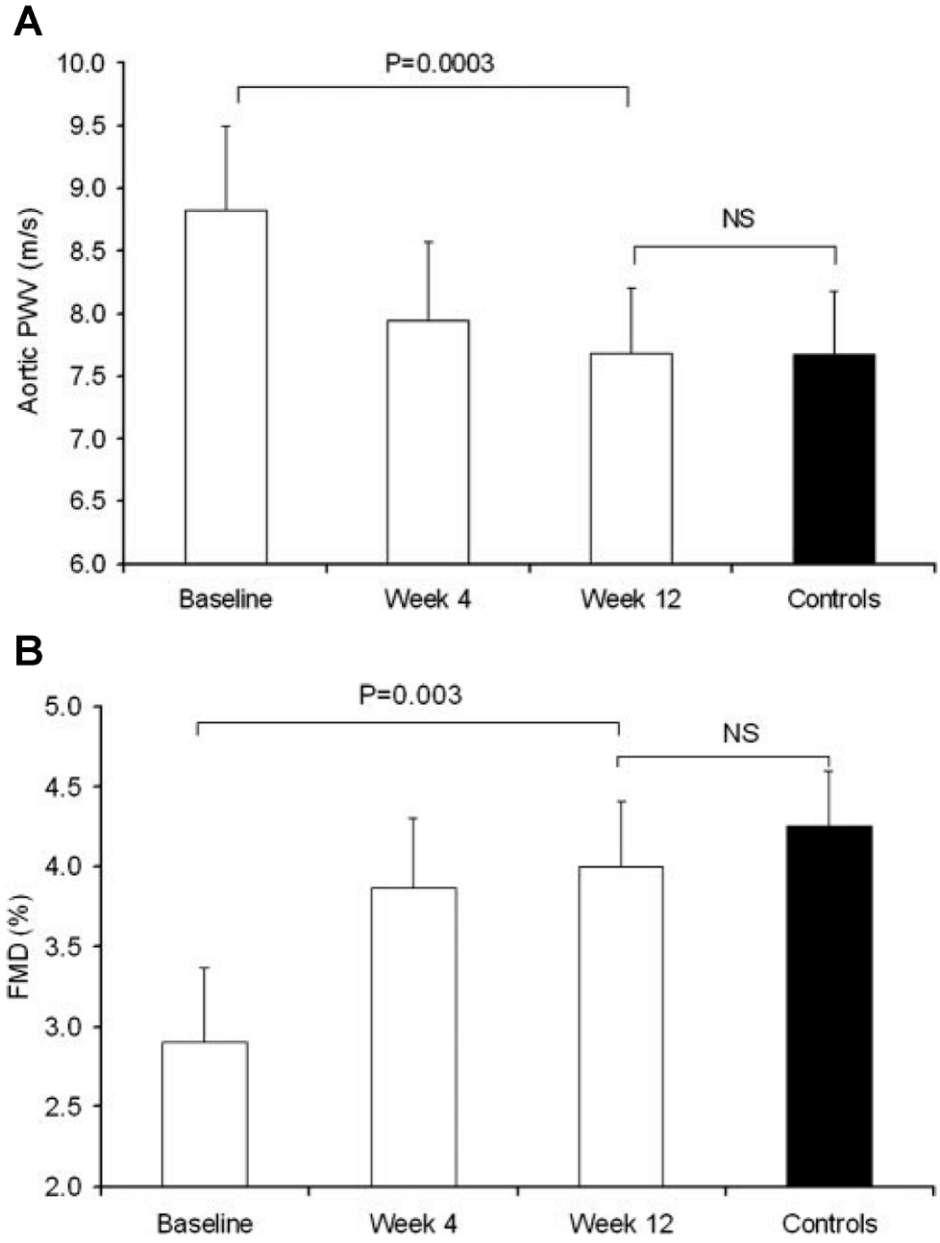

Figure 1.2 legend. The effect of etanercept (anti TNF-α) on aortic PWV (Panel A) and flow-mediated dilatation (Panel B) in patients with Rheumatoid arthritis [20].

Figure 1.3

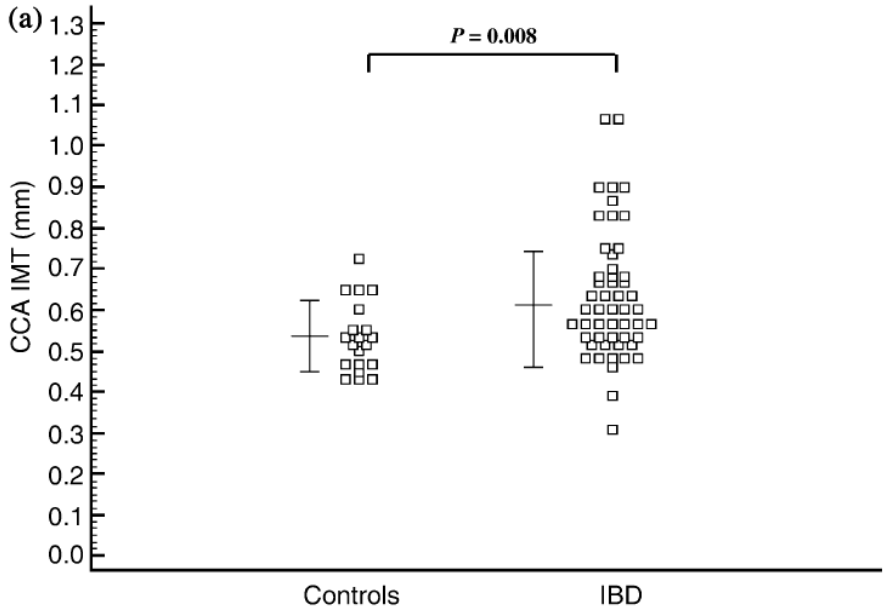

Figure 1.3 legend. Common carotid artery (CCA) intima-media thickness (IMT) in patients with inflammatory bowel disease and controls [66].

