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Effects of exercise training on cardiovascular risk factors and biomarkers of endothelial function / inflammation in coronary artery disease patients.

Fernando Manuel Tavares da Silva Ribeiro

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Dissertação apresentada às provas para obtenção do grau de Doutor em Actividade Física e Saúde, nos termos do Decreto-Lei nº 216/92 de 13 de Outubro, orientada pelo Prof. Doutor José Manuel Fernandes Oliveira e co-orientada pelo Prof. Doutor José Alberto Ramos Duarte

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KEY WORDS: EXERCISE TRAINING; ENDOTHELIAL FUNCTION; INFLAMMATION; CORONARY ARTERY DISEASE; CARDIOVASCULAR RISK FACTORS.

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I wish to dedicate this manuscript in memory of my grandparent Fernando Ribeiro who, with his superb example, taught me to always aim higher and reach farther.

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João Venâncio, my PhD mate, we have build a beautiful friendship that will last forever.

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ABSTRACT

Purpose: to assess in coronary artery disease patients (i) the effects of exercise training on biomarkers of endothelial function and inflammation, and (ii) the contribution of age and changes in traditional risk factors, cardiorespiratory fitness, and left ventricular function to the modification of the endothelial dysfunction and inflammation. **Methods:** Forty-seven patients after acute myocardial infarction participated in this study. Patients were randomly assigned to an exercise-training group (age: 54.3±10.8 yrs) or to a control group (age: 57.0±7.6 yrs). The exercise-training group participated in an 8-week aerobic exercise-training programme (3-sessions a week), whereas the control group received usual medical care. At baseline (1 month after hospital discharge) and after 8 weeks the following parameters were assessed: lipid profile, N-terminal pro-B-natriuretic peptide, endothelial function (soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1) and inflammatory biomarkers [C-reactive protein, interleukin (IL)-6, -10], anthropometrics (weight, height, body composition), resting and peak haemodynamics (heart rate, systolic and diastolic blood pressure), left ventricular ejection fraction, cardiorespiratory fitness, daily physical activity, and dietary intake. **Results:** Exercise training increased the circulating levels of the anti-inflammatory cytokine IL-10 [from 5.82 (2.59-9.05) to 6.36 (4.05-14.66) pg/mL, *P*=.036]. Exercise training also increased heart rate recovery (20.0±6.4 to 24.0±4.7 bpm, *P*=.007), cardiorespiratory fitness (31.4±7.7 to 33.7±8.0 ml/min/kg, *P*=.016), and daily moderate-intensity physical activity (37.1±25.5 to 50.0±32.3 min, *P*=.031), and decreased resting heart rate (68.0±9.2 to 62.6±8.7 bpm, *P*=.03). The improvement in IL-10 was strongly associated with increased moderate-intensity physical activity. In the contrary, control group did not change inflammatory biomarkers and increased the endothelial dysfunction. **Conclusions:** The exercise-training programme revealed an anti-inflammatory effect, expressed by the enhancement of the levels of IL-10, and counteracted the progressive deterioration of the endothelial function. Additionally, the improvement of moderate-intensity physical activity had an important contribution for the anti-inflammatory effect of the exercise-training programme.

KEY WORDS: EXERCISE TRAINING; ENDOTHELIAL FUNCTION; INFLAMMATION; CORONARY ARTERY DISEASE; CARDIOVASCULAR RISK FACTORS.

RESUMO

Objectivo: avaliar em pacientes com doença das artérias coronárias (i) os efeitos de um programa de exercício físico nos biomarcadores de função endotelial e inflamação, e (ii) a contribuição da idade e das alterações nos factores de risco tradicionais, na capacidade cardiorespiratória e na função ventricular para a modificação da disfunção endotelial e da inflamação. **Métodos:** participaram no estudo 47 pacientes com história de enfarte agudo do miocárdio, que foram aleatoriamente divididos em 2 grupos: exercício (idade: 54.3±10.8 anos) e controlo (idade: 57.0±7.6 anos). O grupo de exercício participou num programa de 8 semanas (3 sessões/semana) de treino aeróbio, enquanto o grupo controlo recebeu o seguimento médico habitual. Antes do programa (1 mês após alta hospitalar) e após as 8 semanas foram avaliados os seguintes parâmetros: perfil lipídico, fragmento N terminal do peptideo natriurético tipo B, biomarcadores de função endotelial (molécula de adesão intracelular-1 solúvel e molécula de adesão vascular-1 solúvel) e inflamação [proteína reactiva-C, interleucina (IL)-6, -10], antropometria (peso, altura, composição corporal), variáveis hemodinâmicas em repouso e no pico do exercício (frequência cardíaca, pressão arterial), fracção de ejecção do ventrículo esquerdo, capacidade cardiorespiratória, actividade física, ingestão alimentar. **Resultados:** o exercício físico aumentou os níveis circulatórios da citocina anti-inflamatória IL-10 [5.82 (2.59-9.05) para 6.36 (4.05-14.66) pg/mL, *P*=.036]. Adicionalmente, também melhorou a frequência cardíaca de recuperação (20.0±6.4 para 24.0±4.7 bpm, *P*=.007), a capacidade cardiorespiratória (31.4±7.7 para 33.7±8.0 ml/min/kg, *P*=.016), e os níveis de actividade física de intensidade moderada (37.1±25.5 para 50.0±32.3 min, *P*=.031), e diminuiu a frequência cardíaca de repouso (68.0±9.2 para 62.6±8.7 bpm, *P*=.03). O aumento da IL-10 associou-se fortemente com o aumento da actividade física de intensidade moderada. Pelo contrário, o grupo controlo não melhorou os níveis do biomarcadores inflamatórios e aumentou a disfunção endotelial. **Conclusões:** O programa de exercício físico revelou um efeito anti-inflamatório, expressou pela IL-10, e mitigou a deterioração da função endotelial. Adicionalmente, o aumento da actividade física moderada contribuiu indubitavelmente para o efeito anti-inflamatório do exercício.

PALAVRAS-CHAVE: EXERCÍCIO FÍSICO; FUNÇÃO ENDOTELIAL; INFLAMAÇÃO; DOENÇA DAS ARTÉRIAS CORONÁRIAS; FACTORES DE RISCO CARDIOVASCULAR.

List of abbreviations

ADP: adenosine diphosphate

AMP: adenosine monophosphate

ATP: adenosine triphosphate

BNP: B-type natriuretic peptide

Ca^{2+}: calcium ion

CAD: coronary artery disease

cAMP: cyclic adenosine monophosphate

CAMs: cell adhesion molecules

CECs: Circulating endothelial cells

cGMP: cyclic guanosine monophosphate

Chemokine R: chemokine receptor

CHVNG/E: Centro Hospitalar de Vila Nova de Gaia/Espinho

COX-2: cyclooxygenase-2

CR: cardiac rehabilitation

CRP: C-reactive protein

DBP: diastolic blood pressure

DBPpeak: peak diastolic blood pressure

E-selectin: endothelial-leukocyte adhesion molecule

ECG: electrocardiogram

eNOS: endothelial nitric oxide synthase

EPC: Endothelial progenitor cells

ET-1: endothelin-1

ET_A: endothelin receptor type A

ET_B: endothelin receptor type B

factor-Xa: activated factor X

GM-CSF: granulocyte-macrophage colony-stimulating factor

GTP: guanosine triphosphate

H^+: hydrogen ion

HbA1c: haemoglobin A1c

HDL: high-density lipoprotein

HR: heart rate

HRpeak: peak heart rate

HRR: heart rate recovery

ICAM-1: intercellular adhesion molecule-1

ICAMs: intercellular adhesion molecules

IFN-γ: interferon-γ

IL-1: interleukin-1

IL-10: interleukin-10

IL-4: interleukin-4

IL-6: interleukin-6

IL-8: interleukin-8

IL: interleukin

L-selectin: leukocyte adhesion molecule

LDL: low density lipoprotein

MMP-2: matrix metalloproteinase-2

MMP-9: matrix metalloproteinase-9

MMP: matrix metalloproteinase

mRNA: messenger ribonucleic acid

MS: metabolic syndrome

NADPH: nicotinamide adenine dinucleotide phosphate

˙NO: nitric oxide

NOS: nitric oxide synthase

NT-proBNP: N-terminal pro-B-natriuretic peptide

P-selectin: platelet adhesion molecule

PA index: physical activity index

PAF-R: platelet activating factor receptor

PAF: platelet activating factor

PAI-1: plasminogen activator inhibitor-1

PCI: percutaneous intervention

PECAM-1: platelet-endothelial cell adhesion molecule-1

PGG_2: prostaglandin G_2

PGH_2: prostaglandin H_2

PGI_2: prostacyclin

PLA_2: phospholipase A_2

PPaRα: peroxisome proliferator activated receptor-α

PPaRγ: peroxisome proliferator activated receptor-γ

RCT: randomized controlled trial

RER: respiratory exchange ratio

ROS: reactive oxygen species

RPP: rate-pressure product

RPPpeak: peak rate pressure product

SBP: systolic blood pressure

SBPpeak: peak systolic blood pressure

sE-selectin: soluble endothelial-leukocyte adhesion molecule

sICAM-1: soluble intercellular adhesion molecule-1

sP-selectin: soluble platelet adhesion molecule

sTM: soluble thrombomodulin

sVCAM-1: soluble vascular cell adhesion molecule-1

t-PA: tissue plasminogen activator

TC: total cholesterol

TFPI: tissue factor pathway inhibitor

TM: thrombomodulin

TNF-α: tumor necrosis factor-α

TNF: tumor necrosis factor

VCAM-1: vascular cell adhesion molecule-1

VCAMs: vascular cell adhesion molecules

VCO_2: volume carbon dioxide

V_E: minute ventilation

VLA-4: very late antigen-4

VLDL: very low density lipoprotein

VO_2: volume of oxygen uptake

VO_2peak: peak oxygen uptake

vWF: von Willebrand factor

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

In Portugal, cardiovascular diseases are the main cause of death, accounting for 36% of all deaths (294, 315). Coronary artery disease (CAD) proceeds from the development of atherosclerosis in one or more of the coronary arteries. Atherosclerosis is considered a dynamic and gradual process of endothelial dysfunction and inflammation, being characterized by the accumulation of lipids and fibrous elements in the large arteries, resulting in the increase of arterial stiffening, and ultimately leading to the reduction of blood flow and of the ability to supply oxygen to the tissues (233). The use of traditional cardiovascular disease risk factors to estimate the progression of the pathology and the risk of cardiovascular major event is not completely liable. The majority of the patients with symptomatic cardiovascular disease have at least one risk factor, however the same happens in the majority of the asymptomatic middle-age individuals and in old age individuals without clinical manifestations of cardiovascular disease (133). These facts pushed the scientific community to revisit CAD and consider new strategies for prediction, prevention, and treatment. In this sense, new risk factors called “novel”, “emergent” or “non-traditional” have been investigated in the last decades. The new concepts about atherosclerosis emerged hand-to-hand with the laboratory advances in vascular biology. Thus, serum levels of several biomarkers of endothelial function and inflammation have been investigated and elevated levels have been found to predict cardiovascular risk in a variety of clinical settings (37). The biomarkers mostly studied include the inflammatory biomarkers: C-reactive protein (CRP), and anti- and pro-inflammatory cytokines, including interleukin (IL) -6 and -10; and, the biomarkers of endothelial function: soluble vascular cell adhesion molecule-1 (sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) (273). Taken together the above-mentioned biomarkers provide a wide and more accurate view of the magnitude and progression of atherosclerosis in comparison to the traditional risk factors.

Over the last decades, exercise training assumed a major role in both primary and secondary prevention of CAD. As previously observed, exercise

training has a positive impact on modifiable traditional risk factors and on the regression of clinical symptoms (125, 252, 284, 292). Although the underlying mechanisms are not fully understood, it is widely known that several cardiovascular adaptations contribute to the above-mentioned positive effects, such as the improvement of blood flow and the regression of artery stenosis (125). However, new insights into the atherosclerotic pathophysiology allow raising new explanations for the understanding of the beneficial effects of exercise training. Indeed, it has been proposed that exercise training may improve myocardial perfusion and reduce the risk for CAD by mitigating endothelial dysfunction and vascular wall inflammation. Several studies conducted in CAD patients seem to support this hypothesis (27, 128, 159, 261, 289, 356, 400), showing a reduction of circulating levels of inflammatory biomarkers, namely CRP (128, 261, 289, 400) and IL-6 (128, 400), and an increase in IL-10 (128, 359).

Nevertheless, the majority of studies performed in this field are nonrandomized (58, 127, 128, 208, 260, 261, 289, 355, 359), uncontrolled (58, 127, 128, 208, 289, 359), and merely observational (58, 208, 260, 261). Additionally, the lack of strict inclusion criteria in those studies creates samples with a great variation with regard to the severity of atherosclerosis and co-morbidities (67, 289, 355), introducing potential uncontrolled biases, which preclude its conclusions. Indeed, only three (27, 159, 356) randomized controlled studies have been conducted so far investigating the effects of exercise training on biomarkers of inflammation in CAD patients. However, even in these studies several methodological limitations could be pointed out, including the timing of the baseline assessment post acute event and the use of just one biomarker of inflammation, which might not provide the big picture of the inflammatory reaction underlying CAD (27, 159). These limitations could explain the conflicting results raised by those studies. For instance, Sixt *et al.* (356) and Huffman *et al.* (159) did not found differences in the CRP levels after an exercise program, contrasting with the results of Balen *et al.* (27). Moreover, the above-mentioned studies did not assess any marker of the endothelial functionality, which is paramount to the initiation and progression of

atherosclerosis. Consequently, there is a gap in literature exploring in CAD patients the effects of exercise training on endothelial dysfunction, which can be assessed using the circulating levels of several molecules released from the endothelial cells such as cellular adhesion molecules (e.g. sICAM-1 and sVCAM-1).

Hence, the purpose of the present study is to conduct a randomized controlled study assessing in CAD patients the effects of exercise training (i) on biomarkers of endothelial function, (ii) on biomarkers of inflammation, and additionally (iii) exploring the contribution of age and the changes in traditional risk factors, cardiorespiratory fitness, and left ventricular function to the modification of the endothelial dysfunction and inflammation. Taking into account the fragmented data provided by previous studies, we hypothesize that exercise training mitigates the endothelial dysfunction and inflammation in CAD patients post an acute myocardial infarction even in the absence of significant changes in cardiovascular traditional risk factors.

Six chapters each with a specific objective compose this document. The introduction section (chapter I) intents to justify the purposes and hypothesis of the present work. All the problems succinctly referred in the introduction were deeply analyzed in the review of literature (chapter II). Following the general theoretical background is the methodology section (chapter III), which outlines the key instrumentation and methodology used in this study. After the presentation of the results (chapter IV), the discussion section (chapter V) intents to justify the followed methodological options, to discuss the main results in an attempt to explain them, as well as the perspectives for future research. The chapter VI contains the conclusions and the clinical messages provided by our findings.

CHAPTER II

THEORETICAL BACKGROUND[1]

[1] The theoretical background is accepted for publication in form of two papers (please see Appendix for references) and it is reprinted here with kind permission provided by the editors of *Portuguese Journal of Cardiology* and *International Journal of Cardiology*.

1. THE ENDOTHELIUM AND NORMAL VASCULAR FUNCTION

In recent years, our understanding of vascular physiology has changed radically. The endothelium is no longer seen as an inert blood vessel lining that acts merely as a passive barrier between blood and tissues; it is now recognized as having a specialized and fundamental role in the maintenance of various functions related to vascular homeostasis (304). Under basal physiological conditions, endothelial cell integrity is essential for the normal function of blood vessels and for the maintenance of homeostasis through the secretion (Table 1) or membrane exposure (Table 2) of various molecules that are responsible for the continuous adjustment of vascular tone, control of blood pressure, physiological regulation of leukocyte traffic, and the maintenance of antithrombotic and anticoagulant balance (303, 304).

Table 1. Summary of the main substances released by endothelial cells and their most important effects on vascular homeostasis.

Substances released by endothelial cells	Effects on vascular homeostasis
Plasminogen activator inhibitor	Antifibrinolytic
Tissue plasminogen activator	Fibrinolytic
Protein S	Anticoagulant, profibrinolytic
Tissue factor pathway inhibitor	Anticoagulant
Endothelin-1	Vasoconstrictor
von Willebrand factor	Coagulant (protects factor VIII), platelet adhesion
Nitric oxide	Vasodilator, inhibits platelet aggregation and adhesion
Prostacyclin	Vasodilator, inhibits platelet aggregation and adhesion
Cytokines (including interleukin-1, -6, -8, monocyte chemoattractant protein, and colony-stimulating factor)	Leukocyte function

Table 2. Substances expressed on the surface of endothelial cells and their most important effects on vascular homeostasis.

Exposed on the luminal surface of endothelial cells	Effects on vascular homeostasis
Antithrombin III	Anticoagulant
P_{2y} and ET_B receptors	Vasodilation, platelet inhibition
Angiotensin-converting enzyme	Vasoconstriction
Plasmin receptors	None in the absence of fibrinolysis
Ectonucleotidases	Vasodilation, platelet inhibition
Coagulation factor receptors	None in the absence of fibrinolysis
Thrombin receptors	Anticoagulant, vasodilation, platelet inhibition
Annexin V	Anticoagulant
Heparan sulfate	Anticoagulant
Thrombomodulin	Anticoagulant
Platelet activating factor	Platelet and leukocyte activation
Tissue factor (thromboplastin)	Coagulant
Cell adhesion molecules	Leukocyte adhesion and migration, platelet activation

1.1. Regulation of vascular tone

The endothelium plays a central role in the regulation of blood pressure and flow through continuous modulation of vascular tone, which is under the control of local and systemic factors and results from a delicate balance between vasodilators and vasoconstrictors. Locally, vascular tone is autoregulated mainly in response to mechanical stimuli from pulsatile force and shear stress, which affect the blood vessels as a result of changes in blood flow (397). This regulation is modulated by the synthesis and secretion of two powerful vasodilators with a short half-life that enable instantaneous control of vascular tone and blood pressure: prostacyclin (PGI_2) and nitric oxide ($^{\cdot}NO$).

$^{\cdot}NO$, initially named “endothelium-derived relaxing factor” by Furchgott and Zawadzki (117), is a heterodiatomic free radical generated by nitric oxide synthase (NOS) through oxidation of L-arginine to L-citrulline. The activity of NOS is modulated by various factors including mechanical deformation of the

phospholipid plasma membrane by shear stress or pulsatile force, free calcium (Ca^{2+})-dependent calmodulin activation, reduced nicotinamide adenine dinucleotide phosphate (NADPH), and tetrahydropterin (Figure 1). The functions of ˙NO include an important role in regulating basal arterial tone by its vasodilator action (165), inhibition of platelet aggregation, and control of leukocyte adhesion to the vascular wall (13). It is thus not surprising that disruption of ˙NO synthase due to endothelial dysfunction should have deleterious effects on blood pressure and leukocyte and platelet adhesion to the vascular wall, changes that are known to be associated with vascular pathogenesis in general and atherosclerosis in particular (287).

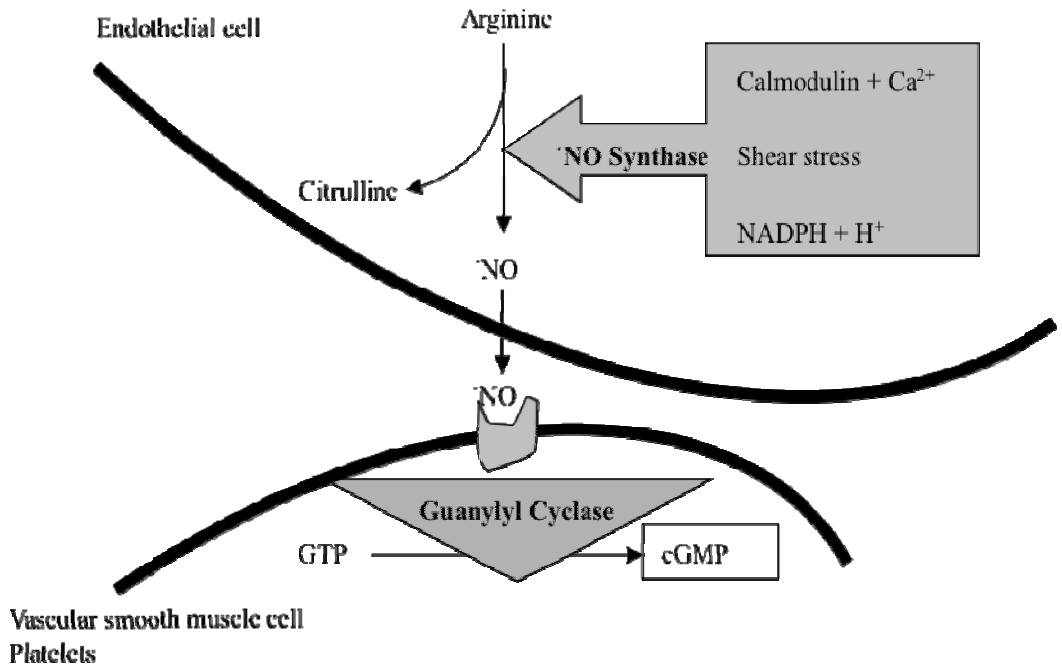


Figure 1. Synthesis of nitric oxide (˙NO) in the endothelial cell and the main factors modulating the activity of NO synthase. The action of ˙NO on smooth muscle and platelets (by activation of adenyl cyclase) is also shown.

Ca^{2+}: calcium ion; cGMP: cyclic guanosine monophosphate; GTP: guanosine triphosphate; H^+: hydrogen ion; NADPH: reduced nicotinamide adenine dinucleotide phosphate.

PGI_2 is one of a family of lipids derived from arachidonic acid, the prostaglandins. It is synthesized by PGI_2 synthase from prostaglandin H_2 (PGH_2), which is produced by hydrolysis of arachidonic acid by cyclooxygenase-2 (COX-2) (267). PGI_2 was described for the first time in 1976 as inhibiting platelet aggregation (266), and was subsequently shown to be a potent vasodilator; these properties are mediated by stimulation of adenyl cyclase in platelets and in smooth muscle respectively (Figure 2) (267). New effects have since been discovered related to inhibition of the proliferation, migration and differentiation of smooth muscle cells in the vascular wall (109, 389).

Endothelial cells synthesize $^{\cdot}NO$ and PGI_2 in response to various substances, including bradykinin, thrombin, adenosine triphosphate (ATP) and adenosine diphosphate (ADP), which increase cytoplasmic Ca^{2+} concentrations (Figure 2), and in response to mechanical deformation of the plasma membrane by shear stress (23, 152). The mechanical action of blood flow on the endothelium via pulsatile force and shear stress induces endothelial $^{\cdot}NO$ production in the absence of significant increases in cytoplasmic Ca^{2+} concentrations. This calcium-independent pathway appears to require the activation of protein kinase B, thus increasing the affinity of NOS for Ca^{2+}/calmodulin (92, 116). This mechanism results from the physical proximity between endothelial NOS (eNOS) and the invaginations, or caveolae, of the endothelial plasma membrane; it has been suggested that the caveolae act as specialized microenvironments, responding to external signals such as shear forces that deform the cytoskeleton (331, 397). Increased shear stress also promotes the release by the endothelium of other vasodilator agonists such as ATP and substance P, which lead to increased cytoplasmic Ca^{2+} in adjacent cells and thus stimulate additional $^{\cdot}NO$ synthesis (397). These observations are in agreement with the previous hypothesis that the Ca^{2+} levels required to activate NO synthesis are lower than those needed to activate the synthesis of PGI_2 (56, 276).

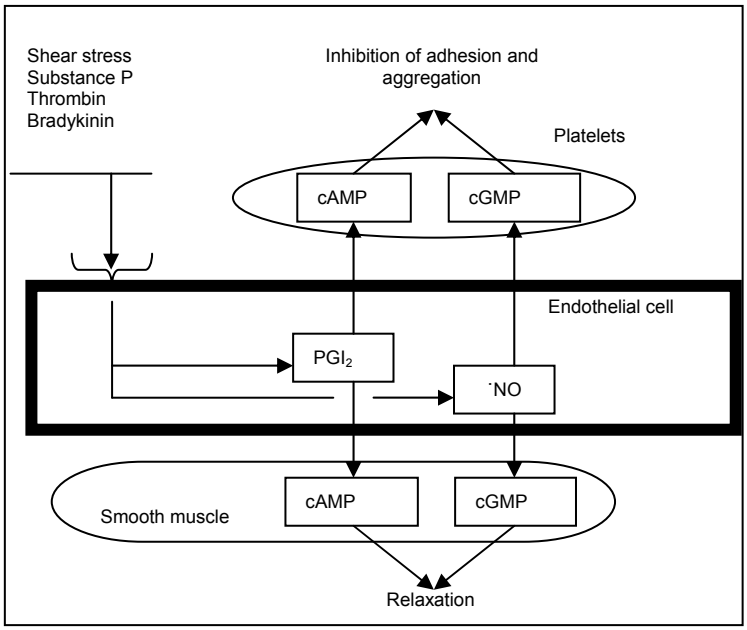


Figure 2. Stimuli for the release of prostacyclin (PGI_2) and nitric oxide ($^{\cdot}NO$) by endothelial cells. High levels of cyclic adenosine monophosphate (cAMP) activate protein kinase A, which then activates myosin light chain kinase, promoting smooth muscle relaxation and vasodilatation. cAMP inhibits undue platelet activation such as may occur with binding to thromboxane A_2. Cyclic guanosine monophosphate (cGMP) acts as a second messenger in a similar fashion to cAMP, by activating intracellular protein kinases.

