UNIVERSITY OF SOUTHAMPTON

THE METABOLIC FATE OF DIETARY LIPID IN HUMAN IMMUNODEFICIENCY VIRUS: IMPLICATIONS FOR THE DEVELOPMENT OF LIPODYSTROPHY

BY

LISA J WARE BSc (Hons)

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE OF HUMAN NUTRITION

FACULTY OF MEDICINE

OCTOBER 2002

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE, HEALTH AND BIOLOGICAL SCIENCES

HUMAN NUTRITION

Doctor of Philosophy

THE METABOLIC FATE OF DIETARY LIPID IN HUMAN IMMUNODEFICIENCY VIRUS – IMPLICATIONS FOR THE DEVELOPMENT OF LIPODYSTROPHY

By Lisa J Ware

During recent years, an HIV-associated lipodystrophy syndrome similar to Metabolic Syndrome X has been reported in HIV patients using highly active antiretroviral therapy (HAART). While the metabolic processes that appear to be dysregulated are similar to those of Metabolic Syndrome X (hyperlipidaemia and an altered insulin sensitivity), the changes appear to be more profound. While it is possible that HIV-associated lipodystrophy is related to the presence of infection plus genetic and environmental factors such as high saturated fat intakes, obesity, smoking and lack of exercise, there is a clear distinction between this and Metabolic Syndrome X as in HIV-associated lipodystrophy there is concurrent depot specific fat accumulation and fat wasting. This would suggest that use of HAART in HIV infection results in altered adipocyte metabolism.

In order to investigate peripheral clearance and uptake of lipid from the circulation, stable isotope labelled fatty acids were given to healthy and HIV subjects with and without HAART and with lipodystrophy associated with specific drug combinations. The results suggest that while HIV infection is associated with delayed clearance of lipid from the circulation, the use of HAART exacerbates this process. In addition, it appears that while PI drugs may delay adipocyte clearance of triglyceride from the circulation, NRTI drugs may reduce adipocyte free fatty acid uptake.

An audit of patients with more than six months exposure to HAART (n=545) showed that 25% of patients had elevated plasma lipid, 10% had elevated plasma glucose, but only 9% had both. While this data did not show an association between specific drug classes and elevated plasma lipid or glucose, there was an association with the duration of therapy. Therefore, it is possible that HAART contributes to the development of HIV-lipodystrophy, but that the cause of this syndrome and the metabolic perturbations are multifactorial.

For Kevin...

"Atque in pepetuum, frater, ave atque vale".
Catullus (c. 84-c. 54_{BC})

Contents

Title.....I
Statement.....II
Abstract.....III
Dedication.....IV
Contents.....V
List of Tables.....XII
List of Figures.....XIX
Publications.....XXV
Acknowledgements.....XXVI
Main abbreviations used in the text.....XXVII

CHAPTER 1. INTRODUCTION
1.1 Background to research.....1
1.2 Outline of thesis.....3

CHAPTER 2. REVIEW OF LITERATURE
2.1 Introduction.....4
2.2 HIV infection and life cycle.....5
2.3 Postprandial lipid metabolism in healthy individuals.....9
2.3.1 Digestion.....9
2.3.2 Absorption.....10
2.3.3 Plasma triacylglycerol.....10
2.3.4 Lipoprotein classification.....11
2.3.5 Entrapment.....14
2.3.6 TAG rich lipoprotein hydrolysis.....16
2.3.7 Fatty acid transport across cell membranes.....16
2.3.7.1 Plasma membrane fatty acid binding protein (FABPpm).....16
2.3.7.2 Fatty acid translocase (FAT/CD36).....17
2.3.7.3 Fatty acid transport proteins.....17
2.3.7.4 Caveolin and caveolae.....18
2.3.8 The fate of the fatty acid within the cell.....18
2.3.8.1 Fatty acid oxidation.....19

2.3.8.2 Re-esterification of fatty acid within the adipocyte.....22
2.3.9 Fatty acid within the circulation.....22
2.3.9.1 Free fatty acids and hepatic lipoprotein synthesis.....23
2.3.9.2 Free fatty acids and insulin secretion.....23
2.4 Stable isotope tracers in postprandial lipid studies.....24
2.5 The host response to HIV infection – The Pre-HAART era.....27
2.5.1 The host immune response.....27
2.5.1.1 Natural immunity.....27
2.5.1.2 Acquired immunity.....27
2.5.1.3 Immunity during the asymptomatic period.....28
2.5.1.4 Immunity during the symptomatic period.....28
2.5.2 Host metabolic response.....31
2.5.2.1 HIV infection and body composition.....32
2.5.2.2 HIV infection and energy expenditure.....33
2.5.2.3 HIV infection and gut function.....34
2.5.2.4 HIV infection, the HPA axis and endocrine disturbances.....35
2.5.2.5 HIV infection and protein turnover.....36
2.5.2.6 HIV infection and glucose homeostasis.....37
2.5.2.7 HIV infection and lipid metabolism.....37
2.5.2.8 Summary of the metabolic effects of HIV Pre-HAART.....39
2.6 Current antiretroviral therapies.....39
2.7 Lipodystrophy.....40
2.7.1 Features of Lipodystrophy.....40
2.7.2 The prevalence of Lipodystrophy.....41
2.7.3 Clinical outcomes of Lipodystrophy.....42
2.7.4 Clinical assessment of Lipodystrophy.....42
2.7.5 Possible therapies to treat Lipodystrophy.....45
2.8 Summary of literature and hypothesis.....46

CHAPTER 3. GENERAL METHODS

3.1 Introduction.....48
3.2 Subjects.....48
3.3 Study protocol.....49
3.4 Stable isotopes as *in vivo* tracers.....50

3.4.1 Units of notation....50
3.4.2 Theory....51
3.5 [1-^{13}C] Palmitic acid in emulsion....52
3.6 The test meal....52
3.7 Breath sampling....53
3.7.1 Breath collection and preparation....53
3.7.2 Breath analysis....53
3.8 Blood sampling....56
3.8.1 Blood collection and preparation....56
3.8.2 Plasma lipid analysis....56
3.8.2.1 Lipid extraction from plasma....56
3.8.2.2 Isolation of TAG and NEFA fractions by solid phase extraction....57
3.8.2.3 Methylation to fatty acid methyl esters....58
3.8.2.4 Gas chromatography combustion isotope ratio mass spectrometry....58
3.8.2.5 Determination of TAG and NEFA recovery from plasma....61
3.8.2.6 Calculation of NEFA concentration....61
3.8.2.7 Calculation of TAG concentration....61
3.8.2.8 Calculation of the [^{13}C] enrichment of palmitic acid in TAG and NEFA....62
3.8.3 Plasma insulin and glucose analysis....65
3.9 Indirect calorimetry....65
3.10 Anthropometry....69
3.10.1 A test of measurement variability within an individual....70
3.10.2 A test of biological variability within an individual....71
3.11 Statistical analysis....76

CHAPTER 4. POSSIBLE FACTORS ASSOCIATED WITH AND THE PREVALENCE OF FASTING PLASMA TRIGLYCERIDE AND GLUCOSE CONCENTRATION IN AN AUDIT OF HAART TREATED PATIENTS

4.1 Introduction....78
4.2 Aims....79
4.3 Methods....80
4.3.1 Data collection and analysis....80
4.4 Results....80

4.4.1 Patient characteristics....80
4.4.2 Patient age, plasma TAG and glucose concentration....80
4.4.3 Patient gender and plasma TAG and glucose concentration....81
4.4.4 Duration of therapy and plasma TAG and glucose concentration....81
4.4.5 Type of therapy and plasma TAG and glucose concentration....81
4.4.6 Viral load and plasma TAG concentration....82
4.4.7 Plasma glucose and TAG concentration....82
4.4.8 Multivariate analysis....83
4.5 Discussion....102
4.5.1 Patient age and plasma TAG and glucose concentrations....102
4.5.2 Patient gender and plasma TAG and glucose concentrations....102
4.5.3 Duration and type of therapy and plasma TAG and glucose Concentrations....103
4.5.4 Viral load and plasma TAG concentration....105
4.5.5 Comparison of the audit population to the study groups....105
4.6 Summary....106

CHAPTER 5. A COMPARISON OF POSTPRANDIAL LIPID METABOLISM IN HIV-POSITIVE THERAPY NAÏVE MALES AND HIV-NEGATIVE MALES
5.1 Introduction....108
5.2 Aims....108
5.3 Methods....109
5.3.1 Subjects....109
5.4 Results....110
5.4.1 Subject characteristics....110
5.4.2 Body composition....110
5.4.3 Energy expenditure....111
5.4.4 Substrate Oxidation....111
5.4.4.1 Endogenous and exogenous lipid oxidation....111
5.4.5 Energy derived from substrates in the postprandial period....112
5.4.6 Plasma triacylglycerol concentration....112
5.4.7 Plasma [^{13}C] triacylglycerol concentration....112
5.4.8 Plasma non-esterified fatty acid....113
5.4.9 Plasma [^{13}C] non-esterified fatty acid concentration....113

5.4.10 Plasma insulin and glucose concentrations....113
5.5 Discussion....139
5.5.1 Subject characteristics and body composition....139
5.5.2 Energy expenditure....140
5.5.3 Substrate oxidation....140
5.5.4 Plasma TAG and NEFA concentration and [^{13}C]enrichment....141
5.5.5 Plasma insulin and glucose concentration....142
5.5.6 Summary....143

CHAPTER 6. A TRIAL COMPARING POSTPRANDIAL LIPAEMIA IN HIV POSITIVE PATIENTS PRIOR TO HAART AND AT 1 MONTH AND 3 MONTHS FOLLOWING THE INITIATION OF HAART INCLUDING A PI

6.1 Introduction....144
6.2 Aims....144
6.3 Methods....145
6.3.1 Subjects....145
6.3.2 Statistical analysis....145
6.4 Results....146
6.4.1 Subject characteristics....146
6.4.2 Body composition....146
6.4.3 Energy expenditure....146
6.4.4 Substrate Oxidation....147
6.4.5 Plasma triacylglycerol concentration....147
6.4.6 Plasma [^{13}C] triacylglycerol concentration....147
6.4.7 Plasma NEFA and [^{13}C] NEFA concentration....148
6.4.8 Plasma insulin and glucose concentration....148
6.5 Discussion....172
6.6 Summary....173

CHAPTER 7. A STUDY TO INVESTIGATE THE INFLUENCE OF COMBINATION THERAPY INCLUDING A PI COMPARED WITH AN ANTIRETROVIRAL DRUG REGIMEN EXCLUDING A PI ON POSTPRANDIAL LIPID METABOLISM IN LIPODYSTROPHIC HIV MALES

7.1 Introduction....175
7.2 Aims....176
7.3 Methods....176
7.3.1 Subjects....176
7.4 Results....176
7.4.1 Subject characteristics....176
7.4.2 Body composition....177
7.4.3 Energy expenditure....177
7.4.4 Substrate oxidation....178
7.4.5 Plasma TAG concentration....178
7.4.6 Plasma [^{13}C] TAG concentration....178
7.4.7 Plasma NEFA concentration....179
7.4.8 Plasma [^{13}C] NEFA concentration....179
7.4.9 Plasma insulin and glucose concentration....179
7.5 Discussion....205
7.5.1 Subject characteristics and body composition....205
7.5.2 Energy expenditure and substrate oxidation....206
7.5.3 Plasma TAG and NEFA concentration and [^{13}C] enrichment....206
7.6 Summary....207

CHAPTER 8. COMBINED SUMMARY OF DATA

8.1 Audit of HIV-positive population on first HAART regimen....209
8.2 HIV-positive males compared with healthy males of similar age....210
8.3 Short term use of PI/NRTI combination....211
8.4 NRTI lipodystrophy compared with PI/NRTI lipodystrophy....212