Figure 1.4

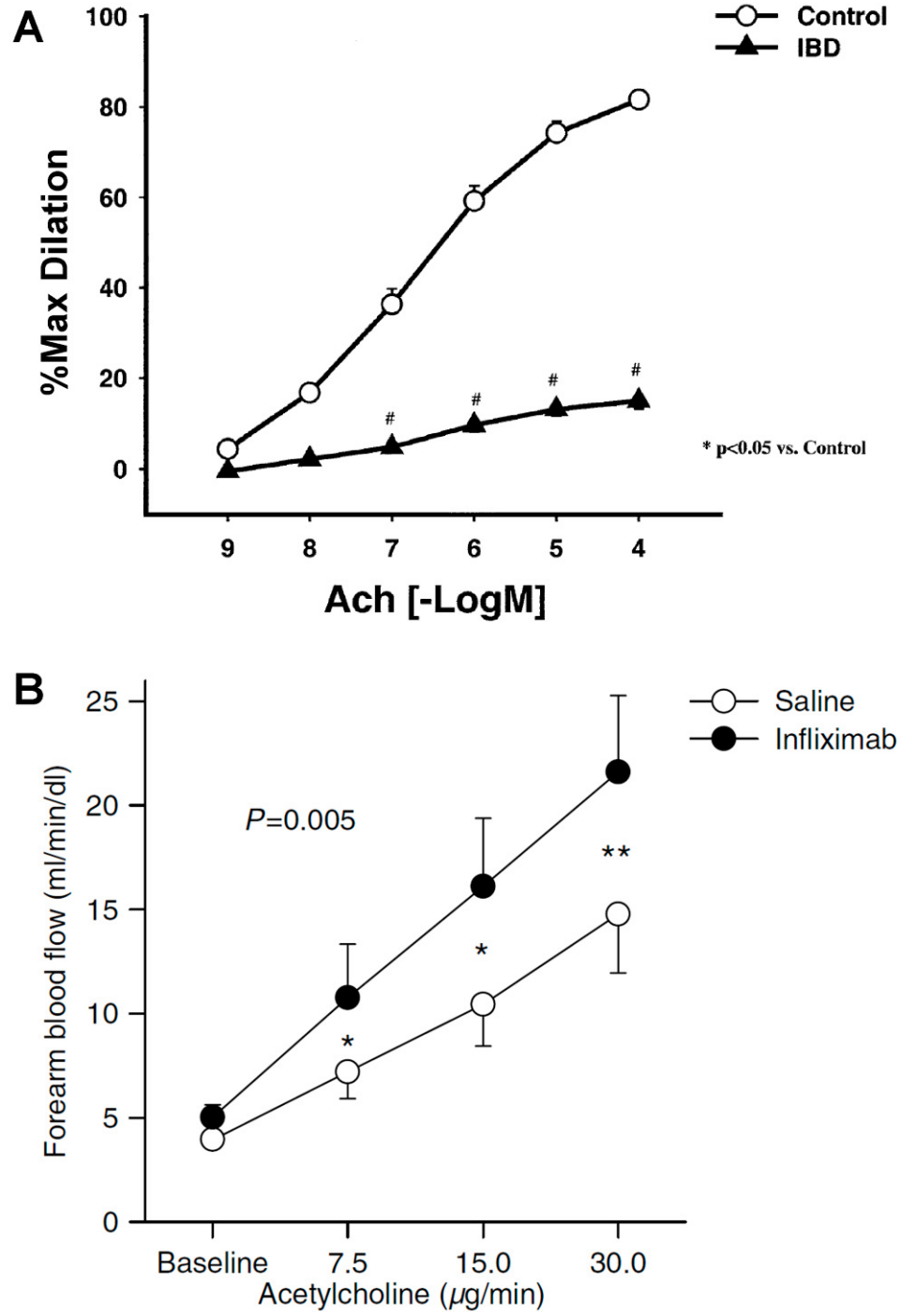

Figure 1.4 legend. Panel A: Responses to Acetylcholine (Ach) in human submucosal intestinal microvessels from patients with inflammatory bowel disease and controls using in vitro videomicroscopy. Microvessels from control tissues dilate in response to increasing doses of Ach, whereas microvessels from IBD tissues showed significantly attenuated dilation to Ach (*P<0.05). Vessels were preconstricted with endothelin 1. The y-axis indicates the percent change from preconstricted diameter. Values are presented as mean ± SEM [68]. **Panel B:** Forearm blood flow values in response to intra-arterial infusion of increasing doses of acetylcholine in patients with Crohn's disease, during concurrent infusion of saline or infliximab (200 mg/min). Values are expressed as mean ± SEM. The P-values refer to the comparison of vascular responses between the treatments by two-way analysis of variance for repeated measures. *P<0.05; **P<0.01 at post hoc pairwise comparisons by Bonferroni t-test [69].

Figure 1.5

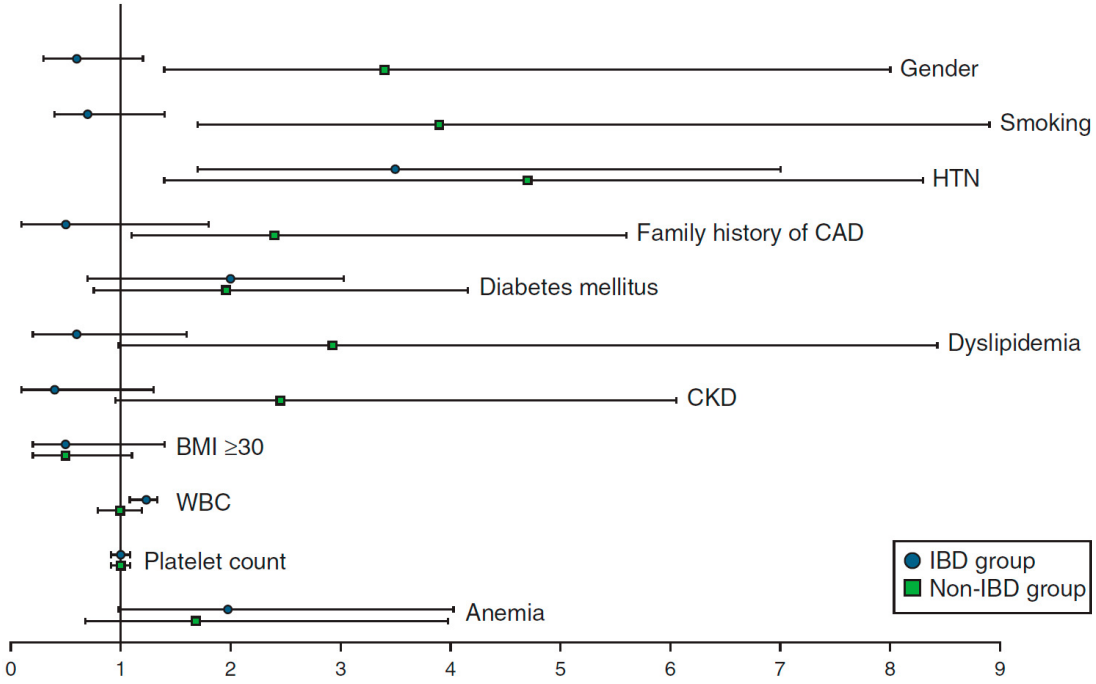

Figure 1.5 legend. Impact of traditional cardiovascular risk factors on combined coronary artery disease events in patients with inflammatory bowel disease and controls. BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; CV, cardiovascular; HTN, hypertension; IBD, inflammatory bowel disease; WBC, serum white blood cell count [71].

