I am the author of the thesis entitled "Relationships of Nutritional and Metabolic Factors to Non-Invasive Indices of Macrovascular Disease in Diabetes" submitted for the degree of Ph.D.

and agree to the thesis being made available for such consultation, loan or photocopying as may be approved by the Chief Librarian provided that no part of the thesis shall be reproduced without the prior approval of the Chief Librarian and with the appropriate acknowledgement of the source.

Signed ........................................

Date  ......................................
TO: ALL USERS OF THIS THESIS

Please sign this form to indicate that you have used this thesis in accordance with the disposition signed by the author of this thesis.

Thank you.

MARGARET CAMERON

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>


RELATIONSHIP OF NUTRITIONAL AND METABOLIC FACTORS

TO NON-INVASIVE INDICES OF MACROVASCULAR DISEASE IN DIABETES

BY

CHE SAM LO
M.D.

(Chung Shan Medical University, China)

Dissertation for the degree of Doctor of Philosophy (1986)

Department of Human Nutrition, Division of Biological and Health Sciences, Deakin University, Victoria, Australia.

Department of Surgery, Prince Henry's Hospital, Monash University, Victoria, Australia.
## CONTENTS

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>xvii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summary</td>
<td>xx</td>
</tr>
<tr>
<td><strong>Chapter 1</strong> Introduction</td>
<td>1</td>
</tr>
<tr>
<td><strong>Chapter 2</strong> Methodology</td>
<td>12</td>
</tr>
<tr>
<td><strong>Chapter 3</strong> General characteristics of controls (healthy subjects) and non-insulin-dependent diabetics</td>
<td>55</td>
</tr>
<tr>
<td><strong>Chapter 4</strong> Arterial wall characteristics of controls (healthy subjects) and non-insulin-dependent diabetics</td>
<td>76</td>
</tr>
<tr>
<td><strong>Chapter 5</strong> Univariate evaluation of biochemical determinants of arterial wall characteristics</td>
<td>92</td>
</tr>
<tr>
<td><strong>Chapter 6</strong> Multivariate evaluation of biochemical determinants of arterial wall characteristics</td>
<td>113</td>
</tr>
<tr>
<td><strong>Chapter 7</strong> Food intake determinants of arterial wall characteristics</td>
<td>125</td>
</tr>
<tr>
<td><strong>Chapter 8</strong> General conclusions</td>
<td>155</td>
</tr>
</tbody>
</table>
CONTENTS

List of Figures xi-xiii
List of Tables xiv-xvi
Acknowledgements xvii
Preface xviii
Abbreviations xix
Summary xx-xxii

Chapter 1 Introduction 1-11
1.1 The problem of macrovascular disease in diabetes 1
1.2 Risk factors for macrovascular disease in diabetes 2
1.3 Non-invasive monitoring of macrovascular disease in diabetes 9
1.4 Hypothesis 10
1.5 Aims and approach 10

Chapter 2 Methodology 12-54
2.1 Introduction 12
2.2 Subjects 12
2.3 Doppler Ultrasound indices 13
2.3.1 The Doppler principle 13
2.3.2 Arterial compliance (AC) 17
2.3.3 Arterial proximal resistance (PR) 24
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>Biochemical indices</td>
<td>30</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Blood collection</td>
<td>30</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Free fatty acids (FFA)</td>
<td>31</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Insulin</td>
<td>35</td>
</tr>
<tr>
<td>2.4.4</td>
<td>Blood glucose</td>
<td>41</td>
</tr>
<tr>
<td>2.4.5</td>
<td>Glycosylated haemoglobin (HbAIC)</td>
<td>42</td>
</tr>
<tr>
<td>2.4.6</td>
<td>Serum total cholesterol</td>
<td>49</td>
</tr>
<tr>
<td>2.4.7</td>
<td>Serum high density lipoprotein cholesterol (HDL-C)</td>
<td>50</td>
</tr>
<tr>
<td>2.4.8</td>
<td>Serum triglycerides (TG)</td>
<td>52</td>
</tr>
<tr>
<td>2.5</td>
<td>Food intake documentation</td>
<td>54</td>
</tr>
<tr>
<td>2.6</td>
<td>Statistical Methods</td>
<td>54</td>
</tr>
</tbody>
</table>

Chapter 3  
General characteristics of healthy subjects and non-insulin-dependent diabetics  
55-75

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>55</td>
</tr>
<tr>
<td>3.2</td>
<td>Subjects and methods</td>
<td>55</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Subjects</td>
<td>55</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Methods</td>
<td>56</td>
</tr>
<tr>
<td>3.3</td>
<td>The age, stature, body weight, body mass index (BMI) and blood pressure of healthy subjects and non-insulin-dependent diabetics</td>
<td>56</td>
</tr>
<tr>
<td>3.4</td>
<td>Blood glucose and glycosylated haemoglobin (HbAIC) of healthy subjects and non-insulin-dependent diabetics</td>
<td>58</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Blood glucose</td>
<td>58</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Glycosylated haemoglobin (HbAIC)</td>
<td>62</td>
</tr>
</tbody>
</table>
3.5 Plasma free fatty acid of healthy subjects and non-insulin-dependent diabetics
3.6 Plasma insulin of healthy subjects and non-insulin-dependent diabetics
3.7 Serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride of healthy subjects and non-insulin-dependent diabetics
3.8 The LDL-C/HDL-C, HDL-C/TG, LDL-C/TG, Insulin/Glucose, FFA/Insulin and FFA/Glucose ratio of healthy subjects and non-insulin-dependent diabetics
3.9 Discussion

Chapter 4 Arterial wall characteristics of healthy subjects and non-insulin-dependent diabetics
4.1 Introduction
4.2 Normal structure and function of arteries
4.3 Arterial compliance (AC) of healthy subjects and non-insulin-dependent diabetics
4.4 Proximal resistance (PR) of healthy subjects and non-insulin-dependent diabetics
4.4.1 Posterior tibial artery (PTA)
4.4.2 Common femoral artery (CFA)
4.5 Correlation between the arterial compliance and proximal resistance

4.6 Discussion

4.7 Conclusion

Chapter 5 Univariate evaluation of biochemical determinants of arterial wall characteristics

5.1 Introduction

5.2 The correlation between arterial compliance and blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid and insulin for non-insulin-dependent diabetics and their healthy controls

5.2.1 The correlation between the arterial compliance and blood glucose level

5.2.2 The correlation between the arterial compliance and glycosylated haemoglobin (HbA1C)

5.2.3 The correlation between the arterial compliance and plasma free fatty acid

5.2.4 The correlation between the arterial compliance and plasma insulin

5.3 The correlation between arterial proximal resistance at the common femoral artery and blood glucose level, glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin for non-insulin-dependent diabetics and their healthy controls
5.3.1 The correlation between arterial proximal resistance of common femoral artery and blood glucose level

5.3.2 The correlation between arterial proximal resistance of common femoral artery and glycosylated haemoglobin (HbA1C)

5.3.3 The correlation between arterial proximal resistance of common femoral artery and plasma free fatty acid

5.3.4 The correlation between arterial proximal resistance of common femoral artery and plasma insulin

5.4 The correlation between arterial proximal resistance at the posterior tibial artery and blood glucose level, glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin for non-insulin-dependent diabetics and their healthy controls

5.4.1 The correlation between arterial proximal resistance of posterior tibial artery and blood glucose level

5.4.2 The correlation between arterial proximal resistance of posterior tibial artery and glycosylated haemoglobin

5.4.3 The correlation between arterial proximal resistance of posterior tibial artery and plasma free fatty acid

5.4.4 The correlation between arterial proximal resistance of posterior tibial artery and plasma insulin

5.4.5 Correlations (r) between arterial indices and each of total cholesterol, HDL-Cholesterol, LDL-Cholesterol and triglycerides for non-insulin-dependent diabetics or their healthy controls.
5.5 Discussion

5.6 Conclusion

Chapter 6 Multivariate evaluation of biochemical determinants of arterial wall characteristics

6.1 Introduction

6.2 Relationships between arterial compliance and blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics

6.3 Relationships between proximal resistance of common femoral artery and blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics

6.4 Relationships between proximal resistance of posterior tibial artery and blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics
6.5 Relationships between arterial compliance and blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for men and women

6.6 Discussion

6.7 Conclusion

Chapter 7 Food intake determinants of arterial wall characteristics

7.1 Introduction

7.2 Methods

7.3 Distribution of food consumption in healthy subjects and non-insulin-dependent diabetics

7.4 Relationships between protective food consumption and arterial compliance (AC), proximal resistance (PR) of common femoral artery and posterior tibial artery in non-insulin-dependent diabetics and their healthy controls

7.4.1 Arterial compliance

7.4.2 Proximal resistance of common femoral artery

7.4.3 Proximal resistance of posterior tibial artery
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>Relationships between fish consumption and arterial compliance (AC), proximal resistance (PR) of common femoral artery and posterior tibial artery in non-insulin-dependent diabetics and their healthy controls</td>
<td>141</td>
</tr>
<tr>
<td>7.5.1</td>
<td>Arterial compliance</td>
<td>141</td>
</tr>
<tr>
<td>7.5.2</td>
<td>Proximal resistance of common femoral artery</td>
<td>141</td>
</tr>
<tr>
<td>7.5.3</td>
<td>Proximal resistance of posterior tibial artery</td>
<td>142</td>
</tr>
<tr>
<td>7.6</td>
<td>Univariate evaluation of food determinants of arterial wall characteristics</td>
<td>145</td>
</tr>
<tr>
<td>7.6.1</td>
<td>Arterial compliance</td>
<td>145</td>
</tr>
<tr>
<td>7.6.2</td>
<td>Proximal resistance of common femoral artery</td>
<td>145</td>
</tr>
<tr>
<td>7.6.3</td>
<td>Proximal resistance of posterior tibial artery</td>
<td>146</td>
</tr>
<tr>
<td>7.7</td>
<td>Multivariate evaluation of food determinants of arterial wall characteristics</td>
<td>150</td>
</tr>
<tr>
<td>7.8</td>
<td>Discussion</td>
<td>150</td>
</tr>
<tr>
<td>7.9</td>
<td>Conclusion</td>
<td>153</td>
</tr>
</tbody>
</table>

Chapter 8 General Conclusion 155-157
Publications arising to data from work presented in this thesis

Aspects of the work presented to date at national and international meetings

References

Appendix I
<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>The Doppler effect.</td>
<td>14</td>
</tr>
<tr>
<td>2.2</td>
<td>The Doppler principle.</td>
<td>16</td>
</tr>
<tr>
<td>2.3</td>
<td>Laplace Transform Damping (Argand diagram).</td>
<td>26</td>
</tr>
<tr>
<td>3.1</td>
<td>Fasting blood glucose in control and NIDD subjects.</td>
<td>59</td>
</tr>
<tr>
<td>3.2</td>
<td>Blood glucose area in control and NIDD subjects.</td>
<td>60</td>
</tr>
<tr>
<td>3.3</td>
<td>Glucose tolerance test in control and NIDD subjects.</td>
<td>61</td>
</tr>
<tr>
<td>3.4</td>
<td>Glycosylated haemoglobin level in control and NIDD subjects.</td>
<td>62</td>
</tr>
<tr>
<td>3.5</td>
<td>Fasting plasma free fatty acid in control and NIDD subjects.</td>
<td>64</td>
</tr>
<tr>
<td>3.6</td>
<td>Plasma free fatty acid area in control and NIDD subjects.</td>
<td>65</td>
</tr>
<tr>
<td>3.7</td>
<td>Plasma free fatty acid level in control and NIDD subjects after glucose load.</td>
<td>66</td>
</tr>
<tr>
<td>3.8</td>
<td>Fasting plasma insulin in control and NIDD subjects.</td>
<td>68</td>
</tr>
<tr>
<td>3.9</td>
<td>Plasma insulin area in control and NIDD subjects.</td>
<td>69</td>
</tr>
<tr>
<td>3.10</td>
<td>Plasma insulin level in control and NIDD subjects after glucose load.</td>
<td>70</td>
</tr>
<tr>
<td>4.1</td>
<td>The development of atheroscleratic plaque.</td>
<td>80</td>
</tr>
<tr>
<td>4.2</td>
<td>Arterial wall compliance at level of common femoral artery in control and NIDD subjects.</td>
<td>82</td>
</tr>
<tr>
<td>4.3</td>
<td>Proximal resistance at level of common femoral artery in control and NIDD subjects.</td>
<td>84</td>
</tr>
<tr>
<td>4.4</td>
<td>Proximal resistance at level of posterior tibial artery in control and NIDD subjects.</td>
<td>85</td>
</tr>
</tbody>
</table>
4.5 Correlation between arterial compliance and proximal resistance at the common femoral artery level in control and NIDD subjects.

4.6 Correlation between arterial compliance and proximal resistance at the posterior tibial artery level in control and NIDD subjects.

4.7 Correlation between proximal resistance at the common femoral artery level and at the posterior tibial artery level in all subjects, control and non-insulin-dependent diabetics.

5.1 Correlation between arterial compliance and blood glucose level.

5.2 Correlation between arterial compliance and glycosylated haemoglobin level.

5.3 Correlation between arterial compliance and plasma free fatty acid.

5.4 Correlation between arterial compliance and plasma insulin.

5.5 Correlation between arterial proximal resistance at the common femoral artery and blood glucose level.

5.6 Correlation between arterial proximal resistance at the common femoral artery and glycosylated haemoglobin level.

5.7 Correlation between proximal resistance at the common femoral artery and plasma free fatty acid.

5.8 Correlation between proximal resistance at the posterior tibial artery and blood glucose level.

5.9 Correlation between proximal resistance at the posterior tibial artery and glycosylated haemoglobin level.
5.10 Correlation between proximal resistance at the posterior tibial artery and plasma free fatty acid. 109
7.1 Prostaglandin synthetic pathways in blood vessels and platelet. 127
7.2 Correlation between arterial compliance and food variety. 150a
7.3 Correlation between proximal resistance of common femoral artery and food variety. 150b
7.4 Correlation between proximal resistance of posterior tibial artery and food variety. 150c
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Coefficient of variation (C.V.%) of arterial compliance.</td>
<td>23</td>
</tr>
<tr>
<td>2.2</td>
<td>Coefficient of variation (C.V.%) of proximal resistance of posterior tibial artery.</td>
<td>28</td>
</tr>
<tr>
<td>2.3</td>
<td>Coefficient of variation (C.V.%) of proximal resistance of common femoral artery.</td>
<td>29</td>
</tr>
<tr>
<td>3.1</td>
<td>The age, stature, body weight, body mass index (BMI) and blood pressure of healthy subjects and non-insulin-dependent diabetics.</td>
<td>57</td>
</tr>
<tr>
<td>3.2</td>
<td>The serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride of healthy subjects and non-insulin-dependent diabetics.</td>
<td>72</td>
</tr>
<tr>
<td>3.3</td>
<td>The LDL-C/HDL-C, HDL-C/TG, LDL-C/TG, Insulin/Glucose, FFA/Insulin and FFA/Glucose ratio of healthy subjects and non-insulin-dependent diabetics.</td>
<td>74</td>
</tr>
<tr>
<td>4.1</td>
<td>Correlation (r) between arterial compliance and proximal resistance for the common femoral arteries in NIDD and healthy subjects.</td>
<td>88c</td>
</tr>
<tr>
<td>4.2</td>
<td>Correlation (r) between arterial compliance and proximal resistance for the posterior tibial arteries in NIDD and healthy subjects.</td>
<td>88c</td>
</tr>
<tr>
<td>4.3</td>
<td>Correlation (r) between the proximal resistance for the common femoral arteries and posterior tibial arteries in NIDD and healthy subjects.</td>
<td>88c</td>
</tr>
<tr>
<td>5.1</td>
<td>Correlation (r) between arterial indices and each of total cholesterol, HDL-Cholesterol, LDL-Cholesterol and triglycerides for NIDD and healthy subjects.</td>
<td>110a</td>
</tr>
</tbody>
</table>
6.1 Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics combined.

6.2 Relationships between proximal resistance of common femoral artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multi-variate analysis for healthy subjects and non-insulin-dependent diabetics combined.

6.3 Relationships between proximal resistance of posterior tibial artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics combined.

6.4 Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for men and women.

7.1 Foods grouped according to biological source.

7.2 Distribution of food consumption per week in healthy subjects and non-insulin-dependent diabetics.

7.3 The arterial compliance (AC), proximal resistance (PR) of common femoral artery and posterior tibial artery of high protective food group and low protective food group in healthy subjects and non-insulin-dependent diabetics.
7.4 The arterial compliance (Ac), proximal resistance (PR) of common femoral artery and posterior tibial artery of fish eating and non-fish eating groups in healthy subjects and non-insulin-dependent diabetics.

7.5 Correlations (r or rs) between arterial compliance and food variety, or protective food core, in healthy subjects and non-insulin-dependent diabetics combined.

7.6 Correlations (r or rs) between proximal resistances of common femoral artery and food variety or protective food score in healthy subjects and non-insulin-dependent diabetics combined.

7.7 Correlations (r or rs) between proximal resistance of posterior tibial artery and food variety or protective food score in healthy subjects and non-insulin-dependent diabetics combined.

7.8 Correlations (r or rs) between arterial indices and food variety in healthy subjects or non-insulin-dependent diabetics.
ACKNOWLEDGEMENTS

The work presented in this thesis was carried out in the Department of Human Nutrition, Deakin University and Department of Surgery, Monash University, Prince Henry's Hospital, during the period 1982-1985. I would like to acknowledge my debt of gratitude to the many people who have assisted me in the performance of these studies.

It is a great pleasure to acknowledge the advice, supervision, encouragement and support of Professor Mark L. Wahlqvist, to whom I am indebted for making available the facilities required to conduct the investigations described. Associate Prof. Kenneth A. Myers afforded the Doppler ultrasound equipment and critical appraisal of the work in progress. Dr. Dan Stroud provided invaluable advice on ultrasound technique. Dr. Richard Simpson referred many of the patients investigated, and his help was essential in several aspects of the work. Mr. Nick Balasz collaborated during several blood analyses, especially in blood glucose, glycosylated haemoglobin, cholesterol, HDL-cholesterol and triglyceride assay. I would also like to acknowledge the help and guidance given to me by Dr. Roger Warne of the National Research Institute of Gerontology and Geriatric Medicine.

I am grateful to the late Mr. Peter Dryden who was Laboratory Manager at Deakin University, Miss Lynne Atley who helped with the plasma insulin assays and Miss Marion Cook, Miss Patricia Apswoude and Miss Jacqui Gillam who bore the task of typing the manuscript.

Finally, I would like to thank the patients and healthy volunteers from the South Melbourne, Middle Park and Footscray Elderly Citizen Clubs, without whose cooperation these studies would have been impossible.
PREFACE

I certify that this thesis entitled "RELATIONSHIP OF NUTRITIONAL AND METABOLIC FACTORS TO NON-INVASIVE INDICES OF MACROVASCULAR DISEASE IN DIABETES" is the result of my own research, it does not incorporate without acknowledgement any material previously submitted for a higher degree to any University and to the best of my knowledge and belief it does not contain any material previously published or written by another person, except where due reference is made in the text.

C. S. L.

CHE SAM LO
M.D. (China)
GLOSSARY OF ABBREVIATIONS

AC   Arterial compliance
BMI  Body mass index
CFA  Common femoral artery
Ch   Cholesterol
CV   Coefficient of variation
FFA  Free fatty acid
G-TT Glucose tolerance test
HbAlc Glycosylated haemoglobin Alc
HDL-C High density lipoprotein cholesterol
LDL-C Low density lipoprotein cholesterol
NIDDM Non-insulin-dependent diabetes
PFS  Protective food score
PR   Proximal resistance
PTA  Posterior tibial artery
QC   Quality control
SD   Standard deviation
SE   Standard error
TG   Triglycerides
SUMMARY

Factors which may account for the high frequency of macrovascular disease in diabetics are age, sex, cigarette smoking, hypertension, obesity, lack of exercise, diet, hyperglycaemia, hyperinsulinaemia, hypercholesterolaemia, hypertriglyceridaemia, low HDL-cholesterol concentration, elevated free fatty acid concentration and enhanced platelet aggregation.

Twenty seven (13 men and 14 women) non-insulin-dependent diabetics and thirty eight age, height and weight matched healthy subjects (10 men and 28 women) were studied. None of the subjects were smokers, or hypertensive. No subject had any clinical evidence of peripheral arterial disease, coronary heart disease or cerebrovascular disease. All had apparently normal peripheral pulses and normal ankle/arm blood pressure indices.

Methods for determining arterial compliance in the segment between the left subclavian artery and each common femoral artery, and proximal resistance at the common femoral artery and posterior tibial artery, have been reviewed and developed. An appropriate food intake methodology for deriving food indices from food records was developed. Biochemical determinants have been made of glucose tolerance, glycosylated haemoglobin, serum total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, plasma free fatty acid and insulin.

A significant decrease in the arterial compliance, and a significant increase in the arterial proximal resistance at the common femoral artery and posterior tibial artery in non-insulin-dependent diabetics, compared with their healthy controls, have been found. Significant negative correlation between arterial compliance and proximal resistance and, a significant positive correlation between the arterial proximal resistance of common femoral artery and posterior tibial artery
were found. Differences between control (healthy subjects) and non-insulin-dependent diabetic groups indicate that preclinical peripheral arterial disease can be recognised even in mild diabetics by non-invasive measurement of arterial compliance or proximal resistance.

There were significant and negative correlations between arterial compliance and each of blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin concentration. There were significant and positive correlations between arterial proximal resistance of common femoral artery and posterior tibial artery and each of blood glucose, glycosylated haemoglobin and plasma free fatty acid concentration. Multivariate analysis to examine each of the biochemical factors including blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin and lipids, showed that the factor which most influenced the arterial compliance and the proximal resistance of posterior tibial artery was the glucose level in the fasting state or the glucose response after a glucose load. In addition, the factors which most influenced proximal resistance of the common femoral artery were free fatty acid level in the fasting state or glucose response after a glucose load. The factors which most influenced arterial compliance were glucose level in men, and the insulin level in the fasting state or the plasma free fatty acid response after a glucose load in women. These findings indicate that blood glucose, plasma free fatty acid and plasma insulin are risk factors for changes in arterial wall characteristic at a stage when no clinical evidence of macrovascular disease is apparent.

