Myogenic response, elasticity and \( K_{\text{ATP}} \) channels: role on the properties and regulation of coronary bypass conduits.

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By

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ABSTRACT

The studies presented in this thesis have demonstrated that the phenomenon of the myogenic response plays a pivotal role in the behaviour of vascular smooth muscle of the blood vessels that are used as conduits during coronary surgery, and that many diverse clinical conditions may affect the myogenic properties of these vessels. To that end I have used the tissue organ bath technique to establish the possible relationship between the various clinical conditions suffered by patients undergoing elective isolated coronary bypass surgery. I have found that internal thoracic artery (ITA), radial artery (RA) and long saphenous vein graft (LSVG) have distinctively different internal diameters and myogenic characteristics. The internal diameter and myogenic responses of RA lay almost halfway between ITA and LSVG. This fact presumably reflects on the degree of wall tension the conduits are being subjected once they are grafted and perhaps this might be the caveat required to explain the differences in longevity of the conduits.

Furthermore in these series of experiments I was able to identify various clinical conditions that appear to have a bearing in formulating the myogenic behaviour of the studied vessels. To that end I was able to demonstrate that reduced left ventricular ejection fraction (LV-EF) and history of myocardial infarction(s) may be associated with larger ITA and LSVG internal diameters and decreased ITA myogenic responsiveness, while unstable angina with smaller RA and LSVG (but not ITA) internal diameters. The myogenic responses of ITA may be augmented in obese and diabetic patients and alleviated by diuretics. ACE inhibitors and \( \text{K}_{\text{ATP}} \) channel openers appeared to have a weakening effect on the myogenic responses of the RA.
Understanding the myogenic properties of coronary bypass conduits and how these properties are affected from the various clinical conditions may be of great importance as it may lead to potential new strategies to prevent perioperative conduit-related complications and long-term graft failure and they deserve further investigation.

Furthermore, these studies have confirmed that the way the elastic properties of the blood vessels are modified by disease states has an impact on the longevity of the conduits and therefore to the outcome of surgery. Accordingly, I was able to demonstrate that Left coronary artery Main Stem (LMS) disease is associated with significantly reduced vascular elasticity when compared to non-LMS coronary disease and also that patients with ischaemic heart disease and reduced arterial elasticity also exhibit increased venous stiffness, proportionate to the age of the patients. I believe that these facts have a serious impact on the behaviour of the conduits and they can in part explain quite satisfactorily the pattern of failure of these vessels. My findings suggest that a defect in vascular elasticity may play a role in the development of LMS disease and can be the explanation for the less satisfactory long-term clinical results in this group of patients. I also concluded that the increased venous stiffness in elderly patients with ischaemic heart disease may well be the cause for the greater incidence of venous thrombosis in this group of patients, reflecting also the higher incidence of failure of the venous grafts. These results indicate that pharmacological modifications of vascular elasticity might be a therapeutic target with significant prognostic implications in the outcome of coronary surgery.
Finally the findings of the studies presented in this thesis demonstrate that the $K_{\text{ATP}}$ channels (both plasmalemmal and mitochondrial) appear to play a significant role in the modulation of the grafts' myogenic behaviour through rather complex mechanisms. I believe that the response of the VSM to the different channel openers and blockers is primarily endothelium independent and probably involves multifarious pathways in the signal transduction. In certain aspects of the plasmalemmal $K_{\text{ATP}}$ channels stimulation, the endothelium appears to play a modulatory role. I think that the elucidation of the role of the $K_{\text{ATP}}$ channels in the conformation of the vascular myogenic response, through a more refined, probably in vivo, model, warrants further consideration.
PUBLICATIONS


PRESENTATIONS


Kotidis K.N., Hadjinikolaou L., Galiñanes M. Venous stiffness progresses with age in patients with ischaemic heart disease and is associated with arterial stiffness. 18th EACTS Annual Meeting, Leipzig, Germany.


Kotidis K. N., Hadjinikolaou L., Galinanes M. Stenotic Disease Of The Left Main Stem Is Associated With Reduced Elasticity Of Extracardiac Vessels. 17th EACTS Annual Meeting, Vienna, Austria.

Kotidis K. N., Hadjinikolaou L., Galinanes M. Stenotic Disease Of The Left Main Stem Is Associated With Reduced Elasticity Of Extracardiac Vessels. Society of Cardiothoracic Surgeons research club. Leicester, U.K.
ACKNOWLEDGEMENTS

This thesis is the result of a large and diverse collection of mental, physical and emotional trials and tribulations, “exercises” and experiences. I had no idea upon embarking on this journey, neither of the devastating frustration that a failed experiment can bring, nor of the enormous elation that a successful one can inspire. I’ve never nurtured any ambition of “groundbreaking” results and to be entirely honest I thank God even for allowing me to finish this work.

Two people played the pivotal role in achieving this feat: my immediate supervisor Mr. Leonidas Hadjinikolaou, who was there to share my insecurities and point out my shortcomings and push me (sometimes even shove me) forward to completion of the task at hand, and Professor Manuel Galíñanes whose vision, leadership and dedication guided me through the labyrinth of laboratory research. To both of them I will be eternally grateful.

I would also like to thank all the surgeons in Glenfield Hospital, namely Messrs M. S. Hickey, R. K. Firmin, J. N. Leverment, A. W. Sosnowski and T. J. Spyt, for allowing me to recruit their patients in my studies and provided me with the appropriate tissue to perform them.

Special thanks are due to professor R. S. Bonser, Mr. R. L. Patel, Mr. F. J. Collins and Mr. P. B. Rajesh for supporting me, through a very busy clinical commitment during my Specialist Registrar rotation, into writing up this thesis.

Finally, for all the omissions and mistakes outlined in this thesis, I am (as always) solely responsible.
DEDICATION

To the sacred and tender memory of my father, whose spirit never ceased to be a compass in my journey...

To my mother whose unconditional love, patience and support I will never come close to repay...

And to my three “raisons d' être”: Niko, Dimitri and above all, to Anthoula...
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ACEI</td>
<td>Angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>C</td>
<td>Compliance</td>
</tr>
<tr>
<td>Ca$$^{++}$$</td>
<td>Calcium</td>
</tr>
<tr>
<td>[Ca$$^{++}$$]_i</td>
<td>Intracellular calcium concentration</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene-related peptide</td>
</tr>
<tr>
<td>CSK</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>CV</td>
<td>Cephalic vein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P-450</td>
</tr>
<tr>
<td>D</td>
<td>Distensibility</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>$\Delta$A</td>
<td>Change in Cross-sectional Area</td>
</tr>
<tr>
<td>$\Delta$P</td>
<td>Change in Pressure</td>
</tr>
<tr>
<td>$\Delta$V</td>
<td>Change in Volume</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
</tr>
<tr>
<td>EDHF</td>
<td>Endothelium-derived hyperpolarizing factor</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>Strain</td>
</tr>
<tr>
<td>GEA</td>
<td>Gastroepiploic artery</td>
</tr>
<tr>
<td>20-HETE</td>
<td>20-hydroxyeicosatetraenoic acid</td>
</tr>
<tr>
<td>5-HD</td>
<td>5-Hydroxy-decanoate</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>IEA</td>
<td>Inferior epigastric artery</td>
</tr>
<tr>
<td>iEmod</td>
<td>Incremental elastic modulus</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>IP(_3)</td>
<td>Inositol triphosphate</td>
</tr>
<tr>
<td>ITA</td>
<td>Internal thoracic artery</td>
</tr>
<tr>
<td>K(^+)</td>
<td>Potassium</td>
</tr>
<tr>
<td>K(_{ATP})</td>
<td>Adenosine triphosphate-sensitive potassium (channels)</td>
</tr>
<tr>
<td>K(_{Ca++})</td>
<td>Calcium-activated potassium (channels)</td>
</tr>
<tr>
<td>K(_{IR})</td>
<td>Inwardly rectifying potassium channels</td>
</tr>
<tr>
<td>K(_V)</td>
<td>Voltage-dependent potassium (channels)</td>
</tr>
<tr>
<td>LAD</td>
<td>Left anterior descending (coronary artery)</td>
</tr>
<tr>
<td>LMS</td>
<td>Left main stem</td>
</tr>
<tr>
<td>LSVG</td>
<td>Long saphenous vein graft</td>
</tr>
<tr>
<td>LV-EF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>Mi</td>
<td>Peak myogenic index</td>
</tr>
<tr>
<td>MLC</td>
<td>Myosin light chain</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>P</td>
<td>Pressure (intraluminal)</td>
</tr>
<tr>
<td>P&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Capillary hydrostatic pressure</td>
</tr>
<tr>
<td>PIP&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phosphatidylinositol-4,5-bisphosphate</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PKG</td>
<td>Protein kinase G</td>
</tr>
<tr>
<td>PLA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Phospholipase A&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>PTK</td>
<td>Protein tyrosine kinase</td>
</tr>
<tr>
<td>PVD</td>
<td>Peripheral vascular disease</td>
</tr>
<tr>
<td>r</td>
<td>Vessel radius</td>
</tr>
<tr>
<td>RA</td>
<td>Radial artery</td>
</tr>
<tr>
<td>σ</td>
<td>Stress</td>
</tr>
<tr>
<td>σθ</td>
<td>Circumferential wall stress</td>
</tr>
<tr>
<td>SACC</td>
<td>Stretch-activated cation channels</td>
</tr>
<tr>
<td>SR</td>
<td>Sarcoplasmic reticulum</td>
</tr>
<tr>
<td>SUR</td>
<td>Sulphonylurea receptor</td>
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<tr>
<td>VGCC</td>
<td>Voltage-gated calcium channels</td>
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<tr>
<td>VIP</td>
<td>Vasoactive intestinal peptide</td>
</tr>
<tr>
<td>VSM</td>
<td>Vascular smooth muscle</td>
</tr>
<tr>
<td>WT</td>
<td>Wall tension</td>
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Chapter 1

Introduction
1.1. CORONARY ARTERY BYPASS CONDUITS

1.1.1. Background

Ischaemic heart disease (IHD) represents the single most common cause of mortality in the Western World (1) and coronary artery bypass grafting (CABG) is the most commonly performed surgical intervention for its amelioration. Alexis Carrel is credited with the first association of angina pectoris and coronary atherosclerotic disease (2), when, in the early 20th century, he developed a canine model for aorto-coronary anastomosis using the animal’s carotid artery as a conduit. However his ideas lay fallow for decades, until an intraoperative misadventure led William Longmire in 1958 to use an internal thoracic artery (ITA) as a graft, in order to restore flow to a coronary artery, following a failed endarterectomy (2).

Later, in 1964 DeBakey and Garrett used the long saphenous vein as an aortocoronary bypass graft which is now recognized as the first successful clinical application of this technique (3). The advent of selective coronary angiography by Mason Sones and his collaboration with René Favaloro, brought about the establishment of CABG, utilizing long saphenous vein grafts (LSVG), as a safe and efficient treatment for single vessel, left main and multivessel coronary disease (4). This development led to an explosion of surgical treatment of angina pectoris by the use of LSVG in aortocoronary anastomoses.

The appreciation of the pedicled left ITA as the most advantageous conduit in coronary surgery was not established until the publication of Floyd Loop’s seminal paper (5), demonstrating the convincing survival benefit afforded by the use of the ITA in the revascularisation of the left anterior descending (LAD) coronary artery.
To that end, it came almost as no surprise, that the usage of bilateral ITA convey also a significant advantage in terms of survival, freedom from angina recurrence and freedom from re-operation (6, 7).

1.1.2. Other conduits used for coronary grafting

The excellent results in long term patency rates of the ITA, combined with the expansion of the pool of surgical candidates to subsets of patients with increased co-morbidities (diabetics, peripheral vascular disease sufferers, patients with severely varicose veins), led to investigate the performance of alternative conduits: radial artery (RA) (8), gastroepiploic artery (GEA) (9, 10), inferior epigastric artery (IEA) (9, 10) and cephalic vein (CV) (11) have been used.

The propensity of the RA to spasm led to its abandonment as a routinely used conduit in the 1970's and 1980's. However, the refinement of its harvesting techniques and the development of a better understanding of its anatomy and physiology (12), along with better antispasmodic agents, have led to its revival since the early 1990's as a popular graft (13).

Nevertheless, there is an increasing volume of literature being published, advising for cautionary usage of the radial arterial conduit, as its patency rates do not appear to be significantly better than that of the LSVG (14, 15) though on the other hand, there is contradicting evidence for the opposite (16), notwithstanding the experience from this institution (17).

The results in terms of patency rates, from the usage of GEA (10), IEA (18) and CV (11) were discouraging with effect these conduits to fall into disrepute.
1.1.3. Factors affecting the patency rates of conduits

The success of CABG surgery is measured by the long-term patency rates of the conduits used. Conceivably, a large array of factors, either extrinsic or intrinsic, may affect their longevity. To date, the early use of aspirin (19), meticulous control of blood glucose levels in diabetics (20) and the early introduction of statins (20-22) have been shown to favourably influence the outcomes in terms of patency rates of the grafted conduits. Intrinsic vascular factors such as the vessel's autoregulation manifested through the vascular myogenic response, the elastic properties of the vessels and the activity of the adenosine triphosphate-sensitive potassium ($K_{\text{ATP}}$) channels, can also be affected by different clinical conditions and play a role in the clinical outcome following surgery.
1.2. MYOGENIC RESPONSE

1.2.1. Definition

Vascular autoregulation represents an important homeostatic mechanism that maintains relatively constant blood flow through the various organs despite large fluctuations in the perfusion pressure, thus allowing for the metabolic demand of a certain organ to be matched by its blood perfusion. Central to the process of autoregulation is the myogenic response, which is defined as the inherent property of the vascular smooth muscle to contract (i.e. reducing the vessel’s diameter), as a response to an increase in the perfusion pressure and to relax (i.e. dilating the vessel), when a reduction in the perfusion pressure is observed. This intrinsic quality of the blood vessels is independent of neural, metabolic and hormonal influences. Figure 1.1 represents the prototypical myogenic response of a cannulated arteriole to a step increase in the blood pressure.

The myogenic response has been demonstrated in moderate- and small-sized arteries, small veins (23) and venules (24) and lymphatics (25), but it is more pronounced in arterioles (25). It has been shown that there exists an inverse relationship between vessel size and myogenic responsiveness of the respective vessels and that the maximum myogenic responsiveness is observed at a pressure near or just slightly higher than its normal pressure as measured in vivo (26). It is worth noting that different studies have demonstrated a variation in the strength of the myogenic response in different vascular beds (27, 28), and even variations in strength within the same vascular bed (29).
Figure 1.1: Example of the myogenic response of a cannulated arteriole to a pressure increase in blood pressure. After the pressure step the vessel initially passively dilates and followed by two phases of constriction, a transient then a sustained. Upon removal of the pressure stimulus the vessel initially collapses and then dilates.
1.2.2. Background

William Maddock Bayliss is credited with the discovery of the myogenic response in 1902, when he recorded large increases in the volume of the dog hindlimb following release of brief aortic occlusions (30). He considered those responses too rapid to be mediated by accumulation of metabolites and thought that the underlying mechanism was the same to the one responsible for the constriction observed in isolated arteries following sudden distension.

Anrep (31) challenged this theory postulating that the hind limb response could be explained by metabolic factors. His persuasive arguments led Bayliss' theory to disrepute, resulting to little work being performed on the myogenic response for the subsequent 45 years and the majority of workers to believe that local vascular regulation should be attributed primarily to chemical and neural mechanisms.

It wasn't until 1949 when Folkow demonstrated that denervated preparations developed pressure-dependent vascular tone (32), and as a result the myogenic response theory regained credibility and attracted the interest of researchers. In the following decades the development of increasingly sophisticated whole organ techniques concluded that this mechanism could account for the significant changes in vascular resistance occurring in vivo (33). At the same time it was demonstrated that vascular smooth muscle (VSM) reacted to quick stretch with active force generation (34). Later, the development of isolated vessel techniques (35) made it possible for the myogenic response to be studied more accurately in different types of vessels (29, 36-38).
1.2.3. Physiological significance

The myogenic response appears to play a major role in two physiologically important functions: \(i\) the development of basal vascular tone and \(ii\) the autoregulation of blood flow and capillary hydrostatic pressure.

\(i\) Basal vascular tone

Basal vascular tone is a sine qua non in order for the autoregulatory dilator influences to a blood vessel, to occur. It represents a function, by which the blood vessels establish a degree of constriction at arteriolar level, that allows for other control mechanisms, producing vasoconstriction or vasodilatation, to take place (39). Its connection to the myogenic response was derived from the finding that denervated whole organ preparations developed a pressure dependent resistance to flow (40, 41).

In studies of the microcirculation in isolated vessels, it was shown that spontaneous tone was developed in nearly all arterial vessels with less than 150 \(\mu\)m in diameter and that excessive levels of anaesthesia, extensive surgical manipulation and trauma would readily compromise it (35). In the same setting it was concluded that the level of tone developed in isolated arteries and arterioles, was comparable to that observed in the same vessels in vivo and required for the vessels to be pressurized to a physiological level of pressure in order for it to develop (26).

\(ii\) Autoregulation of flow and pressure

The fundamental processes of exchange of nutrients for metabolic waste products are taking place at the level of the capillaries. It is therefore paramount for the blood flow and capillary hydrostatic pressure \(P_c\) to be maintained constant as much as possible. Studies conducted by Johnson (42) in whole organ preparations suggested that
changes in arterial inflow or venous outflow pressures, produced changes in arterial resistance that would serve to minimize changes in $P_c$. Furthermore Mellander and colleagues (43, 44) demonstrated that tissue volume of the cat hindlimb skeletal muscle was nearly constant over a wide range of systemic arterial pressures (30-170 mmHg). Assuming that constant tissue volume reflected a constant $P_c$, the conclusion drawn from these experiments was that the autoregulation of $P_c$ was achieved through myogenic adjustments of arteriolar and/or venular tone. A number of experiments attempted to identify whether isolated alterations in arterial perfusion pressure or venous pressure, or alterations in both, made a difference to the degree of contribution of the myogenic response to the regulation of the $P_c$. It was concluded that in the case of selective arterial perfusion pressure reduction, the contribution of the myogenic response to regulation of the $P_c$ was less pronounced than that when inflow and outflow pressures were equally raised or lowered(45, 46).