CHAPTER 9. GENERAL DISCUSSION

9.1 Currently proposed mechanisms for lipodystrophy development.....225

9.1.1 Mechanisms for NRTI induced changes in free fatty acid uptake by the adipocyte.....228

9.1.1.1 Direct effects.....228

9.1.1.2 Indirect effects.....229

9.1.2 Mechanisms for PI induced changes in TAG clearance.....231

9.1.2.1 Direct effects.....231

9.1.2.2 Indirect effects.....232

9.2 Summary and conclusion.....234

CHAPTER 10. APPENDICES

1.0 Patient information sheet.....237

2.0 Study protocol.....238

2.1 Study timeline.....239

3.0 Blood preparation schema.....240

4.0 Bond elut schema.....241

5.0 Addendum following oral examination.....242

5.1 How do current case definitions of lipodystrophy compare with the patients recruited in these studies?.....242

5.2 How does the HIV virus enter the host cell & replicate?.....244

5.3 How do the antiretroviral drugs interact with HIV replication and Could toxicity result?.....248

5.4 Does this work support or refute the mitochondrial toxicity hypothesis?..250

5.5 Do alterations in androgen levels play a role in the aetiology of lipodystrophy?.....253

CHAPTER 11. REFERENCE LIST.....254

List of Tables

Table 2.1 CD4 count and stage of HIV infection.....7

Table 2.2 The lipoprotein properties used to identify lipoproteins.....12

Table 2.3 Cytokine sources and actions.....30

Table 2.4 Preliminary classification of the HIV-associated syndrome.....44

Table 3.1 A test of within and between run variability in breath delta value.....54

Table 3.2 The temperature programme and settings for the GC-C-IRMS.....60

Table 3.3 A test of within sample variability in fasting plasma TAG and NEFA recovery, concentration and enrichment.....63

Table 3.4 A test of within sample variability in postprandial plasma TAG and NEFA recovery, concentration and enrichment.....64

Table 3.5 The volume of O_2 consumed and CO_2 produced in the oxidation of Various fuels.....68

Table 3.6 A test of within individual variability in height, weight, triceps skinfold, mid upper arm circumference, waist circumference, hip circumference and the calculated waist:hip ratio.....72

Table 3.7 A test of within individual variability in bioelectrical impedance (Bodystat 1500).....73

Table 3.8 A test of within individual biological variability in height, weight, triceps skinfold, mid upper arm circumference, waist circumference, hip circumference and the calculated waist:hip ratio.....74

Table 3.9 A test of within individual biological variability in bioelectrical impedance (Bodystat 1500).....75

Table 4.1 Distribution of HAART therapy types and plasma TAG concentration – Data from an audit of 545 HIV-positive patients on HAART.....84

Table 4.2 Distribution of HAART therapy types and plasma glucose Concentration.....85

Table 4.3 Distribution of plasma TAG and glucose concentration – Data from an audit of 405 HIV-positive patients on HAART.....86

Table 4.4 Distribution of hypertriglyceridaemia with and without concurrent hyperglycaemia and analysis of plasma viral load in each treatment group – Data from an audit of 545 HIV-positive patients on HAART.....87

Table 4.5 Patient age and duration of therapy in each treatment group – Data from an audit of 410 HIV-positive patients on HAART.....88

Table 5.1 Viral load, CD4 and CD8 counts of the HIV-positive therapy naïve Group.....114

Table 5.2 Characteristics of subjects including age, height and weight in the HIV-positive and HIV-negative groups.....115

Table 5.3 Results of the bioelectrical impedance analysis in the HIV-positive and HIV-negative groups.....116

Table 5.4 Anthropometry measurements in the HIV-positive and HIV-negative groups.....117

Table 5.5 Resting energy expenditure in the HIV-positive and HIV-negative Groups.....118

Table 5.6 Respiratory exchange ratio and lipid and carbohydrate oxidation prior to the testmeal in the HIV-positive and HIV-negative groups.....119

Table 5.7 Lipid and carbohydrate oxidation during the postprandial period in the HIV-positive and HIV-negative groups.....120

Table 5.8 The calculation of the endogenous and exogenous sources of lipid oxidised over the 7 hour period using the percentage of ^{13}C label recovered on breath over 7 hours in the HIV-positive and HIV-negative groups.....121

Table 5.9 Calculation of the proportion of energy expended over the 7 hour period derived from carbohydrate, endogenous lipid and exogenous lipid oxidation in the HIV-positive and HIV-negative groups.....122

Table 5.10 Fasting plasma TAG expressed as mmol/l and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the area under the curve in the HIV-positive and HIV-negative groups.....123

Table 5.11 Fasting and postprandial plasma NEFA concentration in the HIV-positive and HIV-negative groups.....124

Table 5.12 [^{13}C] palmitic acid in plasma TAG and NEFA fractions in the postprandial period in the HIV-positive and HIV-negative groups.....125

Table 5.13 Plasma insulin and glucose concentration in the postprandial and post-absorptive period in the HIV-positive and HIV-negative groups.....126

Table 6.1 Viral load, CD4 and CD8 counts in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....149

Table 6.2 Therapy details of the HAART combination used in the previously therapy naïve group during the three month study period.....150

Table 6.3 Characteristics of subjects including age, height and weight in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....151

Table 6.4 Results of the bioelectrical impedance analysis in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....152

Table 6.5 Anthropometry measurements in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....153

Table 6.6 Resting energy expenditure in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....154

Table 6.7 Respiratory exchange ratio and lipid and carbohydrate oxidation prior to the test meal in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....155

Table 6.8 Lipid and carbohydrate oxidation during the postprandial period in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....156

Table 6.9 The calculation of the endogenous and exogenous sources of lipid oxidised over the 7 hour period using the percentage of ^{13}C label recovered on breath over 7 hours in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....157

Table 6.10 Calculation of the proportion of energy expended over the 7 hour period derived from carbohydrate, endogenous lipid and exogenous lipid oxidation in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....158

Table 6.11 Fasting plasma TAG expressed as mmol/l and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the area under the curve in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....159

Table 6.12 Fasting plasma NEFA concentration and postprandial plasma NEFA concentration calculated from AUC in the HIV positive group prior to therapy, and at one month and three months following HAART Initiation.....160

Table 6.13 [^{13}C] palmitic acid in plasma TAG and NEFA fractions in the postprandial period in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....161

Table 6.14 Fasting and postprandial plasma insulin and glucose concentrations in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....162

Table 7.1 Viral load, CD4 and CD8 counts of subjects in the PI-lipodystrophy group compared to the DN-lipodystrophy group.....180

Table 7.2 Therapy details at the time of study and previous therapy exposure of the PI-lipodystrophy group and the DN-lipodystrophy group.....181

Table 7.3 Characteristics of the PI-lipodystrophy group and the DN-lipodystrophy group including age, height, weight, BMI and smoking habits.....183

Table 7.4 Results of the bioelectrical impedance analysis in the PI-lipodystrophy and the DN-lipodystrophy groups 184

Table 7.5 Results of anthropometric measures in the PI-lipodystrophy and the DN-lipodystrophy groups.....185

Table 7.6 Resting energy expenditure measured by indirect calorimetry in the PI-lipodystrophy and the DN-lipodystrophy groups.....186

Table 7.7 Respiratory exchange ratio and postabsorptive substrate oxidation calculated from VCO_2 and VO_2 measured by indirect calorimetry in the PI-lipodystrophy and the DN-lipodystrophy groups.....187

Table 7.8 Lipid and carbohydrate oxidation over the postprandial period in the PI-lipodystrophy and the DN-lipodystrophy groups, expressed as grams per 7 hours, calculated by area under the curve and also expressed as the incremental area under the curve, showing the change from baseline in the postprandial period.....188

Table 7.9 The calculation of the endogenous and exogenous sources of lipid oxidised over the 7 hour period using the percentage of ^{13}C label recovered on breath over 7 hours, together with the amount of lipid in the test meal and the total lipid oxidised over the 7 hour period, calculated from the area under the curve in the PI-lipodystrophy and the DN-lipodystrophy groups.....189

Table 7.10 Fasting plasma TAG expressed as mmol/l and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the area under the curve in the PI-lipodystrophy and the DN-lipodystrophy groups.....190

Table 7.11 Plasma NEFA concentration fasting and over the 7 hour period in the PI-lipodystrophy and the DN-lipodystrophy groups.....191

Table 7.12 Plasma [13C] palmitic acid in TAG and NEFA over the 7 hour period in the PI-lipodystrophy and the DN-lipodystrophy groups.....192

Table 7.13 Fasting and postprandial plasma insulin and glucose in the PI-lipodystrophy and the DN-lipodystrophy groups.....193

Table 8.1 Comparison of subject characteristics in all groups.....214

Table 8.2 Comparison of energy expenditure, respiratory exchange ratio and oxidation data in the postabsorptive and postprandial period in all groups.....215

Table 8.3 Comparison of plasma TAG, NEFA, insulin and glucose in the postabsorptive and postprandial period between all groups.....216

List of Figures

Figure 2.1 The replication cycle of a retrovirus.....8

Figure 2.2 Endogenous and exogenous lipid metabolism.....13

Figure 2.3 The principle of fatty acid entrapment.....15

Figure 2.4 Proposed regulation of muscle fatty acid oxidation.....21

Figure 2.5 The principle of using stable isotope labelled fatty acid tracer [1-^{13}C] palmitic acid to determine the metabolic fate of dietary lipid.....26

Figure 2.6 Acquired immunity in response to antigenic stimulation.....29

Figure 3.1 The equation of Watkins et al. 1982 to calculate the percentage of administered dose per hour that is excreted on breath as $^{13}CO_2$.....55

Figure 4.1 The distribution of patient ages - Data from an audit of 545 HIV-positive patients on HAART.....89

Figure 4.2 Distribution of ethnic background - Data from an audit of 545 HIV-positive patients on HAART.....90

Figure 4.3 Association between patient age and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....91

Figure 4.4 Association between patient age and plasma glucose concentration - Data from an audit of 545 HIV-positive patients on HAART.....92

Figure 4.5 Association between patient gender and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....93

Table 4.6 Association between patient gender and plasma glucose concentration - Data from an audit of 545 HIV-positive patients on HAART.....94

Figure 4.7 Association between duration of therapy and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....95

Figure 4.8 Association between duration of therapy and plasma glucose concentration - Data from an audit of 545 HIV-positive patients on HAART.....96

Figure 4.9 Association between therapy type and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....97

Figure 4.10 Association between therapy type and plasma glucose concentration - Data from an audit of 545 HIV-positive patients on HAART.....98

Figure 4.11 Association between viral load and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....99

Figure 4.12 Association between plasma glucose and plasma TAG concentration - Data from an audit of 545 HIV-positive patients on HAART.....100

Figure 4.13 Association between plasma glucose and plasma TAG concentration by therapy type - Data from an audit of 545 HIV-positive patients on HAART.....101

Figure 5.1 Median carbohydrate oxidation during the postprandial period for the HIV-positive and HIV-negative groups.....127

Figure 5.2 Median lipid oxidation during the postprandial period for the HIV-positive and HIV-negative groups.....128

Figure 5.3 Endogenous lipid oxidation during the postprandial period for the HIV-positive and HIV-negative groups.....129

Figure 5.4 Exogenous lipid oxidation during the postprandial period for the HIV-positive and HIV-negative groups.....130

Figure 5.5 Plasma triacylglycerol concentration (mmol/l) during the postprandial period for the HIV-positive and HIV-negative groups.....131

Figure 5.6 Plasma [^{13}C]triacylglycerol concentration (μg/ml) during the postprandial period for the HIV-positive and HIV-negative groups.....132

Figure 5.7 Plasma non-esterified fatty acid concentration (mmol/l) during the postprandial period for the HIV-positive and HIV-negative groups.....133

Figure 5.8 Plasma [^{13}C] non-esterified fatty acid concentration (μg/ml) during the postprandial period for the HIV-positive and HIV-negative groups.....134

Figure 5.9 Fasting plasma TAG concentration and postprandial TAG AUC HIV-positive and HIV-negative groups.....135

Figure 5.10 The association between viral load, CD4 count and BMI in the HIV-positive group.....136

Figure 5.11 Plasma insulin concentration during the postprandial period in the HIV-positive and HIV-negative groups.....137

Figure 5.12 Plasma glucose concentration during the postprandial period in the HIV-positive and HIV-negative groups.....138

Figure 6.1 Resting energy expenditure in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....163

Figure 6.2 The pattern of lipid oxidation over the postprandial period in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....164

Figure 6.3 The pattern of carbohydrate oxidation over the postprandial period in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....165

Figure 6.4 The percentage of energy derived from carbohydrate, endogenous and exogenous lipid oxidation in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....166