Endothelial cells also synthesize a potent vasoconstrictor peptide, endothelin-1 (ET-1), whose biological functions also include stimulating growth and proliferation of smooth muscle cells in the vessel wall (52). ET-1 matures and is stored inside secretory vesicles, enabling endothelial cells to release large quantities in response to a wide range of physical and chemical stimuli that include hypoxia, angiotensin II, growth factors and other cytokines, and reduced blood flow and hence shear stress (237). Synthesis of ET-1 is inhibited by increased shear stress, $^{\cdot}NO$, natriuretic peptides, PGI_2 and heparin (200). In 1990, two receptors with particular affinity for endothelins were identified, ET_A and ET_B (15, 346). ET-1 exerts his biological functions by binding to ET_A receptors in smooth muscle cells, resulting in influx of Ca^{2+} raising its intracellular level (345). By contrast, activation of ET_B receptors in endothelial cells is followed by release of endothelium-derived relaxation factors that induce

vasodilatation (76); it has been shown that the ET_B receptors are upregulated in response to chronic increases in blood flow (28). This vasodilator effect occurs because the interaction of ET-1 with its receptors modulates NOS activity and hence ˙NO production. In normal endothelium there is a constant equilibrium between the production of ˙NO and ET-1, with ˙NO's ability to limit ET-1 production counterbalanced by ET-1's stimulation of endothelial production of ˙NO and other vasodilator peptides (52). There is growing evidence that ET-1 has an important role in cardiovascular homeostasis, its regulatory function depending on a complex balance between the effects mediated by ET_A and ET_B receptors, which in turn depends on the integrity of endothelial function, receptor subtype density, and the efficiency of the receptor-effector pair (52).

1.2. Regulation of platelet function, coagulation and fibrinolysis

Healthy endothelium is anticoagulant and antithrombotic. The vascular homeostasis is maintained by endothelial cells through fine control over platelet aggregation, coagulation and fibrinolysis (303). By limiting platelet adhesion and aggregation, healthy endothelium ensures that platelet aggregation and blood coagulation are restricted to areas of damaged or dysfunctional endothelium. The central role of the endothelial cell in regulating hemostasis and thrombosis depends on the expression and release of various molecules, including PGI_2, ˙NO, platelet activating factor (PAF), ectonucleotidases, von Willebrand factor (vWF), thrombomodulin, tissue factor (thromboplastin), tissue factor pathway inhibitor (TFPI), tissue plasminogen activator (t-PA), and plasminogen activator inhibitor-1 (PAI-1).

PGI_2 and ˙NO act in synergy to inhibit platelet aggregation (320). ˙NO also plays an important role in inhibiting platelet adhesion to the vascular surface (321); its antiaggregant properties are essential for protecting against various prothrombotic conditions. PGI_2 is a potent platelet antiaggregant, dispersing existing aggregations as well as preventing the formation of new ones. Although basal PGI_2 concentrations do not inhibit platelet adhesion to subendothelial collagen exposed by desquamation, thus enabling repair of damaged vessels, they do prevent or attenuate the formation of additional intraluminal thrombi

(320). This antiaggregant action is mediated by stimulation of platelet adenyl cyclase, resulting in increased levels of cAMP (Figure 3).

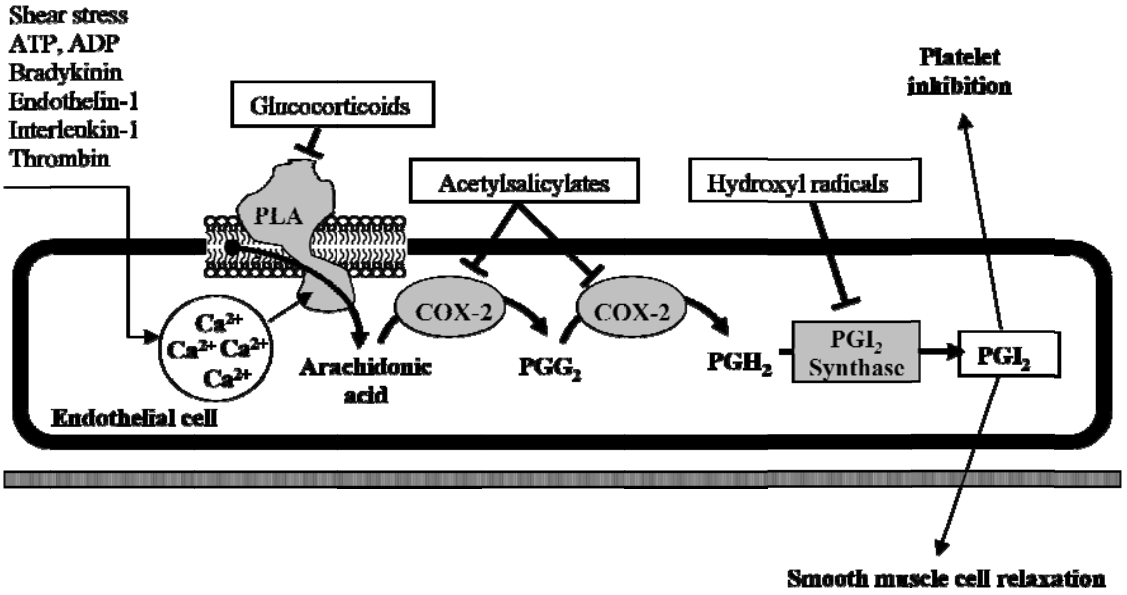


Figure 3. Endothelial synthesis of prostacyclin (PGI_2) is induced by various agonists that raise intracellular calcium (Ca^{2+}) levels. However, the production of PGI_2 is inhibited by glucocorticoids, aspirin and hydroxyl radicals, which thus limit its action on smooth muscle and platelets.

ADP: adenosine diphosphate; ATP: adenosine triphosphate; COX-2: cyclooxygenase-2; PGG_2: prostaglandin G_2; PGH_2: prostaglandin H_2; PLA_2: phospholipase A_2

Just as healthy endothelium is anticoagulant and antithrombotic, non-activated platelets do not adhere to the endothelium or circulating cells. Platelet adhesion to the endothelium results from activation, which is mediated, among other factors, by PAF originating from the endothelium, activated neutrophils and the reactive oxygen species they generate (157). PAF, a powerful phospholipid second messenger with several biological functions, is found in large quantities in endothelial cells (318), where it is synthesized in response to a range of stimuli including thrombin, bradykinin, histamine, ATP, various vasoactive mediators such as angiotensin II and vasopressin, and pro-inflammatory cytokines such as interleukin (IL)-1 and -8, suggesting that PAF amplifies the signals of these mediators (268, 317). It should be noted that PAF

synthesis by endothelial cells is a response to specific agonists and is not affected by nonspecific cellular perturbation (421). Besides its role in platelet adhesion to the endothelium, PAF also increases the permeability of the endothelial barrier and, together with the platelet adhesion molecule (P-selectin), favors leukocyte adhesion to the vessel wall (317). This increased permeability occurs (i) directly by alterations in the cytoskeleton leading to cell retraction and the formation of intercellular fenestrations (55), and (ii) through platelet- and leukocyte-mediated mechanisms (91, 162) that promote endothelial synthesis of vasoactive mediators such as PGI_2, thromboxane A_2 and leukotrienes (75, 101). PAF also stimulates angiogenesis, promoting the synthesis of various angiogenic factors by activating nuclear factor-κB (194), migration of endothelial cells (24) and expression of matrix metalloproteinases (MMP)-2 (24) and -9 (193).

Through the action of ectonucleotides trapped in the glycocalyx (ecto-ATPase, ecto-ADPase and ecto-5'-nucleotidase), circulating ATP, ADP and adenosine monophosphate (AMP) are inactivated on the luminal surface of endothelial cells, reducing platelet activation. ATP and ADP are secreted by activated platelets and trigger P_2 purinergic receptors in the endothelium, promoting the synthesis of PG_2 and $^{\cdot}NO$ (305). However, ADP released by activated platelets, together with thromboxane A_2, promotes the activation and aggregation of more platelets through a positive feedback mechanism (78, 151). This mechanism is largely countered by the activity of ecto-ADPase from endothelial cells, the ADP being dephosphorylated to AMP and then hydrolyzed to adenosine, an inhibitor of platelet aggregation, by ecto-5'-nucleotidase (184, 250). Circulating platelets in the vicinity of endothelial cells cease to respond to their agonists, and the ectonucleotidases on the endothelial cell surface thus fulfil the important function of controlling platelet response. This action is independent of $^{\cdot}NO$ and PGI_2, which suggests that the endothelium is able to preserve some of its anticoagulant and antithrombotic capacity even when the production of $^{\cdot}NO$ and PGI_2 is deficient (250).

vWF is a multimeric circulating protein with adhesive properties synthesized by megakaryocytes and endothelial cells (344). Following synthesis, vWF is

constitutively released into the blood and the subendothelial matrix, some being stored in Weibel-Palade bodies (if synthesized in endothelial cells) and in platelet α-granules (if synthesized in megakaryocytes) (Figure 4).

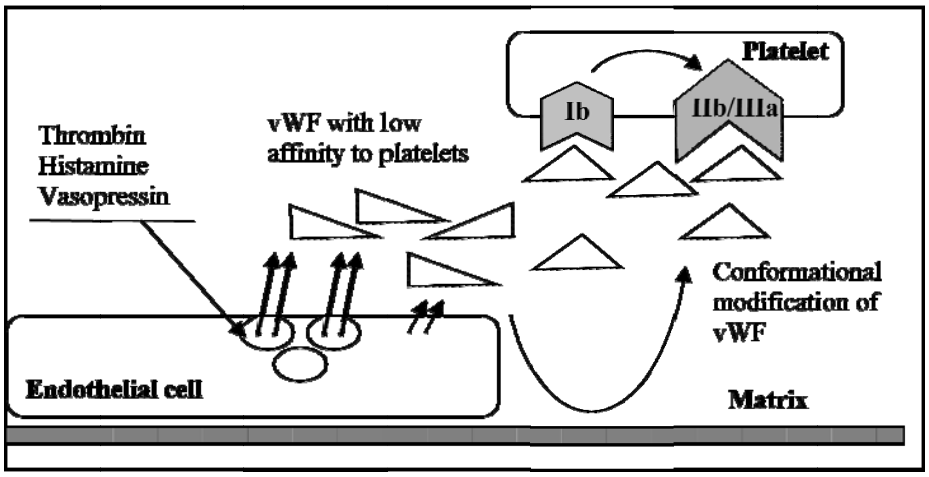


Figure 4. Endothelial release of von Willebrand factor (vWF): constitutive (95%) and inducible by exocytose in response to various stimuli. In the absence of endothelial injury, vWF, due to its the conformational flexibility, has low affinity for circulating platelets. However, in the presence of endothelial desquamation, vWF binds to subendothelial collagen and undergoes conformational modification that enables it to bind to platelets and retain them at the injury site, particularly by interaction with glycoprotein Ib and subsequently binding to the IIb-IIIa complex [for references see (343)].

vWF stored within cells is rapidly released by exocytose following stimulation by various agonists including thrombin, epinephrine, fibrin and vasopressin, which raise intracellular Ca^{2+} concentrations (343, 398). Various cytokines, including IL-1 and tumor necrosis factor-α (TNF-α), also increase vWF secretion, not by directly stimulating constitutive secretion but by enhancing the ability of agonists to promote the release of vWF by Weibel-Palade bodies (295). vWF has essential functions in hemostasis, including (i) mediation of platelet adhesion to sites of vascular injury, (ii) mediation of platelet-platelet interaction, (iii) promotion of platelet aggregation in vessels with elevated shear stress due to rapid blood flow, and (iv) transport of coagulation factor VIII in the plasma, protecting it from proteolytic degradation, and thus prolonging its circulating

half-life and targeting it at sites of exposed subendothelial collagen (343, 344, 398).

Thrombin, a multifunctional plasma protein, is a key enzyme in coagulation and hemostasis, has an important role in various thromboembolic pathologies (74), and is the main effector protease in the coagulation cascade. It is formed by the cleavage of prothrombin to active thrombin by activated factor X (factor-Xa) (74). Its procoagulant action includes the conversion of fibrinogen into fibrin, the activation of factor XIII and cofactors V and VII, reduction of fibrinolysis, and platelet activation and aggregation (74, 348). In endothelial cells, thrombin triggers a wide variety of functional responses including the rapid synthesis and secretion of PAF, rapid secretion of vWF and surface expression of P-selectin (74). The endothelium has two mechanisms that confine the spatial range of thrombin's procoagulant action and prevent systemic effects. One is the inhibition of thrombin by antithrombin, which although synthesized in the liver, binds to glycosaminoglycans in the membrane of intact endothelial cells and then to thrombin, producing an inactive complex that returns to the circulation and thence to the liver (249). The other is dependent on the synthesis and surface expression by endothelial cells of thrombomodulin, an intrinsic membrane protein found exclusively on the luminal surface of undamaged endothelium that has a strong affinity for thrombin. When thrombomodulin binds to thrombin, it causes conformational changes in the latter, reducing its affinity for fibrinogen; this greatly reduces its ability to cleave fibrinogen and increases its capacity to cleave and activate circulating protein C, which together with protein S inactivates factors Va and VIIIa, inhibiting blood clotting (104, 118). This suggests that thrombin has an anticoagulant action in the presence of thrombomodulin (84).

Thromboplastin (tissue factor) is the main physiological activator of the coagulation system. It acts as a receptor for factor VII, thus activating factor X. Unlike extravascular cells, under basal physiological conditions endothelial cells do not express thromboplastin, thereby preserving blood fluidity (79). However, *in vitro* studies show that endothelial cells synthesize and express

thromboplastin at the surface in response to thrombin, cytokines and activated platelets (33, 50).

Endothelial cells also produce TFPI, the most important physiological inhibitor of thromboplastin. This protein is secreted by endothelial cells and is found in plasma or bound to the endothelial cell surface (349). Its mechanism of action is complex, but can be summarized as binding to thromboplastin and factors VIIa and Xa, producing a stable inert quaternary complex and preventing the formation by positive feedback of additional factor Xa and IXa by factor VIIa. How its synthesis is regulated is not fully understood, but circulating TFPI levels rise after administration of bacterial lipopolysaccharides; it remains to be seen whether this is due to the synthesis of more TFPI or decoupling of TFPI bound to the endothelial cell surface (403).

t-PA and its physiological inhibitor, PAI-1, are the principal regulators of fibrinolysis; they are secreted continuously into the bloodstream by endothelial cells (47). t-PA acts on the surface of polymerized fibrin, cleaving the plasminogen molecule to plasmin and initiating the process of fibrinolysis. Besides this continuous secretion, t-PA is also released rapidly into the circulation from small storage granules in the endothelium in response to thrombin, vasopressin (102) and increased shear stress (299). Conversely, decreased shear stress reduces the expression of t-PA, which is consistent with the tendency for thrombi to form in areas of turbulent blood flow and in conditions of low shear stress (299). Secretion of PAI-1 rises after activation of endothelial cells by inflammatory cytokines such as IL-1 and TNF-α, endotoxins, and oxidized or glycated lipoproteins, while the secretion of t-PA is unchanged or reduced (12, 350). This response to inflammatory cytokines and oxidized lipoproteins may help explain the increased procoagulant risk in atherosclerosis.

1.3. Regulation of leukocyte traffic

Under basal physiological conditions, endothelial cells do not express molecules that promote adhesion of circulating leukocytes. However, activation of endothelial cells by thrombin, endotoxins or inflammatory cytokines such as IL-1 and TNF-α induces surface expression of a series of molecules that are

essential for the adhesion, rolling and migration of leukocytes in the bloodstream to damaged tissue (360).

This process is largely mediated by cell adhesion molecules, glycoproteins expressed on the surface of activated cells that are involved in cell-cell and cell-matrix binding. There are four main groups: immunoglobulins, selectins, integrins and cadherins. Only the first three are important in regulation of leukocyte traffic, the cadherins being responsible for cell-cell interactions that help maintain tissue integrity. The immunoglobulin superfamily includes intercellular adhesion molecules and vascular cell adhesion molecules; the selectins includes the endothelial-leukocyte adhesion molecule (E-selectin), P-selectin and the leukocyte adhesion molecule (L-selectin); of the integrin family, the most important for endothelial adhesion are integrin ß1, ß2 and ß7 (99, 418).

The first stage of transendothelial leukocyte traffic is partly mediated by the selectin family and is characterized by reduced flow due to vasodilatation and by adhesion and attachment of circulating leukocytes to the adjacent endothelium (31). Selectins are expressed on the surface of endothelial cells, leukocytes and platelets; in the endothelium their expression is induced by various inflammatory cytokines such as IL-1 and TNF-α (341). Of the selectins, E-selectin is unique in that it derives only from endothelial cells, while the others have multiple sources (31). It stabilizes leukocyte-endothelium interactions by promoting cell-cell adhesion; it is not expressed in inactive cells but is over-expressed in a few hours in response to an inflammatory stimulus. Selectins bind reversibly to leukocytes, capturing them from the bloodstream, slowing their movement and initiating their rolling over the endothelial surface. The second stage, consisting of firm binding of leukocytes to the endothelium, is mediated by the immunoglobulins, namely intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which interact with integrins on the surface of rolling leukocytes and bring about a stable bond to the endothelium (31, 375). Once firmly bound, leukocytes begin the process of transmigration via the capillary wall from the bloodstream to the interstitial space (422). Under basal conditions ICAM-1 is expressed by endothelial cells

and is over-expressed in response to various stimuli such as inflammatory cytokines including IL-1, TNF-α and interferon-γ. VCAM-1, expressed by activated endothelial cells and smooth muscle cells of the vessel wall, promotes firm cell-cell adhesion and subsequent transmigration of inflammatory cells. It acts by binding to integrin α4β1, also known as very late antigen-4 (VLA-4), an intrinsic membrane protein expressed by monocytes, lymphocytes and eosinophils (100). After expression of VCAM-1, cells with VCAM-1 receptors adhere preferentially to the sites of expression, and are subsequently stimulated to migrate through endothelial junctions into the subendothelial space (147). The final stage of leukocyte migration between endothelial cells involves the platelet-endothelial cell adhesion molecule-1 (PECAM-1), which is concentrated at endothelial junctions and facilitates leukocyte diapedesis (285) (Figure 5).

2. THE ENDOTHELIUM AND ATHEROSCLEROSIS

2.1. Pathophysiology of atherosclerosis

Atherosclerosis is a progressive disease with a complex etiology, characterized by the accumulation of lipids and fibrous elements in large arteries (233). Traditionally, it was seen simply as a chronic progressive disease whose manifestations were the result of complete or nearly complete occlusion of the arterial lumen (255). However, evidence that the cardiovascular complications caused by atherosclerosis generally involve non-stenotic lesions has made an important contribution to the conceptual change of what underlies its pathophysiology (223). In recent years, atherosclerosis has ceased to be considered a simple “lipid disease”, and is now understood to be a dynamic and progressive process stemming from endothelial dysfunction and inflammation of the vessel wall that can lead to an acute event due to plaque rupture or thrombosis (336). Atherosclerotic plaque is characterized by asymmetric focal thickening of the arterial intima resulting from the accumulation of lipid-rich necrotic debris and from the migration and proliferation of smooth muscle cells. Inflammatory and immune cells from the bloodstream are also important constituents of the plaque (147, 233). It typically has a fibrous cap composed of

smooth muscle cells and extracellular matrix, which encloses a lipid-rich necrotic core, but can be more complex, with calcification and ulceration of the luminal surface.

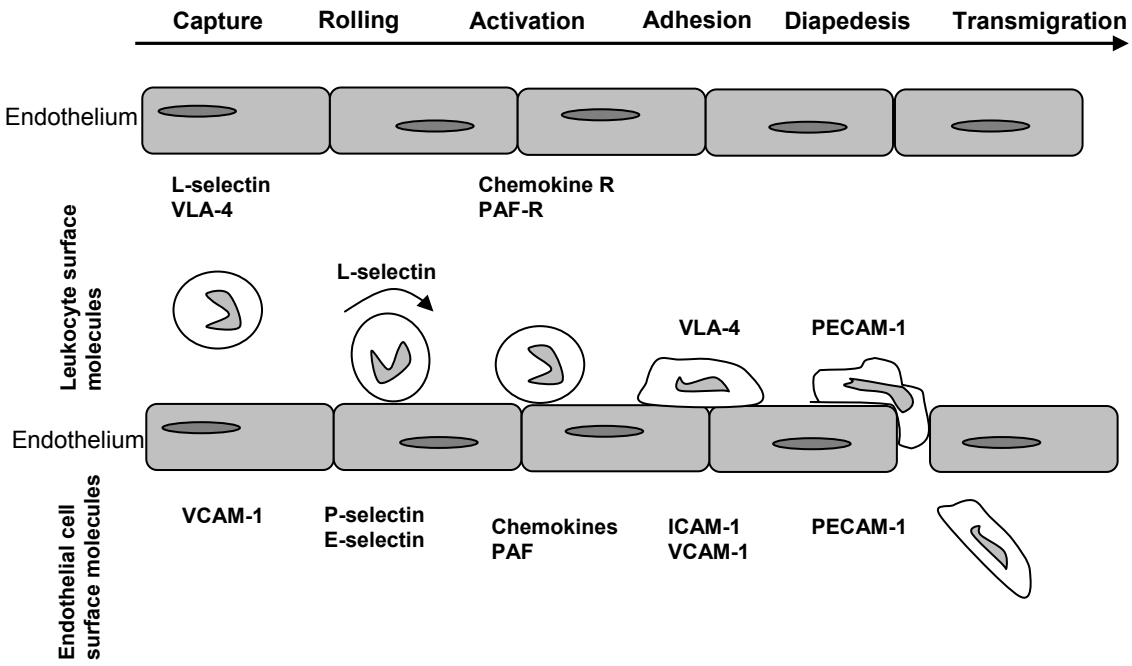


Figure 5. Schematic representation of the different stages of interaction between leukocytes and the endothelium described in the text: capture, rolling, activation, adhesion, diapedesis and transmigration.

Chemokine R: chemokine receptor; E-selectin: endothelial-leukocyte adhesion molecule; ICAM-1: intercellular adhesion molecule-1; L-selectin: leukocyte adhesion molecule; PAF: platelet activating factor; PAF-R: platelet activating factor receptor; PECAM-1: platelet-endothelial cell adhesion molecule-1; P-selectin: platelet adhesion molecule; VCAM-1: vascular cell adhesion molecule-1; VLA-4: very late antigen-4 [adapted from (275)].

Although a plaque may grow until it obstructs blood flow, the main clinical complications of atherosclerosis arise from acute occlusion of the vessel due to the formation of a thrombus, which depending on its location can result in myocardial infarction or stroke (147, 233). The fact that stenotic (constrictive) and non-stenotic (expansive) plaques can be found simultaneously in the same individual suggests that plaque evolution is considerably more complex than a simple accumulation of lipids and narrowing of the vascular lumen. The magnitude of the inflammatory response in the vessel wall to lipid accumulation,

influenced by local factors such as shear stress, systemic factors like hyperlipidemia, and genetic factors, appears to determine plaque evolution (62). A sustained inflammatory response, with constant vascular remodeling, tends to weaken the vessel wall; this gives rise to expansive plaques without constricting the lumen (62). Such plaques, which are much more likely to rupture, are the main cause of acute cardiovascular events. Plaque vulnerability thus appears to depend on the inflammatory process and is determined by the inflammatory content of the necrotic core and the thickness of the fibrous cap (233).

Atherosclerotic lesions can evolve chronically and spread throughout virtually the entire arterial tree, although they are more likely to occur in certain sectors, particularly the coronary arteries (394). As stated above, they are focal lesions and do not affect the whole artery. Differences in blood flow dynamics give rise to areas of hemodynamic tension (turbulent flow) that are particularly susceptible to the development of atherosclerotic lesions. These sites are usually bifurcations in the vessel where laminar flow gives way to turbulent flow, such as the proximal segments of the coronary arteries. The typical hemodynamic pattern of sites vulnerable to atherosclerotic lesions is of low average shear stress but high oscillatory shear stress (211, 255). At these sites, expression and activation of adhesion molecules and inflammatory genes are increased in endothelial cells (77). Given that the entire vascular tree is in general terms equally subject to the harmful effects of risk factors, it is not surprising that it is the hemodynamic pattern that determines where lesions will form. In these sites the endothelium is exposed for longer to various circulating atherogenic agents such as lipoproteins (179). Prolonged and/or repeated exposure of a particular area of endothelium to aggression is likely to exhaust its defenses, leading to endothelial dysfunction at that site and promoting the formation of an atherosclerotic plaque (336).

In atherosclerosis-related pathologies, as in maintenance of vascular homeostasis, the role of the endothelial cell is crucial. Endothelial dysfunction is a key factor in all stages of the progression of atherosclerosis. The term “endothelial dysfunction” refers to an imbalance in the production of mediators that regulate vascular tone, platelet aggregation, coagulation and fibrinolysis

(265). Since vascular tone has been most thoroughly studied, the term is often used to describe impaired endothelial-dependent vasodilatation caused by reduced bioavailability of ˙NO (265). It is characterized by changes in the endothelial phenotype from vasorelaxant, anticoagulant, antiplatelet and profibrinolytic under basal physiological conditions to vasoconstrictive, procoagulant, platelet activating and antifibrinolytic (68), a phenotype that induces lipoprotein oxidation, smooth muscle cell proliferation, matrix deposition or lysis, and vascular inflammation, leading to platelet activation and formation of thrombi (72). In the presence of cardiovascular risk factors that are a source of aggression, endothelial cells release less ˙NO, PGI_2, thrombomodulin and tPA and more ET-1, angiotensin II, PAI-1 and vWF (Figure 6). Tissue factor is also expressed as soon as thrombin production is activated by the binding of activated factor V to factor Xa on the endothelial cell surface (68).