1.2.4. Initiation of the myogenic response

There is compelling evidence that the myogenic response is triggered by changes in vessel wall tension (WT) (47). Laplace’s law defines wall tension as the product of intraluminal pressure ($P$) and vessel radius ($r$). Recently Ahmed et al(48) demonstrated that it is indeed the pressure-induced elevation of wall tension that represents the primary stimulus for the initiation of the myogenic response. The ensuing vasoconstriction and reduction in the vessel diameter, reduces the wall tension, thus acting as a negative feedback mechanism for the attenuation of the response. It was also shown that increases in vessel wall tension during agonist stimulation lead to increases in agonist sensitivity (positive feedback) and decreases in vessel wall tension during agonist stimulation lead to decreases in agonist sensitivity.
Furthermore a number of studies (28, 50) have demonstrated significant correlation of the vessel wall tension changes with the changes in intracellular calcium concentration \([\text{Ca}^{2+}]) and the level of myosin light change (MLC) phosphorylation, two events that have been suggested to be involved in the myogenic response.

Nevertheless, it appears that currently there is no consensus about the sensor element that detects the stimulus of the change in wall tension, during myogenic vasoconstriction/ dilatation. To that end, stretch-activated cation channels (SACC) (51), voltage-gated calcium channels (VGCC) (52), the extracellular matrix (ECM) and the cytoskeleton (CSK) (53), have been investigated. Recently acquired evidence (54, 55) indicates that it is perhaps the VGCC that have the highest probability to be the sensors of the WT changes that lead to the myogenic response.

Finally, it appears that there exists enough evidence to establish that the effectors of the myogenic response are the smooth muscle cells in the vessel wall of arteries, arterioles and venules, and neither the nervous influences from nerve endings in the vessel adventitia (36, 37, 56), nor influences from the endothelium have been shown to play any significant role in the myogenic response (57). However Zimmermann et al (58) postulate that the endothelium, through a tonic nitric oxide (NO) release may play a modulatory role in the myogenic response.

1.2.5. Cellular events associated with the myogenic response

A. Membrane depolarisation

It is well established that vascular smooth muscle (VSM) cells demonstrate depolarisation following an increase in transmural pressure (36). This phenomenon
was shown in vessels of different vascular beds and for a variety of pressure ranges (36, 59-61). This reaction of the VSM cells was always accompanied by vessel contraction, i.e. myogenic response. Several hypotheses have been suggested regarding the mechanism conveying this depolarisation.

**i) Stretch-activated channels**

This type of non-selective cation channels were shown to effect the depolarisation of VSM cell membrane as a response to membrane stretch (51, 62, 63). However, these observations were done by using the patch-clamp technique in isolated single smooth muscle cells, a fact that casts some doubt in the validity of these data. The reason for this is because it has been shown that when the stretch/pressure stimulus applied on the isolated patch of the cell membrane, VGCC could also be activated causing membrane depolarisation (52). Moreover, the application of the stretch/pressure stimulus to isolated cells cannot mimic the change in WT stimulus that initiates the myogenic response to the intact vessels.

**ii) Potassium (K⁺) channels**

A multitude of potassium channels exists in cell membranes, including the VSM cells (64). Current evidence points out that there is a K⁺ channel that counteracts the myogenic response. Komaru et al (65) suggested that this role is advocated by the adenosine triphosphate-sensitive potassium (K_{ATP}) channels based on observations in canine coronary microcirculation, though the evidence from another study (61) failed to confirm this finding. Other researchers suggested that voltage-dependent potassium (Kᵥ) channels play an important role in regulating the myogenic response,
following the finding that $K_V$ channel blockers augment the myogenic response in VSM cells of pressurised arterioles by reducing their membrane potential (61).

Finally, there are conflicting theories about the potential mechanisms by which the calcium-activated potassium ($K_{Ca^{++}}$) channels may regulate the myogenic response: one states that the initial membrane depolarisation characteristic of the myogenic response is caused by inhibition of $K_{Ca^{++}}$ channels and thus a reduction in the outward current of the VSM cells (66) and membrane depolarisation; however there is an alternative model suggesting that the myogenic membrane depolarisation results in increased $[Ca^{++}]_i$, leading to increased frequency of $Ca^{++}$ sparks from the sarcoplasmic reticulum, which in turn activate the $K_{Ca^{++}}$ channels, this causing membrane hyperpolarisation and therefore closure of the $Ca^{++}$ channels that accompanies the membrane depolarisation, thus leading by a negative feedback to a reduction of the myogenic response (67).

\[ iii \] Chloride ($Cl^-$) channels

Current data (68, 69) suggests that there might be a $Cl^-$ channel involved in the membrane depolarisation accompanying the myogenic response. To that end it has been shown that a volume regulated $Cl^-$ channel is expressed in the cell membrane of VSM cells and that it is activated by myogenic membrane depolarisation, effecting $Cl^-$ efflux and thus enhancing the myogenic response. However the fact that there is no selective $Cl^-$ channel blocker puts into question the attribution of the above-mentioned effect to this type of channel.
iv) Calcium (Ca$$^{++}$$) channels

McCarron et al (52) demonstrated in patch-clamp experiments, that membrane stretch can activate VGCC, thus increasing the inward Ca$$^{++}$$ and possibly causing the membrane depolarisation accompanying the myogenic response. However in other series of experiments (61, 70), it was shown that after complete blockade of Ca$$^{++}$$ channels the observed membrane depolarisation was not accompanied by myogenic contraction. The interpretation of these findings was that the calcium influx following the membrane depolarisation was neutralised by an outward current through K$$_{Ca^{++}}$$ channels and it was not per se responsible for the membrane depolarisation; also that the myogenic contraction following the membrane depolarisation required the inward Ca$$^{++}$$ current in order to be effected.

In summary, the myogenic response is initiated by a change in transmural pressure that is accompanied by a membrane depolarisation. However the mechanism conveying this depolarisation is still not fully understood.

B. Intracellular Ca$$^{++}$$ concentration ([Ca$$^{++}$$]$_i$)

During increases in the transmural pressure there is always an increase of the [Ca$$^{++}$$]$_i$ in the VSM cell. This fact has been conclusively established in a multitude of myogenic studies (50, 71, 72). Furthermore it is proven that removal of extracellular Ca$$^{++}$$ completely abolishes the myogenic response (73-75) attesting the necessity of extracellular Ca$$^{++}$$ for its development. McCarron et al postulate that this increase in [Ca$$^{++}$$]$_i$ is through influx of Ca$$^{++}$$ into the cell and not through release of Ca$$^{++}$$ from intracellular stores (52). However in a different study, Watanabe et al (76) demonstrated that the use of ryanodine, a substance known to deplete the intracellular
Ca^{++} stores, reduced the amplitude and rate of development of myogenic contraction after a step up in the transmural pressure. These findings imply that the intracellular Ca^{++} stores though not the primary effectors of the myogenic response, they probably do play a role in its development.

Strong evidence exists that the pathway of Ca^{++} entry into the VSM cell is VGCC, a fact that was demonstrated in numerous experiments by using blockers of these channels, thus producing an inhibition of the myogenic response and abolition of the increase of the [Ca^{++}]_{i}. To the contrary, the use of Bay K 8644, a specific VGCC opener produced an increase in the strength of the myogenic response (77).

There is growing evidence that the increase in [Ca^{++}]_{i} during the myogenic response may activate the MLC kinase, thus phosphorylating the regulatory contractile protein MLC and leading to contraction. Indeed it was shown that elevation in transmural pressure was followed by an increase to the degree of phosphorylation of the MLC (78) and the application of MLC kinase inhibitor resulted in reduction of MLC phosphorylation and failure of increased transmural pressure to elicit the myogenic response, despite an unaffected increase in [Ca^{++}]_{i} (50).

Finally some reports point out indirectly, that there may be extracellular calcium-independent, intracellular second messengers involved in the development of the myogenic response, by increasing the sensitivity of the contractile apparatus in the Ca^{++} influx caused by the membrane depolarisation that initiates the myogenic response (72, 79).
C. Intracellular second messengers.

In recent years significant attention has been drawn to the elucidation of the role of intracellular second messengers and the pathways involved in the development of the myogenic response. As well as the role of the MLC kinase that we briefly touched earlier, other factors investigated were G-proteins, phospholipase C (PLC), inositol triphosphate (IP$_3$), diacylglycerol (DAG), protein kinase C (PKC), protein tyrosine kinase (PTK), arachidonic acid (AA) metabolites and their interaction with the ECM, integrins and the CSK.

i) G-proteins and PLC

G-proteins are heterotrimeric proteins embedded in the plasma membrane and linked with a large number of different receptors. They are known to be activated by a multitude of agonists (adrenaline, glucagon, parathyroid hormone etc) but it was also shown that mechanical stimulation of either skeletal muscle or VSM results in immediate G-protein activation (80-82).

PLC is a cytosolic enzyme that is activated by G-proteins and hydrolyses phosphatidylinositol-4,5-bisphosphate (PIP$_2$) into IP$_3$ and DAG. The use of PLC inhibitors such as U-73122 and neomycin resulted in attenuation of the pressure-induced myogenic contractions (83, 84) providing evidence of its involvement in this pathway of mechanotransduction.

ii) Inositol triphosphate (IP$_3$) and diacylglycerol (DAG)

An important finding in the understanding of the pathways involved in the vascular mechanotransduction was the observation by Narayanan and co-workers that during step increases of intraluminal pressure in conduit arteries, a significant pressure-
related increase in the intracellular concentration of inositol triphosphate (IP$_3$) and diacylglycerol (DAG) (85) was observed. These findings were confirmed in different vascular beds and in isolated VSM cells (86, 87). It is known that the effect of the increased production of IP$_3$ by the cell, leads to the release of Ca$^{++}$ from intracellular stores (i.e. the sarcoplasmic reticulum (SR)) thus increasing the [Ca$^{++}$]$_i$ and effecting the myogenic response as described above. The role of DAG, which remains attached in the inner layer of the plasma membrane, is to recruit the PKC which in turn and as described below, activates and phosphorylates many different intracellular proteins (88).

iii) Protein kinase C (PKC)

PKC is a member of a group of intracellular enzymes that catalyse the reaction of phosphate transfer from ATP to various amino acids. As mentioned above the enzyme is activated by diacylglycerol in the presence of Ca$^{++}$, it is thought to undergo a conformational change upon binding to the membrane and by phosphorylating a variety of target proteins, promotes cellular differentiation and controls cell growth. It appears that its contribution in the myogenic response is multifid. Apart from the already mentioned activation of cation channels (88) it has been implicated in an enhanced MLC phosphorylation (89) as it was shown that the PKC activator indolactam produced constriction by a mechanism involving an increased level of MLC phosphorylation. It was also found that PKC improves the myogenic response by causing Ca$^{++}$ sensitisation of the contractile apparatus (90), an action effected by inhibition of the MLC phosphatase.
iv) Protein tyrosine kinase (PTK)

Another intracellular factor that has been implicated in the regulation of the myogenic response is PTK. It has been shown that the use of specific inhibitors of PTK reduced the pressure-induced contraction of blood vessels from different vascular beds, whereas tyrosine phosphatase inhibitors accentuated the observed response (91). Also Hollenberg (92) has suggested a possible role for mechanisms involving tyrosine phosphorylation in the regulation of smooth muscle contraction. Similarly, these mechanisms have been implicated in activation of mitogen-activated protein kinases (MAPK) (93), which appear to play a role in modulation of Ca^{2+} sensitivity of the contractile apparatus. However the data available so far appear to be inconclusive regarding the order of activation of the different cellular protein kinases and their interaction on the pathway of mechanotransduction (94, 95).

v) Arachidonic acid (AA) metabolites

AA is released from membrane phospholipids into the cytosol, as the result of hydrolysis by different intracellular enzymes, following plasma membrane stimulation by a variety of stimuli including agonist-receptor binding or mechanical stimulation. Cytosolic AA represents a substrate for Cytochrome P-450 (CYP), which metabolises AA, among other metabolites, to 20-hydroxyeicosatetraenoic acid (20-HETE). As early as 1991 the observation that inhibitors of phospholipase A_{2} (PLA_{2}) and CYP attenuated the myogenic response of dog renal arcuate arteries (96), attracted interest in the possibility that the CYP metabolites of AA may play a role in the myogenic response. Since then numerous reports established the pivotal role of 20-HETE in the myogenic contractions produced by intraluminal pressure-changes in various vascular beds (97-100). It is now evident that 20-HETE is involved in a multitude of reactions
and signal transduction pathways. Firstly, endogenous 20-HETE causes membrane depolarisation and a rise in \([\text{Ca}^{++}]_i\) resulting in myogenic constriction of small arteries and arterioles (101). It has also been shown to be involved in the pathway of activation of PKC, PTK (102) and in the activation of MAPK (103). However controversy still exists whether 20-HETE is one of the primary mediators of the myogenic response or it simply modulates the responsiveness of the vessel to intraluminal pressure changes (104).

D. Involvement of ECM, integrins and CSK in the myogenic response.

The VSM cell is embedded in a complex three-dimensional network of ECM proteins, including type III, IV, V, VI collagens, and fibronectin, vitronectin, thrombospondin, elastin, tenascin, osteopontin, and several types of laminin (93). Integrins represent a class of membrane-spanning glycoproteins that link the ECM with the CSK. They are composed of \(\alpha\beta\)-heterodimers with extracellular domains that bind to ECM and short cytoplasmic tails that associate with cytoskeletal contractile proteins (actin, microtubules) (105, 106). It has been proposed that the site of anchoring of the contractile proteins is the area of the dense plaque in the plasma membrane, which represents the mechanical junction between the extracellular and intracellular environments. Upon engagement of integrins by ECM proteins, they recruit the CSK contractile proteins at the dense plaque and by altering their conformation, they cause cell contraction (105).

The predominant integrin subunit combination in the VSM suggests there are extensive interactions with collagens, laminin, and fibronectin in the basement membrane and interstitial matrix (93). It is also known that the ECM can directly transmit force to the VSM cell layer (107) and therefore acute increases in transmural
pressure produces increased circumferential wall tension, thus triggering the myogenic contraction.

Furthermore, there is increasing evidence suggesting the regulatory role of integrins in the vascular myogenic response. The most direct evidence for an integrin role in the myogenic response derives from observations that peptides containing integrin-specific amino acid sequences are potently vasoactive. It has been shown that all the predominant VSM integrins contain the Asp-Gly-Asp (RGD) recognition site and that RGD-containing peptides cause a transient constriction followed by a sustained dilation (108). It is also evident that integrins regulate the [Ca^{++}]_{i} by opening or inhibiting VGCC in the VSM (109) thus interfering with the degree of MLC phosphorylation and the myogenic contractions of blood vessels.

Finally there is emerging evidence that the CSK may play a role in the development of the vascular myogenic reactivity. The prevailing model of the CSK organisation (the tensegrity model) describes the CSK as a highly organized, three-dimensional syncytium of compression-resistant struts (microtubules) suspended among various elastic elements (intermediate filaments and actin filaments) (110). This model predicts that the CSK microtubules would normally oppose the cellular shortening resulting from activation of contractile filaments. Accordingly, disruption of the microtubules would enhance force transmission, thus permitting an increased vascular tone. Therefore the state of microtubule polymerisation can account for the intensity of pressure-induced contractions, as Platts and co-workers (111) showed by using microtubule depolymerising agents, which caused a significant increase in myogenic tone of skeletal muscle arterioles. Conversely taxol, which stabilises microtubules, was shown to inhibit myogenic contractions (111). To that end, Cipolla and Osol
have shown that inhibition of actin polymerisation reduced the vessel ability to react in increased intravascular pressure i.e. to mount a myogenic response (112).

Figure 2 represents a schematic summary of the mechanisms involved in the development of the vascular myogenic response (modified from Schubert R, Mulvany MJ. The myogenic response: established facts and attractive hypotheses. Clin Sci (Lond) 1999; 96 (4): 313-26).
Figure 1.2: Putative mechanisms of the myogenic response. Abbreviations: AA: arachidonic acid, CYT P-450: cytochrome P-450, \( K_{Ca} \): calcium-activated potassium channel, \( \text{PLA}_2 \): phospholipase A\(_2\), SAC: stretch-activated cation channel, Cl: chloride channel, VGCC: voltage-gated calcium channel, IP\(_3\): inositol triphosphate, DAG: diacylglycerol, PTK: Protein tyrosine kinase, ECM: extracellular matrix, CSK: cytoskeleton, MAPK: mitogen-activated protein kinase.
1.3. ELASTICITY

1.3.1. Background

Blood vessels at large serve a variety of functions and have differentiated histologically in order to meet the requirements of their function. To that end, all blood vessels apart from the capillaries of the microcirculation are made up of three layers of tissue, from inside to outside: tunica intima, tunica media and tunica adventitia. The innermost layer (the intima) is made of endothelial cells with a subendothelial connective tissue layer named lamina propria intimae, which comprises of ECM i.e. collagen, elastin, fibroblasts, smooth muscle cells and macrophages (113) which in infants is barely detectable and its thickness increases with age. The border between the intima and tunica media is demarcated by the internal elastic membrane, which is functionally, part of the latter. It is the tunica media that conveys the properties for the vessel to meet its function, as it contains a variable amount of elastin fibres, collagen fibres and smooth muscle cells, allowing for the vessels to either distend and store blood or contract and regulate the blood flow. In general the arrangement of the smooth muscle is circumferentially in a helical fashion, providing circumferential tensile strength. Elastin bears both longitudinal and circumferential loads, while collagen bears primarily circumferential loads (114). Thus large arteries behave primarily in an elastic manner, as their media comprises mainly elastic lamellae, collagen fibres and relatively small numbers of smooth muscle cells (113). To that end, they are able to distend considerably during the systolic phase of the heart cycle, thus “storing” the blood, and then during diastole they gradually return to their original size allowing for the blood flow to become less pulsatile and to reduce the blood pressure requirements (Windkessel function) (115).
It has been actually shown that the elastic and collagen content of the media gradually increases under the influence of the cyclic stretching that these cells are subjected through the cardiac cycle (116). It has also been shown that the elastin and collagen fibres exhibit a differential temporal engagement in different levels of blood pressure (117). In smaller, more muscular arteries and arterioles, the elastic content is less, with increased number of smooth muscle cells that are arranged in such a way in order to function as a syncytium.

In the venous part of the circulation the three tissue layers are also present but less distinct, with less muscular and elastic components but with increased collagen elements, thus allowing for passive distention with less vigorous elastic recoil and muscular contractility and therefore allowing for the venous “capacitance” function to take place.