Table 6.5 The pattern of change in plasma triacylglycerol over the 7 hour period following administration of the test meal, expressed as mmol/l, in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....167

Figure 6.6 The pattern of change in plasma [^{13}C]triacylglycerol over the hour period following administration of the test meal, expressed as μg/ml, in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....168

Figure 6.7 The pattern of change in plasma non-esterified fatty acid concentration over the 7 hour period following administration of the test meal, expressed as mmol/l, in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....169

Figure 6.8 The pattern of change in plasma [^{13}C]non-esterified fatty acid concentration over the 7 hour period following administration of the test meal, expressed as μg/ml, in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....170

Figure 6.9 The pattern of change in plasma insulin and glucose concentrations over the 7 hour period following administration of the test meal, in the HIV positive group prior to therapy, and at one month and three months following HAART initiation.....171

Figure 7.1 Median carbohydrate oxidation during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....194

Figure 7.2 Median lipid oxidation during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....195

Figure 7.3 Recovery of the label on breath in the PI-lipodystrophy and the DN-lipodystrophy groups.....196

Figure 7.4 Endogenous lipid oxidation over the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....197

Figure 7.5 Exogenous lipid oxidation over the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....198

Figure 7.6 Plasma triacylglycerol concentration (mmol/l) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....199

Figure 7.7 Plasma [^{13}C]triacylglycerol concentration (μg/ml) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....200

Figure 7.8 Plasma non-esterified fatty acid concentration (mmol/l) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....201

Figure 7.9 Plasma [^{13}C] non-esterified fatty acid concentration (μg/ml) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....202

Figure 7.10 Plasma insulin concentration (mU/l) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....203

Figure 7.11 Plasma glucose concentration (mmol/l) during the postprandial period for the PI-lipodystrophy and the DN-lipodystrophy groups.....204

Figure 8.1 Postprandial plasma TAG in the HIV-, HIV+, DNL and PIL groups.....217

Figure 8.2 Postprandial plasma [^{13}C]-TAG in the HIV-, HIV+, DNL and PIL Groups.....218

Figure 8.3 Postprandial plasma [^{13}C]-TAG AUC in the HIV-, HIV+, DNL and PIL groups.....219

Figure 8.4 Postprandial plasma NEFA in the HIV-, HIV+, DNL and PIL groups....220

Figure 8.5 Postprandial plasma [^{13}C]-NEFA in the HIV-, HIV+, DNL and PIL Groups.....221

Figure 8.6 Postprandial plasma [^{13}C]-NEFA AUC in the HIV-, HIV+, DNL and PIL groups.....222

Publications

Ware LJ, Morlese J, Burdge GC, Gazzard BG, Wootton SA and Jackson AA. Protease inhibitor associated lipodystrophy in HIV-seropositive men increases postprandial lipid oxidation. *Proceedings of the Nutrition Society*, vol. 59, OCA Spring 2000, 44A.

Ware LJ, Morlese J, Burdge GC, Gazzard BG, Burdge GC, Jackson AA and Wootton SA. Highly active antiretroviral therapy (HAART) in Human Immunodeficiency virus-1 positive men increases the retention time of dietary lipid within the circulation. *Proceedings of the Nutrition Society*, vol. 60, OCA Spring 2001, 31A.

Ware LJ, Morlese J, Gazzard BG, Wootton SA and Jackson AA. Differences in postprandial lipid metabolism in patients with PI-associated and NRTI-associated lipodystrophy. Antiviral Therapy, 2000; 5(suppl 5):13.

Ware LJ, Wootton SA, Morlese J, Gazzard BG and Jackson AA. The paradox of improved antiretroviral therapy in HIV – potential for nutritional modulation. *Proceedings of the Nutrition Society*, 2002; 61(1); 131-136.

Acknowledgements

This work could not have been completed without the efforts of both the team at the Chelsea and Westminster Hospital, particularly Dr John Morlese, and the team at Southampton. All of my colleagues have had some input into this project at some point and the work could not have progressed if it were not for the patience of Dr Graham Burdge in explaining again and again how a GCIRMS works. I also have to thank Dr Amanda Jones and Dr Janet Bennoson for passing on their expertise in lipid research after completing their own PhD theses in this area.

If it weren't for the endless enthusiasm of my supervisor Dr Steve Wootton, this programme of work would probably not have started. For his continued enthusiasm, support and friendship throughout the last five years, I am grateful. Also, I would like to thank Professor Alan Jackson for all he has taught me both professionally and personally. I have to thank Angela May, Dorris Ng, Rachel Sherman, Abbie Cawood, Gary Constable, Angela Hounslow, and Rebecca Dunn for supplying copious amounts of cheer, hugs (and alcohol) when times were tough.

My gratitude also goes to Janice Taylor who always has an ear to listen, a shoulder to cry on and who provides continued support and friendship. I would also like to express my gratitude to Dr John Jackson for being an eternal fountain of knowledge.

Finally, credit must also go to the subjects who gave their time so freely to take part in these trials. While this work has barely scratched the surface of the issue of lipodystrophy for the HIV-positive population, it may act as a building block in the foundations of the work necessary to solve this problem, so that both safe and effective drugs may be developed for the management of this terrible disease in the hope that a cure will be found one day.

Main Abbreviations Used in the Text

3TC	Lamivudine (NRTI)
AIDS	acquired immunodeficiency syndrome
AUC_i	area under the curve measured from baseline
AUC_0	area under the curve measured from zero
AZT	Zidovudine (NRTI)
BMI	body mass index
BMR	basal metabolic rate
CHO	carbohydrate
CM	chylomicron
CO_2	carbon dioxide
CTL	control group
CVD	cardiovascular disease
d4T	Stavudine (NRTI)
ddC	Zalcitibane (NRTI)
ddI	didanosine (NRTI)
DN	dual nucleoside
FFA	free fatty acid
FFM	fat free mass
GC	gas chromatography
GI	gastrointestinal
HAART	highly active anti-retroviral therapy
HDL	high density lipoprotein
HIV	human immunodeficiency virus
HSL	hormone sensitive lipase
IDL	intermediate density lipoprotein
IND	Indinavir (PI)
IRMS	isotope ratio mass spectrometry
LDL	low density lipoprotein
LPL	lipoprotein lipase enzyme
LYP	lipodystrophy
MAG	monoacylglycerol
MUFA	monounsaturated fatty acid
NEFA	non-esterified fatty acid
NIDDM	non-insulin dependent diabetes
NRTI	nucleoside reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine (NNRTI)
O_2	oxygen
PDB	pee dee belemnite
PI	protease inhibitor
PIN	protease inhibitor naïve
PUFA	polyunsaturated fatty acid
RTV	Ritonavir (PI)
SFA	saturated fatty acid
SQV	Saquinavir (PI)

TAG	triacylglycerol
TRL	triglyceride rich lipoprotein
VLDL	very low density lipoprotein
VO_2	whole body oxygen consumption
VCO_2	whole body breath carbon dioxide excretion

Chapter 1 Introduction

1.1 Background to research

In a healthy individual, metabolism is a demand led process. While demand may differ between tissues and over time, the demand must be met in order to maintain structure and function. This demand is satisfied by the availability of nutrients within a circulating pool. The concentration of macronutrients within this pool is maintained within defined levels at all times such that the rate of entry into the pool matches the rate of removal from the pool into the tissues. Therefore, concentration is kept constant despite marked changes in flux or input.

Input is derived from a combination of dietary supply (and availability) and metabolic transformations within the body (to improve the goodness of fit between supply and demand). The relative contribution from each input will change during fasting and feeding. The consequences of a mismatch would be reflected as a change in concentration within the circulating pool, with consequential changes in structure and function.

Infection is associated with a re-ordering of priorities mediated by the infective response. There is a shift in the balance of a pro and anti-inflammatory state, primarily mediated by cytokines with endocrine modulation. This results in a change in dietary supply, metabolic transformations, mobilisation of endogenous stores or the magnitude of demands. If these changes can be accommodated within the normal control, concentration remains constant i.e. if changes are modest. If the changes are too great, this is reflected as a change in circulating macronutrients with deleterious effects. The response to infection is variable between and within individuals and is dependent on infective load and moderated by genotype, programming in early life, lifestyle, plane of nutrition (diet) and body habitus. If left untreated, a change in structure and function can occur, which impacts on morbidity and mortality.

The goal of treatment in HIV is to suppress infective burden, modulate the cytokine response, re-order priorities and re-establish control such that circulating macronutrients return to the normal range. The result is a new state, which reflects the lower infective burden, but this is dependent on the effectiveness of therapy, and may be reflected in the circulating macronutrients. However, therapy may also exert direct effects independent from the effects associated with the reduction of infective burden, either by altering the locus of control or the response to the control system, or via a direct toxic effect. Therapy

results in an altered state, which may influence structure and function with a consequential impact on morbidity and mortality.

This paradigm is exhibited in HIV patients presenting with profound disturbances in macronutrient metabolism expressed as markedly increased plasma triglycerides and glucose, insulin resistance and characteristic changes in body habitus. This is important as the development of these characteristics, termed collectively as "Lipodstrophy", impacts on compliance with therapy and potentially increases the risk of pancreatitis and cardiovascular disease. While the literature contains much descriptive information on this syndrome, it appears that the characterisation of this alteration in macronutrient metabolism is poor.

The purpose of this programme of research is to explore the processes that regulate macronutrient metabolism, specifically lipid metabolism as the most obvious derangements are reflected by changes in body fat mass and distribution, and circulating lipid concentrations. By studying this we will achieve an improved understanding of how these processes are normally controlled and how they may be altered in response to infection and treatment. This may help us establish; how control is achieved, factors that effect control, the mechanism of control, identify when control is lost; predict how therapeutic interventions may affect control and identify ways of improving therapy by moderating these effects or developing new drugs. This work is not only of value to HIV, but also other metabolic disorders of lipid metabolism that may be mediated through common pathways.

As a first step, the aim of the studies reported in this thesis is to use stable isotope methods developed at the Institute of Human Nutrition to measure the metabolic disposal of [1-^{13}C] palmitic acid, administered in a standardised meal, in order to determine the effect of HIV and antiretroviral therapy upon; (1) the contribution of dietary lipid to the postprandial plasma triacylglycerol concentration, (2) the contribution of dietary lipid to the postprandial plasma non-esterified fatty acid concentration and (3) the extent to which the lipid from the meal is oxidised.

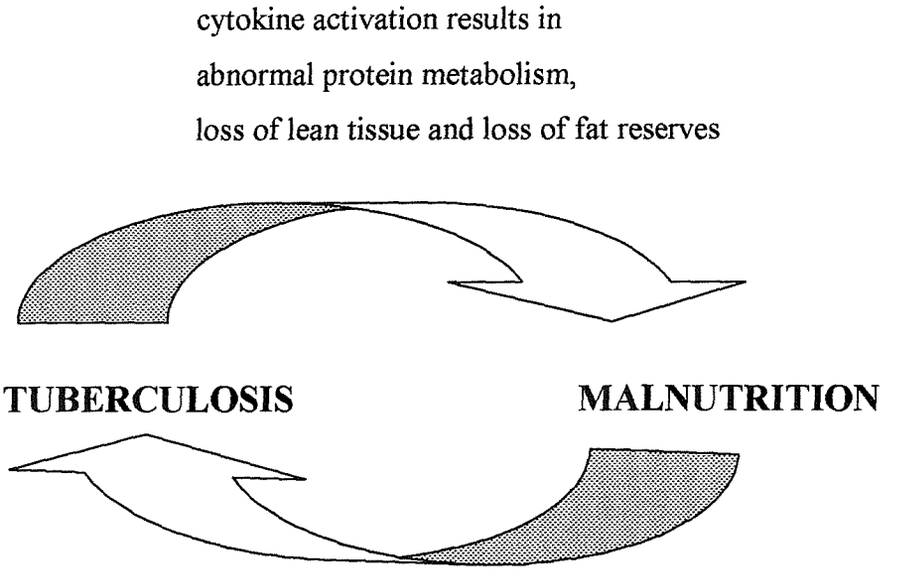
1.2 Outline of Thesis

This thesis describes work carried out to characterise the metabolic disposal of dietary lipid in current and developing hyperlipidaemia associated with HIV infection and the use of the two most predominant antiretroviral drug classes, protease inhibitors (PI) and nucleoside reverse transcriptase inhibitors (NRTI). The report is divided into chapters, starting with a review of the literature describing lipid metabolism in healthy individuals and disease, what is already known of disordered lipid metabolism in HIV and lipodystrophy and how stable isotopes can be used to study this (Chapter 2); the methods used to facilitate the research (Chapter 3); an audit of 545 HIV-positive males to determine factors associated with and the prevalence of metabolic alterations (Chapter 4); the results of a study comparing postprandial lipaemia in HIV-positive to HIV-negative males (Chapter 5); the results of a study examining alterations in postprandial lipaemia before and after short-term HAART exposure in an HIV-positive intervention trial (Chapter 6); a comparison of different HAART therapies on postprandial lipaemia (Chapter 7); and a general discussion (Chapter 8).