Arterial compliance was decreased and the proximal resistance of posterior tibial artery was increased in those with a low intake of protective foods compared with those with a high intake
whether healthy subjects or non-insulin-dependent diabetics. Arterial compliance was decreased in non-fish eaters compared with the fish eaters whether healthy subjects or non-insulin-dependent diabetics. Proximal resistance of the posterior tibial artery in non-fish eaters was increased compared with fish eaters in healthy subjects. Overall, food variety, a protective food score consumption and fish consumption emerge as importance determinants of arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.
CHAPTER ONE

INTRODUCTION

1.1 The Problem of Macrovascular Disease in Diabetes

Macrovascular disease is the most common complication of diabetes. About one-third of the population in developed countries will die of macrovascular disease, and almost three-quarters of all deaths of diabetics are due to macrovascular disease (West, 1978). For example, in 1977 atherosclerosis caused 873,000 deaths in the United States, about half of all deaths in that country, and in the same year cost an estimated $39 billion in health expenditure and lost productivity (Editorial, Arteriosclerosis 1981). Atherosclerosis affects not only life expectancy but also the quality of life. Diabetics not only die at an earlier age than non-diabetics, from macrovascular disease affecting the coronary and cerebrovascular systems, but also suffer considerable morbidity from disease affecting peripheral arteries, renal arteries and, to a lesser extent, the mesenteric arteries (Bryfogle & Bradley 1957; Strandness et al. 1964; Kessler 1971; Kannel & McGee 1979b; Keen & Jarrett 1979; Fuller et al. 1981).

Diabetic macrovascular disease is more common in women than in men. coronary heart disease is 1.7 times more common in diabetic than in non-diabetic men, but 2.7 times more common in diabetic than in non-diabetic women (Garcia et al. 1974; Kannel & McGee 1979a,b). However, not all cardiac disease in diabetics is attributable to macrovascular coronary heart disease. Diabetic cardiomyopathy may result from microvascular
disease, changes in myocardial metabolism or in myocardial membrane properties (Jarrett 1977; Kannel & McGee 1979b; Ledet et al. 1979; Chobanian et al. 1982). Congestive cardiac failure is 2.2 times more common in diabetic than in non-diabetic men and it is 5.4 times more common in diabetic than in non-diabetic women (Kannel & McGee 1979b).

1.2 Risk Factors for Macrovascular Disease in Diabetics

Factors which might account for the high frequency of macrovascular disease in diabetes are age, sex, cigarette smoking, hypertension, obesity, lack of exercise, diet, hyperglycaemia, hypercholesterolaemia, hypertriglyceridaemia, low HDL-cholesterol concentration, elevated free fatty acid concentration, hyperinsulinæmia and enhanced platelet aggregation (Wahlqvist et al. 1984). Diabetics may be affected to differing degrees by each of these risk factors (Pell & D’Alonzo 1970; Beach et al. 1979; Beach & Strandness 1980). The risk of atherosclerotic vascular disease may be different to other arterial wall changes which occur in diabetics (Perrier 1964; Neubauer 1971; Lundback 1973; Ledet 1981). This study, by design, has minimised the effect of other potential risk factors, such as age, body mass, body mass index, blood pressure and smoking on arterial compliance and arterial proximal resistance for both the apparently healthy subjects and non-insulin-dependent diabetics.

Age

The interaction between ageing, diabetes and macrovascular disease is complex. Age itself is an acknowledged and important risk factor for
macrovacular disease in non-diabetic populations; hence it is assumed that the
older diabetic will show more macrovascular disease. Such an age relationship
does not imply that macrovascular disease is part of the ageing process. It seems
likely also that the duration of diabetes will affect the prevalence and
severity of atherosclerotic vascular disease (Deckert, Poulsen & Larsen 1978).

Sex

Diabetic macrovascular disease is more prevalent in women than in men. Also
diabetic women have a relatively greater mortality risk than diabetic men
(Kessler 1971). Siitonen et al reported (1986) that peripheral arterial disease
tended to be somewhat more common in men with newly diagnosed
non-insulin-dependent diabetes than in non-diabetic men, whereas no
difference was found in prevalence of peripheral arterial disease between diabetic
and non-diabetic women.

Cigarette Smoking

In the Framingham study, diabetic men smoked fewer
cigarettes each day than did non-diabetic men. Smoking
added to the risk of cardiovascular disease in
diabetics without appearing to worsen their diabetes
(Kannel & McGee 1979c). Smoking is associated with
accelerated development of atherosclerosis,
particularly in the arteries supplying the lower
limbs. It has also been shown to increase the risk of
late occlusion of femoral popliteal vein grafts and
aorto-femoral dacron grafts by more than three
times (Myers et al. 1978).
Hypertension

Hypertension, particularly elevated systolic blood pressure, is shown by multivariate analysis to be a risk factor for diabetic atherosclerotic vascular disease in population studies where the end point is coronary heart disease mortality or cardiovascular mortality (Kannel & McGee 1979b; Fuller et al. 1980; Jarrett et al. 1982). For juvenile-onset insulin-dependent diabetics the Joslin clinic experience is that hypertension is the major identifiable risk factor for both coronary heart disease and renal disease, and is the major additive risk factor for mortality (Christlieb et al. 1981).

Obesity

Obesity will not only increase the risk and severity of non-insulin-dependent diabetes, but also the likelihood of associated risk factors such as hypertension, hyperlipidaemia and hyperinsulinaemia. Obese women diabetics who sustain a myocardial infarction have a high mortality rate (Tansey et al. 1977).

Lack of Exercise

Physical training in non-insulin-dependent diabetic patients improves glucose, insulin and lipid profiles (Ruderman et al. 1979; Saltin et al. 1979). The exercise hypothesis states that exercise protects against coronary heart disease. The weight of evidence supports the view that exercisers have a lower risk of coronary disease, but that vigorous exercise cannot always prevent progression of coronary atherosclerosis and does increase the risk of sudden death in persons with advanced coronary atherosclerosis. Therefore, the
long-term beneficial effect in terms of preventing the incidence of atherosclerotic vascular disease or favourably influence mortality, has yet to be studied prospectively (Edward et al. 1983).

Food Intake

The role of diet in the pathogenesis of macrovascular disease in diabetics remains unclear. However, where low rates of macrovascular disease are found in diabetic patients, the food intake pattern, both in the diabetic and non-diabetic groups, is characterised by a greater contribution to energy intake from carbohydrates and a lesser contribution from fat (Rudnick & Anderson 1962). For example, there was a much higher incidence of vascular disease in Japanese diabetics who had migrated to Hawaii compared with Japanese diabetics who remained in Hiroshima (Kawate et al. 1979). The Hawaiian diabetics' diet had a much higher fat content than the diet in Japan. Epidemiological comparisons of Greenland Eskimos and mainland Danes have suggested that the diet rich in fish or marine oils may be associated with a reduction in the incidence of occlusive vascular disease (Bang et al. 1980; Kromhout et al. 1985). Studies based on dietary history have suggested that even a small intake of fish may have reduced the incidence of coronary vascular disease in both European and North American populations (Shekelle et al. 1985). The ingestion of some fish or marine oils produces beneficial changes in plasma lipid levels and the development of a mild bleeding disorder thought to be due to a decrease in platelet generation of the potent platelet aggregator and vasoconstrictor thromboxane A2 (Bang & Dyerberg 1972; Hornstra et al.)

Hyperglycaemia

Hyperglycaemia without evidence of clinical diabetes is associated with an increased risk of atherosclerotic vascular disease, intermediate between that for subjects with a normal glucose tolerance and frank diabetes. This applies both to coronary heart disease and peripheral vascular disease (Heinle et al. 1969; Fuller et al. 1981; Keen et al. 1981; Efendic et al. 1982; Keen et al. 1982). In the Whitehall, London, and Paris population studies of cardiovascular mortality, the risk of hyperglycaemia was not a stepwise increase of mortality with increasing degree of glucose intolerance, but rather a "threshold" effect with a sharp doubling of mortality at the 95th percentile of the two hour blood glucose concentration (Eschwege et al. 1980; Fuller et al. 1980).

Hyperinsulinaemia

Diabetics have an exaggerated insulin response to oral glucose. Various experimental studies have supported the hypothesis that elevated levels of circulating insulin per se might predispose to atherosclerotic vascular disease (Ganda 1980; Stout 1981). Administration of insulin promotes development of arterial lesions in animals fed a normal diet (Stout
1970; Stout et al. 1973) and inhibits regression of lesions in cholesterol-fed animals (Stamler et al. 1960). Hyperinsulinaemia is recognised in non-insulin-dependent diabetics and is augmented by sulphonylurea therapy, and may occur in insulin-dependent diabetics where relatively high amounts of injected insulin are given to maintain blood glucose control. Also, insulin antibodies may promote high levels of circulating insulin. Insulin can stimulate smooth muscle cell proliferation in the arterial wall (Stout et al. 1975; Pfeifie et al. 1980, 1981), and also can promote lipid synthesis and mucopolysaccharide metabolism in the arterial wall (Capron et al. 1980, 1981; Sirek et al. 1981; Stout 1981). Population studies in Australia, Scotland and Finland indicate that elevated serum insulin levels are associated with coronary heart disease mortality as an independent risk factor (Logan et al. 1979; Pyorala 1979; Welborn & Wearne 1979). In a study of 7,000 non-diabetic men aged 43-54 years and followed for an average of 63 months, the study found that the fasting serum insulin was predictive of subsequent coronary events as an independent variable; in addition, systolic blood pressure and cigarette smoking were predictive, but not obesity or triglyceride levels (Ducimetiere et al. 1980).

Hyperlipidaemia

Hypercholesterolaemia has long been recognised as one of the cardinal risk factors for atherosclerotic vascular disease in population studies (Kannel et al. 1964). Diabetics are assumed to be at least as likely to show the prevailing hypercholesterolaemia as the non-diabetic population, being susceptible to similar genetic and environmental influences. The metabolic
aberration of diabetes, however, increases triglyceride levels rather than cholesterol levels (Bagdade et al. 1976; Goldberg 1981). Hypertriglyceridaemia is a common metabolic abnormality in diabetes but is not shown to be an independent risk factor (Ducimetiere et al. 1980; Fielding 1981; Steiner 1981).

Irrespective of actual lipid levels, lipoprotein metabolism is altered in diabetes (Ganda 1980) and it has been suggested that diabetes might increase the sensitivity of arteries to risk factors such as LDL-cholesterol levels (Steiner 1981) without marked differences in the actual LDL-cholesterol levels. HDL-cholesterol concentrations can be low in some diabetic populations and the disproportionately low HDL-cholesterol concentration in diabetic women is cited as an explanation for the loss of protection of females from macrovascular disease (Gordon et al. 1977; Reckless et al. 1978).

Free fatty acid concentrations in plasma tend to be higher where there is lack of insulin or insulin action, and this phenomenon is well recognised in diabetics. Experimental data indicate a relationship between plasma free fatty acid concentrations and morphological changes in arteries in animal studies (Rainila 1981). The effects of free fatty acids may be mediated through accelerated prostacyclin degradation in plasma, and thus an increased tendency to platelet aggregation (Mikhailidis et al. 1981). Fatty acid patterns may also be different in diabetics, with possible implications for prostaglandin metabolism (Jones et al. 1983).
Enhanced platelet aggregation

Both platelet adhesion and platelet aggregation are increased in diabetics with vascular disease (Colwell et al. 1981; Kazmir et al. 1981). Two products of the arachidonic acid pathway are important in platelet function (Moncada & Vane 1979; Herold & Kinsella 1986). Thromboxane A2 is found in platelets and causes platelet aggregation and vasoconstriction, while prostacyclin is produced in endothelium cells and prevents platelet aggregation and causes vasodilation.

Increased synthesis of thromboxane B, the stable product of active thromboxane A2, has been found in diabetes (Halushka et al. 1981), while there is evidence that prostacyclin production is decreased in vascular tissue from diabetics (Davis et al. 1981; Wall et al. 1981). The fluid phase of coagulation may contribute in a number of ways to the initiation or propagation of the atherosclerotic process in diabetes.

1.3 Non-Invasive Monitoring of macrovascular Disease in Diabetes

Arteriography has been used to study early macrovascular disease (Blankenhorn 1978), but this is an invasive technique and introduces ethical problems, particularly for large studies or asymptomatic subjects. A non-invasive technique using Doppler ultrasound to study arterial disease is harmless and should be quantitative, reliable, reproducible and simple to perform (Gosling 1976; Skidmore et al. 1980; Baird et al. 1980; Leaun and Gosling 1982). Clinical evidence of arterial disease was significantly more common in those subjects with initially high Laplace
wave form analysis values, than in those with normal wave form values (Campbell et al. 1985). Pulse wave velocity and wave form analysis using Doppler scanning techniques can reveal early evidence of stiffening of the central and peripheral arterial system in diabetes (Wahlqvist et al. 1984; Reif et al. 1986; Lo et al. 1986). The results on this form of assessment are in the following chapters.

1.4 **Hypothesis**

1) That it is possible to recognise preclinical macrovascular disease in those with diabetes using non-invasive Doppler Ultrasound techniques.

2) That risk factors for macrovascular disease in diabetes include blood glucose, glycosylated haemoglobin, plasma free fatty acids, plasma insulin, total cholesterol, HDL-cholesterol and triglycerides.

3) That indices of food intake are predictive of macrovascular disease in diabetes.

1.5 **Aims and Approach**

**Aims:**

1. To identify preclinical macrovascular disease in patients without insulin-dependent diabetes (NIDD) and their healthy controls using computerised Doppler ultrasound technology.

2. To assess differences in Doppler ultrasound indices of the arterial wall.
If arterial disease in diabetes can be detected early, then good metabolic control and other risk factor intervention might delay disease progression. Life expectancy may then be longer, life quality better, and health expenditure reduced.

**Approach**

Chapter 2 develops and reviews the methods for non-invasively determined arterial wall indices, metabolic variables and food intake.

Chapter 3 considers the differences in metabolic variables between non-insulin-dependent diabetics and their healthy controls.

Chapter 4 examines vessel wall characteristics of non-insulin-dependent diabetics and their healthy controls.

In Chapter 5 is a univariate evaluation of biochemical determinants of vessel wall characteristics. The non-invasive indices (arterial compliance and arterial proximal resistance) of macrovascular disease are related to postulated metabolic risk factors such as blood glucose, free fatty acid, and insulin levels.

In Chapter 6 a multivariate evaluation of biochemical determinants of vessel wall characteristics is performed.

Chapter 7 examines food intake determinants of vessel wall characteristics.

Chapter 8 presents general conclusions.
CHAPTER TWO

METHODOLOGY

2.1 Introduction

This chapter describes subject selection and the equipment and techniques used to measure the following indices:

Doppler ultrasound indices:
  a) Arterial compliance (AC)
  b) Arterial proximal resistance (PR)

Biochemical indices:
  a) Plasma free fatty acids (FFA)
  b) Plasma insulin
  c) Glucose tolerance test
  d) Glycosylated haemoglobin (HbAlC)
  e) Total cholesterol, HDL-cholesterol and LDL-cholesterol
  f) Triglycerides

Food Intake techniques.
It also indicates the statistical methods used.

2.2 Subjects

The study was approved by the Ethics Committee of Prince Henry's Hospital in accordance with the statement on human experimentation by the National Health and Medical Research Council of Australia. Informed consent was obtained.
Twenty-seven non-insulin-dependent diabetics (NIDD) treated by diet alone, and 38 age, height and weight matched controls were studied. Those with diabetes were selected consecutively from the diabetics service of Prince Henry's Hospital, Melbourne, and the controls from elderly citizen's clubs in districts serviced by the hospital each gave their informed consent. None of the subjects were hypertensive (blood pressure less than 150/95), or smokers. No subject had any symptoms of peripheral arterial disease, all had apparently normal peripheral pulses and all had normal ankle-arm blood pressure indices. None had present or prior symptoms of coronary artery disease or cerebrovascular disease.

2.3 Doppler Ultrasound Indices

2.3.1 The Doppler principle

The Doppler principle was first recognised by Doppler and Ballot in 1845. They described the change in frequency that occurs when a continuous wave source is moving towards, or away from a detector. The Doppler shift is related to the emitted frequency, the approach velocity of the source and the velocity of sound by equation (2.1).

\[ F = \frac{2fV}{C} \quad (2.1) \]

where \( F \) = change in detected frequency
\( f \) = emitting frequency of the source
\( V \) = velocity of the source relative to the detector
\( C \) = velocity of sound in the particular medium

The cause of the detected change in frequency is represented diagrammatically in Figure 2.1.
Fig 2.1: The Doppler effect.
The first successful attempt to measure blood velocity non-invasively using Doppler principle was reported by Satomura in 1959. Although the equipment used was simple by modern standards, the same basic principles are utilised in ultrasonic blood velocity detectors in present-day equipment. The Doppler probe or ultrasonic pen torch consists of two hemispheric piezoelectric quartz crystals installed at the skin contact end of the probe. One crystal is stimulated electrically to vibrate at a constant frequency, while the other converts reflected sound waves back to electrical pulses. This allows the electrical input to be compared to the electrical output. As expected, any difference is due to the change of frequency or Doppler shift.

A constant electrical input applied to the emitting crystal causes it to produce sound waves of known frequency. When this beam insonates the lumen of an artery or vein, the particulate matter of the blood will vibrate at the frequency of the incident beam. Red blood cells are appropriately sized to vibrate and reradiate the sound (Kato et al. 1962). This reradiated signal is called the backscattered signal in terms of the Doppler equation, the red blood cells become emitters responsible for the Doppler shift. A diagram of the used Doppler probe is shown in Figure 2.2.
Fig 2.2: The Doppler principle, A red cell moving towards the detector gives a shift incident frequency $f$ of $+\Delta f$ away from detector $-\Delta f$. 
If the probe is positioned over the artery and is stationary, the frequency shift will be proportional to the velocity of aggregates of red blood cells moving as part of the blood.

An ultrasonic probe applied to the skin can only insonate vessels at an angle and therefore only detect a vector component of the true blood velocity. This component directly proportional to the ankle of the probe. Hence equation (2.1) becomes:

\[ F = \frac{2fV \cos \theta}{C} \quad (2.2) \]

where
- \( F \) = Doppler shift frequency
- \( f \) = frequency of incident radiation (transmitted or fundamental frequency)
- \( V \) = velocity of the blood cells (moving target)
- \( C \) = velocity of sound in the blood (or tissue)
- \( \theta \) = angle of inclination of the incident wave to the direction of blood flow.

In practice, the best signal possible is obtained without precisely knowing the probe/artery angle. The indices used in this study have been shown to be independent of probe vessel angle (Johnston et al. 1977).

2.3.2 Arterial Compliance (AC)

a) Principle:

The rate at which the pressure pulse wave travels in a non-viscous fluid within an elastic tube of infinite length is described by the Meons-Korteweg equation:
Pulse wave velocity = \sqrt{\frac{Eh}{N^{2}rp}} \hspace{1cm} (2.3)

(PWV)

E = elastic modulus of the wall
h = wall thickness
p = fluid density
r = mean radius of the tube.

The compliance is inversely proportional to the elastic modulus. Such as:
\[ \frac{1}{E} \propto \text{compliance}. \]

Therefore compliance = \frac{1}{(p \cdot w \cdot v)^{2} \cdot 2rp} \cdot h \hspace{1cm} (2.4)

The density of blood is very close to unity (1.055g/cm³) and can be disregarded.
Arterial compliance has been defined as follows (Loagun and Gosling 1982)

\[ C = \frac{1}{Ep} = \frac{\Delta D}{D} / \Delta p \hspace{1cm} (2.5) \]

where
Ep = the pressure-strain elastic modulus of the arterial wall.
D = the lumen diameter at perfusion pressure P
\Delta D = the increase in diameter of the lumen produced by an increment \Delta p in pressure.
Over a given segment length L of a vessel, in the absence of reflected waves, it is the average compliance of the vessel pathway that causes the delay in transit time T found between corresponding points of the proximal and distal pulse waves (of flow and/or pressure) observed for the same heart beat. Thus the Hoen-Korteweg equation gives for the pulse wave velocity:

\[ \text{PWV} = \frac{L}{T} = \sqrt{\frac{E_p}{2\rho}} = \sqrt{\frac{1}{2\rho C}} \] (2.6)

The average compliance C of the vessel pathway is thus given by \( C = \frac{1}{2\rho} \left( \frac{L}{T} \right)^2 \) per unit pulse pressure with T in seconds and L in metres and the average density \( \rho \) of whole blood taken to be \( 10^3 \text{kgm}^{-3} \), we have, for an average pulse pressure of 10 mmHg (1.33 kPa or \( 1.33 \times 10^3 \text{Nm}^{-2} \)).

\[ C = \frac{1.33 \times 10^3}{2 \times 10^3} \left( \frac{T}{L} \right)^2 = 0.667 \left( \frac{T}{L} \right)^2 \]

and when expressed as a percentage

\[ C \% \text{ per } 10 \text{ mmHg} = 66.7 \left( \frac{T}{L} \right)^2. \] (2.7)
The measurement of P.W.V. using the Doppler technique has been used as an indicator of atherosclerotic degeneration of the arterial wall. However, before reviewing these studies, the components of the arterial wall that affect the elastic characteristics of the large conduit arteries should be briefly examined. The arterial wall contains five concentric zones; they are intima, internal elastic lamella, media, external elastic lamella and adventitia. The major contributor to arterial distensibility or compliance is the media and possibly the adventitia (Wolinsky et al. 1964). The media of an elastic artery is composed of a series of concentric elastic lamellae connected by obliquely orientated smooth muscle fibres. The elastic properties of conduit arteries have been well described. The relationship between the elastic modulus and distending pressure is non linear which is a consequence of having both collagen and elastin components in the wall (Roach et al. 1959). The histologic evidence (Wolinsky et al. 1964) from arterial sections fixed at various distending pressure suggests that as the pressure rises an increasing amount of stress is "unloaded" from the elastin to the collagenous components. It is interesting to note that contraction of smooth muscle in the media actually increases the distensibility of the wall (Dobrin et al. 1969). Also the elastic modulus rises along the aorta and its major branches due to the relative rise in collagen content and fall in relative elastin content of the arterial wall (Cleary 1963).
b) Procedure:

The subjects undressed to their underclothes and then rested on the examination couch for 15 minutes in the supine position. Room temperature was controlled at 20 ± 2°C. After a 15 minute rest period, the blood pressure was recorded on two or more occasions until it had stabilised, i.e. the systolic and diastolic pressure had not changed by more than 4 mmHg.