Figure 1.6

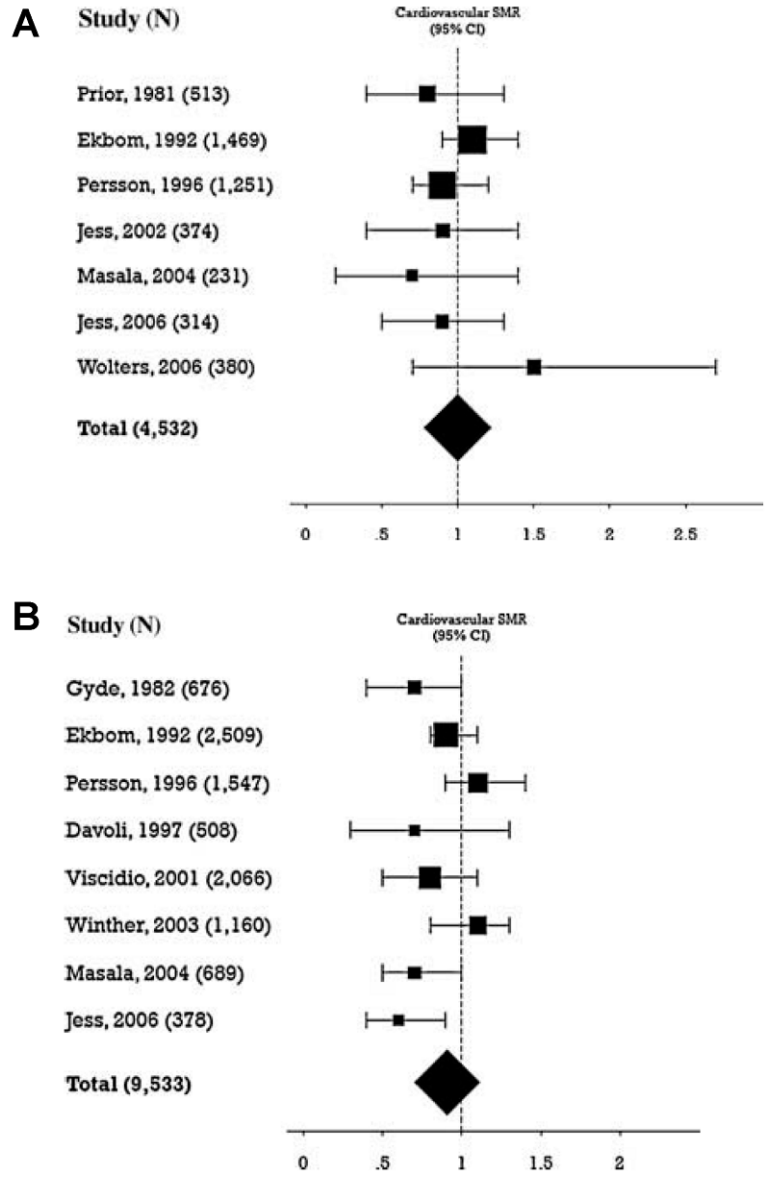

Figure 1.6 legend. Standardized cardiovascular disease mortality ratios in patients with Crohn's disease (**Panel A**) and ulcerative colitis (**Panel B**). [76].

