PLASMA, PLATELET AND RED BLOOD CELL LONG CHAIN FATTY ACIDS IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

BY

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Abstract

The development of large vessel disease (LVD) in non-insulin-dependent diabetic mellitus (NIDDM) may partly be due to the combined effects of dyslipidaemia and altered plasma, platelet and red blood cell fatty acid compositions. These factors could act in a synergistic manner, leading to the increased morbidity and mortality seen in NIDDM. Populations consuming large quantities of (n-3) and monounsaturated fatty acids (MFA) have relatively low incidence of LVD. This phenomenon has been attributed to the beneficial effects of these fatty acids on lipid metabolism, platelet functions and other mechanisms connected to atherogenesis. However, there are relatively little information available on the compositions of plasma lipids and lipoproteins, or plasma, platelet and red blood cell fatty acids in male NIDDM patients with and without LVD, and on the therapeutic values of (n-3) fatty acids and MFA in NIDDM patients without LVD. Thus, this study was designed to study the differences in plasma lipid and lipoprotein compositions and fatty acid profiles (plasma, platelet and red blood cell) between NIDDM patients (20 males with LVD, 20 males without LVD) and normal subjects (n=20). The effects of dietary (n-3) fatty acid (fish oil, n=10) and MFA (olive oil, n=10) supplementations on the lipid and fatty acid profiles in NIDDM patients without LVD were also evaluated.

NIDDM patients, particularly those with LVD, had raised plasma triacylglycerol levels and the excesses may be accommodated in the large VLDL. Although individual lipoprotein cholesterol concentrations were similar in all diabetics, patients with LVD had higher plasma cholesterol levels. This difference may due to the concomitant increased plasma 18:1(n-9) and decreased plasma 18:2(n-6) levels, bringing about an increase in cholesterol synthesis.

Plasma and red blood cell 20:5(n-3) levels were higher in NIDDM patients, whereas their platelets and red blood cells 20:4(n-6) levels were lower. In addition, the platelet saturated fatty acid (SFA) contents were higher. The unexpected plasma and red blood cell 20:5(n-3) levels seen in the diabetics may due to the re-direction of the Δ5 desaturase activity towards the precursor of 20:5(n-3), whereas, higher platelet SFA levels may have undesirable effects on platelet aggregation. Higher
SFA and lower 18:2(n-6) levels were also seen in the red blood cells in NIDDM patients with LVD, reflecting impaired fatty acid acylation with lipids.

NIDDM patients (without LVD) on fish oil increased their plasma HDL\textsubscript{2} levels without concomitant changes in VLDL levels, possibly due to the formation of medium-sized VLDL particles, acting as an extra substrate for HDL\textsubscript{2} formation. Increases in plasma, platelet and red blood cell 20:5(n-3) contents and less markedly 22:6(n-3) were also seen in diabetics on fish oil. These changes occurred at the expense of (n-6) fatty acids. In contrast, olive oil had no effects on plasma, platelet and red blood cell fatty acid compositions. Both oils had produce a slight worsening of glycaemia in diabetics and therefore, should be used with caution as a treatment of dyslipidaemia in NIDDM.
Acknowledgements

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CONTENTS

Abstract
Acknowledgement

1   INTRODUCTION ................................................................. 21
   1.1 Diabetes Mellitus ......................................................... 21
   1.2 Large vessel disease .................................................... 21
   1.3 Epidemiology of LVD in Diabetes ................................. 22
      1.3.1 USA studies ......................................................... 22
      1.3.2 European studies ................................................. 23
      1.3.3 United Kingdom studies ........................................ 24
      1.3.4 Australian study ................................................. 24
      1.3.5 Summary ............................................................. 25
   1.4 Lipoprotein metabolism ................................................. 26
      1.4.1 Intestinal lipoproteins ............................................ 26
         1.4.1.1 Formation of micelles .................................... 26
         1.4.1.2 Absorption of micelles ................................... 26
         1.4.1.3 Intestinal lipid synthesis ................................ 26
         1.4.1.4 Intestinal lipoprotein secretion ....................... 27
      1.4.2 Chylomicron metabolism .......................................... 28
         1.4.2.1 Chylomicrons and lipoprotein lipase .................. 28
         1.4.2.2 Chylomicrons and hepatic lipase ....................... 28
         1.4.2.3 Chylomicron and apoE receptors ...................... 29
      1.4.3 VLDL metabolism .................................................. 30
         1.4.3.1 Hepatic synthesis of VLDL ............................... 30
         1.4.3.2 VLDL catabolism ............................................ 31
         1.4.3.3 Large and small VLDL .................................... 31
         1.4.3.4 VLDL and apoE .............................................. 32
1.4.4 LDL metabolism .......................................................... 32
  1.4.4.1 LDL and apoB,E receptors .................................. 32
  1.4.4.2 LDL and lipid exchange reaction ...................... 33
1.4.5 HDL metabolism .......................................................... 33
1.4.6 Chylomicron abnormalities in diabetes ................. 34
1.4.7 VLDL abnormalities in diabetes ............................ 35
  1.4.7.1 Hyperinsulinaemia-hypertriglyceridaemia hypothesis .... 35
  1.4.7.2 VLDL catabolism in Pima Indians with NIDDM ........ 36
  1.4.7.3 VLDL and apoE in NIDDM ............................... 36
  1.4.7.4 VLDL glycation and degradation by macrophages .... 37
1.4.8 LDL abnormalities in diabetes ............................. 37
  1.4.8.1 LDL levels in NIDDM .................................... 37
  1.4.8.2 FCR and synthetic rate of LDL in NIDDM .......... 38
  1.4.8.3 LDL and glycation in NIDDM ............................ 39
  1.4.8.4 LDL and macrophages in NIDDM ...................... 40
  1.4.8.5 LDL and lipid exchanges in NIDDM ................. 40
1.4.9 HDL abnormalities in diabetes ............................ 41
  1.4.9.1 HDL levels in NIDDM .................................... 41
  1.4.9.2 FCR and synthetic rate of HDL in NIDDM .......... 42
  1.4.9.3 HDL and glycation ........................................... 42
  1.4.9.4 HDL, lipoprotein and hepatic lipase in NIDDM ........ 43
1.5 Long chain fatty acids ................................................. 44
  1.5.1 Fatty acid nomenclature ....................................... 44
  1.5.2 Characteristics of fatty acids in mammalian systems ...... 46
  1.5.3 Fatty acid biosynthesis ....................................... 46
  1.5.3.1 Saturated fatty acids .................................... 46
  1.5.3.2 Unsaturated fatty acids .................................. 48
1.5.3.3 Rules for fatty acid desaturation .............. 49
1.5.3.4 Functions of the desaturases .................. 49
1.5.3.5 (n-7) and (n-9) pathways ..................... 50
1.5.3.6 (n-3) and (n-6) pathways ..................... 52
1.5.3.7 (n-8) pathway .................................. 53

1.5.4 Fatty acid profiles in Diabetes .................. 55
1.5.4.1 Diabetic animal fatty acid profiles .......... 55
1.5.4.2 Human plasma fatty acid profiles .......... 56
1.5.4.3 Human platelet fatty acid profiles ......... 57
1.5.4.4 Human red blood cell fatty acid profiles ... 58

1.6 Fish oil, olive oil and NIDDM ....................... 59
1.6.1 Effects of fish oil on non-diabetic subjects .... 59
1.6.1.1 Plasma lipids and lipoproteins ............ 60
1.6.1.2 Fatty acid profiles ............................ 61
1.6.2 Effects of fish oil on NIDDM patients ......... 62
1.6.2.1 Plasma lipids, lipoproteins and glucose .... 62
1.6.2.2 Fatty acids profiles ........................... 64
1.6.3 Effects of olive oil on non-diabetic subjects .. 64
1.6.3.1 Epidemiological studies .................... 64
1.6.3.2 Dietary studies ................................ 65
1.6.4 Effects of olive oil on NIDDM patients ....... 66

1.7 Outline of the present study ....................... 66

2 METHODS .................................................. 68
2.1 Determination of glycated haemoglobins ........... 68
2.2 Determination of plasma cholesterol and triacylglycerol .. 68
2.3 Determination of lipoprotein cholesterol ........... 69
2.3.1 HDL cholesterol .................................. 69
2.3.2 HDL₂ and HDL₃ cholesterol .................... 69
2.3.3 Estimation of LDL cholesterol .................. 70
2.4 Ultracentrifugal analysis of plasma lipoproteins .... 70
2.4.1 Introduction ..................................... 70
2.4.2 Reagents ................................................................. 71
2.4.3 Centrifuge ............................................................... 72
2.4.4 Ultracentrifugal separation of plasma lipoproteins ... 72
2.4.5 Lipoprotein density adjustment .............................. 73
2.5 Analysis of plasma and platelet fatty acids ................. 76
  2.5.1 Reagents and materials ......................................... 76
  2.5.2 Plasma and platelet separation ............................ 76
  2.5.3 Extraction ............................................................. 77
  2.5.4 Isolation of plasma phospholipids by thin-layer
          chromatography .................................................. 77
  2.5.5 Transesterification and gas-liquid chromatography ... 78
  2.5.6 Quickbasic program for fatty acid calculation ....... 79
2.6 Analysis of red blood cell fatty acids ......................... 81
  2.6.1 Red blood cell separation .................................. 81
  2.6.2 Extraction ............................................................ 81
  2.6.3 Transesterification and gas-liquid chromatography ... 82
2.7 Statistical methods ................................................... 82
2.8 Methods - coefficient of variations ............................ 83

3 CROSS-SECTIONAL STUDIES ON PLASMA LIPID AND
BLOOD FATTY ACID PROFILES IN NIDDM PATIENTS ....... 85
3.1 Plasma lipid and fatty acid profiles in male NIDDM
  patients ................................................................. 85
  3.1.1 Aims ................................................................. 85
  3.1.2 Subjects ............................................................. 85
  3.1.3 Methods ............................................................. 85
  3.1.4 Results ............................................................. 86
    3.1.4.1 Clinical data and plasma lipid levels ............. 86
    3.1.4.2 Plasma total fatty acids ............................. 93
    3.1.4.3 Plasma phospholipid fatty acids ................... 105
  3.1.5 Discussion ......................................................... 110
    3.1.5.1 Plasma lipids ............................................... 110
4.5 Discussion ......................................................... 153

4.5.1 Effects of fish oil and olive oil on HbA1% and lipoprotein lipids ........................................ 153

4.5.2 Effects of fish oil on plasma phospholipid fatty acids ......................................................... 155

4.5.3 Effects of fish oil on platelet fatty acids .......... 156

4.5.4 Effects of fish oil on red blood cell fatty acids ... 156

4.5.5 Effects of olive oil on plasma, platelet and red blood cell fatty acids ................................. 157

5 CONCLUDING REMARKS ........................................ 158

Appendix I .......................................................... 161

Appendix II .......................................................... 162

Appendix III ........................................................ 164

References ......................................................... 177
Tables

Table 1  Fatty acid nomenclature .................................................. 45
Table 2  Physical parameters for KBr at 25 °C ............................... 75
Table 3  Age, body weight, HbA₁% and plasma lipid compositions in normal subjects & NIDDM patients. ........ 86
Table 4  Plasma fatty acid compositions in normal subjects and NIDDM patients. .................................................. 94
Table 5  Fatty acid compositions of plasma phospholipids from normal subjects & NIDDM patients. ..................... 106
Table 6  Platelet fatty acid compositions in normal subjects and NIDDM patients. ............................................... 118
Table 7  Red blood cell fatty acid compositions in normal subjects and NIDDM patients. ......................................... 132
Table 8  Fatty acid compositions of fish oil and olive oil. ................. 140
Table 9  Clinical characteristics of NIDDM patients. ....................... 141
Table 10 Changes in HDL₂ cholesterol with fish oil and olive oil treatment (8 weeks) ................................................. 142
Table 11 Changes in body weight and lipid with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) ......................... 143
Table 12 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) ....... 145
Table 13 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) .......... 146
Table 14 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) ......................... 148
Table 15 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) ......................... 149
Table 16 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) ........................................ 151
Table 17 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) .................................................. 152
Table 18 Age, body weight, HbA1c% and plasma lipid compositions in normal subjects & NIDDM patients with (LVD+) and without (LVD-) large vessel disease. ........................................................................................................ 164
Table 19 Plasma fatty acid compositions in normal subjects and NIDDM patients. ................................................................. 165
Table 20 Fatty acid compositions of plasma phospholipids from normal subjects & NIDDM patients. .................................................. 166
Table 21 Platelet fatty acid compositions in normal subjects and NIDDM patients. ................................................................. 167
Table 22 Red blood cell fatty acid compositions in normal subjects and NIDDM patients. ................................................................. 168
Table 23 Changes in body weight and lipids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) ........................................ 169
Table 24 Changes in body weight and lipids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) ........................................ 170
Table 25 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) .................. 171
Table 26 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) .................. 172
Table 27 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) .................. 173
Table 28 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) .......... 174

Table 29 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks) ................................................................. 175

Table 30 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks) ........................................................................ 176
Figures
Figure 1  Biosynthesis of saturated fatty acids .............................. 47
Figure 2  (n-7) and (n-9) fatty acid metabolic pathways .................. 51
Figure 3  (n-3) and (n-6) PUFA metabolic pathways ....................... 52
Figure 4  (n-8) and Δ5,11 metabolic pathways ............................... 54
Figure 5  A graph of partial specific volume of KBr vs its solution density .......................................................... 75
Figure 6  HbA1% in normal subjects & NIDDM patients ..................... 87
Figure 7  Plasma triacylglycerol levels in normal subjects & NIDDM patients. .............................................................. 88
Figure 8  Plasma cholesterol levels in normal subjects & NIDDM patients. .............................................................. 89
Figure 9  Relationship between LDL and plasma cholesterol in normal subjects & NIDDM patients. ......................... 90
Figure 10 Relationship between LDL cholesterol & plasma triacylglycerol values in normal subjects. ......................... 91
Figure 11 Relationship between log (LDL cholesterol) & plasma triacylglycerol values in normal subjects. ......................... 91
Figure 12 Relationship between HDL₂ cholesterol and plasma triacylglycerol values in normal subjects. ......................... 92
Figure 13  Gas chromatogram of plasma fatty acids .............................. 93

Figure 14  Plasma 16:0 content in normal subjects & NIDDM patients. ......................................................... 95

Figure 15  Plasma 18:1(n-9) content in normal subjects & NIDDM patients. .................................................. 96

Figure 16  Plasma 18:2(n-6) contents in normal subjects & NIDDM patients. ..................................................... 97

Figure 17  Relationship between plasma 18:2(n-6) and SFA+MFA in normal subjects & NIDDM patients. .................... 98

Figure 18  Plasma 20:5(n-3) content in normal subjects & NIDDM patients. ...................................................... 99

Figure 19  Plasma 20:2(n-6) content in normal subjects & NIDDM patients. .................................................... 100

Figure 20  Relationship between plasma triacylglycerol values & plasma 18:1(n-9) in normal subjects & NIDDM patients without LVD. .................................................... 101

Figure 21  Relationship between plasma triacylglycerol & plasma total (n-6) fatty acids in normal subjects & NIDDM patients without LVD. .................................................. 102

Figure 22  Relationship between plasma 18:1(n-9) & 18:2(n-6). Normal subjects & NIDDM patients. ...................... 103
Figure 23  Relationship between plasma 16:0 and 18:2(n-6).
Normal patients & NIDDM patients. ......................... 104

Figure 24  Gas chromatogram of plasma phospholipid fatty acids .............................................. 105

Figure 25  Plasma phospholipid 18:2(n-6) content in normal subjects & NIDDM patients. ................. 107

Figure 26  Plasma phospholipid 20:5(n-3) content in normal subjects & NIDDM patients. ............... 108

Figure 27  Plasma phospholipid 22:6(n-3) content in normal subjects & NIDDM patients. ............... 108

Figure 28  Plasma phospholipid 20:2(n-6) content in normal subjects & NIDDM patients. ............... 109

Figure 29  Gas chromatogram of platelet fatty acids .......................................................... 117

Figure 30  Platelet 16:1(n-7) content in normal subjects & NIDDM patients. ................................. 119

Figure 31  Platelet 16:0 content in normal subjects & NIDDM patients. .................................... 120

Figure 32  Platelet 20:4(n-6) content in normal subjects & NIDDM patients. ............................. 121

Figure 33  Platelet 18:2(n-6) content in normal subjects & NIDDM patients. ............................. 121
Figure 34  Platelet 20:2(n-6) content in normal subjects & NIDDM patients. ................................................................. 122

Figure 35  Relationship between platelet & plasma 22:5(n-3) in normal subjects & NIDDM patients. .............................. 123

Figure 36  Relationship between platelet & plasma 22:6(n-3) in normal subjects & NIDDM patients. .............................. 124

Figure 37  Relationship between platelet & plasma 20:5(n-3) in NIDDM patients. ............................................................ 125

Figure 38  Relationship between platelet & plasma 18:1(n-9) in normal subjects & NIDDM patients. .............................. 126

Figure 39  Relationship between platelet total SFA and 20:4(n-6) in NIDDM patients. ......................................................... 127

Figure 40  Gas chromatogram of red blood cell fatty acids .................................................................................................. 131

Figure 41  Total red blood cell C16 fatty acid contents in normal subjects & NIDDM patients. .............................................. 133

Figure 42  Red blood cell 18:2(n-6) content in normal subjects & NIDDM patients. ........................................................... 134

Figure 43  Red blood cell 20:4(n-6) content in normal subjects & NIDDM patients. ............................................................. 135

Figure 44  Red blood cell 20:5(n-3) content in normal subjects & NIDDM patients. ............................................................. 135
Abbreviations

µg/ml microgram per millilitre
µmol l⁻¹ micromole per litre
ρ density
τ Kendall rank-order correlation coefficient
16:0 palmitic acid (n-hexadecanoic acid)
16:1(n-7) palmitoleic acid (hexadec-9-enoic acid)
18:0 stearic acid (octadecanoic acid)
18:1(n-7) vaccenic acid (octadec-11-enoic acid)
18:1(n-9) oleic acid (octadec-9-enoic acid)
18:2(n-6) linoleic acid (octadeca-9,12-dienoic acid)
18:3(n-3) α-linolenic acid (octadeca-9,12,15-trienoic acid)
18:3(n-6) γ-linolenic acid (octadeca-6,9,12-trienoic acid)
18:4(n-3) parinaric acid (octadeca-9,11,13,15-tetraenoic acid)
20:2(n-6) eicosadienoic acid (eicosa-11,14-dienoic acid)
20:3(n-6) dihomo-γ-linolenic acid (eicosa-8,11,14-trienoic acid)
20:3(n-9) Mead acid (eicosa-5,8,11-trienoic acid)
20:4(n-6) arachidonic acid (eicosa-5,8,11,14-tetraenoic acid)
20:5(n-3) timnodonic acid (eicosa-5,8,11,14,17-pentaenoic acid)
22:1(n-11) cetolic acid (docos-11-enoic acid)
22:4(n-6) adrenic acid (docosa-7,10,13,16-tetraenoic acid)
22:5(n-3) clupanodonic acid (docosa-7,10,13,16,19-pentaenoic acid)
22:5(n-6) docosapentaenoic acid (docosa-4,7,10,13,16-pentaenoic acid)
22:6(n-3) cervonic acid docosa-4,7,10,13,16,19-hexaenoic acid)
A.R. analytical reagent
a-LA α-linolenic acid
ACAT acyl-CoA:cholesterol acyltransferase
ACP acyl carrier protein
ApoA-II apolipoprotein A-II
ApoA-IV apolipoprotein A-IV
<table>
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</tr>
<tr>
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<tr>
<td>b.p.</td>
<td>boiling point</td>
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<tr>
<td>CHD</td>
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<tr>
<td>DHA</td>
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</tr>
<tr>
<td>DHGLA</td>
<td>dihomo-γ-linolenic acid</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
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<td>fatty acid binding protein</td>
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<tr>
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<td>fractional catabolic rate</td>
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<td>3-hydroxy-3-methyl-glutaryl-coenzyme A reductase</td>
</tr>
<tr>
<td>IDDM</td>
<td>insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>IDL</td>
<td>intermediate density lipoproteins</td>
</tr>
<tr>
<td>LCAT</td>
<td>lecithin:cholesterol acyltransferase</td>
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LDL: low density lipoproteins
LVD: large vessel disease
LVD+: diabetic subjects with large vessel disease
LVD-: diabetic subjects without large vessel disease
MFA: monounsaturated fatty acids
mmol l\(^{-1}\): millimole per litre
Mn\(^{2+}\): manganese cations
n: number of subjects/samples
N: normal subjects
NADH: nicotinamide adenine dinucleotide, reduced
NIDDM: non-insulin-dependent diabetes mellitus
p: probability
PUFA: polyunsaturated fatty acids
S\(_{r}\) 0-12: Svedberg flotation rates of LDL
S\(_{r}\) 100-400: Svedberg flotation rates of large VLDL
S\(_{r}\) 20-60: Svedberg flotation rates of small VLDL
S\(_{r}\) 12-100: Svedberg flotation rates of VLDL "remnants"
SFA: saturated fatty acids
t\(_{1/2}\): time for half clearance of lipids from circulation
VLDL: very low density lipoproteins
vs: versus
1 INTRODUCTION

1.1 Diabetes Mellitus

Diabetes mellitus can be defined as a disease of absolute or relative deficiency in insulin production. This pathological condition has a profound effect on carbohydrate metabolism. Insulin deficiency prevents the entry of glucose into cells. As a consequence, blood glucose rises and the cells turn to an alternate source of energy, e.g. fat. Excessive fat catabolism may then lead to the accumulation of keto acids and metabolic acidosis which adversely affects brain function causing the patient to lapse into coma (1).

There are two types of diabetes. The first and more common type is maturity onset, non-insulin dependent diabetes mellitus (NIDDM), also known as non-ketotic or Type 2 diabetes. It often manifests itself after the age of 40 years and is frequently associated with obesity. Patients with NIDDM often have high plasma insulin levels during fasting and the postprandial state. Despite these increases in plasma insulin levels, they are unable to remove glucose from the circulation, possibly due to a defect at the cellular level in the insulin-mediated uptake of glucose. Most NIDDM patients are given oral hypoglycaemic agents to control their plasma glucose levels and do not require insulin therapy. However, there are some NIDDM patients who do not respond to oral hypoglycaemic treatment. For these patients, insulin is prescribed. The second and less common form of diabetes is insulin-dependent diabetes mellitus (IDDM), also known as ketosis-prone or Type 1 diabetes. It often occurs in individuals below the age of 20 years and is rarely associated with obesity. Patients with IDDM are characterised by insulin deficiency due to the autoimmune destruction of pancreatic B cells and require insulin therapy (1).

1.2 Large vessel disease

One of the most serious chronic complications of diabetes is large vessel disease (LVD), particularly affecting the blood vessels supplying the myocardium,
brain and lower limbs resulting in coronary heart disease, cerebrovascular disease and peripheral vascular disease. Coronary heart disease is characterised by angina pectoris, myocardial infarction and sudden death. Cerebrovascular disease may precipitate stroke as a result of either thrombosis or intracranial haemorrhage. Peripheral vascular disease is identified by intermittent claudication, rest pain and gangrene. For the purpose of this thesis, LVD is defined as coronary heart disease, cerebrovascular disease or peripheral vascular disease (2).

1.3 Epidemiology of LVD in Diabetes

1.3.1 USA studies

In the MRFIT study, Stamler et al compared the six year coronary heart disease mortality rates in 5,245 diabetic men and 350,997 non-diabetic men (aged 35-57). The age-adjusted death rates from coronary heart disease and cardiovascular diseases were approximately three-fold higher in diabetics than non-diabetics. The three major risk factors for coronary heart disease and cardiovascular diseases (serum cholesterol, blood pressure and cigarette smoking) had a similar impact in diabetics compared to the non-diabetics. However, the respective mortality rates from these diseases were much higher for diabetics than non-diabetics in the absence of the three risk factors (3).

Another prospective study from Framingham showed that both diabetic men (n=118) and women (n=121) in a sample of 5,209 subjects developed more cerebrovascular disease, coronary heart disease and occlusive peripheral arterial disease than non-diabetics (2). During the sixteen years of follow up, fifty-five diabetics died, forty-two of them as a result of cardiovascular complications. This amounted to almost three times the number of deaths seen in the general Framingham population. Of the forty-two cardiovascular deaths, twenty-six were found to have resulted from coronary heart disease. In order to represent the relative incidence of the three major forms of LVD in these patients, morbidity ratios were used. These ratios were calculated by dividing the number of diabetics observed with vascular complications by the number predicted by statistical theory. The
respective morbidity ratios determined for coronary heart disease, cerebrovascular diseases and intermittent claudication in male diabetics were 164, 235 and 484, while those in female diabetics were 225, 241 and 375. Hence, these results indicated that diabetic patients were more prone to vascular complications.

1.3.2 European studies

There have been several European prospective studies on mortality rates and complications in diabetics. For example, 2,560 predominantly NIDDM patients diagnosed in 1966 in the Erfurt district of the German Democratic Republic were monitored over the following ten years (4). Of the 2,381 diabetics remaining in the study at the end of this period, 1,054 had died. Cardiovascular causes accounted for 63% of these deaths and positively correlated with hypertension. Obesity and age were found to be the two risk factors most likely to promote the development of LVD among the NIDDM subjects.

In another study conducted between 1979 and 1981, 133 newly-diagnosed NIDDM subjects (aged 45-64 at time of diagnosis) and 144 age-matched non-diabetic subjects were recruited from a defined area in the county of Kuopio in Eastern Finland (5). Age-adjusted incidence of myocardial infarction (male: 19.4% v 3.2%; female: 11% v 3%) and claudication (male: 20.3% v 8%; female: 21.8% v 4.2%) were found to be higher in the diabetic group. The cardiovascular risk factors, LDL cholesterol, blood pressure, smoking and HDL cholesterol were found to have no association with the incidence of myocardial infarction in either the diabetic or non-diabetic groups at the end of a five year follow up. However, abnormalities of electrocardiographs (ECG) at the time of diagnosis were found to be a good predictor of myocardial infarction in diabetic men (ECG+ 10.1%, ECG- 0%). Elevated levels of total serum cholesterol, VLDL cholesterol, VLDL and LDL triacylglycerol but lower levels of HDL cholesterol were associated with the appearance of claudication in the diabetics. Using multivariate analysis, only diabetes was shown to have an independent association with myocardial infarction, whereas, smoking, elevated levels of LDL triacylglycerol, VLDL cholesterol and plasma insulin were independently related to claudication.
1.3.3 United Kingdom studies

Mortality rates due to coronary heart disease and other causes were studied over 10 years in the Bedford area (6). The survey included three groups of subjects: newly diagnosed diabetics, borderline subjects and normoglycaemic controls. The 10 year age-adjusted mortality rates from coronary heart disease and all other causes were highest amongst the newly-diagnosed diabetic patients, those with borderline diabetes having intermediate mortality rates. The female diabetics were found to have an excess risk of death over the controls after statistical allowances had been made for baseline differences in age, blood pressure and obesity, much of this excess being due to coronary heart disease. Only blood pressure and cigarette smoking habits were significant predictors of coronary deaths among diabetics.

In a further study, 18,403 male Whitehall civil servants aged between 40 to 64 years were tested for glucose intolerance and followed up for 10 years (7). Subjects with plasma glucose values above the 95th centile (5.4 mmol l\(^{-1}\)) 2 hours after a 50 g oral glucose load were categorised as being glucose intolerant. The mortality rates from coronary heart disease and stroke over the 10 year period showed a significant increase in this group. However, only a small proportion of the increase could be explained in terms of cardiovascular risk factors established for the non-diabetic population. Age and hypertension were the two significant risks related to the subsequent death from coronary heart disease.

1.3.4 Australian study

The prevalence of macrovascular disease among rural diabetics in Western Australia has been recently reported (8). In this cross-sectional study, subjects were more vigorously characterised for NIDDM and of these patients, 53.3% females and 50.2% of males were found to have macrovascular disease. Age was found to be the major time-related risk variable for this disease. The proportion of NIDDM patients diagnosed as having macrovascular disease increased steadily from 10% at the age of 25 to 80% at age 85. Stepwise logistic regression analysis also showed
that glycated haemoglobin, systolic blood pressure difference, plasma cholesterol and HDL cholesterol were important determinants of risk in these patients.

1.3.5 Summary

These epidemiological observations obtained on three continents cover a period of over 50 years. They have provided strong evidence for an increased incidence of LVD in NIDDM. The explanations for the increased risk in vascular disease in NIDDM remains to be fully explained. However, hyperinsulinaemia, hyperglycaemia, abnormalities in lipid and lipoprotein metabolism may have critical roles to play. These important points will be discussed in the following sections.
1.4 Lipoprotein metabolism

1.4.1 Intestinal lipoproteins

1.4.1.1 Formation of micelles

Most of the dietary intake of fats is in the form of insoluble lipid esters. In order to facilitate absorption, dietary fats have to be hydrolysed and emulsified to form soluble micelles in the stomach and bowel. Micelles are spherical, heavily hydrated and charged aggregates. They are formed when the bile salt concentration exceeds the critical micellar concentration i.e. 5-15 μmol ml⁻¹ (9). Bile salt micelles have negatively charged surfaces, with hydroxyl and amino groups facing the surrounding intestinal fluids and steroid hydrophobic cores. Such a structure promotes the transport of hydrolysed dietary products into intestinal cells.

1.4.1.2 Absorption of micelles

It has been shown that in rat and human large intestine, an unstirred water layer occurs adjacent to the brush border of enterocytes (10,11). This layer consists of strata of water lamellae which become progressively more unstirred as they approach the surface of the brush border. Lipid-loaded micelles have to penetrate this unstirred water layer before reaching the cell membrane where lipids enter the cells by passive diffusion.

1.4.1.3 Intestinal lipid synthesis

On entry into the enterocytes, medium length fatty acids (less than 12 carbon atoms) pass directly into the portal circulation where they combine with albumin (12). These free fatty acid-albumin complexes are then transported to the liver and other tissues for use as metabolic fuels. On crossing the enterocyte membrane, long chain fatty acids (greater than 11 carbon atoms) are bound to a specific fatty acid binding protein (FABP) which facilitates their passage across the enterocyte.
membrane. These fatty acids are then transported through the cytosol to the smooth endoplasmic reticulum where they are utilised for the synthesis of lipids (13). Numerous studies have shown that intestinal synthesis of cholesterol and tri-acylglycerol is accelerated in diabetes (14,15). The administration of insulin has been found to restore intestinal cholesterol synthesis to normal levels. The hyperphagia that accompanies poorly controlled diabetes has been suggested to be the main reason for this increase in intestinal cholesterol synthesis (14). It has also been suggested that increased whole body cholesterol synthesis reflects increased intestinal anabolism (15). Dietary carbohydrate is converted to hepatic glycogen and fats via an indirect mechanism involving the sequence: glucose to C₃ unit to glycogen and lipids (16). Since lactate has been shown to convert to fatty acids under the regulatory control of insulin, the increased level of this fatty acid precursor as a consequence of impaired insulin function in diabetes may contribute to increased lipid synthesis (17).

1.4.1.4 Intestinal lipoprotein secretion

Although the mechanisms of intestinal lipoprotein synthesis and secretion are less well understood than that in the liver, similarities between the two systems exist. ApoB-48, apoAI and apoAIV are the major apoproteins synthesised in the rough endoplasmic reticulum, these being incorporated into newly synthesised lipids at the junction of the smooth and rough endoplasmic reticula. These lipid-rich particles are then transported, via the microtubule system, to the Golgi apparatus where they are enclosed in secretory vesicles. On release into the cytosol, the particles migrate to and fuse with the plasma membrane. They are subsequently released into intercellular spaces by reverse pinocytosis (18).
1.4.2 Chylomicron metabolism

1.4.2.1 Chylomicrons and lipoprotein lipase

Newly secreted chylomicrons are transported by the lymphatic system to the circulation by way of the thoracic duct. Chylomicrons on entering the circulation take up apoC (19) and apoE (20) from plasma VLDL and HDL, the triacylglycerol within these modified particles then being rapidly hydrolysed by lipoprotein lipase (EC 3.1.1.34) located at the endothelial surface of blood capillaries to yield free fatty acids (21,22). Human lipoprotein lipase when activated by apoC-II acts preferentially on the 1 and 3 ester bonds of triacylglycerol, subsequent isomerisation of the 2-monoacylglycerol to the 1 isomer being required for complete hydrolysis (23,24). The liberated fatty acids are then taken up by extrahepatic tissues for storage (adipose tissue), oxidation (muscle and brain) and secretion (mammary glands).