The transit time was measured by taking simultaneous readings on the spectrascan from two probes and printing the output at a fast paper speed (200 mm/second) to stretch the time sequence. With a known paper speed, the distance could be converted to time and be divided into the arterial path length to calculate the pulse wave velocity.

The left subclavian artery was insonated in the supraclavicular fossa as it passed over the first rib. The patient was then asked to hold the probe steady with their hand. The left subclavian to common femoral artery tracings were then recorded simultaneously at a chart speed of 200 mm/second onto the U.V. paper for analysis. It is important to note that it was not necessary to obtain clear maximum frequency pictures for the measurement of the transit time; only the clarity of the initial forward flow component was important. This could be obtained by manipulating the patient-held probe until a clear picture was obtained, and allowing them to maintain this position for approximately 30 seconds while recording the transit time for each pulse. After recording the transit times, the arterial path lengths were measured using a tape measure. This was able to be done after
all the Doppler tracings were completed because the slight pressure of applying the probes left a slight indentation on the skin which remained visible for between five and ten minutes after insonation.

c) Calculation

The length of left Subclavian Artery (LSA) down to the Common Femoral Artery (m).

Pulse wave velocity (P.W.V.)

The time difference between two Doppler shifted signals (sec)

Arterial compliance = \frac{1}{(P.W.V.)^2} \times 66.7 \text{ (Gosling, 1976)}

Example: LSA \rightarrow LCF 57.5\text{cm} = 0.575\text{m}

Time difference = (0.072+0.072+0.071+0.071+0.073) \text{ Sec} \div 5
= 0.0718

P.W.V. = 0.575/0.0718 = 8.01\text{m/sec.}

AC = 1/8.01^2 \times 66.7 = 1.04

d) Reproducibility and coefficient Variation (C.V%) of Arterial Compliance (AC. Mean \pm S.E.)
TABLE 2.1: Coefficient of Variation (C.V.%) of Arterial Compliance (Mean ± S.E.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Arterial compliance (AC)</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.72 0.79 0.72</td>
<td>0.74</td>
<td>0.040</td>
<td>5.5</td>
</tr>
<tr>
<td>B</td>
<td>0.32 0.38 0.36</td>
<td>0.35</td>
<td>0.031</td>
<td>8.7</td>
</tr>
<tr>
<td>C</td>
<td>0.35 0.35 0.38</td>
<td>0.36</td>
<td>0.017</td>
<td>4.8</td>
</tr>
<tr>
<td>D</td>
<td>0.27 0.28 0.30</td>
<td>0.28</td>
<td>0.015</td>
<td>5.5</td>
</tr>
<tr>
<td>E</td>
<td>0.23 0.25 0.25</td>
<td>0.24</td>
<td>0.012</td>
<td>4.8</td>
</tr>
<tr>
<td>F</td>
<td>0.41 0.50 0.48</td>
<td>0.46</td>
<td>0.047</td>
<td>10.2</td>
</tr>
<tr>
<td>G</td>
<td>0.61 0.59 0.60</td>
<td>0.60</td>
<td>0.010</td>
<td>1.7</td>
</tr>
<tr>
<td>H</td>
<td>0.30 0.33 0.36</td>
<td>0.33</td>
<td>0.030</td>
<td>9.1</td>
</tr>
<tr>
<td>I</td>
<td>0.34 0.39 0.38</td>
<td>0.37</td>
<td>0.027</td>
<td>7.2</td>
</tr>
<tr>
<td>J</td>
<td>0.68 0.70 0.66</td>
<td>0.68</td>
<td>0.020</td>
<td>2.9</td>
</tr>
<tr>
<td>K</td>
<td>0.61 0.71 0.72</td>
<td>0.68</td>
<td>0.061</td>
<td>9.0</td>
</tr>
<tr>
<td>L</td>
<td>0.69 0.79 0.66</td>
<td>0.70</td>
<td>0.068</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Mean ± S.E. 6.6± 0.80%

From Table 2.1 we can see the range of C.V.% was 1.7% - 10.2%, mean ± S.E. was 6.6 ± 0.80%. The reproducibility was relatively high.
2.3.3 Arterial Proximal Resistance (PR)

a) Principle:

For normal circulation the blood velocity/time waveform has been shown, theoretically, to be described in the frequency domain by three poles:

\[ s = -\alpha, \quad s = -\alpha + j\beta, \quad \text{and} \quad s = -\alpha - j\beta, \quad \text{where:} \]

\[ \beta = \sqrt{\frac{\omega_0^2 - \alpha^2}{N}} \]

\[ \omega_0 \propto \text{stiffness} \]

\[ \alpha \propto \frac{1}{(\text{proximal radius})^2} \quad (2.8) \]

\[ r \propto \frac{1}{(\text{distal radius})^2} \]

The arterial heart - common femoral provides a flow waveform at the common femoral artery which may be described, in the frequency domain, by three poles and one zero, if the visco-elastic effect is taken into account. The position of the poles predict the effect of stiffness, proximal lumen diameter and distal independence. Characterisation of the Doppler waveform shape by a minimum of parameters reflecting the underlying physiological influence was obtained (Skidmore et al. 1980; Clifford et al. 1982). By regarding the arterial pulse as an independent and transient phenomenon it is possible to obtain its Fourier transform and, by computer aided curve fitting techniques, the equivalent Laplace transform. This Laplace transform has been shown to be of the form:
\[
\text{Laplace Transform } (G) = \frac{1}{(S^2 + 2 \delta \omega S + \omega^2)(S + \gamma)} \quad (2.9)
\]

It can be expressed graphically (fig. 2.3)

where: \( \mathcal{R} \) = A function of proximal resistance or proximal lumen diameter. \( \mathcal{R} = \cos \theta \).

It is related to the degree of proximal stenosis.

\( \gamma \) = A function of peripheral resistance of distal impedance (real point). It is related to the degree of vasoconstriction/vasodilatation of the peripheral vascular bed distal to the Doppler probe.

\( \omega_0 \) = A function of arterial wall elasticity or stiffness. It is proportional to the elastic modulus.

\( S = j \omega \). Imaginary number \( j = \sqrt{-1} \).
Fig 2.3: Laplace Transform Damping (Argand diagram). The solutions of the transfer function equation are displayed on an argand diagram. LT Damping $\delta$ is calculated from $\cos \theta$. $\delta$ relates to arterial wall stiffness and $r$ to peripheral resistance.

(from Clifford et al. 1982)
b) Procedure:

Studies were performed in an electrically screened, temperature controlled (20±20C) laboratory after a 15 minute equilibration period. Common femoral artery and posterior tibial artery signals were obtained from supine subjects using a 8MHz continuous wave directional Doppler flowmeter. To avoid artefacts associated with zero-crossing signal processing, the blood maximum velocity/time waveform was obtained using a maximum frequency follower constructed in the laboratory. These signals were analysed by a Medishield Doppler spectra scan Mark II and a Data General Nova II computer.

c) Calculation.

d) Reproducibility and Coefficient of Variation (C.V.% of proximal resistance.)
TABLE 2.2: Coefficient of Variation (C.V.%) of Proximal Resistance of Posterior Tibial Artery (Mean \(\pm\) S.E.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Damping Coefficient</th>
<th>Mean</th>
<th>S.D</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.54</td>
<td>0.60</td>
<td>0.64</td>
<td>0.59</td>
</tr>
<tr>
<td>B</td>
<td>0.45</td>
<td>0.50</td>
<td>0.52</td>
<td>0.49</td>
</tr>
<tr>
<td>C</td>
<td>0.47</td>
<td>0.54</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>D</td>
<td>0.65</td>
<td>0.62</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>E</td>
<td>0.39</td>
<td>0.42</td>
<td>0.44</td>
<td>0.42</td>
</tr>
<tr>
<td>F</td>
<td>0.33</td>
<td>0.38</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>G</td>
<td>0.71</td>
<td>0.75</td>
<td>0.60</td>
<td>0.69</td>
</tr>
<tr>
<td>H</td>
<td>0.62</td>
<td>0.72</td>
<td>0.68</td>
<td>0.67</td>
</tr>
<tr>
<td>I</td>
<td>0.38</td>
<td>0.42</td>
<td>0.33</td>
<td>0.38</td>
</tr>
<tr>
<td>J</td>
<td>0.38</td>
<td>0.37</td>
<td>0.34</td>
<td>0.36</td>
</tr>
<tr>
<td>K</td>
<td>0.47</td>
<td>0.49</td>
<td>0.46</td>
<td>0.47</td>
</tr>
<tr>
<td>L</td>
<td>0.66</td>
<td>0.60</td>
<td>0.60</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Mean: 7.1
\(\pm\): +
S.E: 0.80

From Table 2.2 we can see the range of C.V.% was 2.4% - 11.9%
Mean \(\pm\) S.E. was 7.1 \(\pm\) 0.80%. The reproducibility is relatively high.
Table 2.3: Coefficient of Variation (C.V.% of Proximal Resistance of Common Femoral Artery (Mean + S.E.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Damping Coefficient</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.29</td>
<td>0.33</td>
<td>0.34</td>
<td>0.32</td>
</tr>
<tr>
<td>B</td>
<td>0.28</td>
<td>0.32</td>
<td>0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>C</td>
<td>0.31</td>
<td>0.32</td>
<td>0.34</td>
<td>0.32</td>
</tr>
<tr>
<td>D</td>
<td>0.38</td>
<td>0.40</td>
<td>0.36</td>
<td>0.38</td>
</tr>
<tr>
<td>E</td>
<td>0.45</td>
<td>0.40</td>
<td>0.46</td>
<td>0.44</td>
</tr>
<tr>
<td>F</td>
<td>0.48</td>
<td>0.48</td>
<td>0.42</td>
<td>0.46</td>
</tr>
<tr>
<td>G</td>
<td>0.32</td>
<td>0.37</td>
<td>0.39</td>
<td>0.36</td>
</tr>
<tr>
<td>H</td>
<td>0.55</td>
<td>0.50</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>I</td>
<td>0.43</td>
<td>0.40</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td>J</td>
<td>0.32</td>
<td>0.30</td>
<td>0.36</td>
<td>0.33</td>
</tr>
<tr>
<td>K</td>
<td>0.22</td>
<td>0.24</td>
<td>0.24</td>
<td>0.23</td>
</tr>
<tr>
<td>L</td>
<td>0.34</td>
<td>0.30</td>
<td>0.30</td>
<td>0.31</td>
</tr>
<tr>
<td>M</td>
<td>0.32</td>
<td>0.32</td>
<td>0.30</td>
<td>0.31</td>
</tr>
<tr>
<td>N</td>
<td>0.32</td>
<td>0.32</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>O</td>
<td>0.28</td>
<td>0.27</td>
<td>0.29</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Mean 6.8  
+ +  
S.E. 0.60%

From Table 2.3 we can see the range of C.V.% was 3.2-10.0%. Mean + S.E was 6.8 + 0.60%. The reproducibility is relatively high.
2.4 **Biochemical Indices**

2.4.1 Blood collection

a) Each subject was asked not to eat or drink anything after 8.00 p.m. on the night before the blood test. No breakfast, tea or coffee was allowed in the morning but a glass of water could be taken if necessary.

b) The glucose drink used was "TRUTOL" (100g/100ml). The venoject system was used for all blood collection. This consisted of a sterilised bevelled edge needle which screwed into a plastic barrel. The collection tubes were all evacuated and consisted of:

1 red top tube for total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride estimation

2 grey top tubes containing anticoagulant potassium oxalate/sodium fluoride for fasting, 1 hour and 2 hour glucose estimation;

3 green top tubes containing anticoagulant lithium heparin for fasting, 1 hour and 2 hour free fatty acid estimation;

3 green top tubes containing lithium heparin for fasting, 1 hour and 2 hour insulin estimation

1 purple top tube containing E.D.T.A. for glycosylated haemoglobin estimation.

c) On arrival each subject was asked if they had followed the fasting procedure. Only two had failed to fast as requested, and had to return several days later for blood tests. The first blood sample was collected at 8.30 - 9.00 a.m. when the subjects had been sitting for at least 15 minutes. A fasting blood sample was collected at 8.30 - 9.00 a.m. for total cholesterol,
HDL-cholesterol, LDL-cholesterol, triglyceride, glycosylated haemoglobin, glucose, free fatty acid and insulin estimation. Immediately after collection the subjects were given a 75g glucose loading orally and hourly blood samples were collected for the next two hours for glucose, free fatty acid and insulin estimation. All the blood samples were put into ice as soon as possible. The total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glycosylated haemoglobin and glucose were estimated within 12 hours. The free fatty acid and insulin were estimated within two weeks and stored at -20°C before estimation.

2.4.2. Free fatty acids (Laurell et al. 1967; Felix et al. 1973)

a) Principle

\[
2\text{FFA} + \text{Cu}^{2+} \xrightarrow{\text{CHCl}_3} \text{FPA} + \text{Cu} \xrightarrow{\text{Diphenocarbazide}} \text{Red colour (550 nm)}
\]

b) Reagent

2. Extract mixture.
   chloroform 49: N-heptane 49: Methanol 2
3. pH 6.4 butter solution (M/30)
   M/30 KH₂PO₄: KH₂PO₄ 4.54 g dissolve in 1000 ml.
   deionized water
   M/30 Na₂HPO₄: Na₂HPO₄ .12H₂O 11.94g dissolve in 1000 ml. deionized water.
   M/30 KH₂PO₄ 73.3 ml. + M/30 Na₂HPO₄ 26.7, mix well.
4. Copper reagent: This consists of 0.5M Cu(NO3)2·3H2O 10 ml, 2.0M triethanolamine 5 ml, and diluted to 100 ml. with saturated NaCl, used within a week.

5. Diphenocarbazide solution: This consists of Diphenocarbazide C6H5.NH2CONH.C6H5 40 mg, 0.1M triethanolamine 0.2 ml, and ethanol 10 ml, used immediately.

6. Palmitic acid standard solution (stock) 2000 μmol/L. Palmitic acid 51.3 mg, dissolve in 100 ml, extract mixture.

7. Working standard: 500 μmol/L.
c) Procedure.

1. Label tubes in triplicate as follows:

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Q.C. Samples</th>
<th>Q.C. (low)</th>
<th>Q.C. (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>(ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Palmitic acid (500 μmol/L)
- 0.050 0.100 0.150 0.200 0.300 - - -

Plasma
- - - - - 0.050 0.100 0.100

Deionized Water
0.300 0.250 0.200 0.150 0.100 - 0.250 0.200 0.200

Buffer solution
1.00 1.00 1.00 1.00 1.00 1.00 1.00 1.00

Extract mixture
6.0 6.0 6.0 6.0 6.0 6.0 6.0 6.0 6.0
2. Stopper them and mix well, shake vigorously, vortex for 1 minute. Centrifuge for 10 minutes at 3000 x g.

3. Remove upper part aqueous and protein, take lower part extracted solution 5.0ml.

4. Lower part solution 5.0ml.
   Copper reagent 2.0ml.

5. Stopper again and mix well, shake vigorously, vortex for 1 minute. Centrifuge for 10 minutes at 3000 x g.

6. Take upper part extracted solution 3.0ml., add diphenocarbazide solution 0.5ml. Mix well, wait for 15 minutes.

7. Read the absorbance at 550nm (VS a blank set at zero absorbance).

d). Reproducibility and Coefficient of Variation (C.V.%) of quality control

<table>
<thead>
<tr>
<th>Q.C.</th>
<th>n</th>
<th>Mean (µmol/L)</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>24</td>
<td>685</td>
<td>19.5</td>
<td>2.8</td>
</tr>
<tr>
<td>High</td>
<td>24</td>
<td>1317</td>
<td>41.2</td>
<td>3.1</td>
</tr>
</tbody>
</table>
2.4.3 Insulin (Albano et al. 1972)

a) Principle

\[
\begin{align*}
\text{Insulin in plasma} & \quad \text{Insulin binding reagent (Ab)} \\
\text{or} & \quad \text{Porcine insulin antiserum or}
\text{standard insulin} & \quad \text{guinea pig globulin antiserum}
\end{align*}
\]

\[
\begin{align*}
\text{AgAb} & \quad + \quad \text{125I-insulin} & \quad (\text{Ag}^*_{\text{c}}) \\
\text{Incubated at 40C} & \quad \text{for 65-72 hours}
\end{align*}
\]

\[
\begin{align*}
\text{Ag}^*_{\text{c}} \quad \text{Ab} & \quad + \quad \text{AgAb} \\
\text{Charcoal separation} & \quad \text{and} \\
\text{centrifuge}
\end{align*}
\]

\[
\begin{align*}
\text{Ag}^*_{\text{c}} \quad \text{Ab} & \quad + \quad \text{AgAb} \\
\text{Supernatent} & \quad \text{Precipitate or charcoal} \\
& \quad \text{sediment}
\end{align*}
\]
The essential principle is the reaction of a limited, fixed amount of specific antibody with a mixture of the sample to be assayed and a constant amount of radioactively labelled pure antigen. After the reaction has been allowed to approach completion (65-72 hours incubation at 40°C), the antibody-bound antigen is separated from free antigen and the distribution of radioactivity is determined.

b) Reagents.

   Base Buffer - 29.428 g Na Barbitone
   19.428 g Na Acetate
   0.1 g Thiomersolate
   in 1 L deionized distilled water
   Store at 40°C
   Assay Buffer - 100 ml base buffer
   1900 ml distilled water
   15.4 g NaCl
   6.0 g BSA (Bovine serum albumin)
   pH to 7.4

2. Antibody:

   Wellcome Anti-Insulin Serum (Commercially available - Wellcome Reagents Ltd.)

   Vials containing 0.5 ml freeze-dried guinea-pig antiserum (1:100 dilution) + 2ml H and L Buffer --> 1:400 dilution (can be stored frozen at this strength).

   Working strength: 2ml, storage Ab + 20ml buffer --> 1:44,000 dilution.
3. **Tracer 125labelled Insulin**

   Usually diluted to give approximately 8000 cpn/100 μl (= 0.05 ng/tube). About 50 μl tracer + 80ml buffer.

4. **Insulin Standards:** Weigh out 2mg NOVO crystalline insulin, add

   200 μl 1 M HCl, 800 μl distilled water, 1ml H & L buffer (2mg/2ml) = (A)

   0.100ml (A) + 4.90ml buffer (20,000ng/ml = 500mU/ml) = (B)

   **Insulin storage standard** = 0.05ml (B) --> 2ml buffer

   (10 mU/ml) = (C) (store in 0.5ml aliquots)

   **Insulin working standard** = 250 μl (C) + 20ml buffer

   (12.5 mU/ml) = (D)

   0.2ml (D) + 9.8ml buffer = 2.5 mU/ml 0.1 + 4.9
   0.4ml  + 9.6ml buffer = 5 mU/ml 0.2 + 4.8
   0.8ml  + 9.2ml buffer = 10 mU/ml 0.4 + 4.6
   1.2ml  + 8.8ml buffer = 15 mU/ml 0.6 + 4.4
   1.6ml  + 8.4ml buffer = 20 mU/ml 0.8 + 4.2
   2.4ml  + 7.6ml buffer = 30 mU/ml 1.2 + 3.8
   3.2ml  + 6.8ml buffer = 40 mU/ml 1.6 + 3.4
   4.8ml  + 5.2ml buffer = 60 mU/ml 2.4 + 2.6
   6.4ml  + 3.6ml buffer = 80 mU/ml 3.2 + 1.8

   Store frozen (-20°C) in 0.5ml aliquots
5. Charcoal solution:

5g activated charcoal
0.5g Dextran T70
in 1 L Herbert & Lau buffer (no BSA)
Solution to be made up and stirred on a magnetic stirrer for at least one hour prior to use.
Store at 4°C

6. Insulin Free Plasma:

1g activated charcoal added to every 10ml plasma.
Leave to stir on magnetic stirrer for > 2 hours, preferably overnight at 4°C. Centrifuge 15min., 3000 rpm, 4°C. Repeat spin if still very cloudy.

Freeze plasma for 24 hours (charcoal sticks to fibrinogen). Next day thaw, centrifuge, 10,000 rpm (sorvall). Store frozen in 10ml aliquots.
c) Procedure.

Set up tubes as follows:

<table>
<thead>
<tr>
<th>TUBE NO.</th>
<th>BUFFER</th>
<th>INSULIN</th>
<th>IPP</th>
<th>Ab</th>
<th>TRACER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Total count</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100 μl</td>
</tr>
<tr>
<td>4-6</td>
<td>Non-Specific</td>
<td>600</td>
<td>-</td>
<td>100</td>
<td>100 μl</td>
</tr>
<tr>
<td>7-9</td>
<td>0</td>
<td>500</td>
<td>-</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>10-12</td>
<td>2.5 IU/ml</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>13-15</td>
<td>5</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>16-18</td>
<td>10</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>19-21</td>
<td>15</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>22-24</td>
<td>20</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>25-27</td>
<td>30</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>28-30</td>
<td>40</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>31-33</td>
<td>60</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>34-36</td>
<td>80</td>
<td>400</td>
<td>100</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>37-38</td>
<td>H1</td>
<td>500</td>
<td>50</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>39-40</td>
<td>LI</td>
<td>500</td>
<td>100</td>
<td>-</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>41-42</td>
<td>H1</td>
<td>500</td>
<td>50</td>
<td>100</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>43-44</td>
<td>LI</td>
<td>500</td>
<td>100</td>
<td>-</td>
<td>100 100 μl</td>
</tr>
<tr>
<td>45-?</td>
<td>Unknown</td>
<td>500</td>
<td>100</td>
<td>-</td>
<td>100 100 μl</td>
</tr>
</tbody>
</table>
2. Day 1

Tubes are set up according to table. Buffer and insulin (standard or sample) are added using diluter. Antibody and tracer are added using Hamilton automatic dispenser. Final volume should be 800 μl. Tubes are vortexed and left to incubate for 72 hours at 4°C.