1.3.2. Definitions

A. Elasticity

The term elasticity refers to the relationship between the forces applied to a material and its consequent deformation. More specifically, it is the property of an object or material, which allows it to be restored to its original shape after distortion that was caused by an external or internal (as in concave objects) force(s), upon cessation of the application of the force(s). A material is said to be perfectly elastic when it returns to its original shape without any residual deformation, when the distorting force is removed. Conversely, if it retains part or all of its deformation, it is said to be plastic. If the elastic properties of the material are independent of the direction in which the force is applied, the material is called isotropic.
properties are the same throughout all its parts, is called homogenous. Most homogenous elastic materials respond to deformation by following Hooke’s law (fig. 1.3), which states that there is a linear relationship between the amount of distorting force and the degree of distortion of the material.
**Figure 1.3:** Graphic example of a homogenous elastic material (metal spring) exhibiting Hookean behaviour. $F$ = deformation force, $k$ = spring constant & $x$ = deformation length (from http://hyperphysics.phy-astr.gsu.edu/hbase/permot2.html).
B. Compliance and Distensibility

The ability of a blood vessel wall to expand and contract passively with changes in pressure constitutes an important function of large arteries and veins. This ability of a vessel to distend with increasing transmural pressure is defined as vessel compliance (C), which is the change in volume (ΔV) (or cross-sectional area (ΔA) for others) divided by the change in pressure (ΔP):

\[ C = \frac{\Delta V}{\Delta P} \text{ or } C = \frac{\Delta A}{\Delta P} \]

In other words the compliance can be measured as the first derivative or tangent to the pressure-volume (or pressure-area) curve (118). Figure 1.4 represents a simplified depiction of arterial and venous compliance. It is evident that:

Firstly, the venous compliance as a whole is greater than the arterial one, particularly in the lower range of blood pressure alterations. Therefore veins can accommodate large changes in blood volume with only a small change in pressure. However, at higher pressures and volumes, venous compliance (slope of compliance curve) becomes similar to arterial compliance.

Secondly, the slope of each curve is not linear, which is accounted for by the fact that the blood vessel wall is heterogeneous tissue, indicating that the compliance of each individual vessel varies considerably depending on the cumulative effect of the blood pressure changes in the respective part of the vascular tree (arterial or venous). Conceivably the VSM plays an important role in the dynamic regulation of compliance by means of increasing or decreasing the VSM tone, which in turn produces the opposite effects on the blood vessel wall compliance.
Figure 1.4: Graphic depiction of compliance for arteries and veins. For comment see text.
Furthermore, various exogenous factors (see below) affect the vascular compliance, resulting in its clinical manifestation of hypertension and its sequelae.

Distensibility (D) refers to the fractional change in cross-sectional area for a given change in pressure

\[ D = \frac{\Delta A}{\Delta P} / A \]

where A stands for the diastolic cross sectional area. Therefore D equates to compliance being normalised for the size of the vessel, hence it allows for a more direct comparison of vascular stiffness between blood vessels of different sizes and even different species.

C. Stress, Strain and Incremental Elastic Modulus

In physics, stress (\( \sigma \)) is the internal distribution of forces within a body that balance and react to the loads applied to it. In simpler terms, stress is the sum of forces exerted on a body or area, divided by that area, i.e. \( \sigma = F / A \). Thus circumferential wall stress in a blood vessel can be defined by Lame's equation \( \sigma \theta = (MBP \cdot Dm) / 2hm \), where MBP is mean BP and Dm and hm are the mean values of internal diameter and wall thickness during the cardiac cycle(119), or in a more simplified version \( \sigma \theta = P \cdot r / h \), where P is pressure, r is vessel radius and h wall thickness, respectively (118). The SI unit for \( \sigma \) is Pascal (Pa) equalling 1 Newton/metre\(^2\) (N/m\(^2\)).

Strain (\( \varepsilon \)) is the effect of stress and it is defined as the percentage change in a dimension when a structure is exposed to a stress, divided by the original value of that dimension, i.e. \( \varepsilon = \Delta l / l \) where \( \Delta l \) is the change in length and \( l \) the original length. More specifically for a blood vessel, circumferential wall strain is defined as the
change in the diameter or cross-sectional area or volume, divided by the original
diameter, cross-sectional area or volume, i.e. \( \frac{2\pi r_2^2 - 2\pi r_1^2}{2\pi r_1} \) where \( r_1 \) and \( r_2 \) are the
initial and final vessel radii respectively(118). Notably, strain being a ratio of the
same dimensions, it is in itself dimensionless.

Elastic or Young’s modulus represents the stress-strain relationship in homogenous
elastic materials that their behaviour follows Hooke’s law. More specifically in
Hookean materials the stress: strain ratio (elastic modulus) is exhibiting a linear
relationship and it is constant regardless of the stress or strain that it was measured.
However, the majority of materials, including blood vessels, are not perfectly elastic
and are not homogenous thus their behaviour is non-Hookean. Blood vessels are
made up from a number of elements of widely varying stiffness connected in a
complex manner and stretched under different pressures and in different lengths.
Consequently, the elastic modulus of a material such as the blood vessel wall is not
constant, but rather varies according to the stress: strain variations. Thus the ensuing
relationship between stress and strain is curvilinear with a flat slope at low stress or
strain and a progressively increasing slope at higher stress or strain.

The term incremental elastic modulus (\( i\text{Emod} \)) signifies the tangent or first derivative
of the stress: strain curve (\( \frac{d\sigma}{d\varepsilon} \)) (fig. 1.5). It represents a measure of the vascular
wall stiffness and similarly to compliance, it has to be given at a specific pressure,
stress or strain. It is one of the most widely used parameters for representation of
vascular wall stiffness and various methods have been established for its measurement
(120, 121). Thus it is effortlessly deducible that the \( i\text{Emod} \) is inversely related to
compliance, i.e. a high value of \( i\text{Emod} \) indicates increased vessel wall stiffness and
therefore reduced compliance. However, one should be careful in assuming that
every intervention e.g. pharmacological through a vasodilator drug, that would
Figure 1.5. Graphic depiction of a blood vessel stress: strain curve indicating that the \( iEmod \) is the tangent or first derivative of that curve. As mentioned above the strain is dimensionless.
increase compliance, would automatically result in reduction of iEmod and therefore reduction of vessel wall stiffness. In fact the effect of vasodilatation on the iEmod may be that of increase, decrease or no change at all (122). Figure 1.6 shows a graphic representation of the relationship between compliance and iEmod under baseline conditions and after the administration of a vasodilator drug. After the administration of the latter there is a shift to a new compliance- iEmod curve. From the baseline point A, depending upon the changes in the wall stiffness, vasodilatation may result to reduction in compliance with concomitant increase to iEmod (point B), increase in both compliance and iEmod (point C), or increase in compliance and reduction to iEmod (point D).

Different methods of direct measurement or calculation of the various parameters of vascular elasticity have been developed. Some are based on mathematical models, e.g. calculation of compliance through measurement of the stroke volume and pulse pressure, or through measurement of pulse wave velocity. With the technological advancement in the recent years, imaging techniques, like angiography, functional magnetic resonance imaging (MRI) and ultrasound (M-mode, echo-tracking system or intravascular), have become very accurate to allow for reliable direct measurement of the haemodynamic changes that may affect the elastic properties of blood vessels. Details of these methods would be beyond the scope of this thesis.
Figure 1.6: Graphic illustration of the potential effects of a vasodilator drug on the compliance and iEmod of a blood vessel. After the administration of the latter there is a shift to a new compliance- iEmod curve. From the baseline point A, depending upon the changes in the wall stiffness, vasodilatation may result to reduction in compliance with concomitant increase to iEmod (point B), increase in both compliance and iEmod (point C), or increase in compliance and reduction to iEmod (point D).
1.3.3. Correlation of cardiovascular risk factors with vascular elasticity

There is now good evidence that correlation between cardiovascular risk factors and vascular elasticity exists. This is particularly true for large elastic arteries such as the aorta and the great vessels of the aortic arch. To that end, factors such as gender, age, hypertension, diabetes mellitus (DM), cigarette smoking and hypercholesterolaemia have been shown to have a positive correlation with altered vascular elastic properties. Patients suffering with ischaemic heart disease (IHD), cerebrovascular disease (CVD), peripheral vascular disease (PVD) and renal disease, have been shown to reduce elasticity particularly in the arterial part of their vascular tree.

A. Gender

No difference between sexes exists, in terms of aortic distensibility, for the first ten years of life. However the male arterial distensibility rapidly declines thereafter, only to start converging again with the one in females, around the age of menopause (123, 124). There is a protective effect of the oestrogens on arterial compliance and distensibility, supported by the finding that postmenopausal hormone replacement therapy (HRT) users have higher systemic vascular compliance than non-users (125).

B. Age

Numerous studies have established a highly positive correlation between ageing and a reduction in arterial compliance and distensibility (124, 126, 127). This appears to be a natural phenomenon rather than the effect of atherosclerosis as suggested by studies confirming this finding in populations with reduced cardiovascular risk (128).
C. **Hypertension**

It has been demonstrated that raised arterial blood pressure correlates with reduced arterial distensibility (129, 130). However the degree of hypertension does not appear to be the primary determinant of the degree of cardiovascular risk. It is rather the changes in the wall structure and function that leads to the alteration of the vessel compliance (131, 132). This fact has been the cornerstone principle in developing cardiovascular drugs and therapies that have been proven to modify the arterial compliance and distensibility, thus reducing the cardiovascular event risk (133, 134).

D. **Diabetes Mellitus**

It is established that chronic hyperglycaemia results in a reduction of vascular distensibility, in a manner independent of atherosclerosis, due to the accumulated deposition of advanced glycation end products in the vessel wall (135, 136). These glycation end products contribute to the development of diabetic vascular disease. Furthermore, arterial stiffness has been shown to be elevated in pre-diabetic states (137). The impairment of the endothelial vasodilator function with reduced bioavailability of NO in the arterial tree of diabetic patients may account for the reduced vascular compliance in these patients (138, 139), and stringent glycaemic control in a long term insulin regime has shown encouraging results in the improvement of the vascular elastic properties in such group of patients (140).

E. **Cigarette smoking**

There is conclusive evidence of a positive correlation of smoking with reduction of arterial distensibility (141). It is thought that the adverse effects of smoking on the endothelial function is the cornerstone in the mechanism by which it affects the
vascular elasticity (142, 143), as it appears to impair the basal NO release from the endothelium of this group of people (144). However, the exact mechanism by which cigarette smoking affects vascular elasticity is not as yet fully understood.

F. Hypercholesterolaemia

The evidence regarding the relationship between hypercholesterolaemia and vascular compliance is somewhat conflicting. There are studies that show increased compliance and distensibility with increased plasma cholesterol and low-density lipoproteins but only in the absence of coronary disease (145), whereas a study by Giannattasio et al concluded the opposite (146). By and large the current consensus is that hypercholesterolaemia decreases the release and action of nitric oxide in resistance and coronary arteries (147), which may reduce their elasticity and treatment with cholesterol-lowering medication restores the elastic characteristics of the blood vessels of the treated subjects (148, 149).

G. Ischaemic heart disease

There are a number of studies indicating a strong correlation of coronary artery disease and altered elastic properties of blood vessels (150, 151). From a histological standpoint, there is evidence of a strong association between IHD and aortic atherosclerosis (152). Genetic factors are also implicated as it has been shown that patients with family history of IHD present significant impairment of endothelium-dependent vasodilatation (151).
H. Cerebrovascular Disease

Lehman and co-workers have shown that patients who have suffered a stroke have had reduced aortic distensibility (153). Aortic arch atherosclerosis is associated with reduced aortic elasticity and it is shown to be an independent predictor of ischaemic stroke (154).

I. Peripheral Vascular Disease

Tai et al concluded that PVD leads to significant reduction of vascular compliance and distensibility (155). This fact may represent an important factor in determining graft patency in bypass surgery (156, 157).

J. Renal Disease

In end-stage renal disease, arterial stiffening (as determined by carotid iEmod) has been found to be a strong independent predictor for all-cause and cardiovascular mortality (158). It is established that patients on haemodialysis present low arterial compliance (159) due to primarily vessel calcification (160), leading to left ventricular hypertrophy which in turn represents a strong independent predictor of cardiovascular death in this group of patients (161).
1.4. ATP-SENSITIVE POTASSIUM (K$_{ATP}$) CHANNELS

1.4.1. Background

As discussed earlier, many factors play a role in forming the sum of interactions leading to the moment-to-moment regulation of the VSM tone. The final common pathway for all the stimuli (excitatory or inhibitory) to the VSM cell is the effect they have on the calcium stores in the sarcoplasmic reticulum and its content, i.e. the free calcium that is released into the cytoplasm, which will determine the degree of contraction of the VSM. Also calcium entry into the cytoplasm through VGCC represents a pivotal point in VSM contractility, and any factor that affects the open-state of these channels modifies the VSM cell’s state of contraction.

Activation of K$^+$ channels will have the net effect of membrane hyperpolarisation thus closing the VGCC. Among the various K$^+$ channels, the K$_{ATP}$ channels appear to play an important role as mediators of the response of the VSM to a variety of pharmacological and endogenous vasodilators, as well as to changes in metabolic activity that can directly influence blood flow to various tissues.

1.4.2. K$_{ATP}$ channels and their characteristics

K$_{ATP}$ channels were first discovered by Noma in 1983 when by using the patch-clamp technique he described his observation that mammalian heart cells subjected to hypoxia or treatment with cyanide, exhibited an outward current of potassium. This phenomenon was abolished with the intracellular injection of ATP (162, 163). There is evidence that they are present not only on the cell membrane but also on the mitochondrial membrane; however, this evidence is pharmacological since, to the best of my knowledge, mitochondrial K$_{ATP}$ channels are yet to be cloned.
$K_{ATP}$ channels belong to the same family of the inwardly rectifying potassium channels ($K_{IR}$) channels and they have an octameric structure made up of 4 $K_{IR}$ subunits, each of them associated with a sulphonylurea receptor (SUR) (164). Due to this association with the SUR, and the presence of multiple isoforms of SUR (SUR1, SUR2A, SUR2B), they present significant molecular diversity across species and tissue types and also different tissue density. Though there is some disagreement in the prevailing type (164) it is now thought that the combination of $K_{IR}$6.1/SUR2B is the most prevalent in VSM (165, 166).

$K_{ATP}$ channels serve as a link of the cellular metabolic status to the cellular electrophysiological activity, by participating on the regulation of the membrane potential. This function controls calcium entry into the cells, which in turn regulates various intracellular processes, through the Ca- calmodulin system.

The hallmark and key feature of $K_{ATP}$ channels is the effect that nucleotide phosphates have in their open state. As mentioned above, ATP even in micromolar concentrations, results in channel inhibition. On the other hand adenosine diphosphate (ADP) in the presence of Mg$^{++}$ represents a potent activator (167). It appears that the inhibitory effects of ATP result from binding to the $K_{IR}$ subunit, whereas the stimulation by Mg$^{++}$- ADP is effected through interaction with the SUR (164).

Another key feature of $K_{ATP}$ channels is the inhibition of channel activity by sulphonylurea agents (glibenclamide, gliclazide etc.). These agents appear to be highly selective for the $K_{ATP}$ channels with affinities of these drugs for the channel in the low nmol/L to low μmol/L range (168).
1.4.3. Roles of $K_{ATP}$ channels in VSM

$K_{ATP}$ channels play a central role in the regulation of VSM tone, as they interact with a large array of factors that modify this tone resulting in the regulation of blood flow into the different tissues, which in turn is pivotal in maintaining tissue homeostasis. A brief overview of these factors is given below.

A. Interaction with pharmacological vasodilators

Several studies have established the pharmacological vasodilatation caused by various substances such as cromakalim, pinacidil, diazoxide, nicorandil, etc. (168, 169). It has also been shown that a key feature on the action of these compounds is their ability to effect an increased conductance of $K^+$ in VSM and thereby by hyperpolarisation, exert an inhibitory influence on the vascular tone. The common targets of the above pharmacological vasodilators are the $K_{ATP}$ channels and it has been demonstrated that the open state probability of the channels in the presence of these substances are increased by 10- to 100-fold, depending on the concentration of ATP to which the cells are exposed (170). Another characteristic is that the actions of these compounds are blocked by known $K_{ATP}$ channel blockers as the sulphonylureas (e.g. glibenclamide), but not by blockers of other $K^+$ channels as for example charybdotoxin or tetraethylammonium (171). This hyperpolarisation of VSM has been demonstrated both in intact tissue (172) or in isolated cells (173).

However, it appears that there is some regional heterogeneity in the distribution of $K_{ATP}$ channels in some vascular beds, particularly in the cerebral circulation (174).
B. Interaction with endogenous vasoactive substances

There is large evidence showing the important role that the $K_{\text{ATP}}$ channels play in the mechanism of action of various endogenous vasodilators such as neurotransmitters, endothelial factors and cellular metabolites.

i) Neurotransmitters

Calcitonin gene-related peptide (CGRP) is a potent endogenous vasodilator that is being found in perivascular nerves in many tissues. It has been demonstrated that CGRP activates $K_{\text{ATP}}$ channels in various vascular beds (168, 175), and its effects are partially inhibited by glibenclamide, while the $K_{\text{Ca}^{++}}$ channel blocker charybdotoxin, has no significant effect on it. This indicates that the $K_{\text{ATP}}$ channel activation may only partially account for the induced vasodilatation. We now know that the mechanism involved consists of coupling of the CGRP receptor with adenylyl cyclase via G-proteins and increase of the intracellular cyclic adenosine monophosphate (cAMP), which results in phosphorylation of protein kinase A (PKA). This in turn phosphorylates the $K_{\text{ATP}}$ channels, thus effecting an outward current of potassium and membrane hyperpolarisation, resulting in vasodilatation (176). Similar action is via the cyclic guanosine monophosphate (cGMP) / protein kinase G (PKG) pathway.

Another potent vasodilator that is being found also in the perivascular nerves throughout the cardiovascular system is the vasoactive intestinal peptide (VIP). It appears that it has similar mechanism of action and comparable effects to those of CGRP, as evidenced by the inhibition of its effects by $K_{\text{ATP}}$ channel blockers (170).
ii) Endothelium-dependent vasodilators

It is believed that nitric oxide secreted by vascular endothelial cells relaxes VSM by more than one mechanism. Murphy and Brayden have shown that activation of $K_{ATP}$ channels by nitric oxide may play a role in this phenomenon, as they demonstrated that rabbit mesenteric arteries were hyperpolarised by nitric oxide via such $K_{ATP}$ channel activation (177). Their findings indicate that the predominant pathway in this experiment involves GMP and activation of PKG, but also that cross-activation of PKA might be involved.