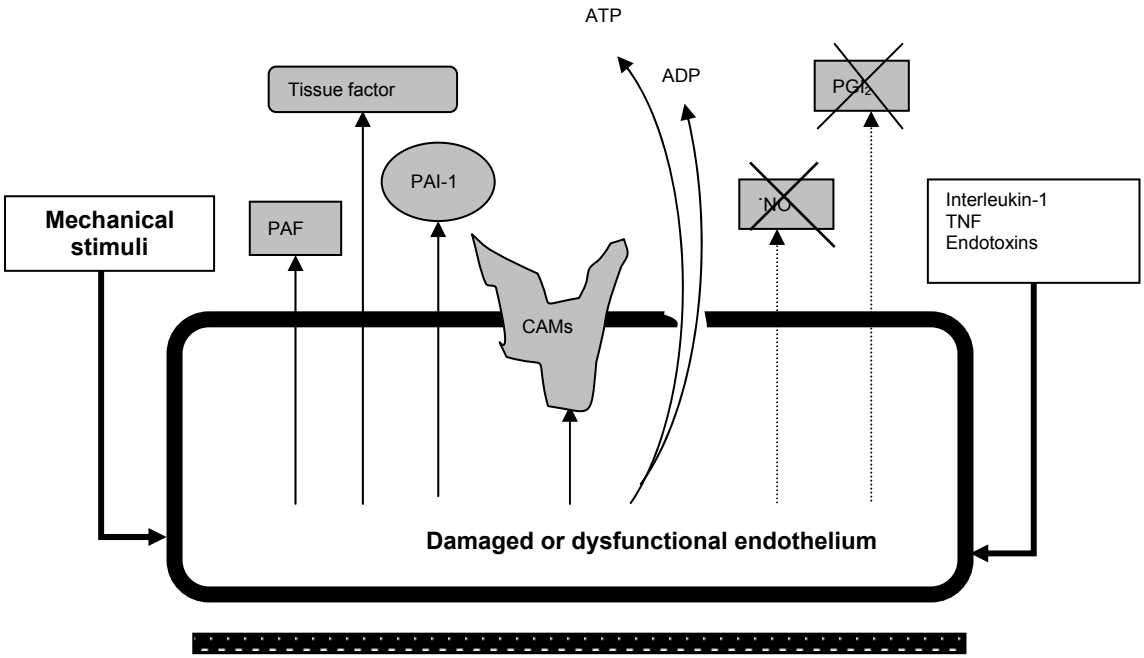


Figure 6. Changes in endothelial functionality following chemical or mechanical stimulation/aggression.

ADP: adenosine diphosphate; ATP: adenosine triphosphate; CAMs: cell adhesion molecules; ˙NO: nitric oxide; PAF: platelet activating factor; PAI-1: plasminogen activator inhibitor-1; PGI_2: prostacyclin; TNF: tumor necrosis factor

The development of the atherosclerotic plaque begins with a focal endothelial cell dysfunction (147, 233). Focal endothelial dysfunction allows the infiltration and retention of serum low-density lipoprotein (LDL) in the arterial intima, initiating an inflammatory response. Endothelial dysfunction includes the overexpression of cellular adhesion molecules, which promote the recruitment of blood mononuclear cells, and the enhancement of the endothelial layer permeability, in turn facilitating the diffusion of low-density lipoproteins (LDL) to the intima (147, 233). Once within the intima, LDL is modified by oxidation or enzyme action, promoting the release of phospholipids that stimulate endothelial cells to express CAMs, such as VCAM-1 and ICAM-1, and to produce growth factors, including granulocyte-macrophage colony-stimulating factor (GM-CSF) (211, 255). CAMs mediate the entry into the vascular wall of specific leukocytes, monocytes and lymphocytes at sites of endothelial damage or dysfunction (147, 233). Within the intima, GM-CSF stimulates monocytes to transform into macrophages, which play an important role in the local inflammatory response by producing inflammatory cytokines, chemokines and free oxygen radicals. The macrophages express scavenger receptors that allow them to engulf and modify oxidized lipoproteins and become foam cells (225, 233) (Figure 7). These lipid-laden phagocytes secrete a number of inflammatory mediators, such as several cytokines (for instance, IL-1, IL-6 and TNF-α), that amplify inflammation in the vessel wall and can contribute to additional leukocyte accumulation, smooth muscle cell proliferation, and extracellular matrix remodelling (221, 233). The local inflammatory response sustained by primary pro-inflammatory cytokines (IL-1 and TNF-α) further stimulates the production of IL-6 in several cell types, including smooth muscle cells and endothelial cells, which magnify the inflammatory response beyond the original focal area of endothelial dysfunction. Therefore, the increased circulating levels of IL-6 will further induce the hepatic synthesis of acute-phase proteins, including fibrinogen, C-reactive protein (CRP) and serum amyloid A. IL-6 acts as an amplifier of the acute-phase response, which is accountable for the augment of the expression/production of adhesion molecules, other cytokines, endothelin-1, endothelial plasminogen activator inhibitor-1, tissue factor in

monocytes, LDL uptake by macrophages, and for the diminution of ˙NO bioavailability (191, 341).

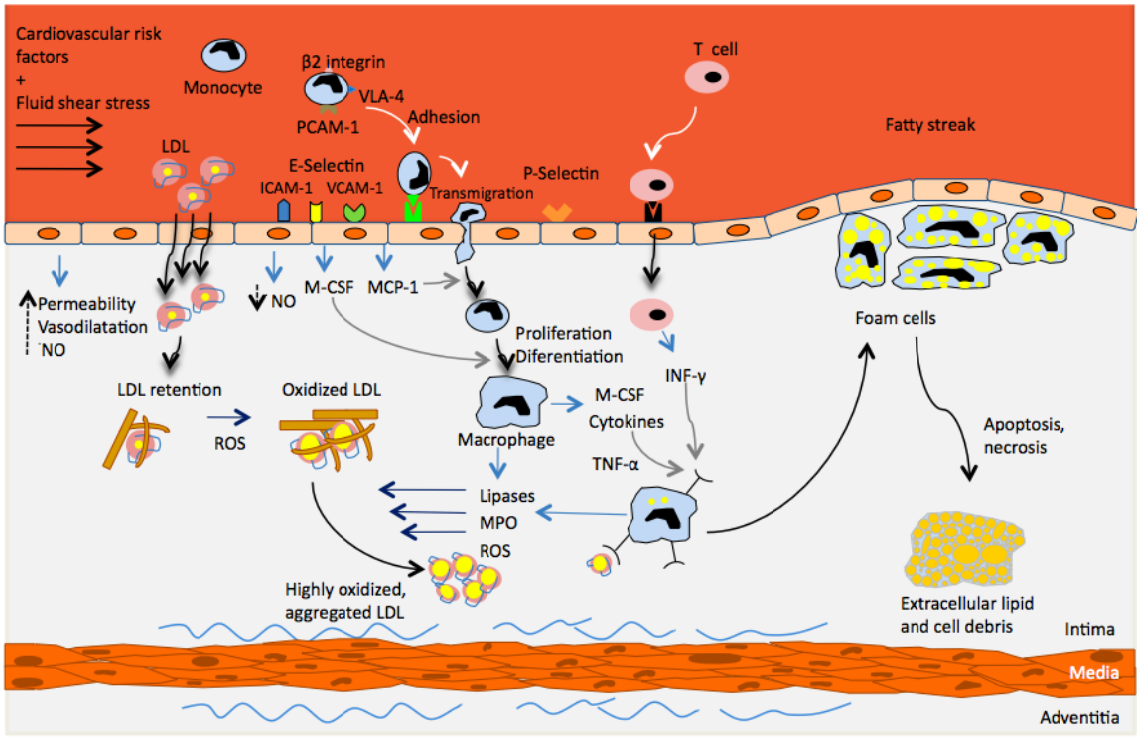


Figure 7. Schematic representation of the atherosclerotic process: from the lesion initiation to the foam cell formation. Haemodynamic forces play a major role in the determination of the locals of lesion predilection; these forces aggravated by cardiovascular risk factors increase the endothelial barrier permeability and consequently the entrance of low-density lipoprotein (LDL) into the intima. Subsequently, LDL is retained as a result of interaction with matrix components and oxidized as a result of interaction with reactive oxygen species (ROS). This process stimulates the overlying endothelial cells to produce several molecules, such as macrophage colony-stimulating factor (M-CSF) and monocyte chemoctatic protein-1 (MCP-1), and express a variety of cellular adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), that participate in the recruitment of monocytes to the vessel wall. Similarly, oxidized LDL decreases the bioavailability of nitric oxide (˙NO). Activated monocytes and T cells express important adhesion molecules, including integrins (VLA-4 and β2) and platelet cell adhesion molecule-1 (PCAM-1), which binds to the endothelial expressed

counterparts. Within the vessel wall M-CSF stimulates the proliferation and differentiation of macrophages, and together with tumour necrosis factor-α (TNF-α) and interferon-γ (INF-γ) promote the expression of several scavenger receptors (for instance CD36 and CD38). Those scavenger receptors recognize the highly oxidized aggregated LDL, which is formed as a result of the action of the ROS and the enzymes lipases and myeloperoxidase (MPO). This fact allows the rapid uptake of LDL particles by macrophages leading to the formation of foam cells. Foam cells will die, forming a mass of extracellular lipids and other cell debris within the vessel wall.

Simultaneously, the inflammatory response inhibits the production of collagen and stimulates macrophage expression of the potent procoagulant tissue factor, contributing to the prothrombotic blood environment nearby the areas of endothelial dysfunction (221, 233). Macrophage activity within the vascular wall may also contribute to plaque vulnerability and enhanced risk of plaque rupture, by elaborating matrix metalloproteinases that degrade the protective collagen structure of the plaque's fibrous cap, and consequently lead to the formation of thrombi that could lead to acute coronary events (225, 233). Hence, endothelial dysfunction and inflammation play a paramount role in the initiation and progression of atherosclerosis as well as in the adverse cardiac complications related to the disease (147, 221, 233). Factors conditioning the grade and the evolution of this inflammatory response may include virtually all the traditional cardiovascular risk factors, such as hypertension, hypercholesterolemia, diabetes mellitus, and obesity (225). All these pro-atherogenic molecules initiate and amplify the cell's response, promoting low-intensity vascular inflammation, thrombosis, progressive intimal thickening and hence the formation and development of the atherosclerotic plaque, which may eventually rupture, causing clinical manifestations (233, 335) (Figure 8). To summarize, altered endothelial function and loss of the endothelium's protective capability is a major factor in the etiology of atherosclerosis, thrombosis and cardiovascular events.

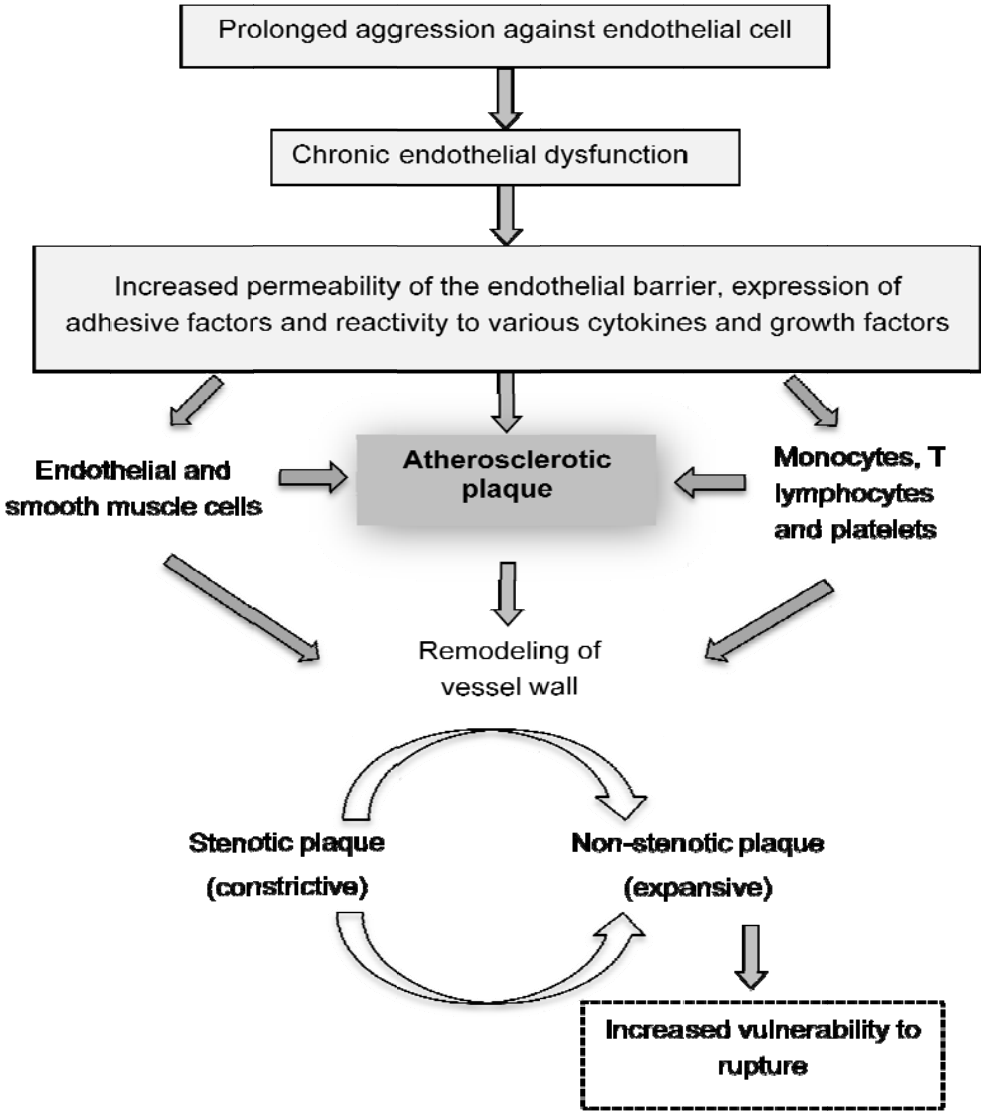


Figure 8. Summary of the main stages in the progression of atherosclerosis

2.2. Biomarkers of endothelial function in atherosclerotic heart disease

Endothelial function *in vivo* can be assessed by measuring the circulating levels of various biomarkers expressed or released by the endothelium. These biomarkers can be useful tools in understanding the evolution and prognosis of atherosclerosis, along with the efficacy of therapeutic modalities. Table 3 summarizes the biomarkers of endothelial function that can be measured in the peripheral circulation and that have clinical usefulness.

Table 3. Biomarkers of endothelial function measured in the peripheral circulation and their origin, function and clinical usefulness

Marker	Origin	Main function	Clinical usefulness
sICAM-1	Endothelial and smooth muscle cells, cardiomyocytes, leukocytes, fibroblasts, and other non-cardiac cells	Promotes stable binding and adhesion of leukocytes to the endothelium	Predicts risk of cardiovascular events (134, 164)
sVCAM-1	Macrophages and endothelial cells	Mediates firm cell-cell adhesion and leukocyte transmigration	Predicts risk of cardiovascular death (39)
sE-selectin	Endothelial cells	Promotes capture of circulating leukocytes and initiates their rolling	Predicts CAD (243) and risk of cardiovascular death (39)
sP-selectin	Platelets and endothelial cells	Mediates rolling, adhesion and binding of leukocytes to the endothelial surface	Predicts risk of cardiovascular events (329)
vWF	Endothelial cells and megakaryocytes	Binds to other proteins, particularly factor VIII, and mediates their adhesion to sites of injury	Predicts risk of cardiovascular death (172)
sTM	Endothelial cells	Binds to thrombin, reducing its affinity for fibrinogen and enabling it to activate protein C	Low levels predict CAD in asymptomatic individuals (347, 415); high levels predict recurrence of cardiovascular events (40)
EPC	Bone marrow	Contribute to repair and post-natal vascular growth	Low levels predict cardiovascular events (351, 406)

CAD: coronary artery disease; EPC: endothelial progenitor cells; sE-selectin: soluble endothelial-leukocyte adhesion molecule; sICAM-1: soluble intercellular adhesion molecule-1; sP-selectin: soluble platelet adhesion molecule; sTM: soluble thrombomodulin; sVCAM-1: soluble vascular cell adhesion molecule-1; vWF: von Willebrand factor

2.2.1. Cell adhesion molecules: ICAM-1, VCAM-1, E- and P-selectin

Various studies have demonstrated significant correlations between serum levels of CAMs and cardiovascular risk factors in apparently healthy adults (87, 332). Of the CAMs, sICAM-1 appears to be the best marker of endothelial damage and to have the strongest correlation with different cardiovascular risk factors in individuals with no clinical manifestations of cardiovascular pathology (87). Chae *et al*. (60), reporting data from the Physicians' Health Study, showed an association between sICAM-1 levels and systolic blood pressure, pulse pressure and mean blood pressure in apparently healthy individuals. Several studies have investigated the association between serum CAM levels and development and progression of CAD and have found elevated levels of sICAM-1 and sVCAM-1 in individuals with atherosclerotic disease (383). Serum sICAM-1, in particular, is elevated in individuals with cardiovascular risk factors but no symptoms and in those with clinical evidence of atherosclerosis, with (119) or without (332) previous cardiovascular events. High levels of sICAM-1 and sVCAM-1 also correlate significantly with angiographic severity of atherosclerosis (333). The predictive value of CAMs for future cardiac events has also been studied; it has been suggested that elevated sICAM-1 is an independent risk factor for myocardial infarction (134) and early restenosis following angioplasty (181). High levels of sVCAM-1 have also been shown to predict cardiovascular death in CAD patients and are now recognized as an important indicator of plaque instability (39).

As with the immunoglobulins, levels of soluble P-selectin (sP-selectin) and soluble E-selectin (sE-selectin), especially the latter, correlate significantly with traditional risk factors for atherosclerosis in apparently healthy individuals (87). Unlike sP-selectin, sE-selectin originates only in the endothelium; it is released into the circulation by enzymatic cleavage or by shedding from damaged endothelial cells (309). Studies have linked elevated sE-selectin with various cardiovascular risk factors, including smoking (87), obesity (243), hypertension (43), hypercholesterolemia (161) and diabetes (364). It is also associated with atherosclerotic lesions in the carotid arteries (164) and their angiographic severity (291), as well as stable (283) and unstable angina (119). In the

evolution of CAD, prospective studies indicate that sE-selectin levels not only provide additional predictive value to traditional risk factors (243), but also predict risk of cardiovascular death (39).

The importance of P-selectin in atherosclerosis is demonstrated by evidence that: (1) it is expressed preferentially in the endothelium of vessels with atherosclerotic plaques (173); (2) P-selectin-deficient mice develop fewer fatty streaks (95); (3) anti-P-selectin antibodies inhibit monocyte rolling and attachment on vascular endothelium (323); and (4) increased sP-selectin is a marker of progression of coronary atherosclerosis following myocardial infarction (42). There have been several studies in animals (254, 323) and in humans (21, 329) that demonstrate the importance of P-selectin in the development of atherosclerosis and in prediction of cardiovascular events. For instance, the risk of cardiovascular events in women in the upper quartile of P-selectin serum levels is 2.2 times higher than for those in the lower quartile, independently of traditional risk factors (329).

2.2.2. von Willebrand factor

vWF plays a crucial role in platelet adhesion to damaged arterial wall (396), and plasma concentrations are a standard indicator of endothelial injury (229). It is associated with various cardiovascular risk factors such as obesity, hypertension and hypercholesterolemia (41). Various studies have also shown that vWF is an independent predictor of cardiovascular disease (271) and cardiovascular death (172).

2.2.3. Soluble thrombomodulin

In basal physiological conditions, thrombomodulin (TM) expressed by endothelial cells has an anticoagulant effect. However, damaged endothelial cells release TM into the bloodstream, and so its soluble form (sTM) can be used as a marker of endothelial injury (45, 169). In healthy individuals, sTM levels reflect its expression in endothelial cells and its normal anticoagulant function, while in those with cardiovascular disease it is a marker of disease severity – the more severe the pathology, the more sTM is released into the

bloodstream (68). There is evidence that low sTM levels are associated with increased risk for CAD in apparently healthy individuals (347, 415), and that high levels predict the rate of progression of atherosclerosis following myocardial infarction (40).

2.2.4. Circulating endothelial cells and endothelial progenitor cells

Circulating endothelial cells (CECs) were described for the first time in the 1970s, but effective techniques for isolating and quantifying them were not developed for many years (90). Endothelial cells are shed from the surface of damaged endothelium and enter the bloodstream; this is caused by various mechanisms including mechanical damage, defective adhesion and detachment induced by cytokines and proteases (44). Very few endothelial cells are detected in the circulation of apparently healthy individuals (414), unlike in those with diagnosed cardiovascular disease, in whom the number is high (44); in patients with atherosclerotic vascular disease, there is a positive correlation between the number of CECs and levels of vWF and TF (242), as well as with the bioavailability of ˙NO (322).

Endothelial progenitor cells (EPC), described by Asahara *et al*. in 1997 (18), are derived from bone marrow and have the potential to differentiate into mature endothelial cells; when mobilized, they are released into the peripheral circulation (399). There is some disagreement in the literature concerning the origin of circulating EPC; they have variously been reported as originating from hematopoietic stem cells, monocytes/macrophages, and mesenchymal stem cells. Their mobilization from bone marrow and subsequent release into the circulation is regulated by various growth factors, enzymes, ligands and surface receptors (399). Bone marrow is the principal reservoir of adult stem cells, which in steady-state conditions remain in the G0 stage of the cell cycle. However, cytokines released in response to various stimuli, including exercise and hypoxia, modify the interaction between stem and stromal cells, inducing the release of MMP-9 and thereby the release of stem cells from the marrow (399). The mobilization of EPC can also be stimulated directly by increased blood flow in the marrow, increasing shear stress in endothelial cells and hence

˙NO production. This enhances eNOS expression in stromal cells and influences the recruitment of EPC and hematopoietic stems cells (8).

EPC in the bloodstream have the ability to repair the endothelium by binding with damaged areas in a process mediated by the expression of adhesion molecules of the integrin family and cytokines (105). This is an important endogenous mechanism for maintaining vessel integrity and has an important role in neovascularization and vascular homeostasis (68). If the process by which EPC are mobilized and recruited to sites of vascular damage is defective, or if EPC reserves are depleted, the endothelium's capacity for regeneration is reduced, leading to loss of vascular homeostasis, permanent endothelial dysfunction, and greater susceptibility to atherosclerosis (371).

In humans, low EPC concentrations are associated with various traditional risk factors (156), emerging risk factors (369) and severity of atherosclerosis (106), and also independently predict cardiovascular events (351, 406). Hill *et al*. (156) demonstrated that the number of circulating EPC in apparently healthy individuals is a better predictor of flow-dependent dilatation than the Framingham risk score. The number of EPC, and hence their regenerative and proliferative capacity, is reduced in patients with CAD (156), probably due to exhaustion of competent EPC, continuing vascular injury (187), or inadequate mobilization from bone marrow (228).

3. EXERCISE AND ENDOTHELIAL DYSFUNCTION

Endothelial cell integrity is essential for preserving vascular homeostasis, allowing the continuous adjustment of vascular tone, the physiological regulation of leukocyte traffic, and the maintenance of blood fluidity (304). In several pathological conditions, such as in atherosclerosis, the endothelial function is chronically disturbed in local areas. Endothelial dysfunction is characterized by an alteration in the basal endothelial phenotype (vasorelaxant, anticoagulant, antiplatelet and profibrinolytic), to one that is vasoconstrictive, procoagulant, platelet-activating and antifibrinolytic (68, 265). The dysfunctional endothelial cells release lower levels of ˙NO, prostacyclin, thrombomodulin and tissue plasminogen activator and meanwhile increase levels of endothelin-1,

angiotensin II, PAI-1 and von Willebrand factor (72). Tissue factor, which is not present on the functional endothelial cell surface, becomes expressed as a result of thrombin production. Thrombin is activated through the binding of activated factor V to activated factor X on the surface of the endothelial cell (68). The utility of these molecules could be to serve as novel biomarkers of "endothelial-health", whereby the effect of various therapeutic strategies could be monitored. Additionally, such strategies could be employed to explore the various mechanisms of exercise training upon the vascular endothelium in states of health and disease.

3.1. Nitric oxide

˙NO is a heterodiatomic free radical generated by the oxidation of L-arginine into L-citruline by the action of NOS. ˙NO has an important role in the regulation of vascular tone, the inhibition of platelet aggregation, and the control of the cytokine adhesion to the vessel wall (13, 165). In vessels with atherosclerotic plaques, the reduction of ˙NO bioavailability is associated with vasoconstriction, platelet adherence and aggregation, leukocytes adherence to the endothelium, and increased proliferation of vascular smooth muscle cells (69). The main pathway responsible for the decrease of ˙NO bioavailability seems to be the degradation of ˙NO through the interaction with reactive oxygen species (ROS) (338). In addition, atherosclerosis also promotes the downregulation of eNOS with a consequent decrease in the production of ˙NO. This enzymatic dysfunction has been studied in endothelial cells exposed to hypercholesterolemia or oxidized LDL, showing an impairment of eNOS isoform activation by calcium/calmodulin (35, 107). Moreover, eNOS exposed to oxidized LDL or hyperglycemia may synthesize superoxide ion, increasing the oxidative stress (61). The NO bioavailability can be indirectly measured via the degree of endothelium-dependent vasodilatation, as has been the aim of previous studies (98, 114, 138, 139, 158). Those studies reported that exercise training, particularly aerobic exercise, promotes favourable adaptations in the endothelial cell function with evident clinical benefits. According to the current knowledge, regular exercise is a non-pharmacological therapeutic modality that

enhances endothelial function in subjects with cardiovascular risk factors, including hypercholesterolemia (218), hypertension (155), metabolic syndrome (209), and type 2 diabetes mellitus (239), and in patients with established CAD (126, 144, 356) and heart failure (142, 158). In a prospective clinical study with CAD patients, 4 weeks of intensive exercise training decreased the coronary artery vasoconstriction, in response to acetylcholine, by 54% (144). Additionally, the follow-up of those patients indicated that the continuation of regular exercise, in a home-based program, could sustain, at least partially, the previously achieved effects on coronary endothelial function (124).