3. Day 4

1ml charcoal suspension is pipetted into each tube (excluding 1-3) and the tubes vortexed. 20 minutes after adding charcoal to the first tube, they are centrifuged at 2,800 rpm for 20 mins at 4°C (Centra 7R). The supernatant is decanted into 10ml (flat bottom) Johns tubes, corked and counted.

NB At all stages charcoal separation should be on ice or at 4°C.

4. Counting

1. The time for counting should be sufficient to allow 10,000 counts difference between total binding and nonspecific binding points (Packard Gamma Counter).
2. When counting make sure the paper tape print-out is operating.
3. Run the paper tape through the computer (Tape #/).
4. Write up assay sheets and enter information into insulin RIA quality control book.
d) Reproducibility and Coefficient Variation (C.V.%) of Quality Control

<table>
<thead>
<tr>
<th>Q.C.</th>
<th>n</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(AU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
<td>10.1</td>
<td>0.73</td>
<td>7.3</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>101.2</td>
<td>7.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

2.4.4 Blood glucose (Passey et al. 1977; Spencer et al. 1978)

a) Principle:

Glucose is measured by the use of an oxygen-sensing electrode and associated electronics that determine the rate of oxygen consumption, resulting in the following series of reactions:

\[
\beta-D-\text{Glucose} + O_2 \xrightarrow{\text{oxidase}} \text{D-glucono-\delta-lactone} + H_2O_2
\]

\[
H_2O_2 + \text{ethanol} \xrightarrow{\text{catalase}} \text{acetaldehyde} + H_2O
\]

\[
H_2O + 2H^+ + 2I^- \xrightarrow{\text{molybdate}} I_2 + 2H_2O
\]

The electrode measures the rate of oxygen consumption in the first reaction while the hydrogen peroxide is captured in the last two reactions.
b) Instrument:

Yellow Spring Instruments Model 23AM - Glucose Analyzer.

c) Coefficient variation was 2.3% at 18.4 mmol L⁻¹, n = 44

2.4.5 Glycosylated haemoglobin (HbA₁C). (Gabbay 1977; Hammans et al. 1982; Lee et al. 1982)

a) Principle:

The principle described by the manufacturers reads as follows:

"The negatively charged resin exhibits an affinity for positively charged molecules. At selected ionic strength and pH, the glycosylated haemoglobins are less positively charged than haemoglobin A. Therefore, the former components bind to the negatively charged resin less tightly than haemoglobin A. With the application of a first developing buffer, Fast Fraction (FF) Developer, the glycosylated haemoglobins are eluted, while the other haemoglobin components are retained. This fraction may be compared to a Total Fraction (TF), or, with the application of a second developing buffer, Slow Fraction (SF) Developer, the remaining haemoglobins (the majority of which is haemoglobin A) are eluted. Following elution the Fast Fraction is compared to the slow Fraction by reading the absorbance of each on a spectrophotometer and calculating the glycosylated haemoglobin percentage."
b) Reagents:

The reagents described by the manufacturers reads as follows:

"The Helena Glycosylated (Fast Fraction) Haemoglobin Quik Column Kit. (Cat. No. 5340) contains 50 Quik Columns prepacked with at least 300 milligrams/column of cation exchange resin equilibrated in phosphate buffer to pH 6.70 with 0.065% KCN. Each kit also contains one bottle of Fast Fraction (FF) Developer (100mL) elution buffer with 0.065% KCN; one bottle of Slow Fraction (SF) Developer (100 mL) elution buffer with 0.065% KCN; one bottle (20 ml) of Hemolysate Reagent-C. All components of the Glycosylated Hemoglobin Quik Column Kit are FOR IN-VITRO DIAGNOSTIC USE ONLY. All components of the kit should be stored at room temperature. The Quik Columns and all reagents are stable for twelve months stored at room temperature.

"The Quik Columns and all reagents for the quantitation of glycosylated haemoglobins should be used in a well ventilated laboratory area. Do not mouth pipette any QUIK column reagents as they contain KCN."

c) Procedure:

Sample collection and preparation. The procedure described by the manufacturers reads as follows:
Whole blood samples should be collected in EDTA and quantitated fresh. Specimens for quantitation should be prepared as follows:

i) Place 20 µl of whole blood collected in EDTA into a small laboratory test tube. A 10-50 µl variable volume Quickvette (Cat. No. 5142) may be used for this step.

ii) Add 300 µl of Hemolysate Reagent-C to the test tube. A 250-1000 µl Variable Volume Quickvette (Cat. No. 5144) may be used for this step.

iii) Vigorously shake the tube to insure complete hemolysis of the sample. Complete lysis is absolutely essential for proper results using the Quik Column.

iv) Let sample(s) stand 5 minutes prior to use. The total volume of each sample = 320 µl.

Total fraction (TF) method.

The procedure described by the manufactures reads as follows:

"All columns and reagents should be at room temperature before performing the assay. The assay should be performed at room temperature of 21-24°C (70 to 74°F) otherwise a temperature correction should be made. (See Temperature Conversion Chart).

i) Place one Quik column per patient quantitation in the column rack. Place one small collection tube (12 x 75mm) and one large collection tube (16 x 125mm) in the rack for each Quik column."
ii) Remove the top cap closure from each column and completely resuspend the contents of the column using a Pasteur Pipette. Remove the bottom cap closure immediately and let the supernatant drain into a small laboratory test tube or directly into a sink. After 5-10 minutes the supernatant will have eluted completely and should be discarded at this point. Align the Quik column over a small collection tube in the column rack. Note: It is very important to completely resuspend the resin and to remove the bottom cap closure quickly in this step of the procedure. Failure to do either step will cause slow flow problems during the performance of the test."

iii) Load 100 µl of sample preparation onto a Quik column using a 100 µl Quickpette (Cat. No. 5164) and immediately add 100 µl of the same preparation to a large collection tube using the same Quickpette. QS the tube to 15ml. This fraction becomes the total fraction (TF).

iv) Following the absorption of the sample into the resin bed, apply 1.5 ml of FF buffer to the column. After the complete elution of the FF, QS the small collection tube to 3 ml. This becomes the FF tube. Invert each tube to mix contents thoroughly.

v) Determine the G-HbX per sample using mode B of the Hemospec or read the absorbance (O.D.) of the contents of both the FF tube and the TF tube. See Results Section.

The contents of the collection tubes are stable for 24 hours.
d) Results:

Determination of test results using the Hemespec. Following the Hemespec Instruction Manual, each small and large collection tube per sample is placed in the unit. Mode A is used for the FF technique (See Alternate Method Section) and Mode B is used for the TF technique. Once the collection tubes are in place, close the lid of the Hemespec. The instrument computes the G-Hb\% and displays the results automatically.

Determination of test results using a standard laboratory spectrophotometer: The following formula should be used to calculate the glycosylated haemoglobin (G-Hb) percent for each sample using the TF method.

\[
\text{O.D. of FF tube} \times 100 = \text{G-Hb\%} \\
5(\text{O.D. of TF tube})
\]

In the formula:

\(\text{G-Hb\%} = \text{percentage of glycosylated haemoglobin in the sample.}\)

\(\text{O.D. of FF Tube} = \text{optical density (absorbance) of the contents of the fast fraction collection tube at a wavelength of 415 nanometers.}\)

\(\text{O.D. of TF Tube} = \text{optical density (absorbance) of the contents of the total fraction collection tube at a wavelength of 415 nanometers.}\)
5 = dilution factor (15 ml of TF tube / 3 ml of FF tube = 5)
100 = percentage conversion factor.
Example: A sample yielding O.D. values of 0.140 for the FF tube and 0.411 for the TF tube is found to have a G-Hb value of 6.8% using the above formula to perform the calculation.

\[
\frac{0.140}{5(0.411)} \times 100 = 6.8\%
\]

d). Coefficient of variation was 7.1% at 6.7% HbA1C.
\( n = 46 \)

2.4.6 Serum total cholesterol (Allain et al. 1974)

a) Principle:

The cholesterol level is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase.

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{fatty acids}
\]

\[
\text{cholesterol} + \text{O}_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestene-3-one} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 4\text{H}_2\text{O}
\]
b) **Reagents:** Human cholesterol liquicolor (enzymatic colourimetric test, Cat. No H-8002).

   i) 4-Aminoantipyrine \( \geq 0.25 \text{ mmol/l} \)
   ii) phenol \( \geq 25 \text{ mmol/l} \)
   iii) peroxidase \( \geq 5 \text{ U/ml} \)
   iv) cholesterolesterase \( \geq 0.15 \text{ U/ml} \)
   v) cholesteroloxidase \( \geq 0.1 \text{ U/ml} \)

   c) **Procedure:** in separate cuvettes pipette.

<table>
<thead>
<tr>
<th>Reagent blank (( \mu l )) Sample (( \mu l ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Reagent</td>
</tr>
</tbody>
</table>

Mix, incubate for 20 minutes at room temperature or 10 minutes at 37°C. Measure the absorbance of the sample against the reagent blank within 60 minutes (\( \Delta E \)). If the test is carried out at 37°C, the samples must be cooled to room temperature after the incubation time (10 minutes).

d) **Coefficient of variation** was 1.6% at 9.2 mmol.L\(^{-1} \) and was 2.1% at 4.8 mmol.L\(^{-1} \).

2.4.7 **Serum high density lipoprotein cholesterol** (HDL-cholesterol) (Allen et al. 1979)

a) **Principle:** Polyethylene Glycol AV M.W. 6000 (PEG) precipitate followed by enzymatic cholesterol determination on ABA-100 Bichromatic Analyzer.
b) Reagents.

i) Polyethylene Glycol Av M.W. 6000 (PEG) (BDH or Merck) - 200 g/L.
   Weigh 2.0 g PEG into a 10ml tube.
   Add 8ml of distilled water and dissolve — make to 10ml
   with additional distilled water and mix.
   Stable 2 - 8oC for 14 days.

ii) Other reagents: as the same as total cholesterol
    analysis.

c) Procedure.: Pipette 1 vol. (250 µl) of serum (or
    plasma) into a clear polystyrene centrifuge tube and
    add an equal volume (250 µl) of PEG solution, using a
    positive displacement pipette, e.g. SMI.
    Mix immediately.
    Let stand 10-30 minutes (not critical) at room
    temperature and centrifuge at approximately 2000g for
    5-10 minutes.
    Remove the CLEAR supernatant (check each tube) and
    analyse for cholesterol.
    Recommended sample to reagent ratio is 1:11 to 1:21 (no
    lower).

d) Coefficient of variation was 3.3% at 0.92 mmol.L⁻¹.
2.4.8. Serum Triglycerides (McCowan et al. 1983).

a) Principle: The triglycerides are determined after enzymatic hydrolysis with lipases. Indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminooantipyrine and 4-chlorophenol under the catalytic influence of peroxidase.

Reaction Principle:

\[
\text{Triglycerides} \xrightarrow{\text{lipases}} \text{glycerol + fatty acids}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate + ADP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone-phosphate + H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-aminooantipyrin} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCl} + 4\text{H}_2\text{O}
\]

b) Reagent composition.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-aminooantipyrine</td>
<td>0.4 mmol/l</td>
</tr>
<tr>
<td>4-chloro-phenol</td>
<td>5.0 mmol/l</td>
</tr>
<tr>
<td>APT</td>
<td>1.0 mmol/l</td>
</tr>
<tr>
<td>Magnesium-ions</td>
<td>5.0 mmol/l</td>
</tr>
<tr>
<td>Lipases</td>
<td>150 U/ml</td>
</tr>
<tr>
<td>Glycerol-kinase</td>
<td>0.4 U/ml</td>
</tr>
<tr>
<td>Glycerol-3-phosphate oxidase</td>
<td>1.5 U/ml</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>0.5 U/ml</td>
</tr>
<tr>
<td>PIPES buffer solution (pH 7.5)</td>
<td>40 mmol/l</td>
</tr>
</tbody>
</table>
c) Procedure:

<table>
<thead>
<tr>
<th>Pipette into Cuvettes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Blank</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Reagent</td>
</tr>
</tbody>
</table>

Mix, incubate for 10 minutes at 20-25°C or 5 minutes at 37°C. Measure the absorbance of the sample against the reagent blank within 60 minutes.

(E_{Sample} - ERB = ΔE_{Sample})

Calculation of the triglycerides concentration

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>c (mg/dl)</th>
<th>c (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>546 nm</td>
<td>1048 x ΔE_{Sample}</td>
<td>11.95 x ΔE_{Sample}</td>
</tr>
<tr>
<td>500 nm</td>
<td>743 x ΔE_{Sample}</td>
<td>8.47 x ΔE_{Sample}</td>
</tr>
</tbody>
</table>

d) Coefficient of variation was 3.6% at 2.15 mmol.L^{-1}
2.5 **Food Intake Documentation**

Each subject (NIDD and controls) completed a seven day dietary record during the week after examination. A clinical nutritionist met with each subject to review the diet record and clarify information necessary for data coding. Food models were used to estimate quantities and types of food consumed. The seven days recorded were coded for a computerised data base. The development of food indices and scores for food are according to biological classification of food is described in chapter 7.

2.6 **Statistical Methods**

For group comparisons, student's T-test was used as a parametric test, and the Mann-Whitney U-test in non-parametric evaluation of group's differences.

Univariate relationships were examined by Pearson's correlation analysis and linear regression analysis for parametric evaluation and, where appropriate, Spearman's rank correlation coefficient ($r_s$) for non-parametric evaluation.

Multivariate analysis was performed using stepwise regression available in SPSS (statistical package for the Social Sciences).
CHAPTER THREE

GENERAL CHARACTERISTICS OF HEALTHY SUBJECTS AND
NON-INSULIN-DEPENDENT DIABETICS

3.1 Introduction

The study, by design, minimised the effect of other
potential risk factors, such as age, body mass, body
mass index, blood pressure and smoking on arterial
compliance and arterial proximal resistance of common
femoral artery and posterior tibial artery for both the
apparently healthy subjects and non-insulin-dependent
diabetics.

This chapter will compare the difference of blood
glucose, glycosylated haemoglobin (HbA1C), plasma free
fatty acids, plasma insulin and lipids between healthy
subjects and non-insulin-dependent diabetics.

3.2 Subjects and Methods

3.2.1 Subjects

The study was approved by the Ethics Committee of
Prince Henry’s Hospital in accordance with the
statement on human experimentation by the National
Health and Medical Research Council of Australia.
Informed consent was obtained.
38 Healthy subjects or controls (10 men and 28 women) and 27 (13 men and 14 women) age, height and weight matched non-insulin-dependent diabetics, were studied. None of the subjects were smokers or hypertensive. No subject had any symptoms of peripheral arterial disease, coronary artery disease or cerebrovascular disease. All of them had apparently normal peripheral pulses and all had normal ankle/arm blood pressure indices.

3.2.2 Methods

In each subject, fasting blood was collected. Immediately after collection, a 75g oral glucose tolerance test was given and blood collected at one and two hours. All blood samples were analysed for glucose, glycosylated haemoglobin (HbALC), insulin, free fatty acid, total cholesterol, HDL-cholesterol and triglyceride concentrations as described in chapter 2.

3.3 The Age, Stature, Body Weight, Body Mass Index (BMI) and Blood Pressure (2) of the Healthy Subjects and Non-Insulin-Dependent Diabetics (NIDD)
### Table 3.1
The age, stature, body weight, body mass index (BMI) and blood pressure (BP) of healthy subjects and NIDD (Mean ± S.D.)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Sex</th>
<th>Age</th>
<th>Stature (cm)</th>
<th>Body Weight (kg)</th>
<th>BMI</th>
<th>BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>10</td>
<td>M</td>
<td>62 ± 5</td>
<td>171 ± 2</td>
<td>77 ± 4</td>
<td>26 ± 1</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>NIDD</td>
<td>13</td>
<td>M</td>
<td>61 ± 3</td>
<td>170 ± 2</td>
<td>79 ± 4</td>
<td>27 ± 1</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Healthy</td>
<td>28</td>
<td>F</td>
<td>69 ± 2</td>
<td>157 ± 1</td>
<td>66 ± 3</td>
<td>27 ± 1</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>NIDD</td>
<td>14</td>
<td>F</td>
<td>69 ± 2</td>
<td>157 ± 2</td>
<td>70 ± 3</td>
<td>28 ± 1</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates that healthy subjects are not significantly different from diabetics (P > 0.05).

1. **BMI** = \( \frac{W}{H^2} \)
2. **Blood Pressure (BP)**
   \[ \text{BP} = \text{Systolic blood pressure} + 2 \times \text{diastolic blood pressure} \]
3.4 The Blood Glucose and Glycosylated Haemoglobin (HbAlC) of Healthy Subjects and Non-Insulin-Dependent Diabetics

3.4.1 Blood glucose

There was a significant increase in the fasting blood glucose and blood glucose area (3) in the NIDD group compared with controls. The mean fasting blood glucose was $10.3 \pm 1.0 \text{mmol.L}^{-1}$ and $5.2 \pm 0.2 \text{mmol.L}^{-1}$ for male ($P<0.001$) and was $10.1 \pm 1.1 \text{mmol.L}^{-1}$ and $5.3 \pm 0.1 \text{mmol.L}^{-1}$ for female ($P<0.001$) respectively. The mean blood glucose area was $30.2 \pm 2.2 \text{mmol.L}^{-1}.h$ and $13.7 \pm 1.0 \text{mmol.L}^{-1}.h$ for male ($P<0.001$) and was $34.1 \pm 2.9 \text{mmol.L}^{-1}.h$ and $15.6 \pm 0.5 \text{mmol.L}^{-1}.h$ for female ($P<0.001$) respectively. Figure 3.1; 3.2; 3.3).

(3) Blood glucose area =

\[
\frac{\text{Fasting blood glucose} + \text{One hour blood glucose}}{2}
\]

\[
+ \frac{\text{One hour blood glucose} + \text{Two hour blood glucose}}{2}
\]
Fig 3.1: Fasting blood glucose in control and NIDD subjects.
Fig 3.2: Blood glucose area in control and NIDD subjects.
Fig 3.3: Glucose tolerance test in control and NIDD subjects.
3.4.2 Glycosylated haemoglobin (HbA1C)

There was a significant increase in the glycosylated haemoglobin (HbA1C) in the NIDD group compared with controls. The mean glycosylated haemoglobin (HbA1C) was $7.6 \pm 0.6 \%$ and $6.0 \pm 0.4 \%$ for male ($P<0.05$) and was $8.2 \pm 0.4 \%$ and $6.0 \pm 0.2 \%$ for female ($P<0.001$) respectively. (Fig. 3.4).