Another endogenous vasodilator derived from endothelial cells, which appears to exert its action through the activation of $K_{ATP}$ channels, is prostacyclin. Jackson et al have conclusively shown that prostacyclin-induced vasodilatation in rabbit hearts is mediated by $K_{ATP}$ channels (178).

However endothelium-dependent relaxations cannot be fully explained by the release of either NO or and prostacyclin. Another unidentified factor(s), which hyperpolarizes the underlying vascular smooth muscle cells, may contribute to endothelium-dependent relaxations, especially in small arteries. One of these factors has been termed endothelium-derived hyperpolarizing factor (EDHF), which is distinct from NO and prostacyclin and it appears to involve activation of $K_{ATP}$ channels in its signal transduction pathway in order to cause vasodilatation (179). Nevertheless this pathway does not appear to be universally present in all species (180).

iii) Adenosine

Adenosine is an endogenous nucleoside present in all cells in the body. It has long been implicated as an important vasodilator in the coronary circulation. Through
experiments involving the use of glibenclamide to attenuate the effects of adenosine on the canine cardiovascular system, Belloni et al have shown that its mechanism of action involves the activation of $K_{\text{ATP}}$ channels (181). Similar activity has been demonstrated in models involving other species (182).

C. **Interactions with vasoconstrictors**

There is mounting evidence that many vasoconstrictor compounds that either have been endogenously isolated or artificially produced, may act by inhibition of $K_{\text{ATP}}$ channels and membrane depolarisation of the VSM cell. In this context, Park and co-workers have recently shown that endothelin-1 acts via protein kinase C to inhibit $K_{\text{ATP}}$ channels in rabbit coronary and pulmonary arterial smooth muscle cells, thus inducing vasoconstriction (183). Similarly, patch clamp techniques were used to demonstrate the effect of vasopressin on the sarcolemmal $K_{\text{ATP}}$ channels, in isolated guinea pig ventricular myocytes (184). It was found that vasopressin inhibited $K_{\text{ATP}}$ channels in a concentration-dependent manner, a phenomenon that may occur via a V1 receptor-related mechanism (184). Finally, Professor Standen's group have conclusively demonstrated that angiotensin II causes vasoconstriction by blockade of $K_{\text{ATP}}$ channels involving the inhibition of PKA and by activation of PKC (185).

Figure 1.6 represents a graphic summary of the factors that appear to be involved in the regulation of VSM tone through interaction with $K_{\text{ATP}}$ channels.
Figure 1.6: Summary of factors influencing the vascular tone through interactions with $K_{ATP}$ channels in VSM. (Modified from: Brayden, JE (186)). For abbreviations see index.
D. Role in the control of resting vascular tone.

During the past decade many researchers have focused their attention on the putative role that the $K_{ATP}$ channels may play on the control of the resting vascular tone. There is now significant amount of evidence indicating that the $K_{ATP}$ channels play a pivotal role in such a control in various vascular beds. Importantly, it appears that $K_{ATP}$ channels are central in the regulation of resting vascular tone in the coronary circulation (187, 188). Both in vitro (188) and in vivo (189) models have concluded that the infusion of glibenclamide greatly increases the coronary vascular resistance, a phenomenon that is reversed when the $K_{ATP}$ channel blocker in question is removed. Other vascular beds' resting VSM tone have also been shown to be influenced by tonically activated $K_{ATP}$ channels (190, 191) with potentially the most clinically relevant being the one from Sheridan and co-workers, implicating the involvement of these channels in the modulation of the vascular tone in pulmonary arterioles (192).

1.4.4. Involvement of $K_{ATP}$ channels in pathophysiology

Several pathophysiological states appear to involve the activation of $K_{ATP}$ channels. Attention has been drawn into hypoxia, acidosis, ischaemia/reperfusion and ischaemic cardiac preconditioning, and septic shock.

A. Hypoxia

There is now sufficient evidence to suggest that during hypoxia many vascular beds, including coronary, cerebral and skeletal muscle, dilate and that $K_{ATP}$ channels mediate this response. Conceivably, this response would play a protective role for the hypoxic tissues and this was concluded recently by Ballanyi in an extensive review
(193) of the mechanisms that potentially convey protection during states of brain hypoxia. Similarly, Cameron and co-workers demonstrated that the activation of $K_{\text{ATP}}$ channels in cardiac VSM results in vasodilatation, a response that may serve a cardioprotective role by helping to conserve ATP or by reducing intracellular Ca$^{2+}$ accumulation (194).

B. Acidosis

$K_{\text{ATP}}$ channels also play an important role in the pathophysiology of states associated with increased tissue $[\text{H}^+]$ i.e. acidosis. Several investigators have concluded that $K_{\text{ATP}}$ channels are very sensitive to changes of tissue or cellular pH and that upon activation they produce vasodilatation, particularly in cerebral (195) and coronary (196) circulations and more recently in skeletal muscle arterioles (197). Importantly, it still remains unclear if this phenomenon is mediated by an endothelium-dependent (195) or endothelium–independent pathway.

C. Ischaemia/ reperfusion and ischaemic preconditioning.

During ischaemia/reperfusion there is Ca$^{2+}$ influx in the cytosol of VSM, which play a detrimental role in recovery of cellular homeostasis (198). This fact along with the activation of neutrophils and the oxygen free radical release in the ischaemic/reperfused tissues, accentuate the associated tissue injury (199). The physiological response to reduce the adverse effects of these phenomena is the reactive hyperaemia observed, which is associated with $K_{\text{ATP}}$ channel activation (200, 201).

To that end it has been observed that brief episodes of myocardial ischaemia followed each time by reperfusion, leads to cardioprotection after further more prolonged
episodes of ischaemia (202). This phenomenon termed ischaemic preconditioning is conveyed by the $K_{ATP}$ channels (203), and we now have evidence that it is not only a exclusive characteristic of the myocardium but of other tissues as well (204).

D. Septic shock

The profound hypotension observed in septic shock, which is reactive to vasopressor agents, has been attributed to activation of $K_{ATP}$ channels (205). In a study by Landry and Oliver the inhibition of $K_{ATP}$ channels by glibenclamide, was able to cause vasoconstriction and restore the blood pressure to normal levels (206).
1.5. **Aim of thesis**

In this thesis I will investigate:

i) The myogenic properties of the different conduits that are predominantly used as bypass grafts in coronary surgery, with an emphasis on how these properties may be modified by clinical conditions such as left ventricular dysfunction, unstable angina, diabetes mellitus, or obesity, as well as various medications.

ii) The possible factors that may be involved in the modulation of the elastic properties of these conduits.

iii) The contributions of the $K_{ATP}$ channels (both plasmalemmal and mitochondrial) in the regulation of the vascular tone of these conduits.

The key aim is to acquire greater insight into the properties of these conduits, with regards to their modulation, with the long-term goal of increasing conduit longevity.
Chapter 2

Methods
2.1. INTRODUCTION

2.1.1. Techniques of studying blood vessels

The expansion of coronary artery bypass surgery in the 1980's led to the development of a growing interest in examining the bypass graft conduits' characteristics under different conditions, in order to potentially refine the techniques used in the whole perioperative spectrum and thus improve the conduits' longevity. In their endeavours to better understand the properties and regulation of the vessels used as bypass grafts, clinicians and scientists developed different methods and models of testing them that can be broadly divided into two major categories: in vivo and in vitro. Both techniques have advantages and disadvantages in terms of producing an accurate prediction of how the human bypass conduits will behave once grafted.

In vivo techniques have the advantage that they test more integrated vascular beds and they can draw conclusions more directly about the stimulus-response mechanism. However, these techniques carry the inherent disadvantage of not being certain whether the response chosen for measurement truly represents the direct effect of the stimulus or is a combination of direct effects plus the system's response to drug perturbation, which raises concerns on the design of the study, the interpretation of the results and perhaps the need for further experimentation for validation (207).

On the other hand, in vitro experimental preparations have the advantage of testing isolated vessels or vascular beds, thus minimizing the potentially complex interactions and confounding influences, which may occur in vivo and also being more easy to reproduce (208). Furthermore, isolated blood vessel bioassays allow for highly controlled experimental designs maximizing analytical power. Nevertheless the problem with in vitro models is that their conclusions have to be extrapolated to the
more integrated (intact) system of a vascular bed and ultimately, the human circulation.

In the literature I studied in preparation of this work, I noted that the vessels being studied were tested in pharmacological isometric tension models, essentially using the vessels as bioassays (209) and thus disregarding the variability resulting from the donor's clinical condition and/or medications. However, I did not come across a model that would attempt to directly test the myogenic properties of the vessels and the way they are affected by size (75), age (210) and the variety of the patient's clinical conditions (58, 211-213). Similarly the elastic properties of coronary vessels in human were not studied in such a setting and the suggestions from the literature were that there is probably a global alteration of these properties in various disease states (151, 214, 215). Finally I identified an increasing interest amongst researchers in the role that the \( \text{K}_{\text{ATP}} \) channels may play in the regulation of the vascular smooth muscle tone (187, 216) in general and thus I decided to investigate these further.

2.2. METHODS

2.2.1. Patient selection

The studies forming part of this thesis were designed and conducted according to the principles and standards set forth by the Helsinki declaration (217) and they were approved by the local ethics committee (ref. 6643) and the University Hospitals of Leicester Research and Development board (ref. 7613). The conduits described in this thesis are internal thoracic arteries (ITA), radial arteries (RA) and long saphenous vein grafts (LSVG). They were obtained from randomly selected patients undergoing elective, primary coronary artery bypass grafting surgery. Patients known to be
taking the known $K_{ATP}$ channel opener Nicorandil were excluded from the relevant studies. After personally counselling each patient, informed written consent was obtained and the patient was supplied with his or her individual patient information leaflet (see appendix 1).

2.2.2 Experimental preparation

The donated conduits were obtained during the operative procedure, immediately after harvesting and having secured that the sufficient conduit length for grafting was achieved. More specifically, ITA were obtained just before its bifurcation at the lower end of the sternum, they were tested for free flow to exclude the possibility of spasm and a average length of 1 cm was donated at each time providing that the remaining conduit would be under no tension when grafted to the native coronary vessel. Similarly the RA was obtained just at the level of the radial styloid process and also 1 cm length was donated after the graft length was judged to be sufficient.

The LSVG were obtained at the upper third of the tibia, just before the Boyd’s perforating vein, as it has been noted that proximally to that level the vessel diameter frequently changes abruptly. Care was taken for the donation to take place with minimal manipulation and without the vein being distended.

The conduits were immediately placed in cold (4° Celsius), oxygenated, HEPES buffered, Krebs-Henseleit solution and transferred to the laboratory in the immediate vicinity of the operating theatre. The Krebs-Henseleit solution used had the following composition (in mmol/l): NaCl (118), KCl (4.8), NaHCO$_3$ (27.2), KH$_2$PO$_4$ (1), MgCl$_2$ (1.2), CaCl$_2$ (1.25), D-Glucose (10), and HEPES (20). It was constituted fresh in the morning of each experiment with the use of de-ionized distilled water and during its preparation it was bubbled with a mixture of gases containing 95% O$_2$ and 5% CO$_2$.
(v/v) and kept at a temperature of 37° Celsius with a pH of 7.4. It was then placed in sterile containers, which during transit time were kept in a Styrofoam box, containing ice.

Once the conduits were taken to the laboratory, they were processed while being immersed in the above physiological solution and with the use of magnifying surgical loupes all loose connective tissue was removed from the vessel. Following this the vessels were cut in rings with the use of a fixed double-blade surgical scalpel providing vascular rings with an average length of 4 mm. Depending on the experimental protocol followed, the vascular endothelium was left intact or removed by gently rubbing the intima with a fine wooden stick. Then the rings were immediately placed in the organ bath for study.

The tissue organ bath (Fig. 2.1) is a device that was designed on the principle of creating a basic physiological environment in order for isolated contractile tissue to be tested. It comprises a 25 ml glass chamber (Linton Glass Company Inc., Indiana, USA) whose walls are part of a closed circuit (Fig. 2.2) where fluid (commonly water) is circulated through a pump/heat exchanger, thus allowing for control of the chamber’s temperature. Appropriate connections at the bottom of the chamber allow for the timely renewal of the chamber’s bathing solution as well as its aeration with the gas mixture mentioned above (95% O₂: 5% CO₂). Within the chamber there are arranged two 0.5 mm stainless steel wires: one fixed at the bottom of the chamber and one at the top, attached on a force transducer (Grass FT03C, Grass Instruments, MA, USA) and a micrometer (Mitutoyo Asia Pacific Pte Ltd, Singapore). It is obvious (Fig. 2.2) that by increasing the distance between the two wires, a certain amount of force will be generated and will be measured by the force transducer, which is
connected with a recording computer with the appropriate software (PowerLab 4/30 4-Channel Data Acquisition System, ADInstruments, USA).

Accepting that the distance between the two wires represents the internal diameter of the blood vessel and measuring the force produced, we are able to calculate various parameters such as wall tension, corresponding pressure, stress, strain and iEmod (see below and in appendix 2).

The vessel ring was suspended between the two wires, bathed in Krebs-Henseleit solution at a temperature of 37° Celsius and pH of 7.4 while the solution was aerated with the afore mentioned gaseous mixture, and it was allowed to equilibrate for one half hour before each experimental protocol was started.

Figure 2.3 represents an archetypal graphic depiction of the force-time curve from the organ bath during the vessel graded distention procedure.

More specifically, for the studies described in chapters 3, 4 & 5, after a number of pilot experiments to validate the experimental setting, the above-mentioned protocol was used without any addition of any pharmacological agents in the bathing solution. Simultaneously the parameters relating to the patients' clinical profile were extracted from the patients' notes.

Furthermore, in chapters 4 and 5, the experiments were conducted by allowing for the maximum wall force to reach 25g and the procedure was repeated 4 times for each vessel ring. The internal circumference and the transmural pressure were also calculated at each step, according to the method described by Angus and Wright (208) and shown in appendix 2. Stress-strain relationships and pressure-diameter relationships were determined for each set of measurements using exponential and 3rd order polynomial regression analysis (58, 214), respectively. In preliminary studies in our laboratory it was demonstrated that the first length-tension curve differed
significantly from the 2nd, 3rd and 4th measurement. Because of this, the first measurement was considered as an accommodation of the vessel to the experimental conditions and, therefore, excluded. In particular, in chapter 4, the compliance, distensibility and incremental elastic modulus (iEmod) were calculated for transmural pressures of 40 and 80mmHg for arteries and at pressure 40mmHg for veins. In chapter 5, the first derivative of the stress-strain curve was used to calculate the incremental elastic modulus (iEmod) at a calculated transmural pressure of 80mmHg. In chapter 6, after exactly the same preparation and equilibration period, and having established their baseline length-tension characteristics, the vessels were tested in an isometric tension model in which they were exposed to incremental concentrations of HMR1098 (plasmalemmal K$_{ATP}$ channel blocker), P1075 (plasmalemmal K$_{ATP}$ channel opener), 5-hydroxy-decanoate (5-HD, mitochondrial K$_{ATP}$ channel blocker) and Diazoxide (mitochondrial K$_{ATP}$ channel opener) every 7.5 minutes. Thus the effective concentration 50% (EC50) for each reagent was established. Following this, twin vessel rings (with and without endothelium) were subjected to the same process of constructing the length tension curves in the presence of a single optimal dose for each of the reagents that the maximum response was achieved in the preliminary set of experiments.

More detailed report about the specific experimental protocols followed will be given on each chapter describing the respective experiment.

2.2.3. Statistical analysis

Statistical analysis was performed with SPSS version 10 and Mathcad version 10. The distribution of each continuous variable was analysed with Kolgomorov-Smirnof test. Relationships between two variables were analysed with linear regression
analysis. Multivariate analysis was performed with multiple linear regression. Results are expressed as mean +/- standard deviation or standard error. Differences were considered significant at a probability level less than 0.05.
Figure 2.1: The tissue organ bath
Figure 2.2: The organ bath detail (from ref. (208)).
Figure 2.3. Organ bath curve during the graded distention of a vessel. Software incompatibility did not allow for a true curve to be recorded.
Chapter 3

Effect of clinical conditions on the size and myogenic properties of bypass graft conduits
3.1. INTRODUCTION

In Chapter 1 I described the pivotal role of the myogenic response in the autoregulation of the VSM. There is now abundant evidence that VSM reacts upon stimulation by changes in biomechanical stresses (flow, pressure, wall tension) and this reaction constitutes the myogenic response and comprises two major components, the basal vascular myogenic tone and the myogenic responsiveness (218).

Myogenic tone represents the underlying contractile status of the vessel at any level of biomechanical stress and it is a determinant of its diameter. Myogenic responsiveness represents the ability of the vessel to contract more vigorously at a particular range of intraluminal pressures and/or flows. The latter is thought to contribute to the ability of a vessel to maintain a relatively constant flow through an organ in spite of fluctuations in pressure (myogenic component of autoregulation). The myogenic response is inherent to smooth muscle and is expressed even in the absence of neural, metabolic, and hormonal influences (219). Extensive ongoing research on the subject provides insights on the mechanisms involved and suggests models of elucidating the process in various vascular beds (220, 221).

The established models to study the myogenic response invariably comprise the isolated in vivo or in vitro blood vessels or vascular beds, with the concomitant application of various vasoreactive agents. Thus the myogenic responsiveness has been studied on in vitro animal experimental preparations using cerebral (222), renal (223), coronary (224) and mesenteric arteries (225), as well as arterioles from various vascular beds (26).

However, the vessels used in these studies have been mostly studied in pharmacological isometric tension models, where the vessels were used as bioassays,
disregarding the variability resulting from the donor's clinical condition and/or medications. I hypothesized that an in vitro study of the myogenic response in the absence of vasoactive agents may be a reflection of structural and/or biochemical changes of the vessel. To that end and based on Angus and Wright (208) as well as other investigators (58), I have developed with the help of my supervisors, an organ bath model in order to describe the myogenic properties of human coronary conduits and their relationships to clinical variables.

3.2. METHODS

3.2.1. Study design

The study design, selection and consent of patients and general experimental setting have been described in chapter 2. A more detailed account of this particular protocol follows.