During the hydrolysis of chylomicrons surface components such as phospholipids, apoA-I, A-II and C apoproteins, are shed from the chylomicron surface and transferred to HDL₃, a prerequisite step for the formation of HDL₄ (25,26). Although there is an increase in apoA-I and apoA-II in the HDL fractions during lipaemia, the plasma contents of these apoproteins remain unchanged suggesting that the apoA-I and A-II are of intestinal origin (27). ApoA-IV, on the other hand, is transferred mainly to the lipoprotein-free fraction of plasma (28). Only apoB-48 and apoE remain with the relatively triacylglycerol-poor, cholesterol-rich remnant particles until their uptake by the liver (29).

1.4.2.2 Chylomicrons and hepatic lipase

A second enzyme involved in chylomicron catabolism is a lipase of hepatic origin (EC 3.1.1.3). This lipase has the combined activities of a triacylglycerol lipase and a phospholipase. Although the major function of hepatic lipase is directed at the HDL₂ particles, some minor activity towards HDL₃ phospholipids has been shown (30). During the HDL-chylomicron apolar-lipid exchange reaction, HDL
cholesterol esters are transferred to the chylomicrons in exchange for triacylglycerol, this reaction being catalysed by lipid transfer proteins (31). The extent of this cholesterol ester-triacylglycerol exchange is governed by the relative abundance of the donor and acceptor lipoprotein particles (32). Consequently, the greater the postprandial lipaemia, the greater is the enrichment of triacylglycerol in HDL\(_2\), this process being at the expense of cholesterol ester. This condition determines whether or not these triacylglycerol enriched HDL\(_2\) particles are converted into HDL\(_3\) by the action of hepatic lipase (33). In normal subjects, the activity of hepatic lipase is inversely proportional to the level of HDL and HDL\(_2\) (34).

Although the action of hepatic lipase on HDL is well established, the role it plays in chylomicron catabolism is less clear. However, there are indications that this enzyme has an important catalytic function in chylomicron catabolism (35,36). The catabolic conversion of chylomicrons to their "remnant" form by the combined activities of lipoprotein and hepatic lipase may be a prerequisite step for the removal of the "remnant" particles from the circulation by the hepatic receptor. Inhibition of hepatic lipase has been shown to bring about the production of LDL containing apoB of intestinal origin (apoB-48) (37). This observation is indicative of the importance of the hepatic lipase in modifying the chylomicron structure for subsequent removal from the circulation.

1.4.2.3 Chylomicrons and apoE receptors

Two types of lipoprotein receptor are found on the surface of hepatocytes, the apoE (remnant) and apoB,E (LDL) receptors. The apoE receptor has an affinity for chylomicron "remnants" whilst the apoB,E receptor prefers LDL and apoE-containing HDL (38). The progressive loss of the apoC peptides during chylomicron catabolism may expose the E apoprotein thus allowing the recognition and binding of the chylomicron "remnants" to the apoE receptor. Direct evidence for the removal of chylomicron "remnants" mediated by the apoE receptor has been obtained in experiments with Watanabe heritable-hyperlipidaemic (WHHL) rabbits (39). These rabbits accumulate VLDL "remnants" and LDL but not chylomicron "remnants". The failure to clear non-chylomicron lipoproteins from the circulation
is due to the lack of apoB,E receptors in these animals. The chylomicron "remnants", on the other hand, are removed from the circulation, clearly establishing the existence of a separate type of receptor for the uptake of these postprandial lipoproteins.

On binding to the hepatic apoE receptor, the chylomicron "remnants" are taken into the cell through the coated pits and are budded off to form multivesicular bodies. The contents of these multivesicular bodies are acidified by an ATP-driven proton pump, uncoupling the receptor-ligand complex (40). Primary lysosomes derived from the Golgi apparatus fuse with the multivesicular bodies and release acid hydrolases. This allows the complete proteolysis and lipolysis of the remnant components, releasing the basic components necessary for metabolic processes within the cell (41). In humans, the clearance of chylomicron "remnants" from the circulation is independent of their rate of formation (42). However, the amount of cholesterol present in these particles may delay their removal from the circulation by limiting triacylglycerol hydrolysis (43).

1.4.3 VLDL metabolism

1.4.3.1 Hepatic synthesis of VLDL

Triacylglycerol is synthesised in hepatic smooth endoplasmic reticulum from fatty acids originating from adipose tissue. Additionally, triacylglycerol may be synthesised from carbohydrates or by de novo synthesis. Although cholesterol is derived mainly from the diet, the liver can synthesise its own by increasing the activity of the enzyme 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (EC 1.1.1.34) (44). This newly synthesised triacylglycerol and cholesterol is found associated with nascent VLDL particles which have been shown to contain very little cholesterol ester due to reduced hepatic ACAT activity (45). ApoB-100, C and E apoproteins are synthesised in the rough endoplasmic reticulum; these are incorporated into the nascent VLDL where the smooth and rough endoplasmic reticula meet. Nascent VLDL particles are transported via the microtubular system to the Golgi apparatus where they are packaged into secretory vesicles. The vesicles
then migrate to and fuse with the plasma membrane of the hepatocytes, releasing their contents into the extracellular space (space of Disse) (18). The secreted substances then enter the circulation via the sinusoidal endothelium of the liver.

1.4.3.2 VLDL catabolism

VLDL catabolism follows a similar pathway to that of the chylomicrons. Nascent VLDL is bound to endothelial lipoprotein lipase where the bulk of its triacylglycerol is hydrolysed (46). This reaction is accompanied by the transferral of VLDL surface components (phospholipids and apoC) to HDL₃ and cholesterol ester from HDL₃ to VLDL. This relocation of lipids and apoproteins allows for HDL₃ to take up a molecular structure similar to that of HDL₂. In addition, LDL cholesterol esters are preferentially transferred to large triacylglycerol-rich VLDL by lipid transfer proteins (47). Thus, smaller and denser VLDL are formed.

The continued catabolism of the smaller and denser VLDL leads to the formation of VLDL "remnants" rich in B-100 and E apoproteins. These "remnants" are catabolized to LDL with further loss of phospholipids, triacylglycerol and apoE. The lipid loss during this final step of the VLDL-"remnants"-LDL metabolic cascade is thought to be facilitated by hepatic triacylglycerol lipase. A decrease in the activity of this enzyme in hyperlipidaemic subjects has been shown to play a part in the accumulation of VLDL "remnants" in the circulation (48).

1.4.3.3 Large and small VLDL

Not all VLDL is converted to LDL. Newly secreted VLDL has been shown to consist of two distinct types of particles, each with their own mode of metabolism. The first type corresponds to a large triacylglycerol-rich particle with a flotation value (S₃) of 100-400. These particles are converted directly to remnant particles (S₁ 12-100) prior removal from the circulation. The second type of nascent VLDL is smaller in size and has a flotation value of 20-60. More than 40% of this species remains in the circulation after its conversion to "remnants". These are destined to become the main precursor of LDL (S₁ 0-12) (78).
1.4.3.4 VLDL and apoE

ApoE is normally associated with nascent VLDL and has an important role to play in the apoB,E-receptor mediated uptake of VLDL "remnant" particles. Degradation of this apoprotein in VLDL by thrombin reduces the binding of the modified lipoprotein by the human fibroblast receptor. When intact apoE is purified and then added to apoE deficient VLDL, the uptake of the lipoprotein is restored. This indicates that apoE is a primary determinant in the removal of VLDL via the receptor-mediated pathway (49).

1.4.4 LDL metabolism

1.4.4.1 LDL and apoB,E receptors

Most of the tissues in the body contain receptors which are capable of recognizing LDL. Since the bulk of the daily input of cholesterol passes through the liver, it would not be unreasonable to propose that the largest numbers of apoB,E receptors are located in this organ. This hypothesis is supported by a study involving a 6 year old girl with severe hypercholesterolaemia who underwent a heart-lung-liver transplant operation; her FCR of intravenously infused $^{125}$I-LDL was restored to near-normal level (0.12 to 0.31 day$^{-1}$) after the operation (50).

Extrahepatic apoB,E receptors were initially discovered in cultured human skin fibroblast (51). They were subsequently found to exist in a variety of cultured extrahepatic cells, including aortic smooth muscle cells, leucocytes, human Hela cells and rat hepatoma cells (51). Although the full spectrum of lipoproteins and their "remnants" are found to bind to the extrahepatic apoB,E receptors in vitro, only LDL is actively bound to these receptors in vivo. The observed differences in the behaviour of the apoB,E receptor may be related to the size of lipoprotein particles. Under in vivo conditions, the size of the lipoprotein particles most likely to pass through the endothelium prior to their binding to extrahepatic apoB,E receptors resembles that of LDL. Lipoproteins with sizes dissimilar to that of LDL may have difficulties traversing the epithelium. This biophysical constraint limits the
type of lipoprotein for binding. In contrast, epithelium is explicitly absent under *in vitro* conditions, therefore, the binding of lipoproteins to extrahepatic receptors is non-specific.

Both hepatic and extrahepatic apoB,E receptors bind LDL in regions of the plasma membrane referred to as coated pits (51). These pits invaginate into the cell and pinch off to form vesicles which are responsible for carrying the LDL to the lysosomes. Cholesterol is liberated from LDL by hydrolytic enzymes within the lysosomes and is used for cellular metabolism. Besides this function, the cholesterol released by lysosomal enzymes also regulates cellular cholesterol contents through three mechanisms. Firstly, it diminishes the activity of HMG CoA reductase, thereby limiting the amount of cellular cholesterol being synthesised. Secondly, it activates the enzyme ACAT which catalyses cellular cholesterol esterification, the esters formed being stored as oil droplets. Thirdly, it prevents further uptake of LDL by suppressing the synthesis of LDL receptors (51).

### 1.4.4.2 LDL and lipid exchanges

Cholesterol ester and triacylglycerol exchange occurs between all the plasma lipoproteins (52). Such exchanges are bidirectional and are mediated by a specific plasma glycoprotein, termed the ‘lipid transfer protein’. Two mechanisms of transfer have been proposed. Firstly, the ‘carrier model’ suggests that the lipid transfer protein binds to a donor lipoprotein, absorbs a lipid molecule, dissociates from the donor lipoprotein and carries the lipid to the acceptor lipoprotein. Secondly, the ‘collision-complex model’ proposes that the transfer of lipid occurs within a ternary collision complex consisting of the donor lipoprotein, acceptor lipoprotein and the lipid transfer protein. Accompanying the net transfer of cholesterol ester from HDL to LDL is an equimolar net transfer of triacylglycerol in the reverse direction (31).

### 1.4.5 HDL metabolism

Animal studies have shown that the liver and intestine are the major source of discoid HDL precursor’s (53). These particles are composed mainly of free
cholesterol, phospholipids, apoAI and apoAII. Once they are secreted into the circulation, they are rapidly acted upon by the enzyme lecithin:cholesterol acyltransferase (LCAT) (EC 2.3.1.43) which catalyses the removal and transfer of the sn-2 acyl chain from 1,2-diacyl-sn-glycerol-phosphatidylcholine to free cholesterol (54). This reaction leads to the formation of mature HDL.

HDL participate in the normal metabolism of chylomicrons and VLDL; HDL₃ serve as an acceptor of phospholipids, apoA-I, apoA-II, apoC and apoE which are important in triacylglycerol metabolism. The transfer of lipids and apoproteins to HDL₃ results in the formation of HDL₂ which are recycled to HDL₃ by hepatic lipase (33).

The equimolar exchange of triacylglycerol for cholesterol ester between lipoproteins has been described in earlier sections. This exchange reaction is mediated by a 'lipid transfer protein' (52). Thus, the major part of the cholesterol ester formed within the HDL fraction is transferred to VLDL and LDL which are subsequently taken up by the liver via the apoB,E receptor.

1.4.6 Chylomicron abnormalities in diabetes

The excessive formation of lipoprotein remnants, formed during chylomicron metabolism, may be involved in the pathogenesis of LVD in non-diabetic subjects (55). Studies have shown that the intestinal cholesterol synthesis, the secretion of chylomicrons containing the newly synthesised cholesterol and the clearance of these chylomicrons from the circulation are increased in diabetic rats (14). These processes may increase the flux of cholesterol in chylomicrons and their remnants and possibly the risk of atherosclerosis in diabetes. In contrast, the chylomicron clearance from the circulation in NIDDM patients (t₁/₂: 22.6 min) is decreased compared to normal human subjects (t₁/₂: 4.6 min) (15).

ApoC-II, which is of HDL₂ origin, has been shown to be a potent activator of lipoprotein lipase (24). Kinetic analysis in hypertriglyceridaemic subjects with normal plasma post-heparin lipoprotein lipase activity indicates that the activity of apoC-II is inhibited by apoC-III (56,57). In type III hyperlipidaemics, apoE has been shown to be increased whereas apoC-II is reduced, resulting in decreased
lipoprotein lipase activity, thereby delaying chylomicron clearance (58). Similar alterations in apoE levels and apoC-II/apoC-III, ratios are also seen in NIDDM patients (59). Chylomicron clearance is also delayed in NIDDM and has been interpreted as being due to reduced lipoprotein lipase activity (60,61). However, insulin resistance may also play a part.

1.4.7 VLDL abnormalities in diabetes

1.4.7.1 Hyperinsulinaemia-hypertriglyceridaemia hypothesis

Many studies have shown that plasma VLDL, expressed as plasma triacylglycerol or VLDL-triacylglycerol, is increased in NIDDM (61,62-66). Most of these increases can be explained in terms of impaired VLDL metabolism. However, for patients with extremely high triacylglycerol levels, other genetic defects may also be involved (67). Hypertriglyceridaemia as a result of enhanced VLDL production may be due to hepatic insensitivity to insulin, effectively resulting in a lack of insulin at the cellular level. Consequently the normal insulin checks on lipoprotein synthesis are removed, resulting in increased VLDL production. In Pima Indians with NIDDM, these increases in VLDL-triacylglycerol production are not seen, probably being related to alterations in plasma free fatty acids rather than to changes in plasma insulin levels (68). The higher basal insulin levels in these ethnic diabetics compared to the non-diabetic controls may be indicative of increased insulin resistance. It has been shown that abnormal VLDL triacylglycerol levels in diabetics are explainable on the basis of a reduction in VLDL clearance (69). Some NIDDM patients with moderate fasting hyperglycaemia show elevated insulin and free FFA levels (65). In these circumstances, insulin-resistance may be either enhancing VLDL production by increasing the availability of FFA for triacylglycerol synthesis or reducing VLDL removal by diminishing adipose tissue lipoprotein lipase activity.

The hyperinsulinaemia-hypertriglyceridaemia hypothesis has been brought into question by studies on VLDL-triacylglycerol metabolism in NIDDM patients with hypertriglyceridaemia (70). These studies have shown that both overproduction and
low fractional catabolic rate (FCR) of VLDL-triacylglycerol occurs in these patients. These kinetic abnormalities were partially corrected by insulin therapy, however normal levels of triacylglycerol were not totally restored. Thus the patients remained hypertriglyceridaemic. In addition, it was found that the responses of the VLDL-triacylglycerol kinetic parameters to insulin treatment were not uniform. Some diabetic subjects showed a greater improvement in the production of VLDL-triacylglycerol than in the FCR whilst others showed the reverse. These observations clearly do not support the hyperinsulinaemia-hypertriglyceridaemia hypothesis and demonstrate the coexistence of primary and secondary forms of hyperlipidaemia in NIDDM. Moreover, the inherited disorder of VLDL synthesis may influence the effect of poor diabetic control on VLDL-triacylglycerol metabolism.

1.4.7.2 VLDL catabolism in Pima Indians with NIDDM

A delay in the clearance of VLDL triacylglycerol and apoB as reflected by a low FCR has been observed in obese NIDDM Pima Indians (70). The result in an increase in plasma triacylglycerol levels, probably being attributable to a decrease in lipoprotein lipase activity. The deficit in lipase activity may be due to alterations in the plasma distribution of apoC confirmed by reduced apoC-II/apoC-III ratios in some of these patients (59). In other studies, however, hypertriglyceridaemia has been shown to be a result of increases in triacylglycerol secretion (71). The triacylglycerol/apoB ratios are also increased in these Pima Indians (72). In addition, the FCR of VLDL-triacylglycerol and VLDL-apoB are reduced and larger amounts of VLDL are removed directly from the circulation without any being converted to LDL. These findings suggest that the rate of clearance of VLDL, mainly of the large and triacylglycerol-rich type, is delayed in NIDDM and may contribute to increased atherogenesis in this disorder.

1.4.7.3 VLDL and apoE in NIDDM

In type III hyperlipoproteinaemia, increased apoE-II concentrations may alter VLDL-receptor binding activity (73). In contrast, in type V hyperlipoproteinaemia,
apoE-II concentrations are unaltered although apoE-IV levels are raised. The precise role of this apoE isomer in the regulation of lipid metabolism is unclear. It has been suggested that in NIDDM, increased plasma apoE-II or apoE-IV levels probably produce hyperlipidaemia (73). The distribution of hyperlipidaemia is found to be unequal among the various apoE phenotypes of diabetic patients (73). The frequency of hyperlipidaemia has been shown to be highest in the apoE3/apoE2 phenotype, followed by the apoE4/apoE3 and apoE3/apoE3 phenotypes. These observations suggest that apoE polymorphism is an important factor in the manifestation of hyperlipidaemia in NIDDM.

1.4.7.4 VLDL glycation and degradation by macrophages

It has been shown that intact VLDL obtained from NIDDM patients is preferentially taken up and degraded by mouse peritoneal macrophages (74). When the influx of VLDL into the macrophage is excessively increased, the formation of foam-like structures within these macrophages is enhanced. These intracellular structures are rich in cholesterol and are found predominantly in atherosclerotic fibrous plaques. Glycation of human VLDL-apoB has been shown to occur in vitro (75) and may occur excessively in NIDDM. In addition, glycated rat VLDL has been shown to be a poor substrate for lipoprotein lipase (76). Taken together, these observations suggest that, in NIDDM, increased glycation of plasma VLDL may delay the clearance of these lipoproteins from the circulation, thus encouraging macrophage foam cell formation and atherogenesis.

1.4.8 LDL abnormalities in diabetes

1.4.8.1 LDL levels in NIDDM

There is a consensus in the scientific community that LDL-cholesterol is a major risk factor for LVD. The extent of risk has been found to correlate positively with plasma total cholesterol and LDL cholesterol. Although increased LDL-cholesterol are commonly observed in non-diabetic subjects with clinical evidence
of LVD, this is not seen in NIDDM patients. Some studies have shown that LDL-cholesterol levels are similar in diabetic and non-diabetic subjects (15,61,66,77). In contrast, NIDDM patients afflicted with claudication have been shown to have higher LDL-cholesterol than those without this complication (61). LDL-cholesterol is also increased in Pima Indians with diabetes, although the concentrations are lower than those seen in Caucasians (78). Thus, the findings in Pima Indians may not be of relevance to observations made in Caucasian diabetics with hyperlipidaemia. Nevertheless, Pima diabetics who have raised LDL-cholesterol have more atherosclerotic disease than Pima controls. Therefore, the mechanism of atherogenesis may be similar in Pima Indians and Caucasians. Incongruous LDL-cholesterol levels have also been observed in a well defined group of NIDDM subjects in Kuopio, East Finland, concentrations again being lower than in normal controls (5).

Other studies have shown that the LDL-triacylglycerol level is consistently increased in diabetic patients (5,61,65,66,72). This abnormality may result from increased triacylglycerol contents in LDL precursor particles. Ultracentrifugal studies of LDL particles obtained from NIDDM patients have shown heterogeneity with respect to molecular weight and density (79). Some of these ‘polydisperse’ LDLs with elevated contents of triacylglycerol and apoB may be poorly recognized by the apoB,E receptor and therefore potentially more atherogenic.

1.4.8.2 FCR and synthetic rate of LDL in NIDDM

The variability in the LDL profiles seen in NIDDM patients may be explained on the basis of changes in lipoprotein synthetic and catabolic rates. The rates of these two opposing processes have been shown to increase equally in NIDDM patients with normal lipaemia, thus resulting in a normal LDL profile (80). The unexpected reduction in plasma LDL in some NIDDM patients with impaired LDL clearance may be attributed to reduced availability of the LDL precursor (small VLDL) (72). In contrast, increased LDL-cholesterol level in moderate to severe NIDDM has been shown to result from reduction in the LDL-apoB FCR with a concomitant increase in the LDL-apoB synthetic rate (80). These changes in catabolic and synthetic rates are under the influence of insulin. Studies have
shown that in mild to moderately severe NIDDM patients, insulin resistance or deficiency alters VLDL catabolism by reducing the ability of the clearance system to respond to increased VLDL loads (65,80).

1.4.8.3 LDL and glycation in NIDDM

Hypertriglyceridaemic subjects with or without diabetes have increased LDL triacylglycerol/protein and decreased LDL cholesterol/protein ratios (81). When mixtures of normal and abnormal LDL particles are presented to cultured fibroblasts, they do not appear to compete with each other for binding to the apoB,E receptor, or interfere with cellular cholesterol synthesis. However, such non-competitive coexistence may be disrupted when the compositional changes in the triacylglycerol-rich LDL becomes extreme. Studies have shown that the ability of these abnormal LDL particles to inhibit normal LDL binding is inversely related to their triacylglycerol/protein ratio and positively related to their cholesterol/protein ratios (81). On its own, the triacylglycerol-rich but cholesterol-poor LDL species reduces the down-regulation of the apoB,E receptor of fibroblasts. The likely reason for this effect may be due to the reduced delivery of these LDL particles via the apoB,E receptor to the fibroblasts. Even if these abnormal LDL particles are taken up by fibroblasts, they contain insufficient cholesterol to down-regulate the receptor.

Apart from abnormal LDL lipid composition, NIDDM patients have been shown to have higher glycated LDL levels than non-diabetics (75). The uptake of glycated LDL by cultured fibroblasts via the apoB,E pathway is completely blocked if around 33% of the LDL ε-lysine residues are glycated (82). Although the extent of LDL glycation in human diabetics is only 2-5%, this is sufficient to reduce its in vivo clearance in male Hartley guinea pigs by 5-25% (83). The uptake and degradation of normal LDL has been shown to inhibit the activity of HMG CoA reductase and to stimulate ACAT activity (82). The effect of normal LDL on these enzymes is to mediate cellular cholesterol homeostasis. In contrast, glycated LDL does not regulate the activity of these enzymes, therefore, increased glycated LDL levels in NIDDM patients may lead to the impairment of cholesterol homeostasis.
The uptake and degradation of glycated LDL by human endothelial cells has been found to be similarly reduced, resulting in an increase in circulating LDL levels (84). This may have repercussions as regards vascular physiology, for example increased cholesterol deposition in vessel walls may occur. Data obtained with the human hepatoma cell line, Hep G2, are consistent with this hypothesis (85). In this study, it was shown that when the incubating medium contains 6% of glycated LDL, a 10% inhibition of LDL catabolism is observed. Changes in LDL catabolism of such magnitude may dramatically increase the risk of atherogenesis in diabetes (85).

1.4.8.4 LDL and macrophages in NIDDM

The recognition, uptake and degradation of chemically modified LDL, for example acetylated LDL or glycated LDL, by the LDL receptor has been shown to be markedly impaired (82,86). In contrast, the receptor-mediated degradation of these modified LDL species by human macrophages is increased, leading to the increased cellular accumulation of cholesterol esters and the formation of foam cells which are commonly observed in atherosclerotic plaques (87). The receptor on the human macrophage responsible for the recognition and binding of these modified LDL is referred to as the scavenger receptor. Glycated LDL taken up by human macrophages is not degraded by the classical LDL pathway but by an independent route (87). The presence of this independent pathway in human macrophages may explain the increased formation of foam cells.

1.4.8.5 LDL and lipid exchanges in NIDDM

The rates of cholesterol ester transfer between lipoproteins obtained from NIDDM patients have been shown to reduced under in vitro conditions (88). This impairment is checked when VLDL and LDL obtained from normal subjects are added to the lipoproteins obtained from diabetics. Similarly, the transfer of cholesterol ester in normal plasma is disturbed by the addition of diabetic VLDL and LDL (88). Accompanying the net transfer of cholesterol ester between
lipoproteins is an equimolar net transfer of triacylglycerol in the reverse direction (31). This process may be reduced in diabetic patients, leaving them with triacylglycerol-rich VLDL and LDL (61,89). In addition, diabetic VLDL and LDL are found to contain higher levels of free cholesterol than their normal counterparts (88). Increased free cholesterol contents of these lipoproteins may change the physical properties of these particles, rendering them unsuitable acceptors of cholesterol ester during the transfer reaction. Therefore, more of these atypical lipoproteins may remain in the circulation thereby contributing to the development of atherosclerosis.

1.4.9 HDL abnormalities in diabetes

1.4.9.1 HDL levels in NIDDM

It has been shown that reduced plasma total HDL and HDL\textsubscript{2} cholesterol concentrations are associated with increased coronary heart disease in non-diabetic populations (90,91). The same conclusion has been drawn from observations in diabetic populations after stepwise logistic regression analysis of risk factors (92). The majority of studies have shown that plasma HDL-cholesterol is lower in NIDDM (5,61,64,66,92-94), the abnormality being confined to the HDL\textsubscript{2} fraction (66,94). However, in some studies, normal HDL-cholesterol levels has been observed in diabetic patients (65,95,96). These discrepancies may be real but, more likely, be related to the non-diabetic controls selected for these studies. As with LDL, the HDL-triacylglycerol level is elevated in NIDDM (64,77,94). Such an increase brings about a reduction in the relative amounts of HDL\textsubscript{2}. Furthermore, increases in HDL-triacylglycerol content may be associated with alterations in the distribution of HDL\textsubscript{2} sub-populations, the proportion of smaller HDL\textsubscript{2} particles being elevated at the expense of larger ones (97).

An abnormally low HDL-apoAI level, particularly that associated with the HDL\textsubscript{2} fraction, is found in NIDDM (64,92). As apoAI is the principle apoprotein for the activation of LCAT, its reduction may increase the free cholesterol content in HDL and may be related to the increased risk of coronary heart disease associated with NIDDM. ApoE is also a constituent of HDL and is involved in hepatic
apoE and peripheral apoB,E receptor recognition and the uptake of HDL particles (98). An impairment of this process may be reflected by the persistently higher plasma apoE and lipid levels seen in the apoE3/2 phenotype of NIDDM (73). Therefore, apoE polymorphism may have an important role in influencing HDL metabolism in NIDDM.

1.4.9.2 FCR and synthetic rate of HDL in NIDDM

Both the FCR and the synthetic rate of apoAI/HDL are increased in NIDDM (99). In addition, a significant inverse correlation between the FCR of apoAI/HDL and plasma HDL-cholesterol and apoAI levels is seen in these patients. The degree of glycaemia also has a positive influence on the FCR of apoAI/HDL. Taken together, these observations support the opinion that the abnormalities in HDL-cholesterol and apoAI contents commonly observed in NIDDM are due to an increase in the catabolic rate of these components, being influenced by plasma glucose and insulin levels (99). In other words, this defect in HDL metabolism is secondary to a fault in carbohydrate metabolism.

1.4.9.3 HDL and glycation

The process of non-enzymatic glycation of HDL is similar to that of LDL. However, unlike LDL, much less is known about the metabolism of glycated HDL. Some information on the catabolic rate of these modified HDL has been obtained from studies on guinea pigs (100). In these experiments, the catabolic rate of the glycated HDL introduced into these animals was shown to increased over that of unmodified HDL. Furthermore, an 8% increase in the rate of catabolism of HDL occurred when only 2% of the lysine residues were glycated. Interestingly, the uptake of glycated HDL by macrophages was reduced, thus indicating that these cells are not instrumental in the degradation of modified HDL. These observations lead one to suggest that the enhanced clearance of glycated HDL may contribute to the decreased HDL levels observed in NIDDM (5,61,64,66,92-94). Indeed, it has been shown that a four fold increase in the degree of HDL glycation occurs in
NIDDM compared with normal controls (101). The level of HDL glycation is positively influenced by the mean blood glucose and phospholipid concentrations and up to 80% of this glycation is associated with apoAI. Although the degree of HDL glycation is weaker than that of other plasma proteins, it may still cause significant functional alterations in processes such as reverse cholesterol transport and receptor-mediated uptake of HDL (101).

1.4.9.4 HDL, lipoprotein and hepatic lipase in NIDDM

Lipoprotein lipase catalyses the degradation of chylomicrons and VLDL with the concomitant transferral of the triacylglycerol-rich lipoprotein surface components to nascent HDL occurring during this process. Thus, lower HDL concentrations in NIDDM patients may be partly due to lower lipoprotein lipase activities (61). In contrast, hepatic lipase appears to exert its physiological role as a phospholipase, using HDL$_2$ directly as a substrate (30). Thus, an increase in hepatic lipase activity may also be partly responsible for the reduced HDL-cholesterol and HDL-apoAI levels seen in NIDDM (56,94,99). Both lipase activities revert to near normality on controlling the level of glycaemia with insulin or sulphonylurea therapy (56,69).
1.5 Long chain fatty acids

A successful living system is dependent on its ability to utilize available raw materials in synthesizing essential components for and to store the surplus during times of plenty. These raw materials include the long chain fatty acids which are important as basic components of membrane phospholipids. Apart from maintaining membrane integrity, long chain fatty acids, when in surplus, are stored in the form of triacylglycerols. Long chain fatty acids may be obtained directly from the diet or produced in the liver by \textit{de novo} synthesis, this latter process being under the control of cytoplasmic acetyl-CoA carboxylase and fatty acid synthetase. The nascent fatty acids produced then undergo further elongation and desaturation to produce variants which are vital to the well being of the organism.

1.5.1 Fatty acid nomenclature

Apart from a few exceptions, the fatty acids described herein are all aliphatic carboxylic acids containing 16 to 22 carbon atoms. They can be classified as saturated (SFA), monounsaturated (MFA) and polyunsaturated fatty acids (PUFA) possessing 0, 1 or more than 1 double bonds respectively. MFA and PUFA are further divided into (n-x) families, where x is an integer denoting where the first double bond occurs relative to the methyl end of the fatty acid molecule.

There are two shorthand conventions commonly adopted to replace the more cumbersome trivial and systemic names of fatty acids. Both systems use the general form of C:D, the number of carbon atoms in the fatty acid chain being represented by C and double bonds by D. However, one system numbers the carbon atoms starting from the carboxyl end of the molecule. The number of double bonds present is represented by Δ and integers denoting the position of carbon atoms (from the carboxyl end of the molecule) where the double bonds occur. The second system numbers the carbon atoms starting from the methyl end of the molecule. In addition, only the position of the double bond closest to the methyl end of the molecule is described and this is represented by (n-x), x being the position of the carbon atom (from the methyl end of the molecule) of the first double bond. Thus,
the two respective shorthand notations for arachidonic acid are $20:4\Delta5,8,11,14$ and $20:4(n-6)$. It should be noted that the latter notation has been used throughout this thesis. Table 1 gives a list of the fatty acids with their trivial names, systematic names and their shorthand notations.