The augment of ˙NO bioavailability induced by exercise could be the result of the increased activity/expression of eNOS, and/or the diminished degradation of ˙NO in result to the reduced interaction with ROS (Figure 9). Studies using animal experiments (353, 413) and cultured endothelial cells (80, 324) suggested that shear stress increases eNOS expression/activity, probably due to the stabilization of eNOS mRNA (80) or the presence of transcription factors in the promoter region of eNOS gene enhancing the synthesis of mRNA (391). Hambrecht *et al*., in 2003 (138), performed the first study demonstrating the positive effects of exercise training on vascular function and eNOS expression in the human vascular system. The authors found a 2-fold increase in eNOS mRNA expression and a 3.2 increase in the phosphorylation of eNOS on serine 1177 residue after 4 weeks of regular exercise training in CAD patients. This led to a rise in the enzymatic activity of eNOS and consequently to an enhanced ˙NO production. Furthermore, regular exercise tends to increase the antioxidant defences and by this way reduce the ˙NO degradation (114). This hypothesis was verified in heart failure patients in whom exercise improved ˙NO-dependent vasodilatation without changes in eNOS expression. This was explained through the increase of antioxidant defences, such as the enhanced activity of Superoxide Dismutase and Glutathione Peroxidase (103). Moreover, in vitro studies showed that the application of laminar shear stress to cultured endothelial cells activates eNOS as well as the cytosolic copper/zinc-containing Superoxide Dismutase pathway (93, 94, 168, 372). This increase in the antioxidant defences, observed both in myocardium and endothelial cells (94,

168, 339, 372), seems to result from repetitive exposure to increased laminar shear stress during acute bouts of exercise training.

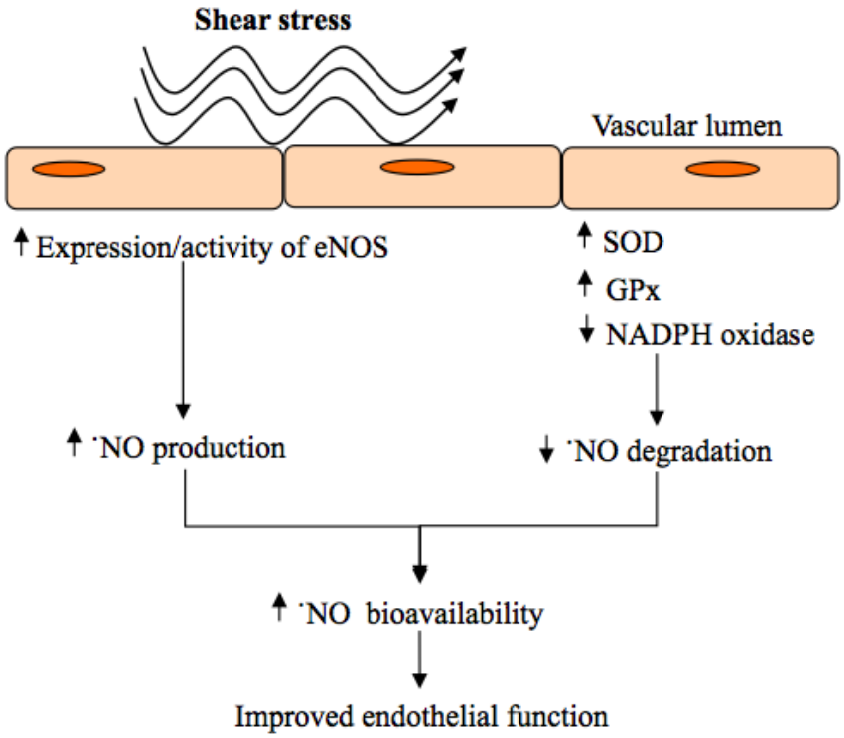


Figure 9. Potential mechanisms explaining the improvement of nitric oxide (·NO) bioavailability induced by exercise: (i) the increase of the activity of endothelial Oxide Nitric Synthase (eNOS), hence enhancing ·NO production; and, (ii) the increase of antioxidant enzymes, Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx), and the decrease of the Nicotinamide Adenine Dinucleotide Phosphate-oxidase (NADPH) activity in infiltrated leukocytes, which lead to a decreased production of superoxide ion, hereby reducing the ·NO degradation.

The increase of antioxidant defences could also contribute to attenuating the formation of foam cells and vascular inflammation through the reduction of a crucial step in the atherogenesis: the LDL oxidation (29, 354). Indeed, the lipid oxidation, as the result of LDL exposure to the oxidative waste of intima layer, seems to be paramount to the foam cells formation, as native LDL is rapidly taken up by macrophages (233).

In summary, regular exercise promotes the acute increase of blood flow and shear stress and, in turn, improves the ˙NO bioavailability, hence increasing the endothelium-dependent vasodilatation. This improvement could represent one of the most important mechanisms explaining the reduction of myocardial ischemia through regular exercise (138, 144). However, it should be noted that exercise intensity seems to be a crucial variable in this response. It is well established that moderate-intensity aerobic exercise augments endothelium-dependent vasodilatation in subjects with impaired endothelial function (132, 240). Nevertheless, recent studies in metabolic syndrome subjects (381) and heart failure patients (411) have shown that high-intensity aerobic interval exercise was better than moderate-intensity aerobic exercise to increase endothelial function and ˙NO availability. Wisløff *et al*. (411) suggested that the superior effect of aerobic interval training could be elicited by higher shear stress during the acute bouts of exercise, which triggers larger responses at the cellular and molecular level.

3.2. Cellular Adhesion Molecules

Under basal physiologic conditions the endothelial cell does not express molecules that induce the adhesion of circulating leukocytes. However, the activation of the endothelial cell by cytokines, oxidized LDL, and ROS induce the endothelial expression of CAMs, such as ICAM-1, VCAM-1, E-selectin, and P-selectin, that are crucial to the recruitment of inflammatory cells to the vessel wall (32, 113). These molecules can be measured in circulation as soluble adhesion molecules since they are released in soluble form into the bloodstream from the proteolytic cleavage of membrane bound molecules. Therefore, these molecules are considered to be important biomarkers of endothelial cell activation and inflammation (113). Exercise training seems to have a positive impact in the circulating CAMs. In subjects at risk of coronary events, two weeks of exercise training reduced circulating levels of sICAM-1 (404). Likewise, in heart failure patients, exercise training decreased the circulating levels of sICAM-1, sVCAM-1 (4), and sP-selectin (34). Also in animal experiments, exercise training performed 5 times per week for 6 to 8 weeks

induced a significant decrease in the expression of P-selectin and VCAM-1 (416, 417). This positive impact of exercise on circulating CAMs could be related to changes in the transcriptional regulation of CAMs induced by shear stress (14). Besides this direct influence in CAMs expression, exercise training might also have indirect favourable effects throughout the reduction of agonists of CAM synthesis, namely inflammatory cytokines (128), ROS (93, 168), and, thus, the oxidation of LDL (29, 354). By reducing the soluble adhesion molecules, which may represent the interaction between activated monocytes/macrophages and endothelial cells, exercise training might be considered an effective non-pharmacological intervention to reduce endothelial adhesiveness.

3.3. Endothelial Progenitor Cells

Endothelial function is dependent on the level of aggression and the vascular capacity to regenerate after injury, which is closely related with the number and function of EPC (207, 217, 390, 405). The EPC are circulating bone marrow-derived stem cells that can differentiate into mature endothelial cells (399). If required, EPC are mobilized from bone marrow and released into peripheral circulation. This process is regulated by several growth factors, enzymes, ligands, and cell surface receptors, as well as by the direct effect of increased blood flow within bone marrow (8, 399). From several therapeutic strategies, which attenuate cardiovascular risk, exercise training seems to be the most effective intervention in stimulating EPC (105). Exercise training has been reported to chronically increase the number of circulating EPC both in healthy subjects (207) and CAD patients (5, 365). Laufs at al. (207) reported an average increase of 280% in the circulating EPC after 4 weeks of regular exercise training. Such an increase could be partially explained by the stimulation of bone marrow as a result of local increase in the bioavailability of $^{\cdot}NO$ (8, 207), in turn favouring the mobilization of EPC (5, 207, 302, 365). In addition to the up-regulation of EPC generation, exercise may increase the number of circulating EPC by decreasing the rate of EPC apoptosis (207). This decrease seems to be mediated by the inhibition of an important pro-apoptotic

enzyme, the Caspase-3 (257). The positive impact of exercise training on the survival, differentiation, and function of EPC may also be indirectly related to the reduction of circulating levels of CRP (393). Independent of the underlying mechanisms, the literature clearly supports the view that exercise is an effective tool to enhance the endothelial regenerative capacity.

4. EXERCISE AND BIOMARKERS OF INFLAMMATION

The anti-inflammatory effect of exercise training in CAD patients has been assessed through the measurement of circulating levels of systemic biomarkers of inflammation, namely circulating cytokines and CRP.

Cytokines are a group of proteins with relatively small molecular weights (286). The pathogenesis of atherosclerosis involves several cytokines from the interleukin group (for instance IL-1, -4, -6, -8, -10) and macrophage associated cytokines such as TNF-α, interferon (IFN)-γ and colony stimulating factors (191). Cytokines can be categorized as pro-inflammatory (pro-atherogenic) and anti-inflammatory (anti-atherogenic). Both pro-inflammatory and anti-inflammatory cytokines play a key role in chronic vascular inflammation, and their balance seems paramount to the atherosclerotic disease (191). The pro-inflammatory cytokines have several biological functions [reviewed in (191)], including: (i) the induction of other pro-inflammatory cytokines and chemokines; (ii) the expression of adhesion molecules on endothelial cells; (iii) the stimulation of cell proliferation and differentiation; (iv) the release of matrix-degrading enzymes; and (v) the regulation of acute-phase reaction. On the other hand, anti-inflammatory cytokines exhibit atheroprotective properties, inhibiting a wide range of immune and inflammatory responses, including the inhibition of pro-inflammatory cytokines. The critical role of cytokines in the pathogenesis of atherosclerosis makes them a pivotal target for the therapeutic strategies. Therefore, the anti-inflammatory effect of exercise in CAD patients has been assessed through the circulating levels of the pro-inflammatory cytokines IL-1, IL-6, IL-8, TNF-α, and IFN-γ, and of the anti-inflammatory cytokine IL-10 (27, 128, 189, 289, 359, 400). Together these studies indicate that exercise training reduces vascular wall inflammation, while increasing the

levels of IL-10 (27, 128, 359) and reducing the levels of pro-inflammatory cytokines (128, 189, 289, 359, 400).

CRP is an acute phase protein largely produced by the liver in response to inflammatory cytokines, primarily IL-6, and, to a lesser extent, IL-1 and TNF-α (57). CRP is an important marker of subclinical chronic vascular inflammation, being considered a strong predictor of cardiovascular events (382). More than a marker, CRP seems to be itself a pro-inflammatory mediator that contributes to the development and progression of atherosclerosis through: (i) the increase of LDL uptake by macrophages (224, 423); (ii) the mediation of ICAM-1 and VCAM-1 expression and the mediation of monocyte chemoctatic protein-1 induction (300, 301); (iii) the induction of tissue factor production by monocytes (59); (iv) the induction of PAI-1 expression (88); and (v) the decrease of the production of ˙NO by endothelial cells (392).

A number of studies have examined the anti-inflammatory potential of exercise in CAD patients through the assessment of circulating levels CRP (Table 4). Taken together the data from those studies indicates that exercise training reduces the circulating levels of CRP. Indeed, several prospective studies (58, 127, 128, 189, 314, 400) examining the influence of exercise training alone or incorporated in cardiac rehabilitation programs on biomarkers of inflammation, have suggested an anti-inflammatory effect of chronic exercise. However, the majority of those studies are nonrandomized, uncontrolled, and observational. Milani *et al*. (260, 261) conducted the first studies assessing the effects of cardiac rehabilitation and exercise training on the plasma levels of CRP. They found a significant reduction in CRP after the 3-month intervention (i) in CAD patients with and without metabolic syndrome (260), (ii) in weight gainers and losers (261), and (iii) in patients with or without statin therapy (261). This data was later confirmed by Caulin-Glaser *et al*. (58), who reported a consistent reduction of CRP in patients who showed no improvement or an increase in triglycerides, body mass index, and weight. Others (128, 314, 400) also observed the statins and weight loss-independent effects of exercise on CRP. In an elegant study, Walther *et al*. (400) randomized 101 patients with stable CAD to either percutaneous intervention with stent or aerobic exercise

training. In a subgroup of 66 patients, after 24 months of training, CRP levels were reduced by 41% whereas no change was observed in the percutaneous intervention group. The effects of exercise were independent of statin therapy. Interestingly, in the patients of the percutaneous intervention group no changes are being observed even in patients on statin therapy. Therefore, this data suggests that cardiac rehabilitation and exercise training has an anti-inflammatory effect independent of statin therapy and weight loss.

As pointed out in the Table 4, the majority of evidence suggests that exercise training is related with ameliorations in biomarkers of systemic low-grade inflammation expressed by the circulating levels of CRP. However, two recent studies (146, 356) failed to confirm this effect. This discrepancy may be due to a number of factors, including differences in subject characteristics and number, the timing of the blood samples taken, and the type, and the intensity and duration of the exercise intervention. Indeed, the duration of the intervention seems to be crucial, since the majority of studies showing improvements in the biomarkers of inflammation included at least a 12-week exercise intervention. The two studies mentioned previously included 4 (356) and 7 (146) weeks of exercise training. It is interesting to note that levels of CRP showed a trend of decreasing following the 7-week intervention. They decreased by 17.4%, although without reaching statistical significance, suggesting that a longer intervention is needed to induce significant improvement in the inflammatory status of CAD patients.

Several potential mechanisms emerge to enlighten the CRP decrease induced by exercise training alone or as a core component of a cardiac rehabilitation program, all of them closely related to the decrease of cytokine production, namely IL-6, IL-1, and TNF-α (182). One of those mechanisms is the reduction of obesity, particularly central obesity, with a consequent decrease in the adipocyte production of inflammatory cytokines. It is well documented that obesity, especially central obesity, is associated with CRP levels (212, 256), possibly due to the increased production of the inflammatory cytokines IL-6 and TNF-α, by the adipocyte (420). Accordingly, exercise could mitigate inflammation by reducing body weight. However, exercise training

decreased the circulating levels of Il-6, Il-1, and CRP levels by 48% independent of changes in body weight or body mass index (128). Likewise, exercise training also reduced the levels of CRP in CAD patients who experience weight gain (58) or who experienced no changes in body fat (261),the exercise-related anti-inflammatory effect. The de suggesting that other factors could contribute to creased production of cytokines in others sites beyond adipose tissue, such as skeletal muscle and mononuclear cells could be pointed out as another possible mechanism mediating the anti-inflammatory effect of exercise. In individuals at risk of developing ischemic heart disease, six months of exercise training diminished the blood mononuclear cell production of inflammatory cytokines (TNF-α, IL-1-beta, and IFN-γ) and increased the production of the anti-inflammatory cytokines IL-10 and IL-4. These changes in cellular function were reflected in the serum levels of CRP, which decreased by 35%, although without reaching statistical significance. Also in patients with heart failure, six months of exercise training reduced the skeletal muscle TNF-α, IL-1-beta and Il-6 expression. However the serum levels of the above mentioned parameters remained unaffected (123), raising the question of whether this local change has physiological repercussions at a systemic level. The enhancement of endothelial function could be another option to justify the decrease of inflammatory status by exercise training, through the reduction of IL-1 and IL-6 released by the endothelium. In fact, several studies have provided evidence supporting the positive impact of exercise training on endothelial function; as earlier explained, exercise training improves ˙NO bioavailability (138) and reduces circulating biomarkers associated with endothelial dysfunction (4, 34). Finally, the favourable impact of exercise training on biomarkers of inflammation could be related to the diminishment of oxidative stress and LDL oxidation, hence reducing monocyte activation and, in this way, attenuating the production of inflammatory cytokines. Indeed, exercise training reduces oxidative stress in skeletal muscle (210) and in the vasculature (114), and reduces the level of circulating biomarkers of oxidative stress (328).

Table 4. Summary of studies that examined the effects of exercise training on CRP levels in CAD patients

Study	Design	Participants	Intervention	Duration	CRP (mg/L) before		CRP (mg/L) after		P Value	Δ%
					Exercise Group	Control group	Exercise group	Control group		
Sixt *et al*. 2008 (356)	Prospective RCT	6 ♀ and 28 ♂	Aerobic exercise/ rosiglitazone/ usual care	4 weeks	3.1 ± 1.4	3.7 ± 2.3/ 5.5 ± 4.4	2.9 ± 1.6	3.6 ± 4/ 3.8 ± 2.9	>0.05	—
Hansen *et al*. 2008 (146)	Prospective	25 ♀ and 109 ♂	Aerobic exercise	7 weeks	4.6 ± 5.8	—	3.8 ± 5.5	—	0.2	—
Walther *et al*. 2008 (400)	Prospective RCT	66 ♂	Aerobic exercise/PCI	24 months	3.1 ± 0.6	2.5 ± 0.4	1.8 ± 0.3	2.3 ± 0.3	0.025†	43.8
Kim *et al*. 2008 (189)	Prospective	11 ♀ and 28 ♂	CR /usual care	14 weeks	3.2 ± 0.5*	3.2 ± 0.8	1.3 ± 0.1	2.3 ± 0.3	<0.0001†	59.4
Pluss *et al*. 2008 (314)	Prospective RCT	49 ♀ and 175 ♂	Expanded CR/Usual CR	12 weeks	3.0 ± 2.8	4.0 ± 3.5	2.1 ± 2.1	2.4 ± 2.5	<0.01‡	30 / 40
Goldhammer *et al*. 2007 (127)	Prospective	14 ♀ and 23 ♂	Aerobic exercise	12 weeks	6.1 ± 4.2	—	3.5 ± 2.4	—	<0.05	42.6
Shin *et al*. 2006 (355)	Prospective	11 ♀ and 28 ♂	CR/CR plus statins/statins	14 weeks	2.8 ± 0.7/ 3.6 ± 0.6	3.2 ± 1.1	1.5 ± 0.2/ 1.1 ± 0.2	2.2 ± 0.3	<0.01‡	58.3
Lavie *et al*. 2006 (208)	Retrospective cohort	29 ♀ and 76 ♂ young; 64 ♀ and 196 ♂ old	CR	12 weeks	4.2 ± 5.4 young 5.6 ± 8.1 old	—	2.8 ± 3.1 young 3.8 ± 5.0 old	—	<0.01‡	33.3 32.1

Huffman *et al*. 2006 (159)	Prospective RCT	89 ♀ and 104 ♂	Aerobic exercise low-amount moderate intensity/low-amount high intensity/ high-amount high intensity/control group	6 months	values not reported	values not reported	values not reported	values not reported	>0.05	—
Niessner *et al*. 2006 (289)	Prospective	14 ♀ and 18 ♂	Aerobic exercise	12 weeks	2.1 ± 0.5*	—	1.9 ± 0.4	—	0.54	—
Goldhammer *et al*. 2005 (128)	Prospective	10 ♀ and 18 ♂	Aerobic exercise	12 weeks	7.5 ± 4.2	—	3.9 ± 3.5	—	<0.001	48
Caulin-Glaser *et al*. 2005 (58)	Retrospective cohort	38 ♀ and 134 ♂	CR	12 weeks	5.7 ± 14.1	—	2.7 ± 6.3	—	0.003	52.6
Milani *et al*. 2004 (261)	Retrospective cohort	75 ♀ and 202 ♂	CR/usual care	12 weeks	5.9 ± 7.7	6.3 ± 6.9	3.8 ± 5.8	6.6 ± 7.0	<0.0001†	35.6
Milani *et al*. 2003 (260)	Retrospective cohort	75 ♀ and 202 ♂	CR/usual care	12 weeks	4.9 ± 6.6 without MS; 7.3 ± 8.7 with MS	6.3 ± 6.9	2.9 ± 3.9 without MS; 4.9 ± 7.4 with MS	Values not reported	<0,001†	40.8 32.9
Smith *et al*. 1999 (359)	Prospective	43 ♀ and 18 ♂	Exercise (different types)	6 months	4.8 ± 1.1*	—	3.1 ± 0.6	—	0.12	—

CRP: C-reactive protein; RCT: randomized controlled trial; PCI: percutaneous intervention; CR: cardiac rehabilitation; MS: metabolic syndrome;

† significant only for the exercise group or CR group; ‡ significant only for CR plus statins group; ǂ significant for both groups; * values are mean± SEM;

5. SUMMARY

Endothelial cells play a crucial role both in basal conditions and in the pathophysiology of atherosclerosis. There is increasing evidence that endothelial dysfunction and vascular wall inflammation are present in all stages of atherosclerosis. Endothelial dysfunction is characterized by changes in the phenotype observed in basal conditions (vasorelaxant, anticoagulant, antiplatelet and profibrinolytic) to one that is vasoconstrictive, procoagulant, platelet activating, and antifibrinolytic. Dysfunctional endothelial cells release less $^{\cdot}NO$, PGI_2, thrombomodulin and tPA and produce more ET-1, angiotensin II, PAI-1 and vWF, as well as expressing tissue factor. Additionally, dysfunctional endothelial cells overexpress CAMs, which promote the recruitment of blood mononuclear cells. The atherosclerotic process also involves a large number of inflammatory mediators, such as several pro-inflammatory cytokines and acute phase reactants. However, atherosclerosis does not have to necessarily progress to an acute clinical event. Several therapeutic strategies exist, such as exercise training, which mitigates endothelial dysfunction and inflammation. Exercise training consistently improves the nitric oxide bioavailability, and the number of endothelial progenitor cells, and also diminishes the level of inflammatory biomarkers, namely pro-inflammatory cytokines and CRP. These positive effects of chronic exercise could be explained by several mechanisms including: the augment of the bioavailability of $^{\cdot}NO$ and antioxidant defences, the decrease in pro-inflammatory cytokines production by the adipose tissue, skeletal muscles, endothelial cells, blood mononuclear cells, and, the increase of the regenerative capacity of endothelium expressed by the number of circulating EPC. Nevertheless, these mechanisms do not fully enlighten all pathways by which exercise can decrease endothelial dysfunction and inflammation, and hence modulate the progression of the underlying disease progress. Future research is needed to provide convincing support to the mechanisms explaining the beneficial effect of exercise in the inflammatory profile of CAD patients.

CHAPTER III

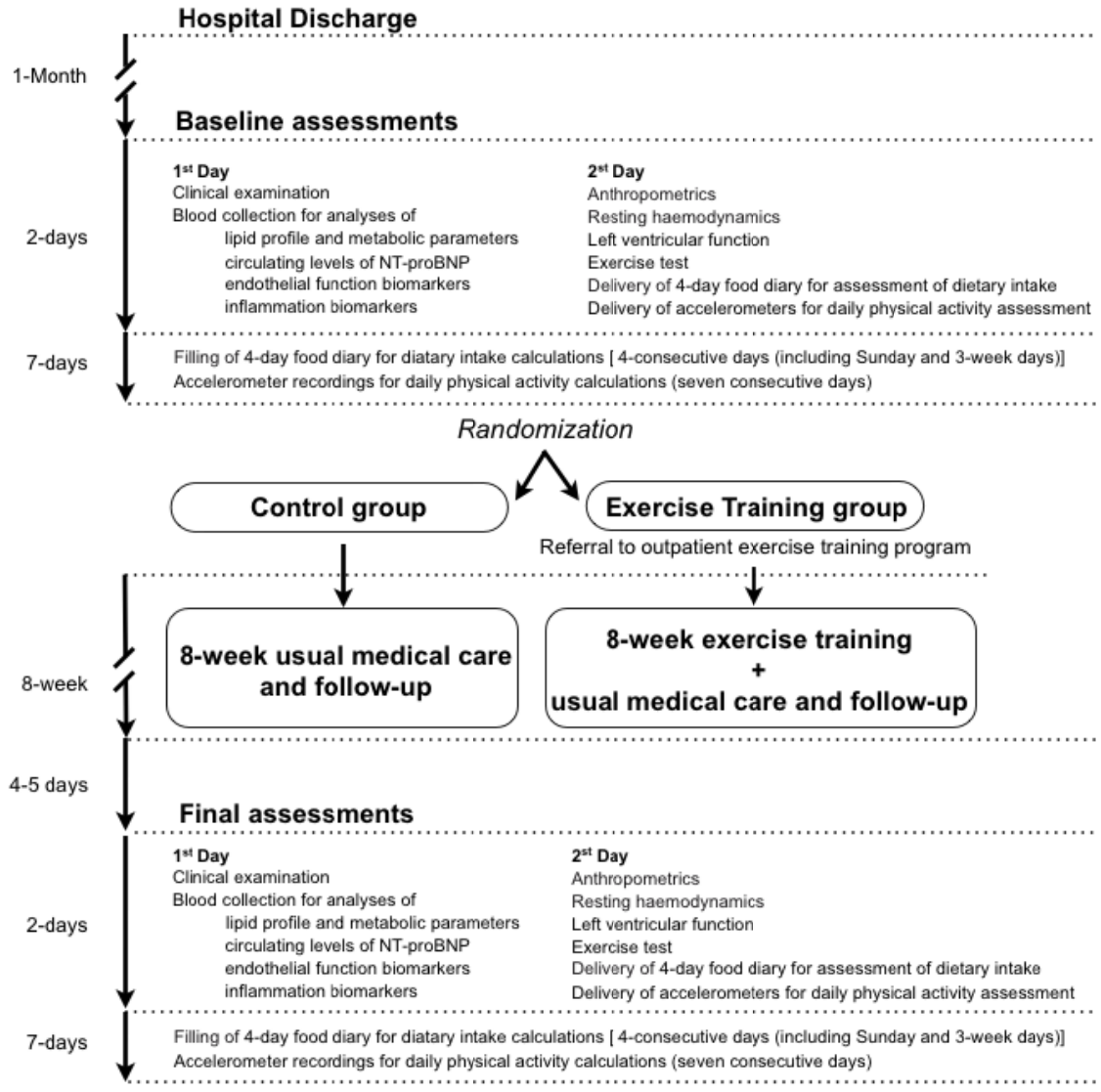
METHODOLOGY

1. Study design, procedures and statistical power

This work is based on a clinical randomized controlled study in CAD patients, conducted in collaboration with the "Serviço de Cardiologia" (cardiology department) and "Serviço de Medicina Física e Reabilitação" (physical medicine and rehabilitation department) of the "Centro Hospitalar de Vila Nova de Gaia/Espinho E.P.E." [Hospital Centre of Vila Nova de Gaia/Espinho (CHVNG/E)]. The hospital ethics committee approved the present study and all the procedures were conducted according to the Declaration of Helsinki. The study design is depicted in Figure 10. To be admitted in the study, patients had to have been referred to the "Serviço de Cardiologia" of the CHVNG/E with the first acute myocardial infarction. From inspection of the clinical files, patients who had hospital discharge and were potential candidates to participate in the study were referred for clinical examination (1 month after hospital discharge).