3.2.2. Experimental preparation

The vessel rings were mounted in the organ bath wires according to the principles described also in Chapter 2, and were allowed to equilibrate unstretched for 30 minutes before undergoing a graded passive distension procedure. The two wires were brought up to touch (distance between the two wires=0) and the micrometer was zeroed. The wires were then moved apart stepwise every minute, while the wall force and the micrometer reading were recorded at each step. The maximum wall force allowed was 25g and the procedure was repeated 4 times for each vessel ring. The internal circumference and the transmural pressure were also calculated at each step, according to the method described by Angus and Wright (208) and shown in appendix
2. Pressure-diameter relationships were determined for each measurement using a third order polynomial regression analysis. The diameters and the myogenic characteristics differed significantly between the first and the three subsequent measurements. Following this observation, the first measurement was considered as an accommodation of the vessel to the experimental conditions and therefore, excluded. The results obtained from each vessel were the mean of the following three measurements.

The diameter at pressures of 40, 80 and 120mmHg were calculated. The area under the pressure-diameter curve between pressures 40 and 80mmHg (AUC\textsubscript{40-80mmHg}) was used as an index for the vessel's internal diameter. The first derivative of the pressure-diameter relationship was used to express the vessels' myogenic responsiveness (myogenic index). The peak myogenic index (Mi), that is inversely related to the maximum strength of myogenic contraction (myogenic responsiveness) and the corresponding trans-mural pressure at peak myogenic index (Mi) were calculated from the first and second derivatives of the pressure-diameter third order polynomial.

Vessel rings employed in the present study were >1.5mm in diameter, underwent minimal manipulation and were carefully handled by surgeons with significant experience. This resulted in consistent preservation of the endothelium, unlike the preparations of very small vessels and arterioles. The integrity of the endothelium in the studied rings was verified at the end of each experiment by pre-constricting the vessels with noradrenaline and observing the endothelium dependent vasodilatation with the application of acetylcholine.
Figure 3.1: graph illustrating a typical pressure-diameter relationship for an individual vessel.
3.2.3. **Statistical analysis**

Statistical analysis was performed with SPSS version 10 and Mathcad version 10. The distribution of each continuous variable was analysed with Kolgomorov-Smirnoff test. Relationships between two variables were analysed with either Student’s t-test or simple linear regression, as appropriate. Multivariate analysis was performed with stepwise forward multiple linear regression. Results are expressed as mean +/- standard deviation. Differences were considered significant at a probability level less than 0.05.

3.3. **RESULTS**

3.3.1. **Patient demographics.**

The demographics and clinical characteristics of the studied population were as following: age 63 +/- 9 years, male gender 74%, body weight (BW) 87 +/- 19 kg, unstable angina 53%, history of myocardial infarction 46%, diabetes mellitus 14%, hypertension 69%, history of smoking 72%, hyperlipidaemia 89%, left main stem disease 32% and reduced left ventricular ejection fraction (LV-EF<50%) 47%. Medications included statines 93%, beta-blockers 73%, angiotensin converting enzyme inhibitors 32%, K$_{ATP}$ channel openers 34%, calcium channel antagonists 58%, nitrates 60% and diuretics 26%.

3.3.2. **Internal diameters and their relation to clinical variables**

The internal diameters of the ITA, RA and LSVG at pressures of 40, 80 and 120 mmHg are presented in Figure 3.2. This shows that LSVG and RA exhibited larger
internal diameters than ITA at all pressures, and that LSVG internal diameters were larger than those of RA at pressures of 40mmHg and 80mmHg.

The univariate and multivariate analyses of the relationships between the vessel internal diameters and the clinical variables, presented in Tables 3/I and 3/II respectively, showed that ITA-AUC_{40-80mmHg} was related to the body weight, the presence of previous acute myocardial infarction(s) and the LV-EF. Since there was a significant association between history of myocardial infarction and LV-EF, these two variables were replaced by a combined variable for the purpose of multivariate analysis. Furthermore, patients with history of myocardial infarction and low LV-EF were often treated with ACE inhibitors, dictating the inclusion of this type of treatment in the multivariate analysis. This analysis revealed that a LV-EF<50% and history of myocardial infarction were strongly associated with larger ITA internal diameters and this association was independent from ACE inhibitors and body weight (Table 3/II). RA-AUC_{40-80mmHg} was inversely related to the presence of unstable angina and this association was also independent from body weight. LSVG-AUC_{40-80mmHg} was directly related to age and severely depressed LV-EF (<30%), and inversely related to unstable angina in univariate analysis (Table 3/I). Multivariate analysis confirmed that severely depressed LV-EF (<30%) was associated with larger LSVG internal diameters while unstable angina with smaller ones, associations that were independent from body weight (Table 3/II). Age, however, was not proved to be a strong predictive factor of the internal diameter of vessels.
Figure 3.2. Pressure-diameter relationships of ITA, RA and LSVG. The box-and-whisker plot (5th, 25th, 50th, 75th and 95th percentile) was calculated for the entire population of vessel rings. The pressure-diameter curves were calculated for vessel rings that presented myogenic response. (* = p<0.05 compared to ITA; ** = p<0.05 compared to RA)
<table>
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<td>No</td>
<td>10</td>
<td>8.7 +/- 1.3</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15</td>
<td>7.5 +/- 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td></td>
<td>0.01 +/- 0.1</td>
<td>0.63</td>
<td>0.01</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>7.5 +/- 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSVG AUC 40-80mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable angina</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>10.6 +/- 1.9</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>9.2 +/- 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV-EF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30%</td>
<td>34</td>
<td>9.7 +/- 1.8</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>&lt;30%</td>
<td>3</td>
<td>13.3 +/- 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td></td>
<td>0.08 +/- 0.04</td>
<td>0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>4.5 +/- 2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coefficient</td>
<td></td>
<td>0.01 +/- 0.02</td>
<td>0.53</td>
<td>0.01</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>9.0 +/- 1.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ITA internal mammary artery, RA radial artery, LSVG long saphenous vein, AUC area under curve, MI myocardial infarction, LV-EF left ventricular ejection fraction, BW body weight, ACE angiotensin converting enzyme.
<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables</th>
<th>Mean+/− SD</th>
<th>p-value</th>
<th>R²</th>
<th>ANOVA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITA AUC 40-80mmHg</td>
<td>MI (+) and/or LV-EF&lt;50%</td>
<td>Coefficient 0.9+/−0.3</td>
<td>0.003</td>
<td>0.23</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>ACE inhibitors</td>
<td>Coefficient 0.2+/−0.3</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BW (kg)</td>
<td>Coefficient 0.02+/−0.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant 4.5+/−0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RA AUC 40-80mmHg</td>
<td>Unstable angina</td>
<td>Coefficient -1.3+/−0.5</td>
<td>0.01</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>BW (kg)</td>
<td>Coefficient -0.002+/−0.01</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant 8.9+/−1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSVG AUC 40-80mmHg</td>
<td>Unstable angina</td>
<td>Coefficient -1.5+/−0.6</td>
<td>0.02</td>
<td>0.28</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>LV-EF &lt;30%</td>
<td>Coefficient 1.3+/−0.5</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Age (years)</td>
<td>Coefficient 0.05+/−0.04</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BW (kg)</td>
<td>Coefficient -0.0008+/−0.02</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant 5.2+/−3.1</td>
<td></td>
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</tr>
</tbody>
</table>

ITA internal mammary artery, RA radial artery, LSVG long saphenous vein, AUC area under curve, MI myocardial infarction, LV-EF left ventricular ejection fraction, BW body weight, ACE angiotensin converting enzyme
3.3.3. **Myogenic responsiveness and its relation to clinical variables**

Myogenic responsiveness was demonstrated in 76.5% [88/115] of all vessels rings studied and there was no overall statistically significant difference between ITA, RA and LSVG (79.2%, 88% and 65%, respectively, p=0.09). The average pressure-diameter curves for ITA, RA and LSVG that presented myogenic activity are shown in Figure 3.1. Mi and pressure at Mi were calculated from the pressure-diameter curves for each individual vessel ring, and are presented in Figure 3.3. ITA myogenic responsiveness (inversely related to Mi) was significantly higher than RA and LSVG, while there was no significant difference between RA and LSVG. There was a statistically significant difference in Mi between ITA, RA and LSVG.

Univariate and multivariate relationships of myogenic index with clinical variables are presented in Table 3/III. Univariate analysis showed that ITA myogenic responsiveness (inversely related to Mi) was related to age, gender and LVEF. Multivariate analysis revealed that female patients and LVEF<50% were associated with decreased myogenic responsiveness, and this relationship was independent from ACE inhibitors. Notably, age, gender and BW were inter-related (men were younger with larger BW, while women were older with smaller BW). RA of patients treated with K\textsubscript{ATP} channel openers had decreased myogenic responsiveness, whereas LSVG myogenic responsiveness was not related to any of the studied clinical variables.

Univariate and multivariate relationships of Mi with clinical variables are presented in Table 3/IV. In both univariate and multivariate analyses, ITA Mi was directly related to diabetes and body weight, and inversely related to treatment with diuretics. RA Mi was influenced only by treatment with ACE inhibitors, and again LSVG Mi was not related to any of the studied clinical variables.
Figure 3.3. The peak myogenic index and the pressure at peak myogenic index internal mammary artery (ITA), radial artery (RA) and long saphenous vein (LSVG). Peak myogenic index is inversely related to maximum myogenic contraction (myogenic responsiveness). Values were calculated only for vessel that presented myogenic response.
Table 3/III. Univariate analysis of the relationships between peak myogenic index and clinical variables

<table>
<thead>
<tr>
<th>ITA Mi</th>
<th>Univariate analysis</th>
<th>n</th>
<th>Mean+/−SD</th>
<th>p-value</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coefficient</td>
<td></td>
<td>0.0001+/−0.0001</td>
<td>0.04</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td></td>
<td>0.0107+/−0.0022</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BSA (m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coefficient</td>
<td></td>
<td>0.0037+/−0.0012</td>
<td>0.005</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td></td>
<td>−0.0015+/−0.0025</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gender Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td></td>
<td>0.0064+/−0.0022</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td></td>
<td>0.0045+/−0.0014</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LV-EF &gt;50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>0.0051+/−0.0018</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;50%</td>
<td></td>
<td>0.0067+/−0.0022</td>
<td></td>
<td></td>
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<td></td>
<td>ACE inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td></td>
<td>0.0055+/−0.0020</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td></td>
<td>0.0068+/−0.0023</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>RA Mi</th>
<th>Univariate analysis</th>
<th>n</th>
<th>Mean+/−SD</th>
<th>p-value</th>
<th>R²</th>
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<tbody>
<tr>
<td></td>
<td>K_ATP channel openers</td>
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</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td>0.0070+/−0.0026</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td></td>
<td>0.0116+/−0.0046</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ITA internal mammary artery, RA radial artery, LSVG long saphenous vein, Mi peak myogenic index, BSA body surface area, LV-EF left ventricular ejection fraction, ACEI angiotensin converting enzyme inhibitors

Note: In this analysis BSA was used instead of body weight, because it was more representative of the different features between men and women.

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Table 3/IV. Relationship between pressure at peak myogenic index and clinical variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
<th>n</th>
<th>Mean±/SD</th>
<th>p-value</th>
<th>R²</th>
<th>ANOVA p-value</th>
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</thead>
<tbody>
<tr>
<td><strong>ITA Mi (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>35</td>
<td>7</td>
<td>90.5±12.3</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>77.7±8.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics</td>
<td>27</td>
<td>14</td>
<td>84.8±12.2</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
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<td>94.4±11.5</td>
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<td>Yes</td>
<td>14</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BW (kg)</td>
<td>Coefficient</td>
<td>Constant</td>
<td>-0.18±0.09</td>
<td>0.05</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>104.6±8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RA Mi (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>No 19</td>
<td>3</td>
<td>74.5±9.3</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89.4±14.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ITA internal mammary artery, RA radial artery, Mi pressure at peak myogenic index, BW body weight, ACE angiotensin converting enzyme
3.4. DISCUSSION

This study has demonstrated that LSVG internal diameters are larger than RA internal diameters, and RA internal diameters larger than ITA internal diameters at a wide range of pressures. Laplace’s law clearly states that wall tension is the product of pressure and diameter, and therefore these data suggest that LSVG is probably subjected to higher wall tensions than RA, and similarly RA is probably subjected to higher wall tensions than ITA. Although, differences in the endothelial properties of coronary grafts (223, 226) have been implicated in differences in their long-term patency, high wall tensions may also be involved in the pathophysiological process and could prove to be a leading cause in the degenerative changes that are detrimental to the grafts’ longevity. In this connection, it has been reported that enclosing the LSVG in an external polyester support reduces the degree of intimal hyperplasia (227), a finding which further supports the latter thesis.

Three out of four vessels in the present study showed myogenic activity. Whilst myogenic responsiveness of RA and LSVG were similar, LSVG showed maximum responsiveness at lower pressures (66mmHg) than RA (75mmHg). Interestingly, ITA presented stronger myogenic responsiveness compared to RA and LSVG but this occurred at a higher pressure (88mmHg). These results demonstrate for the first time that RA and LSVG contract less vigorously to biomechanical stress than ITA, but their contraction occurs at lower pressures, particularly with regard to LSVG. The clinical significance of these observations in terms of autoregulation and long-term patency of the conduits remain to be elucidated. One may stipulate that this property of the ITA could possibly be one of the reasons of the improved longevity of the
vessel as its myogenic contraction at this level of blood pressure, allows perhaps a
more effective regulation of the wall tension and thus slows down the degenerative
process, that ultimately leads to graft failure.

During the past decade we have witnessed an increasing interest from researchers on
the influence that many clinical conditions may have on the development of the
myogenic properties of blood vessels. To that end, it was demonstrated that the small
arteries and arterioles of diabetic rats exhibited increased myogenic tone (58, 211),
and femoral arteries of uraemic rats presented augmented myogenic responsiveness
prior to developing structural changes (212). Age-related impairment of myogenic
properties has been observed in the mesenteric arteries of male mice (228) and in the
human posterior ciliary arteries (210). Notably, none of these studies took into
account the size of the studied subject, since most of them were conducted either in
animals of similar size, or in arterioles, which are thought to be independent from the
subjects' size. In the present study, ITA internal diameters were proportional to body
weight; however, the influence of body weight on the myogenic properties of ITA
was unexpected. Thus, ITA of heavy patients showed a reduced Mi, implying that in
these patients ITA tends to suffer pressure-related contraction at lower pressures.
Interestingly, ITA of diabetic patients showed a similar pattern. This finding in
diabetic patients is in line with the findings reported by Zimmerman and co-workers
(58), as cited above. Indeed, reduced nitric oxide activity has been reported in both
obesity and diabetes (229, 230) and there is evidence that nitric oxide modulates the
myogenic response in various vascular beds, therefore it is expected in these two
conditions for the myogenic response to be impaired. Interestingly, in our series of
experiments, LSVG and RA myogenic properties did not seem to be affected by the
patients' size. Perhaps a possible explanation of this finding could be that the effect of nitric oxide on the modulation of the myogenic activity is differentially applied in various vascular beds.

In the present study depressed LV contractility resulted in dilatation of LSVG and, unexpectedly, ITA. LSVG dilatation, especially in significantly impaired LV function, may be explained through the venous stasis and elevated venous pressures. In contrast, it is well established that heart failure is associated with arterial vasoconstriction due to endothelial dysfunction (231, 232). It would be reasonable to assume that treatment with ACE inhibitors may be the reason for the increased ITA internal diameters and reduced myogenic responses in patients with left ventricular dysfunction. Although a higher proportion of patients with LV impairment were treated with ACE inhibitors than those without (45.7% versus 18.4%, p=0.02), multivariate analysis showed that ACE inhibitors could not explain these differences (Tables II and III). This raises the question whether the systemic vascular remodelling is heterogeneous in ischaemic cardiomyopathy. This thesis is supported by Stassen et al (233), whom have shown that there is significant heterogeneity in the vascular reactivity five weeks after experimental myocardial infarction in rats, including hyper-reactivity in coronary vessels and hypo-reactivity in mesenteric resistance arteries. Atrial (234, 235) and brain (236) natriuretic peptides are also elevated in ischaemic cardiomyopathy and heart failure, and given the altered myogenic responses of blood vessels in these conditions, their role in the internal diameter and the myogenic properties in these patients may deserve further investigation.
Although Imaizumi et al (237) failed to demonstrate alterations in the reactivity of brachial artery in unstable angina, however the present study has shown that unstable angina may be associated with significantly reduced RA and LSVG internal diameters. The most likely mechanism for this may well be the long established systemic inflammatory reaction in unstable angina (238-240). Inflammatory cytokines, especially tumour necrosis factor-alpha, can impair endothelium-dependent reactions and the endothelium may lose its ability to respond to circulating hormones or autacoids (241). The vascular changes seen in unstable angina suggest that the underlying vasoconstrictive mechanism may be present long enough to result in persisting biochemical and/or structural changes in both arteries and veins. It is worth noting that the internal diameters and myogenic properties of the ITA were not affected by unstable angina, highlighting its unique properties among other graft conduits. These observations may have significant implications in the planning of coronary surgery and also in the early postoperative management of unstable patients.

In the present study three types of drugs seemed to affect the myogenic properties of vessels, by decreasing either the strength of myogenic responsiveness or the pressure threshold for the myogenic response. Thus, diuretics decreased the myogenic response of ITA and ACE inhibitors and the $K_{ATP}$ channel openers that of RA. Savage et al (209) have shown that ramipril may reduce the myogenic activity in the femoral artery of uraemic rats but this effect remains untested in humans. Neither calcium antagonists nor other drugs affected the overall internal diameter of the conduits investigated in the present study. These findings suggest that some preoperative medications may have profound effects on the ITA and RA, with implications for their autoregulatory responses. Certainly, double-blind placebo
control trials are required to fully elucidate the interaction of drugs with the myogenic properties of coronary bypass conduits.

3.5. CONCLUSIONS

In summary, ITA, RA and LSVG have distinctively different internal diameters and myogenic characteristics. The internal diameter and myogenic responses of RA lay almost halfway between ITA and LSVG. Reduced LVEF and history of myocardial infarction(s) may be associated with larger ITA and LSVG internal diameters and decreased ITA myogenic responsiveness, while unstable angina with smaller RA and LSVG (but not ITA) internal diameters. The myogenic responses of ITA may be augmented in obese and diabetic patients and alleviated by diuretics. ACE inhibitors and $K_{ATP}$ channel openers may weaken myogenic responses of the RA. Understanding the myogenic properties of coronary bypass conduits and their relationship to clinical conditions may be of great importance as it may lead to potential new strategies to prevent perioperative conduit-related complications and long-term graft failure and they deserve further investigation. It is also important to identify whether alterations in the myogenic properties of bypass conduits may be influenced by the development of arterial atherosclerosis and as a result I embarked in the next study to investigate the possible existence of such an association.
Chapter 4

Correlation of vascular stiffness and coronary artery disease
4.1. INTRODUCTION

Almost two decades ago, Stefanadis and co-workers postulated that the vasa vasorum of the ascending aorta originating from the coronary arteries, may be responsible for the altered aortic elastic properties seen in patients with IHD (151). Since then extensive research on the topic of aortic elasticity has resulted in establishing an association between stiffness of the aorta and coronary atherosclerotic disease (151, 242-244). Furthermore, vascular stiffness was shown to be an independent predictor for cardiovascular morbidity and mortality in patients with coronary disease, hypertension and diabetes (245-247).