<table>
<thead>
<tr>
<th>Trivial name</th>
<th>Fatty acid nomenclature</th>
<th>Systemic name</th>
<th>Shorthand notations</th>
</tr>
</thead>
<tbody>
<tr>
<td>palmitic</td>
<td>n-hexadecanoic</td>
<td>16:0</td>
<td>16:0</td>
</tr>
<tr>
<td>stearic</td>
<td>octadecanoic</td>
<td>18:0</td>
<td>18:0</td>
</tr>
<tr>
<td>palmitoleic</td>
<td>hexadec-9-enoic</td>
<td>16:1(n-9)</td>
<td>16:1(n-7)</td>
</tr>
<tr>
<td>vaccenic</td>
<td>octadec-11-enoic</td>
<td>18:1(\Delta11)</td>
<td>18:1(n-7)</td>
</tr>
<tr>
<td>oleic</td>
<td>octadec-9-enoic</td>
<td>18:1(\Delta9)</td>
<td>18:1(n-9)</td>
</tr>
<tr>
<td>cetolic</td>
<td>docos-11-enoic</td>
<td>22:1(\Delta11)</td>
<td>22:1(n-11)</td>
</tr>
<tr>
<td>linoleic</td>
<td>octadeca-9,12-dienoic</td>
<td>18:2(\Delta9,12)</td>
<td>18:2(n-6)</td>
</tr>
<tr>
<td>eicosadienoic</td>
<td>eicosa-11,14-dienoic</td>
<td>20:2(\Delta11,14)</td>
<td>20:2(n-6)</td>
</tr>
<tr>
<td>(\alpha)-linolenic</td>
<td>octadeca-9,12,15-trienoic</td>
<td>18:3(\Delta9,12,15)</td>
<td>18:3(n-3)</td>
</tr>
<tr>
<td>(\gamma)-linolenic</td>
<td>octadeca-6,9,12-trienoic</td>
<td>18:3(\Delta6,9,12)</td>
<td>18:3(n-6)</td>
</tr>
<tr>
<td>dihomo-(\gamma)-linolenic</td>
<td>eicosa-8,11,14-trienoic</td>
<td>20:3(\Delta8,11,14)</td>
<td>20:3(n-6)</td>
</tr>
<tr>
<td>Mead acid</td>
<td>eicosa-5,8,11-trienoic</td>
<td>20:3(\Delta5,8,11)</td>
<td>20:3(n-9)</td>
</tr>
<tr>
<td>parinaric</td>
<td>octadeca-9,11,13,15-tetraenoic</td>
<td>18:4(\Delta9,11,13,15)</td>
<td>18:4(n-3)</td>
</tr>
<tr>
<td>arachidonic</td>
<td>eicosa-5,8,11,14-tetraenoic</td>
<td>20:4(\Delta5,8,11,14)</td>
<td>20:4(n-6)</td>
</tr>
<tr>
<td>adrenic</td>
<td>docosa-7,10,13,16-tetraenoic</td>
<td>22:4(\Delta7,10,13,16)</td>
<td>22:4(n-6)</td>
</tr>
<tr>
<td>timnodonic</td>
<td>eicosa-5,8,11,14,17-pentaeonoic</td>
<td>20:5(\Delta5,8,11,14,17)</td>
<td>20:5(n-3)</td>
</tr>
<tr>
<td>clupanodonic</td>
<td>docosa-7,10,13,16,19-pentaeonoic</td>
<td>22:5(\Delta7,10,13,16,19)</td>
<td>22:5(n-3)</td>
</tr>
<tr>
<td>docosapentaenoic</td>
<td>docosa-4,7,10,13,16-pentaeonoic</td>
<td>22:5(\Delta4,7,10,13,16)</td>
<td>22:5(n-6)</td>
</tr>
<tr>
<td>cervonic</td>
<td>docosa-4,7,10,13,16,19-hexaenoic</td>
<td>22:6(\Delta4,7,10,13,16,19)</td>
<td>22:6(n-3)</td>
</tr>
</tbody>
</table>
1.5.2 Characteristics of fatty acids in mammalian systems

Only fatty acids containing an even number of carbon atoms are present in substantial amounts in human subjects. This bias is as a consequence of their mode of biosynthesis, namely, the molecules are built up two carbon atoms at a time from acetate units. Saturated and monounsaturated fatty acids can be derived from \textit{de novo} synthesis and the diet, whereas, PUFA are derived solely from dietary 18:2(n-6) and 18:3(n-3). Since PUFA cannot be synthesised by animal tissues, they are often referred to as essential fatty acids (EFA). Chronic deficiency of these EFA in laboratory rats has been shown to produce symptoms of decreased growth rate, development of scaly paws, skin and tail, and fatty liver (102).

In the mammalian system, the configuration about the double bonds is invariably \textit{cis} rather than the more stable \textit{trans}. This particular stereochemistry has an effect which is seemingly trivial but is actually of vital biological importance. The stable conformation of \textit{trans}-unsaturated fatty acids is that of a planar zig-zag structure, which might be expected to fit each other and with saturated fatty acids well into an ordered crystalline array within a lipid bilayer structure. The presence of a \textit{cis} double bond in the fatty acids imparts a bend to the overall conformation. Incorporation of such fatty acids into the ordered array of a bilayer will disrupt the array and make the membrane more fluid. This effect is to provide at physiological temperatures a semi-liquid barrier between the cell and its environment, thereby facilitating normal biological function (103).

1.5.3 Fatty acid biosynthesis

1.5.3.1 Saturated fatty acids

Saturated fatty acids are synthesized via a high molecular weight complex composed of multi-active catalytic sites collectively known as fatty acid synthetase (104). The process begins with the irreversible carboxylation of acetyl-CoA to malonyl CoA, being catalysed by acetyl-CoA carboxylase (EC 6.4.1.2). Both the acetyl-CoA and malonyl-CoA are then transacylated with acyl carrier proteins.
(ACP) to form ACP adducts. The elongation step which follows involves the condensation of the two ACP derivatives to acetoacetyl-ACP. Subsequent to a cycle of reduction, dehydration and a second reduction, the acetoacetyl-ACP is converted to butyryl-ACP. The elongation step and the subsequent cycle of concerted reactions continues to repeat itself until 16:0-ACP is formed. This final adduct does not enter the elongation step again because it is not a substrate for the condensation enzymes, instead it is hydrolysed to 16:0 and ACP (Fig 1).

Figure 1  Biosynthesis of saturated fatty acids

Free 16:0 has been shown to control fatty acid synthesis in two ways. Firstly, it inhibits the activity of fatty acid synthetase. Secondly, it binds to acetyl-
CoA carboxylase and prevents the activation of the enzyme. Thus, it reduces the formation of malonyl-CoA and limits the availability of this precursor for fatty acid synthesis. In contrast, citrate appears to compete with 16:0 for the same carboxylase binding site, thus producing a reverse effect (104).

1.5.3.2 Unsaturated fatty acids

In mammalian systems, unsaturated fatty acids are synthesized by direct oxidative desaturation of long chain fatty acids derived from dietary fat and the products of cytoplasmic fatty acid synthetase and microsomal malonyl-CoA dependent elongase catalysed reactions (105). These long chain fatty acids are acted upon by a microsomal bound desaturase complex consisting of NADH-cytochrome b₅ reductase (EC 1.6.2.2), cytochrome b₅ and a terminal desaturase enzyme.

At least four terminal desaturases exist for the insertion of double bonds at specific positions in the long chain fatty acids (105). The mechanism of desaturation is to selectively remove two hydrogen atoms from the methylene chain of fatty acids to produce the respective unsaturated species. The four desaturases are designated Δ9-, Δ6-, Δ5- and Δ4-desaturase because they act on fatty acid carbon atom numbers 9, 6, 5 and 4 respectively and it has been suggested that only these enzymes are necessary for the synthesis of all known naturally occurring PUFA.

The desaturases require molecular oxygen and a reduced pyridine nucleotide in order to act on substrates which are in the form of fatty acyl-CoA. Interestingly, the Δ5-desaturase appears to exist in two forms in mammalian liver, one being specific for the CoA ester of 20:3(n-6) and the other for the phosphatidylcholine containing the same unsaturated fatty acid (106).

Longer chain unsaturated fatty acids can be formed in elongation reactions similar to those seen with saturated fatty acids. A two-carbon donor in the form of malonyl-CoA is added to the carboxyl end of the existing unsaturated fatty acid. The product of this reaction may then undergo alternate desaturation and elongation to produce the vast assortment of unsaturated fatty acids found in mammalian tissues (105). The rates of elongation of both unsaturated and saturated fatty acids have been shown to be considerably faster than those of desaturation (107).
1.5.3.3 Rules for fatty acid desaturation

A variety of unsaturated fatty acids are found in animal tissues and substrate specificity studies have established certain general rules for their synthesis. These rules have been established from studies carried out with Δ9-desaturase, however, they are also applicable to other desaturases. These rules are now outlined:

1. Double bonds are introduced into the fatty acid substrate at fixed positions from the carboxyl end of the molecule.

2. When the substrate is a saturated fatty acid, the first double bond is formed between carbon atoms 9 and 10, counting from the carboxyl end.

3. When the substrate is an unsaturated fatty acid, the next double bond to be formed occurs between the existing double bond nearest to the carboxyl end and the carboxyl end itself.

4. trans-unsaturated fatty acids are acted upon by the desaturases in the same way as for saturated fatty acids.

1.5.3.4 Functions of the desaturases

Saturated fatty acids derived from dietary carbohydrates are mainly 16:0 and 18:0. These are normally incorporated into lipids as tripalmitoylglycerol and tristearoylglycerol, or as mixed triacylglycerol containing both fatty acids. The lipids are crystalline solids at physiological temperatures, rendering them thermodynamically unfavourable for transport and catabolism. The conversion of these saturated fatty acids to their monoenoic analogues by the Δ9 desaturase lowers the melting points of the lipids in which they occur and facilitates their transport as VLDL to adipose tissue for subsequent metabolism (105).

Unlike Δ9 desaturase, Δ5 and Δ6 desaturases produce PUFA which have different roles to that of the monoenoic acids. The main function of these desaturases is to synthesize PUFA, these being required as precursors for membrane phospholipids (103). PUFA, derived from 18:2(n-6) and 18:3(n-3), are also precursors for many biologically active compounds. These compounds are generally
referred to as prostanoids which include cyclooxygenated and lipooxygenated products derived from 20:3(n-6), 20:4(n-6) and 20:5(n-3) (108-111). Lipooxygenated products of 22:6(n-3) are also found in canine retina (112). The impaired synthesis of these prostanoids may lead to excessive inflammation, thrombosis, atherosclerosis and immune suppression (110,111).

In vitro and in vivo studies have shown that each family of fatty acids consists of a principal precursor from which the remaining members are formed (105). Although the same enzymes are employed by fatty acids of different families for their within-family inter-conversion, they cannot convert fatty acids of one family to the corresponding products of another. Fatty acids of different families are found to compete for the same enzymes during their metabolism, most of this information having been obtained from animal studies. Despite the overall similarities of these biochemical reactions, their rates are dependent upon tissue type and species.

1.5.3.5 (n-7) and (n-9) pathways

Free 16:0, the preferred end product of fatty acid synthetase, is the precursor for both 16:1(n-7) and 18:1(n-9) involving two distinct metabolic pathways, namely (n-7) and (n-9) pathways. For these pathways to proceed the 16:0 must be in the CoA form. The consecutive actions of Δ9 desaturase and elongase convert the activated 16:0 to 16:1(n-7) and 18:1(n-7) via the (n-7) pathway (Fig 2). Although, in theory, 18:1(n-7) can be further metabolized by Δ6 desaturase, this has been shown not to occur (107). In an alternative reaction, the activated 16:0 can be elongated to 18:0 prior to desaturation to 18:1(n-9) via the (n-9) pathway (Fig 2). Chronic deficiency of dietary essential fatty acids leads to the accumulation of an unusual triene acid, 20:3(n-9) (105). The appearance of this acid is indicative of the preference of 18:1(n-9) as an alternate substrate for the Δ6 desaturation during dietary deprivation. If the metabolic cascade continues, the 20:3(n-9) is eventually formed providing indication of malnutrition. Although both (n-7) and (n-9) pathways are utilized by the mammalian system, the latter pathway predominates because 18:0 is the preferred substrate of Δ9 desaturase (113).
Figure 2  (n-7) and (n-9) fatty acid metabolic pathways

$\Delta 9$: $\Delta 9$ desaturase; $\Delta 6$: $\Delta 6$ desaturase; $\Delta 5$: $\Delta 5$ desaturase; e: elongase
1.5.3.6 (n-3) and (n-6) pathways

The (n-3) and (n-6) pathways are shown in Fig 3. The precursors for these metabolic pathways are 18:3(n-3) and 18:2(n-6) respectively. They are converted to 18:4(n-3) and 18:3(n-6) in reactions catalysed by the same Δ6 desaturase (114). The subsequent actions of the elongase (115) and Δ5 desaturase enzymes (116) upon the Δ6 metabolites lead to the production of 20:5(n-3) and 20:4(n-6) respectively. These are in turn elongated and desaturated to yield 22:6(n-3) (117) and 22:5(n-6) (118), via the intermediates 22:5(n-3) and 22:4(n-6).

Figure 3 (n-3) and (n-6) PUFA metabolic pathways

Δ6: Δ6 desaturase; Δ5: Δ5 desaturase; Δ4: Δ4 desaturase; e: elongase; R: retro-conversion
To date there is no evidence of crossover between the (n-3) and (n-6) fatty acid pathways in mammals. However, competition at the enzymatic level between the (n-3) and (n-6) families of PUFA is known to occur both in vivo (119) and in vitro (120). This may have a bearing on the type of PUFA found in plasma and tissue lipids. Retro-conversion of 22:4(n-6) to 20:4(n-6) has been demonstrated to proceed via β-oxidation in rats (121) and this reaction may partly contribute to the plasma and tissue 20:4(n-6) contents seen in these animals. A similar contribution to the plasma 20:5(n-3) contents as a consequence of the increased retro-conversion of 22:6(n-3) to 20:5(n-3) has also been observed in rats fed with cod liver oil. In man, similar findings have been observed after a single dose of 22:6(n-3) ethyl ester (122,123).

Information on the human Δ5, Δ6 and Δ9 desaturases is scarce. The rate of conversion of 18:0 to 18:1(n-9) by Δ9 desaturase in human microsome has been shown to be low as is the conversion of 18:3(n-3) to 18:4(n-4) and 18:2(n-6) to 18:3(n-3) by Δ6 desaturase is (124). Δ5 desaturase activity is reported to be extremely low in human liver homogenate, indicating that the major source of both 20:5(n-3) and 20:4(n-6) is dietary (125). Further evidence for low Δ5 and Δ6 desaturase activities has been obtained from studies in which cod liver oil or linseed oil were given to human subjects (126). Those who received cod liver oil had increased plasma 20:5(n-3) and 22:6(n-3) levels while those receiving linseed oil, rich in 18:3(n-3), showed no increases (126). In contrast with the observations regarding microsomal Δ5 and Δ6 desaturase activities, the Δ6 and Δ9 enzymes have significant activities in cultured human fibroblasts (127). This suggest that desaturase activities vary according to tissue type.

1.5.3.7 (n-8) pathway

An alternative pathway for the formation of 20:3(n-6) from 18:2(n-6) would be possible if a Δ8 desaturase existed which converted 20:2(n-6), formed by chain elongation of 18:2(n-6), to 20:3(n-6) (Fig 4). Evidence for the in vitro conversion of [1-14C]-11,14-20:2 to 8,11,14-20:3 catalysed by such an enzyme have been obtained using normal and cancerous tissues including colon, bladder, ovary and
testes (128,129,130). Δ8 desaturase activity, however, is lacking in rat liver, the Δ5,11 pathway taking over after the initial elongation of 18:1(n-9), 18:2(n-6) and 18:3(n-3) to their respective Δ8 substrates, 20:1(n-9), 20:2(n-6) and 20:3(n-3) (131). This particular route of metabolising the Δ8 substrates results in the formation of the so called 'dead end' products which possess the 5,11 structure (132,133), namely 20:2Δ5,11, 20:3Δ5,11,14, and 20:4Δ5,11,14,17 (Fig 4).

**Figure 4** (n-8) and Δ5,11 metabolic pathways

Δ5: Δ5 desaturase; Δ6: Δ6 desaturase; Δ8: Δ8 desaturase; e: elongase

54
1.5.4 Fatty acid profiles in Diabetes

1.5.4.1 Diabetic animal fatty acid profiles

Alterations in the fatty acid of liver, heart, kidney, adipose tissue, skin, adrenal gland, aorta, platelets, erythrocytes and serum of alloxan- and streptozotocin induced diabetic animals have been demonstrated (134,135). These changes are not uniform, however. Studies in long-term (> 6 weeks) streptozotocin-induced diabetic rats have confirmed that persistent increases in 18:2(n-6) and 20:3(n-6) with concomitant decreases in 20:4(n-6) levels in blood and tissues are the rule (136). Increases in 20:5(n-3) and 22:6(n-3) with reductions in 18:1(n-9) and 22:4(n-6) are also seen in these animals. These results have been ascribed to, but are not proof of, impaired A5, A6 and A9 desaturation (136). The increases in (n-3) fatty acids together with decreases in 20:4(n-6) and 22:4(n-6) seen in this study suggest that the abnormality in fatty acid metabolism probably occurs in the (n-6) pathway and specifically involves abnormalities relating to the A5 and A6 desaturation reactions. The activities of the A5 desaturase and, to a lesser extent, A6 desaturase to the respective (n-6) fatty acid substrates, i.e. 20:3(n-6) and 18:(n-6), may be diminished, leading to the reduction in 20:4(n-6) synthesis. These changes may concomitantly increase A5 and A6 desaturase activities towards the corresponding (n-3) fatty acid substrates, i.e. 18:3(n-3) and 20:3(n-3), leading to an increase in 20:5(n-3) production. The reductions in the blood and tissue levels of 18:1(n-9), on the other hand, are indicative of reduced A9 desaturase activity.

Insulin-treatment of long-term diabetic rats increases plasma, platelet and aorta 16:1(n-7), 18:1(n-9) and 20:3(n-6) with reductions in 18:2(n-6) levels. These effects are in excess of those seen in normal control animals suggesting that insulin-treatment of diabetic rats may have exaggerated actions with respect to the restoration of the A6 and A9 desaturase activities, thereby increasing the synthesis of 16:1(n-7), 18:1(n-9) and 20:3(n-6) (136).

Although short-term (up to 3 weeks) diabetic rats have similar abnormalities in their plasma and red blood cell fatty acid profiles, the corresponding changes in platelets and aorta are not observed (136,137).
1.5.4.2 Human plasma fatty acid profiles

Changes in plasma fatty acid levels are not restricted to diabetic rats. The iodine number, a measure of the degree of fatty acid unsaturation, was found to be decreased in the plasma of human diabetics as long ago as 1928 (138). More detailed studies that followed yielded results consistent with this early observation. Well controlled, elderly diabetics have normal fatty acid levels in their phospholipids (139). However, hyperlipidaemic diabetics have increased amounts of saturated and monounsaturated fatty acids in their phospholipids, as well as in the cholesterol ester and triacylglycerol fractions (140). The PUFA, on the other hand, and in particular 18:2(n-6) and 20:4(n-6), are decreased. When the absolute amounts of the individual fatty acids in the plasma of these diabetic patients are determined, only saturated and monounsaturated fatty acids are increased. The 'essential fatty acid' composition of normolipaemic diabetics is found to be normal whereas in hyperlipidaemic diabetics virtually all serum fatty acid levels are increased. These observations suggest that a non-specific relationship exists between hyperlipidaemia in these diabetics and the levels of the major serum fatty acids. However, these studies did not specify whether the patients were insulin or non-insulin dependent.

When insulin was administered to a group of moderate to severe NIDDM patients, it was found that their saturated and monounsaturated fatty acid levels fell to a greater extent than the PUFA of endogenous origin (141). Under basal conditions, and to a lesser extent during insulin treatment, plasma 18:2(n-6) levels positively correlated with HbA1c, whilst 20:3(n-6) and 20:4(n-6) levels were inversely proportional to HbA1c. These relationships suggest that the levels of (n-6) fatty acids may be dependent on the degree of glycaemic control produced by insulin and that insulin itself may exert an influence on (n-6) fatty acid metabolism in diabetics. In contrast, it has been shown that mild, untreated NIDDM patients have normal levels of (n-6) fatty acids (142). Treatment of these patients with the sulphonylurea glyburide was found to result in only minor changes in saturated fatty acid composition. These studies suggest that in order for fatty acid abnormalities to occur in NIDDM patients, hyperglycaemia needs to be marked.
1.5.4.3 Human platelet fatty acid profiles

Fatty acids exist as phospholipid esters in platelet membranes (136). They are released by phospholipases during platelet activation by substances such as catecholamines and other platelet aggregatory agents (143). PUFA released from phospholipids during platelet activation are metabolized to cyclooxygenase or lipooxygenase products which have been shown to have profound pro-aggregatory effects on platelets, leading to thrombus formation and atherogenesis.

Studies on the fatty acid composition of platelets derived from diabetic patients have produced inconsistent findings. Poorly-controlled NIDDM patients have decreased platelet phospholipid 16:0, 18:0 and 18:2(n-6) contents and increased 20:4(n-6) (144). The elevated 20:4(n-6) content may be due to increased platelet uptake of this fatty acid (145). Platelet 20:3(n-6) and 20:5(n-3) contents are decreased in some diabetic patients (146). Thus, more pro-aggregatory metabolites may be formed from the more readily available 20:4(n-6) and less of the anti-aggregatory metabolites from 20:5(n-3) (147). Therefore, dietary regimes aimed at increasing the proportion of (n-3) fatty acids in platelets may favourably influence the development of vascular disease in diabetics via an effect on platelet function. Correlations exist between plasma and platelet fatty acids, the exception being 20:4(n-6) (146). This anomaly suggests that, in NIDDM, a disproportionate amount of 20:4(n-6) is incorporated into the platelets, possibly being due to increased platelet arachidonoyl-CoA synthetase activity (148).

Other studies have shown that platelet 20:4(n-6) levels in NIDDM patients are similar to those of controls (149,150). Nevertheless, some of these patients were shown to have altered fatty acid distributions in their platelet phospholipid fractions (149). Platelet 18:2(n-6) and 20:4(n-6) are inversely related in normal subjects, suggesting that a precursor-product type of reaction occurs during the metabolic conversion of 18:2(n-6) to 20:4(n-6) (150). However, this is not the case for NIDDM, suggesting that Δ5 and Δ6 desaturase activities in platelets of NIDDM are reduced. However, there is some controversy as to whether Δ5 and Δ6 desaturases exists in human platelets (152-154). Thus, increased platelet 20:4(n-6) in NIDDM may be primarily due to increased uptake from an extracellular source (154).
The platelet content of 20:4(n-6) in diabetics is unrelated to HbA\textsubscript{i}, whereas, that of 18:2(n-6), 20:1(n-9) and 20:3(n-6) is (155). Reduced availability of the 20:4(n-6) precursors, namely 18:2(n-6) and 20:3(n-6), as a consequence of poor diabetic control may contribute to its low platelet levels (156). Thus, improvement of diabetic control may increase 18:2(n-6) and 20:3(n-6) availability with the subsequent formation of 20:4(n-6).

1.5.4.4 Human red blood cell fatty acid profiles

Plasma fatty acid measurements suffer from the disadvantage that they are easily influenced by alterations in dietary intake and fluctuations in triacylglycerol and fatty acid metabolism. In contrast, red blood cell fatty acid composition is relatively stable, responses to dietary changes or altered triacylglycerol metabolism being slower (142). Furthermore, given that the average lifetime of red blood cells is approximately 110 days, measurement of their fatty acids provides a better assessment of a subject's dietary and tissue fatty acid status.

Alterations in red blood cell fatty acid composition have been found in NIDDM patients (157). These changes include increases of 16:0 and decreases in 20:0, total PUFA, particularly 22:6(n-3), and in polyunsaturated/saturated fatty acid (P/S) ratios and are associated with a decrease in membrane fluidity. The daily consumption of 2.7 g of sardine oil over a period of eight weeks has been shown to improve red blood cell membrane fluidity by increasing 20:5(n-3) and 22:5(n-3) contents (157).

Contrary to the above reports, red blood cell fatty acid profiles have been reported to be normal in two recent studies on NIDDM patients (142,158). It has been reported that the fatty acids associated with the phospholipid fractions are similar to those in normal controls. However, it should be noted that the inconsistency of the data may be related to a number of factors including, the small numbers of patients examined (142), the inclusion of both insulin and non-insulin dependent diabetics (158) and that normal plasma lipid levels were seen in some patients (158). Red blood cell membrane acyl:CoA synthetase has been shown to have a role in maintaining fatty acid levels in normal subjects (159). Similar
activities of this enzyme in some diabetics may partly contribute to the normal fatty acid profiles seen in these patients (159).

In NIDDM red blood cell 20:3(n-6) and 20:4(n-6) have been shown to be inversely related to plasma HbA1c whereas 18:2(n-6) is positively related; these relationships are less marked after insulin treatment (141). In addition, the increased conversion of 18:2(n-6) to 20:4(n-6) after controlling diabetes is reflected by increases in the 20:4(n-6)/18:2(n-6) ratios in the red blood cells. Taken together, these observations suggest that insulin may be important in the regulation of (n-6) fatty acid metabolism.

1.6 Fish oil, olive oil and NIDDM

1.6.1 Effects of fish oil on non-diabetic subjects

In the 1970s, studies were undertaken in Greenland Eskimos in which factors such as dietary fat intake and plasma lipid, lipoprotein and fatty acid profiles were examined (160-162). These studies produced the hypothesis that a high dietary intake of marine (n-3) fatty acids may protect against cardiovascular disease. Indeed it has been shown that Greenland Eskimos whose intake of (n-3) fatty acids is approximately 7 g/day have a 92% lower incidence of ischaemic heart disease than the general non-eskimo Danish population (163,164).

A low mortality from cardiovascular disease has also been observed in Okinawans whose dietary intake of fresh fish is high (approximately 150 g/day) (165). Studies of fatty acid composition and platelet aggregation in Japanese fishermen in the Chiba Prefecture have further confirmed the inverse relationship between dietary intake of marine oil and the incidence of ischaemic heart disease (166). The amount of fish consumed by these fishermen (250 g/day) was approximately three times that of their compatriot farmers and produced a significantly higher level of plasma 20:5(n-3) and 22:6(n-3), as well as a reduction in platelet aggregation.

In 1960, a survey of the dietary habits of 852 randomly selected middle-aged Caucasian males without coronary heart disease was carried out in Zutphen,
Netherlands (167). It was found that an inverse relationship between death from coronary heart disease and fish consumption at the onset of the survey persisted during a 20 year follow-up. Mortality from coronary heart disease in the ‘fish-eating’ subjects, who consumed around 20 g of lean fish and 10 g of fatty fish per day, was 50% lower than in those who did not eat fish. Together these observations suggest that although the consumption of fish in Dutch males is considerably lower than that of Japanese fishermen and Greenland Eskimos, the inclusion of one or two fish dishes in the weekly diet may help to prevent coronary heart disease.

1.6.1.1 Plasma lipids and lipoproteins

The effects of fish oil supplements on human plasma lipid and lipoprotein levels have been reviewed recently (168). Dietary studies on normolipidaemic subjects have shown that the increased intake of (n-3) fatty acids results in a decrease in triacylglycerol levels (around 25%), a slight rise in HDL cholesterol and practically no change in total and LDL cholesterol. In similar studies on combined hypercholesterolaemic patients, total cholesterol levels were unchanged while LDL and HDL cholesterol concentrations were increased by 5-7% and triacylglycerol levels decreased by approximately 38% (168).

The most dramatic effects of fish oil have been obtained with hypertriglyceridaemic patients (168). Total cholesterol levels in these patients were decreased by around 8% while LDL and HDL cholesterol levels were increased by 30% and 10% respectively. In contrast, plasma triacylglycerol levels were reduced by 52%. As both LDL and HDL cholesterol levels rose in these patients, it was concluded that the fall in total cholesterol resulted solely from a fall in VLDL cholesterol levels. Thus, an intake of 3-4 g/day of (n-3) fatty acid will probably be suffice to produce a hypotriglyceridaemic effect and a reduction in VLDL levels.

Familial combined hyperlipidaemic patients have increased plasma concentrations of small, dense LDL while patients with hypertriglyceridaemia show increases in the larger and lighter LDL particles (169). The administration of fish oil to the familial combined hyperlipidaemic patients resulted in an increase in plasma LDL cholesterol but not apoB levels (169). In the same study with hypertriglyceridaemic
patients, however, only plasma apoB was significantly increased in response to the fish oil treatment. These observations suggest that fish oil may exert an influence on VLDL catabolism in familial hyperlipidaemics with the consequential production of cholesterol-rich LDL particles whilst in hypertriglyceridaemics, its influence may be restricted to the production of more LDL.

A recent study has shown that fish oil may be anti-atherogenic without being hypocholesterolaemic (170). In this study, increased intake of fatty fish lowered the mortality rate from second heart attacks by 29% in postmyocardial infarction patients without lowering plasma cholesterol levels.

In most studies it has been concluded that the (n-3) fatty acids’ primary beneficial effect is in lowering plasma triacylglycerol levels. The mechanism whereby (n-3) fatty acids produce their hypotriglyceridaemic effect has been examined in human subjects (171-173). It seems that more than one mechanism is involved in the triacylglycerol lowering effects of fish oil. These included the reduction of hepatic triacylglycerol synthesis, VLDL secretion and VLDL catabolism and these factors may be interrelated.

1.6.1.2 Fatty acid profiles

In a number of human intervention studies enrichment of diet with fish oil leads to a lowering of plasma levels of 18:2(n-6) and 20:4(n-6) and an increase in 20:5(n-3) and 22:6(n-3) (111,147,174,175). Plasma 22:6(n-3) levels, however, are raised when the fish oil content of this fatty acid is reduced by one third compared with other studies (176). This indicates that the degree of marine (n-3) fatty acids incorporation into plasma lipids is dependent on the type of fish oil consumed.

Similar changes in platelet 18:2(n-6), 20:4(n-6), 20:5(n-3) and 22:6(n-6) have been shown to occur in subjects consuming a salmon-rich diet (177) or a traditional diet fortified with fish oil (111,174,178,179). These alterations in fatty acid content were confined mainly to phosphatidylcholine although the largest increases in 20:5(n-3) were observed for phosphatidylethanolamine (180). Again, platelet 22:6(n-3) levels were not increased in subjects who had consumed a fish oil deficient in this fatty acid (176).
The red blood cell contents of 20:4(n-6), 20:5(n-3) and 22:6(n-3) are similarly affected in humans who have consumed a diet fortified with fish oil (174,178). Studies have shown that increases in (n-3) fatty acids are mainly associated with phosphatidylcholine, and are entirely at the expense of 18:2(n-6) (175,181). Recently, a survey on the red blood cell composition of Canadian Inuits showed that the red blood cell contents of 20:5(n-3) and 22:6(n-3) is higher compared with non-Inuit controls (182). Since the Inuit diet consisted mainly of mammalian flesh of the ocean, this finding may have a relevance to the amount of (n-3) fatty acids present in the red blood cell. Unlike normal subjects receiving fish oil supplements, however, the changes in the red blood cell (n-3) fatty acids seen in this group are confined mainly to phosphatidylethanolamine. This may have an influence on the dynamic functioning of the red cell membrane, particularly in lowering blood viscosity and increasing malleability of the red blood cell (183).

1.6.2 Effects of fish oil on NIDDM patients

Plasma triacylglycerol, an important risk marker of LVD, is generally raised in NIDDM patients (219). This feature of dyslipidaemia may have led to the early enthusiasm for using fish oils as an alternative therapy. However, other abnormalities such as insulin resistance and hyperglycaemia, are also important in NIDDM and may be influenced by the increased dietary intake of (n-3) fatty acids (6,7). There is evidence indicating that the hypertriglyceridaemic situation in NIDDM patients receiving (n-3) fatty acids in the diet may be improved, however, this is at the expanse of worsened diabetic control.