Figure 1.7

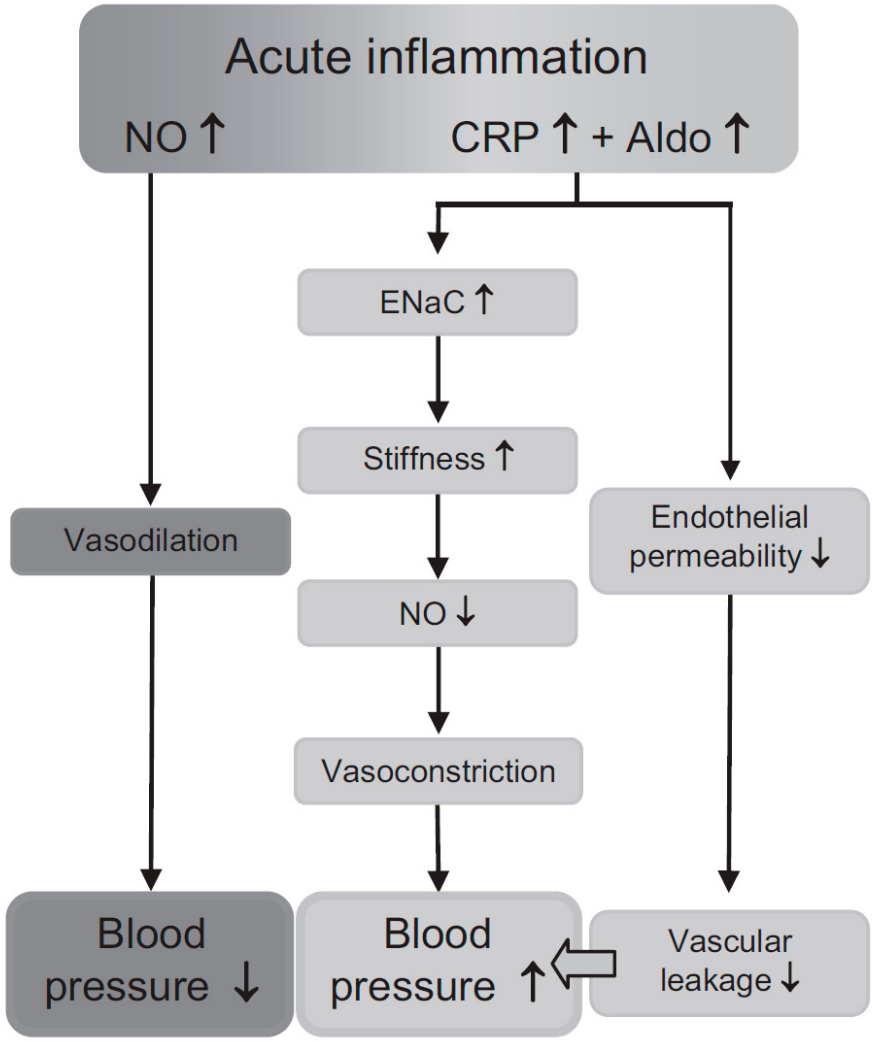

Figure 1.7 legend. How the C-reactive protein (CRP) and aldosterone (Aldo) can stabilize blood pressure during acute inflammatory processes. During inflammation, NO tends to rise, paralleled by a decrease in arterial blood pressure (left side). CRP and aldosterone provide a mechanism of counteraction (center and right side) [89].

Figure 1.8

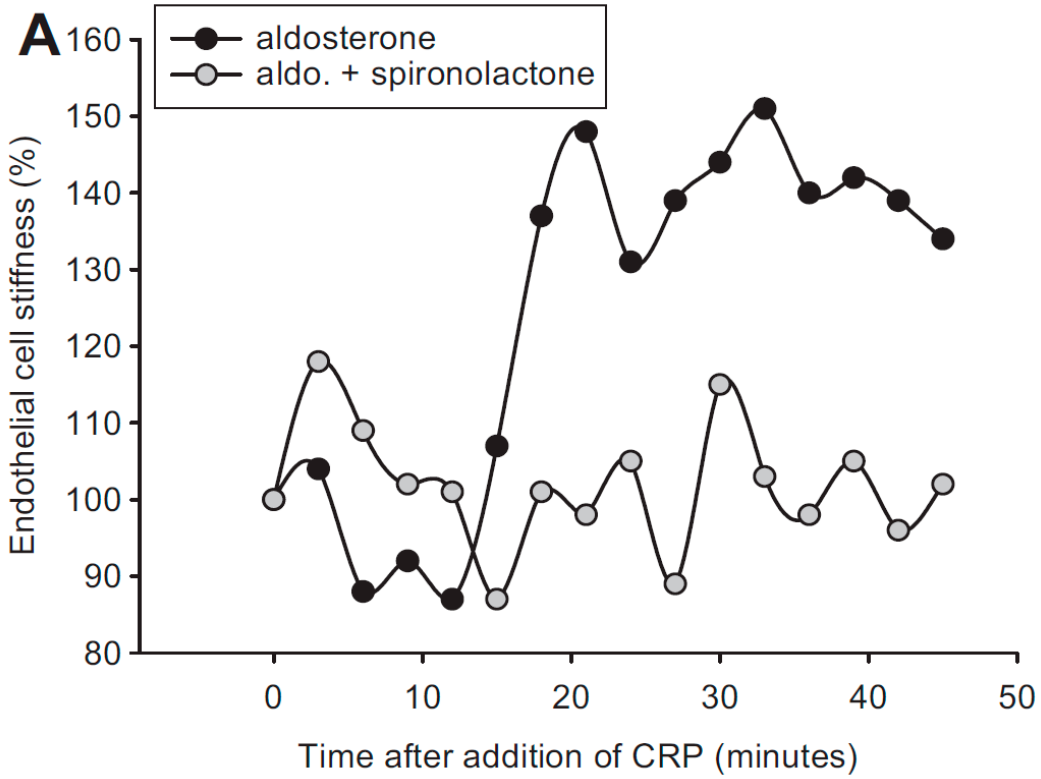

Figure 1.8 legend. Two representative in vitro measurements of endothelial stiffness from cells treated for 24 hours with aldosterone and aldosterone plus spironolactone (start value = 100%) [89].

Figure 1.9

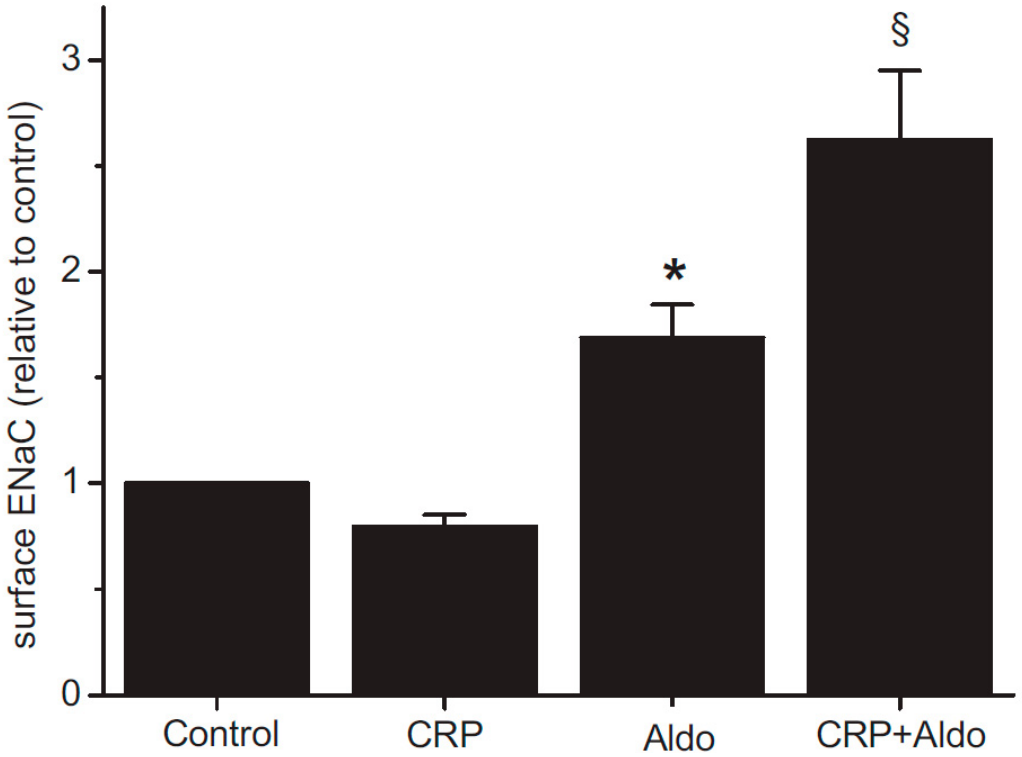

Figure 1.9 legend. Surface epithelial sodium channels (ENaC) measured by immunofluorescence. Amount of mean surface ENaC incubated in CRP, aldosterone (Aldo), and CRP+Aldo is shown relative to the control group (Control).
* P<0.05 compared with Control and CRP; § P<0.05 compared with all other groups [89].