However it appears that we may have a “cause or effect” dilemma on this occasion; it is not entirely clear whether vascular stiffness is a contributory factor to the development of atherosclerosis or a consequence of the atherosclerotic process. In a recent report Trip et al (248) have demonstrated that the occurrence of pseudoxanthoma elasticum, an inborn disorder of the elastic connective tissue, is associated with a fourfold higher risk for coronary disease, an observation that supports the theory that elasticity defects might be in fact a primary event in the pathogenesis of coronary atherosclerosis.

It is now well established that patients with left main stem (LMS) coronary disease have a worse prognosis, in terms of cardiovascular mortality and morbidity, than those with distal coronary disease, with 5-year mortality rate of 50% without surgery (245, 246). Based on the above thesis and also on the fact that in preliminary studies in our laboratory, peripheral arteries and veins from patients undergoing CABG, showed significant variability and a tendency to be stiffer in patients with advanced coronary disease, the working hypothesis in this study was that, if elasticity defect
play a causative role in coronary disease it may be more pronounced in disease of the LMS.

Therefore, I embarked on this study aiming to investigate the elastic properties of medium size arteries and veins in patients with ischaemic heart disease and to examine whether LMS coronary disease was associated with greater elasticity defects compared to non-LMS disease.

4.2. METHODS

4.2.1. Study design

The study design, selection and consent of patients and general experimental setting have been described in chapter 2. In this particular protocol twenty-four patients had LMS disease and 50 patients had distal coronary disease without involvement of the LMS. A more detailed account of this protocol follows.

4.2.2. Experimental preparation

After allowing for equilibration, the organ bath mounted vessel rings were subjected to a stepwise increase of the force applied, as previously described. The maximum wall force allowed was 25g and the procedure was repeated 4 times for each vessel ring. The internal circumference and the transmural pressure were also calculated at each step, according to the method described by Angus and Wright (208) and shown in appendix 2. Stress-strain relationships and pressure-diameter relationships were determined for each set of measurements using exponential and 3rd order polynomial regression analysis (58, 214), respectively. The results obtained from each vessel were the mean of the 2nd, 3rd and 4th measurement, in order to reduce the intra-
The average $R^2$ for the stress-strain relationships were 0.92±0.04, 0.92±0.04 and 0.91±0.05 for ITA, RA and LSV, respectively. The average $R^2$ for the pressure-diameter curves were 0.96±0.04, 0.94±0.04 and 0.92±0.05 for ITA, RA and LSV, respectively.

The compliance, distensibility and incremental elastic modulus (iEmod) were calculated for transmural pressures of 40 and 80mmHg for arteries and at pressure 40mmHg for veins. These pressures were chosen because they fell within the range of reliability of the organ-bath experimental setting. In very small forces, corresponding to pressures less than 30mmHg, the vessel may not be properly stretched and in very high forces, corresponding to pressures more than 90mmHg, the wires may bend and in both situations the circumference calculations may not be reliable. The latter was particularly obvious in large diameter vessels, like saphenous veins. Therefore, in saphenous veins the calculations were performed only for pressure 40 mmHg. The first derivative of the pressure-diameter relationship was used to calculate compliance and distensibility. The first derivative of the stress-strain curve was used to calculate the iEmod. Compliance and distensibility are directly related while iEmod is inversely related to elasticity.

4.2.3. Statistical analysis

Statistical analysis was performed with SPSS version 10 and Mathcad version 10. The Kolgomerov-Smirnoff test was used to examine the assumption that the data followed a normal distribution or not. Relationships between two variables were analysed with either Mann Whitney U-test or chi-square analysis with Yates’ correction, as appropriate. Multivariate analysis was performed with multiple linear
regression. Results are expressed as 25, 50 and 75 percentiles or mean +/- standard error. Differences were considered significant at a probability level less than 0.05.

4.3. RESULTS

The comparison of the clinical, arterial and vein elastic characteristics between patients with and without LMS coronary disease are presented in Table 4/1. Almost all patients suffered hyperlipidaemia (89%) and 93% were treated with statins. Preoperative medications did not significantly differ between the two groups. Notably, neither the research team nor the clinicians had any influence on the chronic medications prior to the study. Although there were no significant clinical differences, the elastic properties of the studied arteries and veins differed significantly between the two groups. Thus, ITA from patients with LMS disease presented significantly lower compliance (-17%) and distensibility (-18%) and significantly higher iEmod (19%) at 80 mmHg than ITA from patients without LMS coronary disease. There were no differences in ITA elastic properties at 40 mmHg of pressure. RA from patients with LMS presented higher iEmod (50%) at 40 mmHg than those without LMS, but failed to reach statistically significant difference at 80 mmHg. LSV from patients with LMS also had reduced compliance (-45%), reduced distensibility (-40%) and increased iEmod (34%) at 40 mmHg compared to patients without LMS. The individual levels for all the study vessels are presented in Figure 4.1.

Table 4/II shows a multiple regression analysis of the iEmod on clinical variables for ITA, RA and LSV. This analysis confirmed the above relationships between LMS disease and iEmod and provides evidence of a higher iEmod of RA at 80 mmHg,
which was not obvious in the univariate analysis. Furthermore, it revealed that additional factors might affect the vascular elasticity. Thus, stiffness was higher in ITA from patients with small body surface area and in RA from patients with reduced left ventricular ejection fraction. The iEmod of ITA was also increased when calcium antagonists were used.
Table 4/1 Comparison of clinical, arterial and vein elastic properties between patients with and without left main stem disease

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Analysis was performed with Mann Whitney-U test and Chi-square analysis with Yates' correction.
LMS left main stem coronary disease, ITA internal thoracic artery, RA radial artery, LSV long saphenous vein, BSA body surface area, LVEF left ventricular ejection fraction, MI myocardial infarction, ACE angiotensin converting enzyme, KATP potassium sensitive ATP channels, Ca calcium, B coefficient, SE standard error, ANOVA analysis of variance.
Figure 4.1. Elastic characteristics (in terms of iEmod) of all the tested vessels in various pressures
Table 4/II  Multivariate analysis on the predictors of arterial and vein incremental elastic modulus in patients with coronary artery disease

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<th>LSV</th>
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<td>SE</td>
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<td>0.06</td>
<td>0.43</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>0.79</td>
<td>1.39</td>
<td>0.57</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>-5.73</td>
<td>1.94</td>
<td>0.005*</td>
</tr>
<tr>
<td>LVEF&lt;50%</td>
<td>0.80</td>
<td>1.06</td>
<td>0.45</td>
</tr>
<tr>
<td>ANOVA</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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</tr>
</tbody>
</table>

Analysis was performed with forward stepwise multiple regression analysis. * Signifies statistical significant difference

ITA internal thoracic artery, RA radial artery, LSV long saphenous vein, BSA body surface area, LMS left main stem coronary disease, LVEF left ventricular ejection fraction, MI myocardial infarction, ACE angiotensin converting enzyme, KATP ATP sensitive potassium channels, Ca calcium, B coefficient, SE standard error, ANOVA analysis of variance
4.4. DISCUSSION

The present study has demonstrated for the first time that patients with LMS coronary disease suffer from higher stiffness in medium size extracardiac arteries and veins compared to patients with distal coronary disease. This finding supports the hypothesis that the loss of vascular elastic properties may have a bearing on either the distribution or the severity of coronary disease or both. In this regard, recent studies have shown an association between hyperlipidaemia and vascular stiffness (249, 250). It is also possible that changes in the vascular structure can make them more susceptible to develop atherosclerotic lesions, a thesis supported by studies showing a genetic predisposition for vascular stiffness and coronary disease (248, 251). An intriguing aspect of the present study was the increased stiffness in the saphenous veins of patients with LMS disease, showing that the defect is global and not limited to the arterial system. It has been reported that factors such as ageing (252, 253), hypertension (254) and heart failure (255) may affect the vein stiffness; however, in this particular study, multivariate analysis of these factors failed to reach statistical significance. Our finding that arteries and veins are stiffer in patients with LMS disease supports the concept that there is a global deficit in the elasticity of vessels in this group of patients.

In univariate analysis there was a marginal difference in age between the two groups, which, however, did not reach statistical significance (p=0.06). In order to exclude the influence of age in the variability of the elastic properties of extracardiac vessels, a multivariate model was used which showed that age was not a predictor of the observed variability.
Patients with LMS disease present earlier graft failure and have a worse long term survival following coronary surgery compared to patients with distal coronary disease. As early as 1989, Rowe et al (256) demonstrated that LMS disease was an independent risk factor for late mortality following coronary surgery. In spite of the extensive use of arterial grafts in the 1990s, LMS disease has continued to be an independent risk factor for late cardiac mortality and for the need for earlier repeat myocardial revascularization (257). The reason for a worse clinical outcome in patients with LMS is unclear; however, reduced elasticity of arteries and veins used as coronary grafts in these patients may be an explanation for the premature graft failure and cardiac deaths.

Our results that RA is stiffer in patients with left ventricular dysfunction contrast with the findings of Kaiser and co-workers (258), who demonstrated decreased iEmod in patients with heart failure. A possible explanation for this discrepancy is that their study was performed in vivo and in the presence of pharmacological agents, while the assessment of vascular elasticity in our study was carried out in vitro and without the use of drugs. In this connection it is worth noting that previous studies have demonstrated that acetylcholine and catecholamines may have an effect on vascular elasticity (255). Our observation that calcium antagonists decrease the elasticity of ITA is of difficult interpretation since the disparate effects of these medications on arterial compliance are well known (259-262). The significance of these findings remains to be elucidated.

A potential limitation of the present study is that vessels from normal subjects could not be obtained, to serve as controls, because of obvious ethical constraints. For that reason the hypothesis that patients without left main stem coronary disease may also have a degree of vascular elasticity deficit compared to normal individuals cannot be
excluded. It may also be argued that our use of an in vitro preparation removes the potential influence of circadian circulating hormones and local metabolites that may influence vascular elastic properties and in fact its use may be considered advantageous. It must be conceded that in vitro experiments may carry a degree of inaccuracy regarding the calculation of 'transmural-pressure' with a relative uncertainty on its correspondence to real arterial pressure, but in compensation they may be more accurate in the calculation of iEmod, since the stress-strain relationships are under direct control.

4.5. CONCLUSIONS

In conclusion, LMS disease is associated with significantly reduced vascular elasticity when compared to non-LMS coronary disease. This finding suggests that a defect in vascular elasticity may play a role in the development of LMS disease and can be the explanation for the less satisfactory long-term clinical results in this group of patients. These results indicate that pharmacological modifications of vascular elasticity might be a therapeutic target with significant prognostic implications.

Having established this relationship, I decided to investigate in more detail factors that may affect venous elasticity, in the next chapter.
Chapter 5

Factors affecting venous elasticity
5.1. INTRODUCTION

In the previous chapter I have elaborated on the factors that play a role in the limitation of vascular elasticity of the conduits most commonly used in CABG. I have concluded that there might be a congenital defect in the vascular elasticity that leads to stiffening of the conduits and, in the case of LMS disease, this might be more pronounced. This thesis is further supported by the recent observation that congenital elasticity disorders are associated with an increased risk of premature coronary artery disease (248).

Furthermore, it is established that pulse pressure is a reliable method to evaluate arterial stiffness, and the latter is an independent predictor of cardiovascular mortality and morbidity (215, 245). To that end, a number of recent studies have shown that pulse pressure assessment plays a pivotal role in quantifying central arterial stiffness and thus predicting the development of clinically relevant cardiovascular pathology (245, 263-265).

Recent reports in the literature alluded to the fact that there might be an association between atherosclerosis and venous pathology, based on the fact that control of factors predisposing to atherosclerosis, i.e. obesity and DM (266) and also hypercholesterolaemia (267), may reduce the incidence of venous thromboembolism. Nevertheless, recently a publication by Prandoni et al (268), reporting a higher incidence of carotid atherosclerosis in patients with spontaneous venous thrombosis, corroborates a strong link between the two pathologies. If this hypothesis is true then alterations in the elastic properties of the peripheral venous system might potentially be a cause of spontaneous venous thrombosis in patients with atherosclerosis. Therefore the confirmation of such a thesis would have serious implications for the
longevity of venous grafts in patients undergoing CABG. Thus, the aim of this study was to investigate whether there is a relationship between central arterial stiffness and the stiffness of LSVG in patients with coronary artery disease, undergoing coronary surgery. The stiffness of the ITA, a vessel usually devoid of atherosclerosis, was used for comparison.

5.2. METHODS

5.2.1. Study design

The study design, selection and consent of patients and general experimental setting have been described in chapter 2. A more detailed account of this particular protocol follows.

A number of segments of internal thoracic arteries (ITA, n=53) and long saphenous veins (LSVG, n=38) to be used as coronary grafts were obtained from 74 patients undergoing primary elective CABG.

Blood pressure values were based upon a single cuff pressure taken at baseline examination. PP was calculated as the difference between systolic and diastolic pressure as determined by standard sphygmomanometry at the time of screening. Body mass index (BMI) was calculated as body weight (kg) divided by the height to the power of two (m^2).

5.2.2. Experimental preparation

This is the same as described before in chapters 2 and 4. As mentioned before, the significant difference between the first length-tension curve and the 2nd, 3rd and 4th curves, led to the conclusion of first measurement to be considered as an
accommodation of the vessel to the experimental conditions and, therefore, excluded. The results obtained from each vessel were the mean of the following three measurements. The first derivative of the stress-strain curve was used to calculate the incremental elastic modulus (iEmod) at a calculated transmural pressure of 80mmHg.

5.2.3. Statistical analysis

Statistical analysis was performed with SPSS version 10 and Mathcad version 10. The distribution of each continuous variable was analysed with Kolgomorov-Smirnov test. Relationships between two variables were analysed with linear regression analysis. Multivariate analysis was performed with multiple linear regression. Results are expressed as mean +/- standard error. Differences were considered significant at a probability level less than 0.05.

5.3. RESULTS

The demographics and disease characteristics of the studied cohort are presented in Table 5/I. As seen in figure 1, venous stiffness progressively increased with age in a way that closely matched the progression of stiffness in the central arterial system (venous iEmod [mN/mm]=-14.7+0.65 x Age [years], p=0.003, R^2=0.22). Interestingly, the elasticity of ITA was not significantly affected by age (arterial iEmod [mN/mm]=17.8+0.10 x Age [years], p=0.18, R^2=0.03). Figure 2 clearly shows that in patients with clinical atherosclerosis the stiffness of veins, but not the stiffness of ITA, is linearly related to the central arterial stiffness (venous iEmod [mN/mm]=9.8+0.27 x pulse pressure [mmHg], p=0.02, R^2=0.14, arterial iEmod [mN/mm]=22.2+0.03 x pulse pressure [mmHg], p=0.50, R^2=0.009).
Multivariate analysis including factors that might play a role in venous pathology, like obesity (expressed as a function of BMI), DM and LV-EF, showed that only advanced age and central arterial stiffness predict changes in venous stiffness (Table 5/II).
### Table 5/I. Demographic and disease characteristics of the studied population

<table>
<thead>
<tr>
<th>Quartiles</th>
<th>50%</th>
<th>25%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.5</td>
<td>55.7</td>
<td>71.0</td>
</tr>
<tr>
<td>Male</td>
<td>74.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.99</td>
<td>1.83</td>
<td>2.07</td>
</tr>
<tr>
<td>LVEF&lt;50%</td>
<td>47.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable</td>
<td>58.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last MI none</td>
<td>54.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30 days</td>
<td>40.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 days</td>
<td>5.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>62.0</td>
<td>50.0</td>
<td>74.0</td>
</tr>
<tr>
<td>Smoking</td>
<td>71.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>68.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>13.5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b-blockers</td>
<td>73.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>32.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K\text{ATP} openers</td>
<td>34.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca antagonists</td>
<td>58.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrates</td>
<td>60.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis was performed with Mann Whitney-U test and Chi-square analysis with Yates' correction.

BSA body surface area, LVEF left ventricular ejection fraction, MI myocardial infarction, ACE angiotensin converting enzyme, K\text{ATP} potassium sensitive ATP channels, Ca calcium.
Figure 5.1. Relationship between age and central arterial, ITA and venous stiffness. Central arterial stiffness was expressed as pulse pressure. The stiffness of ITA, a medium size muscular artery, and LSV were expressed as incremental elastic modulus (iEmod) measured in vitro. Pulse pressure and LSV iEmod were linearly related to age ($y=3.63+0.94x$, $p<0.001$, $R^2=0.25$ and $y=-14.65+0.65x$, $p=0.003$, $R^2=0.22$, respectively). ITA iEmod, however, was not related to age ($y=17.78+0.10x$, $p=0.18$, $R^2=0.03$).

LSV: long saphenous vein, ITA: internal thoracic artery, PP: pulse pressure, iEmod80: incremental elastic modulus at transmural pressure 80mmHg
Figure 5.2. Relationship between central arterial and ITA and venous stiffness. Central arterial stiffness was expressed as pulse pressure. The stiffness of ITA, a medium size muscular artery, and LSV were expressed as incremental elastic modulus (iEmod) measured in vitro. LSV iEmod was linearly related to pulse pressure ($y=4.12+0.35 \times x$, $p=0.02, R^2=0.21$). ITA iEmod, however, was not related to pulse pressure ($y=24.31+0.01 \times x$, $p=0.88, R^2=0.001$).