1.6.2.1 Plasma lipids, lipoproteins and glucose

Early studies into the lipid-lowering potential of 20:5(n-3) oil given daily, for two months, to obese diabetics provided encouraging results (184). The levels of plasma triacylglycerol in these patients were significantly reduced while HDL cholesterol levels increased. Although the beneficial effects of (n-3) fatty acids on plasma triacylglycerol levels were demonstrated in numerous intervention studies,
those on plasma cholesterol were less clear (186,188,189). The plasma cholesterol levels in a group of slightly obese male NIDDM patients on receiving a total of 18 g of marine oil extract rich in (n-3) fatty acids (5.5g) per day for one month, remained unchanged (186). Despite larger amounts of (n-3) fatty acids (8 g) given as dietary supplement to NIDDM patients, only a small reduction of plasma cholesterol levels (7%) was observed at the end of the supplementation (189). Although plasma cholesterol levels of normolipidaemic NIDDM patients were unaffected after receiving 8 weeks of (n-3) fatty acids as supplement, apoB synthesis was increased, thus increasing the production of atherogenic LDL particles (187). This unexpected observation was confirmed in a cross-over study with hypertriglyceridaemic NIDDM patients (188). These diabetics were randomized, one group receiving 4 g of fish oil and the other 12 g safflower oil (control) per day for one month. After a one month washout period, the patients were crossed over to the alternative supplement for another month. At the end of this period, all but one of the patients received 7.5 g fish oil per day for an additional month in order to compare the effects produced by a high and low doses of the fish oil supplement. Differences as regards the effects of fish and safflower oils on LDL apoB and cholesterol levels observed suggest that the production of cholesterol-rich LDL particles may be increased in those patients receiving the fish oil diet, independent of the dose received.

The unexpected effect of (n-3) fatty acids on LDL synthesis has called in to question the usefulness of these PUFA in the treatment of hyperlipidaemia in NIDDM (187,188). The situation with regard to the effect of these fatty acids on diabetic control is worse than first thought. Numerous studies has reported that diabetic control worsened, as indicated by increased fasting glucose and Hba1c concentrations when NIDDM patients were treated with (n-3) fatty acids or fish oil (186,188,189). This adverse effect, however, was abolished on withdrawal of the treatment (186). Surprisingly, in one study, the Hba1c concentrations of untreated NIDDM patients were unchanged despite significant increases in both fasting and meal-stimulated glucose levels (189). This unforeseen condition may be explained by increased hepatic glucose production seen in these diabetics.

The deterioration in diabetic control in NIDDM patients as a result of the increased dietary intake of (n-3) fatty acids by NIDDM, however, has been refuted
in a recent study (185). Patients, having received a course of 8 weeks of (n-3) fatty acid supplement (3 g per day) were infused with glucose and insulin and the rate of glucose clearance calculated. The glucose clearance rate, after a 150 minute combined infusion of glucose (33 mmol/kg/min) and insulin (50mU/kg/min), was found to be significantly increased in these patients compared to diabetic controls. Thus, at least in this study, diabetic control may be improved by the inclusion of a lower dose of (n-3) fatty acids in the diet.

1.6.2.2 Fatty acids profiles

Most studies in the effects of fish oil on NIDDM patients are in agreement as regards increases in plasma and red blood cell contents of the (n-3) fatty acids, 20:5(n-3) and 22:6(n-3) (185,186,189). However, the daily dietary supplementation of NIDDM patients with 2.7 g of sardine oil (25% 20:5(n-3), 7.3% 22:6(n-3)) resulted in a significant increase of the red blood cell 20:5(n-3) and 22:5(n-3) but not 22:6(n-3) (157). This unexpected observation may be explained on the basis of either 20:5(n-3) and 22:5(n-3) are preferentially incorporated into red blood cells during their synthesis or 22:6(n-3) is selectively retro-converted to 22:5(n-3) in vivo, thus reducing the availability of 22:6(n-3) for red blood cell synthesis.

1.6.3 Effects of olive oil on non-diabetic subjects

1.6.3.1 Epidemiological studies

A major study involving seven countries including the USA, Japan and several countries in both southern and northern Europe has shown that plasma cholesterol and coronary heart disease are related to the dietary intake of saturated fatty acids (190). This is particularly true in Crete where the rate of coronary heart disease is low despite the intake of high levels of dietary fat. Most of the fat is in the form of olive oil which is rich in 18:1(n-9) as is the case for many of the Mediterranean countries. This particular dietary bias is reflected in the enrichment of 18:1(n-9) in plasma, red blood cell and platelet fatty acid fractions (191).
1.6.3.2 Dietary studies

Epidemiological studies have suggested that the increased intake of olive oil may partly explain the reduced incidence of coronary heart disease in the Mediterraneans. Based on this hypothesis, dietary studies have been designed to investigate the effects of a diet rich in 18:1(n-9) (olive oil) on coronary risk factors in non-diabetic subjects. In one study, healthy normolipidaemic men were asked to consume 3 different types of cholesterol-free diet, each containing different amounts of monounsaturates, for separate periods of 4 weeks duration with a single break of 2 weeks or more between the different types of diet (192). Eight of the subjects had a break between the first two dietary periods while the remaining 4 took the break between the second and third dietary periods. The results obtained showed that changes in plasma and LDL cholesterol are dependent on the type of fatty acid consumed; the most marked reduction in these coronary risk factors occurred for the diet containing the highest proportion of monounsaturates (40%). This phenomenon was also observed in patients with angiographic evidence of peripheral arterial disease who has replaced their corn oil diet (high in 18:2(n-6) content) with one which rich in olive oil (195). Animal studies have shown that saturated fatty acids suppress hepatic receptor-mediated uptake of LDL, resulting in high plasma LDL cholesterol levels (194). This effect may however be overcome by replacing dietary saturated fatty acids with 18:2(n-6). Unfortunately, there is evidence that the concentration of plasma HDL cholesterol concentration, a major independent risk factor for coronary heart disease, may be reduced by high dietary intakes of 18:2(n-6) (193). Although this suggestion was repudiated in another study, the discrepancy may primarily be due to the lower (n-6) fatty acid contents in the diet (192). Since the usefulness of 18:2(n-6) as a cholesterol lowering agent was uncertain, that of 18:1(n-9) may prove more useful. There are sufficient evidence that this monounsaturated fatty acid may prove more useful in the concomitant reduction of LDL and increased HDL cholesterol levels (193,195,196).
1.6.4 Effects of olive oil on NIDDM patients

It has been shown that the administration of PUFA supplements or a low fat, high carbohydrate diet to NIDDM patients does not significantly improve their blood glucose and HDL cholesterol levels (185,186,188,189,197,198). As the main aim of dietary therapy in diabetes is to improve diabetic control and reduce the risk of coronary heart disease, care should be taken in selecting the type of diet for these patients.

Numerous studies with NIDDM patients have shown that the effects produced by olive oil on diabetic control and dyslipidaemia were favourable (198,199). Results from one of these studies have shown that olive oil is a better alternative dietary supplement for diabetics because it does not increase blood glucose and HbA1c concentration (198). In the other study, two different diets were administered to NIDDM patients in a cross-over fashion, each lasting for 28 days (199). The first diet contained 60% carbohydrate and 25% fat (9% saturates, 9% monounsaturates and 6% polyunsaturates) whilst the second was a monounsaturate-rich diet, containing 35% carbohydrate and 50% fat (10% saturates, 33% monounsaturates and 7% polyunsaturates). The main source of monounsaturates in the second diet was derived from olive oil. At the end of the study, HDL cholesterol levels were found to be increased by 13% on the low carbohydrate, high monounsaturates diet. Plasma glucose, insulin requirements, plasma triacylglycerol and VLDL cholesterol were found to be reduced. Total and LDL cholesterol levels, however, were unaffected by either diet. Therefore, these observations provide evidence for the beneficial effects of olive oil as a dietary treatment for dyslipidaemia in NIDDM without compromising diabetic control.

1.7 Outline of the present study

The scientific data reviewed in this chapter suggests that enhanced protein glycation and platelet activity, as a result of hyperinsulinaemia and hyperglycaemia, are probably important explanations as regards cardiovascular risk attributable to NIDDM per se. In addition, alterations in apoproteins, lipid and lipoprotein
metabolism leading to hypertriglyceridaemia, low HDL-cholesterol concentrations and alterations in the composition of lipoprotein particles may play a crucial role. Recent studies into a possible link between PUFA and coronary heart disease have provided evidence that non-diabetic populations with a low incidence of CHD have higher concentrations of 18:2(n-6) and (n-3) fatty acids in adipose tissue (151), plasma (162,165), platelets (177) and red blood cells (182).

Therefore, in order to establish the contribution made by individual fatty acids to the dyslipidaemia in NIDDM patients with and without LVD, plasma, platelet and red blood cell fatty acids, and plasma lipid levels were measured in NIDDM patients and normal subjects. The lipid lowering effects of (n-3) fatty acids (MaxEPA fish oil) and olive oil were evaluated in NIDDM patients without clinical evidence of LVD. The effects of these oils on plasma phospholipid, platelet and red blood cell fatty acid compositions were also examined.
2 METHODS

2.1 Determination of glycated haemoglobins

Glycated haemoglobin consists of four components, HbA_{i1}, HbA_{i2}, HbA_{i3} and HbA_{i4}, which represent respectively roughly 0.2, 0.2, 0.4 and 3% of the total haemoglobin in non-diabetic individuals. These haemoglobin variants appear to arise as a result of the non-enzymatic glycation of haemoglobin, HbA_{i0}. Using agar buffered at pH 6.3 as the stationary phase, the four components of glycated haemoglobin move as a single band (HbA_{i}) cathodic to HbA_{i0}. The proportion of HbA_{i} in a blood sample is a useful long-term index of glucose/carbohydrate status, commonly being used to assess the effectiveness of dietary treatments, exercise, insulin and oral hypoglycaemic agents in diabetics (200).

In the present study HbA_{i} fraction was determined using agar electrophoresis supplied by Coming Ltd (Coming Medical and Scientific, England). One part of whole blood was mixed with 3 parts of haemolysing reagent, 1 μl of the haemolysate then being electrophoresed for 40 min at 60V. The agar film was dried and scanned at 420 nm and HbA_{i} expressed as a % of total haemoglobin.

2.2 Determination of plasma cholesterol and triacylglycerol

Plasma Cholesterol and triacylglycerol were determined using diagnostic kits supplied by Boehringer-Mannheim (201).

For cholesterol determination the reaction began with the hydrolysis of cholesterol esters to free cholesterol by cholesterol esterase. The free cholesterol was then oxidised by cholesterol oxidase to Δ⁴-cholestenone, hydrogen peroxide, produced in this reaction was then coupled with 4-aminophenazone and phenol in the presence of peroxidase to produce a chromogen, 4-(p-benzoquinone-monoimino) phenazone, which has a maximum absorption at 500 nm (201).

The reaction for triacylglycerol determination began with its hydrolysis to glycerol by an esterase. The glycerol was then converted to glycerol-3-phosphate in a reaction catalysed by glycerol kinase, which in turn was oxidised to dihydroxy-
acetone phosphate by the enzyme glycerol phosphate oxidase. Again, hydrogen peroxide produced in this reaction was coupled with 4-aminophenazone and 4-chlorophenol in the presence of peroxidase and the absorption measured at 500 nm (201).

2.3 Determination of lipoprotein cholesterol

2.3.1 HDL cholesterol

ApoB containing lipoproteins (VLDL and LDL) were selectively precipitated from plasma by sulphated polysaccharides and divalent cations i.e. heparin and manganese respectively, cholesterol then being estimated in the resulting HDL fraction as outlined above (203). Thus, 225 μl of sodium heparin solution (5000 units/ml, source: porcine mucous, CP Pharmaceutical Ltd., UK) and 300 μl of 1 M manganese chloride (MnCl₂·4H₂O) were added to 3 ml of plasma sample. After mixing, the sample was left to stand at 0°C (ice-ammonium chloride mixture) for 30 min. The sample was then centrifuged at 1600 g for 30 min at 4°C and the clear supernatant, containing HDL, was used for total HDL cholesterol determination and HDL₂ precipitation (202).

2.3.2 HDL₂ and HDL₃ cholesterol

The method adopted for HDL₃ cholesterol determination was that of Gidez et al (203). An aliquot (200 μl) of 1.43% of dextran sulphate (M, 15,000, Sochibo, Boulogne, France) was added to 2 ml of heparin-Mn²⁺ supernatant (section 2.3.1). After standing at room temperature for 20 min, the sample was centrifuged at 1600 g for 30 min at 4°C. HDL₃ cholesterol was determined in the supernatant and HDL₂ cholesterol concentration obtained by subtracting the HDL₃ value from that of total HDL.
2.3.3 Estimation of LDL cholesterol

Plasma LDL cholesterol concentration was obtained using the Friedewald equation (204):

\[
(LDL\ chol) = (Total\ Chol) - (T/2.186) - (HDL\ chol)
\]

where \( chol = \) cholesterol (mmol l\(^{-1}\))
\( T = \) triacylglycerol (mmol l\(^{-1}\))

Using this method, adequate estimates of LDL cholesterol were obtained, although inaccuracies have been reported in samples in which triacylglycerol levels exceeded 4.5 mmol l\(^{-1}\) (205). In none of the subjects studied did concentrations exceed this value.

2.4 Ultracentrifugal analysis of plasma lipoproteins

2.4.1 Introduction

Plasma lipoproteins have lower hydrated densities than plasma proteins. Thus, it is possible to use flotation techniques for their isolation and classification. Modern ultracentrifugal procedures are based on the original method described by de Lalla and Gofman in 1954 (206). These involve the use of various combinations of potassium bromide or sodium bromide solutions with sodium chloride solution, or the direct addition of solid sodium bromide or potassium bromide to raise the solvent density of plasma and lipoprotein fractions prior to ultracentrifugation. The peak concentrations of lipoproteins are located at distinct ranges of solvent density, namely at \( \rho<1.006 \) (VLDL), \( \rho=1.006-1.063 \) (LDL), \( \rho=1.063-1.125 \) (HDL\(_2\)) and \( \rho=1.125-1.21 \) (HDL\(_3\)). Many workers choose to regard plasma and solvent density as being one and the same thing. However, the validity of this assumption has been disputed and other workers have suggested that the solvent volume should be taken as being 94% of the plasma volume (207). The remaining 6% is then taken as
corresponding to proteins and other solids which should not be included when defining lipoprotein densities. As human plasma proteins have been shown to vary in weight by between 6 to 8%, this may contribute to variations as regards plasma density (208). Such discrepancies have been overcome by designating the solution densities of LDL, HDL₂ and HDL₃ as being 1.073, 1.125 and 1.21 respectively (209). These densities were adopted in this study and solid potassium bromide used, in place of sodium bromide, to raise the plasma density. Potassium bromide was used because it has been suggested that it causes less lipoprotein particle deformability than sodium bromide (207).

2.4.2 Reagents

0.15 M sodium chloride: A solution containing 8.775 g of A.R. sodium chloride was made up to 1 litre with distilled water.

Stock density solution (p = 1.346): A solution containing 153 g of A.R. sodium chloride and 354 g of A.R. potassium bromide was made up to 1 litre with distilled water.

Density solution 1 (p = 1.063): A solution containing 10 ml of stock density solution and 50 ml of 0.15 M sodium chloride was prepared prior to use.

Density solution 2 (p = 1.125): A solution containing 27 ml of stock density solution and 50 ml of 0.15 M sodium chloride was prepared prior to use.

Density solution 3 (p = 1.210): A solution containing 75 ml of stock density solution and 50 ml of 0.15 M sodium chloride was prepared prior to use.

Potassium bromide: Analytical grade potassium bromide was dried in a 100 °C oven overnight and cooled to room temperature in a vacuum desiccator prior to use.
2.4.3 Centrifuge

All ultracentrifugal separations of plasma lipoproteins were carried out on a MSE Europe 55M preparative ultracentrifuge using a 6 x 14 ml swing out titanium rotor.

2.4.4 Ultracentrifugal separation of plasma lipoproteins

A weighed sample of plasma (10 ml) was delivered into a 15 ml ultracentrifuge tube and 0.15 M sodium chloride carefully applied to the surface of the plasma so as to provide a protein-free medium for the flotation of VLDL. Samples were centrifuged at 100,000 g for 18 hours at 16 °C. After the first centrifugation, 2 ml of the supernatant (VLDL) was made up to 5 ml with 0.15 M sodium chloride. This was thoroughly mixed and stored in a 5 ml EDTA blood tube at 4 °C prior to VLDL cholesterol determination. The subnatant containing LDL and HDL was mixed thoroughly and made up to 15 ml with 0.15 M sodium chloride. The procedures for the subsequent sequential ultracentrifugal separations of LDL, HDL₂ and HDL₃ were similar to that described for VLDL but with the following modifications. The weight of 10 ml of lipoprotein solution was used to calculate the amount of potassium bromide required to raise its solvent density to 1.073, 1.125 or 1.21 (section 2.4.5). The calculated amount of potassium bromide was then dissolved in the lipoprotein solution. This adjusted lipoprotein solution was finally layered with the appropriate salt solution (density solution 1, 2 or 3), i.e. one having a solvent density similar to that of the adjusted lipoprotein solution, prior to centrifugation.
2.4.5 Lipoprotein density adjustment

The equation used to calculate the amount of potassium bromide required to adjust the solvent density of lipoprotein fractions was derived as follows:

Density of plasma or infranate  \( = \rho_{\text{solute}} \)
Weight of plasma or infranate before addition of potassium bromide  \( = m_0 \)
Volume of plasma or infranate before addition of potassium bromide  \( = V_0 \)
Density of potassium bromide  \( = \rho_{\text{KBr}} \)
Weight of potassium bromide required to adjust lipoprotein solvent density  \( = m_{\text{KBr}} \)
Volume of potassium bromide added to lipoprotein solution  \( = V_{\text{KBr}} \)
Partial specific volume of KBr  \( = v_s \)

\[
\begin{align*}
\rho_{\text{solute}} &= \frac{(m_0 + m_{\text{KBr}})/(V_0 + V_{\text{KBr}})}{\rho_{\text{KBr}}} \\
&= \frac{(m_0 + m_{\text{KBr}})/(V_0 + m_{\text{KBr}}/\rho_{\text{KBr}})}{\rho_{\text{KBr}}} \\
&= \frac{(m_0 + m_{\text{KBr}})/(V_0 + m_{\text{KBr}}/v_s)}{\rho_{\text{KBr}}} \\
\rho_{\text{solute}}(V_0 + m_{\text{KBr}} v_s) &= (m_0 + m_{\text{KBr}}) \\
m_{\text{KBr}}(1 - \rho_{\text{solute}} v_s) &= (\rho_{\text{solute}} V_0 - m_0) \\
m_{\text{KBr}} &= \frac{(\rho_{\text{solute}} V_0 - m_0)(1 - \rho_{\text{solute}} v_s)}{1 - \rho_{\text{solute}} v_s} \quad (1)
\end{align*}
\]
The partial specific volume of potassium bromide, \( v_s \), was derived as follows:

- Density of KBr solution = \( \rho_{\text{soln}} \)
- Density of solid KBr = \( \rho_{\text{KBr}} \)
- Weight of solid KBr = \( m_{\text{KBr}} \)
- Weight of water to dissolve \( m_{\text{KBr}} \) = \( m_w \)
- Volume of the solid KBr = \( V_{\text{KBr}} \)
- Volume of water to dissolve \( m_{\text{KBr}} \) = \( V_w \)
- Partial specific volume of KBr = \( v_s \)

\[
\rho_{\text{soln}} = \frac{(m_{\text{KBr}} + m_w)}{(V_{\text{KBr}} + V_w)}
\]
\[
= \frac{(m_{\text{KBr}} + m_w)}{V_{\text{KBr}}/\rho_{\text{KBr}} + V_w}
\]
\[
= \frac{(m_{\text{KBr}} + m_w)}{m_{\text{KBr}}/\rho_{\text{KBr}} v_s + V_w}
\]

\[
\rho_{\text{soln}}(m_{\text{KBr}} v_s + V_w) = (m_{\text{KBr}} + m_w)
\]
\[
(m_{\text{KBr}} v_s + V_w) = (m_{\text{KBr}} + m_w)/\rho_{\text{soln}}
\]
\[
m_{\text{KBr}} v_s = (m_{\text{KBr}} + m_w)/(\rho_{\text{soln}}) - V_w
\]
\[
v_s = \frac{[(m_{\text{KBr}} + m_w)/(\rho_{\text{soln}}) - V_w] / m_{\text{KBr}}}{2}
\]

The partial specific volume of potassium bromide was calculated using equation 2 and data provided by Baxter et al (210). Using the values from table 2, a graph of partial specific volume of potassium bromide versus its corresponding densities was drawn (Fig 5). The partial specific volumes of potassium bromide at the LDL (\( \rho = 1.073 \)), HDL2 (\( \rho = 1.125 \)) and HDL3 (\( \rho = 1.21 \)) solvent densities were then estimated using the graph shown in Figure 5.
Table 2  Physical parameters for KBr at 25 °C

<table>
<thead>
<tr>
<th>$\rho_{KBr}$</th>
<th>$m_{KBr}$</th>
<th>$m_w$</th>
<th>$v_w$</th>
<th>$v_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.01627</td>
<td>27.113</td>
<td>990.268</td>
<td>993.178</td>
<td>0.2919</td>
</tr>
<tr>
<td>1.03554</td>
<td>27.113</td>
<td>488.753</td>
<td>490.189</td>
<td>0.2940</td>
</tr>
<tr>
<td>1.07386</td>
<td>27.113</td>
<td>238.967</td>
<td>239.670</td>
<td>0.2991</td>
</tr>
<tr>
<td>1.18625</td>
<td>27.113</td>
<td>90.665</td>
<td>90.931</td>
<td>0.3082</td>
</tr>
<tr>
<td>1.37090</td>
<td>27.113</td>
<td>40.752</td>
<td>40.872</td>
<td>0.3184</td>
</tr>
</tbody>
</table>

Figure 5  A graph of partial specific volume of KBr vs its solution density
2.5 Analysis of plasma and platelet fatty acids

2.5.1 Reagents and materials

*Extraction solvent (containing 0.05% butylated hydroxytoluene as antioxidant):*

Two volumes of chloroform and one volume of methanol were mixed thoroughly prior to the extraction of lipids.

*Pure solvent upper phase:*

Forty volumes of redistilled chloroform (b.p. 60-62° C), 24 volumes of redistilled methanol (b.p. 64-65° C) and 15 volumes of distilled water were mixed thoroughly and the emulsion allowed to separate into two phases, the upper phase then being transferred into a brown bottle. This reagent was prepared just before use.

*Fat-free non-absorbent cotton wool:*

Non-absorbent cotton wool was soaked in peroxide-free diethyl ether for half an hour. The ether was then decanted and the cotton wool soaked in fresh peroxide-free diethyl ether for a further 15 minutes. The ether was then removed by suction and the cotton wool dried in vacuo.

2.5.2 Plasma and platelet separation

Forty ml of EDTA-treated blood was centrifuged for 10 minutes at 200 g. The supernatant (platelet-rich plasma) was then removed and centrifuged for 10 minutes at 1600 g to obtain a platelet pellet. The supernatant (platelet-poor plasma) was then separated and stored in glass tubes at 4 °C. Meanwhile, the platelet pellet was resuspended in 10 ml of EDTA-saline (0.015M EDTA in 0.15M sodium
chloride) and centrifuged at 200 g for 10 minutes to sediment any remaining leucocyte and erythrocyte contaminants. It was established using a Coulter Thrombocounter that less than 10 leucocytes or erythrocytes occurred per $10^6$ platelets in these suspensions. Platelet suspensions were then centrifuged for 15 minutes at 1600 g, the packed platelets obtained being suspended in 1 ml of EDTA-saline and stored at -20 °C until time of analysis.

2.5.3 Extraction

Plasma total lipids were extracted according to the method of Folch et al (211). Plasma (0.4 ml) was added dropwise into a glass tube containing 7.6 ml of extraction solvent. The mixture was then shaken intermittently for 10 minutes and filtered through fat-free non-absorbent cotton wool into a glass centrifuge tube. Distilled water (1.6 ml) was added to the filtrate which was mixed thoroughly and then centrifuged for 15 minutes at 1600 g resulting in the formation of a two phase system; if the phases were cloudy, the mixture was centrifuged for a further 10 minutes. The supernatant was then carefully removed by a glass pasteur pipette and the surface of the subnatant rinsed gently with 0.6 ml of the pure solvent upper phase then being discarded. The rinsing procedure was then repeated a second time and the subnatant stored at 4° C for subsequent transesterification.

The procedures for the extraction of plasma phospholipids and platelet total lipids were essentially the same as that described for plasma total lipids except with respect to the scale of the extractions, extraction volumes being 2.5 times that being used for plasma total lipids extraction. It should be noted that platelet samples were subjected to sonication prior to extraction, thus ensuring their homogeneity.

2.5.4 Isolation of plasma phospholipids by thin-layer chromatography

Thin-layer chromatography of plasma lipids was conducted using pre-coated silica gel plates (20 x 20 cm, 0.25 mm thickness gel, BDH) that had previously been dried by heating at 100°C for 45 min and then cooled to ambient temperature in a vacuum desiccator. Extracts containing plasma lipids were evaporated to
dryness on a rotary evaporator and the residues redissolved in a smaller volume of extraction solvent (100 μl per sample). These were then spotted onto thin layer plates with a Hamilton microsyringe, under a stream of nitrogen, so as to form narrow bands, each band being 3 cm wide and 1.5 cm from the lower edge of the plate. The flasks holding the lipids were rinsed twice with extraction solvent (100 μl per sample per rinse) and the rinses also applied to the thin layer plates. A known mixture of phospholipids, triglycerides and cholesterol esters was also applied to the plates, serving as lipid markers. Thin layer plates were then run in air tight chromatography tanks containing n-hexane, diethyl ether and glacial acetic acid (80:20:1). The partitioned lipid fractions were then visualized by exposing thin layer plates to iodine crystals and the bands corresponding to phospholipids, triglycerides and cholesterol esters then scraped into 10 ml glass tubes for transesterification and gas liquid chromatography.

2.5.5 Transesterification and gas-liquid chromatography

Esterified fatty acids were transesterified to methyl esters according to the method of Morrison et al (212). Plasma and platelet lipid extracts were evaporated to dryness under a jet of oxygen-free nitrogen and were left overnight at room temperature to react with 14% Boron trifluoride-methanol complex (1 ml per sample) to form fatty acid methyl esters. The reaction was stopped by the addition of 1 ml of distilled water and the fatty acid methyl esters extracted into 2 ml of petroleum ether (40-60°). The emulsion formed as a consequence of these additions was then centrifuged for 15 minutes at 1600 g to obtain a two phase system. The lower phase was extracted with a further 2 ml of petroleum ether (40-60°), the supernatants then being combined and evaporated to dryness under nitrogen. N-heptane containing 0.05% butylated hydroxytoluene was then added to the dry residue and an aliquot of the extract (0.2 μl) injected into the chromatograph. A Pye Unicam GCV chromatograph fitted with two flame ionization detectors and a splitter injector system was used to separate fatty acid methyl esters. The chromatograph was equipped with a 50 x 0.25 mm fused silica WCOT CP™ Sil88 capillary column (Chrompack UK Ltd), the column temperature being set at 220°C. Injection
and detector temperatures were both set at 270°C. The carrier gas was oxygen-free nitrogen and the flow rate was set at 0.7 ml per min. Fatty acid methyl ester qualitative standards and NHI reference mixtures (Supelco, UK) were used to test the chromatographic system to ensure its reliability for quantitative analysis (a chromatogram of these fatty acid standards was included in appendix I). Individual fatty acids were expressed as [(mole of fatty acid)/(sum of moles of all individual fatty acids)]\times100. Using the Quickbasic compiler, a PC program was written to achieve this calculation.

2.5.6 Quickbasic program for fatty acid calculation

```
5 REM***LING25/PC*APR*1990
10 CLEAR: SCREEN 0: COLOR 0, 3: CLS 0: GOSUB 1800
30 FOR Z = 1 TO A: READ A$(Z): NEXT Z
40 FOR Z = 1 TO B: READ B$(Z), B(Z): NEXT Z: GOSUB 1500
100 INPUT " USER NAME " ; LS$(1): A$(10) = A$(10) + " : " + LS$(1)
110 INPUT " CUSTOMER " ; LS$(2): A$(11) = A$(11) + " : " + LS$(2)
120 INPUT " EXPERIMENT " ; LS$(3): A$(12) = A$(12) + " : " + LS$(3)
130 INPUT " DATE " ; LS$(4): A$(13) = A$(13) + " : " + LS$(4)
140 PRINT: RS = "LPT1;"
200 INPUT " NO OF SAMPLES " ; C: CLS 0: DIM A(C, B), C$(C)
210 FOR Z = 1 TO C: E = 1: COLOR 0, 3
220 PRINT A$(14); SPC(3); : INPUT C$(Z)
230 GOSUB 620
240 FOR X = 1 TO B
250 GOSUB 600: PRINT X; SPC(4); B$(X); SPC(16 - LEN(B$(X))); GOSUB 510: A(Z, X) = A(Z, X) / B(X)
260 D = D + A(Z, X): GOSUB 600
270 GOSUB 800: COLOR 14, 1: E = 1: CLS 0: PRINT A$(14); SPC(3); C$(Z)
280 PRINT 510: PRINT A(Z, X) / D * 100
290 A(Z, X) = INT(A(Z, X) * 100 + .5) / 100
300 FOR V = 1 TO 38: PRINT : NEXT V: PRINT : RETURN
310 FOR X = 1 TO B
320 GOSUB 700: A(Z, X) = A(Z, X) / D * 100
330 A(Z, X) = INT(A(Z, X) * 100 + .5) / 100
340 PRINT X; SPC(4); B$(X); SPC(16 - LEN(B$(X))); GOSUB 510: GOSUB 700
350 GOSUB 800: COLOR 14, 1: E = 1: CLS 0: PRINT A$(14); SPC(3); C$(Z)
360 NEXT X
370 GOSUB 800: COLOR 0, 3: CLS 0: D = 0: NEXT Z
380 GOTO 1000
500 FOR V = 1 TO 38: PRINT "="; : NEXT V: PRINT : RETURN
510 FOR V = 1 TO 38: PRINT "="; : NEXT V: PRINT : RETURN
600 IF X <> E * 10 THEN 630
610 E = E + 1: CLS 0: PRINT A$(14); SPC(3); C$(Z)
620 PRINT "PEAK PEAK NAME": SPC(8); "PEAK AREA COUNT": GOSUB 500
630 PRINT "PEAK PEAK NAME": SPC(8); "PEAK AREA COUNT": GOSUB 500
630 RETURN
700 IF X <> E * 10 THEN 730
710 E = E + 1: GOSUB 800: COLOR 14, 1: CLS 0: PRINT A$(14); SPC(3); C$(Z)
720 PRINT "PEAK PEAK NAME": SPC(9); "MOLE": GOSUB 500
730 RETURN
800 COLOR 31, 4: PRINT "PRESS RETURN"
810 DO: LOOP WHILE INKEY$ = ""
820 RETURN
```

79
900 X$ = STR$(A(Z, X))
910 FOR I = 1 TO 3
920 IF MID$(X$, I, 1) <> "," AND MID$(X$, I, 1) <> "" THEN 930 ELSE 940
930 NEXT I: RETURN
1000 GOSUB 1500: INPUT " DO YOU WANT A PRINTOUT OF THE RESULT (Y/N) "; HS
1010 IF LEFT$(HS, 1) = "Y" THEN GOSUB 1900
1020 PRINT ": PRINT : PRINT : RETURN
1800 PRINT ": GLC FATTY ACIDS DETERMINATION"
1820 PRINT ": THE CALCULATING PROGRAM"
1830 PRINT ": K L E L I N G"
1840 PRINT ": RETURN
1900 CLS 0
1910 PRINT ": PRINT : PRINT " IS YOUR PRINTER CONNECTED TO LPT1 (Y/N) "; PS
1920 IF LEFT$(PS, 1) = "Y" THEN GOSUB 2000: RETURN
1930 PRINT ": PRINT : PRINT " ABORT PRINTING (A) OR CONNECT PRINTER PORT (C) "; PS
1940 IF PS = "A" THEN CLS 0: RETURN
1950 PRINT ": PRINT : PRINT " NAME OF PRINTER PORT "; RS
1960 GOSUB 3500: RETURN
2000 OPEN R$ FOR OUTPUT AS #1
2010 FOR Z = 1 TO 7: PRINT #1, A$(Z): NEXT Z
2020 PRINT #1, PRINT #1, " 
2030 PRINT #1, A$(8); SPC(IO); A$(9): PRINT #1, " 
2040 FOR X = 1 TO B
2045 PRINT #1, A$(15)
2060 CLOSE #1: IF G$ <> "" THEN RETURN: PRINT : PRINT
2200 CLS 0: PRINT ": PLACE PAPER IN PRINTER"; PRINT
2210 PRINT ": THEN PRESS RETURN"
2220 DO: LOOP WHILE INKEY$ = ""
2230 PRINT ": PRINT : PRINT : IF LL <> 0 THEN 2260
2240 PRINT " : NO OF COPIES REQUIRE "; LL
2250 IF LL = 0 THEN RETURN
2260 LL = LL - 1: GOTO 2000
2300 CLS 0: COLOR 5, 7: GOSUB 1500
2310 INPUT " FILENAME OF THE CURRENT RESULTS "; A$ 
2320 PRINT ": PRINT : PRINT " STORE IN WHICH DRIVE "; BS
2330 G$ = BS + A$ 
2340 OPEN G$ FOR OUTPUT AS #1
2350 GOSUB 2500: RETURN
2500 FOR Z = 1 TO (15 + C * 12): PRINT #1, ";": NEXT Z: PRINT "; RETURN: PRINT ";"
2.6 Analysis of red blood cell fatty acids

2.6.1 Red blood cell separation

Blood (20 ml) was collected into EDTA and centrifuged for 15 minutes at 1600 g. Supernatant and buffy coat fractions, together with 1 ml of packed red blood cells were then removed. The remaining red blood cells were suspended in an equal volume of EDTA-saline and subjected to a washing procedure consisting of two consecutive centrifugations so as to free them of plasma and other blood cells. The first centrifugation was at 200 g for 10 min, the second being at 1600 g for 15 min. After each of these spinnings, supernatant and 1 ml of the subnatant were discarded.