Patients were asked to come to the hospital in two different days. On the first day, patients were invited to participate in the study and were informed about the purposes, procedures and risks underlying their participation in the study. Those who gave their written consent were included in the study. After clinical examination by a cardiologist, blood was collected for further biochemical determination of lipid profile (total cholesterol, high- and low-density lipoprotein cholesterol, triglycerides), metabolic parameters [fasting plasma glucose and haemoglobin A1c (HbA1c)], N-terminal pro-B-natriuretic peptide (NT-proBNP), and endothelial function (sICAM and sVCAM) and inflammatory biomarkers (CRP, IL-6, IL-10). For the purpose of blood collection, patients were previously informed to observe an overnight fast of at least 12 hours. On the second day (2-3 days after the first visit), patients underwent several evaluations by the following order: anthropometric measurements (weight, height, body mass index, body composition), resting haemodynamics (heart rate, systolic and diastolic blood pressure), 2-dimensional echocardiography for assessment of left ventricular function (left ventricular ejection fraction at rest), and a maximal or symptom-limited exercise testing for the assessment of

cardiorespiratory fitness (VO_2 peak) and haemodynamics at peak exercise (peak heart rate, peak systolic and diastolic blood pressure).



NT-proBNP: N-terminal pro-B-natriuretic peptide

Figure 10. Flowchart depicting the study design.

Patients also were instructed to fill in a four-day food diary, providing details about their diet in the days that followed the second visit to the hospital, and to wear an accelerometer (motion sensor that monitors and evaluates physical activity) during the following 7-days. Patients also were asked to report to the hospital after wearing the accelerometer for 7 days, in order to return both the

4-day food diary and the accelerometer. Following all the baseline assessments, patients were randomly assigned to an exercise-training group or a control group. Complete randomization was performed by allowing the patients to choose one of two sealed numbered envelopes containing the allocation to the exercise training group or control group. The exercise-training group participated in an 8-week outpatient exercise-training programme, whereas the control group received only usual medical care and follow up. The usual care and follow up comprised regular appointments with a cardiologist and medication. After the completion of the 8-week period and within no more than 4-5 days, patients of both groups underwent the final assessment, which included the same evaluations and procedures performed at baseline. All the assessments (including those at baseline and at the end of the study) were performed in the morning by the same examiners and followed the exact same order of assessment.

The statistical power (G*Power 3, University Düsseldorf, Germany) for the matched pairs analysis was computed *a priori* to determine the number of patients required for the study. This computation was based on normative data collected from previous studies, which analyzed the effect of cardiac rehabilitation and exercise training programs on biomarkers of inflammation and endothelial function (4, 27, 127, 128, 189, 208, 260, 314, 355, 359, 400, 404). Taken together, data from those studies provided information to calculate the effect sizes for CRP, IL-10, IL-6, sICAM-1, and sVCAM-1. A sample size calculation revealed that it was required 10 to 18 subjects to detect a large effect size in CRP, IL-10, and IL-6 with a power of 90% and $\alpha = 0.0167$ (0.05/3). Concerning sICAM-1 and sVCAM-1, the sample size required to detect a large effect with a power of 90% and $\alpha = 0.025$ (0.05/2) was 7 subjects. A target of 24 subjects for each group was identified to accommodate an expected drop out rate of 20%.

2. Patients

In order to be included in the study all patients had to be referred to the hospital due to acute myocardial infarction. Patients were excluded from the

study if they met the following criteria: ventricular tachyarrhythmia; uncontrolled hypertension (systolic blood pressure >180 mmHg or diastolic blood pressure >100 mmHg); significant valvular disease; unstable angina pectoris; reduced left ventricular function (ejection fraction < 40%); abnormal hemodynamic response, myocardial ischemia and/or severe ventricular arrhythmias during baseline exercise testing; uncontrolled metabolic disease (e.g. uncontrolled diabetes or thyroid disease); presence of pulmonary and renal comorbidities; peripheral artery disease and/or orthopaedic limitations. Additionally, were excluded from the final statistical analysis patients who changed the medication during the study or those in the exercise-training group who failed to attend to at least 80% of the exercise sessions. Forty-seven consecutive CAD patients (age, 55.7 ± 11.0 years; weight, 79.8 ± 15.1 kg; body mass index, 28.1 ± 4.7 kg/m^2) participated in this study. Patients were revascularized by percutaneous coronary intervention (74.5%) or received non-invasive treatment (25.5%), depending on of the severity of coronary stenosis,

3. Measurements

3.1. Anthropometrics

Height and weight measurements were attained using a standard wall-mounted *stadiometer and scale, respectively.* Body mass index was calculated from the ratio of weight (kg) to squared height (m^2). Percentage of fat mass and fat free mass were calculated by bioelectrical impedance analysis using the tetrapolar body composition analyzer (Bodystat QuadScan 4000, Bodystat Ltd, Isle of Man, UK). Patients were analyzed in the supine position, without shoes and stockings. The patients' upper and lower limbs were positioned at an angle of approximately 45º from the midline. Electrodes were connected to the body extremities (upper limbs: one electrode at the dorsal surface of the wrist so that the upper border of the electrode bisected the head of the ulna, and the other at the base of the third metacarpal-phalangeal joint; and lower limb: one electrode at dorsal surface of the ankle so that the upper border of the electrode bisected the medial and lateral malleoli, and the other at the base of the third metatarsal-

phalangeal joint), after cleaning the areas with alcohol according to the instructions of the manufacturer.

3.2. Lipid profile and metabolic parameters

Twelve-hour fasting blood samples were collected by venipuncture of the antecubital vein for analysis of the following parameters: fasting plasma glucose, total cholesterol, high-density lipoprotein cholesterol, triglycerides, and HbA1c by enzymatic methods (Beckman Synchron LX 20 Analyzer; Beckman Coulter Inc., Fullerton, California, USA). Low-density lipoprotein cholesterol was calculated using the Friedewald equation, except if triglycerides > 400 mg/dL.

3.3. Resting haemodynamics

Resting systolic and diastolic blood pressure and heart rate were measured using a digital automatic blood pressure monitor (Omron M6 Comfort, Omron Healthcare Co., Ltd, Kyoto, Japan). Patients were sat down, resting their arm on a table so the brachial artery was levelled with the heart. Two measurements were then obtained at intervals of 1 minute and their average was recorded. If there was more than 5 mmHg of difference between the two readings, one more reading was obtained for average. The resting heart rate was monitored for 15 s and multiplied by 4 to obtain the resting heart rate for 1 minute. Two readings were made at intervals of 1 minute and values were averaged.

Resting rate-pressure product was computed by multiplying resting heart rate by resting systolic blood pressure.

3.4. Daily physical activity

Physical activity was objectively measured during 7 consecutive days using the ActiGraph accelerometer (model GT1M, Florida, USA). Subjects were asked to wear the accelerometer on the waist using an elastic strap with placement aligned with the right anterior iliac crest during all waking hours (except when bathing or swimming). Additionally, subjects were instructed to call the study coordinator if they had questions. The dual-axis accelerometer measures vertical acceleration and deceleration. The acceleration signal is

filtered and digitized by an 8-bit analog-digital converter at 10 samples per second. The analog-digital converter measures the magnitudes of the accelerations (counts), which are then summed over a given period of time (epoch). For this study the accelerometers were programmed to record data in 60 seconds epochs (counts/min). At the end of the 7-day recording period, patients returned to the hospital with their accelerometers and a study investigator used a reader interface unit to download the accelerometer data into a desktop computer, which was stored until further analysis. A computer program (ActiLife Software, ActiGraph, Florida, USA) was then used to sum the accelerometer counts/min over 7 days and to compute the average min/day spent at different intensities of physical activity. In this study, the cut points of Freedson *et al*. (112) relating counts/min to the intensity of physical activity were used: resting/light (0-1951 counts/min) moderate (1952-5724 counts/min), vigorous (5725-9497 counts/min), and very vigorous (≥9498 counts/min). For analysis, periods of 10 consecutive minutes with zero counts were excluded, assuming that the device was not being worn in such periods. Thus, data were normalized for each patient in order to adjust for inter-individual differences in the total monitoring time. In this sense, a physical activity index (counts/min/day) was created, and calculated by dividing the average of the total daily counts by the average of daily time effectively monitored (excluding periods of time with zero counts).

The accelerometer data in the present study are presented as minutes per day spent in light, moderate, vigorous and very vigorous categories, and average total counts per day. In addition, the number of 10-min bouts of activity per day was calculated. A 10-min bout was defined as 10 or more consecutive minutes where the intensity fell continuously within the range for accelerometer counts that represented 3–6 METs for moderate intensity (9).

3.5. Dietary intake

Dietary intake was assessed using a 4-day food diary as representative of the usual intake according to the methodology described previously (71). Patients were asked to provide detailed information concerning the food and

beverages intake in four consecutive days (Sunday and 3-week days). Subjects also were instructed to call the study coordinator if they had questions. Nutrient intake data were obtained by multiplying the frequency of consumption of each food item by the nutrient content of the specified portion size, with once a day equaling to one. For detailed nutrient analysis, the Food Processor Plus Program version 7.02 (ESHA Research, Salem, OR, USA), based on values from the US Department of Agriculture, was used. Additionally, values for typical Portuguese foods were computed using the Portuguese tables of food composition, typical recipes, and data from previous studies (71). In the present study, the dietary intake is summarized in the following parameters: total energy intake, consumption of protein, carbohydrates, total fibre, sodium, total fat, saturated fat, monounsaturated fat, cholesterol, and n-3 and n-6 fatty acids.

3.6. Left ventricular function

Circulating levels of NT-proBNP and left ventricular ejection fraction were used as indicators of left ventricular function. Plasma circulating levels of NT-proBNP were measured by means of a 1-step enzyme immunoassay based on electrochemiluminescence (Elecsys; Roche Diagnostics, Mannheim, Germany). The plasma used to derivate NT-proBNP was collected by venipuncture from the antecubital vein after a 12-hour fasting period. Left ventricular ejection fraction was assessed with 2-dimensional echocardiography (HP Sonos 7500, Philips, Amsterdam, NL) using the biplane Simpson's method. The subjects underwent a standard echocardiographic examination. The same experienced technician performed all the echocardiograms. Images were videotaped at the end of the expiration phase of normal respiration. A standard protocol was used based on apical fourand two-chamber views according to the recommendations of the American Society of Echocardiography (129).

3.7. Biomarkers of endothelial function and inflammation

The circulating levels of sICAM-1 and sVCAM-1 were measured at baseline and at the end of the study, as biomarkers of endothelial function. Similarly, the circulating levels of CRP, IL-6 and IL-10 were assessed before and after the 8

weeks and were used as inflammatory biomarkers. To assess these biomarkers, 10ml of blood was collected, before exercise testing, from the antecubital vein and transferred into serum separator tubes. Samples were allowed to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Then, the serum was aliquoted and stored at -20ºC for future biochemical analysis. Using commercially available assay kits (R&D Systems, Minneapolis, MN, USA), the serum levels of sICAM-1 (catalogue number DCD540), sVCAM-1 (catalogue number DVC00), CRP (catalogue number DCRP00), IL-10 (catalogue number D1000B), and IL-6 (catalogue number D6050) were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions and read at 450 nm using a microplate reader (Labsystems iEMS MF controlled by Ascent software v. 2.4, Dynex Labsystems). All samples were assayed in duplicate. All patients were screened and excluded from biomarkers assessment if infection and/or any acute inflammatory process was reported or detected.

3.8. Cardiorespiratory fitness and haemodynamics at peak exercise

Maximal or symptom-limited treadmill exercise testing was conducted using the modified Bruce protocol (122). All subjects were encouraged to exercise to exhaustion. However, exercise test end-points were considered according to the guidelines for exercise testing of the American College of Sports Medicine and the American College of Cardiology/American Heart Association (1, 122). Pulmonary gas exchange analysis was performed throughout the exercise protocol, using the Cardiovit CS-200 Ergo Spiro (Schiller, Baar, Switzerland) measuring system. Oxygen uptake (VO_2), carbon dioxide production (VCO_2), minute ventilation (V_E), and respiratory exchange ratio (RER) were collected breath-by-breath. VO_2 peak was defined as the highest VO_2 achieved by the patient during the test. Values for VO_2 were indexed to body weight. Electrocardiogram (ECG), heart rate, and blood pressure were continuously monitored and recorded during each stage of exercise testing. Using the 12-lead ECG readings, heart rate was monitored during the test and averaged every 10 s. Peak heart rate was considered the highest 10 s average value

achieved during the test. Blood pressure was measured with a mercury sphygmomanometer at rest, during the last 45 s of each stage at exercise, and in the last 15 s of exercise when the patients approached the end of the exercise testing. Peak systolic and diastolic blood pressures were recorded as the highest value achieved during the exercise testing. Peak rate-pressure product was computed by multiplying peak heart rate by peak systolic blood pressure. A post-exercise cool-down period was provided, after heart rate recovery has been recorded for 1 minute standing. The cool-down consisted of walking for 2 minutes at 2 km/hour (1.243 mile/hour) and 0% grade and then standing for at least 4 minutes. Heart rate recovery was determined in response to the treadmill exercise test by calculating the difference between heart rate at peak exercise and heart rate at 1st minute after completion of exercise (120), and represented the drop in heart rate during that time interval.

4. Exercise training programme

Patients in the exercise-training group participated in an outpatient aerobic exercise-training programme following the exercise prescription guidelines of the American Association of Cardiopulmonary Prevention and Rehabilitation (325). The supervised exercise-training programme was performed 3 days per week for a total of 8 weeks. Each exercise session included 10 minutes of warm up (including stretching and callisthenics), 35 minutes of aerobic exercise, and 10 minutes of cool down. Aerobic exercise involved 35 minutes of exercise on cycloergometer or treadmill. The exercise intensity for aerobic training was calculated as 65-75% of maximal heart rate achieved in the treadmill exercise testing. Individualized exercise prescription was periodically adjusted to encourage a gradual increase in overall exercise performance. Participants were instructed to adjust their work rate to meet their target intensity range, using perceived exertion [i.e., somewhat hard (score of 13) to hard (score of 15)] as an adjunctive intensity modulator. In addition to the supervised exercise sessions, each patient was encouraged to daily exercise outside the formal exercise programme.

5. Data Analysis

Data was analyzed using SPSS 17.0 software programme for windows. Exploratory analysis was performed to test for the normality of the distribution. Data is reported as absolute values and as percentage of change [(final value – baseline value/baseline value) * 100]. Variables that were normally distributed are reported as mean ± standard deviation, and those that were not are reported as median (percentile 25^{th} – percentile 75^{th}). Student's independent t-test, Mann–Whitney U test and Chi-square test were used to compare the baseline values and the percentage of change between the two groups in normally distributed, not normally distributed, and nominal data, respectively. Student's paired t-test and Wilcoxon signed-rank test were performed for intra-individual comparisons within groups for normally and not normally distributed data, respectively. Additionally, each group was further divided into two sub-groups based on the baseline blood pressure (pre-hypertension/hypertension versus normotension), daily physical activity (non-active versus active), and glycaemic control (diabetic versus non-diabetic); in order to assess the influence of the baseline values on the effects of exercise training on blood pressure, daily levels of moderate-intensity physical activity, and glycaemic control, respectively. The criteria to define the sub-groups were: systolic blood pressure >120 mmHg, and/or diastolic blood pressure >80 mmHg to define pre-hypertension/hypertension (245); daily levels of moderate-intensity physical activity <30 minutes to define non-active lifestyle (26); and, fasting blood glucose level > 125 mg/dL or current treatment with insulin or oral antidiabetic agents to define diabetes (131).

Pearson's correlation coefficient was used to analyze the association of percentage changes in endothelial function and inflammatory biomarkers with age and changes in anthropometrics, cardiorespiratory fitness, haemodynamics at peak exercise, left ventricular function, dietary intake, and daily physical activity. Variables with $P<0.10$ were included in a multiple linear regression analysis (method: enter) to assess the extent to which they influence the percentage changes in biomarkers of endothelial function and inflammation. In the first step of the regression analysis, the variable group was forced into the

model. In the second step, the group was forced into the model together with the variables with $P<0.10$. $P\leq0.05$ was considered statistically significant.

CHAPTER IV

RESULTS

1. Patients Characteristics

From the forty-seven patients that initially met the criteria for enrolment in the study, seven patients terminated the study prematurely not being considered for data analysis. Indeed, in the control group, one patient died during the study period, three declined to participate in the final assessment and one moved to another geographical area; and, in the exercise-training group, one patient withdrew for personal reasons (moved to abroad) and other for orthopaedic problems (broken leg). Additionally, despite participating in the final assessment, two patients were excluded from the statistical analysis because they only complied with approximately 50% of the exercise sessions (Figure 11).

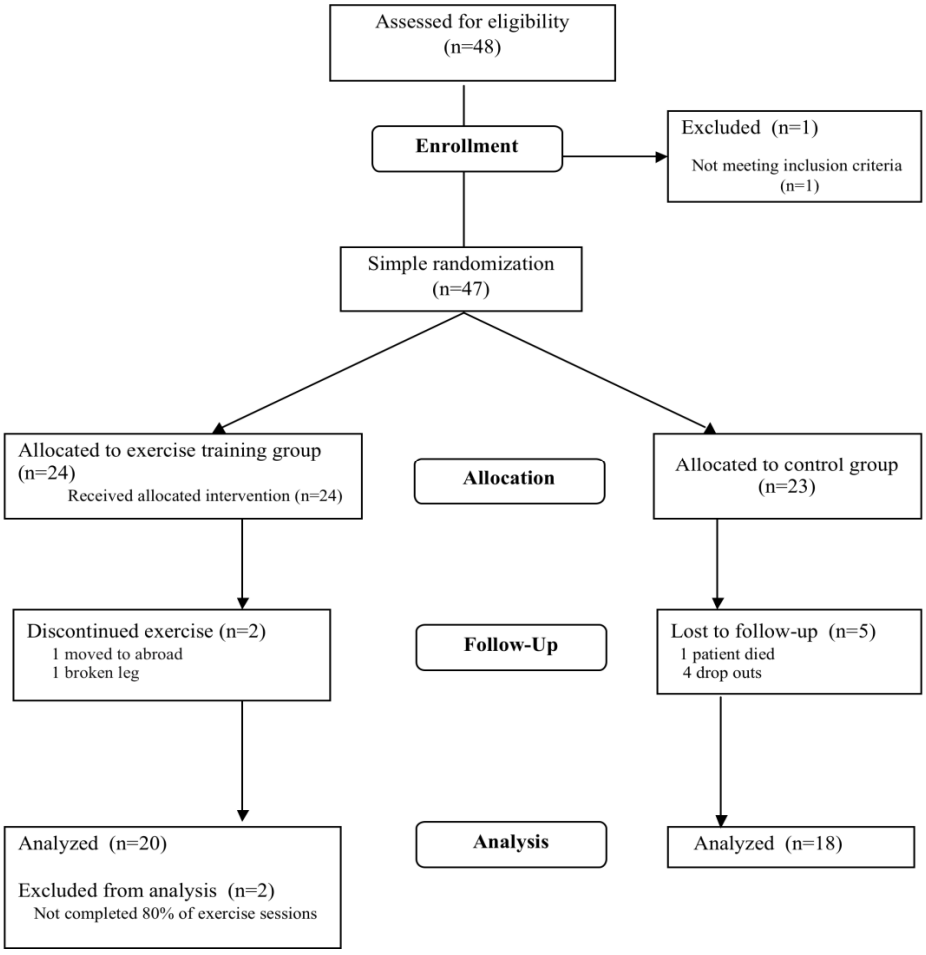


Figure 11. Flow diagram providing information concerning the four stages of the study: enrolment, intervention allocation, follow-up, and analysis.

The thirty-eight patients included in the analysis (age 55.6 ± 9.4 years old, body mass index 27.5 ± 4.3 kg/m^2) were revascularized by percutaneous coronary intervention (77.8%) or received non-invasive treatment (22.2%). Patients were medicated with β-blockers (89.5%), nitrates (7.9%), diuretics (15.8%), Ace-inhibitors (73.7%), antiplatelets (81.6%), Angiotensin II receptor antagonist (7.9%), and lipid-lowering drugs (100%). During the study the medication remained unchanged. General patients' characteristics are reported in Table 5.

On average, hospital discharge occurred 5.2 ± 2.2 and 5.0 ± 0.9 days after hospital admission, respectively for control and exercise-training group (*P*=0.703). Patients in the exercise-training group attended to 24 ± 0.67 exercise sessions. On average, the time interval from hospital discharge to the first assessment was 35.8 ± 3.5 and 35.9 ± 3.9 days, respectively for exercise-training and control group (*P*=0.929). Likewise, the interval from hospital discharge to the second assessment was similar for both groups, respectively 126.7 ± 11.8 and 125.4 ± 17.1 days for exercise-training and control group (*P*=0.796). Both groups were mostly men and were statistically similar regarding the prevalence of cardiovascular disease risk factors, cardiac intervention and medication prescription (Table 5).

2. Anthropometrics

At baseline, the body weight of patients in the exercise-training group was significantly higher than that of the patients in the control group (*P*=0.007, Table 6). However, regarding body mass index, percentage of fat mass and fat free mass no significant differences were presented between groups at baseline (Table 6). After 3 months none of these parameters changed significantly (Table 6).

Table 5. Baseline general patient characteristics in both groups

	Exercise-training group	Control Group	*P* value
General Features			
n	20	18	
Age (years)	54.3 ± 10.8	57.0 ± 7.6	0.366
Sex (% male)	90.0	72.2	0.158
Cardiovascular disease risk factors			
Currently smoking (%)	55.6	55.6	1.000
Diabetes mellitus (%)	15	5.6	0.603
Hypertension (%)	95.0	100	1.000
Hyperlipidemia (%)	100	100	1.000
Familiar history (%)	22.2	5.6	0.338
Obesity (%)	22.2	25.0	1.000
Cardiac pathology/intervention			
Referred to hospital with			
Acute myocardial infarction (%)	100	100	1.000
Coronary arteries treated with			
Percutaneous coronary intervention (%)	83.3	72.2	0.691
Non-invasive treatment (%)	16.7	27.8	0.691
Medication prescription			
β-blockers (%)	90.0	88.9	1.000
Ace inhibitors (%)	75.0	72.2	1.000
Antiplatelets (%)	80.0	83.3	1.000
Diuretics (%)	15.0	16.7	1.000
Nitrates (%)	0.0	16.7	0.097
Angiotensin II receptor antagonist (%)	5.0	11.1	0.595
Lipid-lowering drugs (%)	100	100	1.000

Criteria for diabetes are based on fasting blood glucose level > 125 mg/dL or current treatment with insulin or oral antidiabetic agents, hypertension are based on seated blood pressure >140/90 mmHg or antihypertensive treatment, obesity are based on body mass index > 30 kg/m^2, and hyperlipidemia are based on fasting total cholesterol > 175 mg/dL or use of antilipidemic medication.

3. Resting haemodynamics

At baseline, resting systolic and diastolic blood pressure, resting heart rate, and rate-pressure product in the exercise-training group were similar to those in the control group (Table 6). From baseline to the final assessment, resting systolic and diastolic blood pressure, and resting rate-pressure product were not significantly changed in both groups (P>0.05). Nevertheless, a trend for decreased resting rate-pressure product was found in the exercise-training group (P=0.078). An average decrease of 5.5 beats per minute in the resting heart rate was found after the training (P=0.03). Conversely, no changes were observed in the resting heart rate of patients in the control group (P>0.05, Table 6).

From a total of 38 patients that composed both groups, 5 in the control group and 9 in the exercise-training group fulfil the criteria to be classified as pre-hypertensive/hypertensive. In these patients, the exercise-based intervention was effective by reducing significantly the systolic blood pressure (from 135 ± 7.1 to 125.6 ± 11.3 mmHg, P=0.012). However, the diastolic blood pressure remained unchanged (from 80.6 ± 10.1 to 77.2 ± 9.4 mmHg; P=0.360). The normotensive patients in the exercise-training group increased the systolic (from 110.9 ± 8.0 to 120.0 ± 14.1 mmHg, P=0.022) and maintained the diastolic blood pressure (from 75.0 ± 5.0 to 78.5 ± 6.7 mmHg, P=0.173). In the control group, both systolic (from 134 ± 5.5 to 130 ± 14.1 mmHg, P=0.477) and diastolic (from 76 ± 5.5 to 85 ± 15.8 mmHg, P=0.181) blood pressure remained unchanged in the pre-hypertensive/hypertensive patients. In this group, the normotensive patients increased the systolic (from 111.2 ± 7.7 to 117.7 ± 7.7 mmHg, P=0.041) but not the diastolic (from 73.5 ± 7.5 to 73.9 ± 8.7 mmHg, P=0.883) blood pressure.