LSV: long saphenous vein, ITA: internal thoracic artery, iEmod 80: incremental elastic modulus at transmural pressure 80mmHg
Table 5/II. Predictors of LSV-iEmod 80

Dependent Variable: LSV iEmod 80

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficients</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>0.35</td>
<td>0.12</td>
<td>0.007</td>
</tr>
<tr>
<td>LV-EF</td>
<td>-1.36</td>
<td>3.57</td>
<td>0.71</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-9.42</td>
<td>4.62</td>
<td>0.051</td>
</tr>
<tr>
<td>Body mass index (in kg/m2)</td>
<td>0.69</td>
<td>0.39</td>
<td>0.09</td>
</tr>
<tr>
<td>Constant</td>
<td>-13.78</td>
<td>15.62</td>
<td></td>
</tr>
</tbody>
</table>

$R^2$ adjusted: 0.16

ANOVA p-value: 0.05

LSV long saphenous vein, iEmod incremental elastic modulus at 80mmHg
LV-EF left ventricular ejection fraction, SE standard error
5.4. DISCUSSION

The present study has shown for the first time that the increase in venous stiffness in patients with clinical manifestations of atherosclerosis, parallels the increased stiffness in the central arterial system and has demonstrated that they are associated with aging alone. These results may suggest that increased venous stiffness is the cause for the recently reported spontaneous venous thrombosis in patients with atherosclerosis (268). It has been previously shown in a number of studies that the reduction in visco-elasticity of the peripheral venous system is age related and thought to be due to the chronic effect of elevated hydrostatic pressure (252, 253, 269), a thesis supported by the finding that the venous compliance in the lower extremities is increased after prolonged alleviation of the effect of gravity on the leg veins (270). However in our study, hypertension and heart dysfunction that result in venous hypertension and stasis, thus altering the properties of the vein walls, did not affect venous elasticity in either univariate or multivariate analyses. Therefore, if aging leads to elasticity defects in both arteries and veins, in atherosclerotic patients, it is reasonable to assume that those changes are the cause for observed greater incidence of venous thrombosis in this population.

It is worth noting that in spite of the increased stiffness in the central arterial system, the elastic properties of the internal thoracic artery remained unaffected. It has been previously shown that the ITA is free of atherosclerotic disease regardless of the patient's age (271).

Sims(272) showed that, whereas coronary arteries exhibit significant defects in the internal elastic lamina, the ITA does not. The reason for this selectivity is not fully understood and it deserves further investigation.
Previous reports implicated common risk factors in the pathogenesis of atherosclerosis and deep venous thrombosis. Thus obesity, diabetes mellitus (266) and hypercholesterolaemia (267) have been shown to be associated with both pathologies. However, in the present study obesity and diabetes mellitus were not found to significantly affect venous elasticity and hypercholesterolaemia was afflicting the vast majority of patients, thus its potential role in the decrease of venous elasticity could not be evaluated. I believe that to elucidate these issues larger studies may be required.

There were two major limitations in the present study. First, the lack of a control group (atherosclerosis-free and matched for age and gender): this would be difficult to overcome due to ethical constraints in obtaining vein specimens from normal individuals. Second, the index used for central arterial stiffness was PP, and because the assessment of PP depends on stroke volume and peak aortic flow, it could be reduced in patients with severe heart failure; however, none of the patients in this study had severe heart failure (LV-EF<30%) and the measured degree of heart dysfunction was unrelated to arterial stiffness. PP is easily obtained and has been widely used as an index of stiffness by most studies searching for a relationship between coronary atherosclerosis and central arterial stiffness (245, 263-265). Unfortunately alternative methods such as echocardiography have not yet been tested in large series.

5.5. CONCLUSIONS

In conclusion, these data suggest that patients with ischaemic heart disease and reduced arterial elasticity also exhibit increased venous stiffness, proportionate to the
patients' age. This fact may well be the cause for the greater incidence of venous thrombosis in this group of patients, reflecting also the higher incidence of failure of the venous grafts.

As I discussed in the introduction, there has been implication of a putative role of the $K_{\text{ATP}}$ channels in the modulation of the vascular myogenic response. To that end I decided this to be the next step in my investigation on the properties of bypass conduits.
Chapter 6

On the role of $K_{ATP}$ channels on the myogenic tone of vascular smooth muscle in humans
6.1. INTRODUCTION

The role of the adenosine triphosphate-sensitive potassium (K\textsubscript{ATP}) channels in the myogenic tone of vascular smooth muscle, in the absence of neurohumoral stimulation, has been uncertain. During the past decade many researchers focused their attention on the putative role that the K\textsubscript{ATP} channels may play on the control of the resting vascular tone. The suggestion by Komaru et al (65) that K\textsubscript{ATP} channels play a role in the autoregulatory responses of the coronary micro-vessels is in contradiction with the findings by Knot and Nelson (61), as they failed to show a significant effect of K\textsubscript{ATP} channel antagonists on the depolarisation of isolated small rabbit cerebral arteries at any pressure.

Nonetheless, there is now significant amount of evidence indicating that the K\textsubscript{ATP} channels play a pivotal role in such a control in various vascular beds. Importantly, it appears that K\textsubscript{ATP} channels are central in the regulation of resting vascular tone in the coronary circulation (187, 188). Both in vitro (188) and in vivo (189) models have concluded that the infusion of glibenclamide greatly increases the coronary vascular resistance, a phenomenon that is reversed when the K\textsubscript{ATP} channel blocker is removed. A very important study published recently by Broadhead et al, demonstrated very elegantly the central role of K\textsubscript{ATP} channels on the ischaemic preconditioning of human vascular endothelium (273).

The resting VSM tone in other vascular beds has been shown to be influenced by tonically activated K\textsubscript{ATP} channels (190, 191) with potentially the most clinically relevant study being the one from Sheridan and co-workers, implicating the involvement of these channels in the modulation of the vascular tone in pulmonary arterioles (192). Furthermore, Jackson and co-workers established that K\textsubscript{ATP} channels

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are involved in the mediation of the vasodilatory effect of endothelium derived prostacyclin (178). More recently, a study by Gozalov et al has clearly shown that \( K_{ATP} \) channels are involved in the regulation of the diameters of endocranial arteries in rats (274).

Recently it has been suggested that there is a differential role for plasmalemmal and mitochondrial \( K_{ATP} \) channels in cardiac myocytes (275), bringing about proposals from some investigators that there is also a differential role for plasmalemmal and mitochondrial \( K_{ATP} \) channels in vascular smooth muscle (276-278). The effect of plasmalemmal and mitochondrial \( K_{ATP} \) channel openers and blockers has not yet been fully evaluated in the human conduit arteries. I hypothesized that mitochondrial \( K_{ATP} \) channels may play a role in the control of the basal myogenic tone of vascular smooth muscle in human conduit arteries.

The aim of this study was to investigate the effect of certain plasmalemmal and mitochondrial \( K_{ATP} \) channel openers and blockers on the basal myogenic tone of human conduit arteries and their potential interaction with transmural pressures and endothelium.

6.2. METHODS

6.2.1. Study design

The study design, selection and consent of patients and general experimental setting have been described in chapter 2. In this particular study I recruited 63 patients whom I consented to obtain segments of ITA. I excluded patients suffering of DM. A more detailed account of this particular protocol follows.
6.2.2. Experimental preparation

After being allowed to equilibrate as described before, the vessels were subjected in a graded passive distension procedure in the standard manner established previously, in order to determine their baseline length-tension characteristics. Stress-strain relationships and pressure-diameter relationships were determined for each set of measurements using an exponential and a 3\textsuperscript{rd} order polynomial regression analysis (58, 214), respectively. As I had previously demonstrated, the first length-tension curve differed significantly from the 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} measurement (150). Because of this, the first measurement was considered as an accommodation of the vessel to the experimental conditions and, therefore, excluded. The 2\textsuperscript{nd} measurement of length-tension was considered as the baseline measurement. The average $R^2$ for the baseline stress-strain relationships was 0.92±0.04 and the average $R^2$ for the pressure-diameter curves was 0.96±0.04. The pressure-diameter relationship was used to calculate the distance between the wires corresponding to 90mmHg.

\textit{i)} Isometric tension model

The vessel was allowed to equilibrate for 30 minutes at a transmural pressure 90mmHg, under continuous monitoring of the wall tension. Following this period the vessel ring was reset to a baseline transmural pressure 90mmHg and consequently exposed to incremental concentrations of HMR1098 (plasmalemmal K\textsubscript{ATP} channel blocker), P1075 (plasmalemmal K\textsubscript{ATP} channel opener), 5-hydroxy-decanoate (5-HD, mitochondrial K\textsubscript{ATP} channel blocker) and Diazoxide (mitochondrial K\textsubscript{ATP} channel opener) every 7.5 minutes. Concentration-response curves were constructed for each agent and the effective concentration 50\% (EC50) and maximum response were calculated for each vessel. A single optimal dose for use in the isobaric model was
calculated from the cumulative concentration-response curves for each agent (Figure 1). Concentration-contraction curves were constructed by using a sigmoid function, shown in the appendix.

ii) Length-tension model

The methodology for the construction of the length-tension curves was similar to the methodology used for the baseline length-tension characteristics. The studies were conducted in twin vessel rings from the same vessel with and without endothelium, which was achieved (the endothelial removal) by gently rubbing a surgical thread in the lumen of the vessel ring. The successful removal of the endothelium was tested by evaluating the relaxing effect of acetylcholine ($3 \times 10^{-4}$M) after pre-constriction with noradrenaline ($10^{-5}$M) at the end of the experiment. Relaxation less than 30% was considered to be associated with endothelial disruption (279). Length-tension measurements were undertaken in the presence of a single optimal dose of each agent, based on the cumulative concentration response curves of the previously conducted isometric experiments. The concentration corresponded to the first dose which achieved maximum response and was $10^{-6}$M for HMR1098, $10^{-4}$M for P-1075, $3 \times 10^{-4}$M for 5-HD and $5 \times 10^{-4}$M for diazoxide. Stress-strain relationships and pressure-diameter relationships were determined for each set of measurements using an exponential and a 3rd order polynomial regression analysis, respectively. The first derivative of the stress-strain relationship was used to calculate the incremental elastic modulus at 80mmHg, before and after the application of agents. The area under the curve of the pressure-diameter relationship between 60mmHg and 100mmHg divided by 40 was used as an index for the cumulative change in the diameter of the vessel (symmetrically around 80mmHg). This method provides the average change in
diameter in this range of transmural pressures. This range of pressures was chosen because it fell within the range of reliability of the organ-bath experimental setting. In very small forces, corresponding to pressures less than 30mmHg, the vessel may not be properly stretched and in very high forces, corresponding to pressures more than 140mmHg, the wires may bend and in both situations the circumference calculations may not be reliable.

6.2.3. Statistical analysis

Statistical analysis was performed with SPSS version 10 and Mathcad version 10. Non-linear regression analysis was used to evaluate the best-fit curves for stress-strain, pressure-diameter and concentration-contraction relationships. The non-linear functions used in the non-linear regression analysis are shown in the Appendix. The statistical methods used for the analysis of the results were: 1. one sample T-test, 2. paired t-test, 3. one-way analysis of variance and 4. repeat measures analysis (general linear model). Results were expressed as mean +/- standard deviation (standard error). Differences were considered significant at a probability level 0.05.

6.3. RESULTS

6.3.1. Isometric model

The effective concentrations 50% (EC$_{50}$) and the maximum responses for the HMR1098, P1075, 5-HD and diazoxide, in the isometric tension studies, are presented in Table 6/I. With a baseline transmural pressure of 90mmHg, HMR1098 caused an increase in the myogenic tone, while the P1075 a decrease. Both 5-HD and diazoxide caused reduction of myogenic tone, which, however, failed to reach statistical
significance in this model. Figures 6.1a and 6.1b show the cumulative concentration-response curves for HMR1098, P1075, 5-HD and diazoxide in a comparable range of concentrations. These cumulative relationships were used to select a single optimal dose for the length-tension model. HMR1098 at concentrations above $10^{-4}$ mol/l almost completely reversed the contraction observed at lower concentrations.

6.3.2. Length-tension model

In the length-tension studies HMR1098 caused a reduction in the average diameter for a range of pressures between 60 and 100mmHg by $-4.8 +/- 2.3\%$ ($p=0.05$). Conversely, P1075 and diazoxide caused an increase by $6.7 +/- 1.8\%$ ($p=0.009$) and $9.2 +/- 3.8\%$ ($p=0.002$), respectively. 5-HD did not significantly affect the diameter in this model ($-1.9 +/- 3.0\%$, $p=0.20$). Endothelium-denuded vessels showed similar changes in diameters: $-2.0 +/- 1.3\%$ ($p=0.02$), $9.2 +/- 6.6\%$ ($p=0.003$), $6.8 +/- 2.8\%$ ($p=0.002$) and $-1.3 +/- 6.7\%$ ($p=0.24$) for HMR1098, P1075, diazoxide and 5-HD, respectively (Figure 6.2a). There was no significant difference in the diameter changes between endothelium intact and endothelium denuded vessels. Diazoxide was the only agent that caused significant changes in the incremental elastic modulus (Figure 6.2b).

6.3.3. Interaction between agents, transmural pressure and endothelium

The interaction between the transmural pressure, the endothelium and the three agents, that significantly influenced the myogenic tone, are shown in Figure 6.3. The changes caused by HMR1098 and diazoxide were affected by rising transmural pressures, while those of P1075 were not. The changes caused by HMR1098 were weaker above transmural pressures 70-90mmHg and those of diazoxide above 90-110mmHg. These differences were endothelium independent. Also, there was a
significant difference in the potency (magnitude of constriction or dilation) of these agents to modify myogenic tone at different pressures. At low pressures (30-50 mmHg) the potency of all three agents was similar in both endothelium-intact and endothelium-denuded vessels. At higher pressures (70-110 mmHg), however, the potency of diazoxide was higher than that of HMR1098 in both endothelium-intact and endothelium-denuded vessels. Furthermore, at higher pressures (70-110 mmHg) the potency of P1075 was similar to that of HMR1098 in endothelium-intact vessels. In endothelium-denuded vessels, however, the potency of P1075 was higher than that of HMR1098.
Effective concentrations and the maximum response of the distal internal mammary artery to plasmalemmal and mitochondrial $K_{\text{ATP}}$ channel openers and blockers

Table 6/I.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$n$</th>
<th>$\text{mean}$</th>
<th>SD</th>
<th>p-value</th>
<th>$\text{mean}$</th>
<th>SD</th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMR1098</td>
<td>10</td>
<td>21.2</td>
<td>27.0</td>
<td>0.035</td>
<td>-7.48</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>P-1075</td>
<td>7</td>
<td>-9.2</td>
<td>4.7</td>
<td>0.002</td>
<td>-5.18</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>5-HD</td>
<td>12</td>
<td>-7.3</td>
<td>20.6</td>
<td>0.245</td>
<td>-5.95</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Diazoxide</td>
<td>12</td>
<td>-8.0</td>
<td>18.5</td>
<td>0.162</td>
<td>-5.76</td>
<td>1.19</td>
<td></td>
</tr>
</tbody>
</table>

The statistical difference of the maximum response from the value 0 was tested by one-sample t-test.

$EC_{50}$: effective concentrations 50%, $R_{\text{max}}$: maximum response of the vascular ring expressed as percentage of the baseline.

* p-value<0.05 compared to the other compounds
Figure 6.1. Concentration-response curves for plasmalemmal and mitochondrial $K_{ATP}$ channels blockers and openers. The large dots show the concentrations selected for the length–tension model. HMR1098: plasmalemmal $K_{ATP}$ channel blocker, P1075: plasmalemmal $K_{ATP}$ channel opener, 5-HD: mitochondrial $K_{ATP}$ channel blocker, Diazoxide: mitochondrial $K_{ATP}$ channel opener.
Figure 6.2. The effect of plasmalemmal and mitochondrial K\textsubscript{ATP} channels blockers and openers on the diameter (a) and incremental elastic modulus (b) of internal thoracic arteries from a length-tension model. *: p<0.05 in rejecting the null-hypothesis by one-sample t-test.
Figure 6.3. The interaction between transmural pressure, endothelium and the three $K_{ATP}$ channel agents that influence the basal myogenic tone.

END: endothelium intact. DEN: endothelium denuded. * $p<0.05$ compared to the value at the previous pressure step. † $p<0.05$ compared to corresponding values of P1075 and diazoxide in endothelium-denuded vessels.
6.4. DISCUSSION

6.4.1. Consideration of methodology and utility of the model

Myogenic tone has been documented in arteries, arterioles, veins and lymphatic vessels, mainly in in-vitro studies, which eliminate confounding neural and metabolic factors (24, 26, 56). The commonest method to study the myogenic tone has been the isolated pressurized vessels (280, 281). This preparation has the advantage of maintaining normal geometry of the vascular wall and the disadvantage of the need to cannulate a vessel of considerable length. The latter makes the use of this model difficult in studying human vascular physiology, because the length of vascular specimens is usually restricted. An alternative method has been the isometric wire-mounted ring preparations (208, 282). In the present study two different methodologies were used to eliminate variability inherent to the experimental protocols. The isometric-tension model facilitated the concentration-response studies and the length-tension model made possible the study of the effect of different levels of wall-stress, in terms of tension and trans-mural pressure.

6.4.2. Plasmalemmal $K_{ATP}$ channels

In this study I have shown that plasmalemmal $K_{ATP}$ channels may play a role in the myogenic tone of human conduit arteries. Previous studies have shown a vasodilatory effect of $K_{ATP}$ channel openers in pre-contracted human internal mammary arteries; however, the role of plasmalemmal $K_{ATP}$ channels in the baseline myogenic tone of human conduit arteries has not been tested. In 1991 Komaru et al (65) showed that in ischaemic in-vivo conditions $K_{ATP}$ channels caused dilatation of coronary microvessels (less than 100 microns); however, they did not affect the myogenic tone.
of arterioles larger than 100 microns. In 1995 Knot and Nelson (61) conducted
electrophysiological studies on vascular smooth muscle of animal cerebral arteries
and concluded that the depolarisation caused by gradual intraluminal pressure
elevation was abolished by voltage-gated potassium channels and not by $K_{\text{ATP}}$
channels blockers. It was therefore concluded that $K_{\text{ATP}}$ channels did not play a role
in the myogenic tone of vascular smooth muscle. In the present study the vascular
rings where studied in two different models. In the isometric model the baseline
transmural pressure was set to 90 mmHg, while in the length-tension model the wall
tension was gradually elevated, covering a range of transmural pressures from
30mmHg to 140mmHg. Both models provided comparable results, showing that the
myogenic tone was decreased by opening the plasmalemmal $K_{\text{ATP}}$ channels with
P1075, and increased by closing them with HMR 1098. These results are in contrast
to the results of Knot and Nelson, and support the hypothesis that $K_{\text{ATP}}$ channels may
play a significant role in the myogenic tone of human vascular smooth muscle. If this
mechanism is independent from membrane depolarisation, this may strengthen the
hypothesis that basal myogenic tone is mediated by more complex mechanisms and
not exclusively by membrane depolarisation and calcium-calmodulin myosin light-
chain phosphorylation. In this respect, Osol et al (220) have suggested that calcium
sensitisation may play a role in the basal myogenic tone especially at high transmural
pressures.