Fig. 3.4: Glycosylated haemoglobin level in control and NIDD subjects.
3.5 **Plasma Free Fatty Acid of Healthy Subjects, and Non-Insulin-Dependent Diabetics**

There was a significant increase in the fasting plasma free fatty acid and free fatty acid area(4) in the NIDD group compared with controls (healthy subjects). The mean fasting plasma free fatty acid was $424 \pm 30 \mu\text{mol.L}^{-1}$ and $329 \pm 32 \mu\text{mol.L}^{-1}$ for male (P<0.05) and was $461 \pm 36 \mu\text{mol.L}^{-1}$ and $353 \pm 17 \mu\text{mol.L}^{-1}$ for female (P<0.01) respectively. The mean plasma free fatty acid area(4) was $647 \pm 51 \mu\text{mol.L}^{-1}.\text{h}$ and $357 \pm 41 \mu\text{mol.L}^{-1}.\text{h}$ for male (P<0.001) and was $687 \pm 59 \mu\text{mol.L}^{-1}.\text{h}$ and $415 \pm 22 \mu\text{mol.L}^{-1}.\text{h}$ for female (P<0.001) respectively. (Fig. 3.5; 3.6; 3.7).
Fig 3.5: Fasting plasma free fatty acid in control and NIDD subjects.
Fig 3.6: Plasma free fatty acid area in control and NIDD subjects.
Fig 3.7: Plasma free fatty acid level in control and NIDD subjects after glucose load.
Free fatty acid area

\[
\text{Fasting plasma} \quad \text{one hour plasma}
\]
\[
\frac{\text{free fatty acid}}{2} + \frac{\text{free fatty acid}}{2}
\]

\[
\text{One hour plasma} \quad \text{two hour plasma}
\]
\[
\frac{\text{free fatty acid}}{2} + \frac{\text{free fatty acid}}{2}
\]

3.6 Plasma Insulin of Healthy Subjects and Non-lnsulin-Dependent Diabetics.

There was a significant increase in the fasting plasma insulin in the NIDD group compared with controls (healthy subjects). The mean fasting plasma insulin was 20.6 ± 2.8 μU/ml-l and 10.5 ± 1.9 μU/ml-l for male (P<0.01) and was 22.5 ± 2.6 μU/ml-l and 9.4 ± 0.9 μU/ml-l for female (P<0.001) respectively. The plasma insulin area(5) was 136.1 ± 23.2 μU/ml-l.h and 116.8 ± 22.6 μU/ml-l.h for male and 155.3 ± 19.5 μU/ml-l.h and 108.7 ± 11.8 μU/ml-l.h for female (P<0.05) respectively. (Fig 3.8; 3.9; 3.10)
Fig 3.8: Fasting plasma insulin in control and NIDD subjects.
Fig 3.9: Plasma insulin area in control and NIDD subjects.
Fig 3.10: Plasma insulin level in control and NIDD subjects after glucose load.
Plasma Insulin Area

\[
= \frac{\text{Fasting plasma insulin} + \text{One hour plasma insulin}}{2} + \frac{\text{One hour plasma insulin} + \text{Two hour plasma insulin}}{2}
\]

3.7 Serum Total Cholesterol, HDL-cholesterol, LDL-cholesterol and Triglyceride of Healthy Subjects and Non-Insulin-Dependent Diabetics

There was no significant difference in the total cholesterol and LDL-cholesterol level in the NIDD group compared with controls (healthy subjects). The mean fasting total cholesterol was 6.2 ± 0.4 mmol.L\(^{-1}\) and 6.6 ± 0.3 mmol.L\(^{-1}\) (NS) and the mean fasting LDL-cholesterol was 4.1 ± 0.3 and 4.5 ± 0.2 (NS) respectively. There was a significant decrease in the fasting HDL-cholesterol in the NIDD group compared with controls (healthy subjects). The mean fasting HDL-cholesterol was 1.06 ± 0.06 mmol.L\(^{-1}\) and 1.46 ± 0.08 mmol.L\(^{-1}\) (P<0.001) respectively. There was a significant increase in the fasting triglyceride in the NIDD group compared with controls (healthy subjects). The mean fasting triglyceride was 2.5 ± 0.2 mmol.L\(^{-1}\) and 1.8 ± 0.1 mmol.L\(^{-1}\) (P<0.01) respectively. (Table 3.2)
<table>
<thead>
<tr>
<th>Subject n</th>
<th>Sex</th>
<th>Total Cholesterol (mmol.L⁻¹)</th>
<th>HDL-Cholesterol (mmol.L⁻¹)</th>
<th>LDL-Cholesterol (mmol.L⁻¹)</th>
<th>Triglyceride (mmol.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIDD</td>
<td>27 M+F</td>
<td>6.2±0.4</td>
<td>1.06±0.05</td>
<td>4.1±0.3</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Control</td>
<td>38 M+F</td>
<td>6.6±0.3</td>
<td>1.46±0.08</td>
<td>4.5±0.2</td>
<td>1.8±0.1</td>
</tr>
</tbody>
</table>

P NS <0.001 NS <0.01
The LDL-C/HDL-C, HDL-C/TG, LDL-C/TG, Insulin/Glucose,
FFA/Insulin, and FFA/Glucose Ratio of Healthy Subjects
and Non-Insulin-Dependent Diabetics

There was no significant difference in the LDL-C/HDL-C
ratio in the NIDDD group compared with controls (healthy
subjects). The mean LDL-C/HDL-C was 4.37 ± 0.62 and
3.50 ± 0.30 (NS). There was a significant decrease in
the HDL-C/TG, LDL-C/TG, Insulin/Glucose and FFA/Glucose
ratio in the NIDDD group compared with controls (healthy
subjects). The mean HDL-C/TG was 0.54 ± 0.06 and
0.99 ± 0.10 (P<0.01) respectively. The mean LDL-C/TG
was 1.86 ± 0.14 and 2.89 ± 0.21 (P<0.001)
respectively. The mean Insulin/Glucose was 5.0 ± 0.6
and 7.3 ± 0.6 (P<0.001) respectively and the mean
FFA/Glucose was 21.8 ± 1.5 and 27.1 ± 1.3 (P<0.01)
respectively. On the other hand, there was a
significant increase in the FFA/Insulin ratio in the
NIDDD group compared with controls (healthy subjects).
The mean FFA/Insulin ratio was 7.1 ± 1.2 and 4.6 ± 0.4
(P<0.05) respectively. (Table 3.3)
Table 3.3  THE LDL-C/HDL-C, HDL-C/TRIGLY, LDL-C/TRIGLY, INSULIN/GLUCOSE, FFA/INSULIN AND FFA/GLUCOSE RATIO OF HEALTHY SUBJECTS AND NON-INSULIN-DEPENDENT DIABETICS (MEAN ± S.E.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>n</th>
<th>Sex</th>
<th>LDL-C/HDL-C</th>
<th>HDL-C/TG</th>
<th>LDL-C/TG</th>
<th>Insulin/Glucose</th>
<th>FFA/Insulin</th>
<th>FFA/Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIDD</td>
<td>27</td>
<td>M</td>
<td>4.37±0.62</td>
<td>0.54±0.06</td>
<td>1.86±0.14</td>
<td>5.0±0.6</td>
<td>7.1±1.2</td>
<td>21.8±1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>4.37±0.62</td>
<td>0.54±0.06</td>
<td>1.86±0.14</td>
<td>5.0±0.6</td>
<td>7.1±1.2</td>
<td>21.8±1.5</td>
</tr>
<tr>
<td>Control</td>
<td>38</td>
<td>M</td>
<td>3.50±0.30</td>
<td>0.99±0.10</td>
<td>2.89±0.21</td>
<td>7.3±0.6</td>
<td>4.6±0.4</td>
<td>27.1±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>3.50±0.30</td>
<td>0.99±0.10</td>
<td>2.89±0.21</td>
<td>7.3±0.6</td>
<td>4.6±0.4</td>
<td>27.1±1.3</td>
</tr>
<tr>
<td>F</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.9 Discussion

In Chapter 1 the factors which might account for the high frequency of macrovascular disease in diabetes were said to be hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia, elevated free fatty acid concentration, low HDL-cholesterol concentration etc. The study showed a significant increase in blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acid, plasma insulin, triglyceride and a significant decrease in HDL-cholesterol level in non-insulin-dependent diabetics. There were also significant decreases in the HDL-C/TG, LDL-C/TG. Insulin/Glucose, FFA/Glucose ratios in non-insulin-dependent diabetics. The non-insulin-dependent diabetics investigated in this study could, therefore, have these as risk factors for macrovascular disease.
CHAPTER FOUR

ARTERIAL WALL CHARACTERISTICS OF CONTROLS
(HEALTHY SUBJECTS) AND NON-INSULIN-DEPENDENT DIABETICS

4.1. Introduction

Vascular disease is the most important cause of morbidity and mortality in diabetics. Diabetic vascular disease can be divided into three categories: large, medium and microsize-vessel disease. Disease of the large and medium vessels results in myocardial infarction, stroke, and gangrene of the lower limbs. Microsize-vessel disease leads to diabetic retinopathy and nephropathy. This chapter will deal primarily with macrovascular disease affecting the lower limb or peripheral vascular disease (PVD). Peripheral vascular disease predicts an increased risk of future myocardial infarction and stroke (Kannel et al. 1979). If arterial disease could be detected early, then good metabolic control and other risk factor intervention might delay disease progression. There is evidence that non-invasive techniques using Doppler ultrasound can demonstrate early changes in the arterial wall. Such changes probably reflect early disease.

Gosling (1976) has shown that arterial compliance can be measured accurately by recording the pulse wave velocity down an arterial segment, from the time difference between the start of the pulse wave at each end, using sensitive Doppler ultrasound techniques. Skidmore and Woodcock (1980) have developed a
mathematical technique to assess the form of the velocity wave obtained by the Doppler ultrasound, using Fourier analysis to derive an index of proximal arterial resistance.

This chapter reports the results of both techniques in a group of non-insulin-dependent diabetics who have no symptoms or signs of arterial disease in the lower limbs or at any other site. The aim was to use these Doppler studies to determine whether the diabetics had more disease in the aorto-iliac and femoro-tibial segments than matched non-diabetic controls. Statistical comparisons of groups were made using students t-test and the correlations were examined by linear regression analysis.

4.2. Normal Structure and Function of Arteries

The arterial wall has three layers: the intima, media and adventitia. The adventitia consists of adipose and connective tissue, and plays no part in the development of atherosclerosis. The media consists of smooth muscle cells concentrically and longitudinally arranged, and is the main structural support for the artery and also provides the artery with its contractile properties. The intima lines the luminal surface of the artery and consists of a single layer of endothelial cells and a layer of connective tissue. The connective tissue contains a small number of smooth muscle cells which tend to increase in number as the artery ages. The intima is bounded by the internal elastic lamina, as distinct fenestrated structure.

Endothelial cells have at least three functions (Cimbrone 1979). They act as a blood compatible
container allowing the free flow of blood and preventing clotting within the vessel. This is accomplished by both the physical characteristics of endothelial cells and by the synthesis and secretion of a potent platelet anti-aggregatory agent, prostacyclin.

Endothelial cells also act as a selective permeability barrier allowing entry into the inner parts of the artery of selected plasma constituents and excluding others. This is an active process requiring energy. Endothelial cells synthesise, metabolise and secrete a number of important substances, such as angiotensin-converting enzyme, factor VIII, plasminogen activator, von Willebrand factor, prostacyclin, thromboxane, fibronectin, collagen (type IV), alpha-2-macroglobulin, lipoprotein lipase and hormone receptors (including adrenergic, insulin, oestrogen and thrombin) (Stout 1982).

Arterial smooth muscle cells also have a variety of functions (Chamley-Campbell et al. 1979). As well as providing the main structural support and contractile properties of the artery, they also have major synthetic functions. As smooth muscle is the only cell type in the arterial media, these cells synthesise, metabolise and secrete a number of important substances such as actin, myosin, collagen, elastin, microfibrillar proteins, proteoglycans, and lipids (Stout 1982). Smooth muscle cells are also capable of endocytosis of foreign material and lipoproteins.

A major development in atherosclerosis research has been the description of ways of growing both endothelial and smooth muscle cells in culture. Endothelial cells of human origin can be conveniently grown from umbilical vein or artery (Jaffe et al. 1973;
Cimbrone et al. 1974) and of animal origin from appropriate vessels (Schwartz et al. 1981). Human arterial smooth muscle cells have been grown from small pieces of arterial tissue obtained at surgery (Bierman & Albers 1975) and they may also be cultured from primates and other animals (Ross 1971). Cell culture allows investigation of the biology of arterial cells, and their ability to react to external stimuli can be studied under carefully controlled laboratory conditions. Caution must be exercised in the interpretation of results from cell culture experiments, as the environmental conditions are different from those which occur in vivo. Nevertheless, cell culture experiments in the last decade have provided a considerable amount of information on possible mechanisms in the development of atherosclerosis.

The earliest identifiable change in the development of the atherosclerotic lesion is an accumulation of smooth muscle cells in the arterial intima (Fig. 4.1). These cells result from replication of cells already in the intima or may come from proliferation and migration of smooth muscle cells from the media. In more advanced stages of the disease the smooth muscle cells are seen to contain lipid, and later extracellular lipid is found. As this process continues, extracellular connective tissue is formed. Eventually calcification, haemorrhage, ulceration and superimposed thrombosis, the characteristics of the complicated lesion, occur. The characteristic cell of the advanced atherosclerotic lesion is the lipid engorged foam cell. The exact origin of foam cells is uncertain, but they may originate from smooth muscle cells which have become laden with lipid or from circulating monocyte-macrophages (Ross 1981).
Fig. 4.1: The development of the atherosclerotic plaque.

The normal artery (1) consists of an epithelial-like endothelium, the media consisting of smooth muscle cells and the connective tissue adventitia. An early change in atherogenesis (2) is an accumulation of smooth muscle cells in the intima. These cells become filled with lipid (3) which also accumulates extracellularly (4).

The complicated lesion (5) also contains fibrous tissue, calcification and superimposed thrombus.

(From Stout, Bierman & Brunzell, 1975 by kind permission of MRP Press).
4.3. **Arterial Compliances (AC) of controls (healthy subjects) and Non-Insulin-Dependent Diabetics**

There was a significant decrease in the arterial compliance in the non-insulin-dependent diabetic group compared with controls (healthy subjects). The mean arterial compliance of all subjects (male and female) was $0.55\pm0.03$ and $0.91\pm0.04$ respectively ($p<0.001$). The mean arterial compliance of males was $0.55\pm0.06$ and $1.02\pm0.07$ respectively ($p<0.001$). The mean arterial compliance of females was $0.56\pm0.03$ and $0.89\pm0.05$ respectively ($p<0.001$) (Fig 4.2).
Fig 4.2: Arterial wall compliance at level of common femoral artery in control and NIDD subjects.
4.4. **Proximal Resistance (PR) of controls (healthy subjects) and Non-Insulin-Dependent Diabetics**

4.4.1 **Common femoral artery (CFA)**

There was a significant increase in the proximal resistance at the common femoral artery level in the non-insulin-dependent diabetic group compared with controls (healthy subjects). The mean proximal resistance of all subjects (male and female) was 0.45±0.03 and 0.34±0.02 respectively (p<0.01). The mean proximal resistance of males was 0.42±0.04 and 0.29±0.02 respectively (p<0.05). The mean proximal resistance of females was 0.47±0.03 and 0.36±0.02 respectively (p<0.01) (Fig 4.3).
Fig 4.3: Proximal resistance at level of common femoral artery in control and NIDD subjects.
4.4.2 Posterior tibial artery (PTA)

There was a significant increase in the proximal resistance at the posterior tibial artery level in the non-insulin-dependent diabetic group compared with controls (healthy subjects). The mean proximal resistance of all subjects (male and female) was 0.52±0.02 and 0.38±0.02 respectively (p<0.001). The mean proximal resistance of males was 0.51±0.04 and 0.35±0.02 respectively (p<0.005). The mean proximal resistance of females was 0.53±0.02 and 0.40±0.02 respectively (p<0.001) (Fig 4.4).

![Graph showing proximal resistance at level of posterior tibial artery in control and NIDD subjects.](image)

Fig 4.4: Proximal resistance at level of posterior tibial artery in control and NIDD subjects.
4.5. Correlation between the Arterial Compliance and Proximal Resistance

There was a significant negative correlation between arterial compliance and proximal resistance of the common femoral arteries in all subjects, diabetics and healthy subjects. The correlation coefficient (r) for males was -0.68 (p<0.001). The correlation coefficient (r) for females was -0.58 (p<0.001). Fig 4.5.

Fig 4.5: correlation between arterial compliance and proximal resistance at the common femoral artery level in control and NIDD subjects.
There was a significant negative correlation between the arterial compliance and proximal resistance of the posterior tibial arteries in all subjects, diabetics and controls. The correlation coefficient (r) for males was -0.70 (p<0.001) and the correlation coefficient (r) for females was -0.57 (p<0.001). Fig 4.6.

Fig 4.6: Correlation between arterial compliance and proximal resistance at the posterior tibial artery level in control and NIDD subjects.
There was a significant positive correlation between the proximal resistance for the common femoral arteries and posterior tibial arteries in all subjects, diabetics and healthy subjects. The correlation coefficient \( r \) for males was 0.59 \((p<0.01)\) and the correlation coefficient \( r \) for females was 0.58 \((p<0.001)\). Fig 4.7.

\[\begin{align*}
\text{Proximal Resistance (Posterior Tibial Artery, PTA)} & \quad \text{Proximal Resistance (Common Femoral Artery, CFA)} \\
\end{align*}\]

\[\begin{align*}
\text{Male} & \\
\text{Female} & \\
\end{align*}\]

\[\begin{align*}
r & = 0.59 \ (p<0.01) \\
& \quad \text{NIDD} \\
\end{align*}\]

\[\begin{align*}
r & = 0.58 \ (p<0.001) \\
& \quad \text{NIDD} \\
\end{align*}\]

**Fig 4.7:** Correlation between proximal resistance at the common femoral artery level and at the posterior tibial artery level in all subjects, control and non-insulin-dependent diabetics.
There was a significant negative correlation between the arterial compliance and proximal resistance for the common femoral arteries in diabetics and healthy subjects. The correlation coefficient ($r$) for healthy diabetics was $-0.38 \ (p<0.05)$ and the correlation coefficient ($r$) for healthy subjects was $-0.63 \ (p<0.001)$. Table 4.1.

There was a significant negative correlation between the arterial compliance and proximal resistance for the posterior tibial arteries in diabetics and healthy subjects. The correlation coefficient ($r$) for diabetics was $-0.43 \ (p<0.05)$ and the correlation coefficient ($r$) for healthy subjects was $-0.43 \ (p<0.01)$. Table 4.2.

There was a significant positive correlation between the proximal resistance for the common femoral arteries and posterior tibial arteries in diabetics and healthy subjects. The correlation coefficient ($r$) for diabetics was $0.37 \ (p<0.05)$ and the correlation coefficient ($r$) for healthy subjects was $0.58 \ (p<0.001)$. Table 4.3.
Table 4.1 Correlation ($r$) between arterial compliance and proximal resistance for the common femoral arteries in NIDD and healthy subjects.

<table>
<thead>
<tr>
<th></th>
<th>NIDD (n=27)</th>
<th>HEALTHY SUBJECTS (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>-0.38'</td>
<td>-0.63</td>
</tr>
<tr>
<td>$p$</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4.2 Correlation ($r$) between arterial compliance and proximal resistance for the posterior tibial arteries in NIDD and healthy subjects.

<table>
<thead>
<tr>
<th></th>
<th>NIDD (n=27)</th>
<th>HEALTHY SUBJECTS (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>-0.43</td>
<td>-0.43</td>
</tr>
<tr>
<td>$p$</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 4.3 Correlation ($r$) between the proximal resistance for the common femoral arteries and posterior tibial arteries in NIDD and healthy subjects.

<table>
<thead>
<tr>
<th></th>
<th>NIDD (n=27)</th>
<th>HEALTHY SUBJECTS (n=38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>0.37</td>
<td>0.58</td>
</tr>
<tr>
<td>$p$</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
4.6. Discussion

Non-invasive techniques using Doppler ultrasound to measure arterial compliance are harmless, simple to perform and reproducible. Laogun and Gosling (1982) measured compliance in the aorto-iliac and femoro-tibial segments in 600 normal volunteers. They found in the aorto-iliac segment, compliance was high in the first two decades then fell to a level which only slightly decreased thereafter. In females compliance was significantly higher than males until menopause, after which the difference was lost. Compliance was not affected by the mean blood pressure until it exceeded 95 mmHg, after which there was an appreciable fall. They also found in the femoro-tibial segments that there was far less difference according to age, no significant differences between the sexes and no significant relation to mean blood pressure. Wahlqvist et al (1984) reported that arterial compliance was significantly and negatively correlated to age, and also showed this was independent of other risk factors, as was in healthy subjects (Relf et al. 1986). A similar technique has been used to show reduced compliance in the femoral to tibial artery segment in asymptomatic diabetic adults (Cairns et al. 1978), although another study found changes only in diabetic patients with foot ulcers (Scarpello et al. 1980).
Skidmore and Woodcock (1980) used the technique of Fourier analysis of the velocity wave to measure proximal resistance to distinguish patients with normal, mildly diseased and severely diseased iliac arteries. Baird et al (1980) used the technique to detect haemodynamically significant iliac lesions in patients with femoral artery disease. Campbell et al (1984; 1984; 1985) used the technique to evaluate lower limb arterial disease in symptomatic individuals. They reported that clinical evidence of arterial disease was significantly more common in those subjects with initially high Laplace wave form analysis values than in those with normal wave form results.

The present study showed a significant decrease in compliance and increase in proximal resistance in the NIDD group compared to controls (healthy subjects). There was a good correlation between the two quite different techniques used to assess disease in the proximal arteries. Probably these changes are due to pre-clinical atherosclerosis. However, there are other possible explanations for the changes in arterial compliance such as glycosylation of proteins in the arterial wall (Wieland et al, 1983). In diabetes an increase in non-enzymatic glycosylation of arterial wall proteins has been demonstrated (Kohn et al. 1984; Brownlee et al. 1984; Cerami et al. 1985; Chang et al. 1985). In fact the increase can be shown to correlate with the degree of vascular disease. Recent studies have shown that the glucose molecule after attachment to lysine becomes further rearranged to form a group of fluorescent products collectively named "melanoidins". These substances have important properties such as increasing the cross-linking of proteins, affecting the absorbative properties of collagen and altering the recognition of proteins by macrophages. These
end-products of glycosylation may be important in the behaviour of arterial wall proteins and may play a role in the increased incidence of atherosclerosis in diabetes (Yue et al. 1983; Yue 1985).

The results indicate that both aorto-iliac and femoro-tibial disease are present in the diabetic group to a greater degree than in healthy subjects. This more extensive disease was apparent in diabetics who were otherwise apparently normal. The only defined differences between NIDD men and healthy subjects which might lead to these arterial changes were in carbohydrate status or related variables. There was no differences in body mass index, blood pressure, and all subjects were non-smokers. The two groups were age-matched.

4.7. **Conclusion**

This study showed a significant decrease in the arterial compliance, and a significant increase in the arterial proximal resistance of common femoral artery and posterior tibial artery in non-insulin-dependent diabetics. The study showed a significant negative correlation between the arterial compliance and proximal resistance and, on the other hand, showed a significant positive correlation between the arterial proximal resistance of common femoral artery and posterior tibial artery. The significant difference between the controls (healthy subjects) and non-insulin-dependent diabetic groups indicate that pre-clinical peripheral arterial disease can be recognised even in mild diabetics by non-invasive measurement of compliance or proximal resistance.
CHAPTER FIVE

UNIVARIATE EVALUATION OF BIOCHEMICAL DETERMINANTS OF
ARTERIAL WALL CHARACTERISTICS

5.1. Introduction

Non-invasive techniques using Doppler ultrasound to study arterial wall compliance and proximal resistance are harmless, simple to perform and reproducible (Wahlqvist et al., 1984; Lo et al., 1986). Reif et al. (1986) reported that arterial compliance is significantly and negatively correlated to age, blood pressure, serum total cholesterol and serum triglyceride and also significantly and positively correlated to serum HDL-cholesterol in healthy men. However, the correlation between arterial compliance or arterial proximal resistance and other risk factors are unknown. This chapter reports the results of the relationships between arterial compliance, arterial proximal resistance and blood glucose, glycosylated haemoglobin (HbAlC), plasma free fatty acid and insulin in controls (healthy subjects) and non-insulin-dependent diabetics. Observations for healthy subjects and non-insulin-dependent diabetics have been pooled so as to allow compliance to be correlated with a wide spectrum of glucose, glycosylated haemoglobin (HbAlC), free fatty acid and insulin values. The relationship between arterial compliance (Ac), proximal resistance (PR) of common femoral artery and posterior tibial artery and their determinants was examined by correlation analysis, both parametric (r) and non-parametric (rs, Siegel, S. 1974).
5.2. The correlation between arterial compliance and each of blood glucose, glycosylated haemoglobin, plasma free fatty acid and plasma insulin for non-insulin-dependent diabetics and their healthy controls

5.2.1 The correlation between the arterial compliance and blood glucose level

There was a significant negative correlation between the arterial compliance and blood glucose level. The correlation coefficient ($r$) for arterial compliance and fasting blood glucose level was $-0.46$ ($p<0.001$) and the correlation coefficient ($r$) for arterial compliance and blood glucose area was $-0.52$ ($p<0.001$). The Spearman rank correlation coefficient ($rs$) for the arterial compliance and fasting blood glucose level was $-0.64$ ($p<0.001$) and the Spearman rank correlation coefficient ($rs$) for the arterial compliance and blood glucose area was $-0.67$ ($p<0.001$). Fig 5.1.
Fig 5.1: Correlation between arterial compliance and blood glucose level.
5.2.2 The correlation between the arterial compliance and glycosylated haemoglobin (HbA1C).