Chapter 2 Review of the literature

2.1 Introduction

It has long since been recognised that infection can alter the plane of nutrition and vice versa. HIV in Africa is often referred to as "Slim Disease" as it results in a severe wasting of the body [Serwadda et al. 1985]. Where Tuberculosis (TB) and HIV co-exist in a patient, the magnitude of wasting is further elevated. A mechanism for this interaction between infection and nutrition has been proposed by Macallan (1999) i.e.



This suggests that the way in which infection alters the host plane of nutrition is mediated principally by cytokine activation, occurring as part of the host immune response to the invading pathogen. This can result in increased energy expenditure, reduced energy intake (anorexia) and altered substrate metabolism. In the example of TB, treatment with chemotherapy may lead to a repletion of nutritional status fuelled either by an increased energy intake, reduced energy demands or by more efficient substrate utilisation [Macallan 1999a].

Therefore, it could be postulated that a reduction in viral burden during HIV infection would result also in a nutritional repletion with increased energy intake, reduced energy demands and improved substrate utilisation. However, in some effectively treated

HIV-positive patients, a reduction in viral load is coupled with a wasting of fat tissue in the periphery, a central accumulation of body fat, elevated plasma glucose and lipid and insulin resistance. It is unclear if this "Lipodystrophy" syndrome is a result of ameliorated infective load, a direct toxic effect of the antiretroviral drugs used or an interaction of both.

This literature review is aimed at providing the reader with a basic understanding of the HIV virus and how it interacts with the host. As this thesis is specifically aimed at investigating the deranged lipid metabolism observed in this population and in order to better understand lipid metabolism per se, this chapter includes a review of lipids and what is known of how lipids are metabolised in healthy subjects. Once lipid metabolism has been reviewed, the chapter discusses the immune and metabolic responses of the host to HIV infection with specific reference to alterations in the handling of lipid. The classes of antiretroviral drugs currently available and sites at which these work is also then discussed, followed by a more detailed presentation of the literature published on Lipodystrophy syndrome.

2.2 HIV infection and life cycle

The Human Immunodeficiency Virus is of the subfamily *Lentivirinae* and is a retrovirus containing ribonucleic acid (RNA) instead of deoxyribonucleic acid (DNA). A viral reverse transcriptase enzyme synthesises viral DNA from the viral RNA upon cell infection (Figure 2.1). The provirus or viral DNA becomes incorporated into the host DNA and either remains latent, being passed to daughter cells as the host cell replicates or replicates within the cell to produce large numbers of viral proteins which assemble to form virions (immature virus particles). After release from the cell, the virions mature and infect other cells, repeating the process.

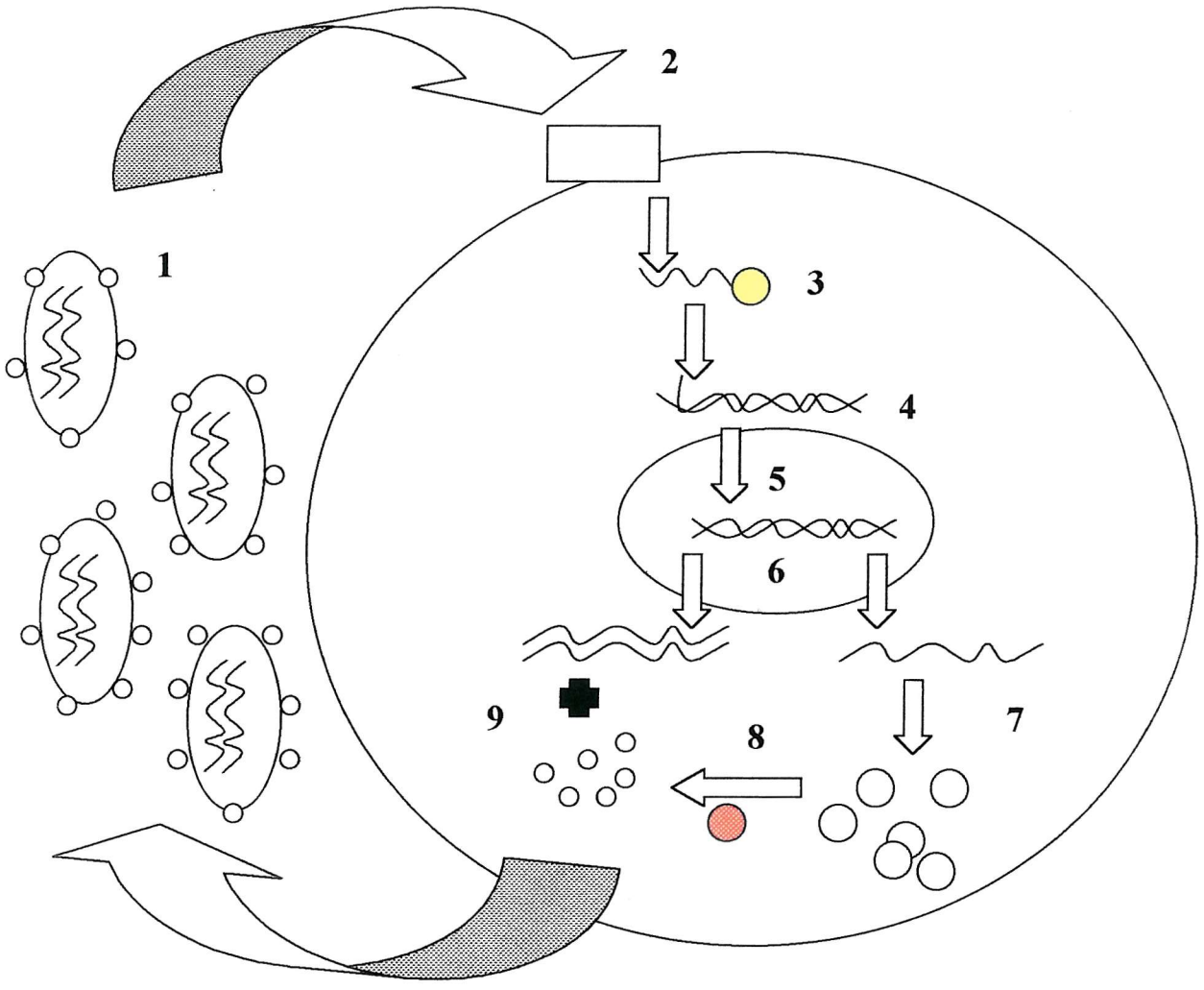
HIV infection is transmitted through sexual contact, injecting drug use, receipt of contaminated blood or blood products and from mother to infant, mainly through childbirth and possibly breastfeeding where the virus is carried in infected CD4 T cells or as free virus in blood, semen, vaginal fluid or milk. All routes of infection eventually result in the virus being established within lymph nodes [Gotch 1999]. The HIV-1 virus has a specific affinity for CD4 T lymphocytes (a subset of T cells that express the CD4 molecule on the cell surface) causing a reduction in CD4 T cell population and function. However, binding of the HIV virus to CD4 surface protein is not sufficient to allow the virus to enter the cell, secondary coreceptors, such as transmembrane G-coupled receptors are required. Seven coreceptors have been identified and include CCR5, CCR2b and CCR3 (all β-chemokine

receptors), and CXCR4 (an α-chemokine receptor). Thus β-chemokines such as RANTES may play a role in resistance to infection through competition for receptor binding [Gotch 1999]. The CD4 T cells are helper T cells, which activate both the B cell and cytotoxic T cell immune responses. The function of the T cell surface receptor is to bind antigens that are complexed with the body's own plasma membrane proteins called major histocompatability complex (MHC) proteins. There are two classes of MHC proteins, class I (found on the surface of all nucleated cells) and class II (only found on the surface of macrophages, B lymphocytes and several macrophage like cells i.e. dendritic cells and keratinocytes). Helper T cells are only activated by antigens bound to MHC Class II proteins [Vander, Sherman & Luciano 1994]. Upon activation, the helper T cells secrete protein messengers called cytokines, which act on cytotoxic T cells and B cells that have also bound antigen. Without cytokine stimulation, binding of the antigen to the cytotoxic T cells and B cells is not usually enough to cause activation of these cells. Once the cells are activated, the cytotoxic T cells bind to the antigen presenting cells and release cytotoxic chemicals, while the B cells produce antibodies that guide phagocytes, complement and natural killer cells to the antigen presenting cells or free antigen. HIV-1 virus binding causes impairment in this process, known as cellular immunity, resulting in the reduced destruction of virus infected cells, parasites and cancer cells. CD4 cell count is used in conjunction with viral load and the presence of opportunistic infections to establish the stage of HIV disease (Table 2.1) and to determine which therapy or therapies may be most effective. Various opportunistic infections and tumours such as Pneumococcal pneumonia, pulmonary tuberculosis, and Kaposi's sarcoma and B cell lymphoma are associated with each stage of HIV infection. In addition to directing the immune response to viral or pathogen challenge, cytokines alter host metabolism, including lipid metabolism possibly in order to fuel this immune process.

Table 2.1 **CD4 count, stage of HIV infection and the approximate duration of each stage (adapted from Fahey J. & Fleming G. AIDS/HIV Reference Guide for Medical Professionals 4th Ed. Baltimore: Williams & Wilkins, 1996:106)**

Stage of HIV	CD4 Count Range	Duration
Acute infection	1000-750	1-4 weeks
Asymptomatic	750-200	2-15+ years
Early symptomatic	500-100	1-5+ years
Late symptomatic	50-200	1-4+ years
Advanced disease	50-0	0-2+ years

Figure 2.1 **The replication cycle of a retrovirus within the host including entry into a host cell, replication and release back into the circulation (adapted from Weiss, 1988).**



1. The virus particle has a diploid RNA genome
2. Infection occurs via specific cell surface receptor proteins such as CD4 and CCR5*
3. Reverse transcriptase makes a DNA copy of the viral RNA genome
4. The provirus enters the nucleus
5. The provirus is integrated into the host's chromosomal DNA
6. The provirus is transcribed to RNA genomes and messenger RNA (mRNA)
7. mRNA is transcribed to viral proteins
8. Viral protease enzyme modifies the viral proteins for incorporation into virions
9. The RNA genomes are packaged into progeny virions

* For a more detailed description of virus/host interactions, see Appendix 5.2

2.3 Postprandial lipid metabolism in healthy individuals

In order to discuss hyperlipidaemia, it is first important to understand what happens to lipid in a healthy individual. As the majority of our day is spent in the postprandial period, the metabolic response to a meal is likely to give more information on metabolic competence than a single fasting value, especially as fasting TAG and glucose levels are influenced by preceding diet, alcohol consumption [Mann & Chisholm, 1999] and exercise, among other factors. Just as the glycaemic response to feeding is used to assess diabetes control, the lipaemic response to feeding could be used to assess disordered lipid metabolism. Before discussing the lipaemic response to a meal, the processes of lipid digestion, absorption and transport in the circulation will be discussed, followed by a brief review of the cellular uptake and metabolism of fatty acids from the circulation.