Aliquots (0.5 ml) of the packed red blood cells were mixed thoroughly with an equal volume of EDTA-saline and counted on a Coulter Thrombocounter C. All red cell preparations isolated in this manner were found to contain not more than 20 platelets and 30 leucocytes per 10^6 red cells. Packed red cells were then frozen at -20°C for subsequent lipid extraction.

2.6.2 Extraction

The method of Folch et al. (211) was not suitable for red blood cell extraction due to the solubility of haem in the extraction solvent. A procedure which gives minimal haem contamination of extracts was therefore chosen (212). Aliquots (1 ml) of packed red cells were haemolysed by treatment with an equal volume of distilled water at room temperature for 15 minutes. Propan-2-ol (11 ml,
A.R. grade) containing 0.05% butylated hydroxytoluene was then added dropwise to the haemolysed solution and left to stand at room temperature for an hour, with intermittent mixing. This was followed by the addition of redistilled chloroform (7 ml, A.R. grade) containing 0.05% butylated hydroxytoluene, the whole mixture then being left for a further hour at room temperature, with periodic mixing. The extract obtained was then filtered through fat-free non-absorbent cotton wool into a glass tube containing approximately 0.5 g of anhydrous sodium sulphate. This was left at 4°C for approximately 30 minutes in order to remove the water fraction from the lipid extract.

2.6.3 Transesterification and gas-liquid chromatography

The method for the transesterification and gas-liquid chromatography of red blood cell fatty acids was essentially the same as that described for plasma and platelet fatty acids. The only difference was that the extracts were evaporated to dryness under vacuum prior to transesterification.

2.7 Statistical methods

Pearson's coefficient of variation (CV% = 100*standard deviation/mean) was used to determine the within-batch variation of assays.

All data were initially tested for normality using the Wilk-Shapiro test. As the data were found not to be normally distributed despite log transformation of data, nonparametric procedures were used for statistical analyses.

In the cross-sectional studies, the data were expressed as the medians and 95% confidence intervals (46). The Kruskal-Wallis one-way analysis of variance was used to test for the equality of medial values (lipid and fatty acids) across the three groups (normal subjects, NIDDM patients with and without large vessel disease). If significant differences were found pairs of groups were compared using the Wilcoxon rank sum test (214).

In the MaxEPA and olive oil studies, data were expressed as the means and 95% confidence intervals. Paired Student's t-test was used to analyse the
changes in each variable in the two treatment groups (fish oil and olive oil) between baseline (0 week) and 4/8 weeks. Unpaired Student's t test was used to compare between-group differences in each variable at baseline (0 week) and also to compare changes in each variable in the fish oil group between baseline (0 week) and 4 weeks and baseline (0 week) and 8 weeks with those in the olive oil (control) group.

The Kendall rank-order correlation coefficient (τ) was used to measure the degree of association between two variables (214).

The box and whisker plot was used to give a graphical representation of the medians (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (outlier points).

Scatter diagrams were used to give a pictorial representation of the association between two variables.

All graphics were created by the Statgraphics V3.0 statistics package (STSC Inc). Statistic significance was quoted at the p<0.05, p<0.02 or p<0.001 levels.

2.8 Methods - coefficient of variations

Individual method is checked for variability by repeating the procedures 6 or 10 times with a randomly selected sample. Platelet samples were pooled from ten subjects. The Pearson's coefficient of within-batch variation of assays (CV%) were shown on the next page.
<table>
<thead>
<tr>
<th>Methods</th>
<th>n</th>
<th>within-batch CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA₁</td>
<td>10</td>
<td>5.8</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
<td>2.6</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>10</td>
<td>3.7</td>
</tr>
<tr>
<td>THDL</td>
<td>10</td>
<td>4.8</td>
</tr>
<tr>
<td>HDL₂</td>
<td>10</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Ultracentrifugal analysis of lipoprotein cholesterol**

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>n</th>
<th>within-batch CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>6</td>
<td>3.2</td>
</tr>
<tr>
<td>LDL</td>
<td>6</td>
<td>4.0</td>
</tr>
<tr>
<td>HDL₂</td>
<td>6</td>
<td>5.1</td>
</tr>
<tr>
<td>HDL₃</td>
<td>6</td>
<td>5.7</td>
</tr>
</tbody>
</table>

**Gas-liquid chromatographic analysis of fatty acids (within-batch cv%):**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>n</th>
<th>CV%(plasma/PL)</th>
<th>CV%(PLT)</th>
<th>CV%(RBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>10</td>
<td>3.1/3.3</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>18:0</td>
<td>10</td>
<td>2.7/3.0</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>10</td>
<td>3.7/4.0</td>
<td>4.1</td>
<td>3.4</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>10</td>
<td>3.2/3.5</td>
<td>2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>10</td>
<td>2.1/2.4</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>10</td>
<td>3.2/5.3</td>
<td>3.0</td>
<td>4.4</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>10</td>
<td>4.2/4.9</td>
<td>3.8</td>
<td>3.5</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>10</td>
<td>2.8/3.1</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>10</td>
<td>2.9/3.5</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>10</td>
<td>3.1/4.3</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>10</td>
<td>4.7/4.9</td>
<td>5.4</td>
<td>4.5</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>10</td>
<td>4.2/5.3</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>10</td>
<td>3.2/3.5</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>10</td>
<td>3.3/4.5</td>
<td>3.1</td>
<td>2.5</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>10</td>
<td>2.7/3.4</td>
<td>2.8</td>
<td>2.3</td>
</tr>
</tbody>
</table>
3 CROSS-SECTIONAL STUDIES ON PLASMA LIPID AND BLOOD FATTY ACID PROFILES IN NIDDM PATIENTS

3.1 Plasma lipid and fatty acid profiles in male NIDDM patients

3.1.1 Aims

Studies have shown that plasma PUFA levels are related to the risk of developing LVD (164,190,215,216) and that NIDDM increases this risk (6,217). Dyslipidaemia is common in NIDDM (218-221) and may be linked to differences in plasma fatty acid levels. In order to verify this hypothesis, plasma lipids and fatty acid levels have been measured in male NIDDM patients with and without clinical evidence of LVD. Age-matched non-diabetic subjects served as controls.

3.1.2 Subjects.

Forty male NIDDM patients were recruited from the diabetic clinic at University College Hospital. The optical fundi of these patients were carefully examined following dilation of the pupil to exclude retinopathy. The patients had normal renal function and no proteinuria and showed no evidence of clinical neuropathy. Twenty of the NIDDM patients suffered with angina pectoris or myocardial infarction as confirmed by resting electrocardiograms. The duration of diabetes in these patients was 1 to 17 years. The other 20 patients were free of LVD, the duration of diabetes in this group ranging between 1 and 37 years. All NIDDM patients were being treated with oral hypoglycaemic drugs (Appendix II). Twenty age-matched healthy male subjects with no family history of diabetes were selected from hospital personnel to serve as controls.

3.1.3 Methods

All subjects were asked not to alter their diet and to refrain from taking any anti-inflammatory drugs for at least a week prior to the blood sampling.
Subjects, after an overnight fast, were asked to sit for at least 15 minutes before blood was collected into tubes containing EDTA (1 mg per ml blood). Plasma was separated for lipid analysis as described in sections 2.2 and 2.3. Plasma total and plasma phospholipid fatty acid compositions were determined (section 2.5). Glycated haemoglobin (HbA1%) was estimated as detailed in section 2.1. Statistical differences were assessed by tests outlined in section 2.7.

3.1.4 Results

3.1.4.1 Clinical data and plasma lipid levels

The median age and body weight of NIDDM patients with (LVD+) and without (LVD-) clinical evidence of LVD and normal controls (N), together with their plasma lipid levels are shown in table 3. There were no differences in the age and body weight (and BMI) between NIDDM patients and normal subjects.

Table 3 Age, body weight, HbA1% and plasma lipid compositions in normal subjects & NIDDM patients with (LVD+) and without (LVD-) large vessel disease.

<table>
<thead>
<tr>
<th></th>
<th>Normals (N, n=20)</th>
<th>NIDDMs (LVD-, n=20)</th>
<th>NIDDMs (LVD+, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (range)</td>
<td>median (range)</td>
<td>median (range)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>46 (23-82)</td>
<td>55 (30-75)</td>
<td>60 (36-70)</td>
</tr>
<tr>
<td>weight (kg)</td>
<td>73 (55-108)</td>
<td>77 (62-96)</td>
<td>81 (70-96)</td>
</tr>
<tr>
<td>HbA1% (mmol/l)</td>
<td>&lt;0.001</td>
<td>6.9 (6.6-7.1)</td>
<td>9.7 (8.7-11.2)***</td>
</tr>
<tr>
<td>Trig (mmol/l)</td>
<td>&lt;0.001</td>
<td>1.06 (0.89-1.49)</td>
<td>1.63 (1.26-2.20)**</td>
</tr>
<tr>
<td>VLDL-C (mmol/l)</td>
<td>&lt;0.001</td>
<td>0.49 (0.41-0.68)</td>
<td>0.75 (0.58-1.01)</td>
</tr>
<tr>
<td>T Chol (mmol/l)</td>
<td>&lt;0.02</td>
<td>5.72 (5.28-6.21)</td>
<td>5.45 (4.94-6.05)</td>
</tr>
<tr>
<td></td>
<td>KW(p) median (95% C.I.)</td>
<td>median (95% C.I.)</td>
<td>median (95% C.I.)</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>6.9 (6.6-7.1)</td>
<td>9.7 (8.7-11.2)***</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>1.06 (0.89-1.49)</td>
<td>1.63 (1.26-2.20)**</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>0.49 (0.41-0.68)</td>
<td>0.75 (0.58-1.01)</td>
</tr>
</tbody>
</table>

1: p values, using Kruskal-Wallis one-way ANOVA; N.S.: not significant.
***: p<0.001, **: p<0.02, compared to values of normal subjects.
++: p<0.02, ++: p<0.05, compared to values of NIDDM without LVD.
Complete lipid and lipoprotein profiles of normal subjects and NIDDM patients are shown in appendix III, table 18.
The HbA1% levels seen in NIDDM patients were significantly higher than in controls despite their treatment with oral hypoglycaemic agents and dietary control (LVD-, LVD+ vs N, p<0.001; Fig 6). Plasma triacylglycerol levels were also significantly higher in the patients than in normal subjects (LVD- vs N, p<0.02; LVD+ vs N, p<0.001; Fig 7), the highest levels being seen in those with LVD (LVD+ vs LVD-, p<0.02; Fig 7).

Figure 6: HbA1% in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: p<0.001). Median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Figure 7  Plasma triacylglycerol levels in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD- vs N, p<0.02; ***: LVD+N vs N, p<0.001; ++: LVD- vs LVD+, p<0.02).

median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Plasma total and individual lipoprotein cholesterol concentrations were similar in NIDDM patients without LVD and normal subjects. In contrast, NIDDM patients with LVD had significantly higher total plasma cholesterol levels than normal controls and also patients without LVD (LVD+ vs N, \( p<0.02 \); LVD+ vs LVD-, \( p<0.02 \); Fig 8). Individual lipoprotein cholesterol levels in these patients, however, were not unlike those of normal subjects.

Figure 8 Plasma cholesterol levels in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD+ vs N, \( p<0.02 \); ++: LVD- vs LVD+, \( p<0.02 \)). median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
The LDL cholesterol levels were found to correlate significantly with total plasma cholesterol in NIDDM patients and normal controls (N: τ=0.7230, p<0.001; LVD−: τ=0.7158, p<0.001; LVD+: τ=0.6455, p<0.001; Fig 9).

![Figure 9](image)

**Figure 9** Relationship between LDL and plasma cholesterol in normal subjects (1) & NIDDM patients with (3) and without (2) LVD (1: τ=0.7230, 2: τ=0.7158, 3: τ=0.6455; p<0.001).

A positive relationship was found to exist between LDL cholesterol and plasma triacylglycerol concentrations in normal subjects (N: τ=0.3430, p<0.05; Fig 10). The log (LDL cholesterol) were seen to reach a steady state in those subjects with a plasma triacylglycerol greater than 1.5 mmol/l (Fig 11).
Figure 10  Relationship between LDL cholesterol & plasma triacylglycerol values in normal subjects ($r=0.3430$, $p<0.05$).

Figure 11  Relationship between log (LDL cholesterol) & plasma triacylglycerol values in normal subjects.
An inverse correlation between HDL₂ cholesterol and plasma triacylglycerol concentrations was also seen in normal subjects (N: $\tau=-0.3253$, $p<0.05$; Fig 12). Similar associations were however not observed in NIDDM patients.

Figure 12  Relationship between HDL₂ cholesterol and plasma triacylglycerol values in normal subjects ($\tau=-0.3253$; $p<0.05$).
3.1.4.2 Plasma total fatty acids

Although more than fifteen fatty acids were detected by gas chromatography, only those with retention times comparable to authentic standards were examined. A typical chromatogram of plasma fatty acids is shown in Fig 13.

![Gas chromatogram of plasma fatty acids](image)

**Figure 13** Gas chromatogram of plasma fatty acids

<table>
<thead>
<tr>
<th>1: BHT</th>
<th>6: 18:2(n-6)</th>
<th>11: 20:4(n-6)</th>
<th>16: 22:6(n-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2: 16:0</td>
<td>7: 18:3(n-6)</td>
<td>12: 20:5(n-3)</td>
<td></td>
</tr>
<tr>
<td>3: 16:1(n-7)</td>
<td>8: 18:3(n-3)</td>
<td>13: 22:4(n-6)</td>
<td></td>
</tr>
<tr>
<td>4: 18:0</td>
<td>9: 20:2(n-6)</td>
<td>14: 22:5(n-6)</td>
<td></td>
</tr>
<tr>
<td>5: 18:1(n-9),(n-7)</td>
<td>10: 20:3(n-6)</td>
<td>15: 22:5(n-3)</td>
<td></td>
</tr>
</tbody>
</table>
Plasma fatty acid levels in normal subjects and NIDDM patients are shown in Table 4.

### Table 4 Plasma fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects (N) (n=20)</th>
<th>NIDDM patients (LVD-) (n=20)</th>
<th>NIDDM patients (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol%</td>
<td>KW(p)† median (95% C.I.)</td>
<td>median (95% C.I.)</td>
<td>median (95% C.I.)</td>
</tr>
<tr>
<td>16:0</td>
<td>&lt;0.001 24.84 (23.48-25.78)</td>
<td>26.19 (25.27-27.34)**</td>
<td>27.51 (26.08-29.13)**</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>0.05 20.06 (19.16-21.65)</td>
<td>21.27 (19.13-23.00)</td>
<td>22.14 (20.76-23.70)**</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.001 32.16 (27.99-34.54)</td>
<td>26.24 (25.21-29.45)**</td>
<td>26.02 (21.87-27.61)**</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.05 0.21 (0.11-0.34)</td>
<td>0.39 (0.20-0.58)**</td>
<td>0.31 (0.22-0.37)*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.001 0.59 (0.44-0.74)</td>
<td>1.11 (0.62-2.03)**</td>
<td>0.91 (0.77-1.13)**</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>&lt;0.001 39.47 (36.57-42.93)</td>
<td>35.45 (33.08-39.30)**</td>
<td>33.42 (30.53-36.01)**</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.001 43.70 (41.07-45.94)</td>
<td>39.89 (38.92-43.46)*</td>
<td>37.71 (34.68-41.80)**</td>
</tr>
<tr>
<td>ΣΩ16</td>
<td>&lt;0.001 27.56 (26.75-29.20)</td>
<td>29.62 (28.19-31.02)**</td>
<td>30.69 (29.34-31.18)**</td>
</tr>
<tr>
<td>ΣΩ18</td>
<td>&lt;0.05 28.46 (27.05-30.14)</td>
<td>29.44 (27.68-31.49)</td>
<td>31.68 (29.13-32.79)**</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.001 56.30 (54.07-58.93)</td>
<td>60.11 (56.54-61.08)*</td>
<td>62.29 (58.90-65.32)**</td>
</tr>
</tbody>
</table>

N.S.: not significant.

†: p values, using Kruskal-Wallis one-way ANOVA.

***: p<0.001, **: p<0.02, *: p<0.05, compared to values of normal subjects.

Complete data are shown in appendix III, Table 19.
Total plasma SFA and MFA contents were significantly higher in all NIDDM patients studied (LVD- vs N, p<0.05; LVD+ vs N, p<0.001). The main contribution to this elevation was made by 16:0 (LVD- vs N, P<0.02; LVD+ vs N, p<0.001; Fig 14). To a smaller extent, 18:1(n-9) was also a significant contributor to the increase in total SFA and MFA levels observed in NIDDM patients with clinical evidence of LVD (LVD+ vs N, p<0.02; Fig 15).

**Figure 14** Plasma 16:0 content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD (**: LVD- vs N, p<0.02; ***: LVD+ vs N, p<0.001). Median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
The plasma total PUFA content was decreased in all NIDDM patients studied (LVD- vs N, p<0.05; LVD+ vs N, p<0.001). This reduction was entirely due to the decreased in 18:2(n-6) (LVD- vs N, p<0.02; LVD+ vs N, p<0.001; Fig 16). From the fatty acid data on the NIDDM patients and normal controls, an inverse correlation was found between plasma total (n-6) fatty acids (N: $\tau=-0.8842$, $p<0.001$; LVD-: $\tau=-0.6632$, $p<0.001$; LVD+: $\tau=-0.9053$, $p<0.001$), particularly 18:2(n-6) (N: $\tau=-0.8421$, $p<0.001$; LVD-: $\tau=-0.6316$, $p<0.001$; LVD+: $\tau=-0.7579$, $p<0.001$) and plasma SFA plus MFA (Fig 17).
Figure 16 Plasma 18:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD (**: LVD- vs N, p<0.02; ***: LVD+ vs N, p<0.001).

median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Figure 17  Relationship between plasma 18:2(n-6) and SFA+MFA in normals(1) & NIDDM patients with(3) and without(2) LVD (1*:τ=-0.8421;2*:τ=-0.6314;3*:τ=-0.7579;*:p<0.001).
Despite similarities in the levels of total plasma (n-3) fatty acids in normal and NIDDM subjects, NIDDM patients had an increased proportion of plasma 20:5(n-3) (LVD- vs N, p<0.02; LVD+ vs N, p<0.001; Fig 18).

Although 20:2(n-6) can be synthesised from the 18:2(n-6), this particular metabolic pathway does not occur in the liver under normal circumstances. Thus as expected, a minimal amount of this fatty acid was found in the plasma of normal subjects. In contrast NIDDM patients had significant, though variable, increases in this dienoic acid (LVD- vs N, p<0.02; LVD+ vs N, p<0.05; Fig 19).
Plasma triacylglycerol was found to correlate positively with total plasma SFA + MFA content (N: $\tau=0.3895$, $p<0.02$; LVD-: $\tau=0.4500$, $p<0.02$), and plasma 18:1(n-9) content (N: $\tau=0.4316$, $p<0.02$; LVD-: $\tau=0.3600$, $p<0.05$; Fig 20), in both NIDDM patients without LVD and normal controls. In contrast, an inverse correlation was found between plasma triacylglycerol and plasma total PUFA content (N: $\tau=-0.3895$, $p<0.02$; LVD-: $\tau=-0.4500$, $p<0.02$), particularly the (n-6) fatty acids (N: $\tau=-0.3789$, $p<0.02$; LVD-: $\tau=-0.3852$, $p<0.02$; Fig 21), in the same subjects. Similar correlations were not seen in the LVD+ group of NIDDM patients.
Figure 20  Relationship between plasma triacylglycerol values & plasma 18:1(n-9) in normal subjects (1) & NIDDM patients without (2) LVD (1: $\tau=0.4316$, $p<0.02$; 2: $\tau=0.3600$, $p<0.02$).
Combining the data for the normal and diabetic groups, an consistent inverse correlation was found between plasma 18:1(n-9) and 18:2(n-6) (N: \( \tau = -0.5263, p < 0.02; \) LVD-: \( \tau = -0.5013, p < 0.02; \) LVD+: \( \tau = -0.5646, p < 0.001; \) Fig 22). In addition, plasma 16:0, the precursor of 18:1(n-9) was also found to correlate inversely with plasma 18:2(n-6) (N: \( \tau = -0.6000, p < 0.001; \) LVD-: \( \tau = -0.3895, p < 0.02; \) LVD+: \( \tau = -0.5579, p < 0.001; \) Fig 23).
Figure 22: Relationship between plasma 18:1(n-9) & 18:2(n-6). Normal subjects (1: $\tau=-0.5263, p<0.02$); NIDDM patients with (3: $\tau=-0.5646, p<0.001$) & without (2: $\tau=-0.5013, p<0.02$) LVD.
Figure 23  Relationship between plasma 16:0 and 18:2(n-6). Normal subjects (1:τ=−0.6000, p<0.001); NIDDM patients with (3:τ=0.5579, p<0.001) & without (2:τ=−0.3895, p<0.02).
3.1.4.3 Plasma phospholipid fatty acids

A typical chromatogram of plasma phospholipid fatty acids is shown below (Fig 24).

Figure 24  Gas chromatogram of plasma phospholipid fatty acids

1: BHT  
2: 16:0  
3: 16:1(n-7)  
4: 18:0  
5: 18:1(n-9),(n-7)  
6: 18:2(n-6)  
7: 18:3(n-6)  
8: 18:3(n-3)  
9: 20:2(n-6)  
10: 20:3(n-6)  
11: 20:4(n-6)  
12: 20:5(n-3)  
13: 22:4(n-6)  
14: 22:5(n-6)  
15: 22:5(n-3)  
16: 22:6(n-3)
The fatty acid compositions of plasma phospholipids obtained from normal subjects and NIDDM patients are shown in Table 5.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Fatty acid compositions of plasma phospholipids from normal subjects &amp; NIDDM patients.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (N) (n=20)</td>
</tr>
<tr>
<td>mmol%</td>
<td>KW(p)</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PUFA</td>
<td>N.S.</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

N.S.: not significant.

1: p values, using Kruskal-Wallis one-way ANOVA.

**:p<0.02, *:p<0.05, compared to values of normal subjects.

Complete plasma phospholipid fatty acid profiles of normal subjects and NIDDM patients are shown in appendix III, Table 20.
Contrary to the levels observed in whole plasma, plasma phospholipid contents of SFA and MFA were similar in normal and NIDDM subjects. PUFA contents were also similar. However, a significant decrease in 18:2(n-6) (LVD- vs N, \( p<0.02; \) LVD+ vs N, \( p<0.02; \) Fig 25) together with an increase in 20:5(n-3) (LVD-, LVD+ vs N, \( p<0.02; \) Fig 26) was observed in the plasma phospholipids of NIDDM patients.

**Figure 25** Plasma phospholipid (PL) 18:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD (**: LVD+ and LVD- vs N, \( p<0.02).\) median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Figure 26

Plasma phospholipid (PL) 20:5(n-3) content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD (**: LVD+ and LVD- vs N, p<0.02).

Figure 27

Plasma phospholipid (PL) 22:6(n-3) content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range (‘whiskers’ extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Significant increases in 20:2(n-6) levels were found in plasma phospholipids of all NIDDM patients (LVD- vs N, $p<0.02$; LVD+ vs N, $p<0.02$; Fig 28).

**Figure 28** Plasma phospholipid (PL) 20:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) and without (LVD-) LVD (**: LVD+ and LVD- vs N, $p<0.02$). median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
3.1.5 Discussion

3.1.5.1 Plasma lipids

The literature on epidemiological association between plasma triacylglycerol and CHD is not consistent (283). Many cross-sectional and prospective non-diabetic studies have demonstrated an univariate triacylglycerol-CHD association. In many but not all studies using multivariate statistical technique after controlling for plasma cholesterol or LDL-cholesterol, triacylglycerol remains a significant predictor of CHD. However, the least consistent results are obtained if HDL-cholesterol level is taken into account during multivariate analysis. Thus, the position of hypertriglyceridaemia as a risk factor is still uncertain.

In the WHO multinational study of vascular disease in diabetes, serum triacylglycerol concentrations appear to be more strongly related to the prevalence of CHD than serum cholesterol, particularly in obese, NIDDM patients (219). This observation may, however, simply be a marker of some other more direct determinant of CHD susceptibility, e.g. HDL-cholesterol.

A recent report on a long-term (11 years) prospective study of 943 Parisian men with impaired glucose tolerance or diabetes have shown that using multivariate regression analysis, plasma triacylglycerol is the only factor positively and significantly associated with coronary death in diabetes (221). This new epidemiological evidence increases the importance of triacylglycerol as an important predictor of CHD mortality and marker of CHD risk in diabetes.

The plasma triacylglycerol level, as well as that of cholesterol in NIDDM is influenced by the extent of glycaemia (221,222). In this study, all NIDDM patients had elevated levels of plasma triacylglycerol and HbA1%. Those patients with clinically confirmed LVD had the highest levels of these two substances, together with increased plasma cholesterol. Therefore, the combination of poor diabetic control and dyslipidaemia in these patients may be partly responsible for their vascular complications.

Both plasma and LDL cholesterol are major risk factors for CHD in the non-diabetic population (223). The importance of these factors with respect to the
risk of atherogenesis in diabetics is less certain. Numerous studies have shown that
the levels of plasma LDL cholesterol in diabetics with and without vascular
complications are similar (15,61,66,77) or lower (5) to those of normal controls.
The plasma LDL cholesterol levels reported in the latter study, however, was not
related to the frequency of MI. In contrast, diabetic patients with claudication have
been shown to have increased LDL cholesterol levels (5). There is also evidence
suggesting that the risk of coronary heart disease is increased in middle-aged
diabetic patients with plasma cholesterol levels greater than 7.3 mmol l\(^{-1}\) (224).
These contradictory findings have contributed to the uncertainty regarding
cholesterol as an atherogenic risk factor in diabetes.

The European Atherosclerosis Society has recommended that plasma
cholesterol should be maintained at a concentration less than 5.2 mmol l\(^{-1}\) (225).
According to this guideline, 90% of the NIDDM patients with clinical evidence of
LVD examined in the present study were hypercholesterolaemic. Interestingly, the
plasma cholesterol levels seen in 60% of NIDDM patients without LVD and 70% of
the normal subjects were also greater than 5.2 mmol l\(^{-1}\). The high incidence of
hypercholesterolaemia, as defined by the European Atherosclerosis Society, seen in
the latter two groups may lead to increased morbidity and mortality from LVD in
these populations. This study also showed that plasma cholesterol levels was mainly
determined by LDL cholesterol. Therefore, plasma total and LDL cholesterol can
still be accepted as reliable indicator of risk for LVD in NIDDM populations.

In fasting humans, VLDL is the major carrier of triacylglycerol (226). Thus,
elevations in triacylglycerol primarily reflect increases in VLDL triacylglycerol.
Under normal metabolic conditions, LDL are formed in the circulation from VLDL
by a precursor-product type reaction (226). In this study an association between
plasma triacylglycerol and LDL cholesterol was observed in normotriglyceridaemic
subjects. However, this relationship was absent in subjects with plasma
triacylglycerol greater than 1.5 mmol l\(^{-1}\). The excess triacylglycerol may be
accommodated in the large VLDL particles which are not converted to LDL but
are taken up by the liver via an apoE receptor system (78,72).

Although LDL cholesterol levels seen in NIDDM patients were similar to
those of normal controls, their rate of anabolism and catabolism may be impaired.
Previous study has shown that in NIDDM patients, the concomitant reductions in the conversion of VLDL to LDL and clearance of LDL from the circulation may explain the normal LDL levels seen in these patients (72). Other studies with familial hypercholesterolaemic patients has shown that a lipoprotein particle with the density of IDL or LDL is secreted into the circulation by the liver (227). This process may also occur in the present study of NIDDM patients with LVD, thus contributing to the increased plasma cholesterol levels seen in these patients.

The significant inverse correlation between plasma triacylglycerol and HDL\textsubscript{2} cholesterol reported previously was also noted in the normal subjects studied (228,229). Similar correlations have also been shown in diabetics (64,94,230), however, this was not the case in this study. The inverse relationship seen in normal subjects is indicative of lipid and protein exchange between lipoproteins during their metabolism. In NIDDM, this process may be disrupted due to reduced lipoprotein lipase and enhanced hepatic lipase activities. The combined impairment of the two lipases may increase plasma triacylglycerol levels and reduce the availability of HDL for lipid exchange reaction in NIDDM (231).

In this study, NIDDM patients had normal total HDL and HDL\textsubscript{2} cholesterol levels. However, HDL composition may differ between diabetics and normals subjects. Numerous studies have shown that HDL are composed of discrete populations of particles of varying size and composition (232,233) and the formations of these HDL particles are dependent on VLDL concentrations (234). Thus, differences in the distribution and the composition of HDL and their subfractions may impair VLDL and HDL metabolism, producing the dyslipidaemia seen in NIDDM.

### 3.1.5.2 Plasma fatty acids

It is thought that long chain fatty acids have both structural and functional roles when they are an integral part of membrane phospholipids. Long chain fatty acids are normally esterified in the sn\textsubscript{2} position of the phospholipids. During a prolonged period of deprivation of dietary essential fatty acid, 18:2(n-6), an increased synthesis of 20:3(n-9) from 18:1(n-9) will occur. Thus, the appearance of
20:3(n-9) and an increase in the 20:3(n-9)/20:4(n-6) ratio in plasma phospholipids have been suggested as being indicative of essential fatty acid deficiency (235,236). Therefore, measurements of plasma phospholipid fatty acids may provide useful information regarding abnormal lipid metabolism.

The present study provided information on both total plasma fatty acids and plasma phospholipid fatty acid levels in NIDDM patients and normal controls. The combined plasma levels of SFA and MFA in NIDDM patients were significantly increased, whereas, their plasma phospholipid levels were similar to those in normals. Hence, the observed difference rested with the apolar lipid fractions. This conclusion is endorsed by a recent study in which the proportions of SFA plus MFA were found to increase in serum cholesterol esters of diabetic subjects (238). Both total plasma 16:0 and 18:1(n-9) contents were increased in the NIDDM patients with LVD, these fatty acid abnormalities possibly being due to the combined effects of increased dietary intake and hepatic de novo synthesis.