Part II

Research Work

Chapter 2. Arterial stiffness in patients with inflammatory bowel disease

Contents

2.1 List of abbreviations

AIx_{75} = Augmentation index corrected for a steady heart rate of 75 beats/min
CI = confidence interval
DBP = diastolic blood pressure
IBD = Inflammatory bowel disease
PP = pulse pressure
PWV = Pulse wave velocity
SBP = systolic blood pressure

2.2 Abstract

Background & Aims. Recent studies have reported early atherosclerosis in patients with inflammatory bowel disease (IBD). In these patients, the chronic low grade inflammation may predispose to vascular remodelling and arterial stiffening. We aimed at studying arterial stiffness in IBD patients.

Methods. 32 IBD patients without cardiovascular risk factors and 32 matched controls were enrolled (age 19-49 years). SphygmoCor device (AtCor Medical) was used to measure carotid-femoral and carotid-radial (muscular artery) pulse wave velocity (PWV), augmentation index, and central blood pressure.

Results. Carotid-femoral PWV was higher in IBD patients than in controls (6.6±1.4 m/s vs. 6.0±0.8, respectively, $P<0.05$), as well as carotid-radial PWV (8.5±1.2 m/s vs. 7.2±1.0, $P<0.001$). Central pulse pressure was higher in IBD than in controls (32±6 mm Hg vs. 28±7 mm Hg, $P<0.05$). Aging was an important determinant of carotid-femoral PWV in both groups and carotid-radial PWV only in IBD patients. In fully adjusted model performed in both groups of patients considered as a whole, age was positively associated with carotid-femoral PWV ($R^2=0.10$; +0.05 m/s per 1 year of aging, 95% CI 0.01-0.08 m/s, $P<0.05$), as well as IBD ($R^2=0.10$; +0.72 m/s if IBD present, 95% CI 0.19-1.26 m/s, $P<0.05$). In IBD patients, carotid-radial PWV was positively associated with the disease duration ($R^2=0.20$; +0.11 m/s per 1 year of aging, 95% CI 0.03-0.19 m/s, $P<0.05$).

Conclusions. Arterial stiffness is increased in patients with IBD independently of conventional cardiovascular risk factors.

2.3 Keywords

inflammation; pulse wave velocity; central blood pressure.

2.4 Introduction

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. In IBD, intestinal microvascular endothelial cells are damaged by an abnormal immune response resulting in chronic inflammation [1]. Recent studies reported early atherosclerosis [2], altered high density lipoprotein [3], increased carotid intima-media thickness [4], elevated homocysteine [5], and insulin resistance [6] in patients with IBD. In addition, endothelium-dependent vasodilation is impaired [7] and a novel prostaglandin-mediated vasodilatory mechanism has been described in the gut of patients with IBD [8]. To our knowledge few data are available on the arterial elastic properties in IBD [9], although various clinical models of chronic inflammatory diseases are associated with an increased arterial stiffness [10,11].

The role of arterial stiffness in the development of cardiovascular diseases is well known [12]. Arterial elastic properties are increasingly used for stratifying the cardiovascular risk in several populations; aortic pulse wave velocity (PWV) has predictive value for cardiovascular events and all-cause mortality independent of classic cardiovascular risk factors in the general population and in patients at high cardiovascular risk [13-16]; aortic stiffness is listed as a target organ damage to be detected in clinical practice, in the 2007 European guidelines for the management of hypertension and guidelines for cardiovascular disease prevention [17,18].

We hypothesized that IBD, which is characterized by both a chronic, subclinical, systemic inflammation and episodes of acute systemic

inflammation during the reactivation of the disease, is associated with an increased arterial stiffness. Our objective was thus to demonstrate that patients with IBD have a higher arterial stiffness than matched healthy controls, and that the systemic inflammation plays an important role in this process.

2.5 Methods

2.5.1 Study population

A total of 32 young IBD patients (16 patients with Crohn's disease and 16 patients with ulcerous colitis, age 19-49 years) without cardiovascular risk factors were enrolled and paired to 32 matched healthy controls. Exclusion criteria were: hypertension, as defined by blood pressure ≥140/90 mm Hg and/or use of antihypertensive medication; hyperlipidemia and/or use of lipid-lowering medication; diabetes mellitus and/or use of antidiabetic medication; heart failure, as defined by ventricular ejection fraction <50%; smoking; chronic kidney disease, defined as glomerular filtration rate <60ml/min/1.73m^2; obesity, defined as body mass index ≥30 kg/m^2; history of past cardiovascular or cerebrovascular events. The protocol was approved by the local ethics committee, in accordance with the Helsinki Declaration, and all participants gave written informed consent.

2.5.2 Study design

The diagnosis of IBD was based on established criteria of clinical, radiological, endoscopic, and histological findings. Patients with IBD who met the inclusion criteria were included in this analysis. A control group was constituted by healthy subjects matched for age, sex, brachial blood pressure, heart rate, weight and height (case/control ratio: 1/1). The medical history of the patients, including the disease duration, was collected; a routine physical examination was conducted.

2.5.3 Hemodynamic measurements

The non-invasive investigation was performed in a dedicated room after 15 minutes of recumbent rest following the recommendations for standardization of subject conditions [12]. Brachial blood pressure measurements were taken every 2 minutes (Dinamap ProCare 100; GE Healthcare). Central pressures were recordered noninvasively by applanation tonometry (SphygmoCor; AtCor Medical, Sydney, Australia), as previously described and validated by comparison with simultaneous invasive pressure recordings [19-21]. Tonometry uses a transfer function from the radial to the aortic site, for estimating central blood pressure, and requires an absolute calibration performed with brachial cuff measurements of diastolic and mean blood pressure in the contralateral arm in order to determine the aortic pressure waveform [12].