2.3.1 Digestion

Most of the lipid in the diet is in the form of triacylglycerol (TAG), consisting of three fatty acids with a glycerol backbone. In the small intestine, pancreatic lipase catalyses the removal of the fatty acids from position 1 and 3 on the glycerol backbone to produce 2-monoacylglycerol (MAG) and two free fatty acids [Tso 1985]. Due to the hydrophobic nature of the lipid, TAG in the small intestine congregates into large lipid droplets. As the pancreatic lipase is a water-soluble enzyme, it is only able to work on the outside or hydrophilic surface of the lipid droplet. This would make lipid digestion very slow and therefore, emulsification occurs to produce smaller lipid particles and provide the enzyme a larger substrate surface area [Frayn, 1997]. The lipid emulsion is achieved by mechanically disrupting the large lipid droplets through contraction of the lower portion of the stomach and small intestine, while bile salts and phospholipids from the diet and from bile act as emulsifying agents. These amphipathic emulsifiers associate with the non-polar interior of the smaller lipid droplet while the polar region remains at the droplet surface to repel other lipid droplets, preventing the smaller lipid droplets aggregating into larger droplets. These small, emulsified lipid droplets are typically 1 μm in diameter allowing the pancreatic lipase to hydrolyse the TAG. Following this hydrolysis, smaller molecules are formed (4-6 nm) called mixed micelles consisting of MAG, free fatty acids, bile salts, phospholipids and fat-soluble vitamins [Frayn, 1997].

2.3.2 Absorption

Micelle formation allows the movement of hydrophobic material through the aqueous environment within the gut to the surface of the absorptive cells. Fatty acid binding proteins have been identified within the enterocyte, but it remains unclear whether these proteins have a role in the uptake of fatty acids from the gut lumen or if fatty acids cross the intestinal cell membrane by simple diffusion [Frayn, 1997].

Within the enterocyte, lipid is re-esterified to TAG and packaged with phospholipids, cholesterol, fat-soluble vitamins and apoprotein B48. The enterocyte membrane then surrounds these compounds and buds off into the interstitial fluid to form chylomicrons. These are large, buoyant lipoproteins (approximately 1μm in diameter) that pass through the large slit pores of the lacteal wall into the lymph and are then transported to the systemic circulation via the lymphatic system.

2.3.3 Plasma triacylglycerol

TAG within the circulation is carried in lipoproteins and the measurement of total plasma TAG concentration incorporates the TAG in the lipoprotein fractions produced either by the gut and containing dietary lipid or the liver. In the fasting state, the majority of plasma TAG would probably be found in liver derived lipoproteins. In the postprandial period, some contribution to the plasma TAG would be expected by gut derived lipoproteins. The postprandial lipaemic response to a meal represents the influx of gut-derived lipoproteins containing dietary lipid into the circulation and the subsequent clearance of these particles from the circulation. Measurements of plasma TAG concentration following a meal in healthy individuals have indicated that the magnitude of this postprandial response increases with age and is influenced by the macronutrient composition of the meal and the previous diet [Bennoson 2000]. The body composition of the individual and the duration and timing of exercise relative to eating [Gill *et al.* 2001] as well as whether or not a person smokes [Mero *et al.* 1997] have also been shown to influence this response.

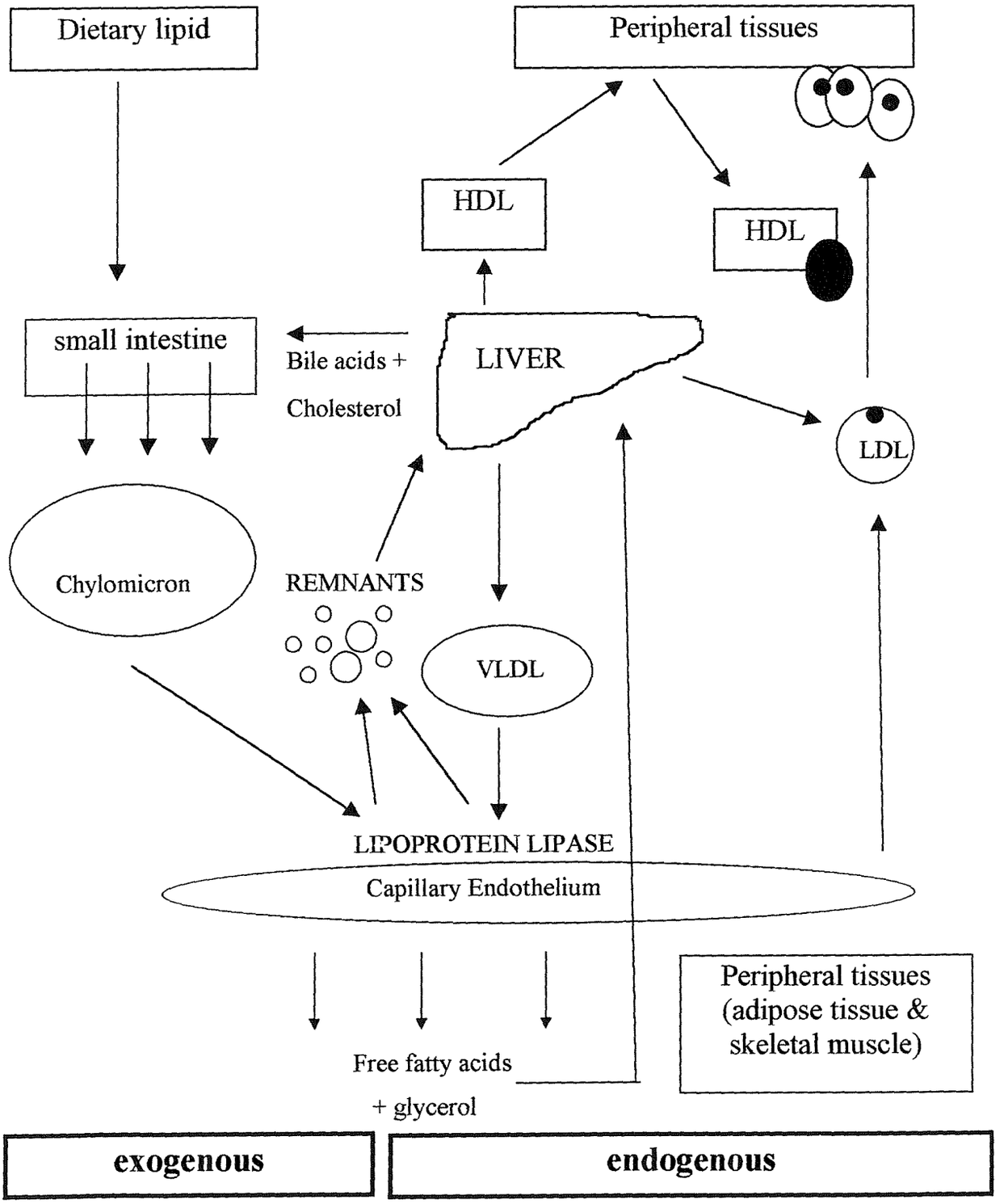
2.3.4 Lipoprotein classification

A typical lipoprotein consists of a hydrophobic core containing TAG, cholesteryl ester, and the hydrophobic regions of amphipathic phospholipids, free cholesterol and apolipoproteins, with a hydrophillic surface composed of the hydrophillic regions of the amphipathic molecules [Frayn 1997]. The lipoprotein classes can be isolated from plasma on the basis of their size, density and content (Table 2.2). The removal of the TAG-rich chylomicron and very low-density lipoproteins (VLDL) from the circulation involves the removal of the TAG core by enzymatic hydrolysis. However, following hydrolysis not all TAG is removed from these lipoprotein particles and the generated 'remnants' of both chylomicrons and VLDL can be mistaken for other lipoprotein classes. While chylomicrons are derived from the enterocyte and transport dietary lipid to the tissues, VLDL are synthesised by the liver and carry TAG from the liver to the tissues (Figure 2.2). However, both lipoproteins are referred to as TAG-rich lipoproteins. The smaller lipoproteins, LDL, IDL and HDL, are more involved with the transport of cholesterol to and from cells [Frayn 1997].

Table 2.2 **Lipoprotein properties used to identify isolated lipoproteins, such as the diameter and density of the lipoprotein, and the typical composition and apolipoproteins associated with chylomicrons (CM), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).**

PROPERTY	CM	VLDL	IDL	LDL	HDL
Diameter (nm)	100-500	30-80	25-30	20-35	5-10
Density (kg/l)	<0.95	<1.006	1.006-1.019	1.019-1.063	>1.063
COMPOSITION Approx. %					
TAG	90	65	35	10	5
Cholesterol	5	20	40	50	35
Phospholipid	5	10	15	20	35
Protein	1	5	10	20	25
APOLIPOPROTEIN COMPOSITION					
Listed in descending order of amount	C,B,E (A)	C,B,E (A)	B (C,E,A)	B	A,C,E (B)

Figure 2.2 **Endogenous and exogenous lipid metabolism showing the integration of the lipoprotein fractions (adapted from Smith 1998).**



Cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL,

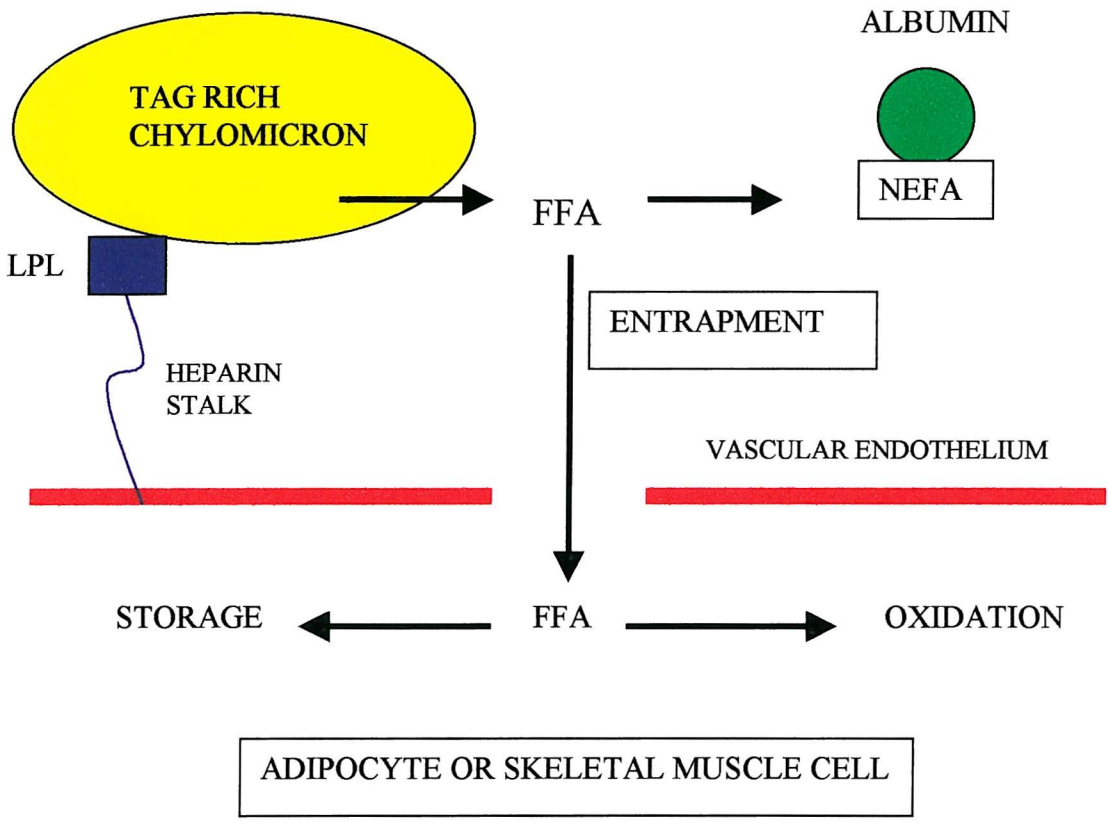
Cholesterol; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL,

2.3.5 Entrapment

The partitioning of lipid within the body in both the postabsorptive and postprandial state, requires the effective integrated regulation of several different systems operating concurrently in different tissues within the body. At the heart of this regulation lies the balance between the processes of TAG hydrolysis resulting in fatty acid release into the circulation, their subsequent uptake into tissues and the re-esterification of these fatty acids into TAG (storage) or metabolism (mitochondrial or peroxisomal oxidation) within tissues. Hyperlipidaemia represents an imbalance between the rate at which lipid enters the circulation and its clearance by peripheral tissues such as muscle and adipose tissue, or removal by the liver.