In the present study, there were some noteworthy discrepancies regarding HMR1098
and P1075. If they simply influenced a specific pathway, their response should be
mirror image. However, HMR1098 appeared to affect the myogenic tone at much
lower concentrations than P1075 and, surprisingly, at higher doses it reversed the
initial constriction through an unclear mechanism (Figure 1a). The effect of P1075 was endothelium-independent and not influenced by differences in transmural pressures, while the effect of HMR1098 was influenced by differences in transmural pressures, showing less potency above 70-90mmHg. Also, there is some indirect evidence that the mechanism of HMR1098 was influenced by the presence of endothelium. Although there was no difference in the vasoconstrictive effect of HMR1098 between endothelium-intact and endothelium-denuded vessels (p-value=0.17), there was a difference between the potency of HMR1098 and P1075 in endothelium-denuded vessels for pressures 70-110mmHg (Figure 6.3). These observations may suggest that in human smooth muscle HMR1098 is not simply a plasmalemmal $K_{\text{ATP}}$ channel blocker, since its effect may be modulated by mechano-transduction pathways, which are known to include calcium influx via membrane depolarisation, PKC and rho-kinase pathways (70, 88, 283). Furthermore, it may have a different effect on the endothelium, explaining thus the reversal of the initial vasoconstriction at higher doses and the paradoxically reduced vasoconstrictor potency in endothelium-denuded vessels.

6.4.3. Mitochondrial $K_{\text{ATP}}$ channels

In 1997 Garlid and colleagues (284) and later Liu et al (275, 285) suggested that the cardioprotective effect of ischaemic preconditioning is mediated by mitochondrial $K_{\text{ATP}}$ channels, which are opened by diazoxide and blocked by 5-HD. Recently, Kopustinskiene et al (276) suggested that levosimedan is acting through mitochondrial and not plasmalemmal $K_{\text{ATP}}$ channels in the vascular smooth muscle. Another study in support of this thesis, by Holmuhamedov and co-workers, has shown that the mitochondrial $K_{\text{ATP}}$ channels are the effectors of the cellular protection
conferred by reduction of Ca\textsuperscript{2+} uptake by the mitochondria, thus reducing its deleterious effect on them \cite{286}. Furthermore, de Klaver et al \cite{277, 278} showed that 5-HD, but not HMR 1098, abolished the protective effect of isoflurane and lidocaine on human vascular smooth muscle cells exposed to cytokines. In addition, diazoxide mimicked the time course of isoflurane-induced protection in all cell lines. These observations support the hypothesis that, similarly to the cardiac myocytes, there is a differential role for plasmalemmal and mitochondrial K\textsubscript{ATP} channels in human vascular smooth muscle. In the present study, blocking the K\textsubscript{ATP} channels with 5-HD affected neither the myogenic tone nor the elasticity. Diazoxide, however, significantly reduced the myogenic tone in the length-tension model, although it did not affect the myogenic tone in the isometric-tension model. The explanation for this may be that in the isometric tension model the baseline transmural pressure was 90mmHg and this is exactly the level above which diazoxide shows less effect on the myogenic tone. The reduction of the elasticity caused by diazoxide may be due to the difference in the potency of this agent to alter the myogenic tone at different transmural pressures. Although HMR1098 also showed different potency at different transmural pressures, its effect especially at high pressures was considerable less than that of diazoxide, and this may explain why HMR1098 did not alter the elasticity. It is worth noting that in cardiac myocytes the concentration of diazoxide that causes intracellular oxidative stress by opening the mitochondrial K\textsubscript{ATP} channels is 100 \mu M \cite{287, 288}. In the length-tension experiments of the present study the concentration of diazoxide was 5 \times 100\mu M, which is considerably higher than that used for the cardiac myocytes. Whether this difference in concentrations play a role in the activation or de-activation of different pathways remains to be investigated.
6.4.4. **Limitations**

The major limitation of the present study lies with the assumption that mitochondrial \( \text{K}_{\text{ATP}} \) channels exist in smooth muscle cells. This assumption relied on indirect evidence provided by numerous studies(275-278, 284, 285). However, the problem lies with the fact that some of the pharmacological agents used to test this hypothesis, are actually also active against the plasmalemmal \( \text{K}_{\text{ATP}} \) channels (289) demonstrating a great variability in their specificity and sensitivity between tissues (166). Furthermore, the current consensus with regards to the presence of \( \text{K}^+ \) channels in the inner mitochondrial membrane points to the direction of the co-existence of at least three \( \text{K}^+ \) channels: \( \text{K}_{\text{Ca}^{++}}, \text{K}_V \) and \( \text{K}_{\text{ATP}} \) channels (289). The absence of the molecular characterisation of the mitochondrial \( \text{K}_{\text{ATP}} \) channels conveys the inherent difficulty of interpreting the actions of putative channel blockers and openers. The solution to this problem might be the advances made to the field of transgenic studies which may allow for the structure of the mitochondrial \( \text{K}_{\text{ATP}} \) channels to be identified (166).

6.5. **CONCLUSIONS**

Plasmalemmal \( \text{K}_{\text{ATP}} \) channels may play a role in the myogenic tone of vascular smooth muscle in human conduit arteries. Blocking the plasmalemmal \( \text{K}_{\text{ATP}} \) channels with HMR1098 may involve a complex mechanism, which is affected by mechano-transduction and, possibly, endothelium. Diazoxide relaxes the myogenic tone and increases the stiffness of the smooth muscle independently from endothelium, but at doses higher than those known to cause oxidative stress in cardiac myocytes. The effect of diazoxide is also affected by mechano-transduction. P1075 relaxes the myogenic tone in an endothelium- and mechanotransduction independent pathway,
and its potency is similar to that of diazoxide. 5-HD may not have an effect on the myogenic tone of vascular smooth muscle in human conduit arteries.
Chapter 7

Conclusions and future directions
7.1. CONCLUSIONS

The present studies have demonstrated that the myogenic response is a phenomenon and a property present in moderate size arteries and veins, which we commonly use as grafts during coronary surgery and plays a pivotal role in regulation of the ITA, RA and LSVG. The absence of myogenic responsiveness in 25% of the vessels I have tested, in my opinion should be attributed to perhaps a learning curve in handling the experimental preparation and possibly to factors that I failed to identify as relevant to the conducted study. I have also shown that the tissue organ bath, notwithstanding its limitations, can be a fairly reliable method to study the myogenic properties of these vessels.

More specifically, the first useful conclusion that I was able to draw from the first series of my experiments was that ITA, RA and LSVG have distinctively different internal diameters and myogenic characteristics. The internal diameter and myogenic responses of RA lay almost halfway between ITA and LSVG. This fact presumably reflects on the degree of wall tension the conduits are being subjected once they are grafted and perhaps this might be the caveat required to explain the differences in longevity of the conduits.

Furthermore in these series of experiments I was able to identify various clinical conditions that appear to have a bearing in formulating the myogenic behaviour of the studied vessels. To that end I was able to demonstrate that reduced LV-EF and history of myocardial infarction(s) may be associated with larger ITA and LSVG internal diameters and decreased ITA myogenic responsiveness, while unstable angina with smaller RA and LSVG (but not ITA) internal diameters. The myogenic responses of ITA may be augmented in obese and diabetic patients and alleviated by
diuretics. ACE inhibitors and $K_{\text{ATP}}$ channel openers appeared to have a weakening effect on the myogenic responses of the RA.

Understanding the myogenic properties of coronary bypass conduits and how these properties are affected from the various clinical conditions may be of great importance as it may lead to potential new strategies to prevent perioperative conduit-related complications and long-term graft failure and they deserve further investigation.

With regards the elastic properties of the conduits, I was able to demonstrate that LMS disease is associated with significantly reduced vascular elasticity when compared to non-LMS coronary disease and also that, patients with ischaemic heart disease and reduced arterial elasticity also exhibit increased venous stiffness, proportionate to the patients' age. I believe that these facts have a serious impact on the behaviour of the conduits and they can in part explain quite satisfactorily the pattern of failure of these vessels. My findings suggest that a defect in vascular elasticity may play a role in the development of LMS disease and can be the explanation for the less satisfactory long-term clinical results in this group of patients. I also concluded that the increased venous stiffness in elderly patients with ischaemic heart disease may well be the cause for the greater incidence of venous thrombosis in this group of patients, reflecting also the higher incidence of failure of the venous grafts. These results indicate that pharmacological modifications of vascular elasticity might be a therapeutic target with significant prognostic implications in the outcome of coronary surgery.

In the final part of my studies I demonstrated that the $K_{\text{ATP}}$ channels (both plasmalemmal and mitochondrial) appear to play a significant role in the modulation of the grafts' myogenic behaviour through rather complex mechanisms. My results
seem to indicate that the response of the VSM to the different channel openers and blockers is primarily endothelium independent and probably involves multifarious pathways in the signal transduction. In certain aspects of the plasmalemmal $K_{\text{ATP}}$ channels stimulation, the endothelium appears to play a modulatory role. I think that the elucidation of the role of the $K_{\text{ATP}}$ channels in the conformation of the vascular myogenic response, through a more refined, probably in vivo, model, warrants further consideration.

7.2. FUTURE DIRECTIONS

Despite the advances in our understanding of the vascular myogenic response, many questions remain to be answered and many controversies need to be resolved. Certainly, differences in species, tissue, vessel size, and method of study contribute to the variability in many of the results described above. However, it would seem unlikely that a phenomenon as basic as the myogenic response, present in almost every type of vessel, would utilize different signalling pathways in different types of vessels.

I believe that today there are still a number of questions awaiting for an answer with regards the complex mechanisms that form the behaviour of vascular smooth muscle. For example and with respect to mechanical properties of smooth muscle, is the myogenic behaviour of arterioles simply an extension of the same mechanical response to length changes seen in striated muscle? If so, this might diminish the importance of studies related to specific membrane-bound receptors and contractile proteins.
Furthermore and with respect to electromechanical coupling, is depolarisation sufficient to account for myogenic behaviour? The roles of a multitude of channels need to be clarified. This will require the development and careful testing of specific pharmacological antagonists for the respective channels, along with parallel or simultaneous measurements of electrophysiological and mechanical responses of arterioles. Also specific problems related to the experimental technique, either being an in vivo vascular bed or a single vessel or even a single VSM cell need to be overcome and evaluate the physiological relevance of each method.

The role of $K^+$ channels needs further clarification. I think that the function of the stretch-activated $K^+$ channels, identified using single-channel techniques, requires further elucidation. Furthermore the potential participation of the $K_{ATP}$ channels in the modulation of the vascular myogenic response warrants further investigation. Similarly, the exact role of AA metabolites in the generation and sustentation of the myogenic response needs to be investigated further.

With respect to second messenger pathways involved in arteriolar myogenic signalling, future studies must elucidate the degree of participation of each of them to the modulation of the myogenic response. Moreover, the way by which integrins transmit the forces associated with the triggering of myogenic behaviour and the exact role of the VSM cytoskeleton, are questions to be addressed. Specifically pertinent to this issue is the question of whether the cultured VSM (currently required to obtain sufficient quantities of protein for molecular assays) an adequate model of intact VSM. I believe that studies on mechanotransduction through the extracellular matrix-integrin-cytoskeleton axis are likely to be an important area of research over the next decade.
In this way I believe that it will be possible to determine which signalling mechanisms in VSM are fundamental to myogenic contraction and which represent parallel modulatory pathways. By doing so, the vascular myogenic response along with the - in my opinion- inseparable issue of vascular elasticity, will be understood much more conclusively. This understanding will allow us to treat alterations in these properties as part of the disease process in blood vessels.

In this context it may be possible to develop clinically applicable methods of in vivo direct measurement of changes in the myogenic and elastic properties of the cardiovascular system, as early warning signs of disease. Thus, we may be able to develop targeted therapies, which perhaps we could initiate early to prevent progression of the disease to more advanced and probably irreparable, stages.
Appendix 1

PATIENT INFORMATION LEAFLET

"Investigating the Properties and Regulation of Vascular Smooth Muscle in Vessels Used as Coronary Grafts."

Principal Investigator: Professor M. Galiñanes, Professor of Cardiac Surgery

You may contact Professor M. Galiñanes at the Department of Cardiac Surgery, Glenfield Hospital NHS trust, Groby Road, Leicester LE3 9PQ. (0116) 256 3032

You are invited to participate in the above study, which is being carried out at the Glenfield Hospital. Before you decide it is important for you to understand why the research is being done and what it will involve.

1. What is the purpose of the study?

The purpose of the study is to investigate the properties and the mechanisms that affect them, of the vessels used as grafts during coronary artery bypass surgery.

During coronary artery bypass graft surgery the surgical team is harvesting blood vessels from different areas of the body, in order to use them as grafts to bypass the narrowed areas on the coronary arteries. The commonly used vessels are the internal thoracic artery (behind the chest wall), the radial artery (from the forearm) and the long saphenous vein (from the lower limb). After harvesting, the grafts are
measured to the necessary length and sutured on the coronary arteries in such way that
the areas of the heart that are beyond the narrowing, are supplied again with blood
necessary for their normal function. Invariably there are small pieces of vessels left
after measuring, which are discarded as clinical waste for incineration.

However, the grafts do not remain patent forever. We do not know how these
vessels behave once they have undergone the process of grafting and we also do not
know if and how this behaviour is influenced by other factors (e.g. drugs used
during surgery, hormones produced by the body during surgery and pre-existing
disease conditions).

Using an established laboratory model we will undertake a series of experiments to
investigate the properties and the factors affecting the behaviour of the vessels used as
coronary grafts.

What will be involved if I take part in the study?

We will ask you to allow us to use the pieces of vessels that are left after measuring
the exact length required for the bypass procedure. As mentioned above, almost
invariably after harvesting and measuring these vessels, small pieces of vessel (up to
one inch) are being left as redundant. The reason this happens is because it is
essential for the grafts not to be short (as this would result to tension), but on the
other hand it is necessary for them not to be excessively long to avoid kinking.
These pieces of tissue would normally be discarded at the end of the operation, as
clinical waste for incineration.

We intend to undertake laboratory experiments to the redundant pieces of vessels try
and identify the way they behave when subjected to different conditions. By doing
so we may be able to develop techniques to modify this behaviour and thus achieving a more favourable outcome of surgery.

After completion of the experiments, tissue will be discarded within a period of six months maximum, following your donation. There are strict guidelines, which will be adhered to in the destruction of human tissue.

A variety of different types of experiments will be undertaken over a period of time, however all different types of research projects using our experimental model will have first been given approval by a Local Research Ethics Committee. Should you wish to know the laboratory staff would be able to advise you of the current types of laboratory studies which your tissue may be used for.

We may also need to obtain blood samples during your hospital stay in order to measure the levels of various hormones in the blood stream. This may

Nothing will be done to you as part of this research that would not otherwise have happened for your routine care; the redundant piece of vessel will only be taken to the laboratory after the necessary lengths of grafts have been measured.

Please note that the results of this research will not benefit you either clinically or financially, however it is hoped that this research may benefit patients in the future.

3. Will information obtained in the study be confidential?

Your participation in the study will be treated with the usual degree of confidentiality under the data protection act. No details, which could identify you, will be attached to the sample used for this research. Non-clinical researchers may be involved in
collecting vital information from your personal medical records, however they are bound by the same confidentiality requirements as other medical staff.

4. What if I am harmed by the study?

Medical research is covered for mishaps in the same way, as for patients undergoing treatment in the NHS i.e. compensation is only available if negligence occurs. Should you wish to register a complaint, please contact Mr Vaughn Marsden, Senior Complaints Officer, Balmoral Building, level 3, Leicester Royal Infirmary or telephone (0116) 258 6118.

What happens if I do not wish to participate in this study or wish to withdraw from the study?

If you do not wish to participate in this study or if you wish to withdraw from the study at any time, you may do so without justifying your decision and your future treatment will not be affected.

Thank you for taking the time to read this information sheet.
PATIENT CONSENT FORM

“Investigating the Properties and Regulation of Vascular Smooth Muscle in Blood
Vessels Used as Coronary Grafts.”

Principal Investigator: Prof M Galiñanes, Professor of Cardiac Surgery. This form
should be read in conjunction with the Patient Information Leaflet.

I agree to take part in the above study as described in the Patient Information Sheet.

I understand that I may withdraw from the study at anytime without justifying my
decision and without affecting my normal care and medical management.

I understand that members of the research team may wish to view relevant sections of
my medical records, but all the information will be treated as confidential.

I understand that medical research is covered for mishaps in the same way as for
patients undergoing treatment in the NHS i.e. compensation is only available if
negligence occurs.

I have read the patient information leaflet on the above study and have had the
opportunity to discuss the details with ........................................................ and
ask any questions. The nature of the tests to be undertaken have been explained to
me and I understand what will be required if I take part in the study.

Signature of patient..........................................................Date...................

(Name in BLOCK CAPITALS)..................................................................

I confirm I have explained the nature of the Trial, as detailed in the Patient
Information Sheet, in terms that in my judgement are suited to the understanding of
the patient.

Signature of Investigator..........................................................Date.............

(Name in BLOCK CAPITALS)..................................................................
Appendix 2

Formula 1

\[ C = [(\pi + 2) \cdot d] + 2L \]

Where \( C \) = Internal circumference (mm), \( d \) = wire diameter (mm), \( L \) = distance between the inner surface of the wires (mm) and \( \pi = 3.14 \)

Formula 2

\[ d = \frac{C}{\pi} \]

as above

Formula 3

\[ WT = 9.807 \cdot \frac{F}{2l} \]

Where \( WT \) = Wall tension (mN/mm), \( F \) = force (g), \( l \) = vessel length (mm) and 9.807 = tissue constant (mN/g)

Formula 4

\[ P = 7.403 \cdot 2\pi \cdot \frac{WT}{d} \]

Where \( P \) = Transmural pressure mmHg and 7.403 = tissue constant (mmHg/mN)

Formula 5 - Sigmoid function

\[ AD = b_0 \times \text{atan} \left( \frac{\text{concentration} \cdot \text{log} [\text{M} + 10] - \text{EC}_{50} \cdot \text{log} [\text{M} + 10]}{b_1} \right) + R_{50} \]

Where \( AD \) = Percentage diameter change \( EC_{50} \): effective concentration 50%, \( R_{50} \): 50% of the maximum response, \( b_0 \) and \( b_1 \) coefficients calculated by non-linear regression analysis


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