An important physics principle is that the pulse travels at a higher velocity in a stiff vessel and more slowly in an elastic vessel. PWV, an established index of arterial stiffness [12,13,22] was measured by a well accepted device (SphygmoCor; AtCor Medical, Sydney, Australia) using the foot-to-foot velocity method. Briefly, waveforms were obtained transcutaneously over the common carotid artery and the right femoral or radial artery, and the delay was measured between the feet of the two waveforms. The distance covered by the waves was estimated subtracting the distance from the carotid location to the sternal notch from the distance between the sternal notch and the femoral or radial site of measurement [12]. The equation used in the present report for calculating PWV is as follows:

PWV = subtracted distance (metres) / delay (seconds) [12].

Both carotid-femoral (aortic) and carotid-radial (muscular artery) PWV were measured.

The augmentation index represents a composite measure of the magnitude of wave reflection and arterial stiffness which affects timing of wave reflections. The augmentation index was measured on the central pressure waves determined by applanation tonometry, averaged from 10-12 successive waves and corrected for a steady heart rate of 75 beats/min (AIx_{75}) [23].

2.6 Results

The matching process worked well, patients and controls were comparable for age, sex ratio, blood pressure, heart rate, weight and height (Table 2.1).

2.6.1 Clinical characteristics of patients with IBD

The characteristics of the populations are presented in Table 1. IBD patients were relatively young (30±9 years) and predominantly males (19 [59%] males and 13 [41%] females). The mean disease duration was 63±61 months (min-max 0-267 months). Healthy controls were well matched and there was no significant difference between IBD and controls regarding demographics (Table 2.1).

Among IBD patients, 50% (n=16) had ulcerative colitis and 50% (n=16) had Crohn's disease. There were no differences between patients with ulcerative colitis and Crohn's disease regarding demographics and arterial parameters. Of IBD patients, 88% (n=28) were in remission, whereas 12% (n=4) had active disease; among patients with IBD, 66% (n=21) had no complication, whereas 22% (n=7) had stricture, 25% (n=8) had fistula, and 22% (n=7) had abscess. Among IBD patients, 38% (n=12) were treated with only salicylates, while the remaining 62% (n=20) were treated by salicylates and steroids or immunosuppressors.

2.6.2 Arterial parameters

Brachial blood pressure and heart rate were comparable in patients with IBD and in controls. IBD patients had a higher central pulse pressure than controls (32±6 mm Hg vs. 28±7 mm Hg, respectively, P<0.05; Figure 2.1, panel A) and a higher carotid-femoral PWV (6.6±1.4 m/s vs. 6.0±0.8 m/s, P<0.05; Figure 2.1, panel B). AIx_{75} was not significantly increased in IBD. Carotid-femoral PWV was higher in IBD than in controls (6.6 m/s vs. 6.0 m/s, P<0.05). A significant relationship between age and carotid-femoral PWV was observed in both patients with IBD and controls (Figure 2.2, panel B), whereas a significant relationship between age and carotid-radial PWV was observed only in IBD (PWV=5.94 + 0.09.age, P<0.001) (Figure 2.2, panel B). Carotid-radial PWV was higher in patients with IBD than in controls (8.5 m/s vs 7.2 m/s, P<0.001). A significant relationship was observed between the disease duration and carotid-radial PWV (Figure 2.3, panel A). No significant difference in carotid-femoral PWV was observed between patients with ulcerative colitis and Crohn's disease (6.8 m/s vs 6.5 m/s, respectively, NS) and in carotid-radial PWV (8.7 m/s vs 8.3 m/s, NS).

In multiple regression analysis involving the entire population (Table 2.2), IBD was significant determinant of carotid-femoral PWV, explained 10% of its variance, even after adjustment for age. The presence of an IBD shifted the age-PWV relationship upward (Figure 2.2, panel B), by 0.72 m/s. In fully adjusted model performed in both groups of patients considered as a whole, age was positively associated with carotid-femoral PWV (R^2=0.10; +0.05 m/s per 1 year of aging, 95% CI 0.01-0.08 m/s, P<0.05), as well as IBD (R^2=0.10; +0.72 m/s if IBD present, 95% CI 0.19-1.26 m/s, P<0.05).

2.7 Discussion

This is the first study designed to determine arterial stiffness in young IBD patients without known cardiovascular risk factors. The major result of this study is that the stiffness of elastic and muscular arteries is increased in IBD patients compared with matched healthy controls.

2.7.1 Interpretation of the data

The stiffness of both elastic and muscular arteries is increased in patients with IBD. Several mechanisms can play a role in this process. It is well known that the level of inflammation could be related to both carotid-femoral and carotid-radial PWV [24-26]. Recent studies have reported an association between chronic low grade inflammation and arterial stiffening [10,11]. Systemic inflammation thus appears as an emerging causal factor for increased arterial stiffness in chronic inflammatory disease states such as systemic vasculitis [11], systemic lupus erythematosus [10], rheumatoid arthritis [10], and human immunodeficiency virus [27]. Moreover, it has been reported that even an acute, mild, transient inflammatory stimulus may lead to deterioration of large artery elastic properties [28]. However, the arterial stiffening in chronic inflammatory disorders can be independent of the presence of atherosclerosis and related to disease duration [10] or, alternatively, can be a manifestation of vascular disease preceding. Several mechanisms by which a systemic inflammatory state can accelerate the atherosclerotic process have been suggested. Cytokine-mediated damaging of the endothelium, immune cell activation and activation of the coagulation

cascade have all been implicated. IBD seems to be the result of a combination of environmental, genetic, and immunologic factors where an uncontrolled immune response within the intestine leads to inflammation in genetically predisposed individuals [29]. Dysfunctions of the intestinal immune system and cross-reactivity against host epithelial cells have been implicated as major mechanisms by which inflammation occurs [30]. Early atherosclerosis is a clinical feature common to several inflammatory and immunological diseases [30]. Several reports have suggested that IBD is associated with premature atherosclerosis by demonstrating IMT thickening [2,4] and endothelial dysfunction [7]. The latter seems to improve after administration of TNF-alpha antagonist [31].