There was a significant negative correlation between the arterial compliance and glycosylated haemoglobin. The correlation coefficient (r) was -0.40 (p<0.001). The Spearman rank correlation coefficient (rs) for the arterial compliance and glycosylated haemoglobin was -0.45 (p<0.001). (Fig 5.2).

Fig 5.2: Correlation between arterial compliance and glycosylated haemoglobin level.
5.2.3 The correlation between arterial compliance and plasma free fatty acid.

There was a significant negative correlation between the arterial compliance and plasma free fatty acid. The correlation coefficient (r) for the arterial compliance and fasting free fatty acid was -0.31 (p < 0.05) and the correlation coefficient (r) for the arterial compliance and free fatty acid area was -0.50 (p < 0.001). The Spearman rank correlation coefficient (rs) for the arterial compliance and fasting free fatty acid was -0.39 (p < 0.01) and the spearman rank correlation coefficient (rs) for the arterial compliance and free fatty acid area was -0.59 (p < 0.001). Fig 5.3.
Fig 5.3: Correlation between arterial compliance and plasma free fatty acid.
5.2.4 The correlation between arterial compliance and plasma insulin

There was a significant negative correlation between the arterial compliance and plasma insulin level. The correlation coefficient (r) of the arterial compliance and fasting plasma insulin was −0.39 (p<0.01) and the correlation coefficient (r) of the arterial compliance and plasma insulin area was −0.26 (p<0.05). The Spearman rank correlation coefficient (rs) for the arterial compliance and fasting plasma insulin was −0.45 (p<0.001) and the Spearman rank correlation coefficient (rs) for the arterial compliance and plasma insulin area was −0.38 (P<0.01). (Fig 5.4).
Fig 5.4: Correlation between arterial compliance and plasma insulin.
5.3. The correlation between arterial proximal resistance at
the common femoral artery and blood glucose,
glycosylated haemoglobin (HbA1C), plasma free fatty
acid and insulin for non-insulin-dependent diabetics
and their healthy controls.

5.3.1 The correlation between arterial proximal resistance at
the common femoral artery and blood glucose

There was a significant positive correlation between
the arterial proximal resistance of common femoral
artery and blood glucose. The correlation coefficient
(r) for the arterial proximal resistance of common
femoral artery and fasting blood glucose level was 0.29
(p<0.05) and the correlation coefficient (r) for the
arterial proximal resistance of common femoral artery
and blood glucose area was 0.37 (p<0.01). (Fig 5.5).
Fig 5.5: Correlation between proximal resistance at the common femoral artery and blood glucose level.
5.3.2 The correlation between arterial proximal resistance at the common femoral artery and glycosylated haemoglobin (HbA1C)

There was a significant positive correlation between the arterial proximal resistance of common femoral artery and glycosylated haemoglobin (HbA1C). The correlation coefficient (r) was 0.27 (p<0.05). (Fig 5.6).

Fig 5.6: Correlation between proximal resistance at the common femoral artery and glycosylated haemoglobin level.
5.3.3 The correlation between arterial proximal resistance at the common femoral artery and plasma free fatty acid.

There was a significant positive correlation between the arterial proximal resistance of common femoral artery and plasma free fatty acid level. The correlation coefficient ($r$) for the arterial proximal resistance of common femoral artery and fasting plasma free fatty acid level was 0.29 ($p<0.05$) and the correlation coefficient ($r$) for the arterial proximal resistance of common femoral artery and plasma free fatty acid area was 0.33 ($p<0.01$). (Fig 5.7).
Fig 5.7: Correlation between proximal resistance at the common femoral artery and plasma free fatty acid.
5.3.4 The correlation between arterial proximal resistance at the common femoral artery and plasma insulin. There was no significant correlation between the arterial proximal resistance of common femoral artery and plasma insulin level.

5.4 The correlation between arterial proximal resistance at the posterior tibial artery and blood glucose, glycosylated haemoglobin (HbA1C) level, plasma free fatty acid and insulin for non-insulin-dependent diabetics and their healthy controls.

5.4.1 The correlation between arterial proximal resistance at the posterior tibial artery and blood glucose level.

There was a significant positive correlation between the arterial proximal resistance of posterior tibial artery and blood glucose level. The correlation coefficient (r) for the arterial proximal resistance of posterior tibial artery and fasting blood glucose level was 0.43 (p<0.001) and the correlation coefficient (r) for the arterial proximal resistance of posterior tibial artery and blood glucose area was 0.46 (p<0.001). (Fig 5.8).
Fig 5.8: Correlation between proximal resistance at the posterior tibial artery and blood glucose level.
5.4.2 The correlation between arterial proximal resistance at the posterior tibial artery and glycosylated haemoglobin (HbA1C) level

There was a significant positive correlation between the arterial proximal resistance of posterior tibial artery and glycosylated haemoglobin (HbA1C) level. The correlation coefficient \( r \) was 0.42 \((p<0.01)\). (Fig 5.9).

There was a significant positive correlation between the arterial proximal resistance of posterior tibial artery and glycosylated haemoglobin (HbA1c) in controls, the correlation coefficient was 0.33 \((n=38, p<0.05)\).

Fig 5.9: Correlation between proximal resistance at the posterior tibial artery and glycosylated haemoglobin level.
5.4.3 The correlation between arterial proximal resistance at
the posterior tibial artery and plasma free fatty acid

There was a significant positive correlation between
the arterial proximal resistance of posterior tibial
artery and plasma free fatty acid. The correlation
coefficient (r) for the arterial proximal resistance of
posterior tibial artery and fasting plasma free fatty
acid was 0.35 (p<0.01) and the correlation coefficient
(r) for the arterial proximal resistance of posterior
tibial artery and plasma free fatty acid area was 0.44
(p<0.001). (Fig 5.10).

There was a significant positive correlation between
the arterial proximal resistance of posterior tibial
artery and free fatty acid area in controls, the
correlation coefficient was 0.41 (n=38, p<0.01).
Fig 5.10: Correlation between proximal resistance at the posterior tibial artery and plasma free fatty acid.
5.4.4 The correlation between arterial proximal resistance at the posterior tibial artery and plasma insulin: There was no significant correlation between the arterial proximal resistance of posterior tibial artery and plasma insulin level.

5.4.5 There was no significant correlation between arterial indices and each of total cholesterol, HDL-Cholesterol, LDL-Cholesterol and triglycerides for non-insulin-dependent diabetes or their healthy controls (Table 5.1).

5.5. Discussion

Arterial compliance was significantly and negatively correlated to blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin immunoreactivity. On the other hand, arterial proximal resistance of common femoral artery and posterior tibial artery was significantly and positively correlated to blood glucose, blood glycosylated haemoglobin (HbA1C) and plasma free fatty acid.

It should be emphasised that this study has, by design, minimised the effect of other potential risk factors, such as age, body mass, body mass index and blood pressure on compliance and proximal resistance for both the apparently healthy subjects and non-insulin-dependent diabetics.

We cannot say that measurement of arterial compliance and arterial proximal resistance in this study necessarily reflects atherosclerosis. However, the observation that these might be a risk factor for arterial wall change is of particular interest in the light of studies of atherogenesis. Free fatty acid can be incorporated into cholesterol ester in atherosclerotic lesions (Wahlqvist et al. 1969). For insulin, epidemiological evidence points to it as a
Table 5.1 Correlations (r) between arterial indices and each of total cholesterol, HDL-Cholesterol, LDL-Cholesterol and triglycerides for non-insulin-dependent diabetes or their healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Total Cholesterol</th>
<th>HDL-Cholesterol</th>
<th>LDL-Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy 38</td>
<td>NIDDM 27</td>
<td>Total 65</td>
<td>Healthy 38</td>
</tr>
<tr>
<td>Arterial compliance</td>
<td>-0.13</td>
<td>-0.11</td>
<td>-0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Proximal resistance of</td>
<td>0.11</td>
<td>0.15</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>common femoral artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal resistance of</td>
<td>0.10</td>
<td>0.15</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>posterior tibial artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n is the number of subjects and is shown in parenthesis.

r is the correlation coefficient.

The significance of a correlation coefficient is indicated by NS(p>0.05) *(p<0.05) **(p<0.01)
risk factor for coronary arterial disease (Ducimetiere et al. 1980; Logan et al. 1978; Welborn et al. 1979) and experimental evidence points to it influencing atherogenesis (Stout, 1981).

Clinical study showed that elevated insulin level, either fasting or in response to oral glucose, have a predictive role in the development of cardiovascular disease. In experimental animals, insulin deficiency retards the development of diet-induced arterial disease. Insulin stimulates lipid synthesis in isolated arteries and stimulates proliferation and lipid accumulation in cultured arterial smooth muscle cells (Stout, 1985).

Considering glycosylated haemoglobin and glucose, an increase in non-enzymatic glycosylation of arterial wall proteins has been demonstrated in diabetics (Brownlee et al. 1984; Kohn et al. 1984; Cerami 1985). In fact the increase can be shown to correlate with the degree of vascular disease (Yue 1985).

5.6. Conclusion

The study showed a significant and negative correlation between the arterial compliance and blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin concentration in all subjects, healthy and non-insulin-dependent diabetics. The study also showed a significant and positive correlation between arterial proximal resistance of common femoral artery and posterior tibial artery and blood glucose, blood glycosylated haemoglobin and plasma free fatty acid concentration in all subjects, healthy subjects and non-insulin-dependent diabetics.
These results indicate that blood glucose, blood glycosylated haemoglobin, plasma free fatty acid are risk factors for changes in arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.
CHAPTER SIX

MULTIVARIATE EVALUATION OF BIOCHEMICAL DETERMINANTS
OF ARTERIAL WALL CHARACTERISTICS

6.1. Introduction

In chapter 4, it was shown that arterial compliance (Ac) is significantly lower in non-insulin-dependent diabetics than in apparently healthy subjects, and also shown that proximal resistance of the common femoral artery and posterior tibial artery is significantly higher in non-insulin-dependent diabetics than healthy subjects. We found a significant negative correlation between arterial compliance and age, blood pressure, and serum cholesterol and triglycerides levels, and a significant positive correlation with high density lipoprotein levels in apparently healthy men (Relf et al. 1986). Other studies have reported that age is an important factor associated with reduced arterial compliance in normal subjects (Loagun and Gosling, 1982; Avolio et al. 1983; Avolio et al. 1985) and that normotensive adult subjects who follow a low salt diet have reduced arterial stiffness independent of blood pressure (Avolio et al. 1986).

In chapter 5, it was shown that there was a significant negative correlation between arterial compliance and blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin concentration in all subjects, healthy and non-insulin-dependent diabetics. The study also showed a significant and positive correlation between arterial proximal
resistance of the common femoral artery and posterior tibial artery and blood glucose, blood glycosylated haemoglobin and plasma free fatty acid in all subjects, healthy subjects and non-insulin-dependent diabetics. Multivariate analysis was used to determine the relative influence of free fatty acid, insulin, glucose and lipid levels on changes in arterial compliance (Ac) and proximal resistance of the common femoral artery and posterior tibial artery. Multivariate analysis was performed by stepwise regression.

6.2. Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics.

Multivariate analysis examined each of the biochemical factors studied which could have independently influenced arterial compliance. The best equation, on the basis of the regression coefficients of independent variables, showed that the factor which most influenced the result was the glucose level in the fasting state or the glucose area after the glucose load. (Table 6.1)
Table 6.1: Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics combined (n=65).

<table>
<thead>
<tr>
<th>Multivariate Analyse</th>
<th>B0</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
<th>B7</th>
<th>T</th>
<th>P</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>1.10</td>
<td>-0.023</td>
<td>-0.024</td>
<td>-0.0002</td>
<td>-0.005</td>
<td>-0.04</td>
<td>-0.014</td>
<td>0.057</td>
<td>4.9</td>
<td>0.0001</td>
<td>0.33</td>
</tr>
<tr>
<td>Best</td>
<td>1.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14.2</td>
<td>0.0001</td>
<td>0.21</td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>1.09</td>
<td>-0.014</td>
<td>-0.008</td>
<td>-0.004</td>
<td>-0.0007</td>
<td>-0.031</td>
<td>-0.013</td>
<td>0.028</td>
<td>5.2</td>
<td>0.0001</td>
<td>0.39</td>
</tr>
<tr>
<td>Best</td>
<td>1.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.5</td>
<td>0.0001</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Complete Y = B0+B1X1+B2X2+B3X3+B4X4+B5X5+B6X6+B7X7

Best Y = B0+BX

Y = Arterial Compliance or Proximal resistance

X = Risk factors

B = Regression coefficients

Significance of regression coefficients is indicated by *(p<0.05) **(p<0.01)
6.3. **Relationships between proximal resistance of common femoral artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics.**

Multivariate analysis examined each of the biochemical factors studied which could have independently influenced proximal resistance of the common femoral artery. The best equation, on the basis of the regression coefficients of independent variables, showed that the factor which most influenced the result was the free fatty acid level in the fasting state or the glucose area after the glucose load. (Table 6.2)
Table 6.2: Relationships between proximal resistance of common femoral artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics combined (n=65).

<table>
<thead>
<tr>
<th>Multivariate</th>
<th>B0</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
<th>B7</th>
<th>T</th>
<th>P</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyse</td>
<td>G-Hb</td>
<td>Glucose</td>
<td>FFA</td>
<td>Insulin</td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>HDL-C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fasting</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>0.205</td>
<td>0.012</td>
<td>0.007</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.01</td>
<td>0.007</td>
<td>-0.024</td>
<td>1.7</td>
<td>N.S.</td>
<td>0.15</td>
</tr>
<tr>
<td>Best</td>
<td>0.268</td>
<td>0.0003**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Area</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>0.225</td>
<td>0.0025</td>
<td>0.0039</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0035</td>
<td>0.013</td>
<td>-0.032</td>
<td>1.8</td>
<td>N.S</td>
<td>0.17</td>
</tr>
<tr>
<td>Best</td>
<td>0.289</td>
<td>0.0044**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
6.4. Relationships between proximal resistance of posterior tibial artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics.

Multivariate analysis examined each of the biochemical factors studied which could have independently influenced proximal resistance of the posterior tibial artery. The best equation, on the basis of the regression coefficients of independent variables, showed that the factor which most influenced the result was the glucose level in the fasting state or the glucose area after the glucose load. (Table 6.3)
Table 6.3: Relationships between proximal resistance of posterior tibial artery and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for healthy subjects and non-insulin-dependent diabetics combined (n=65).

<table>
<thead>
<tr>
<th>Multivariate B0</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
<th>B6</th>
<th>B7</th>
<th>T</th>
<th>P</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyse</td>
<td>G-Hb</td>
<td>Glucose</td>
<td>FFA</td>
<td>Insulin</td>
<td>Cholesterol</td>
<td>Triglyceride</td>
<td>HDL-C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>0.255</td>
<td>0.018</td>
<td>0.010</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.007</td>
<td>0.009</td>
<td>-0.008</td>
<td>2.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Best</td>
<td>0.330</td>
<td></td>
<td>0.015**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>0.281</td>
<td>0.011</td>
<td>0.003</td>
<td>0.0002</td>
<td>0.003</td>
<td>0.026</td>
<td>0.001</td>
<td>-0.005</td>
<td>2.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Best</td>
<td>0.326</td>
<td></td>
<td>0.005**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6.5. **Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for men and women.**

Multivariate analysis examined each of the biochemical factors studied which could have independently influenced arterial compliance. The best equation, on the basis of the regression coefficients of independent variables, showed that the factor which most influenced the result was the glucose level in men, and the insulin level in the fasting state or the free fatty acid area after the glucose load in women. (Table 6.4)
Table 6.4: Relationships between arterial compliance and blood glucose, glycosylated haemoglobin, plasma free fatty acid, plasma insulin and lipids assessed by multivariate analysis for men and women.

<table>
<thead>
<tr>
<th>Multivariate Analysis</th>
<th>B0</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G-Hb</td>
<td>Glucose</td>
<td>FFA</td>
<td>Insulin</td>
<td>Cholesterol</td>
<td></td>
</tr>
</tbody>
</table>

**Males - Fasting**

- **Complete**
  - 0.907
  - -0.031
  - -0.0003
  - -0.0044
  - -0.061

- **Best**
  - 1.05
  - -0.041

**Males - Area**

- **Complete**
  - 1.18
  - -0.0055
  - -0.017
  - -0.0001
  - -0.0002
  - -0.0081

- **Best**
  - 1.11
  - -0.017

**Females - Fasting**

- **Complete**
  - 1.11
  - -0.015
  - -0.018
  - -0.0004
  - -0.0051
  - -0.049

- **Best**
  - 0.940
  - -0.012

**Females - Area**

- **Complete**
  - 1.18
  - -0.0066
  - -0.0051
  - -0.0005
  - -0.0011
  - -0.025

- **Best**
  - 1.11
  - -0.0007
<table>
<thead>
<tr>
<th>B6</th>
<th>B7</th>
<th>T</th>
<th>P</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>HDL-ch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.023</td>
<td>0.084</td>
<td>1.7</td>
<td>0.12</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.2</td>
<td>&lt;0.0001</td>
<td>0.29</td>
</tr>
<tr>
<td>-0.041</td>
<td>0.066</td>
<td>1.9</td>
<td>0.08</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.5</td>
<td>&lt;0.0001</td>
<td>0.41</td>
</tr>
<tr>
<td>-0.061</td>
<td>0.073</td>
<td>3.3</td>
<td>0.002</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.9</td>
<td>&lt;0.0001</td>
<td>0.18</td>
</tr>
<tr>
<td>0.018</td>
<td>0.036</td>
<td>4.0</td>
<td>0.0004</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.7</td>
<td>&lt;0.0001</td>
<td>0.27</td>
</tr>
</tbody>
</table>
6.6. Discussion

Chapter 5 showed that arterial compliance (AC), proximal resistance of the common femoral artery and the posterior tibial artery were significant correlated with blood glucose, glycosylated haemoglobin (HbA1C) plasma free fatty acid and plasma insulin level.

This chapter showed that the factor which most influenced the result of arterial compliance (AC) and proximal resistance of the posterior tibial artery was the glucose level in the fasting state or the glucose area after the glucose load. In addition, the factor which most influenced the result of the proximal resistance of the common femoral artery was the free fatty acid level in the fasting state or the glucose area after the glucose load. Considering glycosylated haemoglobin and glucose, an increase in non-enzymatic glycosylation of arterial wall proteins has been demonstrated in diabetics. In fact the increase can be shown to correlate with the degree of vascular disease (Yeu, 1985).

It has been shown that apparently healthy women have higher fasting insulin concentration than normal men, and a greater rise in insulin response to a glucose load (Peter et al, 1980). This was not confirmed in the studies, either for apparently healthy subjects or diabetics, but such a difference would be relevant in the understanding of differences in determinants of compliance between sexes. However the best equation was produced where glucose was the independent variable for men, and where fasting insulin or free fatty acid area the independent variable for women.
The finding that free fatty acid is an independent risk factor which affects arterial wall characteristics is of particular interest in the light of previous studies of atherogenesis. Wahlqvist et al (1967) showed that free fatty acid can be incorporated into cholesterol ester in atherosclerotic lesions. Epidemiological evidence points to insulin as a risk factor for coronary artery disease (Logan et al. 1978; Welborn et al. 1979; Ducimetiere et al. 1980) and experimental evidence shows that it influences atherogenesis (Stout, 1981). However, we cannot say that the arterial compliance and proximal resistance measured in this study necessarily reflects the presence and severity of atherosclerosis.

6.7. Conclusion

The study showed that the factor which most influenced the results of arterial compliance (AC) and proximal resistance was the glucose level in the fasting state and glucose area after the glucose load in all subjects, healthy and non-insulin-dependent diabetics.

The study also showed that the factors which most influenced the results of arterial compliance (AC) were the glucose level in men, and the insulin level in the fasting state or the free fatty acid area after the glucose load in women. These results indicate that blood glucose, plasma free fatty acid and plasma insulin are risk factors for changes in arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.
CHAPTER SEVEN

FOOD INTAKE DETERMINANTS OF ARTERIAL WALL CHARACTERISTICS

7.1 Introduction

Epidemiological comparisons of Greenland Eskimos and mainland Danes have suggested that diet rich in fish or marine oil may reduce the incidence of occlusive vascular disease (Bang et al. 1980; Krombou et al. 1985). Low death rates from coronary heart disease are also found in Japanese (Keys 1980; Kagawa et al. 1982). One reason may be the relatively high fish intake of the Japanese. In a study carried out in Chiba Prefecture in Japan, a fishing and a farming village were compared. Mortality from coronary heart disease was significantly lower in the fishing village than in the farming area (Hirai et al. 1980). Studies based on dietary history have suggested that even a small intake of fish may reduce the incidence of macrovascular disease in both European and North American populations (Shekelle et al. 1985).

The ingestion of some fish or marine oils produces beneficial changes in plasma lipid levels and the development of a mild bleeding disorder thought to be due to a decrease in platelet generation of the potent platelet aggregator and vasoconstrictor thromboxane A2 (Bang and Dyerberg 1972; Dyerberg et al. 1978; Brux et al. 1981; Goodnight et al. 1981; Sanders et al. 1981; Bradlow et al. 1983; Lorent et al. 1983; Singer et al. 1983; Phillipson et al. 1985). Eicosapentaenoic acid,
a polyunsaturated fatty acid of the omega-3 series is believed to be important in mediating this effect (Dyerberg and Bang 1979; Hirai et al. 1980; Hay et al. 1982; Knapp et al. 1986; Herold and Kinsella, 1986).