Plasma 18:2(n-6) contents were significantly decreased in NIDDM patients. This reduction was compensated for by increases in both 16:0 and 18:1(n-9). In ex vivo animal studies, 18:2(n-6) has been shown to limit A9 desaturase activity with respect to the precursors of 18:1(n-9) (239). Thus, a reduction of 18:2(n-6) may increase the action of A9 desaturase on 18:0, leading to an increased synthesis of 18:1(n-9). The inverse correlation between plasma 18:2(n-6) and 18:1(n-9) observed with NIDDM patients supports this hypothesis. As the activity of β-HMG-CoA reductase, the rate-limiting enzyme for endogenous cholesterol synthesis, is dependent on the amount of 18:1(n-9) present, an excess of this fatty acid may bring about an increase in cholesterol synthesis (240). The combination of these events could lead to a subsequent increase in plasma cholesterol levels in NIDDM patients with clinical evidence of LVD.

One of the major consequences of incorporating monoenoic acids into lipids is to reduce their melting points, thus facilitating their transport from the liver to other tissues for metabolism. Thus, a surplus of hepatic 18:1(n-9) may encourage an accelerated release and transport of lipids in the NIDDM patients with LVD. The results from this study do not confirm that plasma 18:1(n-9) was incorporated into plasma triacylglycerol or cholesterol in these patients. However, Miettinen et
al has shown that 18:1(n-9) is elevated in plasma triacylglycerol of non-diabetic subjects who have suffered episodes of myocardial infarction (215). In contrast, Wang et al has shown that increases in 18:1(n-9) in non-diabetic hypercholesterolaemic subjects are confined to the plasma cholesterol esters (241).

The plasma total PUFA content in the diabetics studied was reduced. However, the individual (n-6) and (n-3) fatty acids were not necessary reduced, e.g. 18:2(n-6) was indeed diminished, whereas, 20:5(n-3) was increased. Mammalian tissues cannot synthesise 18:2(n-6), this essential fatty acid being normally derived from the diet. Thus, a reduced proportion of 18:2(n-6) and its metabolites in the plasma may reflect either reduced dietary intake of these acids or impaired uptake in the gut. Neither of these possibilities is applicable in the NIDDM patients studied as they had normal proportions of plasma 18:2(n-6) metabolites, despite a significant reduction of the substrate itself. Since the plasma contents of (n-6) fatty acid metabolites were similar in all the subjects studied, normal (n-6) fatty acid metabolism would appear to be preserved in NIDDM. In contrast, the plasma concentrations of 20:5(n-3), a metabolic product of 18:3(n-3), were significantly raised, this finding being consistent with the hypothesis that (n-3) fatty acid metabolism, with respect to A5 desaturation, may be accelerated in NIDDM. Such a change in desaturase activity may lead to increased 20:5(n-3) synthesis at the expense of 20:4(n-6). However, plasma 20:4(n-6) content in NIDDM patients was similar to that in normal controls, possibly indicating that alternative biochemical processes are altered in NIDDM thereby preserving the plasma levels of this (n-6) fatty acid. A possible mechanism may involve the preferential incorporation of 20:4(n-6) into lipid molecules, primarily at the expense of 18:2(n-6).

Plasma concentrations of 20:2(n-6) were significantly raised in NIDDM patients, although these concentrations were extremely low and variable. Thus, NIDDM patients, or at least some of them, would appear to have the ability to elongate 18:2(n-6) to 20:2(n-6) which may be subsequently converted to 20:3(n-6) by the A8 desaturase. The contribution of this pathway to the overall formation of 20:4(n-6) may be significant, particularly under conditions where the classical pathway is impaired.
3.1.5.3 Plasma lipids and fatty acids

Exogenous long chain fatty acids, in the form of lipid esters, enter the systemic circulation as chylomicrons. These large postprandial lipoproteins are catabolized to smaller remnant particles which are subsequently removed from the circulation by hepatic apoE receptors (38,39). The absorbed remnants are further catabolized to provide a source of exogenous fatty acids for hepatic synthesis of lipid esters. In addition, dietary carbohydrates also contribute to the hepatic fatty acid pool through their conversion via the process of lipogenesis. During fasting, the supply of hepatic fatty acids are maintained by the hydrolysis of lipids in the adipose tissue. Thus, the types of fatty acids and their availabilities influence the cellular synthesis of lipid esters and their release as VLDL to the circulation.

In this study, a positive correlation was found between plasma triacylglycerol concentration and the relative proportions of plasma total SFA and MFA in normal subjects and NIDDM patients without LVD. This finding is consistent with the known association between a high dietary intake of SFA and hypertriglyceridaemia (242,243). Although it has been shown that (n-3) fatty acids have a hypotriglyceridaemic effect as compared with (n-6) fatty acids (173,185,244), an inverse relationship between plasma triacylglycerol concentration and marine PUFA was not observed in this study. Instead, negative correlations were found between plasma triacylglycerol and total (n-6) fatty acids in both normal subjects and NIDDM patients without LVD. These findings can not be explained on the basis of dietary factors alone, but rather reflect a complex and indirect relationship between long chain fatty acids, particularly PUFA, and plasma triacylglycerol. Furthermore, such intricacies could be further complicated by the presence of vascular abnormalities in NIDDM.

The present study suggests that the dietary regime tested may have failed to control the plasma contents of long chain fatty acids and triacylglycerol in NIDDM. This failure may be due to a combination of defects in intestinal and hepatic uptake, cellular metabolism and the release of these lipids into the circulation. In addition, distinct differences in the distributions of these lipids between NIDDM patients with and without LVD were observed, suggesting that dietary regimes
should perhaps be tailored to individual patients or groups of patients rather than
prescribing a generalised diet. Such a strategy may, in the long run, be a more
effective means of reducing the incidence of LVD in NIDDM.

3.2 Platelet fatty acids in NIDDM patients

3.2.1 Aims

Blood platelets have been shown to play an important part in haemostasis. Some thrombotic tendencies and haemorrhagic syndromes may be linked to platelet hyper- and hypoactivity respectively (245). The mechanisms leading to changes in platelet activity are not fully understood. However, it is known that the liberation of long chain fatty acids from platelet membrane phospholipids with their subsequent oxygenation to pro- and anti-aggregatory prostanoids is involved in mediating platelet activity (109,110,246-248). The levels of some of these platelet fatty acids have been shown to alter in patients who have suffered an episode of myocardial infarction (249,250). Numerous studies involving the measurement of platelet fatty acid levels in diabetics without complications have been conducted (144-146,149, 150). However, similar studies in diabetics with vascular disease have not been carried out. In this study platelet fatty acid profiles were obtained in diabetics with and without LVD, and normal subjects.

3.2.2 Subjects

The normal and NIDDM individuals recruited for the previous study (section 3.1.2) also participated in this study.
3.2.3 Methods

The platelets were isolated from 40 ml of blood and the fatty acid composition determined as described in section 2.5. Results are expressed as median and range and were analysed statistically as described in section 2.7.

3.2.4 Results

A typical chromatogram obtained for platelet fatty acids is shown in Fig 29.

![Chromatogram of platelet fatty acids](image)

**Figure 29** Gas chromatogram of platelet fatty acids

1: BHT  
2: 16:0  
3: 16:1(n-7)  
4: 18:0  
5: 18:1(n-9),(n-7)  
6: 18:2(n-6)  
7: 18:3(n-6)  
8: 18:3(n-3)  
9: 20:2(n-6)  
10: 20:3(n-6)  
11: 20:4(n-6)  
12: 20:5(n-3)  
13: 22:4(n-6)  
14: 22:5(n-3)  
15: 22:5(n-6)  
16: 22:6(n-3)
Table 6  Platelet fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th>mmol%</th>
<th>Normal (N=20)</th>
<th>NIDDM (LVD-) (n=20)</th>
<th>NIDDM (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mW(p)</td>
<td>median</td>
<td>(95% C.I.)</td>
</tr>
<tr>
<td>16:0</td>
<td>&lt;0.05</td>
<td>20.53</td>
<td>(19.07-21.75)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>&lt;0.05</td>
<td>1.46</td>
<td>(1.37-1.58)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.02</td>
<td>8.58</td>
<td>(8.32-9.05)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.02</td>
<td>0.40</td>
<td>(0.38-0.56)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>&lt;0.05</td>
<td>22.33</td>
<td>(21.37-23.35)</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>&lt;0.05</td>
<td>36.04</td>
<td>(34.84-36.81)</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.001</td>
<td>39.99</td>
<td>(38.95-41.01)</td>
</tr>
<tr>
<td>ΣC16</td>
<td>&lt;0.05</td>
<td>22.07</td>
<td>(21.36-23.08)</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.001</td>
<td>60.01</td>
<td>(58.99-61.05)</td>
</tr>
</tbody>
</table>

N.S.: not significant.

*: p values, using Kruskal-Wallis one-way ANOVA.

***: p<0.001, **: p<0.02, *: p<0.05, compared to values of normal subjects.

Complete platelet fatty acid profile of normal subjects and NIDDM patients are shown in appendix III, Table 21.
Platelet total SFA and MFA content was significantly raised in all NIDDM patients (LVD- vs N, \( p<0.001 \); LVD+ vs N, \( p<0.02 \)). The increases were confined mainly to those fatty acids made up of sixteen carbon atoms, 16:1(n-7) being elevated in the platelets of the LVD- group (LVD- vs N, \( p<0.02 \) Fig 30) and 16:0 in the LVD+ group (LVD+ vs N, \( p<0.02 \); Fig 31).

Figure 30 Platelet (PLT) 16:1(n-7) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD-vs N, \( p<0.02 \)). median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
There was a general decrease in platelet PUFA content in the diabetics (LVD- vs N, p<0.001; LVD+ vs N, p<0.02), mainly of the (n-6) fatty acid family (LVD- vs N, p<0.02; LVD+ vs N, p<0.001). The platelet levels of 20:4(n-6) were significantly lowered in both NIDDM groups (LVD- vs N, p<0.05; LVD+ vs N, p<0.05; Fig 32). In addition, the platelet content of 18:2(n-6) content in the LVD- group was significantly reduced while that of the LVD+ group just failed to reach statistical significance (LVD- vs N, p<0.02; LVD+ vs N, p=0.051; Fig 33).
Figure 32
Platelet (PLT) 20:4(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (*: LVD-and LVD+ vs N, p<0.05).

Figure 33
Platelet (PLT) 18:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD- vs N, p<0.02).
Platelet 20:2(n-6) contents in NIDDM patients were significantly raised although there was considerable inter-individual variation (LVD- vs N, p<0.02; LVD+ vs N, p<0.05; Fig 34).

![Figure 34](image)

**Figure 34**  Platelet (PLT) 20:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD- vs N, p<0.02; *: LVD+ vs N, p<0.05). Median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).

In all the subjects studied, platelet total (n-3) fatty acids were found to correlate positively with that in plasma (N: τ=0.3958, p<0.02; LVD-: τ=0.6211, p<0.001; LVD+: τ=0.5397, p<0.001). Individual (n-3) fatty acids were also found to correlate in the two fractions: 22:5(n-3) (N: τ=0.3957, p<0.02; LVD-: τ=0.3549, p<0.02; LVD+: τ=0.3191, p<0.05; Fig 35) and 22:6(n-3) (N: τ=0.3936, p<0.02;
LVD-: $\tau=0.6138$, $p<0.001$; LVD+: $\tau=0.4063$, $p<0.02$; Fig 36). Platelet and plasma 20:5(n-3) correlated positively in both NIDDM groups (LVD-: $\tau=0.7018$, $p<0.001$; LVD+: $\tau=0.3747$, $p<0.02$; Fig 37).

**Figure 35**  Relationship between platelet (PLT) & plasma 22:5(n-3) in normal subjects (1) & NIDDM patients with (3) & without (2) LVD (1: $p<0.02$; 2: $p<0.02$; 3: $p<0.05$).
Figure 36  Relationship between platelet (PLT) & plasma 22:6(n-3) in normal subjects (1) & NIDDM patients with (3) & without (2) LVD (1: \( p<0.02 \); 2: \( p<0.001 \); 3: \( p<0.02 \)).
Figure 37  Relationship between platelet (PLT) & plasma 20:5(n-3) in NIDDM patients with (3) & without (2) LVD (3: $\tau=0.3747$, $p<0.02$; 2: $\tau=0.7018$, $p<0.001$).
A correlation between platelet and plasma total SFA plus MFA was not observed. However, platelet and plasma 18:1(n-9) showed a significant positive correlation. (N: τ=0.5895, \( p<0.001 \); LVD-: τ=0.4802, \( p<0.02 \); LVD+: τ=0.5119, \( p<0.02 \); Fig 38)

Figure 38  Relationship between platelet (PLT) & plasma 18:1(n-9) in normal subjects (1) & NIDDM patients with (3) & without (2) LVD (1: \( p<0.001 \); 2: \( \tau= p<0.02 \); 3: \( p<0.02 \)).
A significant inverse correlation was found between platelet total (n-6) fatty acids and total SFA in NIDDM patients (LVD-: τ=-0.5895, p<0.001; LVD+: τ=-0.4737, p<0.02). The (n-6) fatty acid found to be the major contributor to this correlation was 20:4(n-6) (LVD-: τ=-0.5858, p<0.001; LVD+: τ=-0.4908, p<0.02; Fig 39).

Figure 39  Relationship between platelet (PLT) total SFA and 20:4(n-6) in NIDDM patients with (3) and without (2) LVD (1: p<0.001; 2: p<0.02).
3.2.5 Discussion

It has recently become apparent that changes in platelet fatty acid composition may be related to platelet sensitivity and vascular disease (143). Hence, this study was designed to examine the combined effects of NIDDM and LVD on platelet fatty acid composition. Significant changes were noted in the platelet fatty acid composition in both groups of NIDDM patients; total SFA concentration was higher and PUFA, 18:2(n-6) and 20:4(n-6), levels were lower than normals. These findings are similar to those of Jones et al (252), although in other studies the levels of these (n-6) fatty acids in diabetics have been found to be similar (253) or even higher than those of normal subjects (144,146,251). These inconsistencies may be due to the differences regarding patient selection. Indeed in some of the studies, IDDM and NIDDM patients have been examined as a single group (251-253). Nevertheless, the present findings support the hypothesis that changes in platelet fatty acid composition are independent of LVD and occur before the onset of macroangiopathy in diabetes (251). Therefore the underlying mechanisms responsible for these changes are likely to be similar in the two group of patients.

Although the bulk of platelet fatty acids have their origins in the megakaryocytes, the fatty acid profile of the mature platelet does not necessarily resemble that of its precursor (254). Thus, other biochemical reactions must occur in platelets in order to modify fatty acid composition during and after their maturation. Platelets have been shown to possess all the enzymes required for the de novo fatty acid biosynthesis (255). Washed human platelets are able to incorporate PUFA and MFA from the plasma. These are then elongated prior to acylation with phospholipids (256). Thus, it is not unreasonable to expect that the levels of fatty acids in platelets are related to their concentrations in plasma. The positive correlations between 18:1(n-9), 20:5(n-3), 22:5(n-3) and 22:6(n-3) levels in platelets and their concentrations in plasma demonstrated in this study supports this hypothesis.

In diabetes, the de novo biosynthesis of fatty acids is impaired although the elongation mechanism appears to be unaltered (257). It has been reported that mature platelets from non-diabetic subjects exhibit minimal activities of the Δ6 desaturase (154). In the same study it was also concluded that changes in the
platelet 20:4(n-6) levels did not arise from reduced desaturation and elongation of (n-6) fatty acid substrates, but from reduced uptakes of extracellular 20:4(n-6). The present study has shown that there were no significant differences between NIDDM patients and normal subjects in the plasma concentrations of 20:4(n-6), whereas, platelet 20:4(n-6) levels were reduced in NIDDM patients. Therefore, it is likely that the uptake of plasma 20:4(n-6) into platelets is reduced in NIDDM, this impairment possibly occurring as described below.

Free 20:4(n-6) associated with plasma HDL (258) may be reduced in NIDDM as a consequence of impaired hydrolysis of its ester by phospholipase A_2. Such a change in 20:4(n-6) content may reduce its inhibitory effect on the platelet uptake of other fatty acids (259) and lead to the increased platelet SFA levels seen in this study. The preferential incorporation of 20:4(n-6) in the presence of other fatty acids (153,260-262) is due to the existence of a second acyl CoA synthase which is specific for this (n-6) fatty acid (148,263). This enzyme may be defective in NIDDM and changes in its activity may explain the lower platelet 20:4(n-6) levels seen in this study. An alternative source of 20:4(n-6) for uptake by platelets is from plasma phosphatidylcholine (258,264). When this phospholipid is taken up by platelets it undergoes transacylation with other platelet phospholipids to rid itself of 20:4(n-6) in order to favour the uptake of more plasma phosphatidylcholine-bound 20:4(n-6) (258). Therefore, impairment of these biochemical processes in NIDDM may limit the uptake of 20:4(n-6) containing phosphatidylcholine, contributing to reduced platelet 20:4(n-6) levels reported in this study.

In this study, increased platelet SFA levels may have occurred at the expense of 20:4(n-6) in NIDDM patients, as shown by the inverse correlation between these fatty acids. Abnormally high levels of plasma and platelet SFA seen in these patients may have undesirable effects on platelet aggregation (265). In addition, the PUFA released as a result of hydrolytic cleavage of platelet phospholipids by phospholipase A_2 during aggregation are subjected to oxygenation by both platelet cyclooxygenase and lipoxygenase (108,110,266). These oxygenated metabolites are in turn involved in the physiology and pathophysiology of platelets. In diabetes, an imbalance of these compounds may produce a situation where platelets aggregate more readily, leading to thrombus formation and atherogenesis.

129
3.3 Red blood cell fatty acid profiles in NIDDM patients

3.3.1 Aims

The measurement of plasma fatty acids provides a convenient method for the assessment of fatty acid metabolism but is subjected to variations as a consequence of changes in diet and in lipoprotein turnover. Although platelet fatty acid levels are less influenced by diet, their measurement is not particularly useful as an indicator of fatty acid status because of the uniqueness of platelet 20:4(n-6) metabolism. Red blood cell (RBC) fatty acids are also not influenced to a great extent by dietary habits (267,268). These cells also lack the enzymes required for the elongation or desaturation of fatty acids occurring in their cellular membrane (269). Thus, measurement of red blood cell fatty acids provides an alternative and useful means of assessing fatty acid status. Altered red blood cell membrane fluidity, deformability, microviscosity and sodium transport has been reported in NIDDM (157,270-272), however, studies of fatty acid compositions in these patients, particularly those with vascular complications have been limited. In this study red blood cell fatty acid composition was examined in NIDDM patients with and without LVD and compared to that in normals.

3.3.2 Subjects

The same normal and NIDDM individuals recruited for the studies in section 3.2 were used in this study.

3.3.3 Methods

Blood (20 ml) was collected after an overnight fast into EDTA as described in section 3.2. Red blood cells were isolated and their fatty acid compositions determined as described in section 2.6. Results are expressed as median and range and were statistically analysed as described in 2.7.
3.3.4 Results

A typical fatty acid chromatogram obtained with a red blood cell extract is shown in Fig 40.

![Gas chromatogram of red blood cell fatty acids](image)

**Figure 40**  Gas chromatogram of red blood cell fatty acids

1: BHT  
2: 16:0  
3: 16:1(n-7)  
4: 18:0  
5: 18:1(n-9),(n-7)  
6: 18:2(n-6)  
7: 18:3(n-6)  
8: 18:3(n-3)  
9: 20:2(n-6)  
10: 20:3(n-6)  
11: 20:4(n-6)  
12: 20:5(n-3)  
13: 22:4(n-6)  
14: 22:5(n-6)  
15: 22:5(n-3)  
16: 22:6(n-3)
Red blood cell fatty acid compositions for normal subjects and NIDDM patients are shown in Table 7.

### Table 7  Red blood cell fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th>mmol%</th>
<th>Normal (N) (n=20)</th>
<th>NIDDM (LVD-) (n=20)</th>
<th>NIDDM (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.05 13.77 (12.84-14.85)</td>
<td>12.83 (10.95-13.74)</td>
<td>12.25 (10.60-13.40)**</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>&lt;0.05 14.02 (12.32-14.26)</td>
<td>12.45 (10.37-13.63)**</td>
<td>12.30 (11.20-12.97)**</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.001 0.88 (0.69-1.00)</td>
<td>1.69 (1.53-2.01)***,++</td>
<td>1.17 (1.02-1.43)**</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>&lt;0.05 31.96 (30.16-32.82)</td>
<td>29.71 (27.84-32.44)</td>
<td>29.64 (26.27-31.17)**</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.05 39.73 (39.23-40.83)</td>
<td>39.19 (38.09-40.88)</td>
<td>37.56 (35.43-40.36)**</td>
</tr>
<tr>
<td>ΣC16</td>
<td>&lt;0.05 26.60 (25.68-27.24)</td>
<td>27.55 (26.19-29.11)</td>
<td>27.88 (27.21-29.14)**</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.05 60.27 (59.17-60.77)</td>
<td>60.81 (59.12-61.97)</td>
<td>62.44 (59.64-64.57)**</td>
</tr>
</tbody>
</table>

N.S.: not significant; †: p values, using Kruskal-Wallis one-way ANOVA.

***: p<0.001, **: p<0.02, *: p<0.05, compared to values of normal subjects.

++: p<0.02, NIDDM without LVD compared to NIDDM with LVD.

Complete red blood cell fatty acid profiles of normal subjects and NIDDM patients are shown in appendix II, Table 22.
The red blood cell SFA plus MFA levels in LVD+ group of NIDDM patients were significantly higher than normal subjects. These increases were confined to those fatty acids with sixteen carbon atoms (LVD+ vs N, p<0.02; Fig 41). In contrast, the concentrations of SFA and MFA were unaltered in the LVD- group of NIDDM patients.

**Figure 41** Total red blood cell (RBC) C16 fatty acid contents in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD+ vs N, p<0.02). Median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).

Red blood cell PUFA levels were reduced in NIDDM patients with LVD (LVD+ vs N, p<0.02), reductions being particularly marked for the (n-6) fatty acids, 18:2(n-6) (LVD+ vs N, p<0.02; Fig 42) and 20:4(n-6) (LVD+ vs N, p<0.05; Fig 43). In contrast, the proportion of 20:5(n-3) was significantly increased in this group of patients (LVD+ vs N, p<0.02; Fig 44). This increase was, however, insufficient to normalize the red blood cell PUFA content.
Although similar changes in 20:4(n-6) (LVD- vs N, p<0.05; Fig 43) and 20:5(n-3) (LVD- vs N, p<0.001; Fig 44) were observed in the red blood cells of NIDDM patients without LVD, the 18:2(n-6) content was similar to that in normals. These patients also had the highest RBC content of 20:5(n-3) observed in the three groups (LVD- vs LVD+, p<0.02; Fig 44).

**Figure 42** Red blood cell (RBC) 18:2(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD+ vs N, p<0.02). median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range ("whiskers" extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
Figure 43
Red blood cell (RBC) 20:4(n-6) content in normal subjects (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (*: LVD+ and LVD- vs N, p<0.05).

Figure 44
Red blood cell (RBC) 20:5(n-3) content in normals (N) & NIDDM patients with (LVD+) & without (LVD-) LVD (**: LVD+ vs N, p<0.02; ***: LVD- vs N, p<0.001; ++: LVD- vs LVD+, p<0.02).

* median (line within the central box), interquartile range (central box), minimum and maximum values within 1.5 x the interquartile range (*whiskers* extending out to the extremes) and values occurring far away from the bulk of the data (separate points).
3.3.5 Discussion

Conflicting data have been reported regarding red blood cell fatty acid compositions in diabetics. These differing results may be due to a variety of factors, including the type of patients studied; in some studies IDDM patients were studied (273,274), whereas in others NIDDM patients (142,158,237, 272). Other factors which may also influence the findings include gender, lipid type analysed, diet, analytical methods employed and the numbers of patients studied. With respect to the latter, a study involving a small number of patients has shown that phospholipid fatty acids in red blood cell membranes were significantly different from those in normals (272), whereas, in another study involving a larger group of patients no differences were observed (158). In contrast, higher red blood cell contents of 16:0 (142,157), 18:2(n-6), 20:4(n-6) and 22:6(n-3) (237) in NIDDM patients have been reported.

In the present study, the red blood cell levels of 20:4(n-6) and 20:5(n-3) in both groups of NIDDM patients were reduced and increased respectively. The reduction in 20:4(n-6) may due to the re-direction of the action of Δ5 desaturase away from its precursor, 20:3(n-6), towards 18:4(n-3) with the consequent increased production of 20:5(n-3).

It is proposed that 20:5(n-3) is anti-atherogenic (246,266), however, in this study there was no clear relationship between red blood cell 20:5(n-3) and LVD; the levels of 20:5(n-3) was increased to the same extent in both groups of patient. Therefore, it is probable that in order to circumvent premature atherosclerosis in NIDDM even higher levels of 20:5(n-3) will need to be attained in these patients.

Raised red blood cell contents of 16:0 and reduced contents of 18:2(n-6) were also seen in NIDDM patients with LVD. The concentrations of these fatty acids have been shown to be dependent on their dietary levels (182,267). Thus, increased dietary intake of 16:0 at the expance of 18:2(n-6) may have occurred due to the competition between the two fatty acids for absorption in the gut. Although the reduction of red blood cell levels of 18:2(n-6) may be as a result of reduced gastric absorption, the possibility that decreased utilization of 18:2(n-6) for red blood cell production cannot be overlooked.
Some investigators speculate that a rise in red blood cell fatty acid concentrations is due to an increase in their uptake from plasma (269). In this study, comparable alterations in the red blood cell and plasma 20:4(n-6) were not seen in either group of NIDDM patients. Therefore, a reduction in the red blood cell but not plasma 20:4(n-6) may indicate a decreased uptake of this (n-6) fatty acid from plasma. In contrast, both the plasma and red blood cell contents of 20:5(n-3) were increased, thus, the contribution made by plasma 20:5(n-3) to red blood cell concentrations as a result of uptake may probably be minimal.
4 FISH OIL AND OLIVE OIL STUDIES

4.1 Aims

The results obtained in the cross-sectional studies indicated that lipid and fatty acid abnormalities occur in NIDDM patients with and without LVD. One of the striking observations was that NIDDM patients were hypertriglyceridaemic despite high plasma and red blood cell levels of (n-3) fatty acids. Therefore, (n-3) fatty acid levels seen in these patients may have to increase further in order to bring about reductions in triacylglycerol levels. According to the guidelines set out by the European Atherosclerosis Society, 60% of the NIDDM patients without LVD examined in the present study were considered to be hypercholesterolaemic, and a reduction of their cholesterol levels would therefore be desirable. Thus, a controlled study was designed to investigate the usefulness of fish oil as a lipid modifying agent when given as a supplement to the patients' home diet and the effects produced on platelet and red blood cell fatty acid contents as a consequence of olive oil supplement were also evaluated.

4.2 Subjects

Twenty NIDDM patients without LVD (diagnostic criteria as defined in section 3.1.2) were entered into this study having established by laboratory screening that plasma triacylglycerol >1.5 mmol l\(^{-1}\), HbA1% <13% and BMI <30%. Informed consents were obtained from each patients. Ethical approval was obtained from the College Ethical Committee. The study was carried out according to the principles of the Declaration of Helsinki.

The patients were randomized on a double-blind basis to receive 8 weeks of either fish oil (MaxEPA, Seven Seas Health Care Limited, UK) or olive oil (control) (MaxEPA, Seven Seas Health Care Limited, UK) supplement in the form of 9 capsules per day (9.9 g of oil per day). Ten NIDDM patients (10 males, mean age 62 years) received the fish oil while the remaining patients (7 males, 3 females, mean age 55 years) received olive oil. The oil supplements were taken at
meal times. Each patient had been seen by a dietician and was given a diet (following the guideline of the new British Diabetic Association nutrition committee report) consisting 30% of calories as fat and 50% as carbohydrate. The diet was composed of high fibre and low fat foods i.e. wholemeal bread or pasta, brown rice, pulses, fresh vegetables and fruits, wholegrain cereals, low fat spread, skimmed or semi-skimmed milk and lean meats; sugar was to be avoided.

4.3 Methodology

The fatty acid composition of the fish oil and the olive oil were determined by gas-liquid chromatography. Blood samples were obtained as described in section 3.1.3. Plasma, platelets and red blood cells were separated as described in sections 2.5 and 2.6. Plasma cholesterol and triacylglycerol levels were determined as described in section 2.2. VLDL, LDL, HDL₂ and HDL₃ were separated by ultracentrifugation (section 2.4) and the cholesterol levels determined. HbA1% was measured by agarose gel electrophoresis (section 2.1). Total plasma phospholipids, and platelet and red blood cell fatty acids were measured (sections 2.5 and 2.6).

Results are expressed as mean and 95% confidence interval. Paired Student’s t-test was used to analyse the changes in each variable in the two treatment groups (fish oil and olive oil) between baseline (0 week) and 4/8 weeks. Unpaired Student’s t test was used to compare between-group differences in each variable at baseline (0 week) and also to compare changes in each variable in the fish oil group between baseline (0 week) and 4 weeks and baseline (0 week) and 8 weeks with those in the olive oil (control) group. The minimum number of patients required to detect a 20% change of plasma triglyceride between the groups at the 5% level with a 80% power of detecting such a difference was estimated to be 10.
4.4 Results

4.4.1 Fatty acid compositions of fish oil and olive oil

The fatty acids contained in the fish oil and olive oil were extracted and transesterified to their methyl esters and determined by gas liquid chromatography (see section 2.5). The results obtained are shown in Table 8.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Fish oil (mole%)</th>
<th>Olive oil (mole%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>22.43</td>
<td>13.12</td>
</tr>
<tr>
<td>18:0</td>
<td>5.69</td>
<td>2.27</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>11.52</td>
<td>1.10</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>15.24</td>
<td>71.45</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>2.16</td>
<td>10.99</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.56</td>
<td>0.70</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>19.46</td>
<td>0.00</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>3.27</td>
<td>0.00</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>17.31</td>
<td>0.00</td>
</tr>
</tbody>
</table>

4.4.2 Effects of fish oil and olive oil on NIDDM

4.4.2.1 Clinical characteristics of subjects

The fish oil and the olive oil (control) group of patients were matched for age, smoking habits, duration of diabetes and mode of treatment (Table 9).
Table 9  Clinical characteristics of NIDDM patients.

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>male/female</td>
<td>10/10</td>
<td>7/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 (55,69)(^3)</td>
<td>55 (49,60)(^3)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>76 (72,80)(^3)</td>
<td>82 (76,88)(^3)</td>
</tr>
<tr>
<td>Treatment:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Medical history:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAD(^1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PVD(^2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>1 to 12 years</td>
<td>1 to 15 years</td>
</tr>
<tr>
<td>Current smokers</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Ex smokers</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ex alcohol users</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^1\): coronary artery disease; \(^2\): peripheral vascular disease
\(^3\): mean value (95% confidence interval)
4.4.2.2 HbA1%, plasma lipid and lipoprotein composition

NIDDM patients found the fish oil and olive oil capsules acceptable and palatable and had completed the study without complaining of flatulence or abdominal pain. Fasting HbA1%, lipid, plasma phospholipid, platelet and red blood cell fatty acid contents did not differ significantly between the two groups at baseline (Appendix III, Tables 23 - 30). The major effect of 8 weeks of fish oil supplement on plasma lipids was on the cholesterol content of HDL\(_2\), which was increased over the baseline diet. When the change in the HDL\(_2\) cholesterol content in the fish oil group was compared with the change in the olive oil (control) group, the difference attributable to fish oil was not significant (Table 10).

There was a weight gain after 4 weeks on fish oil, whereas, plasma total and VLDL triacylglycerol levels were lowered (Table 11). These changes, however, were only transient and became insignificant at the end of the study (Appendix III, Table 23). An 8% reduction of plasma cholesterol was observed in the olive oil (control) group at week 4 of the study, thus reducing the usefulness of olive oil as a control oil. Half the NIDDM patients in the fish oil and the olive oil (control) group showed a small but insignificant rise in HbA1% level at the end of the study.