Many studies reported that the prevalence of classical cardiovascular risk factors is lower in patients with IBD than in the general population [3,32-35]. Low body mass index and lipid levels were previously seen in IBD patients [32-35]. IBD patients had also significantly lower rates of hypertension, diabetes, and obesity [35]. Therefore, given the risk profile of patients with IBD, cardiovascular morbidity and mortality should be lower in these patients than in the general population. However, a meta-analysis reported that the standardized mortality ratio is not reduced in IBD patients [36]; recent studies reported an increased risk of coronary artery disease in IBD patients [37,38]. We think that IBD represents a useful model to study the effect of chronic low-grade inflammation in the development of cardiovascular diseases. In patients with IBD, the low cardiovascular risk associated with the low prevalence of cardiovascular risk factors may offset

the increased cardiovascular risk associated with chronic inflammation. A better comprehension of these concomitant and inverse effects, mostly not considered in the cardiovascular risk stratification of IBD patients, could help to clarify whether IBD is associated or not with an increased cardiovascular risk. At this regards, the arterial stiffening could represents a link between chronic inflammation and cardiovascular risk in IBD patients.

Another important finding of this report is the significant increase of carotid-radial PWV according with the disease duration. This finding is clinically relevant and may help to understand the association between inflammation and arterial stiffening. Disease duration can be considered a marker of inflammation; therefore, patients with longer disease duration were exposed to a significantly higher amount of inflammation than patients with short disease duration. Interestingly carotid-radial PWV, but not carotid-femoral PWV, was significantly increased according with the disease duration. These findings suggest different mechanisms in the stiffening of elastic and muscular arteries in response to aging and inflammation.

Indeed, in the present study, chronic inflammation (i.e. IBD) increased aortic stiffness at any given age, suggesting that arterial stiffening provided by IBD was additive to that of normal aging, representing a 14 years acceleration. Aging is associated with a number of molecular changes of the load-bearing media of elastic arteries: the orderly arrangement of elastic fibers and laminae is gradually lost over time, and thinning, splitting, fraying and fragmentation are observed. The degeneration of elastic fibers is associated with an increase in collagenous material and in ground substance, often

accompanied by calcium deposition in ground substance and in degenerate elastic fibers [40,41]. By contrast, muscular arteries, like the brachial and radial arteries, do not stiffen with aging in normal subjects [42-44]. The present results suggest that the stiffening process induced by IBD and associated inflammation differs from that of aging (Figure 2.2, panel A).

In clinical practice, measuring carotid-radial stiffness in IBD may help to estimate the amount of damage induced by inflammation on the arterial system. Measuring carotid-femoral stiffness may help to better predict the cardiovascular risk in these patients. Indeed, arterial stiffness, which is increased in high cardiovascular risk populations such as in patients with chronic kidney disease, hypertension, diabetes, hypercholesterolemia, and smoking [45-50], has a predictive value for CV events and all-cause mortality independent of classic CV risk factors in the general population and in patients at high cardiovascular risk [13-16]. The carotid-femoral PWV of patients with IBD enrolled in the present study, expressed according to the reference value project [39], was above the 75th percentile of the normal value reported in healthy people with comparable age and blood pressure levels [39]. This finding is consistent with the results of the present report and supports the hypothesis that the cardiovascular risk of patients with IBD is increased.

In the present study, central PP was increased in patients with IBD, very likely as a result of an increased aortic stiffness, favouring the early return of wave reflection. Central blood pressure is now well accepted as the true

load damaging target organs and being responsible for cardiovascular events.

The increased arterial stiffness detected in the present work in patients with IBD and in other chronic inflammatory disease [10,11] and the recent evidences of early atherosclerosis in IBD patients [2,3] support the role of inflammation in the pathogenesis of cardiovascular diseases. It is estimated that only one half of the risk for cardiovascular disease is explained by conventional risk factors, including blood pressure. Indeed, newly individualized risk factors are not taken into account, particularly markers of small and large artery damage, including small artery remodelling, carotid intima-media thickening, endothelial dysfunction, and arterial stiffening. All of these parameters have demonstrated their predictive value for cardiovascular events in high cardiovascular risk patients. Larger epidemiological studies are needed in patients with IBD to confirm the results of the present report and to further clarify whether the chronic inflammation and the arterial stiffening are associated with the cardiovascular risk of patients with non conventional risk factors.

2.7.2 Methodological issues

The present study has several strengths. First, because age, gender, blood pressure, heart rate, weight, height and many cardiovascular risk factors are important determinants of arterial stiffness, we compared IBD patients to controls of similar age, sex ratio, brachial blood pressure, heart rate, weight and height, and excluded from this analysis IBD patients and controls with

significant cardiovascular risks and open cardiovascular diseases. Second, this is the first study which has performed a comprehensive measurement of the elastic and muscular artery stiffness in patients with IBD. Third, we used the gold standard method for assessing arterial stiffness, and measured carotid-femoral PWV with a high-fidelity applanation tonometer (SphygmoCor; AtCor Medical, Sydney, Australia) [12].

This study also has some limitations. The current study is a cross-sectional one; therefore, causation cannot be determined for any of the observed relationships. Nonetheless, the findings show strength of association, temporality, consistency, biological plausibility and gradient, coherence with previous studies, and are analogous to the results reported in other population with chronic inflammation. These features make it probable that the findings reflect a biological phenomenon [51].

Our study population is small limiting the ability to generalize the findings to other clinical settings. Despite the small study population, post hoc power analysis revealed that the examined sample size provided adequate power for multiple regression analysis (94%) with a type 1 error rate <0.05.

2.8 Conclusions

In conclusion, the present study documents, for the first time, increased aortic and muscular artery stiffness in IBD patients and provides evidences demonstrating the potential contribution of inflammation to the arterial stiffening.

2.9 Perspectives

Larger epidemiological studies are needed in patients with IBD to confirm the results of the present report and to further clarify whether the stiffness of muscular and elastic arteries is influenced by chronic low-grade inflammation. Another important open question is the impact that the treatment of chronic low-grade inflammation (i.e. with anti-inflammatory medicaments, statins and anti-TNFα) has on the reduction of arterial stiffness and cardiovascular risk in patients with IBD. Last, the study of the arterial function with a high resolution echotracking device will help to clarify the effect of classical and new cardiovascular risk factors on arterial stiffness in patients with chronic low-grade inflammation.