Peripheral clearance requires a combination of endothelial LPL to release fatty acids from circulating TAG-rich particles and the effective trapping of the fatty acids that are released by hydrolysis, a process known as entrapment (Figure 2.3). Efficient entrapment is likely to occur through a demand led process, co-ordinated by the cell in response to the intracellular metabolism of fatty acids. The signalling processes that link the endothelial phase of TAG hydrolysis and the subendothelial phase of fatty acid uptake and metabolism remain unclear, but an impairment in the co-ordinated control of these processes could lead to less efficient entrapment of fatty acids from the circulation, resulting in an increased flux of NEFA to the liver. This would provide fatty acids for VLDL synthesis and export back into the circulation, serving to maintain or elevate plasma lipid concentrations, depending on the extent of dysregulation.

Figure 2.3 **The principle of fatty acid entrapment. TAG-rich chylomicrons in the circulation are hydrolysed by the lipoprotein lipase enzyme, which is attached to the vascular endothelium by a heparin stalk. The generated free fatty acids are released from the chylomicron into the circulation and diffuse down a concentration gradient towards the cell. The cell probably through a combination of diffusion and protein-mediated transport can then take up fatty acids. Once inside the cell, the fatty acids can be stored or oxidised to generate energy.**



2.3.6 TAG rich lipoprotein hydrolysis

In the circulation, the chylomicron or other TAG rich lipoprotein is exposed to hydrolysis and release of TAG by the enzyme lipoprotein lipase (LPL), which is stimulated by insulin [Frayn 1997]. This enzyme is particularly abundant on the vascular endothelial surface of adipocytes and skeletal muscle cells and is attached to the surface by a heparin stalk. Chylomicrons have been shown *in vitro* to have a higher binding affinity for the LPL enzyme than VLDL, which in turn has a higher binding affinity for LPL than LDL [Xiang *et al.* 1999]. It was thought that LPL availability was one of the key determinants of TAG clearance from the circulation [Sniderman *et al.* 1997]. However, it has been shown that LPL activity is greatly in excess of normal requirements and that the correlation between local LPL activity and TAG storage in adipose tissue is poor [Montague *et al.* 1998].

Following LPL action the generated free fatty acids can be taken up by the adipocyte or skeletal muscle cell. This process is thought to be a combination of diffusion and a carrier-mediated process involving specific fatty acid binding proteins, fatty acid translocase and fatty acid transport protein [Bonen *et al.* 1998; Turcott *et al.* 1997].

2.3.7 Fatty acid transport across cell membranes

The relative importance of diffusion or protein-mediated transport for the movement of fatty acids across cell membranes remains in dispute. However, increasing numbers of proteins are being associated with this process. The following sections review what is known of these proteins, their regulation and the environment in which they function.

2.3.7.1 Plasma membrane fatty acid binding protein (FABPpm)

This is a 40 kDa protein expressed on the plasma membrane of liver, adipose, cardiac muscle, intestine and vascular endothelial tissue, as well as on internal membranes [Stremmel *et al.* 1985a,b; Sorrentino *et al.* 1988; Schwieterman *et al.* 1988; Calles-Escandon *et al.* 1996]. The FABPpm protein sequence has been found to be identical with that of mitochondrial aspartate aminotransferase (mAspAT) [Berk *et al.* 1990; Zhou *et al.* 1995] and the capacity of this protein to take up fatty acids is increased three-fold in the presence of zinc [Isola et al. 1995]. Antibodies to FABPpm can partially inhibit fatty acid

uptake in various cell types [Stremmel 1988; Stremmel *et al.* 1986; Zhou *et al.* 1994; Berk *et al.* 1990; Luiken *et al.* 1999] suggesting FABPpm has a role in this process but is not the sole mechanism of uptake. There is some evidence that increases in plasma free fatty acid and muscle fatty acid oxidation can increase FABPpm concentration [Turcotte et al. 1997]. *In vitro*, alcohol has been shown to increases FABPpm resulting in the increased accumulation of lipid droplets within chronically treated cells [Zhou *et al.* 1998] but the regulation of this protein *in vivo* is still unclear.

2.3.7.2 Fatty acid translocase (FAT)/CD36

This 88 kDa protein was identified in adipocytes [Harmon et al. 1991, 1992] but is also expressed in vascular endothelium, heart, intestine, monocytes, macrophages, platelets, spleen and skeletal muscle [Abumrad *et al.* 1993; Greenwalt *et al.* 1995; Martin *et al.* 1997; Memon *et al.* 1998; Poirier *et al.* 1996]. This protein stretches across the membrane and has a large extracellular hydrophobic domain, which is thought to act as a fatty acid binding pocket [Abumrad *et al.* 1993]. FAT/CD36 knockout mice have a normal lifespan, fertility and phenotype but exhibit reduced macrophage function, decreased adipocyte fatty acid uptake and reesterification with elevated plasma HDL cholesterol, VLDL-TAG and free fatty acids, and low plasma fasting glucose concentrations [Febbraio *et al.* 1999]. In contrast, mice that over express this protein in muscle show a reduced body fat, lower plasma TAG and free fatty acids with an elevated plasma glucose concentration and fatty acid oxidation during exercise [Ibrahimi *et al.* 1999]. FAT/CD36 is upregulated in response to PPARγ agonists, such as troglitazone, and PPARα agonists [Martin *et al.* 1997], long chain fatty acids [Han *et al.* 1999], oxidised LDL, retinoic acid receptor (RXR) activators [Amri *et al.* 1995] and cellular cholesterol [Nagy *et al.* 1998].

2.3.7.3 Fatty acid transport proteins

There are currently six of these proteins identified in humans (hsFATP1-6) following discovery in the mouse (mmFATP) [Hirsch *et al.* 1998]. There appear to be site-specific differences in the expression of these proteins and hsFATP4 is found in high concentrations within jejunum and ileum [Fitcher *et al.* 1998] suggesting a role in the uptake of fatty acids from the gut. Upregulation of mmFATP1 occurs in response to PPARα activators, such as fenofibrate, in mouse liver and the PPARγ activators,

troglitazone and pioglitazone in adipose tissue [Motojima *et al.* 1998]. The naturally occurring ligands of PPARγ-RXR, 9-cis retinoic acid and linoleic acid, can also upregulate mmFATP1 [Frohnert et al. 1999] and mmFATP1 is downregulated in adipose tissue in response to insulin [Man *et al.* 1996], TNFα and IL-1 [Memon *et al.* 1998].

2.3.7.4 Caveolin and Caveolae

Caveolin is a 22kDa protein with a high binding affinity for fatty acids, shown to be integral in the membrane of preadipocytes and adipocytes [Gerber *et al.* 1993; Trigatti *et al.* 1991] and a structural part of caveolae, omega shaped invaginations of the plasma membrane with a diameter of 50-100nm found predominantly in adipocytes, fibroblasts, myocytes and capillary endothelium [Gumbleton et al. 2000]. Caveolae are free cholesterol rich domains and caveolin binds cholesterol to regulate cellular cholesterol homeostasis [Fielding & Fielding 2000]. Cholesterol depletion in adipocytes results in the gradual destruction of caveolae structures and reduced insulin stimulated uptake of glucose by the cell, as the caveolae have been found to be associated with the tyrosine phosphorylation of the insulin receptor substrate, IRS-1 [Parpal *et al* 2001]. In addition, membrane cholesterol concentration can regulate signal transduction [Fielding 2001] and caveolae compartmentalise and integrate a wide range of signal transduction processes [Campbell et al. 2001]. The signalling proteins that reside in the caveolae are regulated, to an extent by caveolin proteins [Schlegel & Lisanti 2001]. Some pathogens, including viruses such as the Simian Virus 40, can enter the cell via the caveolae resulting in their transport intact into the endoplasmic reticulum [Pelkmans et al. 2001] and an altered host-pathogen interaction [Norkin 2001]. Although caveolin has been shown to have a high binding affinity for fatty acids, it is unclear if, additionally, free fatty acids enter the cell via the caveolae unbound to caveolin.

2.3.8 The fate of the fatty acid within the cell

Fatty acids that are taken up by the cell can either be oxidised to produce energy, reesterified to TAG and stored for later use, incorporated into the cell membrane or used in the synthesis of lipid signalling molecules or other lipid structures. While there is little known of the regulation of intracellular fatty acid partitioning, a reasonable body of work exists describing the control of fatty acid oxidation and several theories exist for the

regulation of this process. The potential role for the acylation stimulating protein (ASP) in the regulation of fatty acid reesterification is also discussed.

2.3.8.1 Fatty Acid Oxidation

Fatty acids are oxidised primarily within the liver, cardiac and skeletal muscle. Upon entering the cell, the enzyme fatty acyl-CoA synthetase esterifies the FFA's to produce acyl coenzyme A (ACoA), which is then transported across the mitochondrial membrane. This transport is mediated by carnitine and the enzyme carnitine palmitoyltransferase I (CPT-I), which transports long chain fatty acids into the mitochondria (figure 2.4). Inside the mitochondria, acyl CoA is converted to acetyl CoA by the enzymes of the pathway. ATP is then generated through oxidative phosphorylation from the energy produced by β-oxidation of fatty acids and the oxidation of acetyl CoA in the tricarboxylic acid cycle. The rate of FFA oxidation is regulated at the tissue level [Rasmussen & Wolfe 1999] as, at rest, approximately 70% of the FFA released by adipocytes into the blood are recycled back into TAG [Wolfe *et al.* 1990]. This TAG-FFA cycling allows rapid changes in fatty acid oxidation, for example at the onset of exercise, without the need for rapid changes in lipolysis within the adipocyte.

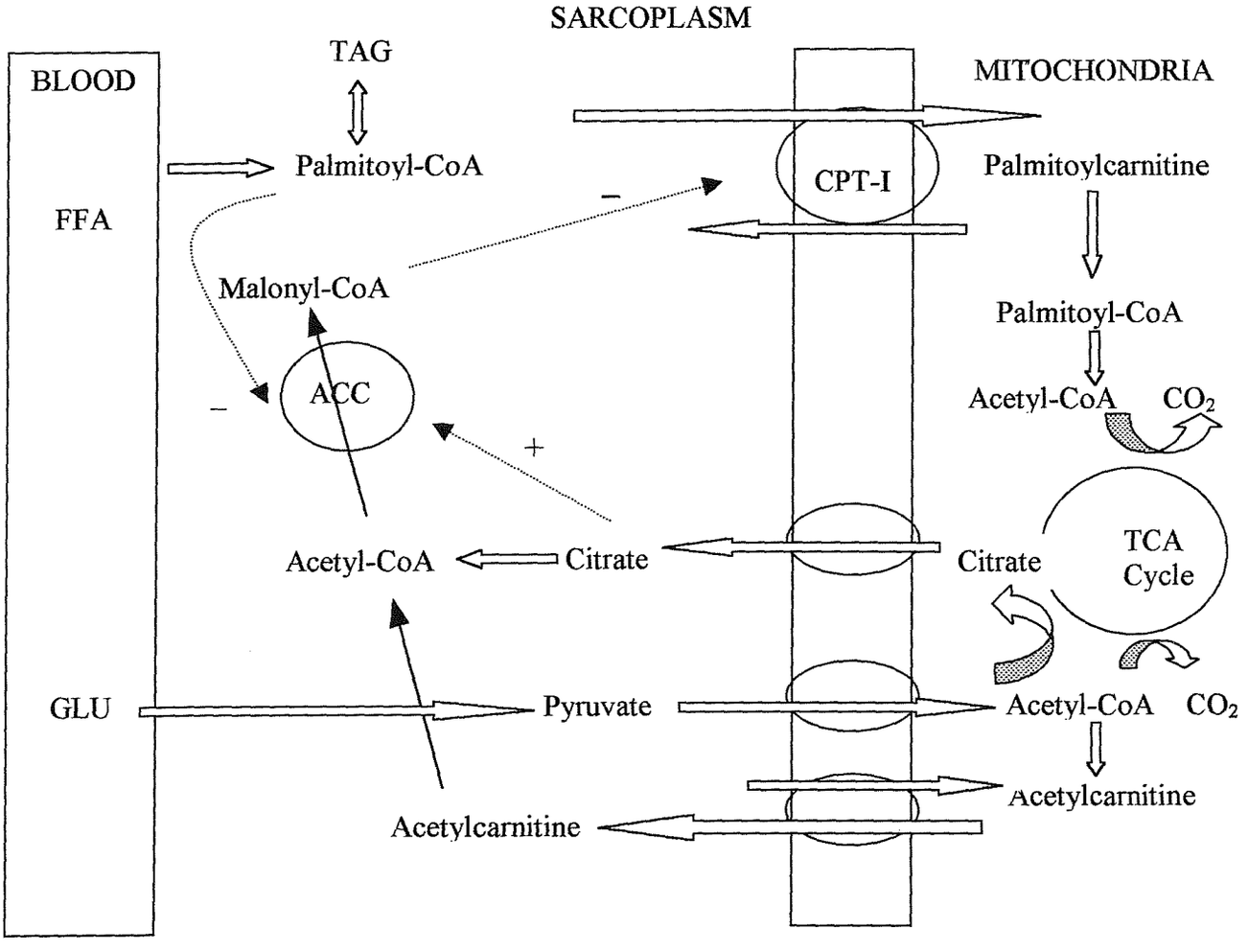
Regulation of FFA oxidation by skeletal muscle can occur at three potential sites; (1) the uptake of fatty acids by the cell, (2) enzymatic regulation, particularly of CPT-I, or (3) enzymatic regulation of the β-oxidation enzymes within the mitochondria. Research has shown that during exercise, fatty acid uptake by muscle is a saturable process [Turcotte *et al.* 1992] and prior treatment of cells with protease reduces FFA uptake [Stremmel *et al.* 1985] indicating that uptake of FFA is a carrier mediated process. In addition to the fatty acid binding proteins in the plasma membrane ($FABP_{PM}$), fatty acid binding proteins have been identified within the cytosol ($FABP_C$) which may play a role in the distribution of the fatty acid once inside the cell, although the physiological relevance of these proteins is yet to be determined [Rasmussen & Wolfe 1999]. However, an increase in plasma FFA concentration has been shown to cause a corresponding increase in FFA uptake without increasing FFA oxidation [Issekutz *et al.* 1967]. Therefore, it is more likely that FFA oxidation is controlled by factors within the cell.