Table 10 Changes in HDL\(_2\) cholesterol with fish oil and olive oil treatment (8 weeks)

<table>
<thead>
<tr>
<th>HDL(_2) cholesterol (mM/l)</th>
<th>Fish oil</th>
<th>Olive oil (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 week</td>
<td>0.34 (0.18,0.50)</td>
<td>0.42 (0.24,0.60)</td>
</tr>
<tr>
<td>8 week</td>
<td>0.47 (0.32,0.62)</td>
<td>0.47 (0.29,0.66)</td>
</tr>
<tr>
<td>p value</td>
<td>0.002*</td>
<td>0.77</td>
</tr>
</tbody>
</table>

| Change attributable to treatment | 0.08 (-0.08,0.24) |
| p value                         | 0.33          |

Values are mean (95% confidence interval)
*: statistically significant (paired t test - changes between week 8 and baseline)
Table 11  Changes in body weight and lipids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th></th>
<th>Olive oil (control)</th>
<th></th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>p</td>
<td>week 0</td>
<td>week 4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 ( 71.8, 79.7)</td>
<td>76.9 ( 72.8, 81.0)</td>
<td>0.02*</td>
<td>82.1 ( 76.3, 87.9)</td>
<td>83.0 ( 77.7, 88.4)</td>
</tr>
<tr>
<td>T Chol¹</td>
<td>6.10 ( 5.38, 6.82)</td>
<td>6.22 ( 5.50, 6.95)</td>
<td>0.48</td>
<td>5.93 ( 5.20, 6.66)</td>
<td>5.46 ( 5.03, 5.90)</td>
</tr>
<tr>
<td>TG²</td>
<td>2.33 ( 1.74, 2.92)</td>
<td>2.02 ( 1.43, 2.60)</td>
<td>0.03*</td>
<td>2.19 ( 1.66, 2.72)</td>
<td>2.17 ( 1.69, 2.65)</td>
</tr>
<tr>
<td>VLDL-T³</td>
<td>1.27 ( 0.88, 1.66)</td>
<td>1.01 ( 0.64, 1.38)</td>
<td>0.02*</td>
<td>1.06 ( 0.55, 1.56)</td>
<td>1.16 ( 0.66, 1.67)</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval)

¹: total plasma cholesterol (mM/l);
²: plasma triacylglycerol (mM/l);
³: VLDL triacylglycerol (mM/l)

*: statistically significant (paired t test - changes between week 4 and baseline)

*: statistically significant (unpaired t test - changes between treatment groups)
4.4.2.3 Plasma phospholipid fatty acid composition

Fish oil treatment was associated with significant changes in plasma (n-3) phospholipid fatty acid values. The degree of enrichment of (n-3) fatty acids after 8 and 4 weeks of fish oil supplement when compared to baseline were shown in Tables 12 and 13 respectively.

The major changes were substantial increases in 20:5(n-3), 22:5(n-3) and 22:6(n-3) levels at the end of 8 weeks on fish oil (Table 12). The increase in 20:5(n-3) in 7 of the 10 patients varied between 260% and 459%. The remaining 3 patients showed smaller increases in 20:5(n-3), namely 8%, 64% and 69%. The effect of 8 weeks on fish oil on 22:6(n-3) content, however, was less dramatic, increases in 7 of the patients varying between 56% and 110%. As before, 3 of the patients showed smaller increases in this fatty acid, namely -15%, 1% and 34%. Interestingly, these were the same patients that gave small increases in 20:5(n-3).

Fish oil appeared to reduce the amounts of (n-6) fatty acids associated with plasma phospholipids (Tables 12 and 13). These reductions were significant as early as week 4 on the supplement and affected the 18:2(n-6), 20:4(n-6) and 22:4(n-6) species (Table 13). Although 20:3(n-6) levels were also influenced by fish oil, statistically significant effects were only observed after 8 weeks of supplementation (Table 12).

When changes in these (n-3) and (n-6) fatty acids in the fish oil group were compared with those in the olive oil (control) group, the difference attributable to fish oil treatment reached similar significance (Tables 12 and 13). In addition, reductions in 16:0 and 18:1(n-9) levels were also associated with 4 weeks of fish oil treatment (Table 13).
### Table 12 | Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 8</td>
<td>p</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>21.68 (18.66,24.70)</td>
<td>17.22 (14.75,19.70)</td>
<td>0.001*</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>2.59 (1.94,3.24)</td>
<td>1.74 (1.28,2.20)</td>
<td>0.003*</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>8.83 (7.39,10.28)</td>
<td>7.92 (7.11,8.73)</td>
<td>0.049*</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>0.23 (0.17,0.29)</td>
<td>0.11 (0.07,0.15)</td>
<td>0.008*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>2.17 (0.99,3.36)</td>
<td>6.19 (4.64,7.74)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.87 (0.75,0.99)</td>
<td>1.39 (1.12,1.66)</td>
<td>0.007*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.19 (3.41,4.97)</td>
<td>6.25 (5.20,7.30)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>34.53 (32.32,36.74)</td>
<td>27.94 (25.34,30.53)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>7.60 (5.78,9.42)</td>
<td>14.16 (11.62,16.69)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 8 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
Table 13  Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>week 0</td>
</tr>
<tr>
<td>16:0</td>
<td>30.56 (29.29,31.82)</td>
<td>32.00 ( 30.88, 33.12)</td>
<td>0.06</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>11.08 ( 9.24,12.93)</td>
<td>10.11 ( 8.97, 11.25)</td>
<td>0.06</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>21.68 (18.66,24.70)</td>
<td>17.17 ( 14.47, 19.86)</td>
<td>0.004*</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>8.83 ( 7.39,10.28)</td>
<td>8.09 ( 7.03, 9.16)</td>
<td>0.047*</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>0.23 ( 0.17, 0.29)</td>
<td>0.10 ( 0.06, 0.14)</td>
<td>0.002*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>2.17 ( 0.99, 3.36)</td>
<td>5.83 ( 4.26, 7.40)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.87 ( 0.75, 0.99)</td>
<td>1.51 ( 1.23, 1.79)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.19 ( 3.41, 4.97)</td>
<td>5.87 ( 4.96, 6.79)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>34.53 (32.32,36.74)</td>
<td>28.62 ( 25.84, 31.40)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>7.60 ( 5.78, 9.42)</td>
<td>13.56 (11.23, 15.89)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 4 and baseline) 
*: statistically significant (unpaired t test - changes between treatment groups)
4.4.2.4 Platelet fatty acid composition

There was a significant increase in total platelet (n-3) fatty acids in NIDDM patients receiving 8 weeks of fish oil supplement (Table 14). The fatty acid species affected were 20:5(n-3), 22:5(n-3) and 22:6(n-3). Changes in these individual fatty acids were detected as early as 4 week on the fish oil supplement (Table 15). The changes in the (n-3) fatty acids seen at the end of the study varied tremendously: 20:5(n-3), -27% to +577%; 22:5(n-3), -25% to +205% and 22:6(n-3), -29% to +345%. Surprisingly, reductions in these individual platelet (n-3) fatty acids were observed in one patient whose plasma phospholipid contents of these (n-3) fatty acids were also reduced, the one exception being 20:5(n-3) which was increased by 8%.

Platelet 22:4(n-6) content was significantly reduced at 4 and 8 weeks on fish oil when compared to baseline (Tables 15 and 14), whereas, 22:5(n-6) remained unchanged. These findings contrast with those obtained with plasma. In addition, a transient reduction of 20:2(n-6) was also observed at week 4. When changes in the (n-3) fatty acids and 22:4(n-6) in the fish oil group were compared to those in the olive oil (control) group, differences attributable to fish oil reached similar significance.

There was a small but significant reduction of 16:0 content in NIDDM patients after completing the olive oil study (Table 14).
Table 14  Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 8</td>
<td>p</td>
</tr>
<tr>
<td>16:0</td>
<td>22.25 (21.71,24.18)</td>
<td>21.71 (19.24, 24.18)</td>
<td>0.71</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>1.38 (1.08, 1.68)</td>
<td>0.90 (0.66, 1.15)</td>
<td>0.008*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.01 (0.54, 1.49)</td>
<td>3.07 (2.13, 4.02)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.29 (1.13, 1.44)</td>
<td>2.14 (1.69, 2.58)</td>
<td>0.002*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>1.65 (1.37, 1.93)</td>
<td>2.60 (1.87, 3.33)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>4.19 (3.42, 4.96)</td>
<td>8.01 (6.06, 9.96)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 8 and baseline)

#: statistically significant (unpaired t test - changes between treatment groups)
Table 15  Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>p</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.99 (0.54, 1.43)</td>
<td>0.68 (0.34, 1.01)</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>1.38 (1.08, 1.68)</td>
<td>0.84 (0.70, 0.98)</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.01 (0.54, 1.49)</td>
<td>2.81 (1.95, 3.68)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.29 (1.13, 1.44)</td>
<td>2.09 (1.74, 2.44)</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>1.65 (1.37, 1.93)</td>
<td>2.31 (2.07, 2.55)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>4.19 (3.42, 4.96)</td>
<td>7.42 (6.04, 8.81)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 4 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
4.4.2.5 Red blood cell fatty acid composition

The effects of fish oil on red blood cell fatty acid compositions were found to be similar to those seen in plasma phospholipids and platelets. The major impact was associated with (n-3) fatty acids, significant increases in 20:5(n-3), 22:5(n-3) and 22:6(n-3) occurring after only 4 weeks on fish oil (Table 17).

The absolute increases in 20:5(n-3), 22:5(n-3) and 22:6(n-3) recorded at the end of the study period varied enormously between patients (20:5(n-3), 40% to 256%; 22:5(n-3), 5% to 394%; 22:6(n-3), -21% to +196%). The unexpected decrease in red blood cell 22:6(n-3) concentration in two of the patients studied was not mirrored by changes in plasma and platelet levels, furthermore, changes in this fatty acid were not significant (Table 16).

Although total red blood cell (n-6) fatty acid levels remained unaltered after treatment with fish oil, the levels of 18:2(n-6) were significantly reduced (Tables 16 and 17). In addition, transient reductions of 18:3(n-6), 20:3(n-6) and 20:4(n-6) were also seen in the fish oil group (table 17).

Parallel changes in 18:2(n-6), 20:5(n-3) and 22:5(n-3) were observed when the effects of 8 weeks of fish oil supplement on these fatty acids were compared to those of olive oil (control) (Table 16).
Table 16 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 8</td>
<td>week 0</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>11.71 (10.20, 13.22)</td>
<td>10.04 ( 8.90, 11.17)</td>
<td>0.01*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.77 ( 0.91, 2.63)</td>
<td>3.69 ( 2.63, 4.74)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>2.04 ( 1.37, 2.71)</td>
<td>2.70 ( 2.04, 3.35)</td>
<td>0.002*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.75 ( 3.52, 5.99)</td>
<td>5.19 ( 4.50, 5.87)</td>
<td>0.20</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>27.40 (23.82,30.98)</td>
<td>24.60 ( 21.88, 27.32)</td>
<td>0.08</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>8.82 ( 6.61,11.02)</td>
<td>11.88 ( 9.77,14.00)</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 8 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>week 0</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>11.71 (10.20,13.22)</td>
<td>10.24 ( 8.91,11.56)</td>
<td>0.02*</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.41 ( 0.30, 0.52)</td>
<td>0.36 ( 0.27, 0.46)</td>
<td>0.02*</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.32 ( 1.05, 1.60)</td>
<td>1.16 ( 0.89, 1.44)</td>
<td>0.03</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>11.49 ( 9.12,13.86)</td>
<td>10.62 ( 8.53,12.72)</td>
<td>0.004*</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.77 ( 0.91, 2.63)</td>
<td>3.21 ( 2.23, 4.20)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>2.04 ( 1.37, 2.71)</td>
<td>2.46 ( 1.98, 2.93)</td>
<td>0.03</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.75 ( 3.52, 5.99)</td>
<td>5.24 ( 4.00, 6.49)</td>
<td>0.03</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>27.40 (23.82,30.98)</td>
<td>24.70 (21.85,27.54)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>8.82 ( 6.61,11.02)</td>
<td>11.16 ( 8.68,13.64)</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 4 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
4.5 Discussion

4.5.1 Effects of fish oil and olive oil on HbA1c and lipoprotein lipids

The effects of fish oil consumption on circulating lipids and lipoproteins have been recently reviewed (168). Most of the findings are encouraging with regard to the treatment of hyperlipidaemia, particularly hypertriglyceridaemia (185,186,188,275). Many studies have evaluated lipid-lowering effects of fish oil in non-diabetic subjects; fewer have investigated the effects of dietary supplements with (n-3) PUFA and (n-9) MFA on lipid and fatty acid profiles in male NIDDM patients. Epidemiological studies indicate that diets rich in these fatty acids lower plasma triacylglycerol and cholesterol levels (168,190). These effects, however, were not evident in the present study although patients receiving the prescribed daily dosage of (n-3) fatty acids in the form of fish oil concentrates (MaxEPA) had transient reductions of plasma and VLDL triacylglycerol levels, furthermore, olive oil produced a transient improvement of plasma total cholesterol of NIDDM patients. At the end of the study, the only lipid parameter which is significantly increased by fish oil was HDL<sub>2</sub> cholesterol, however the change was not significant when compared to that in the olive oil (control) group.

The plasma concentration of HDL<sub>2</sub> is normally dependent on VLDL catabolism (46). In NIDDM, insulin resistance may affect lipoprotein lipase activity, leading to reduced transfer of VLDL components to HDL<sub>2</sub> required for the formation of HDL<sub>3</sub>. Thus, the increased in HDL<sub>2</sub> levels due to fish oil treatment seen in this study are indicative of increased VLDL catabolism and should, in theory, accompany a decrease in VLDL levels. However, VLDL triacylglycerol levels in these patients remained unaltered. This seemingly aberrant finding could not be explained in terms of improved VLDL catabolism. Therefore, other metabolic activities might have occurred under the influence of fish oil to produce this effect.

Two types of VLDL particles varying both according to size and metabolism are released from the liver to the circulation (78). The smaller particles have a lower rate of clearance from the circulation and are therefore more easily converted to LDL. In contrast, large VLDL particles are removed directly from the circulation.
However, other types of VLDL particle, possibly intermediate in size between the small and large VLDL particles may exist. Given the findings obtained in this study with respect to plasma VLDL and HDL\textsubscript{3} concentration, it could be suggested that under the influence of fish oil such an intermediate or medium-sized particle may be formed at the expense of the small and large species. This medium-sized form of VLDL might then undergo conversion to smaller 'remnant' particles with the concomitant release of lipid and protein components to HDL\textsubscript{3}, thereby providing an extra pathway for HDL\textsubscript{2} formation. The smaller 'remnant' particles, however, might not continue to convert to LDL but remain in the circulation, thus providing a plausible explanation for the negative effects of fish oil on VLDL and LDL levels seen in this study.

Alternatively, the observed increases in HDL\textsubscript{2} levels may be due to the influence of fish oil on hepatic lipase activity. In diabetes, the activity of this enzyme is reported to be raised (277), presumably leading to reduced HDL levels (220). The NIDDM patients taking part in this study were recruited from the earlier cross-sectional study and all of them presented with HDL\textsubscript{2} cholesterol levels similar to those of the control subjects. Thus, the increases in HDL\textsubscript{2} levels after fish oil treatment may be due to a reduction of hepatic lipase activity induced by (n-3) fatty acids, particularly 20:5(n-3) and 22:6(n-3).

Both proposed mechanisms regarding the effects of fish oil on HDL\textsubscript{2} levels may occur synergistically. Improved HDL\textsubscript{2} levels have been proposed to indicate increased cholesterol transport from peripheral tissues to the liver for catabolism (276). This 'anti-atherogenic' process may be enhanced by increasing the dietary intake of fish oil and contribute to the reduction of atherosclerosis in NIDDM.

The beneficial influence produced by fish oil on HDL\textsubscript{2} cholesterol levels did not extend to glycaemic control in the NIDDM patients. Glycaemic control, indicated by HbA1\% levels, had worsened by the end of the study in 5 patients, whereas, in the remaining patients glycaemia were marginally improved. The variations in glycaemic control seen in this study may be due to the fact that patients were given a moderate daily dose of fish oil (9.9 g/day). In previous studies, higher doses of fish oil, e.g. 18 g/day, have been shown to worsen glycaemic control to an even greater extent (186).
The effectiveness of olive oil as an agent for improving glycaemic control in NIDDM (198,199) was questioned because responses towards this oil was not uniform; HbA1% contents were raised in 5 of the 10 patients studied. Previous studies have shown that olive oil decreased plasma total and LDL cholesterol and increase HDL cholesterol in diabetics (198,199) and non-diabetics (192,193,195, 196). Although these effects were not observed in the present study, 6 of the 10 patients on olive oil showed a slight improvement of HDL₂ cholesterol. This small but insignificant effect may be sufficed to obscure that provided by the fish oil.

4.5.2 Effects of fish oil on plasma phospholipid fatty acids

After 8 weeks on fish oil NIDDM patients showed striking increases in plasma phospholipid 20:5(n-3), 22:5(n-3) and 22:6(n-3), and corresponding decreases in the (n-6) fatty acids. Surprisingly, 4 weeks of fish oil supplement produced insignificant changes in 20:4(n-6) levels when compared to olive oil (control). As both the (n-3) fatty acids and 20:4(n-6) are believed to compete for the same sn2 position on the phospholipid molecule (105), the incorporation of these PUFA into phospholipids may be selective in NIDDM during the first 4 weeks of the supplement.

Although the amounts of 20:5(n-3) and 22:6(n-3) in the fish oil preparation were similar, the distribution of these (n-3) fatty acids in the plasma phospholipids of the NIDDM patients after fish oil treatment differed considerably. The amounts of 20:5(n-3) incorporated into phospholipid were approximately 1.8 times that of 22:6(n-3), implying that 20:5(n-3) may be the preferred substrate for the acylation of phospholipids. In addition, some of the 22:6(n-3) may have been retro-converted in the liver (123), thus providing increased amounts of 20:5(n-3) for phospholipid formation. Only small amounts of 22:5(n-3) were found in the fish oil preparation, however, considerable increases of this (n-3) fatty acid were seen in the plasma phospholipids of NIDDM patients after treatment. This finding provides further support for the notion that retro-conversion of 22:6(n-3) to 20:5(n-3) occurs in these patients as well as the elongation of 20:5(n-3) to 22:5(n-3).
4.5.3 Effects of fish oil on platelet fatty acids

The mechanisms by which changes in platelet fatty acids produce reductions in platelet activity are not known. However, it has been suggested that changes in 20:5(n-3) and 20:4(n-6) levels are particularly important in this regard and hence for the development of LVD (266). Increased dietary intake of (n-3) fatty acids leads to the platelet production of thromboxane A₃ which is less proaggregatory than thromboxane A₂ (147). Under these circumstances, the formation of thromboxane A₂ from 20:4(n-6) is also reduced (111). However, it should be noted that apart from its beneficial actions, replacement of platelet 20:4(n-6) with 20:5(n-3) may produce adverse physiological effects such as prolonging bleeding time (180).

In this study, the observed increases in platelet 20:5(n-3), 22:5(n-3) and 22:6(n-3) were not accompanied by alterations in 18:2(n-6) and 20:4(n-6). Previous studies have shown that platelet sensitivity are reduced in normal subjects fed with a 18:2(n-6) rich diet (279). Since the levels of 18:2(n-6) in the NIDDM patients were already lower than that of normal controls, a further reduction in this fatty acid may increase the platelet sensitivity thereby increasing the risk of thrombosis.

The increases in platelet 20:5(n-3), 22:5(n-3) and 22:6(n-3) levels were not related to the amounts in the fish oil preparation or the levels measured in the plasma phospholipids of the NIDDM patients. Therefore, the uptake of these (n-3) fatty acids may be selective as have been reported for non-diabetic subjects (262,264).

4.5.4 Effects of fish oil on red blood cell fatty acids

The raised levels of 20:5(n-3) and 22:5(n-3) seen in the plasma phospholipids and platelets of the patients after fish oil treatment were also detected in the red blood cells. Increased red blood cell contents of these (n-3) fatty acids were accompanied by a significant drop in the 18:2(n-6) level. Less marked increase in the red blood cell 22:6(n-3) contents were also observed (157). In fact, 2 of the 10 patients had reduced red blood cell 22:6(n-3) contents at the end of the study. The unexpected reduction in red blood cell 22:6(n-3) in these 2 patients was not
mirrored by changes in their plasma phospholipids and platelets of this fatty acid. Taken together, these observations suggest that 22:6(n-3) may not be the fatty acid species of choice for the synthesis of red blood cells and changes occurring in 18:2(n-6) and other (n-3) fatty acid levels may be more important in improving red blood cell physiology in NIDDM.

4.5.5 Effects of olive oil on plasma, platelet and red blood cell fatty acids

Although the olive oil preparation used in this study contained over 70% of 18:1(n-9), the plasma phospholipid, platelet and red blood cell levels of this fatty acid, as well as other fatty acids was not affected by the daily consumption of this oil (9.9 g/day) over an 8 week period. The only exception was a slight but significant reduction of 16:0 in the plasma phospholipid fraction. On the basis of this observation, 18:1(n-9), at the dosage prescribed in this study, could be conservatively regarded as 'neutral' in its effects on NIDDM blood fatty acid profiles, i.e. it had neither improved nor worsened the blood fatty acid profiles in these patients.

What this study cannot determine is whether the levels of 18:1(n-9) seen in NIDDM patients at the end of the study was due to poor absorption or increased catabolism of olive oil. Both these possibilities, however, may not be as harmful as that if the major proportion of the exogenous 18:1(n-9) are retained in the tissues. The effect of increased tissue 18:1(n-9) contents may enhance cholesterol synthesis by altering the activity of β-HMG-CoA reductase, the rate-limiting enzyme for endogenous cholesterol synthesis (240). Thus, given the possible adverse effect on cholesterol synthesis and the unpredictable influence on glycaemic control, olive oil should be used with caution as a treatment for hyperlipidaemia in NIDDM.
5 CONCLUDING REMARKS

In conclusion, this study indicates that plasma triacylglycerol concentrations are increased in male NIDDM patients, particularly those with LVD. The excess triacylglycerol may be preferentially incorporated into larger VLDL particles and the subsequent removal of these triacylglycerol-rich particles from the circulation though the mediation of apoE (72) could lead to a reduction of VLDL materials available for HDL2 formation. Therefore, excessive production of this type of VLDL in NIDDM may alter the inverse correlation between plasma triacylglycerol and HDL2 cholesterol levels previously seen in normal subjects (228,229). Indeed, this relationship is absent in the NIDDM patients studied, suggesting that VLDL of the larger type predominates in the circulation. Past studies have also shown that prolonged presence of this VLDL subfraction in the circulation may contribute to the increased incidence of atherosclerosis in NIDDM (72). Thus, reductions of these atherogenic particles is of prime importance in the control of vascular complications in NIDDM. In this study, a medium-sized VLDL with a composition and metabolism unlike those of the large and small particles may have been produced by fish oil when taken as a dietary supplement. This alternative type of VLDL may undergo partial lipolysis prior to direct clearance from the circulation, thus providing a source of VLDL materials for HDL2 formation without affecting LDL production. Therefore, in order to further our understandings of the mechanism of atherogenesis in NIDDM and the effects of fish oil on VLDL and HDL metabolism, more detailed studies on the distribution and composition of VLDL and their subfractions should be conducted.

Since the plasma cholesterol levels in most of the NIDDM patients studied were greater than those recommended by the European Atherosclerosis Society (225), these patients can be regarded as being hypercholesterolaemic. It would therefore seem prudent to lower their cholesterol levels. Unfortunately, the hypocholesterolaemic effects of olive oil reported by others were not repeated here in the present study (192,193,195,196,198,199). This study also showed that the effects on glycaemic control on supplementing the NIDDM patients’ diets with olive oil were variable. Thus, the dosage of olive oil used in this study (9.9 g/day)
may not be useful for lowering cholesterol in NIDDM patients and may prove positively unfavourable as regards glycaemic control. However, the effects of lower doses of olive oil on dyslipidaemia and glycaemia in NIDDM should be investigated in future studies before discarding totally the use of olive oil in the treatment of dyslipidaemia in NIDDM.

The present data on plasma and red blood cell fatty acids suggest that changes occurring in fatty acid composition are due to the combined effects of impaired fatty acid desaturation and acylation. The unexpected increase in the 20:5(n-3) levels in NIDDM patients may due to the re-direction of the action of Δ5 desaturase towards the precursor of this (n-3) fatty acid, leading to a reduction in 20:4(n-6). However, comparable reductions in plasma and red blood cell 20:4(n-6) levels were not seen in this study. This anomaly may have occurred if 20:4(n-6) is preferentially incorporated into lipid molecules prior to their release into the circulation. Similar asymmetric distribution of 22:6(n-3) in plasma phospholipids and red blood cells was also seen in NIDDM patients with vascular disease. Thus, it can be inferred that changes in fatty acid composition seen in this study are due to altered fatty acid metabolism, and not as a consequence of dietary influence. This conclusion is further strengthened by the observation that red blood cell SFA plus MFA levels seen in the two groups of NIDDM patients were different.

Apart from their beneficial role with regard to atherogenesis, PUFA are subject to free-radical peroxidation (280). High PUFA levels, when insufficiently protected by anti-oxidants, may increase peroxidation, leading to the increased lipid peroxidation products seen in patients with occlusive arterial disease (281) and NIDDM (282). Therefore, further studies of the factors that determine or influence the reaction of peroxidation are needed to evaluate the exact contribution of this reaction to atherosclerosis.

Changes in red blood cell physiology are known to be influenced by the type of fatty acids present in their membranes (157,270-272). In addition, fatty acids may also determine the structural and biochemical properties of blood vessels, and affect plasma viscosity. Thus, questions regarding the combined effects of these factors on blood rheology should be addressed in future research.
The data on the fatty acid composition of platelets in NIDDM patients suggests that the platelet incorporation of 20:4(n-6) and SFA may be affected by dietary factors and increased 20:4(n-6) metabolism, thus influencing the formation of prostaglandins and other compounds (147). It has been shown that platelets in diabetics are characterised by increased sensitivity to various agonists, and this has been explained on the basis of the increased production and release of thromboxane A$_2$, a powerful aggregating agent (147). On the other hand, prostacyclins, i.e. PGI$_2$ and PGI$_3$, generated in blood vessel walls from 20:4(n-6) and 20:5(n-3), counteract the deleterious effects of thromboxane A$_2$. The positive effects of fish oil on the platelet levels of 20:5(n-3) seen in the present study may increase the formation of thromboxane A$_3$, a compound with little or no aggregating effects (174,266). Other metabolic products of PUFA produced by the lipooxygenase pathway, may also influence platelet function (143). Thus, efforts designed to identify and measure these biologically active compounds should be pursued in order to understand their relations to the pathogenesis of vascular complications in NIDDM.
Appendix I

A typical chromatogram obtained with a mixture of fatty acid standards is shown in Fig 56.

Figure 56  Gas chromatogram of fatty acid standards

1: BHT  
2: 16:0  
3: 16:1(n-7)  
4: 18:0  
5: 18:1(n-9),(n-7)  
6: 18:2(n-6)  
7: 18:3(n-6)  
8: 18:3(n-3)  
9: 20:2(n-6)  
10: 20:3(n-6)  
11: 20:4(n-6)  
12: 20:5(n-3)  
13: 22:4(n-6)  
14: 22:5(n-6)  
15: 22:5(n-3)  
16: 22:6(n-3)
Appendix II

Characteristics of patients entered into the cross-sectional study

NIDDM patients

<table>
<thead>
<tr>
<th>Medication</th>
<th>with LVD</th>
<th>without LVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glibenclamide</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Metformin</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Atenolol</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medical history</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD&lt;sup&gt;1&lt;/sup&gt;</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>PVD&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension&lt;sup&gt;3&lt;/sup&gt; + CAD</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal ECG&lt;sup&gt;4&lt;/sup&gt;</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>1 to 37 years</td>
<td>1 to 17 years</td>
</tr>
<tr>
<td>Current smokers</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Ex smokers</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Alcohol users</td>
<td>18</td>
<td>16</td>
</tr>
</tbody>
</table>

<sup>1</sup>CAD (coronary heart disease) was diagnosed if the patient had symptoms of angina pectoris, a history of or the occurrence of myocardial infarction or evidence of heart failure and hypertension.

<sup>2</sup>PVD (peripheral vascular disease) was diagnosed if it was impossible to feel either of the pulses in one foot, being asymptomatic or associated with intermittent claudication, foot ulceration, dry gangrene or rest pain.
Hypertension was defined as the repeated finding of a diastolic pressure greater than 100 mg.

Electrocardiographs (ECG) were recorded and coded according to the Minnesota Code. Positive ECG signs were taken as one or more of the following Minnesota code items:

- Q/QS waves (codes 1.1-1.3)
- ST depression (codes 4.1-4.4)
- T wave inversion or flattening (codes 5.1-5.3)
- Left bundle-branch block (code 7.1)
### Table 18

Age, body weight, HbA1c% and plasma lipid compositions in normal subjects & NIDDM patients with (LVD+) and without (LVD-) large vessel disease.

<table>
<thead>
<tr>
<th></th>
<th>Normals (N, n=20)</th>
<th>NIDDMs (LVD-, n=20)</th>
<th>NIDDMs (LVD+, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>median (range)</td>
<td>median (range)</td>
<td>median (range)</td>
</tr>
<tr>
<td><strong>Age (years)</strong>*</td>
<td>46 (23-82)</td>
<td>55 (30-75)</td>
<td>60 (36-70)</td>
</tr>
<tr>
<td><strong>weight (kg)</strong></td>
<td>73 (55-108)</td>
<td>77 (62-96)</td>
<td>81 (70-96)</td>
</tr>
<tr>
<td><strong>KW(p)</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c%</td>
<td>&lt;0.001</td>
<td>6.9 (6.6-7.1)</td>
<td>9.7 (8.7-11.2)***</td>
</tr>
<tr>
<td>T Chol</td>
<td>&lt;0.02</td>
<td>5.72 (5.28-6.21)</td>
<td>5.45 (4.94-6.05)</td>
</tr>
<tr>
<td>Trig</td>
<td>&lt;0.001</td>
<td>1.06 (0.89-1.49)</td>
<td>1.63 (1.26-2.20)**</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>&lt;0.001</td>
<td>0.49 (0.41-0.68)</td>
<td>0.75 (0.58-1.01)</td>
</tr>
<tr>
<td>LDL-C</td>
<td>N.S.</td>
<td>3.88 (3.46-4.12)</td>
<td>3.38 (3.06-4.21)</td>
</tr>
<tr>
<td>THDL</td>
<td>N.S.</td>
<td>1.20 (1.04-1.43)</td>
<td>1.03 (0.80-1.34)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>N.S.</td>
<td>0.31 (0.17-0.48)</td>
<td>0.29 (0.19-0.37)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>N.S.</td>
<td>0.91 (0.64-1.10)</td>
<td>0.84 (0.34-1.02)</td>
</tr>
</tbody>
</table>

---

1: $p$ values, using Kruskal-Wallis one-way ANOVA; N.S.: not significant.