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2.11 Tables of Chapter 2

Table 1. Main clinical data of study population

Parameters	**IBD M (SD)**	**Controls M (SD)**	**P value**
Patients, n	*32*	*32*	
Age, years	30 (9)	31 (7)	NS
Male gender, %	59	59	NS
Weight, Kg	67.1 (14.0)	69.0 (12.6)	NS
Height, m	1.68 (0.10)	1.68 (0.10)	NS
BMI, Kg/m^2	23.5 (3.6)	24.3 (2.8)	NS
Heart rate, b/min	68 (9)	65 (10)	NS
Brachial SBP, mm Hg	115 (10)	113 (11)	NS
Brachial DBP, mm Hg	66 (10)	68 (8)	NS
Brachial PP, mm Hg	49 (10)	45 (11)	NS
Central SBP, mm Hg	99 (11)	97 (8)	NS
Central DBP, mm Hg	67 (11)	69 (9)	NS
Central PP, mm Hg	32 (6)	28 (7)	<0.05
Carotid-femoral PWV, m/s	6.6 (1.4)	6.0 (0.8)	<0.05
Carotid-radial PWV, m/s	8.5 (1.2)	7.2 (1.0)	<0.001
Augmentation index, %	7.4 (10.5)	1.5 (15.4)	NS

SBP, systolic blood pressure; DBP, diastolic blood pressure, PP, pulse pressure; PWV, pulse wave velocity.

Table 2. Determinants of arterial stiffness in IBD patients and controls considered as a whole.

Parameters	R^2 increment	Beta coeff.	Lower CI	Upper CI	P-value
Dependent variable: carotid-femoral PWV					
Age, years	0.10	0.05	0.01	0.08	<0.05
IBD	0.10	0.72	0.19	1.26	<0.05
R^2=0.19					

Beta unit: m/s. IBD, inflammatory bowel disease; PWV, pulse wave velocity; CI, confidence interval.

2.12 Figures of Chapter 2

Figure 2.1

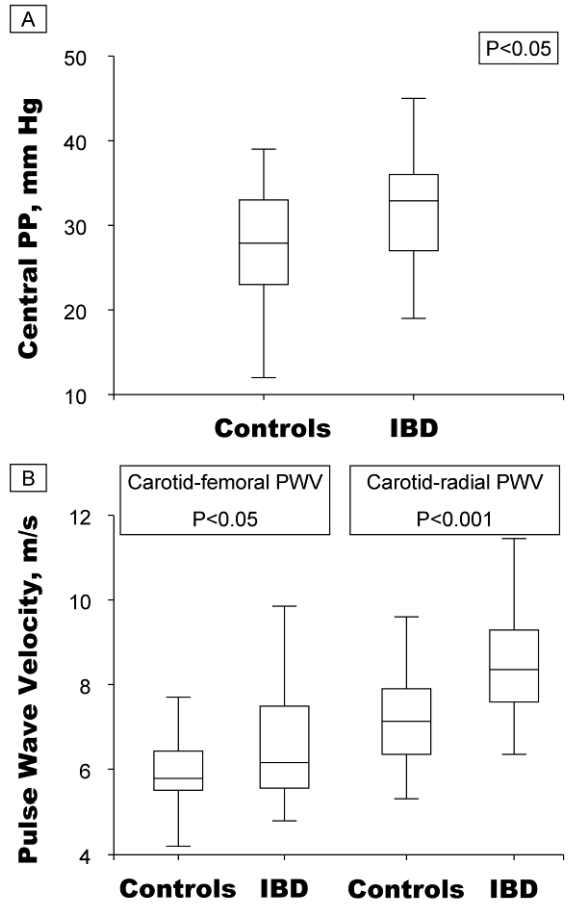

Figure 2.1 Legend. Central pulse pressure (Panel A), and arterial stiffness (Panel B) in patients with inflammatory bowel disease and in controls.

Figure 2.2

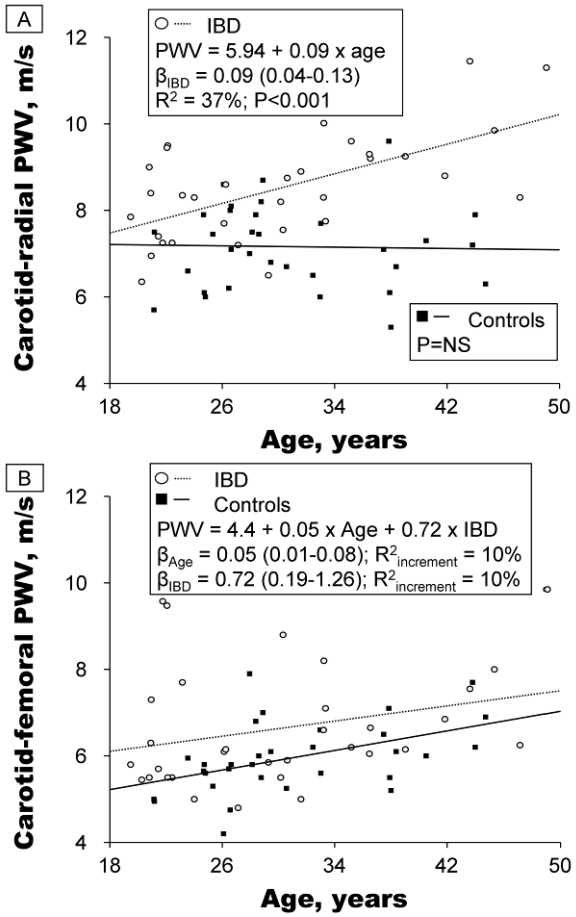

Figure 2.2 Legend. Relationship between age of the patients and arterial stiffness. Panel A: carotid-radial (muscular artery) PWV; Panel B: carotid-femoral (aortic) PWV.

Figure 2.3

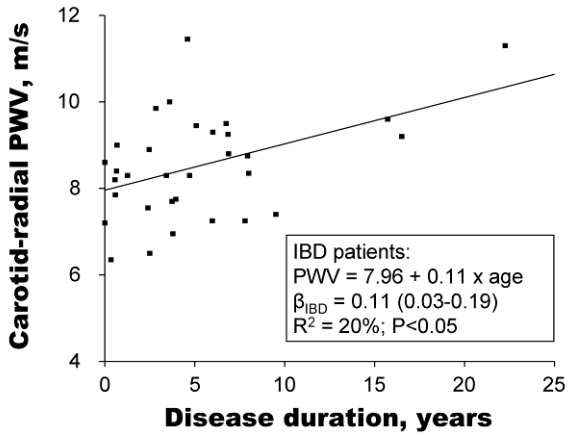

Figure 2.3 Legend. Relationship between disease duration and carotid-radial (muscular artery) PWV.