Table 6. Changes in anthropometrics, resting haemodynamics, and lipid profile and metabolic parameters

	Exercise-training group			Control Group		
	Baseline Assessment	**Final Assessment**	**Δ%**	**Baseline Assessment**	**Final Assessment**	**Δ%**
Anthropometrics						
Body weight (kg)	83.4 ± 14.1	83.0 ± 13.8	-0.33 ± 5.4	72.0 ± 10.1[#]	71.3 ± 10.3	-0.99 ± 2.8
Body mass index (kg/m^2)	28.4 ± 4.0	28.2 ± 4.0	-0.33 ± 5.4	26.6 ± 4.6	26.4 ± 4.5	-0.99 ± 2.8
Fat mass (%)	27.5 ± 4.5	26.8 ± 5.5	-2.5 ± 11.0	29.6 ± 8.3	29.3 ± 8.3	-0.97 ± 9.9
Fat free mass (%)	72.5 ± 4.5	73.1 ± 5.5	0.69 ± 3.2	70.4 ± 8.3	70.7 ± 8.3	0.33 ± 3.1
Resting haemodynamics						
HR (beats/min)	68.0 ± 9.2	62.6 ± 8.7[†]	-7.1 ± 13.9	67.5 ± 9.7	66.1 ± 9.6	-1.1 ± 15
SBP (mmHg)	121.6 ± 14.4	122.5 ± 13.0	1.3 ± 11.7	117.5 ± 12.6	121.0 ± 14.1	3.4 ± 10.1
DBP (mmHg)	78.0 ± 8.2	77.5 ± 7.9	-0.1 ± 15.8	74.2 ± 6.9	76.9 ± 11.8	4.0 ± 14.3
RPP (10^3)	8.3 ± 1.5	7.6 ± 1.3	-6.1 ± 15.8	7.9 ± 1.5	8.0 ± 1.4	2.8 ± 23.3
Lipid profile and metabolic parameters						
TC (mg/dL)	138.8 ± 24.6	146.6 ± 27.3[†]	5.4 ± 9.9	132.4 ± 28.5	140.3 ± 29.5[†]	10.8 ± 17.6
HDL (mg/dL)	41.8 ± 8.8	46.1 ± 8.6[†]	9.6 ± 15.7	40.9 ± 15.0	45.0 ± 13.1[†]	14.6 ± 22.6
LDL (mg/dL)	71.0 ± 18.0	74.1 ± 19.7	4.4 ± 21.2	67.1 ± 19.6	73.3 ± 21.8[†]	19.5 ± 27.9
TC-HDL ratio	3.3 ± 0.8	3.2 ± 0.9	-2.7 ± 11.5	3.5 ± 1.1	3.5 ± 1.9	-0.1 ± 21.0
Triglycerides (mg/dL)	123.1 ± 54.8	128.4 ± 67.7	5.4 ± 31.7	125.6 ± 50.2	116.8 ± 69.8	-1.4 ± 39.0
Fasting glucose (mg/dL)	98.3 ± 19.2	100.7 ± 15.3	3.8 ± 11.3	101.2 ± 24.1	91.9 ± 13.5	-7.5 ± 12.3[#]
HbA1c (%)	5.7 ± 0.5	5.7 ± 0.3	-3.4 ± 11.4	5.8 ± 0.7	5.6 ± 0.4	-2.5 ± 7.3

HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; RPP: rate-pressure product; TC: total cholesterol; HDL: high-density lipoprotein; LDL: Low-density lipoprotein; HbA1c: haemoglobin A1c; Δ%: percentage of changes

[#] Significantly different from exercise-training group, P<0.05;

[†] Significantly different from baseline assessment, P<0.05

4. Lipid profile and metabolic parameters

There were no differences between groups at baseline in the lipid profile and metabolic parameters (P>0.05, Table 6). In the exercise-training group, significant increases were found in blood plasma total cholesterol (P=0.048) and HDL cholesterol (P=0.040), but not in LDL cholesterol (P>0.05, Table 6). In the control group, significant increases from baseline to the final assessment were observed in blood plasma total cholesterol (P=0.046), HDL (P=0.031) and LDL (P=0.022) cholesterol. In both groups, the circulating levels of triglycerides, fasting glucose, total cholesterol-HDL ratio, and HbA1c remained unchanged (P>0.05). The percentage of change in blood plasma total cholesterol and HDL cholesterol was similar in both groups (P>0.05).

To analyse the effect of the intervention in the glycaemic control of patients with diabetes, the three diabetic patients in the exercise-training group were analyzed separately. Despite no changes were observed in fasting blood glucose levels (from 113.7 ± 38 to 114.7 ± 18 mg/dL, P=0.953), there was a significant decrease of 1.87 ± 0.57 % in the levels of HbA1c (from 7.93 ± 1.03 to 6.07 ± 0.87 %, P=0.030). Conversely, the non-diabetic patients maintained unchanged the levels of HbA1c (from 5.55 ± 0.31 to 5.66 ± 0.22 %, P=0.214).

5. Daily physical activity level

At the beginning of the study, no differences between groups were observed in the daily physical activity index, time spent at light and moderate intensity physical activity, and number of bouts (10-minute periods performing continuously physical activity of moderate intensity) (P>0.05, Table 7). Neither the exercise-training group nor the control group spent time in vigorous- or very vigorous-intensity physical activity in the two assessment moments. In the control group the above-mentioned parameters remained unchanged (P>0.05, Table 7). Although no changes were observed in the daily physical activity index and in the number of bouts, the exercise-training group increased significantly the time spent in light (P=0.024) and moderate (P=0.017) physical activity. In the exercise-training group the time spent in light and moderate physical activity increased in average 10% and 65%, respectively.

Table 7. Changes in daily physical activity and dietary intake

	Exercise-training group			Control Group		
	Baseline Assessment	**Final Assessment**	**Δ%**	**Baseline Assessment**	**Final Assessment**	**Δ%**
Daily physical activity						
Light (min/day)	593.5 ± 108.8	646.9 ± 120.4[†]	10.0 ± 16.9	641.2 ± 114.4	624.9 ± 99.5	-1.7 ± 11.6[#]
Moderate (min/day)	37.1 ± 25.5	50.0 ± 32.3[†]	64.6 ± 123	39.8 ± 23.8	38.7 ± 26.3	-3.7 ± 34.7[#]
Bouts*	0.46 (0.06 – 1.13)	0.71 (0.22 – 1.11)	0 (-28.6 – 99.9)	0.71 (0 – 1.29)	0.43 (0.13 – 0.79)	-11.1 (-61.3 – 15.3)
PA index (counts/min/ day)	428.7 ± 167.0	480.1 ± 208.6	11.6 ± 26.8	430.7 ± 165.2	434.3 ± 184.3	4.3 ± 32.3
Dietary intake						
Energy (kcal)	1624 ± 355.4	1651 ± 502.9	0.62 ± 20.6	1678.1 ± 367.2	1774.3 ± 419.8	6.2 ± 16.9
Protein (g)	86.5 ± 12.8	82.6 ± 25.4	-6.1 ± 19.5	82.9 ± 16.0	84.2 ± 15.6	4.2 ± 24.8
Carbohydrates (g)	197.7 ± 57.5	200.8 ± 64.9	2.2 ± 20.0	189.2 ± 43.1	201.0 ± 53.4	7.0 ± 20.8
Total Fibre (g)	18.6 ± 7.2	17.0 ± 7.2	-4.3 ± 26.7	17.4 ± 6.0	18.4 ± 5.8	13.0 ± 41.7
Sodium (g)	1.39 ± 0.46	1.60 ± 0.78	17.2 ± 49.4	1.51 ± 0.74	1.56 ± 0.50	18.7 ± 65.1
Total fat (g)	53.4 ± 13.0	55.7 ± 19.8	6.7 ± 43.2	62.3 ± 15.0	65.6 ± 15.9	7.4 ± 22.9
Saturated fat (g)*	14.7 (12.2 – 19.1)	17.6 (12.5 – 20.7)	0 (-11.6 – 26.5)	16.8 (14.2 – 19.9)	17.2 (14.4 – 21.6)	0 (-10.8 – 3.3)
Monounsaturated fat (g)*	23.5 (18.1 – 28.9)	22.5 (17.3 – 34.1)	0 (-29.5 – 20.2)	26.5 (22.6 – 37.4)	28.4 (24.2 – 36.2)	0 (-11.1 – 23.5)
Polyunsaturated fat (g)*	8.1 (7.0 – 10-6)	9.5 (7.2 – 11.2)	0 (-11.5 – 19.4)	10.9 (8.9 – 12.8)[#]	12.0 (9.1 – 13.3)	0 (-12.5 – 20.0)
Cholesterol (mg)*	211 (179 – 234)	200 (156 – 231)	0 (-32.8 – 2.1)	216 (190 – 283)	208 (183 – 267)	-0.8 (-16.2 – 7.2)
n-3 fatty acids (g)*	0.97 (0.74 – 1.24)	0.95 (0.71 – 1.27)	0 (-27.3 – 24.4)	0.88 (0.78 – 1.33)	1.2 (0.8 – 1.5)	4.8 (-5.1 – 49.1)
n-6 fatty acids (g)	7.2 ± 1.9	7.6 ± 2.7	9.6 ± 38.3	9.4 ± 2.3[#]	9.1 ± 2.9	-0.4 ± 26.4

PA index: physical activity index; Δ%: percentage of changes

* Values are median (Percentile 25th – Percentile 75th)

[#] Significantly different from exercise-training group, P<0.05;

[†] Significantly different from baseline assessment, P<0.05

In opposition, the control group decreased in average 1.7% in light and 3.7% in moderate intensity physical activity (Table 7). These percentage changes were significantly higher in the exercise-training group, either for physical activity of light- (P=0.025) as moderate-intensity (P=0.031).

To analyse the effect of exercise training on those patients with levels of moderate-intensity physical activity below the recommendations they were analysed in separate. Nonetheless, the results followed the same pattern as for the entire groups. In the eight patients of the control group who performed at baseline less than 30 minutes of moderate intensity physical activity no differences were observed (from 16.9 ± 8.2 to 21.7 ± 13.6 min, P=0.280). In contrast, the nine patients of the exercise-training group, which at baseline performed less than 30 minutes, enhanced the time spent in moderate intensity activities by 13 minutes, that is, from 11.7 ± 8.1 to 24.7 ± 18.3 min (P=0.05). In both groups, the patients that at baseline performed more than 30 minutes did not increase the time spent in moderate-intensity physical activity (control group: 52.9 ± 19.6 to 51.1 ± 24.3 min, P=0.746; exercise-training group: 55.0 ± 16.8 to 68.2 ± 27.2 min, P=0.100).

6. Dietary intake

At baseline the total energy intake, consumption of protein, carbohydrates, total fibre, sodium, total fat, saturated fat, monounsaturated fat, cholesterol, and n-3 fatty acids was similar in both groups (P>0.05). A significantly higher intake of polyunsaturated fat (P=0.009) and n-6 fatty acids (P=0.002) was found in the control group compared to the exercise-training group at baseline (Table 7). No changes were observed in the dietary intake of both groups from baseline to the final assessment (P>0.05, Table 7).

The percentage of calories derived from daily macronutrients' intake in the exercise-training group was (respectively at baseline and final assessment): from proteins, 21.8 ± 3.4% and 20.4 ± 4.4%; from carbohydrates, 48.0 ± 7.3% and 48.6 ± 6.5%; from total fat, 29.7 ± 4.2% and 30.1 ± 4.3%; from saturated fat, 8.9 ± 1.9% and 9.0 ± 2.1%; from monounsaturated fat, 13.3 ± 2.3% and

13.5 ± 2.2%; from polyunsaturated fat, 4.9 ± 1.1% and 5.1 ± 0.9%; from n-3 fatty acids, 0.59 ± 0.2% and 0.60 ± 0.2%; from n-6 fatty acids 4.0 ± 0.9% and 4.2 ± 0.8%. In the control group the percentage of calories derived from daily macronutrients' intake was (respectively at baseline and final assessment): from proteins, 20.0 ± 2.3% and 19.4 ± 2.3%; from carbohydrates, 45.4 ± 6.3% and 45.4 ± 6.4%; from total fat, 33.5 ± 4.7% and 33.5 ± 4.0%; from saturated fat, 9.4 ± 1.7% and 9.5 ± 1.7%; from monounsaturated fat, 15.4 ± 3.1% and 15.0 ± 1.9%; from polyunsaturated fat, 6.0 ± 1.1 % and 5.9 ± 1.3%; from n-3 fatty acids, 0.57 ± 0.2% and 0.63 ± 0.3%; from n-6 fatty acids, 5.1 ± 1.1% and 5.4 ± 6.4%.

7. Cardiorespiratory fitness and haemodynamics at peak exercise

At baseline, no significant differences were present between groups in VO_2 peak, peak heart rate, peak systolic and diastolic blood pressure, and rate pressure product (P>0.05, Table 8). At baseline, the control group showed a significant higher heart rate recovery than the exercise-training group (26 ± 7 vs. 20 ± 6 beats/min, P=0.008). After the exercise training an improvement in VO_2 peak by 8.5% was observed, from 31.4 ± 7.7 to 33.7 ± 8.0 ml/min/kg (P=0.016). In contrast, patients in the control group did not change VO_2 peak. No changes of peak heart rate, peak systolic and diastolic blood pressure, and peak rate pressure product were found (P>0.05). Regarding heart rate recovery at first minute, the exercise-training group increased significantly the recovery from 20 ± 6 to 24 ± 5 beats per minute (P=0.007), whereas the control group remained unchanged. The percentage of changes in heart rate recovery was significantly different between groups (P=0.009).

Table 8. Changes in cardiorespiratory fitness and haemodynamics at peak exercise

	Exercise-training group			Control Group		
	Baseline Assessment	**Final Assessment**	**∆%**	**Baseline Assessment**	**Final Assessment**	**∆%**
Cardiorespiratory fitness and haemodynamics at peak exercise						
VO_2peak (ml/min/kg)	31.4 ± 7.7	33.7 ± 8.0†	8.5 ± 14.9	32.4 ± 5.7	33.0 ± 7.2	1.8 ± 13
HRpeak (beats/min)	137 ± 20	134 ± 18	-1.1 ± 10.0	134 ± 12	136 ± 13	1.7 ± 8.8
SBPpeak (mmHg)	164.6 ± 28.1	176.6 ± 27.9	7.1 ± 21.1	149.7 ± 16	152.7 ± 19.9	3.0 ± 11.9
DBPpeak (mmHg)	78.9 ± 7.5	77.5 ± 10.2	-1.8 ± 11.1	74.4 ± 8.6	76.5 ± 12.3	3.4 ± 16.6
RPPpeak (10^3)	23.0 ± 5.9	23.6 ± 5.2	9.7 ± 25.6	20.1 ± 2.9	20.8 ± 3.7	4.1 ± 14.8
HRR (beats/min)	20.0 ± 6.4	24.0 ± 4.7†	35.4 ± 50.1	26.1 ± 7.2#	25.9 ± 9.7	-2.5 ± 25#

VO_2peak: peak oxygen uptake; HRpeak: peak heart rate; SBPpeak: peak systolic blood pressure; DBPpeak: peak diastolic blood pressure; RPPpeak: peak rate pressure product; HRR: heart rate recovery; ∆%: percentage of changes

Significantly different from exercise-training group, P<0.05;

† Significantly different from baseline assessment, P<0.05

8. Left ventricular function

Left ventricular ejection fraction and circulating levels of NT-proBNP were identical at baseline in both groups (Table 9). In both groups, left ventricular ejection fraction remained unchained during the study. Conversely, a significant decrease in NT-proBNP levels was observed in both groups (*P*=0.008 and *P*=0.023, respectively in exercise-training group and control group). The percentage of changes was no different between the two groups (*P*=0.531).

Table 9. Changes in left ventricular function

	Exercise-training group			Control Group		
	Baseline Assessment	**Final Assessment**	**Δ%**	**Baseline Assessment**	**Final Assessment**	**Δ%**
Left ventricular function						
Ejection fraction (%)	55.1 ± 7.7	56.8 ± 4.4	4.5 ± 13.6	55.5 ± 6.8	55.2 ± 6.2	-0.1 ± 6.9
NT-proBNP (pg/mL)	302.1 ± 207.5	111.9 ± 40.6†	-52.0 ± 22.9	246.0 ± 203.5	126.1 ± 108.8†	-45.2 ± 26.3

NT-proBNP: N-terminal pro–Brain natriuretic peptide; Δ%: percentage of changes

† Significantly different from baseline assessment, P<0.05

9. Inflammatory biomarkers

At baseline no significant differences were observed between groups in the circulating levels of C-reactive protein, IL-6, and IL-10 (*P*>0.05, Table 10). The category classification according to the clinical predictive value of C-reactive protein for future cardiovascular events of low (<1 mg/L), moderate (1-3 mg/L) and high (> 3mg/L) indicated that at baseline 52.6% (n=20) of the patients exhibited C-reactive protein levels inferior to 1 mg/L, 34.2% levels between 1-3 mg/L (n=13) and only 13.2% (n=5) levels superior to 3 mg/L. The circulating levels of CRP and IL-6 did not change from baseline to the end of the study in both groups (*P*>0.05). An increase in IL-10 levels was found after the exercise-training programme (*P*=0.036). Conversely, no changes of IL-10 levels were found in the control group (*P*=0.268). The percentage of changes in the exercise-training group was significantly superior to that observed in the control group (*P*=0.025, Table 10).

10. Endothelial function biomarkers

At baseline, the endothelial function, expressed by the circulating levels of sVCAM-1 and sICAM-1, was similar in both groups (*P*>0.05, Table 10). Nevertheless, the circulating levels of sVCAM-1 and sICAM-1 remained unchanged after the exercise intervention, while a significant increase in both sVCAM-1 (*P*<0.001) and sICAM-1 (*P*=0.001) was observed in the control group. Although there were no differences in the percentage changes of sVCAM-1 and

sICAM-1, the control group showed a greater trend to increase than the exercise-training group (Table 10).

Table 10. Changes in circulating biomarkers of inflammation and endothelial function

	Exercise-training group			Control Group		
	Baseline Assessment*	**Final Assessment***	**Δ%**	**Baseline Assessment***	**Final Assessment***	**Δ%**
Inflammatory biomarkers						
CRP (mg/L)	1.09 (0.50 – 2.17)	1.36 (0.47 – 2.35)	45.7 ± 86.3	0.89 (0.46 – 1.68)	0.85 (0.67 – 1.90)	44.7 ± 112
IL-6 (pg/mL)	2.48 (1.57 – 3.16)	3.13 (2.13 – 4.99)	37.2 ± 79.1	3.64 (1.69 – 6.53)	3.48 (2.77 – 4.66)	56.0 ± 150
IL-10 (pg/mL)	5.82 (2.59 – 9.05)	6.36 (4.05 – 14.66)†	115 ± 195	7.69 (4.36 – 17.17)	7.26 (2.93 – 10.14)	1.6 ± 71.2#
Endothelial function biomarkers						
sICAM-1 (ng/mL)	169.4 (143 – 209)	187.3 (154 – 201)	8.2 ± 25.6	179.8 (147 – 219)	206.3 (169 – 242)†	16.3 ± 13.3
sVCAM-1 (ng/mL)	297.0 (223 – 377)	340.4 (269 – 418)	20.4 ± 33.2	244.8 (179 – 304)	340.9 (231 – 424)†	36.3 ± 47.3

CRP: C-reactive protein; IL: interleukin; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; Δ%: percentage of changes

* Values are median (Percentile 25th – Percentile 75th)

Significantly different from exercise-training group, P<0.05;

† Significantly different from baseline, P<0.05

11. Relationship of biomarkers of endothelial function and inflammation with age, anthropometrics, haemodynamics, cardiorespiratory fitness, dietary intake, and daily physical activity

Since percentage of changes in inflammatory and endothelial function biomarkers was the primary outcome, its association with the change in parameters that could potentially have influence on them, namely age, changes in anthropometrics, haemodynamics, cardiorespiratory fitness, dietary intake, and daily physical activity, was ascertained. Percentage of changes of IL-10

levels was positively correlated with percentage of changes of moderate-intensity physical activity (r=0.606, *P*<0.001). The effect size of this association is large, as the coefficient of determination (r^2) indicates that 36.7% of the variance in percentage of change of IL-10 is shared with the percentage of changes of moderate-intensity physical activity. No correlation was found between the percentage of changes in IL-10 levels and age, measures of cardiorespiratory fitness, haemodynamics, dietary intake, and anthropometrics. Percentage of changes in the other biomarkers (CRP, IL-10, sVCAM-1, sICAM-1) was not correlated with age and daily physical activity, cardiorespiratory fitness, haemodynamics, dietary intake and anthropometrics.

From these findings, a linear regression model was constructed including the variable group and the percentage of changes of moderate-intensity physical activity as independent variables. The linear regression analysis indicated that when the variable group was evaluated as a predictor, the adjusted r^2 was 0.10. When percentage changes in moderate-intensity physical activity was considered, it accounted for 35.3% of the variance in percentage changes of IL-10 levels (*P*=0.001). This association was independent of the variable group. In fact, group is not significantly related to the percentage of changes in IL-10 levels after adjusting for the percentage of changes in moderate-intensity physical activity (*P*=0.260, Table 11).

Table 11. Summary of regression analysis

	Beta	**Standard Error of Beta**	***P* value**
Model 1			
Group	119.7	56.2	0.041
Model 2			
Group	58.0	50.6	0.260
∆% Moderate-intensity physical activity	0.91	0.25	0.001

CHAPTER V

DISCUSSION

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DISCUSSION

In the present investigation, we evaluated the effects of an exercise-training programme on the biomarkers of endothelial function and inflammation in patients with a history of recent myocardial infarction. We have hypothesized that exercise training attenuates the endothelial dysfunction and inflammation in CAD patients, after an acute myocardial infarction, independent of significant changes in cardiovascular traditional risk factors.

Exercise training enhanced the circulating levels of the anti-inflammatory cytokine IL-10. The improvement in IL-10 was strongly associated with the improvement of daily levels of moderate-intensity physical activity. On the other hand, there were no changes in the circulating levels of biomarkers of endothelial dysfunction, sICAM-1 and sVCAM-1, and inflammation, CRP and IL-6, in the exercise-training group. In addition, resting heart rate and systolic blood pressure were reduced, and heart rate recovery, cardiorespiratory fitness, and daily physical activity were enhanced as result of the exercise-training programme. To the contrary, the control group did not change IL-10, CRP and IL-6, while increasing the endothelial dysfunction expressed by increased circulating levels of sICAM-1 and sVCAM-1. These results confirmed our hypothesis that exercise training mitigates the endothelial dysfunction and inflammation in CAD patients.

1. Methodology discussion

The benefits of exercise training in modifiable cardiovascular risk factors, cardiorespiratory fitness and exercise tolerance of CAD patients are well known. However, the scientific evidence seems to be weak regarding effectiveness of exercise training in the mitigation of the endothelial dysfunction and vascular wall inflammation. The majority of studies performed in this field are nonrandomized (58, 127, 128, 208, 260, 261, 289, 355, 359), uncontrolled (58, 127, 128, 208, 289, 359), merely observational (58, 208, 260, 261), and lack strict inclusion criteria (67, 289, 355). In addition, (i) most of these studies assessed only a single inflammatory biomarker, which does not provide a

comprehensive picture of the vascular inflammation underlying CAD (27, 58, 146, 159, 260, 261); (ii) they did not control modifications in diet, daily physical activity, and body fat, and hence did not account for the contribution of these potentially biased factors to the alterations in endothelial dysfunction and vascular inflammation (27, 128, 146, 314), and, consequently, (iii) did not observe the independent effect of exercise in the attenuation of the atherosclerotic process.

Thus, taking into consideration the above-mentioned limitations, we performed a randomized controlled study in a CAD population, trying to eliminate the selection and referral bias. On the other hand, the inclusion of a control group receiving only the standard care (provided to all the patients) allowed us to differentiate the effects of exercise training *per se.*

The number of patients enrolled in a randomized controlled trial is always a matter of concern. Based on an *a priori* power analysis, we ensured that the number of patients enrolled in this study was sufficient enough to reveal intra-group differences if they actually existed. The number of participants in our study is according to the number of patients enrolled in previous randomized controlled studies conducted in this field (189, 356). Additionally, it should be noted that the number of patients recruited to the present study represents a substantial part of all the patients receiving cardiac rehabilitation and exercise training per year in Portugal; according to the data from a National Survey published recently (376), our sample represents 15.1% of all the patients receiving cardiac rehabilitation and exercise training *per* year in Portugal.

The defined inclusion and exclusion criteria were elaborated to recruit patients with similar clinical characteristics, without contraindications or/and co-morbilities that could influence/preclude exercise participation and physical activity, and with low risk for cardiac events during exercise participation (407). We decided to enrol only patients with history of acute myocardial infarction in an attempt to restrict the inclusion to patients with permanent damage in the myocardial cells. This approach avoid comparing patients with different presentations of CAD (angina pectoris and acute myocardial infarction) and, consequently, with differences, for instance, in the presentation of

atherosclerotic lesions within the coronary arteries (402), and in the circulating level of several biomarkers (including IL-6, IL-10, CRP, sICAM-1, and sVCAM-1) (115, 163, 412).

The timing of the baseline assessment was designed to establish the baseline circulating levels of inflammatory and endothelial function biomarkers at a time point where it could be assumed they were unaffected by the inflammatory reaction (202) to necrosis and/or by revascularization procedures (stent implantation) (342). Therefore, it better reflects the patient's basal inflammatory status. Indeed, after myocardial infarction, the levels of inflammatory markers, namely CRP, dramatically increase, returning to baseline levels in 3–4 weeks (202). Previous studies assessing the effects of exercise training on inflammatory biomarkers did not take the timing of biomarker measurement into consideration (27, 146, 189, 261, 314, 355). Therefore, they provided values that could be overestimated (influenced by the acute event and/or by the revascularization procedure) and have a natural tendency to decrease. This is particularly relevant in non-controlled studies, in which the effects of exercise training could be overstated.