The CPT-I enzyme is inhibited by malonyl-CoA, which is synthesised from acetyl-CoA by the enzyme acetyl-CoA carboxylase (ACC) [McGarry *et al.* 1983]. This synthesis is the first step in lipogenesis within the liver, however, skeletal muscle is a non-lipogenic

tissue and it is thought that malonyl-CoA exists in skeletal muscle specifically to regulate FFA oxidation via inhibition of CPT-I [Winder *et al.* 1989]. ACC is activated in the presence of glucose and citrate [Saha *et al.* 1997] and inhibited by AMPK activity. AMPK is a protein kinase, which increases activity when muscle contracts [Hutber *et al.* 1997] in response to elevated free levels of AMP and reduced ATP within the cell [Hardie & Carling, 1997].

The Randle Hypothesis [Randle *et al.* 1963] proposes a glucose-fatty acid cycle whereby FFA oxidation directly inhibits glycogen and glucose oxidation. However, Rasmussen & Wolfe (1999) have suggested that glucose availability determines the mix of FFA oxidation and glucose oxidation, as glucose inhibits carnitine acyltransferase, thus reducing the entry of fatty acids into the mitochondria. It is possible that glucose and fatty acids interact synergistically in both of these ways, as these two studies were carried out under different physiological conditions (clamp versus mixed meal), suggesting that regulation of this process may alter with both the oxidative demand and substrate availability.

Figure 2.4 **Proposed regulation of muscle fatty acid oxidation in the fed state. The three potential sites for fatty acid oxidation regulation are; the uptake of fatty acids by the cell, the enzymatic regulation of CPT-I, and the enzymatic regulation of β-oxidation by mitochondrial enzymes (adapted from Rasmussen & Wolfe, 1992)**



GLU, glucose; TCA cycle, tricarboxylic acid cycle; CPT-I, carnitine palmitoyl transferase-I; ACC, acetyl-CoA carboxylase, FFA, free fatty acids; TAG, triacylglycerol

2.3.8.2 Reesterification of fatty acids within the adipocyte

This process is stimulated by insulin and inhibited by growth hormone and leptin [Kersten 2001] possibly through the action of acylation stimulating protein (ASP). Intraperitoneal injection of ASP in normal and obese mice accelerated TAG clearance following an orally administered fat load as well as reducing postprandial glucose levels [Saleh *et al.* 2001]. ASP is a 76 amino acid fragment of the third component of complement (C3), which is generated by the interaction of adipsin and factor B with C3. C3-deficient mice, and therefore ASP-deficient mice, given oral fat loads show; a delayed postprandial triglyceride clearance more pronounced in males compared to females and in both males and females, the differences were more pronounced in the second half of the postprandial period; and elevated fasting and postprandial free fatty acid (FFA) [Murray *et al.* 1999b]. There are two functional domains in ASP, one that is responsible for the initial binding to the cell surface receptor, and a second domain that activates and increases triacylglycerol synthesis stimulation [Murray et al. 1999a]. Chylomicrons, but not the other plasma lipoproteins or fatty acids, activate the generation of ASP by cultured human adipocytes and ASP may interact with LPL to regulate the release of fatty acids from the chylomicron [Sniderman et al 1997]. Therefore, ASP can potentially regulate both the supply of fatty acids to the adipocyte and the uptake of these fatty acids via maintenance of a concentration gradient for diffusion within the cell by channelling fatty acids into TAG synthesis. The relative importance of this protein in the regulation of fatty acid hydrolysis, uptake and intracellular trafficking is, however, largely unknown.

2.3.9 Fatty acids in the circulation

The efficiency with which the cell entraps the fatty acids determines how much free fatty acid remains in the circulation. In order to regulate the transport of these hydrophobic molecules, the majority of free fatty acids that are not taken up by the cell bind to the protein albumin in the circulation to become non-esterified fatty acids (NEFA's). A chylomicron or other TAG rich lipoprotein remnant is also formed following the LPL action. NEFA's can be taken up by the liver or cells in the periphery and there is some evidence that plasma NEFA and free fatty acid concentrations can act as signals to regulate insulin secretion.

2.3.9.1 Free fatty acids and hepatic lipoprotein synthesis

NEFA's and lipoprotein remnants can be removed from the circulation by the liver, which uses unesterified cholesterol from the lipoprotein remnant plus the NEFAs (reesterified to TAG) along with apoproteins B and E to synthesise very low-density lipoprotein (VLDL). A fatty acid binding protein specific to the liver (L-FABP) takes up fatty acids from the circulation into the liver [Huang *et al* 2002] and there is a direct correlation between hepatic L-FABP concentration and the rate of hepatic fatty acid uptake [Wolfram *et al.* 1999] and reesterification [Prows *et al.* 1995]. Studies in animal models show that an increase in plasma fatty acid concentration also increases the rate at which the liver takes up fatty acids from the circulation [Bucki *et al.* 1997], suggesting that the circulating fatty acid concentration may upregulate L-FABP expression. Chylomicron remnant and VLDL remnant uptake is mediated by binding with the LDL receptor related protein (LRP) and the LDL receptor [van Berkel *et al.* 1995] and hydrolysis by hepatic LPL [Hussain *et al* 1997].

Increased hepatic cholesteryl ester (CE) synthesis catalysed by the enzyme Acetyl coenzyme A: cholesterol acyl transferase (ACAT-1) results in increased CE incorporation into VLDL and increased hepatic release of VLDL. Spady *et al.* (2000) hypothesised that fatty acids regulate ACAT-1 activity through the LDL receptor pathway. VLDL secreted into the circulation can then be hydrolysed by LPL in the periphery allowing transport of the lipid from the liver back to the tissues.

2.3.9.2 Free fatty acids and insulin secretion

As insulin is known to regulate the action of lipoprotein lipase, it is an important determinant of fatty acid uptake into the cell. While insulin can influence this process, there is also debate on the influence of plasma free fatty acid concentration on the synthesis and secretion of insulin from the pancreas. The literature on free fatty acids and insulin secretion appears very confusing. The *in vivo* studies and the *in vitro* studies appear to conflict in places, although there does appear to be both a direct action of free fatty acids on the pancreatic β-cells that synthesize insulin and an indirect effect of free fatty acids via alterations in glucose production and utilisation.

In vitro, pre-exposure of β-cells to elevated FFA levels for 48 hours reduces the insulin response to 27mmol/l glucose by 40% [Zhou & Grill 1995]. While this is obviously

not a physiological dose of glucose, the authors did find that the inhibition was partially prevented by etomoxir, a CPT-1 inhibitor suggesting that FFA's require transport into the β-cell mitochondria to exert at least part of this inhibitory effect. *In vivo*, acute elevation of plasma NEFA achieved either through a TAG and heparin infusion [Hennes *et al.* 1997; Chalkley *et al.* 1998] or a high fat meal plus heparin [Balasse & Ooms 1973] were found to enhance glucose-induced insulin secretion. This may indicate different responses of the β-cell to chronic and acute elevation of plasma NEFA. However, in patients with NIDDM, hyperinsulinaemia is observed in the presence of chronically elevated plasma NEFA [Reaven *et al.* 1998]. This apparent inconsistency may be explained by the direct actions of FFA on glucose metabolism and thus glucose-stimulated insulin response. Elevated plasma FFA concentrations are known to reduce whole body glucose uptake, oxidation and glycogen synthesis [Roden *et al.* 1996; Boden *et al.* 1999]. These effects are thought to be due to FFA inhibiting membrane transport of glucose, glucose phosphorylation and glycogen synthase [Randle *et al.* 1963], in addition to inhibition of pyruvate dehydrogenase to reduce glucose oxidation and promote the release of gluconeogenic precursors into the circulation for hepatic gluconeogenesis in the so-called glucose fatty acid cycle [Randle *et al.* 1963]. Other studies have reported that reduced insulin-mediated glucose disposal [Garg *et al.* 1988] and hyperinsulinaemia [Steiner *et al.* 1984] are associated with elevated plasma TAG concentrations supporting a role for FFA generated from lipoproteins in insulin secretion and action.

2.4 Stable Isotope Tracers in Postprandial Lipid Studies

In order to study the alteration in lipid metabolism that occurs with lipodystrophy *in vivo*, a method is required that allows the investigator to follow the metabolism of a substrate within the body without influencing the metabolic fate of that substrate. Stable isotope labelled substrates provide this and can be administered in such small amounts so as not to provide extra substrate and therefore alter the macronutrient composition presented to the gut. Fatty acids labelled with stable isotopes of carbon allow a non-invasive method of studying lipid metabolism *in vivo* that does not carry the risks associated with the use of radioactive isotopes.