Research on the metabolism of arachidonic acid and its role as precursor of the prostaglandins produced in platelets and vessel walls (i.e. thromboxane and prostacyclin) has provided an attractive explanation. (Fig 7.1)
Figure 7.1  POSTAGLANDIN SYNTHETIC PATHWAYS IN BLOOD VESSELS AND PLATELETS

DIETARY LINOLEIC ACID (18:2n6)

(Diagram of pathways involving dietary linoleic acid, leading to various eicosanoids and their functions)

(From Herold & Kinsella, 1986)
Relationships between dietary intake and cardiovascular disease have received a great deal of attention (Morris et al. 1977; Kromhout et al. 1982; Temple 1983; Lanzola et al. 1983; Lapidus et al. 1986). Diets high in cereal grains, legumes and vegetables are consistently associated with a reduced incidence of cardiovascular disease (Edwards et al. 1971; Brewster and Jacobson, 1983). Additional effects of the high carbohydrate diets from vegetable sources may be caused by certain plant fibres including lignin, pectin and gums. (Jenkins et al. 1975; Sertori et al. 1977; Brown and Karmally 1985). The therapeutic effects of Chinese mushroom on hyperlipidemia in male adults have been investigated in China, where it is reported that after three months of increased intake of mushroom, the mean values of serum total cholesterol and triglyceride were significantly decreased (Xiao et al. 1986).

This chapter reports the relationships between consumption of fish and other protective foods (such as cereal grains, legumes, mushroom, citrus and tropical fruit) and the arterial indices of compliance and proximal resistance in non-insulin-dependent diabetics and their healthy controls.

7.2 Methods

Each subject (healthy and non-insulin-dependent diabetic) completed a seven day dietary record during the week after examination. A clinical nutritionist met with each subject to review the diet record and clarify information necessary for data coding. Food models were used to estimate quantities and types of food consumed. Food variety, protective food score and
food units were analysed. An index of food variety was developed from the biological classification of food for human nutrition, derived by David Briggs and Mark Wahlqvist (1985). The categories of food were as follows:-
<table>
<thead>
<tr>
<th>GROUP</th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ANIMAL</strong></td>
<td><strong>CEREALS AND GRAINS</strong></td>
</tr>
<tr>
<td>Eggs</td>
<td>Morning cereal</td>
</tr>
<tr>
<td>Milk</td>
<td>Corn</td>
</tr>
<tr>
<td>Dairy (e.g. Chees, Yogurt)</td>
<td>Oats/Porridge</td>
</tr>
<tr>
<td>Fish</td>
<td>Rye</td>
</tr>
<tr>
<td>Shellfish (e.g. mussels,</td>
<td>Rice</td>
</tr>
<tr>
<td>oysters)</td>
<td>Pastry</td>
</tr>
<tr>
<td>Crustaceans (e.g. prawns,</td>
<td>Biscuits</td>
</tr>
<tr>
<td>lobster)</td>
<td>Cake</td>
</tr>
<tr>
<td>Ruminants (e.g. sheep, cattle</td>
<td>Pasta</td>
</tr>
<tr>
<td>Monogastric (e.g. ham, pig)</td>
<td>Bread - white</td>
</tr>
<tr>
<td>Poultry (e.g. chicken, duck</td>
<td>Bread - wholemeal</td>
</tr>
<tr>
<td>turkey)</td>
<td></td>
</tr>
<tr>
<td>Game (e.g. Rabbit, Bird,</td>
<td></td>
</tr>
<tr>
<td>Kangaroo)</td>
<td></td>
</tr>
<tr>
<td><strong>FRUITS</strong></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Citrus (e.g. oranges,</td>
</tr>
<tr>
<td></td>
<td>lemons)</td>
</tr>
<tr>
<td>Brain</td>
<td>Tropical Fruit (e.g. mango,</td>
</tr>
<tr>
<td></td>
<td>papaya, banana)</td>
</tr>
<tr>
<td>Giblets (e.g. kidneys,</td>
<td>Stone Fruit (e.g. plums,</td>
</tr>
<tr>
<td>heart, intestines)</td>
<td>apricots, cherries, peaches)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PLANT</strong></td>
<td></td>
</tr>
<tr>
<td>Vegetables:</td>
<td></td>
</tr>
<tr>
<td>Root, white (potatoes)</td>
<td></td>
</tr>
<tr>
<td>Root, yellow (carrots)</td>
<td></td>
</tr>
<tr>
<td>Leafy (e.g. spinach, cabbage)</td>
<td></td>
</tr>
<tr>
<td>Marrow</td>
<td></td>
</tr>
<tr>
<td>Flowers (e.g. broccoli,</td>
<td>Apples</td>
</tr>
<tr>
<td>cauliflower</td>
<td>Pears</td>
</tr>
<tr>
<td>Stalks (celery)</td>
<td>Berries (e.g. strawberries,</td>
</tr>
<tr>
<td></td>
<td>raspberries)</td>
</tr>
<tr>
<td>Onion-like (e.g. spring</td>
<td></td>
</tr>
<tr>
<td>onions)</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
</tr>
<tr>
<td>Peppers (capsicum)</td>
<td></td>
</tr>
<tr>
<td>Legumes (e.g. beans, peas,</td>
<td></td>
</tr>
<tr>
<td>lentils</td>
<td></td>
</tr>
<tr>
<td>GROUP</td>
<td>GROUP</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
<tr>
<td><strong>Confectionery</strong></td>
<td></td>
</tr>
<tr>
<td>lollies</td>
<td></td>
</tr>
<tr>
<td>chocolate</td>
<td>Tea</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td><strong>Coffee</strong></td>
</tr>
<tr>
<td><strong>Jam</strong></td>
<td><strong>Alcohol</strong></td>
</tr>
<tr>
<td><strong>Added Fat</strong></td>
<td><strong>Softdrink</strong></td>
</tr>
<tr>
<td><strong>Added Sugar</strong></td>
<td>Water</td>
</tr>
</tbody>
</table>
Maximal variety (m) in a week's food consumption would have been the use of each food category on at least one occasion. The food variety score was a fraction of the total (X/m) for convenience expressed as X. The total food units (TFU) eaten in a week was the total number of average serving sizes (according to Davis Briggs and Mark Wahlqvist, 1985) from any category of food eaten in a week. It reflects total amount of food eaten. For any food category, the frequency of consumption is expressed as the number of average servings eaten per week.

In order to consider the independent predictive power of various aspects of food intake, food indices and categories were included in a stepwise regression analysis with an arterial wall index as the dependent variable, using SPSS (Statistical Package for the Social Sciences).

For an index of foods potentially protective against macrovascular disease, the number of servings that those food were eaten per week was aggregated:

Fish (Kromhout et al. 1985)
Lentils (Simpson et al. 1981)
Whole grain cereal based food (Morris and Marr. 1977)
Mushroom and fungi (Xiao et al. 1986)
Citrus and tropical fruits (Jenkins. 1975)

These foods were chosen because they have been shown to alter presumed risk factors of one kind or another for macrovascular disease. The risk factors involved include platelet function (fish), serum lipids (fish, lentils, fungi, citrus and tropical fruits) and blood glucose or serum insulin (lentils, whole grain cereals,
citrus and tropical fruits). The concept of this expression is that the coalescence of these foods in the diet may be synergistic or interactive or even operative in unique ways, not in evidence with a consideration of one food type by itself or a component of that food.

In order to consider the relative importance of plant-derived and animal-derived foods, foods were given aggregate scores in each of those categories. These were either an index of variety or the total food units consumed per week in a plant or animal-derived food category.

For foods categorised according to biological source (e.g. monogastric meat, ruminant meat), the total number of average serving sizes eaten per week was referred to as that food's "Unite". In most cases this figure approximates to the number of occasions eaten per week, but in some cases it exceeds it.

The rationale of combining non-insulin-dependent diabetics with their healthy controls for correlation analysis is to provide sufficient amplitude in arterial wall indices to recognise determinants. It should also be remembered that impaired glucose tolerance (IGT), intermediate between what is regarded as normal and diabetes, is also seen epidemiologically as a situation of increased risk for macrovascular disease or its outcomes, (Lo et al. 1986; Relf et al. 1986; Wahlqvist et al. 1986). What distinguishes diabetes from IGT, by W.H.O. criteria (W.H.O. 1980; Zimmet 1982) is the propensity to microvascular disease such as background retinopathy. Thus it is reasonable, and indeed desirable, to consider a continuum of metabolic
abnormality from normality, through IGT to type II diabetes managed only on diet. We are not considering type II diabetes managed by oral agents or type I diabetes, which is insulin dependent diabetes.

As far as food intake in this diet with type II diabetes is concerned, we know that adherence to dietary recommendations is low about 50% of diabetics population at Prince Henry’s Hospital (Truswell 1975; Blau 1979; Kouris and Wahlqvist 1985).

Thus to a large extent the dietary pattern of diabetics in this study will reflect their usual pre-diabetic diet. Some, however, will have changed their diet. No account of change in diet in any subjects has been taken in this study. Therefore, it must be stressed, that the analysis of food intake relationships here is in respect of current and not past diet, whether in diabetics or their controls. Furthermore, there was no matching of patients or controls in food habits, so that the usual range of food habits in each group can be expected to have been reflected.

Statistical comparison of groups for differences in arterial wall indices were made using student’s T-test and where required, the Mann-Whitney U-test. The relationships between food intake and arterial wall indices were examined by parametric (r) and, where required, non-parametric correlation analysis ($r_s$).

7.3 Distribution of Food Consumption in Non-Insulin-Dependent Diabetics and their Healthy Controls
Table 7.2  DISTRIBUTION OF FOOD CONSUMPTION PER WEEK IN
NON-INSULIN DEPENDENT DIABETICS AND THEIR HEALTHY
CONTROLS.

<table>
<thead>
<tr>
<th>Food Consumption</th>
<th>Food Variety</th>
<th>Food Unit</th>
<th>Protective Food Score</th>
<th>Protective Food Unit</th>
<th>Plant Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low  High</td>
<td>Low  High</td>
<td>Low  High</td>
<td>Low  High</td>
<td>Low  High</td>
</tr>
<tr>
<td></td>
<td>14-23  24-36</td>
<td>76-130  131-205</td>
<td>2-3  4-5</td>
<td>2-25  26-47</td>
<td>11-18  19-68</td>
</tr>
<tr>
<td>Median</td>
<td>24.0</td>
<td>131.0</td>
<td>4.0</td>
<td>26.0</td>
<td>19.0</td>
</tr>
<tr>
<td>Frequency</td>
<td>26  27</td>
<td>27  26</td>
<td>23  30</td>
<td>26  27</td>
<td>26  27</td>
</tr>
<tr>
<td>%</td>
<td>49.1  50.9</td>
<td>50.9  49.1</td>
<td>43.4  56.6</td>
<td>49.1  50.9</td>
<td>49.1  50.9</td>
</tr>
<tr>
<td>Mean ± S.E</td>
<td>23.3 ± 0.7</td>
<td>129.7 ± 3.6</td>
<td>3.49 ± 0.10</td>
<td>26.0 ± 1.3</td>
<td>19.3 ± 1.1</td>
</tr>
</tbody>
</table>

<p>|                  | 53 | 53 | 53 | 53 | 53 |</p>
<table>
<thead>
<tr>
<th>Food Consumption</th>
<th>Plant Unit</th>
<th>Bread Unit</th>
<th>Legumes Unit</th>
<th>Mushroom Unit</th>
<th>Citrus &amp; Tropical fruit unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>52-105</td>
<td>106-181</td>
<td>0-14</td>
<td>15-35</td>
<td>0-2</td>
<td>3-14</td>
</tr>
<tr>
<td>Median</td>
<td>106.0</td>
<td>15.0</td>
<td>3.0</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Frequency</td>
<td>26</td>
<td>27</td>
<td>26</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>%</td>
<td>49.1</td>
<td>50.9</td>
<td>49.1</td>
<td>50.9</td>
<td>84.9</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>103.4 ± 3.7</td>
<td>16.8 ± 1.2</td>
<td>3.32 ± 0.43</td>
<td>0.189 ± 0.07</td>
<td>5.13 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Food Consumption</td>
<td>Tea Unit</td>
<td>Coffee Unit</td>
<td>Dairy Unit</td>
<td>Ruminant Unit</td>
<td>Fish Unit</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>-------------</td>
<td>------------</td>
<td>---------------</td>
<td>-----------</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>0-9</td>
<td>10-45</td>
<td></td>
<td>0-4</td>
<td>5-14</td>
<td>1-6</td>
</tr>
<tr>
<td>Median</td>
<td>10.0</td>
<td>6.0</td>
<td>5.0</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Frequency</td>
<td>26</td>
<td>27</td>
<td>25</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>%</td>
<td>49.1</td>
<td>50.9</td>
<td>47.2</td>
<td>52.8</td>
<td>45.3</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>12.3 ± 1.6</td>
<td>10.6 ± 1.7</td>
<td>4.94 ± 0.50</td>
<td>8.32 ± 1.5</td>
<td>1.17 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>53</td>
</tr>
</tbody>
</table>
7.4 Relationships Between Protective Food Consumption and Arterial Compliance (AC), Proximal Resistance of Common Femoral Artery and Posterior Tibial Artery in Non-insulin-Dependent Diabetics and their Healthy Controls.

7.4.1 Arterial Compliance

There was a significant decrease in the arterial compliance in the low protective food group compared with the high protective food group in healthy subjects. The mean arterial compliances were $0.80 \pm 0.07$ and $0.96 \pm 0.03$ respectively ($P<0.05$). (Table 7.3).

There was a significant decrease in the arterial compliance in the low protective food group compared with the high protective food group in non-insulin-dependent diabetics. The mean arterial compliances were $0.47 \pm 0.04$ and $0.60 \pm 0.03$ respectively ($P<0.05$). (Table 7.3).

7.4.2 Proximal resistance of the common femoral artery

There was no significant difference in the proximal resistance of the common femoral artery in the low protective food group compared with the high protective food group of non-insulin-dependent diabetics or their healthy controls. The mean proximal resistance of the common femoral artery was $0.51 \pm 0.05$ and $0.41 \pm 0.04$ respectively for non-insulin-dependent diabetics (NS). The mean proximal resistance of common femoral artery was $0.39 \pm 0.04$ and $0.32 \pm 0.02$ respectively for healthy subjects (NS) (Table 7.3).
7.4.3 Proximal resistance of posterior tibial artery

There was a significant increase in the proximal resistance of the posterior tibial artery in the low protective food group compared with the high protective food group in healthy subjects. The mean proximal resistance of posterior tibial artery was $0.59 \pm 0.03$ and $0.49 \pm 0.03$ respectively ($P<0.025$) (Table 7.3).

There was a significant increase in the proximal resistance of posterior tibial artery in the low protective food group compared with the high protective food group in non-insulin-dependent diabetics. The mean of proximal resistance of posterior tibial artery was $0.44 \pm 0.04$ and $0.34 \pm 0.01$ respectively ($P<0.025$) (Table 7.3).
Table 7.3 The arterial compliance (AC), proximal resistance (PR) of common femoral artery and posterior tibial artery of high protective food group and low protective food group in healthy subjects and non-insulin-dependent diabetics. (Mean ± S.E)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Age</th>
<th>Arterial Compliance (AC)</th>
<th>Proximal Resistance (PR)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HNID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high(1)protective</td>
<td>12</td>
<td>66.5</td>
<td>0.60 ± 0.03</td>
<td>0.41 ± 0.04</td>
<td>0.49 ± 0.03</td>
</tr>
<tr>
<td>food score</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>2.7</td>
</tr>
<tr>
<td>low (2)protective</td>
<td>10</td>
<td>66.0</td>
<td>0.47 ± 0.04</td>
<td>0.51 ± 0.05</td>
<td>0.59 ± 0.03</td>
</tr>
<tr>
<td>food score</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>2.6</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td></td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td>Healthysubjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>high protective</td>
<td>18</td>
<td>69.4</td>
<td>0.96 ± 0.03</td>
<td>0.32 ± 0.02</td>
<td>0.34 ± 0.01</td>
</tr>
<tr>
<td>food score</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>low protective</td>
<td>13</td>
<td>67.5</td>
<td>0.80 ± 0.07</td>
<td>0.39 ± 0.04</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>food score</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td></td>
<td>&lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.025</td>
</tr>
</tbody>
</table>
(1) We have suggested a protective food index which included 5 kinds of food: (a) fish, (b) wholemeal bread, (c) legumes, (d) mushroom and fungi, (e) citrus and tropical fruit (papaya, mango, banana etc.). Those in the high protective food group consumed 4-5 kinds of protective food per week, as determined in Table 7.2.

(2) Those in the low protective food group consumed: 2-3 kinds of protective food per week.

7.5 The relationships between fish consumption and Arterial Compliance (AC), proximal resistance of Common Femoral Artery and Posterior Tibial Artery in Non-Insulin-Dependent Diabetics and their Healthy Controls.

7.5.1 Arterial Compliance

There was a significant decrease in the arterial compliance in the non-fish eating group compared with the fish eating group in healthy subjects. The mean arterial compliances were 0.79 ± 0.07 and 0.96 ± 0.03 respectively (P<0.025). Table 7.3

There was significant decrease in the arterial compliance in the non-fish eating group compared with the fish eating group in non-insulin-dependent diabetics. The mean arterial compliances were 0.46 ± 0.04 and 0.59 ± 0.03 respectively (P<0.05). (Table 7.4).

7.5.2 Proximal Resistance of Common Femoral Artery

There was no significant difference in the proximal resistance of the common femoral artery in the non-fish eating group compared with the fish eating group in
healthy subjects or in non-insulin-dependent diabetics. The mean proximal resistance of the common femoral artery was $0.40 \pm 0.04$ and $0.32 \pm 0.02$ respectively for healthy subjects (NS). The mean proximal resistance of the common femoral artery was $0.49 \pm 0.06$ and $0.44 \pm 0.04$ respectively for non-insulin-dependent diabetics (NS). (Table 7.4).

7.5.3 Proximal resistance of the posterior tibial artery

There was a significant increase in the proximal resistance of the posterior tibial artery in the non-fish eating group compared with the fish eating group in healthy subjects. The mean proximal resistances of the posterior tibial artery were $0.44 \pm 0.04$ and $0.35 \pm 0.01$ respectively ($P<0.025$). (Table 7.4).

There was no significant difference in the proximal resistance of posterior tibial artery in the non-fish eaters compared with fish eaters in non-insulin-dependent diabetics. The mean proximal resistances of the posterior tibial artery were $0.54 \pm 0.06$ and $0.51 \pm 0.04$ respectively (NS). (Table 7.4).
Table 7.4  The Arterial Compliance (AC), and Proximal Resistances of Common Femoral Artery and Posterior Tibial Artery of the Fish Eating and Non-Fish Eating Groups of Healthy Subjects and Non-Insulin-Dependent Diabetics (Mean ± S.E.).

<table>
<thead>
<tr>
<th>Group</th>
<th>Age</th>
<th>Arterial Compliance (Ac)</th>
<th>Proximal Resistance (PR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Common Femoral artery</td>
</tr>
<tr>
<td>Fish</td>
<td>13</td>
<td>62.5 0.59 ± 0.03</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.51 ± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>Non-Fish</td>
<td>9</td>
<td>68.0 0.46 ± 0.04</td>
<td>0.49 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>0.54 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

| Mediterranean  |     |                           |                          |
| Fish           | 19  | 69.5 0.96 ± 0.03          | 0.32 ± 0.02              |
|                |     | +                         | 0.35 ± 0.01              |
|                |     | 1.5                       |                          |
| Non-Fish       | 12  | 67.2 0.79 ± 0.07          | 0.40 ± 0.04              |
|                |     | +                         | 0.44 ± 0.04              |
|                |     | 3.2                       |                          |
| P              | NS  | <0.025                    | NS                       |
| Healthy subjects |   |                           |                          |
(3) Fish eaters: Fish intake 1-5 servings per week per person, about 100g-500g per week.

(4) Non-fish eaters: no fish intake in a week.
7.6 Univariate Evaluation of Food Determinants of Arterial Wall Characteristics

7.6.1 Arterial compliance

Univariate analysis showed significant and positive correlations between arterial compliance and food variety and also protective food score in all subjects combined, healthy subjects and non-insulin-dependent diabetics. The correlation coefficient ($r$) of the arterial compliance and food variety was 0.36 ($P<0.01$) and the correlation coefficient ($r$) of the arterial compliance and protective food score was 0.35 ($P<0.01$). (Table 7.5). Non-parametric correlation coefficients ($r_s$) confirmed the relationships (Table 7.5). There was no significant correlations between the arterial compliance and food variety in healthy subjects or non-insulin-dependent diabetics (Table 7.8).

7.6.2 Proximal resistance of the common femoral artery

Univariate analysis showed a significant and negative correlation between the proximal resistance of the common femoral artery and food variety and also protective food score in all subjects combined, healthy subjects and non-insulin-dependent diabetics. The correlation coefficient ($r$) of the proximal resistance of the common femoral artery and food variety was $-0.36$ ($P<0.01$) and the correlation coefficient ($r$) of the proximal resistance of the common femoral artery and protective food score was $-0.37$ ($P<0.01$). (Table 7.5). Non-parametric correlation coefficients ($r_s$) confirmed these relationships. (Table 7.6). There was no significant correlations between the proximal resistance of common femoral artery and food variety in healthy subjects or non-insulin-dependent diabetics (Table 7.8).
7.6.3 Proximal resistance of the posterior tibial artery

Univariate analysis showed a significant and negative correlation between the proximal resistance of the posterior tibial artery and food variety and protective food score consumption in all subjects, healthy subjects and non-insulin-dependent diabetics. The correlation coefficient ($r$) of the proximal resistance of posterior tibial artery and food variety was $-0.43$ ($P<0.01$) and the correlation coefficient ($r$) of the proximal resistance of posterior tibial artery and protective food score was $-0.35$ ($P<0.01$). (Table 7.7). Non-parametric analysis correlation coefficients ($r_s$) confirmed these relationships (Table 7.7). There was no significant correlations between the proximal resistance of posterior tibial artery in healthy subjects (Table 7.8), but there was a significant negative correlation between the proximal resistance of posterior tibial artery in non-insulin-dependent diabetics (Table 7.8). The correlation coefficient ($r$) was $-0.54$ ($p<0.01$). Non-parametric analysis correlation coefficient ($r_s$) confirmed this relationship (Table 7.8).
Table 7.5  Correlations (r or rs) between arterial compliance and food variety or protective food score in healthy subjects and non-insulin-dependent diabetics combined.