***: $p<0.001$, **: $p<0.01$, compared to values of normal subjects.

++: $p<0.02$, +: $p<0.05$, compared to values of NIDDM without LVD.
Table 19: Plasma fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th>mmol%</th>
<th>Normal subjects (N) (n=20)</th>
<th>NIDDM patients (LVD-) (n=20)</th>
<th>NIDDM patients (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KW(p)</td>
<td>median (95% C.I.)</td>
<td>median (95% C.I.)</td>
</tr>
<tr>
<td>16:0</td>
<td>&lt;0.001</td>
<td>24.84 (23.48-25.78)</td>
<td>26.19 (25.27-27.34)**</td>
</tr>
<tr>
<td>18:0</td>
<td>N.S.</td>
<td>8.34 ( 7.50- 8.87)</td>
<td>8.31 ( 7.58- 9.28)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>N.S.</td>
<td>3.28 ( 2.81- 3.62)</td>
<td>3.25 ( 2.63- 3.87)</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>0.05</td>
<td>20.06 (19.16-21.65)</td>
<td>21.27 (19.13-23.00)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.001</td>
<td>32.16 (27.99-34.54)</td>
<td>26.24 (25.21-29.45)**</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>N.S.</td>
<td>0.61 ( 0.52- 0.68)</td>
<td>0.61 ( 0.48- 0.76)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.05</td>
<td>0.21 ( 0.11- 0.34)</td>
<td>0.39 ( 0.20- 0.58)**</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>N.S.</td>
<td>1.28 ( 1.14- 1.51)</td>
<td>1.41 ( 1.16- 1.63)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>N.S.</td>
<td>5.79 ( 5.00- 6.05)</td>
<td>5.79 ( 4.71- 6.60)</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>N.S.</td>
<td>0.12 ( 0.05- 0.18)</td>
<td>0.10 ( 0.08- 0.15)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>N.S.</td>
<td>0.04 ( 0.00- 0.09)</td>
<td>0.08 ( 0.00- 0.10)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>N.S.</td>
<td>0.83 ( 0.64- 0.91)</td>
<td>0.90 ( 0.67- 1.23)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.001</td>
<td>0.59 ( 0.44- 0.74)</td>
<td>1.11 ( 0.62- 2.03)**</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>N.S.</td>
<td>0.48 ( 0.37- 0.55)</td>
<td>0.51 ( 0.46- 0.65)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>N.S.</td>
<td>2.03 ( 1.78- 2.40)</td>
<td>2.08 ( 1.57- 2.66)</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>&lt;0.001</td>
<td>39.47 (36.57-42.93)</td>
<td>35.45 (33.08-39.30)**</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>N.S.</td>
<td>4.07 ( 3.41- 4.60)</td>
<td>4.45 ( 4.05- 5.88)</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.001</td>
<td>43.70 (41.07-45.94)</td>
<td>39.89 (38.92-43.46)*</td>
</tr>
<tr>
<td>ΣC16</td>
<td>&lt;0.001</td>
<td>27.56 (26.75-29.20)</td>
<td>29.62 (28.19-31.02)**</td>
</tr>
<tr>
<td>ΣC18</td>
<td>&lt;0.05</td>
<td>28.46 (27.05-30.14)</td>
<td>29.44 (27.68-31.49)</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.001</td>
<td>56.30 (54.07-58.93)</td>
<td>60.11 (56.54-61.08)*</td>
</tr>
</tbody>
</table>

N.S.: not significant.

1: p values, using Kruskal-Wallis one-way ANOVA.

***:p<0.001, **:p<0.02, *:p<0.05, compared to values of normal subjects.
Table 20: Fatty acid compositions of plasma phospholipids from normal subjects & NIDDM patients.

<table>
<thead>
<tr>
<th>mmol%</th>
<th>Normal (N) (n=20)</th>
<th>NIDDM (LVD-) (n=20)</th>
<th>NIDDM (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>N.S.</td>
<td>31.57 (30.73-32.73)</td>
<td>32.51 (30.35-34.05)</td>
</tr>
<tr>
<td>18:0</td>
<td>N.S.</td>
<td>13.85 (13.09-14.32)</td>
<td>13.81 (13.19-14.12)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>N.S.</td>
<td>1.43 (1.00-1.64)</td>
<td>1.35 (1.14-1.89)</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>N.S.</td>
<td>11.82 (10.57-12.70)</td>
<td>11.09 (10.30-12.39)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.001</td>
<td>23.99 (23.28-26.07)</td>
<td>20.87 (17.90-22.13)**</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>N.S.</td>
<td>0.29 (0.25-0.33)</td>
<td>0.31 (0.27-0.41)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.001</td>
<td>0.26 (0.22-0.33)</td>
<td>0.33 (0.27-0.51)**</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>N.S.</td>
<td>2.36 (2.01-2.59)</td>
<td>2.54 (2.16-2.90)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>N.S.</td>
<td>8.18 (6.99-9.43)</td>
<td>9.17 (8.08-10.03)</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>N.S.</td>
<td>0.21 (0.18-0.22)</td>
<td>0.21 (0.16-0.24)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>N.S.</td>
<td>0.13 (0.07-0.20)</td>
<td>0.13 (0.08-0.27)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>N.S.</td>
<td>0.35 (0.27-0.49)</td>
<td>0.35 (0.28-0.40)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.05</td>
<td>1.04 (0.89-1.40)</td>
<td>1.54 (1.15-2.57)**</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>N.S.</td>
<td>0.79 (0.70-0.89)</td>
<td>1.00 (0.78-1.10)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>N.S.</td>
<td>3.43 (3.18-3.80)</td>
<td>3.59 (3.41-4.06)</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>N.S.</td>
<td>35.68 (34.59-37.65)</td>
<td>33.72 (32.03-34.96)</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>N.S.</td>
<td>5.90 (4.82-6.17)</td>
<td>6.54 (5.94-7.87)</td>
</tr>
<tr>
<td>PUFA</td>
<td>N.S.</td>
<td>41.38 (40.74-42.36)</td>
<td>40.60 (39.65-42.68)</td>
</tr>
<tr>
<td>ΣC16</td>
<td>N.S.</td>
<td>33.22 (31.99-33.79)</td>
<td>34.44 (32.52-35.50)</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>N.S.</td>
<td>58.62 (57.64-59.26)</td>
<td>59.40 (57.32-60.35)</td>
</tr>
</tbody>
</table>

N.S.: not significant.

1: p values, using Kruskal-Wallis one-way ANOVA.

**:p<0.02, *:p<0.05, compared to values of normal subjects.
Table 21  Platelet fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th></th>
<th>Normal (N) (n=20)</th>
<th>NIDDM (LVD-) (n=20)</th>
<th>NIDDM (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol%</td>
<td>KW(p)</td>
<td>median (95% C.I.)</td>
<td>median (95% C.I.)</td>
</tr>
<tr>
<td>16:0</td>
<td>&lt;0.05</td>
<td>20.53 (19.07-21.75)</td>
<td>21.05 (19.91-22.36)</td>
</tr>
<tr>
<td>18:0</td>
<td>N.S.</td>
<td>19.98 (18.78-20.50)</td>
<td>19.64 (18.96-20.07)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>&lt;0.05</td>
<td>1.46 ( 1.37- 1.58)</td>
<td>1.92 ( 1.62- 3.52)**</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>N.S.</td>
<td>18.49 (16.74-19.56)</td>
<td>19.64 (18.00-20.05)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.02</td>
<td>8.58 ( 8.32- 9.05)</td>
<td>7.75 ( 7.21- 8.48)**</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>N.S.</td>
<td>1.02 ( 0.91- 1.22)</td>
<td>1.03 ( 0.79- 1.19)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>&lt;0.02</td>
<td>0.40 ( 0.38- 0.56)</td>
<td>0.66 ( 0.48- 1.05)**</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>N.S.</td>
<td>1.40 ( 1.35- 1.54)</td>
<td>1.44 ( 1.12- 1.51)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>&lt;0.05</td>
<td>22.33 (21.37-23.35)</td>
<td>20.72 (19.38-22.57)*</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>N.S.</td>
<td>1.36 ( 1.29- 1.75)</td>
<td>1.45 ( 1.14- 1.73)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>N.S.</td>
<td>0.14 ( 0.12- 0.19)</td>
<td>0.08 ( 0.00- 0.16)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>N.S.</td>
<td>0.29 ( 0.20- 0.43)</td>
<td>0.27 ( 0.21- 0.41)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>N.S.</td>
<td>0.55 ( 0.43- 0.91)</td>
<td>0.62 ( 0.46- 1.07)</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>N.S.</td>
<td>1.34 ( 1.17- 1.57)</td>
<td>1.26 ( 1.16- 1.49)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>N.S.</td>
<td>1.73 ( 1.66- 1.97)</td>
<td>1.46 ( 1.29- 1.88)</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>&lt;0.05</td>
<td>36.04 (34.84-36.81)</td>
<td>32.94 (32.46-34.24)**</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>N.S.</td>
<td>4.18 ( 3.90- 4.61)</td>
<td>3.86 ( 3.14- 4.85)</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.001</td>
<td>39.99 (38.95-41.01)</td>
<td>37.34 (35.98-38.54)**</td>
</tr>
<tr>
<td>ΣC16</td>
<td>&lt;0.05</td>
<td>22.07 (21.36-23.08)</td>
<td>23.39 (22.40-26.12)*</td>
</tr>
<tr>
<td>ΣC18</td>
<td>N.S.</td>
<td>38.04 (37.09-39.06)</td>
<td>39.06 (37.88-40.19)</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.001</td>
<td>60.01 (58.99-61.05)</td>
<td>62.66 (61.46-64.02)**</td>
</tr>
</tbody>
</table>

N.S.: not significant.

1: p values, using Kruskal-Wallis one-way ANOVA.

***: p<0.001, **: p<0.02, *: p<0.05, compared to values of normal subjects.
### Table 22  
Red blood cell fatty acid compositions in normal subjects and NIDDM patients.

<table>
<thead>
<tr>
<th>mmol%</th>
<th>Normal (N) (n=20)</th>
<th>NIDDM (LVD-) (n=20)</th>
<th>NIDDM (LVD+) (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:0</td>
<td>N.S. 17.08 (16.69-17.25)</td>
<td>16.78 (15.96-17.30)</td>
<td>16.93 (16.72-17.26)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>N.S. 1.25 (1.19-1.46)</td>
<td>1.50 (1.25-1.89)</td>
<td>1.74 (1.17-2.45)</td>
</tr>
<tr>
<td>18:1(n-9),(n-7)</td>
<td>N.S. 16.29 (15.74-17.63)</td>
<td>16.54 (15.25-18.52)</td>
<td>17.95 (16.65-18.79)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>&lt;0.05 13.77 (12.84-14.85)</td>
<td>12.83 (10.95-13.74)</td>
<td>12.25 (10.60-13.40)**</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>N.S. 0.42 (0.37-0.44)</td>
<td>0.34 (0.31-0.47)</td>
<td>0.38 (0.32-0.44)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>N.S. 0.36 (0.28-0.44)</td>
<td>0.39 (0.16-0.35)</td>
<td>0.39 (0.28-0.51)</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>N.S. 1.48 (1.40-1.72)</td>
<td>1.37 (0.20-1.95)</td>
<td>1.45 (1.30-1.55)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>&lt;0.05 14.02 (12.32-14.26)</td>
<td>12.45 (10.37-13.63)*</td>
<td>12.30 (11.20-12.97)*</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>N.S. 2.09 (1.65-2.32)</td>
<td>1.65 (1.05-2.06)</td>
<td>1.78 (1.51-2.10)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>N.S. 0.28 (0.23-0.37)</td>
<td>0.24 (0.17-0.34)</td>
<td>0.22 (0.17-0.32)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>N.S. 0.26 (0.19-0.32)</td>
<td>0.19 (0.29-0.48)</td>
<td>0.30 (0.19-0.41)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>&lt;0.001 0.88 (0.69-1.00)</td>
<td>1.69 (1.53-2.01)<strong>+, ++1.17 (1.02-1.43)</strong></td>
<td></td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>N.S. 2.10 (1.94-2.42)</td>
<td>2.24 (1.94-2.62)</td>
<td>2.13 (1.71-2.39)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>N.S. 4.80 (4.13-5.48)</td>
<td>4.65 (4.20-5.59)</td>
<td>4.63 (3.90-5.12)</td>
</tr>
<tr>
<td>(\Sigma)(n-6)</td>
<td>&lt;0.05 31.96 (30.16-32.82)</td>
<td>29.71 (27.84-32.44)</td>
<td>29.64 (26.27-31.17)**</td>
</tr>
<tr>
<td>(\Sigma)(n-3)</td>
<td>N.S. 8.26 (7.48-8.84)</td>
<td>9.10 (8.57-9.73)</td>
<td>7.88 (6.89-9.48)</td>
</tr>
<tr>
<td>PUFA</td>
<td>&lt;0.05 39.73 (39.23-40.83)</td>
<td>39.19 (38.09-40.88)</td>
<td>37.56 (35.43-40.36)**</td>
</tr>
<tr>
<td>(\Sigma)C16</td>
<td>&lt;0.05 26.60 (25.68-27.24)</td>
<td>27.55 (26.19-29.11)</td>
<td>27.88 (27.21-29.14)**</td>
</tr>
<tr>
<td>(\Sigma)C18</td>
<td>N.S. 33.56 (33.19-34.38)</td>
<td>33.74 (32.66-34.67)</td>
<td>34.81 (33.11-35.78)</td>
</tr>
<tr>
<td>SFA + MFA</td>
<td>&lt;0.05 60.27 (59.17-60.77)</td>
<td>60.81 (59.12-61.97)</td>
<td>62.44 (59.64-64.57)*</td>
</tr>
</tbody>
</table>

N.S.: not significant; \(^\dagger\): \(p\) values, using Kruskal-Wallis one-way ANOVA.

***: \(p<0.001\), **: \(p<0.01\), *: \(p<0.05\), compared to values of normal subjects.

++: \(p<0.02\), NIDDM without LVD compared to NIDDM with LVD.
Table 23 Changes in body weight and lipids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 8</td>
<td>p</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 (71.8, 79.7)</td>
<td>76.1 (72.3, 79.9)</td>
<td>0.55</td>
</tr>
<tr>
<td>HbA1%</td>
<td>8.7 (6.5, 10.9)</td>
<td>10.4 (8.8, 11.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>T Chol(^a)</td>
<td>6.10 (5.38, 6.82)</td>
<td>6.19 (5.38, 6.99)</td>
<td>0.59</td>
</tr>
<tr>
<td>TG(^b)</td>
<td>2.33 (1.74, 2.92)</td>
<td>2.05 (1.46, 2.64)</td>
<td>0.08</td>
</tr>
<tr>
<td>VLDL-C(^c)</td>
<td>0.88 (0.60, 1.16)</td>
<td>0.71 (0.42, 1.00)</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-C(^c)</td>
<td>3.93 (3.28, 4.58)</td>
<td>4.03 (3.37, 4.69)</td>
<td>0.66</td>
</tr>
<tr>
<td>THDL(^d)</td>
<td>0.96 (0.74, 1.18)</td>
<td>1.07 (0.92, 1.23)</td>
<td>0.17</td>
</tr>
<tr>
<td>HDL(_2)-C(^e)</td>
<td>0.34 (0.18, 0.50)</td>
<td>0.47 (0.32, 0.62)</td>
<td>0.002(^*)</td>
</tr>
<tr>
<td>HDL(_3)-C(^f)</td>
<td>0.62 (0.39, 0.85)</td>
<td>0.60 (0.37, 0.83)</td>
<td>0.85</td>
</tr>
<tr>
<td>HDL(_3)-C(^g)</td>
<td>0.62 (0.39, 0.85)</td>
<td>0.60 (0.37, 0.83)</td>
<td>0.85</td>
</tr>
<tr>
<td>VLDL-T(^h)</td>
<td>1.27 (0.88, 1.66)</td>
<td>1.14 (0.79, 1.48)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval)

\(^a\): total plasma cholesterol (mM/l); \(^b\): plasma triacylglycerol (mM/l); \(^c\): VLDL cholesterol (mM/l); \(^d\): LDL cholesterol (mM/l);
\(^e\): Total HDL cholesterol (mM/l); \(^f\): HDL\(_2\) cholesterol (mM/l); \(^g\): HDL\(_3\) cholesterol (mM/l);
\(^h\): VLDL triacylglycerol (mM/l)

\(^*\): statistically significant (paired t test - changes between week 8 and baseline)

169
Table 24 Changes in body weight and lipids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>p</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 (71.8, 79.7)</td>
<td>76.9 (72.8, 81.0)</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1%</td>
<td>8.7 (6.5, 10.9)</td>
<td>9.5 (8.0, 10.9)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Chol(^1)</td>
<td>6.10 (5.38, 6.82)</td>
<td>6.22 (5.50, 6.95)</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG(^2)</td>
<td>2.33 (1.74, 2.92)</td>
<td>2.02 (1.43, 2.60)</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-C(^3)</td>
<td>0.88 (0.60, 1.16)</td>
<td>0.77 (0.41, 1.13)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C(^4)</td>
<td>3.93 (3.28, 4.58)</td>
<td>4.02 (3.41, 4.63)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THDL(^5)</td>
<td>0.96 (0.74, 1.18)</td>
<td>1.00 (0.84, 1.16)</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL(_2)-C(^6)</td>
<td>0.34 (0.18, 0.50)</td>
<td>0.43 (0.29, 0.58)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL(_3)-C(^7)</td>
<td>0.62 (0.39, 0.85)</td>
<td>0.57 (0.38, 0.75)</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-T(^8)</td>
<td>1.27 (0.88, 1.66)</td>
<td>1.01 (0.64, 1.38)</td>
<td>0.02*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval)

\(^{1}\): total plasma cholesterol (mM/l); \(^{2}\): plasma triacylglycerol (mM/l); \(^{3}\): VLDL cholesterol (mM/l); \(^{4}\): LDL cholesterol (mM/l); \(^{5}\): Total HDL cholesterol (mM/l); \(^{6}\): HDL\(_2\) cholesterol (mM/l); \(^{7}\): HDL\(_3\) cholesterol (mM/l); \(^{8}\): VLDL triacylglycerol (mM/l)

*: statistically significant (paired t test - changes between week 4 and baseline)

*= statistically significant (unpaired t test - changes between treatment groups)
## Table 25 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>week 0</strong></td>
<td><strong>week 8</strong></td>
<td><strong>p</strong></td>
</tr>
<tr>
<td>16:0</td>
<td>30.56 (29.29, 31.82)</td>
<td>31.54 (30.35, 32.74)</td>
</tr>
<tr>
<td>18:0</td>
<td>14.47 (13.38,15.56)</td>
<td>14.57 (13.96, 15.18)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>1.76 (1.26, 2.25)</td>
<td>1.45 (1.18, 1.73)</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>11.08 (9.24,12.93)</td>
<td>10.34 (8.83, 11.84)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>21.68 (18.66,24.70)</td>
<td>17.22 (14.75, 19.70)</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.38 (0.20, 0.57)</td>
<td>0.24 (0.18, 0.31)</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.53 (0.21, 0.84)</td>
<td>0.56 (0.19, 0.92)</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>2.59 (1.94, 3.24)</td>
<td>1.74 (1.28, 2.20)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>8.83 (7.39,10.28)</td>
<td>7.92 (7.11, 8.73)</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>0.23 (0.17, 0.29)</td>
<td>0.11 (0.07, 0.15)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.29 (0.03, 0.54)</td>
<td>0.14 (0.06, 0.22)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.37 (0.24, 0.50)</td>
<td>0.32 (0.22, 0.43)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>2.17 (0.99, 3.36)</td>
<td>6.19 (4.64, 7.74)</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.87 (0.75, 0.99)</td>
<td>1.39 (1.12, 1.66)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.19 (3.41, 4.97)</td>
<td>6.25 (5.20, 7.30)</td>
</tr>
<tr>
<td>(\Sigma)(n-6)</td>
<td>34.53 (32.32,36.74)</td>
<td>27.94 (25.34, 30.53)</td>
</tr>
<tr>
<td>(\Sigma)(n-3)</td>
<td>7.60 (5.78, 9.42)</td>
<td>14.16 (11.62, 16.69)</td>
</tr>
<tr>
<td>SFA+MFA</td>
<td>57.87 (55.48,60.27)</td>
<td>57.90 (55.79, 60.02)</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between treatment groups)  
*: statistically significant (unpaired t test - changes between week 8 and baseline)
Table 26 Changes in plasma phospholipid fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>p</td>
</tr>
<tr>
<td>16:0</td>
<td>30.56 (29.29,31.82)</td>
<td>32.00 (30.88,33.12)</td>
<td>0.06</td>
</tr>
<tr>
<td>18:0</td>
<td>14.47 (13.38,15.56)</td>
<td>14.53 (13.58,15.47)</td>
<td>0.90</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>1.76 (1.26,2.25)</td>
<td>1.18 (0.60,1.77)</td>
<td>0.07</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>11.08 (9.24,12.93)</td>
<td>10.11 (8.97,11.25)</td>
<td>0.06</td>
</tr>
<tr>
<td>16:0</td>
<td>21.68 (18.66,24.70)</td>
<td>17.17 (14.47,19.86)</td>
<td>0.004*</td>
</tr>
<tr>
<td>18:0</td>
<td>0.38 (0.20,0.57)</td>
<td>0.25 (0.20,0.30)</td>
<td>0.07</td>
</tr>
<tr>
<td>18:0</td>
<td>0.53 (0.21,0.84)</td>
<td>0.54 (0.16,0.92)</td>
<td>0.84</td>
</tr>
<tr>
<td>18:0</td>
<td>2.59 (1.94,3.24)</td>
<td>2.30 (1.48,3.12)</td>
<td>0.21</td>
</tr>
<tr>
<td>18:0</td>
<td>8.83 (7.39,10.28)</td>
<td>8.09 (7.03,9.16)</td>
<td>0.047*</td>
</tr>
<tr>
<td>18:0</td>
<td>0.23 (0.17,0.29)</td>
<td>0.10 (0.06,0.14)</td>
<td>0.002*</td>
</tr>
<tr>
<td>18:0</td>
<td>0.29 (0.03,0.54)</td>
<td>0.17 (0.02,0.32)</td>
<td>0.11</td>
</tr>
<tr>
<td>18:0</td>
<td>0.37 (0.24,0.50)</td>
<td>0.34 (0.27,0.42)</td>
<td>0.66</td>
</tr>
<tr>
<td>18:0</td>
<td>2.17 (0.99,3.36)</td>
<td>5.83 (4.26,7.40)</td>
<td>0.001*</td>
</tr>
<tr>
<td>18:0</td>
<td>0.87 (0.75,0.99)</td>
<td>1.51 (1.23,1.79)</td>
<td>0.001*</td>
</tr>
<tr>
<td>18:0</td>
<td>4.19 (3.41,4.97)</td>
<td>5.87 (4.96,6.79)</td>
<td>0.004*</td>
</tr>
<tr>
<td>18:0</td>
<td>34.53 (32.32,36.74)</td>
<td>28.62 (25.84,31.40)</td>
<td>0.001*</td>
</tr>
<tr>
<td>18:0</td>
<td>7.60 (5.78,9.42)</td>
<td>13.56 (11.23,15.89)</td>
<td>0.001*</td>
</tr>
<tr>
<td>18:0</td>
<td>57.87 (55.48,60.27)</td>
<td>57.82 (55.96,59.68)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 4 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
Table 27 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 8</td>
<td>p</td>
</tr>
<tr>
<td>16:0</td>
<td>22.25</td>
<td>21.71</td>
<td>0.71</td>
</tr>
<tr>
<td>18:0</td>
<td>20.90</td>
<td>19.73</td>
<td>0.18</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>2.68</td>
<td>2.56</td>
<td>0.74</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>18.15</td>
<td>18.47</td>
<td>0.78</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>7.68</td>
<td>7.28</td>
<td>0.42</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>1.06</td>
<td>1.06</td>
<td>0.99</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.36</td>
<td>1.30</td>
<td>0.46</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>19.17</td>
<td>17.85</td>
<td>0.56</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>1.38</td>
<td>0.90</td>
<td>0.008*</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.17</td>
<td>0.14</td>
<td>0.56</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.24</td>
<td>0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.01</td>
<td>0.37</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.29</td>
<td>1.24</td>
<td>0.002*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>1.65</td>
<td>1.79</td>
<td>0.02*</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>31.81</td>
<td>30.26</td>
<td>0.25</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>4.19</td>
<td>8.01</td>
<td>0.001*</td>
</tr>
<tr>
<td>SFA+MFA</td>
<td>63.99</td>
<td>61.73</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 8 and baseline)
*: statistically significant (unpaired t test - changes between treatment groups)
Table 28 Changes in platelet fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
</tr>
<tr>
<td>16:0</td>
<td>22.25  (21.71,24.18)</td>
<td>21.66  (20.41,22.92)</td>
</tr>
<tr>
<td>18:0</td>
<td>20.90  (13.5,17.86)</td>
<td>19.07  (18.39,19.75)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>2.68   (1.25,4.11)</td>
<td>2.66   (1.43,3.90)</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>18.15  (16.40,19.91)</td>
<td>19.08  (18.18,19.97)</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>7.68   (6.93,8.43)</td>
<td>7.80   (6.07,9.53)</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>1.06   (0.87,1.25)</td>
<td>1.03   (0.76,1.30)</td>
</tr>
<tr>
<td>18:4(n-6)</td>
<td>0.99   (0.54,1.43)</td>
<td>0.68   (0.34,1.01)</td>
</tr>
<tr>
<td>18:3(n-9)</td>
<td>1.36   (1.15,1.58)</td>
<td>1.66   (1.16,2.16)</td>
</tr>
<tr>
<td>18:4(n-6)</td>
<td>19.17  (16.61,21.74)</td>
<td>17.90  (16.76,19.04)</td>
</tr>
<tr>
<td>18:5(n-6)</td>
<td>1.38   (1.08,1.68)</td>
<td>0.84   (0.70,0.98)</td>
</tr>
<tr>
<td>18:5(n-6)</td>
<td>0.17   (0.10,0.23)</td>
<td>0.19   (0.09,0.29)</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.24   (0.13,0.35)</td>
<td>0.21   (0.11,0.32)</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.01   (0.54,1.49)</td>
<td>2.81   (1.95,3.68)</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.29   (1.13,1.44)</td>
<td>2.09   (1.74,2.44)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>1.65   (1.37,1.93)</td>
<td>2.31   (2.07,2.55)</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>31.81  (28.69,34.94)</td>
<td>30.09  (28.55,31.63)</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>4.19   (3.42,4.96)</td>
<td>7.42   (6.04,8.81)</td>
</tr>
<tr>
<td>SFA+MFA</td>
<td>63.99  (60.82,67.17)</td>
<td>62.48  (60.36,64.60)</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 4 and baseline)

*: statistically significant (unpaired t test - changes between treatment groups)
Table 29 Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (8 weeks)

<table>
<thead>
<tr>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>17.38 (16.35, 18.41)</td>
<td>17.06 (15.73, 18.40)</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>1.91 (1.40, 2.42)</td>
<td>1.87 (1.45, 2.28)</td>
</tr>
<tr>
<td>18:0</td>
<td>17.00 (15.03, 18.98)</td>
<td>17.01 (15.33, 18.68)</td>
</tr>
<tr>
<td>16:1(n-9)</td>
<td>11.71 (10.20, 13.22)</td>
<td>10.04 (8.90, 11.17)</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.68 (0.30, 0.52)</td>
<td>0.57 (0.28, 0.86)</td>
</tr>
<tr>
<td>20:5(n-6)</td>
<td>2.00 (1.34, 2.68)</td>
<td>1.96 (1.34, 2.58)</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>4.75 (3.52, 5.99)</td>
<td>5.19 (4.50, 5.87)</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>27.40 (23.82, 30.98)</td>
<td>24.60 (21.88, 27.32)</td>
</tr>
<tr>
<td>SFA+MFA</td>
<td>63.79 (58.67, 68.90)</td>
<td>63.52 (59.59, 67.44)</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between week 8 and baseline)

*: statistically significant (unpaired t test - changes between treatment groups)
Table 30  Changes in red blood cell fatty acids with fish oil (MaxEPA) and olive oil (control) treatment (4 weeks)

<table>
<thead>
<tr>
<th></th>
<th>Fish oil (MaxEPA)</th>
<th>Olive oil (control)</th>
<th>Change attributable to treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 0</td>
<td>week 4</td>
<td>p</td>
</tr>
<tr>
<td>16:0</td>
<td>27.49  (24.58,30.40)</td>
<td>27.69  (25.30, 30.10)</td>
<td>0.48</td>
</tr>
<tr>
<td>18:0</td>
<td>17.38  (16.35,18.41)</td>
<td>17.24  (15.99, 18.49)</td>
<td>0.42</td>
</tr>
<tr>
<td>16:1(n-7)</td>
<td>1.91   ( 1.40, 2.42)</td>
<td>2.20   ( 1.56, 2.84)</td>
<td>0.19</td>
</tr>
<tr>
<td>18:1(n-9)</td>
<td>17.00  (15.03,18.98)</td>
<td>17.00  (15.65, 18.34)</td>
<td>0.99</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>11.71  (10.20,13.22)</td>
<td>10.24  ( 8.91,11.56)</td>
<td>0.02*</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.41   ( 0.30, 0.52)</td>
<td>0.36   ( 0.27, 0.46)</td>
<td>0.02*</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.68   ( 0.30, 1.06)</td>
<td>0.57   ( 0.27, 0.86)</td>
<td>0.30</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>1.32   ( 1.05, 1.60)</td>
<td>1.16   ( 0.89, 1.44)</td>
<td>0.03</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>11.49  (9.12,13.86)</td>
<td>10.62  ( 8.53,12.72)</td>
<td>0.004*</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>1.55   ( 1.15, 1.95)</td>
<td>1.49   ( 1.08, 1.91)</td>
<td>0.75</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.23   ( 0.14, 0.32)</td>
<td>0.25   ( 0.17, 0.33)</td>
<td>0.38</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.25   ( 0.15, 0.35)</td>
<td>0.24   ( 0.16, 0.33)</td>
<td>0.63</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>1.77   ( 0.91, 2.63)</td>
<td>3.21   ( 2.23, 4.20)</td>
<td>0.001*</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>2.04   ( 1.37, 2.71)</td>
<td>2.46   ( 1.98, 2.93)</td>
<td>0.03*</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>4.75   ( 3.52, 5.99)</td>
<td>5.24   ( 4.00, 6.49)</td>
<td>0.03*</td>
</tr>
<tr>
<td>Σ(n-6)</td>
<td>27.40  (23.82,30.98)</td>
<td>24.70  (21.85, 27.54)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Σ(n-3)</td>
<td>8.82   ( 6.61,11.02)</td>
<td>11.16  ( 8.68,13.64)</td>
<td>0.003*</td>
</tr>
<tr>
<td>SFA+MFA</td>
<td>63.79  (58.67,68.90)</td>
<td>64.14  (59.72, 68.56)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Values are means (95% confidence interval); *: statistically significant (paired t test - changes between treatment groups)
*: statistically significant (unpaired t test - changes between week 4 and baseline)
References


70. Dunn FL, Raskin P, Bilheimer DW, Grundy SM. The effect of diabetic control on very low-density lipoprotein-triglyceride metabolism in patients with Type II diabetes mellitus and marked hypertriglyceridemia. *Metabolism* 1984; **33**:117-123.


185
ω-3 fatty acids upon plasma and cellular lipids, platelet function, and

112. Bazan NG, Birkle DL, Reddy TS. Docosahexaenoic acid (22:6(n-3)) is


114. Brenner RR, Peluffo RO. Effect of saturated and unsaturated fatty acids on
the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acid.

115. Nugteren DH. The enzymatic chain elongation of fatty acids by rat-liver

116. Brenner RR. The oxidative desaturation of unsaturated fatty acids in animals.

117. Steinberg G, Slaton Jr WH, Howton DR, Mead JF. Metabolism of essential
224:841-849.

linoleic, arachidonic, and docosa-7,10,13,16-tetraenoic acids in rat testicles.

The influence of dietary manipulation with n-3 and n-6 fatty acid on liver

synthesis of arachidonic acid in rat kidney cells. *Biochim Biophys Acta* 1983;
750:465-471.

121. Sprecher H. The total synthesis and metabolism of 7,10,13,16-docosatetraeno-
ate in the rat. *Biochim Biophys Acta* 1967; 144:296-304.

122. Stoffel W, Ecker W, Assad H, Sprecher H. Enzymatic studies on the
mechanism of the retroconversion of C_{22}-polyenoic acids to their C_{20} homologues. *Hoppe-Zeiler's Z Physiol Chem* 1970; 351:1549-1554.

acid is retroconverted in man to eicosapentaenoic acid, which can be quickly


**Publications related to this thesis**

1. Delamothe AP, Ling KLE, Betteridge DJ. MaxEPA supplementation in non-insulin dependent diabetes mellitus - preliminary results *The N-3 fatty acid conference, Reading University, July 1984*.


5. Ling KLE, Dickson AC, Betteridge DJ. The distribution of fatty acids in erythrocytes from Type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1988; 31:515A.