In addition to biomarkers of endothelial function and inflammation, other variables were assessed in order to provide clinical information and/or to be included in the regression analysis to ascertain their contribution to the expected modification of the endothelial dysfunction and inflammation. For instance, diet (230) and daily physical activity (215) seem to play an important role in the development of CAD; thus, any modification to these risk factors could favourably alter the endothelial dysfunction and vascular inflammation underlying atherosclerosis (138, 182). Accordingly, by controlling diet and daily physical activity we could observe the independent effect of exercise training. Additionally, fat and fat free mass were assessed using tetrapolar bioimpedance, the method which best agrees with the dual-energy X-ray absorptiometry (379); physical activity was objectively measured by accelerometry, which is of great value since self-reported measures of physical activity correlate poorly to direct measures (319). Another important contribution of the present study design is the assessment of body composition, using the

percentage of body fat as main parameter, instead of body mass index. So far, clinical trials yielded conflicting results with regards to the role of weight loss in the reduction of inflammation. Researchers have found decreases in circulating inflammatory biomarkers with or without weight loss (58, 128, 261). However, those studies did not evaluate the reduction of body fat, which could provide more insight into the mechanism by which exercise exerts beneficial effects, since the adipocyte is an important source of several pro-inflammatory molecules (e.g. IL-6) (420).

Furthermore, our exercise capacity data was not indirectly estimated on the basis of the speed and grade of the treadmill, although this type of estimation is the most common clinical measure of exercise tolerance, but instead was measured directly. Cardiorespiratory fitness was assessed directly by measuring peak oxygen consumption, which is known to be a more accurate and reproducible measure of exercise tolerance (277), as well as a more robust predictor of outcomes (278, 366).

To provide insight into cardiac function, we decided to use two indicators: left ventricular ejection fraction and the circulating levels of the inactive NT-pro-BNP. The natriuretic peptide family consists of a group of structurally similar peptide hormones that play a major role in the regulation of cardiovascular, endocrine, and renal homeostasis (201). The predominant stimulus controlling the synthesis and release of natriuretic peptides from cardiac atria and ventricles is wall stretch (190, 248). The natriuretic peptides have several physiological actions targeting the heart, blood vessels, kidneys, other neurohormonal axes, and the central nervous system. Those actions include (i) arterial and venous vasorelaxation, (ii) modulation of cell growth, apoptosis, and proliferation in vascular smooth muscle cells and cardiomyocytes, (iii) suppression of cardiac fibroblast proliferation, (iv) inhibition of the renin–angiotensin–aldosterone system, (v) inhibition of renin release, (vi) natriuretic and diuretic effects, (vii) inhibition of arginine vasopressin and endothelin-1 production, and (viii) inhibition of sympathetic outflow (136, 201). B-type natriuretic peptide (BNP), a peptide hormone released predominantly from cardiac ventricles, is synthesized as an inactive pro-hormone that is split into

the active hormone BNP and the inactive NT-pro-BNP (201). The predominant stimulus controlling the synthesis and release of BNP from cardiomyocites is wall stretch, but other stimuli can also motivate the expression and release of BNP, including norepinephrine, angiotensin II, ET-1, glucocorticoids, and pro-inflammatory cytokines (201). The main pathophysiological process that underlies increased plasma levels of BNP and NT-pro-BNP is regional or global impairment of left ventricular systolic or diastolic function, leading to increased left ventricular wall stress (248).

The rationale for assessing sVCAM-1 and sICAM-1 as biomarkers of endothelial function and CRP, IL-6 and IL-10 as inflammatory biomarkers rests primarily on the importance they have in the atherosclerotic process. Among the growing number of inflammatory biomarkers described in the literature, CRP has received the most focus and appears to provide the most consistent association to clinical end-points. CRP seems to have a pro-inflammatory role in the atherosclerosis, for instance, mediating the expression of cellular adhesion molecules, tissue factor production and decreasing $^{\cdot}NO$ production by endothelial cells (59, 301, 392). In comparison to other acute-phase proteins, CRP has proven to be a more robust analyte than fibrinogen or PAI-1 (222). Since the hepatic production of acute-phase proteins is mainly controlled by IL-6, which can also induce the expression of cellular adhesion molecules in vascular endothelial cells, it was also evaluated in our study. Furthermore, from a theoretical point of view, IL-6 should be a stronger biomarker than CRP, a downstream biomarker in comparison to IL-6 (222). Changes in IL-6 induced by exercise training should therefore be accompanied by changes in CRP levels. In addition to these two pro-inflammatory molecules, we have also decided to assess an anti-inflammatory cytokine, the IL-10, which has a potential regulatory role for the development and progression of the atherosclerotic lesion (85, 86, 244, 269). Moreover, elevated serum levels of IL-10 were associated with significantly improved outcomes in patients after an acute myocardial infarction, even in those with elevated CRP levels (153). These data seem to indicate that exercise training could have an anti-inflammatory effect just by increasing the serum levels of IL-10. Taken together, these data clearly

reinforce the importance of assessing more than one biomarker of inflammation when looking for the anti-inflammatory effects of exercise training.

ICAM-1 and VCAM-1 are involved in the recruitment of inflammatory cells from the circulation and in their transendothelial migration. There is accumulating evidence, from prospective studies, for the predictive role of elevated circulating levels of sICAM-1 in initially healthy people, and of sVCAM-1 in patients at high risk or with diagnostic of CAD (38, 164, 181, 383, 386). Consequently, the study of both sVCAM-1 and sICAM-1 could be a point of interest. VCAM-1 is focally and prematurely expressed (by endothelial cells in which the monocyte had first accumulated) in the fatty streak formation sites; and ICAM-1 is expressed by many cells of haemotopoietic lineage and fibroblasts. Therefore, sICAM-1 may be a less specific biomarker than sVCAM-1, which is mainly expressed on atherosclerotic plaques by activated endothelial cells and smooth muscle cells. Thus, the predictive value of sVCAM-1 may be limited to those with more advanced atherosclerosis at the time of measurement (36).

Taken together, the above-mentioned biomarkers could provide a comprehensive portrait of the atherosclerotic process underlying CAD, as the recruitment of inflammatory cells from the blood to the vessel wall, continuing through the promotion, amplification and perpetuation of vascular inflammation, could ultimately promote the destabilization of the atherosclerotic plate.

The duration, intensity, and mode of the exercise intervention provided in our study followed the recommendations for the exercise component of an outpatient cardiac rehabilitation programme (325). In general, the exercise training programmes provided to CAD patients range between 8 and 12 weeks; however, positive effects on endothelial function and inflammation have been observed with shorter (3 and 4 weeks) (27, 356) and longer lengths (14 weeks) (189, 355). Thus, in accordance with the purpose of our study, we decided to provide an exercise programme with a length of 8 weeks, well within the guidelines for a phase II cardiac rehabilitation and exercise training programme (325).

2. Results discussion

2.1. Patients' characteristics at baseline

This study enrolled patients one month after acute myocardial infarction and assigned them to a programme of exercise training or to standard care and follow-up. At the time of group allocation, all patients were engaged in optimal medical therapy and generally presented few cardiovascular traditional risk factors. As a whole, they showed good left ventricular function, low levels of inflammation and endothelial dysfunction, very good cardiorespiratory fitness, and optimal blood pressure and lipid profile. In addition, in most patients, the dietary intake and physical activity pattern were already within the targets recommended by the American Heart Association and the European Society of Cardiology (26, 131, 149, 226, 385).

Even though our patients suffered from acute myocardial infarction, at baseline they showed good left ventricular function, assessed by left ventricular ejection fraction. Average circulating levels of NT-proBNP were only slightly raised in comparison to an aged-matched healthy population (40 to 75 years old), which upper limits range from 190 to 300 ng/L (241). It is known that following an acute myocardial infarction there is an increased production of cardiac natriuretic peptides (137, 272, 293, 327), caused by the myocardial stretch secondary to left ventricular dysfunction, increased heart rate, hypoxia, ischemia *per se*, as well as by the stimulatory effects of catecholamines, angiotensin II, and endothelins (201). The rise in circulating levels of BNP and NT-proBNP, secondary to a reversible increase in regional wall stress induced by myocardial ischemia, may be sustained over several weeks after an acute coronary syndrome and correlates with infarct size (16, 63, 282). A second peak in plasma levels of NT-proBNP after acute myocardial infarction may also appear weeks thereafter, as result of impaired left ventricular function (272). Therefore, our findings are not unexpected, since all patients in the present study had no impaired left ventricular ejection fraction, were under optimal medical treatment, were in a clinically stable condition, and were enrolled in this study several weeks after the acute myocardial infarction.

As mentioned previously, the patients presented good left ventricular function; however, this was not a good indicator of the severity of the pathology underlying acute myocardial infarction. Indeed, as atherosclerosis proceeds from endothelial dysfunction and vascular wall low-grade inflammation (147, 233, 335), the best indicators of the pathophysiological process that led to the cardiac acute event should be the biomarkers of endothelial function and inflammation. In our study the circulating levels of CAMs were used to assess the endothelial dysfunction. CAMs play a crucial role in the recruitment of inflammatory cells to the site of atheroma development; regarding ICAM-1 and VCAM-1, they mediate the stronger attachment of the circulating leucocytes to the site of dysfunction/injured endothelium (31, 375). It was possible to observe that baseline levels of sVCAM-1 were higher than the levels of sICAM-1. As explained previously, sVCAM-1 may be a better indicator in subjects with more advanced atherosclerosis, since it is primarily expressed on atherosclerotic plaques (36). Since an acute myocardial infarction usually occurs due to atherosclerotic plaque instability, which is often related to the aggravation of the inflammatory process, our results regarding the higher average values of sVCAM-1 comparatively to those of sICAM-1 are not completely unexpected. In our study the average baseline levels of sICAM-1 and sVCAM-1 are below those observed in subjects at risk for coronary artery disease (404), as well as in patients with CAD (39), with peripheral arterial disease (17) or with heart failure (4). The relatively low level of endothelial dysfunction in our patients, compared to the above-mentioned studies, is accompanied and in agreement with the observed low levels of vascular wall inflammation expressed by the circulating levels of pro-inflammatory biomarkers.

While not specific biomarkers of vascular wall inflammation, the circulating levels of CRP and cytokines can provide valuable insight into the phenomenon. The low levels of inflammation are clearly illustrated by the baseline values of CRP (median 1.09 and 0.89 mg/L in exercise training and control group, respectively), given that the 50^{th} percentile of CRP distribution for the American and European populations are about 1.5 and 1.6 mg/L, respectively (111, 167, 330). These low levels of pro-inflammatory biomarkers may be explained by the

characteristics of the patients, optimal medical therapy, and the timing of the measurement. As explained earlier in the discussion, at 1 month post-hospital discharge the baseline circulating levels of inflammatory biomarkers are unaffected by the inflammatory reaction to necrosis and/or by revascularization procedures, and hence reflect the patient's basal inflammatory status. Furthermore, the small number of obese patients in our sample could also have contributed to the observed levels of biomarkers of endothelial function and inflammation. Moreover, at baseline, the percentage of fat of our patients is only slightly elevated in comparison to reference values for an aged-matched Caucasian population in the Mediterranean area (65). Indeed, it is well documented that obesity is associated with endothelial dysfunction (10, 238) and inflammation (212, 256), possibly due to the increased production of pro-inflammatory cytokines (namely, IL-6 and TNF-α) by the adipocyte (420). These cytokines in turn amplify inflammation in the vessel wall, induce the hepatic synthesis of acute-phase proteins (e.g. CRP), and contribute to additional leukocyte accumulation, smooth muscle cell proliferation, and extracellular matrix remodelling (220, 233). In this sense, we can speculate that the body composition of our sample might also have had favourable influence both on the circulating levels of pro-inflammatory cytokines and the CRP measured at baseline.

Additionally, at baseline, patients already had dietary intake and levels of daily physical activity close to the recommendations (26, 131, 149, 226, 385). These modifiable risk factors could also have had a positive impact in the endothelial function and inflammatory status exhibited by our patients (182, 231, 296). In fact, diet and physical activity are important components of an overall healthy lifestyle. An overall healthy diet, rich in vegetables, fruits, fish, whole-grain, and higher-fibre foods, is part of the master plan for cardiovascular disease risk reduction (226). In addition, the intake of salt, saturated fat, cholesterol, beverages and foods with added sugars should be minimized (226). The recommendations of the American Heart Association Nutrition Committee concerning a healthy diet are summarized in Table 12 (149, 226, 385). In general, at baseline both groups reported a daily dietary pattern close

to the recommendations. When we compare the adequacy of the percentage of calories derived from daily macronutrients' intake, fibre intake, cholesterol intake and sodium intake with the recommendations, it is possible to conclude that most patients in both groups followed their diet according to the recommendations.

Table 12. Recommendations of the American Heart Association Nutrition Committee for dietary intake of macronutrients (percentage of daily calories derived from), fibre, cholesterol and sodium

	Recommendations
Protein (% Kcal)	10-35%
Carbohydrates (% Kcal)	45-65%
Total fat (% Kcal)	20-35%
Saturated fat (% Kcal)	<7%
Monounsaturated fat (% Kcal)	Up to 20%
Polyunsaturated fat (% Kcal)	Up to 10%
n-3 fatty acids (% Kcal)	0.6-1.2%
n-6 fatty acids (% Kcal)	5-10%
Cholesterol (mg)	<300 mg/day
Total Fibre (g)	20-30 g/day
Sodium (g)	1.5 g/day*

*Since a reduction in sodium intake to 1.5 g/day is not easily achievable, the American Heart Association Nutrition Committee provides a short-time goal of 2.3 g/day (226)

Regarding the intake of polyunsaturated fatty acids (exercise training group, 4.9 ± 1.1%; control group, 6.0 ± 1.1 %), particular interest is given to the intake of n-3 (exercise training group, 0.59 ± 0.2%; control group, 0.57 ± 0.2%) and n-6 fatty acids (exercise training group, 4.0 ± 0.9%; control group, 5.1 ± 1.1%), as a high intake is associated with lower cardiovascular morbidity and

mortality (11, 20, 54). Furthermore, the intake of n-3 and n-6 fatty acids protect from fatal events in patients who have suffered a previous myocardial infarction (131, 149). The most important mechanisms explaining the protective role of polyunsaturated fatty acids include the transcriptional down-regulation of pro-inflammatory cytokines and vascular surface expression of CAMs (82, 188). In fact, it has been suggested that these fatty acids may inhibit the production of pro-inflammatory cytokines by binding to the peroxisome proliferator activated receptors (PPaR)α and PPaRγ, which regulate the transcription of target genes (176, 177, 188). PPaRs might also repress gene transcription by interfering with signaling molecules, such as nuclear factor-κB, therefore inhibiting the production of pro-inflammatory cytokines (253, 363). Additionally, it was also suggested that the unsaturated double bond of n-3 or n-6 fatty acids inactivate ROS and prevent their interaction with nuclear factor-κB (82, 83). In this way, n-3 and n-6 fatty acids have been reported to be strongly and independently associated with lower levels of pro-inflammatory biomarkers (for instance, IL-6 and TNF-α) (108, 311, 316).

Another important feature of a healthy diet is the daily intake of total fat (exercise training group, 29.7 ± 4.2%; control group, 33.5 ± 4.7%) and cholesterol (exercise training group, median = 211 mg; control group, median = 216 mg); for our patients, these elements were also within the recommendations. The intake of cholesterol is particularly important, as it raises the concentrations of LDL cholesterol (226). In contrast, the intake of fibre (exercise training group, 18.6 ± 7.2 g; control group, 17.4 ± 6.0 g) has been associated to the decrease of LDL (49). This, together with lipid-lowering medication, could also help to explain why, at baseline, our patients showed average levels of LDL and total cholesterol under the most optimistic recommendation of the European Society of Cardiology (131) for those with clinically established atherosclerotic cardiovascular disease. Actually, our patients had average levels of total cholesterol below 155 mg/dL and LDL cholesterol below 80 mg/dL. Low circulating levels of LDL cholesterol are an important achievement *per se*, as LDL infiltration and retention within the arterial intima is a major feature of the atherosclerotic process (233). Since our patients

presented low circulating levels of LDL cholesterol, they were less prone to express CAMs because of the lower LDL infiltration with further oxidation (147, 233), helping to justify the reduced levels of soluble CAMs detected in circulation at baseline.

A reduced sodium intake (exercise training group, 1.39 ± 0.46 g; control group, 1.51 ± 0.74 g) was also observed at baseline. This is particularly important to prevent hypertension in nonhypertensive individuals, to facilitate the control of hypertension, and also to lower blood pressure in the setting of antihypertensive medication (226). The low intake of sodium, together with the antihypertensive medication, support the values of resting blood pressure observed in our patients, which were about average according to the recommendations for the classification and management of arterial hypertension (245). Therefore, the dietary intake reported by our patients, namely n-3 and n-6 fatty acids, total fat, cholesterol and fibre, could also be an additional factor explaining the favourable status of endothelial function and inflammation observed at their baseline.

A sedentary lifestyle has been recognized as a major modifiable risk factor for cardiovascular diseases (378), as the risk of developing CAD is decreased by 20% to 50% in those subjects who regularly accumulate a moderate amount of physical activity (1). Nevertheless, to maximize the health-related benefits of physical activity, the target should be to enhance not only the amount of physical activity but also the time spent performing physical activity of moderate intensity. Presently, patients with CAD are recommended to accumulate a minimum of 30 to 60 minutes per day of moderate-intensity physical activity at least 5 days per week (26). This goal can be achieved during daily life and leisure, in activities such as household or yard tasks, stair climbing, walking, and bicycling (361).

It should be emphasized that even at baseline, patients performed levels of moderate-intensity physical activity related to health benefit recommendations. This suggests that patients were already conscious about the benefits of being physically active. The underlying reason for their levels of physical activity may be related to the information that was provided during the hospitalization

highlighting the importance of being physically active. The active lifestyle of our patients could also be called upon to explain the low endothelial dysfunction and inflammation observed in our patients. Numerous studies have shown that the physical activity is inversely related to the circulating levels of inflammatory biomarkers, including IL-6 and CRP (22, 145, 270, 297, 312, 419). However, the biological mechanisms of physical activity that influence inflammatory factors have not been fully elucidated. It is likely that the effects of physical activity on decreasing inflammation are likely to result from multiple mechanisms. In this sense, the mitigation of the inflammatory process through increased physical activity has been ascribed to several factors, including enhanced endothelial function, improved lipid profile, and reduction in adipose tissue (53). Indeed, by enhancing the production of ˙NO in the endothelial cells, physical activity may have an important impact on the regulation of vascular tone, the inhibition of platelet aggregation, and the control of the cytokine adhesion to the vessel wall (13, 165). Furthermore, physical activity promotes the regenerative capacity of the endothelium by increasing the number and the function of EPC (207, 217, 390, 405). After mobilization from bone marrow, EPC have the capacity to migrate to the sites of desquamated endothelium and differentiate into adherent mature endothelial cells, contributing to postnatal vessel growth and repair, and consequently improving endothelial function (19, 306, 399). In turn, enhanced endothelial function will further decrease the expression of CAMs, the production of IL-6, and consequently the hepatic production of CRP. Physical activity also ameliorates endothelial function and systemic inflammation by increasing the blood and endothelial antioxidant defences, then reducing the degradation of ˙NO (114), and attenuating the formation of foam cells and vascular inflammation through the reduction of a crucial step in the atherogenesis: the LDL oxidation (29, 354). Additionally, physical activity seems to reduce visceral obesity (264) and triglyceride levels and to increase HDL cholesterol (298, 357). By reducing body fat, physical activity decreases the adipocyte production of numerous pro-inflammatory factors, including CRP, IL-6 and TNF-α (135), which seem to be in agreement with our results. The reduction of these factors, namely TNF-α, may increase

the hepatic production of adiponectin, which in turn could improve endothelial function, inhibit vascular smooth muscle cell proliferation, suppress macrophage-to-foam cell transformation, and ameliorate hyperglycemia and hyperinsulinemia (387). Physical activity also seems to be associated with the upregulation of anti-inflammatory cytokines (eg, IL-10) (171), a result that seems to be corroborated by our baseline results. Indeed, the baseline values of our patients are higher than those observed at baseline in other studies similar to ours (27, 128), highlighting the importance of the amount of time spent performing physical activity at intensities related to cardiovascular health benefits. Taken together, the above-mentioned mechanisms explaining the positive impact of daily physical activity provide valuable insight to enlighten our results regarding endothelial function, inflammatory status, and traditional risk factors.

The baseline cardiorespiratory fitness (above 31 ml/kg/min) of our sample, which can be considered as very good, is in accordance with the data from daily physical activity. Indeed, the patients were in the category of “average cardiorespiratory fitness” for apparently healthy individuals between 50-59 years old (70); furthermore, they had higher peak values of VO_2 than those seen in other studies of patients entering cardiac rehabilitation (7). In a study conducted with the purpose of establishing normative values for cardiorespiratory fitness of patients entering cardiac rehabilitation, the data from 2896 patients indicated that men (61 ± 11 years of age) and women (62 ± 11 years of age) present an average VO_2 peak of 19.3 ± 6.1 and 14.5 ± 3.9 ml/kg/min, respectively (7). The values reported in that study are unquestionably much lower than those observed in our patients. Our results could have several possible explanations. The limitations that affect the performance in a maximal or symptom-limited exercise test could be cardiovascular, pulmonary and/or musculoskeletal (70). Since our patients had no muscle and/or pulmonary diseases, it can be assumed that the major limitations to peak VO_2 in the exercise test were related to cardiovascular limitations associated to the repercussions of myocardial ischemia and/or medication (namely, beta blockers and hypertensive medication). In this sense, apart from methodological issues, the discrepancy

between our baseline values and those reported in the above-mentioned study could be related to differences in sample characteristics, such as presence of orthopaedic and pulmonary co-morbidities, size/location of myocardial infarction, age, levels of physical activity, and medication. In fact, those values are very low and indicative of severe pathology, especially in women (73, 246). Comparing our results to those of studies conducted in similar populations, it is possible to observe that the cardiorespiratory fitness of our patients continues to be a little bit higher (189, 355, 356, 400).

In summary, several factors could have contributed to the favourable results observed at baseline in both groups, including: optimal medication, the weeks elapsed since the myocardial infarction, and the counselling and information concerning healthy lifestyle (healthy diet, the importance of being physical active and compliance with medication) that was provided during hospitalization.

2.2. Effects of exercise training

Taken together, the data from the baseline assessment clearly indicates that our patients had little room for improvement through exercise training in the majority of the parameters assessed. Similar to previous studies conducted in similar populations (127), we could not demonstrate any significant change in left ventricular ejection fraction as a result of exercise training. Our results were not surprising, given that even in patients with reduced left ventricular ejection fraction the great majority of studies corroborate the absence of meaningful training-induced changes of resting ejection fraction (259, 388). Nevertheless, our results concerning the concentrations of NT-proBNP showed significant reductions in both groups. This reduction can be explained, at least, by the medication that was prescribed to both groups. For instance, it is known that the blockade of angiotensin II type 1 receptors suppressed stretch-induced BNP gene transcription by 50% in cultured neonatal ventricular cells (219). Additionally, the low levels of pro-inflammatory cytokines observed in both groups at the end of the study period could also contribute to justify NT-proBNP reduction, since high levels of IL-6 were shown to stimulate the BNP synthesis

(201). Surprisingly, the NT-proBNP changes were identical in both groups, indicating that exercise training did not have an additive effect on medication. In fact, our results are not in agreement with those reported by other (121, 251), who described significant reductions in NT-proBNP induced by exercise training. However, it must be emphasized that the baseline levels of NT-proBNP reported in the above-mentioned studies were higher than those observed in our patients. In this sense, we can hypothesize that the huge magnitude of variations observed in those studies allows a better differentiation among the effects of exercise training and medication.

In our study, circulating CAMs remained unchanged in patients who followed exercise training; in contrast, sICAM-1 and sVCAM-1 increased in the control group, which seems to support, at least partially, the protective effect of exercise in CAD patients. Our results seem to sustain the assumption that exercise training favourably alters the progression of the atherosclerotic process (150, 288, 352), as circulating levels of CAMs are associated with the extent of this pathology (81, 307). Few studies have been conducted to assess the effects of exercise in the circulating levels of sVCAM-1 and sICAM-1. Wegge *et al.* (404) observed a significant reduction in sICAM-1, but not in sVCAM-1, after a diet and exercise intervention in subjects at risk of coronary events. Likewise, in heart failure patients, a 12-week exercise-training programme decreased the circulating levels of sICAM-1 (367 ± 31 vs. 314 ± 29 ng/ml) and sVCAM-1 (1247 ± 103 vs. 1095 ± 100 ng/ml) (4). However, it should be noted once again that the baseline levels reported in those studies (4, 404) were much higher than that observed in our study. Thus, we can speculate that a positive effect of exercise is more apparent when the baseline levels are higher, indicating a more pronounced endothelial dysfunction. Even in the absence of a reduction in the circulating levels of CAMs, our results showed a positive impact of exercise in the endothelial adhesiveness of CAD patients, because the control group increased the endothelial dysfunction in the same period of time. Several mechanisms have been suggested to explain the positive impact of exercise on circulating CAMs. First, it was reported that by increasing shear stress, exercise training could favourably change the transcriptional regulation of CAMs (14).

Additionally, exercise training could also have a positive impact in the endothelial dysfunction by increasing the bioavailability of ˙NO. Indeed, in vessels with atherosclerotic plaques, the reduced ˙NO bioavailability is related to vasoconstriction, platelet adherence and aggregation, leukocytes adherence to the endothelium, and increased proliferation of vascular smooth muscle cells (69). Exercise training can increase the bioavailability of ˙NO by increasing the activity/expression of eNOS and/or decreasing its degradation by ROS (103, 114, 138). This assumption has been illustrated in several investigations conducted in CAD patients, in which regular aerobic exercise enhanced ˙NO bioavailability and thus improved endothelial function (126, 144, 356). Finally, our results could also have had a contribution from EPC. Indeed, exercise training can also improve endothelial function by increasing the number and function of EPC. In CAD patients a 2.9 ± 0.4-fold increase was reported in the number of circulating EPC, as a result of exercise training; this was positively associated with increased synthesis of ˙NO and flow-mediated vasodilatation (365).