<table>
<thead>
<tr>
<th></th>
<th>Food Variety (n=53)</th>
<th>Protective Food Score (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>r</strong></td>
<td>0.36</td>
<td>0.35</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>rs</strong></td>
<td>0.38</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Table 7.6  The correlation ($r$ or $rs$) between proximal resistance of common femoral artery and food variety or protective food score in healthy subjects and non-insulin-dependent diabetics.

<table>
<thead>
<tr>
<th></th>
<th>Food Variety ($n=53$)</th>
<th>Protective Food Score ($n=53$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>-0.36</td>
<td>-0.37</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>$rs$</td>
<td>-0.38</td>
<td>-0.39</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt;0.01$</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>
Table 7.7 The correlations (r or rs) between proximal resistance of posterior tibial artery and food variety or protective food score in healthy subjects and non-insulin-dependent diabetics.

<table>
<thead>
<tr>
<th>Food Variety (n=53)</th>
<th>Protective Food Score (n=53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.43</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rs</td>
<td>-0.44</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 7.8 The correlation (r or rs) between arterial indices and food variety in healthy subjects and non-insulin-dependent diabetics.

<table>
<thead>
<tr>
<th>Arterial Indices</th>
<th>Healthy (n=31)</th>
<th>NIDD (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>rs</td>
</tr>
<tr>
<td>Arterial compliance</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Proximal resistance of common femoral artery</td>
<td>-0.19</td>
<td>-0.17</td>
</tr>
<tr>
<td>Proximal resistance of posterior tibial artery</td>
<td>-0.10</td>
<td>-0.20</td>
</tr>
</tbody>
</table>

(p<0.01)(p<0.01)
**Fig. 7.2** Correlation between arterial compliance and food variety.
Fig. 7.3 Correlation between proximal resistance of common femoral artery and food variety.
Fig. 7.4 Correlation between proximal resistance of posterior tibial artery and food variety.
7.7 Multivariate Evaluation of Food Determinants of Arterial Wall Characteristics

Multivariate analysis was used to examine each of the following food factors: food variety, protective food score, legumes units, citrus and tropical fruit units, bread units, mushroom units, fish units, dairy units, ruminants units, tea units and coffee units. This analysis showed that the factor which most influenced arterial compliance, proximal resistance of common femoral artery and posterior tibial artery was the food variety. The relationship is shown in Figure 7.2, 7.3 and 7.4.

Discussion

The relationship between dietary intake and arteriosclerotic cardiovascular disease has received a great deal of attention (Morris et al. 1977; Dyerberg 1981; Dyerberg and Bang 1982; Lauzola et al. 1983; Arutzenius et al. 1985; Glueck et al. 1986; Lipidus et al. 1986). In recent years we have seen that arteriosclerotic cardiovascular disease is the most frequent cause of premature death in Western industrialised nations. Cross-cultural studies have shown a strong association between dietary fat (total fat, saturated fat and cholesterol) intake and both the prevalence and incidence of myocardial infarction and sudden death (Keys 1979; Stamler and Stamler 1984).

Recently, studies in man and animals have demonstrated that diets high in cereal grains, legumes, vegetables and fruits are associated with a reduced incidence of cardiovascular disease. This appears to be due primarily to the associated reduction in serum cholesterol and low density lipoprotein cholesterol (Brown et al. 1984; Gotto et al. 1984; Brown and Karmally 1985).
The effects of the high carbohydrate diets from vegetable sources may be partly attributed to certain plant fibres including lignin, pectin and gums. Reduction in total cholesterol and LDL-cholesterol by certain plant fibres including pectin, gums and lignin are observed more consistently than others (Keys et al. 1961; Palmer and Dixon 1965; Durrington et al. 1976). It has been shown that, after three months increased intake of Chinese mushroom, the mean values of serum total cholesterol and triglycerides are significantly decreased (Xiao et al. 1986).

The present study showed a significant decrease in the arterial compliance in those in the low protective food group compared with the high protective food group whether healthy subjects or non-insulin-dependent diabetics. The study also showed a significant increase in the proximal resistance of the posterior tibial artery in the low protective food group compared with high protective food group in healthy subjects and in non-insulin-dependent diabetics. There was a significant and positive correlation between arterial compliance and both food variety and protective food score in all subjects combined, in healthy subjects and in non-insulin-dependent diabetics. The study also showed a significant and negative correlation between the proximal resistance of the common femoral artery and posterior tibial artery and food variety and protective food score in all subjects combined, in healthy subjects and in non-insulin-dependent diabetics. These findings indicate the importance of a protective food (including fish, cereal grains, legumes, mushroom, citrus and tropical fruit) and food variety consumption in respect of arterial wall characteristics.
What the present study emphasises is the value of aggregate food indices, rather than single foods or components of foods, in predicting a health outcome, in this case pre-clinical macrovascular disease. More work can be done with the data bases available from those investigations with, for example, meals and eating patterns, but for the moment, food variety and potentially protective foods are of particular interest because these categories are being incorporated into national dietary guidelines in countries like Australia which have an excess of chronic disease such as cardiovascular disease, type II diabetes and large bowel cancer, in an effort to reduce the prevalence of these diseases. The present approach is a novel way to test the recommendations.

The relationship between fish intake and arteriosclerotic cardiovascular disease has also received a great deal of attention (Kromhout et al. 1985; Herold and Kinsella 1986; Knapp et al. 1986; Weiner et al. 1986). Kromhout et al (1985) reported that mortality from coronary heart disease was more than 50 percent lower among those who consumed at least 30g of fish per day than among those who did not eat fish. The consumption of as little as one or two fish dishes per week may be of preventive value in relation to coronary heart disease. Knapp et al. (1986) reported that populations that consume a diet rich in marine lipids may have a lower risk of atherosclerotic vascular disease. Studies based on dietary history have suggested that even a small intake of fish may have reduced the incidence of coronary vascular disease in both European and North American populations (Shekelle et al. 1985). Weiner et al. (1986) reported that significantly less disease was seen in arterial sections from pigs that were fed cod-liver oil. The mean lesion area per vessel, mean luminal encroachment
per vessel, and mean maximal luminal encroachment per vessel were reduced in animals that were fed cod-liver oil, as compared with controls. They conclude that, in their animal model, dietary cod-liver oil retarded the development of coronary artery disease, possibly through changes in prostaglandin metabolism.

Herold and Kinsella (1986) compared the findings from animal and human trials. They found that dietary n-3 polyunsaturated fatty acids (n-3PUFAs), abundant in marine organisms, may reduce the development of cardiovascular disease. The data from laboratory animals and human volunteers show many similar responses in certain parameters (such as serum lipids, lipoproteins, triglycerides, cholesterol) to the consumption of n-3PUFAs. The biochemical and metabolic changes observed are generally consistent with reduced development of arteriosclerotic cardiovascular disease.

The study has shown a significant decrease in arterial compliance in a non-fish eating group compared with fish eating group whether healthy subjects or non-insulin-dependent diabetics. The study also showed a significant increase in the proximal resistance of the posterior tibial artery in the non-fish eating group compared with fish eating group of healthy subjects. These findings also indicate a potential role for fish intake between about 0 and 70 g per day in the determination of arterial wall characteristics.

7.9 Conclusion

This study showed a significant and positive correlation between arterial compliance and food variety, protective food score in all subjects
combined, in healthy subjects and in non-insulin-dependent diabetics. The study also demonstrated a significant and negative correlation between the proximal resistance of the common femoral artery and the posterior tibial artery with food variety and protective food score in all subjects combined, in healthy subjects and non-insulin-dependent diabetics. These findings indicate a relationship between the food variety and protective food score and arterial wall characteristics.

A significant decrease in the arterial compliance in the low protective food score group compared with the high protective food score group in healthy subjects and in non-insulin-dependent diabetics was observed. The study also showed a significant increase in the proximal resistance of the posterior tibial artery in the low protective food group compared with high protective food group in healthy subjects and non-insulin-dependent diabetics. The significant difference between the low protective food group and the high protective food group indicates a potentially important role for protective food consumption in relation to arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.

There was a significant decrease in the arterial compliance in non-fish-eaters compared with fish-eaters whether healthy subjects or non-insulin-dependent diabetics. A significant increase in the proximal resistance of the posterior tibial artery in non-fish eaters compared with fish eaters in healthy subjects was observed. These significant differences between non-fish eaters and fish eaters also indicate the potential importance of fish consumption for arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.
CHAPTER EIGHT

GENERAL CONCLUSION

The study showed a significant decrease in the arterial compliance, and a significant increase in the arterial proximal resistance of the common femoral artery and posterior tibial artery in non-insulin-dependent diabetics. The study showed a significant negative correlation between the arterial compliance and proximal resistance and, on the other hand, showed a significant positive correlation between the arterial proximal resistance of the common femoral artery and the posterior tibial artery. The significant difference between the healthy and non-insulin-dependent diabetic groups indicates that pre-clinical peripheral arterial disease can be recognised even in a mild diabetic by non-invasive measurement of compliance or proximal resistance.

Univariate analysis was used to examine each of the biochemical factors showed a significant and negative correlation between the arterial compliance and blood glucose, blood glycosylated haemoglobin (HbA1C), plasma free fatty acid and plasma insulin concentration in all subjects, healthy subjects and non-insulin-dependent diabetics. The study also showed a significant and positive correlation between arterial proximal resistance of the common femoral artery and the posterior tibial artery and blood glucose, blood glycosylated haemoglobin and plasma free fatty acid concentration in all subjects, healthy subjects and non-insulin-dependent diabetics. These findings indicate that plasma insulin, plasma free fatty acid,
blood glucose and blood glycosylated haemoglobin are risk factors for changes in arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.

Multivariate analysis of the metabolic variables showed that, the factor which most influenced the arterial compliance and proximal resistance of the posterior tibial artery was the glucose level in the fasting state or the glucose area after the glucose load. In addition, the factors which most influenced the proximal resistance of the common femoral artery were plasma free fatty acids in the fasting state or the blood glucose response to an oral glucose load. The study also showed that the factors which most influenced arterial compliance were the glucose level in men, and the insulin level in the fasting state or the free fatty acid response after an oral glucose load in women. These findings also indicate that blood glucose, plasma free fatty acid and plasma insulin are risk factors for changes in arterial wall characteristics at a stage when no clinical evidence of macrovascular disease is apparent.

There was a significant decrease in the arterial compliance and a significant increase in the proximal resistance of the posterior tibial artery in the low users of protective foods compared with high users whether healthy subjects or non-insulin-dependent diabetics. The study showed a significant decrease in the arterial compliance in the non-fish eater compared with the fish eater whether healthy subjects or non-insulin-dependent diabetics. There was also a significant increase in the proximal resistance of the posterior tibial artery in the non-fish eater compared with the fish eater amongst healthy subjects. These findings indicate the potential importance of food
variety, a protective food score (such as cereal, grains, legumes, mushroom, citrus and tropical fruit) and fish consumption in as far as arterial wall characteristics are concerned at a stage when no clinical evidence of macrovascular disease is apparent.

I consider, therefore, that of the original hypothesis, the following have been fulfilled.

1) **It is possible to recognise pre-clinical macrovascular disease in non-insulin-dependent diabetics, using non-invasive Doppler ultrasound techniques.**

2) Each of blood glucose, glycosylated haemoglobin (HbA1C), plasma free fatty acids, plasma insulin, but not serum total cholesterol, HDL-cholesterol and triglycerides are predictive of arterial wall indices in non-insulin-dependent diabetics considered together with their healthy controls by univariate parametric and non-parametric analysis.

3) **The food indices of variety and of putatively protective foods are predictive of macrovascular disease in non-insulin-dependent diabetics.**
Publications arising to data from work presented in this thesis are:


Aspects of the work presented to date at national and international meetings:


REFERENCES


Pyorala, K. (1979). Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: Results from two population studies in Finland. Diabetes Care 2, 131-41.


Appendix 1: General characteristics of non-insulin-dependent diabetics and healthy subjects

<table>
<thead>
<tr>
<th>NIDD No.</th>
<th>SEX</th>
<th>AGE (yr)</th>
<th>HEIGHT (cm)</th>
<th>WEIGHT (kg)</th>
<th>BLOOD PRESSURE (mmHg)</th>
<th>SMOKING</th>
<th>DURATION (YR-MONTH)</th>
<th>TREATED BY</th>
<th>MEDICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>72</td>
<td>170</td>
<td>65</td>
<td>130/80</td>
<td>NO</td>
<td>3-0</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>55</td>
<td>166</td>
<td>88</td>
<td>130/82</td>
<td>NO</td>
<td>1-2</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>68</td>
<td>170</td>
<td>73</td>
<td>127/86</td>
<td>NO</td>
<td>1-1</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>66</td>
<td>183</td>
<td>97</td>
<td>131/82</td>
<td>NO</td>
<td>4</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>74</td>
<td>173</td>
<td>71</td>
<td>167/93</td>
<td>NO</td>
<td>10</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>67</td>
<td>172</td>
<td>77</td>
<td>126/80</td>
<td>NO</td>
<td>9</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>36</td>
<td>169</td>
<td>109</td>
<td>128/83</td>
<td>NO</td>
<td>2</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>66</td>
<td>175</td>
<td>65</td>
<td>130/82</td>
<td>NO</td>
<td>3-0</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>53</td>
<td>176</td>
<td>84</td>
<td>111/73</td>
<td>NO</td>
<td>1</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>48</td>
<td>161</td>
<td>64</td>
<td>123/83</td>
<td>NO</td>
<td>2-6</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>NIDD No.</td>
<td>SEX</td>
<td>AGE</td>
<td>HEIGHT (cm)</td>
<td>WEIGHT (kg)</td>
<td>BLOOD PRESSURE (mmHg)</td>
<td>SMOKING</td>
<td>DURATION YEAR</td>
<td>MONTH</td>
<td>TREATED BY DIET ALONE</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>-----</td>
<td>-------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>---------</td>
<td>---------------</td>
<td>--------</td>
<td>----------------------</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>59</td>
<td>164</td>
<td>82</td>
<td>129/81</td>
<td>NO</td>
<td>3</td>
<td>6</td>
<td>YES</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>57</td>
<td>176</td>
<td>94</td>
<td>135/91</td>
<td>NO</td>
<td>3</td>
<td>8</td>
<td>YES</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>65</td>
<td>157</td>
<td>58</td>
<td>124/73</td>
<td>NO</td>
<td>3</td>
<td>4</td>
<td>YES</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>62</td>
<td>168</td>
<td>91</td>
<td>130/72</td>
<td>NO</td>
<td>3</td>
<td>8</td>
<td>YES</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>76</td>
<td>156</td>
<td>67</td>
<td>135/71</td>
<td>NO</td>
<td>5</td>
<td>0</td>
<td>YES</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>66</td>
<td>157</td>
<td>70</td>
<td>132/86</td>
<td>NO</td>
<td>5</td>
<td>0</td>
<td>YES</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>70</td>
<td>153</td>
<td>59</td>
<td>130/82</td>
<td>NO</td>
<td>1</td>
<td>10</td>
<td>YES</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>64</td>
<td>153</td>
<td>71</td>
<td>132/82</td>
<td>NO</td>
<td>4</td>
<td>6</td>
<td>YES</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>56</td>
<td>165</td>
<td>97</td>
<td>147/89</td>
<td>NO</td>
<td>8</td>
<td>0</td>
<td>YES</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>68</td>
<td>162</td>
<td>64</td>
<td>138/80</td>
<td>NO</td>
<td>6</td>
<td>1</td>
<td>YES</td>
</tr>
<tr>
<td>NIDD No.</td>
<td>SEX</td>
<td>AGE</td>
<td>HEIGHT (cm)</td>
<td>WEIGHT (kg)</td>
<td>BLOOD PRESSURE (mmHg)</td>
<td>CIGARETTE SMOKING</td>
<td>DURATION YEAR MONTH</td>
<td>TREATED BY DIET ALONE</td>
<td>MEDICATIONS</td>
</tr>
<tr>
<td>---------</td>
<td>-----</td>
<td>-----</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>----------------------</td>
<td>----------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>78</td>
<td>151</td>
<td>89</td>
<td>148/88</td>
<td>NO</td>
<td>6 0</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>67</td>
<td>164</td>
<td>77</td>
<td>130/80</td>
<td>NO</td>
<td>4 10</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>66</td>
<td>163</td>
<td>80</td>
<td>130/86</td>
<td>NO</td>
<td>4 9</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>83</td>
<td>162</td>
<td>63</td>
<td>135/70</td>
<td>NO</td>
<td>4 0</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>59</td>
<td>147</td>
<td>69</td>
<td>140/82</td>
<td>NO</td>
<td>2 11</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>83</td>
<td>152</td>
<td>61</td>
<td>138/78</td>
<td>NO</td>
<td>5 1</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>27</td>
<td>F</td>
<td>61</td>
<td>152</td>
<td>70</td>
<td>123/70</td>
<td>NO</td>
<td>5 3</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>HEALTHY SUBJECTS No.</td>
<td>SEX</td>
<td>AGE (cm)</td>
<td>HEIGHT (kg)</td>
<td>WEIGHT</td>
<td>BLOOD PRESSURE mmHg</td>
<td>CIGARETTE SMOKING</td>
<td>MEDICATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>----------</td>
<td>-------------</td>
<td>--------</td>
<td>---------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>41</td>
<td>169</td>
<td>69</td>
<td>134/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>67</td>
<td>172</td>
<td>65</td>
<td>134/83</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>78</td>
<td>169</td>
<td>91</td>
<td>157/84</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>60</td>
<td>171</td>
<td>90</td>
<td>179/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>72</td>
<td>177</td>
<td>61</td>
<td>130/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>71</td>
<td>167</td>
<td>74</td>
<td>140/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>68</td>
<td>178</td>
<td>78</td>
<td>126/81</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>67</td>
<td>180</td>
<td>78</td>
<td>128/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>63</td>
<td>159</td>
<td>95</td>
<td>138/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>37</td>
<td>172</td>
<td>68</td>
<td>110/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject No.</td>
<td>SEX</td>
<td>AGE (cm)</td>
<td>HEIGHT (cm)</td>
<td>WEIGHT (kg)</td>
<td>BLOOD PRESSURE (mmHg)</td>
<td>CIGARETTE SMOKING</td>
<td>MEDICATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>-----</td>
<td>----------</td>
<td>-------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>63</td>
<td>148</td>
<td>57</td>
<td>122/78</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>73</td>
<td>157</td>
<td>64</td>
<td>128/67</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>76</td>
<td>149</td>
<td>60</td>
<td>131/68</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>72</td>
<td>166</td>
<td>79</td>
<td>148/92</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>69</td>
<td>166</td>
<td>96</td>
<td>141/89</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>70</td>
<td>152</td>
<td>43</td>
<td>136/68</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>74</td>
<td>161</td>
<td>62</td>
<td>130/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>75</td>
<td>162</td>
<td>79</td>
<td>139/84</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>93</td>
<td>159</td>
<td>74</td>
<td>130/74</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>77</td>
<td>157</td>
<td>89</td>
<td>138/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEALTHY SUBJECTS</td>
<td>SEX</td>
<td>AGE</td>
<td>HEIGHT (cm)</td>
<td>WEIGHT (kg)</td>
<td>BLOOD PRESSURE</td>
<td>CIGARETTE SMOKING</td>
<td>MEDICATIONS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-----</td>
<td>-----</td>
<td>-------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>58</td>
<td>166</td>
<td>80</td>
<td>134/84</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>81</td>
<td>150</td>
<td>54</td>
<td>140/78</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>70</td>
<td>167</td>
<td>82</td>
<td>128/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>64</td>
<td>160</td>
<td>73</td>
<td>140/76</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>72</td>
<td>159</td>
<td>52</td>
<td>115/74</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>64</td>
<td>145</td>
<td>59</td>
<td>132/64</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>F</td>
<td>57</td>
<td>158</td>
<td>62</td>
<td>130/78</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>F</td>
<td>70</td>
<td>148</td>
<td>47</td>
<td>130/82</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>F</td>
<td>63</td>
<td>151</td>
<td>67</td>
<td>142/78</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>80</td>
<td>144</td>
<td>56</td>
<td>120/68</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEALTHY SUBJECTS No.</td>
<td>SEX</td>
<td>AGE (cm)</td>
<td>HEIGHT (kg)</td>
<td>BLOOD PRESSURE (mmHg)</td>
<td>CIGARETTE SMOKING</td>
<td>MEDICATIONS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>-----</td>
<td>----------</td>
<td>-------------</td>
<td>-----------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>F</td>
<td>66</td>
<td>161</td>
<td>76</td>
<td>139/78</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>63</td>
<td>159</td>
<td>53</td>
<td>124/68</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>F</td>
<td>61</td>
<td>152</td>
<td>52</td>
<td>142/70</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>F</td>
<td>57</td>
<td>157</td>
<td>68</td>
<td>126/80</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>F</td>
<td>62</td>
<td>161</td>
<td>61</td>
<td>141/74</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>F</td>
<td>73</td>
<td>161</td>
<td>60</td>
<td>125/71</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>F</td>
<td>68</td>
<td>164</td>
<td>76</td>
<td>138/75</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>F</td>
<td>64</td>
<td>149</td>
<td>64</td>
<td>115/73</td>
<td>NO</td>
<td>NO</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>