Similarly to the results observed in CAMs, exercise training did not induce any relevant change in circulating levels of CRP and IL-6. These findings are unexpected to some degree, since more than 20 cross-sectional studies consistently demonstrated an inverse relationship between circulating levels of CRP and both cardiorespiratory fitness and physical activity (182, 313, 410). Additionally, data from nonrandomized prospective and retrospective studies generally indicate that aerobic exercise training reduces CRP levels in patients with CAD (58, 128, 189, 208, 260, 261, 355). However, the few prospectively randomized controlled studies focusing on the effects of exercise on inflammatory biomarkers in CAD patients provide conflicting results (159, 356, 400). For instance, Walther *et al.* (400) randomized 101 patients with stable CAD to either percutaneous intervention with stents or aerobic exercise training, and reported that 24 months of exercise training reduced CRP levels by 41%. In contrast, Sixt *et al.* (356), employing a 4-week exercise intervention, observed a significant improvement in the endothelium-dependent vasodilatation, but not in the inflammatory biomarkers. One reason for these conflicting results could be

related to the duration of the exercise intervention. Actually, most of the studies conducted to date that show a reduction in circulating levels CRP and IL-6 comprise a 12-week exercise training intervention. Indeed, two recent studies involving CAD patients that included less than 12 weeks of exercise training failed to demonstrate a decrease in CRP levels (146, 356). Considering all trials together, it may be speculated that a period of training lasting only 8 weeks, as ours did, may not be long enough to produce detectable changes in CRP and IL-6 levels, especially in those patients exhibiting low baseline levels of pro-inflammatory biomarkers. As previously discussed, the baseline values of CRP were low on average, leaving little room for improvement. These assumptions were confirmed by a meta-analysis of randomized controlled clinical trials, showing that aerobic exercise training does not reduce basal CRP levels in apparently healthy adults (185). Additionally, the only study in CAD patients that presented baseline levels of CRP (median 1.2 mg/L) close to the values exhibited by our patients also did not find any significant decrease in CRP and IL-6 levels (289). Indeed, studies reporting significant decreases in CRP levels enrolled CAD patients with high baseline levels of CRP (58, 127, 128, 189, 208, 260, 261, 314, 400). None of the several potential mechanisms offered by the literature as explanations for the reduction in CRP as a result of exercise training (182) were present in our study. In fact, reductions in CRP were associated with (182): (i) the reduction of fat mass with reduced adipocyte-derived IL-6 and TNF-α production; (ii) the reduction of cytokines production from other sites besides adipose tissue, such as skeletal muscles and mononuclear cells; and, (iii) improvement of endothelial dysfunction with lower release of IL-6 by the endothelial cells. Actually, we observed neither a reduction in fat mass nor any significant change in the circulating levels of IL-6. Another potential explanation is the influence of genetics on the response of CRP to exercise training. CRP has been associated with strong genetic determinants (30, 192) and researchers have described a CRP polymorphism influencing the response to an inflammatory stimulus (51). Changes in CRP with exercise training may also be influenced by similar genetic variants. Future studies evaluating the role of genetic influences in the response of CRP to

exercise training are clearly necessary to clarify this topic.

On the other hand, in contrast to the results found in CRP and IL-6 levels, in our study the exercise-training programme did induce a significant improvement in the circulating levels of IL-10. This is of great interest, since IL-10 has strong deactivating properties in macrophages and T cells, and modulates many cellular processes that may interfere with the development and stability of the atherosclerotic plaque (85, 86, 244, 269). IL-10, which is mainly secreted by lymphocytes of the Th2 subtype and macrophages, is an anti-inflammatory cytokine with several recognized antiatherogenic properties (244, 269). Those properties include: inhibition of the prototypic pro-inflammatory transcription factor nuclear factor-κB leading to suppressed cytokine production (213, 401); inhibition of matrix-degrading metalloproteinases (204); reduction of tissue factor (178, 227) and cyclooxygenase-2 expression (258); promotion of the phenotypic switch of lymphocytes into the Th2 phenotype (310); and inhibition of T-cell death (64). Cross sectional studies in healthy subjects indicate that physical activity and/or cardiorespiratory fitness are positively associated with levels of IL-10 (53, 171). In the context of cardiovascular diseases, the response of IL-10 to exercise training has been poorly investigated. Smith *et al.* (359) reported a 36% increase in the production of IL-10 by cultured mononuclear cells from patients at a high risk of developing cardiovascular disease, following 6 months of exercise training, compared with baseline levels. Goldhammer *et al.* (128), in a prospective non-controlled study, observed an increase of 30% in the circulating levels of IL-10 following a 12-week exercise training intervention in CAD patients. These results were recently confirmed in patients with history of myocardial infarction (27). In our study, a superior increase of IL-10 was observed as a result of a shorter exercise intervention. The mechanisms whereby exercise training increases IL-10 production are not clear. However, it has been proposed that exercise training downregulates nuclear factor-κB activity and increases IL-10 secretion by monocytes and Th2 lymphocytes (180, 362). The elevation of circulating levels of IL-10 is of great interest, as low serum levels of IL-10 are indicative of a poor prognosis even after the occurrence of an acute ischemic event (153), and

increased IL-10 serum levels in patients with CAD are associated with improved systemic endothelial vasoreactivity even in patients with elevated serum levels of CRP (110).

Interestingly, changes in IL-10 were independently associated with the increase in moderate-intensity physical activity, reinforcing the importance of physical activity performed on a daily basis. Our data indicates that the improvement in moderate-intensity physical activity could be paramount to enhancing the circulating levels of IL-10. Previous studies have shown that patients enrolled in cardiac rehabilitation and exercise-training programmes usually fail to improve physical activity levels on days away from the program (25, 174). Our findings are in contrast with the above-mentioned reports. However, they agree with a previous report showing an increase on daily physical activity levels as a result of a cardiac rehabilitation and exercise-training programme (292). In our study, the exercise-training group increased daily moderate-intensity physical activity, despite having no structured component of education and counselling, suggesting that patients were aware of the benefits of exercise. Additionally, we can speculate that exercise could have increased the feelings of well-being and overall state of health of the patients, thus impelling them to increase the daily levels of physical activity. It is interesting to note that even though both groups had performed levels of moderate-intensity physical activity related to health benefit recommendations, at the end of the study, only the exercise-training group achieved a weekly amount of activity correspondent to a reduced risk of myocardial infarction and death. Indeed, the average of the weekly volume of moderate physical activity accumulated by the exercise-training group (5.8 hours per week vs. 4.5 hours per week in the control group) at the end of the 8 weeks met the recommended level of 5 to 6 hours of moderate physical activity per week to reduce the risk for recurrent myocardial infarction and death (1, 143).

As previously mentioned, one of the reasons determining the lack of changes in pro-inflammatory biomarkers in the exercise training group could be the absence of any significant change in body weight, body mass index, and percentage of fat and fat free mass. Similar findings have also been shown in

previous reports encompassing exercise training alone (128, 400) or incorporated in cardiac rehabilitation programmes (314). Our results could be related to the short duration of the exercise-training programme. Indeed, it has been shown that longer intervention periods (>20 weeks) were more effective in reducing body mass index and body fat percentage (409). Diet-only and exercise-only interventions have been shown to promote weight loss (48), but the combination of both diet and exercise interventions appears to be the most effective (170, 262). Nonetheless, our results indicate that, in the absence of dietary changes, a short-term exercise intervention does not induce significant modifications in anthropometrics.

Since diet and medication remained unchanged, the positive effects observed in blood pressure should have been the result of exercise training. Our study was performed in patients given optimal medical therapy, whose average values of resting blood pressure fell near healthy goals, according to the recommendations for the classification and management of arterial hypertension (245). This clearly leaves little room for improvement through exercise intervention. This fact was demonstrated when patients with values of blood pressure in the normal range (systolic <120 and diastolic <80 mmHg) were analysed together with patients with values within prehypertensive (systolic of 120 to 139 mmHg or diastolic of 80 to 89 mmHg) and hypertensive range (systolic ≥140 or diastolic ≥90 mmHg). When prehypertensive and hypertensive patients were considered exclusively, the exercise training induced a significant reduction of the systolic blood pressure (in average 9.4 mmHg). These results are in agreement with a very elegant meta-analysis evaluating the effectiveness of exercise-based rehabilitation in patients with CAD (374). The authors reported an average reduction in systolic blood pressure of 3.2 mmHg and no changes in diastolic blood pressure. The effects of exercise on resting blood pressure have been explained by enhanced endothelial dependent vasodilatation in result of increased $^{\cdot}$NO biovailability, decreased ET-1 concentrations, and changes in angiotensin system (6, 235, 338). Indeed, exercise training elicits improvements in endothelial dependent vasodilatation in hypertensive rats (130) and humans with both essential hypertension (155) and

CAD (130, 138). The expression and activity of pathways influencing ˙NO bioavailability have been suggested to illuminate these findings. In fact, exercise training increases the expression and activity of vascular eNOS and antioxidant enzymes, and induces adaptations in vascular NADPH oxidase, that, in concert, favour enhanced ˙NO bioavailability (6, 198, 338, 339). Exercise training also seems to modulate the vasoconstrictor effects of ET-1. ET-1 is a potent vasoconstrictor peptide synthesized by the endothelial cells (52) in conditions of reduced blood flow and low shear stress (237). In turn, its synthesis is inhibited by increased shear stress and ˙NO (200), which seems to be supported by studies showing that aerobic exercise training decreases plasma ET-1 concentration (234, 236) as well as ET-1 mediated vascular tone (235). These changes are closely related to increased endothelial function, to reduced ET-1 sensitivity, and to the re-balance between the production of ˙NO and ET-1 (52, 377). Regarding the angiotensin system, exercise training seems to moderate the vascular expression of the AT_1 receptor and angiotension II-induced vasoconstriction, as well as to increase AT_2 receptor mRNA (6). The tissue actions of angiotensin II mediated by AT_2 receptors are antagonists to its classical actions mediated by AT_1 receptors (337). Therefore, promoting a vasodilatory response that seems to be dependent on bradykinin production in turn results in eNOS activation and ˙NO production (337). All of these changes associated with exercise training seem to act in concert to decrease blood pressure in non-normotensive patients. Despite not having assessed in our patients, all these explanations could be suitable to explain our results.

Exercise training also decreased patients' resting heart rate by 7.1% and induced a trending decrease in resting rate-pressure product, a sensitive index of myocardial oxygen consumption, by 6.1%. This effect in resting heart rate could be associated with increased parasympathetic nervous system tone and/or decreased sympathetic drive induced by exercise training (206). In CAD patients, exercise training has been shown to increase resting arterial baroreflex sensitivity (203, 263), to decrease muscle sympathetic nerve activity (263), and to reduce the circulating levels of catecholamines (370). The decrease in resting heart rate has undeniably positive prognostic value. In fact, pathophysiological

studies indicated that high resting heart rate is associated with several detrimental effects, on elements such as: (i) the progression of coronary atherosclerosis (160); (ii) the stability of pre-existing atherosclerotic plaques (154); (iii) arterial stiffness (247, 340); (iv) the occurrence of myocardial ischemia (increasing oxygen demand and reduces diastolic perfusion time) (373) and ventricular arrhythmias (46, 326); and, (v) left ventricular function (281). High resting heart rate seems to directly affect the arterial wall due to the mechanical pulsatile stress, and possibly by involving the pro-inflammatory actions of oscillatory fluid shear stresses acting on the vascular endothelium (384). Nevertheless, despite being statistically significant, one may ask if decreasing the resting heart rate from 68 to 62 beats per minute, as reported in our study, is clinically significant. We believe so, because recent studies have demonstrated a parallel increase in cardiovascular risk with heart rate, at least for values above approximately 60 beats/min (89, 175).

The positive effect of exercise training in the autonomic nervous system suggested above is supported by the faster heart rate recovery observed in the exercise-training group. Heart rate recovery is an indirect and simple marker of autonomic function and refers to the decline of heart rate after acute exercise (205, 206). Heart rate recovery is thought to reflect the function of parasympathetic nervous system, and it has been recognized as an arguably more simple method of assessing parasympathetic tone (205, 206). In apparently healthy subjects and in athletes, the heart rate rapidly falls after exercise termination (166). During a graded exercise test, heart rate increases as a result of withdrawal of parasympathetic tone and increased sympathetic tone (166). Immediately after exercise, heart rate decreases quickly, mainly because of the rapid reactivation of the parasympathetic nervous system (166). This ability to recover heart rate following exercise, associated with the capacity of the cardiovascular system to reverse autonomic nervous system (withdrawal of vagal activity) and baroreceptor (detection of changes in blood pressure and inhibition of sympathetic discharge) adaptations, is often termed vagal reactivation (166, 334). Similar to previous studies (120, 148, 368), we observed an improvement of 4 beats/min at 1 minute into recovery after the

exercise training intervention. For instance, Giallauria *et al.* (120) reported that heart rate recovery at 1 minute post-exercise improved by approximately 6 beats/min after 3 months of exercise training in patients after myocardial infarction; also, Streuber *et al.* (368) reported an improvement in 5 beats/min after 12 weeks of exercise in heart failure patients. The faster heart rate recovery described in literature and also observed in our exercise training group is of particular interest, since the rate of recovery following exercise has been shown to be inversely associated with the occurrence of cardiac events and all-cause mortality (66, 274, 280, 290, 395).

Exercise training is also essential for improving cardiorespiratory fitness in cardiac patients (216). Supervised exercise training for 3 to 6 months has been reported to augment patients' VO_2 peak by 11% to 36%, with the greatest improvements in the most deconditioned patients (216). In our study, patients significantly improved their cardiorespiratory fitness with a shorter duration programme (8-week exercise intervention). Additionally, it is important to note that baseline the VO_2 peak in our sample was already high. Notwithstanding, the extent to which VO_2 peak improved with the exercise training in our study (8.5%) is in line with that observed in previous studies (260, 261), in which 12 weeks of exercise training enhanced VO_2 peak by 9% (16.6 ± 5.1 to 18.1 ± 5.9 ml/kg/min) (261) and 7.4 % (16.2 ± 4.9 to 17.5 ± 5.4 ml/kg/min) (260). Due to the relatively strong prognostic value of VO_2 peak, which was found to be the single best predictor of both cardiac and all-cause deaths among patients with established cardiovascular disease (183, 279), patients who make only modest gains in cardiorespiratory fitness could nevertheless obtain significant prognostic and functional benefits. For instance, Myers *et al.* (279) reported that every 3.5 ml/kg/min increase in VO_2 peak is associated with a 12% improvement in survival. Moreover, the increase in VO_2 peak suggests that after the intervention the patients in the exercise-training group can maintain the same submaximal exercise at lower functional reserve (232). Considering the history of acute myocardial event presented by our patients, we can theorize that the observed increase in VO_2 peak induced by exercise training can be related to changes both in cardiovascular functionality and peripheral muscle

mass. In this particular, the positive effects of exercise observed in the exercise training group can be attributed to increased stroke volume during exercise and to reduced vascular resistance therefore enhancing blood flow, and to skeletal muscle adaptations, including increased capillary density, mitochondrial volume density, muscular coordination, and motor unit recruitment (97, 140, 141, 186, 308).

A reduced rate-pressure product at a given workload is compatible with a training effect, and suggests increased exercise tolerance that most likely reflects improved cardiorespiratory fitness. In the present study we did not evaluate the rate-pressure product at a given sub-maximal workload, but we observed, as result of the exercise training, the maintenance of the peak rate-pressure product at a higher VO_2 peak. Improved VO_2 peak in association with reductions in resting heart rate and systolic blood pressure results in lower myocardial oxygen requirements during moderate-to-vigorous daily living activities (216). This is according to the recognized anti-ischemic potential of aerobic exercise training (216), which can reduce myocardial ischemia in patients with cardiovascular disease by decreasing myocardial oxygen demands during physical exertion, thereby raising the ischemic threshold (214, 378).

At their baseline values, the lipid profile and metabolic parameters of most of the patients were within the target goals for cardiovascular disease prevention (2, 131, 226), therefore leaving little room for improvement through exercise training. However, despite having optimal baseline levels of HDL cholesterol, both groups further increased their HDL cholesterol. Increasing HDL cholesterol is clinically important, as HDL levels are inversely associated with the risk of developing cardiovascular disease (2). HDL cholesterol protects against the development of atherosclerosis through the transportation of cholesterol from peripheral tissues to the liver for subsequent metabolism or excretion, the so-called reverse cholesterol transport (2). However, as observed in previous studies, it is difficult to increase HDL levels *via* exercise training in cardiac patients (146, 314, 374). Since the levels of HDL cholesterol increased in both groups, we can speculate that the enhancement in HDL cholesterol is

not related to the exercise training *per se*, but to the maintenance, by both groups, of a pattern of daily physical activity related to health benefits. This hypothesis seems to be supported by cross-sectional studies, which provide strong evidence indicating that subjects who are more physically active have higher levels of HDL cholesterol (96, 199, 408). In a meta-analysis that included 35 randomized trials and evaluated the effects of exercise on HDL levels, the authors concluded that exercise duration, rather than frequency or intensity, is correlated with rises in HDL levels (195). It was estimated that exercise should be no less than 120 minutes a week in order to raise HDL levels. Interestingly, in our study, both groups performed well beyond the 120 minutes of moderate physical activity a week. Hypothesizing about the mechanisms underlying the observed effects on HDL cholesterol levels is challenging, although there is evidence indicating that modifications in lipid stores, whole body metabolism, and insulin action in muscle, liver, and adipose tissue can contribute to the rise in HDL levels (358). However, caution should be taken when interpreting this data, since the patients in the control group showed a parallel deterioration of endothelial function with the enhanced HDL concentration.

On the other hand, LDL cholesterol remained unchanged in the exercise-training group, while it increased significantly, by 19.5%, in the control group. The clinical significance of the 19.5% increase might be questionable, since the absolute values of LDL cholesterol still remain below the cut-off for optimal levels (131). However, once again it is important take into account the enhanced expression of CAMs in these patients, which does not presume a favourable course of pathology even in the presence of a normal lipid profile. Patients in the present study were treated with lipid-lowering drugs; therefore, variations in the LDL levels could be related to small variations in the dietary determinants of LDL cholesterol, such as intake of saturated fat (226).

In addition, in the exclusive group of diabetic patients who participated in the exercise training, there was a clinically significant decrease in HbA1c (reduction of 1.87 ± 0.57 %). Despite the reduced number of patients (*N*=3), it should be noted that all these patients decreased their levels to values below the recommended treatment targets (HbA1c <6.5%) (131). These results are

particularly interesting since the UK Prospective Diabetes Study (3, 367) demonstrated that a 0.9% reduction in HbA1c was associated with a 16% risk reduction for myocardial infarction. Future studies, encompassing a large number of diabetic patients, are clearly needed to strengthen our findings.

A final note about the lack of differences reported in the dietary intake. As expected, exercise-based intervention alone, without nutritional counselling, did not change the dietary intake. Moreover, even when nutritional advice is given during cardiac rehabilitation, it is difficult for cardiac patients to improve their dietary intake (380). Several studies have pointed out many reasons for these findings, including eating in company, having too much food available, the perception that healthy foods do not taste good, and social influence (196, 197). Fortunately, the absence of differences in dietary intake in both groups during the study period has facilitated the identification of the effects of exercise training *per se*.

3. Study Limitations

Some limitations of the current study should be kept in mind. First, we investigated patients with a low risk for recurrent cardiac events, under optimal medical therapy, and with the majority of the measured parameters under the recommended levels at baseline, which limits the generalization of our findings towards high-risk cardiac patients. Second, rather low levels of biomarkers of endothelial function and inflammation with high relative intraindividual variations may have limited the ability to find a significant change, given the sample size of our cohort. Third, since the impact of aerobic exercise in the bioavailability of $^{\cdot}NO$ is well established, the measurement of endothelium-dependent vasodilatation would have enabled us to draw more robust conclusions about the role of exercise training in endothelial function.

4. Perspectives for Future Research

There are some interesting points within our results that shall be addressed to indicate issues of interest for future research. They refer to the anti-inflammatory role of short-term exercise training programmes and daily physical

activity. Since our results indicated that the improvement in IL-10 was dependent on the daily levels of moderate-intensity physical activity, future studies should clarify whether moderate-intensity physical activity improves IL-10 levels *per se* or if its effects are additive to the exercise sessions.

Furthermore, further randomized controlled studies are needed to evaluate the effects of exercise in patients that consistently have high levels of inflammatory markers at 6-8 weeks post-myocardial infarction. The effect of genetic variability on the anti-inflammatory response to exercise training also warrants examination. Additionally, the role of EPC in the mediation of the positive effects of exercise training in the endothelial function should be further investigated.

CHAPTER VI

CONCLUSIONS AND CLINICAL MESSAGES

1. CONCLUSIONS

Considering the purposes defined for the present study and based on our findings, it seems reasonable to emphasize the following conclusions:

(i) The endothelial function in the control group showed a progressive deterioration during the study period, even under optimal medication and in the presence of optimal management of traditional cardiovascular risk factors. In contrast, the exercise-training programme pronouncedly counteracted this trend.

(ii) Reinforcing the current knowledge regarding the important role of exercise in mitigating inflammation, our exercise-training programme revealed an important anti-inflammatory effect, expressed by the enhancement of the levels of IL-10, which is an indicator of better prognosis.

(iii) The improvement of daily levels of moderate-intensity physical activity induced by exercise training significantly accounted for the anti-inflammatory effect of the exercise-training programme.

(iv) The improvement of functional capacity and exercise tolerance, expressed by VO_2 peak and peak rate-pressure product, as a result of the participation in exercise training reflects positive cardiac adaptations and supports the assumption of the anti-ischemic effects of exercise.

(v) Exercise training also had important repercussions in resting heart rate, resting systolic blood pressure, and heart rate recovery, suggesting an improvement of autonomic function.

The present results support our hypothesis that exercise training mitigates the endothelial dysfunction and inflammation in CAD patients after an acute myocardial infarction, even in the absence of significant changes in traditional cardiovascular risk factors.

In summary, the findings of this study may contribute to enlarging the spectrum of studies supporting the positive impact of exercise training on endothelial dysfunction and inflammation in CAD patients, although suggesting that daily moderate-intensity physical activity might also be involved in the training-induced increase of anti-inflammatory cytokines.

2. CLINICAL MESSAGES

- Standard care and follow up were shown to be inadequate to modify the course of the pathology
- Even patients with a good profile, after an acute myocardial infarction, showed considerable improvements with exercise, justifying the referral to exercise training programmes
- Our short-term exercise training programme was showed to be safe and promise valuable clinical outcomes, thus being a potential therapeutic tool for clinical application
- Traditional cardiovascular risk factors *per se* do not provide adequate sensitive information to monitor the beneficial effects of exercise training on the cardiovascular system, as well as to monitor the progression of the atherosclerosis
- As atherosclerosis is a complex phenomenon, and lacks a single and specific biomarker that represents all the stages of the endothelial dysfunction and inflammation that underlies its pathophysiology, we recommend the use of a set of biomarkers representing different pathways and evolutionary stages of the pathology.

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APPENDIX

List of the conference communications and journal papers related with this work

Papers accepted for publication in national and international journals

1. Fernando Ribeiro, Alberto J. Alves, José A. Duarte, José Oliveira. *Is exercise training an effective therapy targeting endothelial dysfunction and vascular wall inflammation?* International Journal of Cardiology 2009 (accepted for publication)

2. Fernando Ribeiro, Alberto J. Alves, Madalena Teixeira, Vasco Ribeiro, José A. Duarte, José Oliveira. *Endothelial function and atherosclerosis: circulatory markers with clinical usefulness.* Portuguese Journal of Cardiology 2009 (epub ahead of print)

Abstracts published in internatinal journals

1. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alberto J. Alves, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *Phase I cardiac rehabilitation, physical activity levels and exercise capacity in coronary artery disease patients*. Medicine & Science in Sports & Exercise 2009; 41(Supp 5):S442-3
2. Fernando Ribeiro, Fátima Miranda, Madalena Teixeira, Cristina Azevedo, Alberto J. Alves, José A. Duarte, José Oliveira. *Effects of phase II cardiac rehabilitation programme on daily physical activity levels of coronary artery disease patients.* European Journal of Cardiovascular Prevention and Rehabilitation 2009; 16 (Supp 1): S74.
3. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alberto J. Alves, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *The effect of cardiac rehabilitation and exercise training on exercise capacity and circulatory power in coronary artery disease patients: a randomised controlled trial.* European Journal of Cardiovascular Prevention and Rehabilitation 2009; 16 (Supp 1): S74.

Abstracts published in conference procedings

1. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alberto J. Alves, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *Níveis de actividade física diária em pacientes com doença das artérias coronárias e sua relação com a capacidade cardiorespiratória e composição corporal.* Caderno de Resumos do XII Congresso de Ciências do Desporto e Educação Física dos Países de Língua Portuguesa, 17-20 de Setembro de 2008, Porto Alegre, Brasil, pp.337

Poster presentations

1. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alberto J. Alves, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *Phase I cardiac rehabilitation, physical activity levels and exercise capacity in coronary artery disease patients*. American College of Sports Medicine 56th Annual Meeting, 27th-30th of May 2009, Seattle, Washington, USA
2. Fernando Ribeiro, Fátima Miranda, Madalena Teixeira, Alberto J. Alves, Cristina Azevedo, José A. Duarte, José Oliveira. *Effects of phase II cardiac rehabilitation program on daily physical activity levels of coronary artery disease patients.* EuroPRevent 2009 - Meeting of the European Association for Cardiovascular Prevention and Rehabilitation, 6th-9th of May 2009, Stockholm, Sweden
3. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alves JA, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *The effect of cardiac rehabilitation and exercise training on exercise capacity and circulatory power in coronary artery disease patients: a randomized controlled trial.* EuroPRevent 2009 - Meeting of the European Association for Cardiovascular Prevention and Rehabilitation, 6th-9th of May 2009, Stockholm, Sweden
4. Fernando Ribeiro, Madalena Teixeira, Fátima Miranda, Alberto J Alves, Mónica Pinho, Cristina Azevedo, José A. Duarte, José Oliveira. *Níveis de actividade física diária em pacientes com doença das artérias coronárias e sua relação com a capacidade cardiorespiratória e composição corporal.* XII congresso de Ciências do Desporto e Educação Física dos Países de Língua Portuguesa, 17-20 de Setembro de 2008, Porto Alegre, Brasil