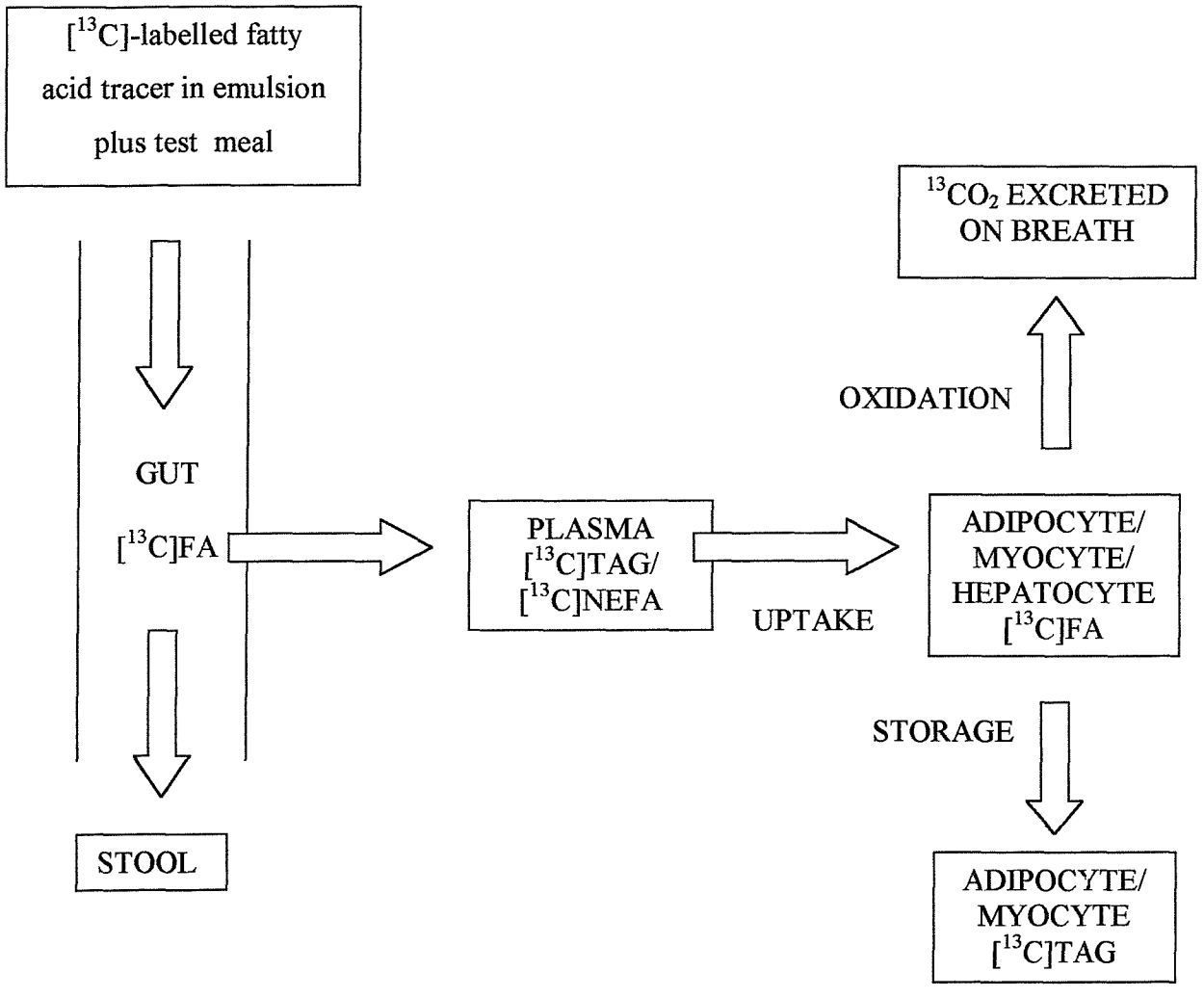
Most elements exist in a commonly found form, for example [^{12}C], and less common forms or isotopes, for example, [^{13}C] and [^{14}C]. The heavy isotope [^{14}C] is not stable and decays, emitting radioactivity. While this isotope has been previously used to

label compounds fed to animals, it is not ideal for use in humans. Therefore, the stable isotope of carbon, [^{13}C], is now commonly used to label compounds administered to human subjects. The metabolic fate of compounds such as fatty acids (figure 2.5) can then be traced by measuring the change in the [^{12}C:^{13}C] ratio in plasma lipid fractions, on breath or in the body compartment of interest over time, using a gas chromatography combustion isotope ratio mass spectrometer (GC-C-IRMS) [Brenna *et al.* 1992].

Previous studies have used [^{13}C] labelled fatty acids to examine the extent of elongation or desaturation of specific dietary fatty acids following digestion and absorption from the gut [Emken *et al.* 1993]. Other studies have used [^{13}C] labelled fatty acids to examine the influence of fatty acid chain length and degree of saturation on the absorption and metabolic fate of specific fatty acids [Jones *et al.* 1999]. Isotopically labelled compounds have also been used in clinical tests for the diagnosis of bile acid malabsorption [Fromm & Hoffman 1971], fat malabsorption [Schwabe et al. 1962], lactose intolerance [Sasaki *et al.* 1970] and liver function [Lauterburg & Bircher 1976]. While these clinical tests employed primarily [^{14}C], with the recommendations of Schoeller *et al.* (1977), the safer isotope [^{13}C] can also be used to conduct these tests.

While there is a growing body of literature discussing the use of stable isotopically labelled lipids to examine lipid metabolism within healthy and diseased subgroups of the population, there is as yet no reported use of this technique within the HIV population to characterise the HIV treatment associated hyperlipidaemia.

Figure 2.5 **The principle of using stable isotope labelled fatty acid tracer [1-^{13}C]palmitic acid to determine the metabolic fate of dietary lipid. The tracer is administered orally as part of a standardised test meal and changes in the [^{13}C:^{12}C] ratio in plasma TAG and NEFA, and in breath CO_2 are measured using GC-C-IRMS.**



2.5 The host response to HIV infection – The Pre-HAART Era.

This section aims to describe what is known of the integrated response of the host to HIV infection and includes both the immune response in terms of the cells and mediators such as cytokines directly involved in host defence and the resultant alterations in metabolism that may occur as a result of both these immune mediators and the presence of HIV infection. This section does not discuss the influence of antiretroviral therapy on this process.

2.5.1 Host immune response

2.5.1.1 Natural immunity

The natural immune defences are those that protect against foreign matter without having to recognise it and these are activated within hours of HIV infection. The responses include inflammation, activation of macrophages, natural killer cell activation, the complement cascade and cytokine release. Acute infection with HIV causes flu-like symptoms with abundant virus in the blood and a transient drop in CD4 T cells. Viral load reaches a maximum value approximately 4 to 8 weeks after the onset of symptoms and then often decreases rapidly [Gotch 1999]. It is thought that during this time both natural and acquired immune responses work to keep the virus in check.

2.5.1.2 Acquired immunity

Seroconversion or the time at which antibodies to the virus can be measured in the blood usually occurs within 12 weeks of infection. Both B and T lymphocytes (Figure 2.6) mediate the acquired immune response and, unlike natural immunity, the lymphocytes have to recognise the specific foreign matter or antigen to be attacked. B cells are primed following antigenic stimulation and, with cytokine stimulation from helper T cells, antibodies to both the envelope proteins of the virus and to internal viral proteins are produced. This usually occurs at the same time as the reduction in viraemia, or viral load, and the rebound in CD4 count. Specific T cell responses are also primed at this time and include CD8 cytotoxic T cells that recognise viral epitopes presented on the surface of target cells, then bind to and lyse the infected cells. In addition, CD8 T cells release

soluble factors such as interferon-γ, the interleukins IL-10, IL-13, IL-16 (Table 2.3) and the β-chemokines MIP-1α, MIP-1β and RANTES, which have anti-HIV effects [Gotch 1999]. The response described in figure 2.5 has also been referred to as a Th1/Th2 response, where the CD4 helper T Cells are divided into two subsets based upon the cytokine profile and predominant function. Type 1 (Th1) cells produce IFN-γ, TNF-α, TNF-γ and IL-2 (but not IL-4, IL-5 or IL-10) and regulate immune reactions centred around macrophage activation and T-cell mediated immunity. Type 2 (Th2) cells produce IL-4, IL-5, IL-6 and IL-10 and regulate B-cell antibody mediated immunity. Both the Th1 and Th2 response are activated in HIV infection.

2.5.1.3 Immunity during the asymptomatic period

The asymptomatic period may last for up to 15 years with the viral load remaining low or undetectable. During this time, the CD8 cytotoxic T cells remain at a level that is inversely proportional to the viral load but the CD4 T cells show a gradual reduction in number and function. Throughout this period the virus can evade the CD8 cells and antibodies by several mechanisms. Firstly, the virus becomes integrated into the host DNA where it remains invisible to the immune system. Secondly, because the viral reverse transcriptase that converts viral RNA to DNA for incorporation into the host genome has no proofreading capacity, mutations in viral DNA lead to the production of viral strains not previously recognised by the immune system. In addition, the virus is capable of producing immunosuppressive proteins that cause apoptosis of CD4 cells [Gotch 1999].

2.5.1.4 Immunity during the symptomatic period

In addition to the decrease in CD4 T cells, during the symptomatic period, there is also a decline in circulating antibodies and cytotoxic T cells. This is thought to be due to viral infiltration of the lymph nodes and production of large amounts of immunosuppressive viral proteins. The viral load increases at this time and due to the reduction in immune capacity, opportunistic infections, malignancies and diseases of the central nervous system can occur [Gotch 1999].

Figure 2.6 **Acquired immunity in response to antigenic stimulation. CD4 T lymphocytes produce cytokines, which enhance the production of antibodies by B lymphocytes and the cytotoxic action of CD8 T lymphocytes, in order to remove antigen bearing cells or free antigen from the host (adapted from Human Physiology, Vander, Sherman & Luciano 1994, 6th Edition).**

A: encounter and recognition

B: activation

C: attack

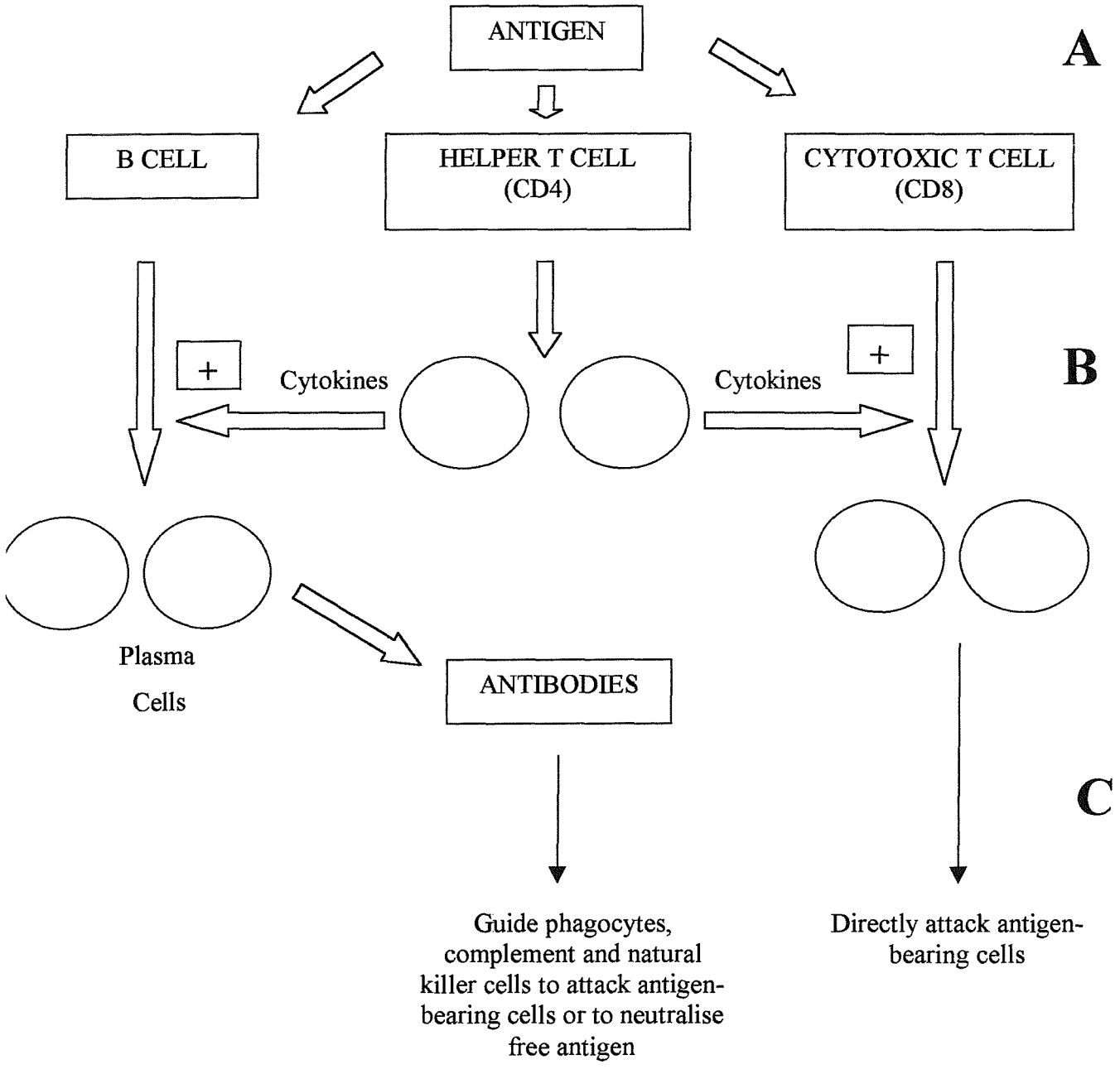
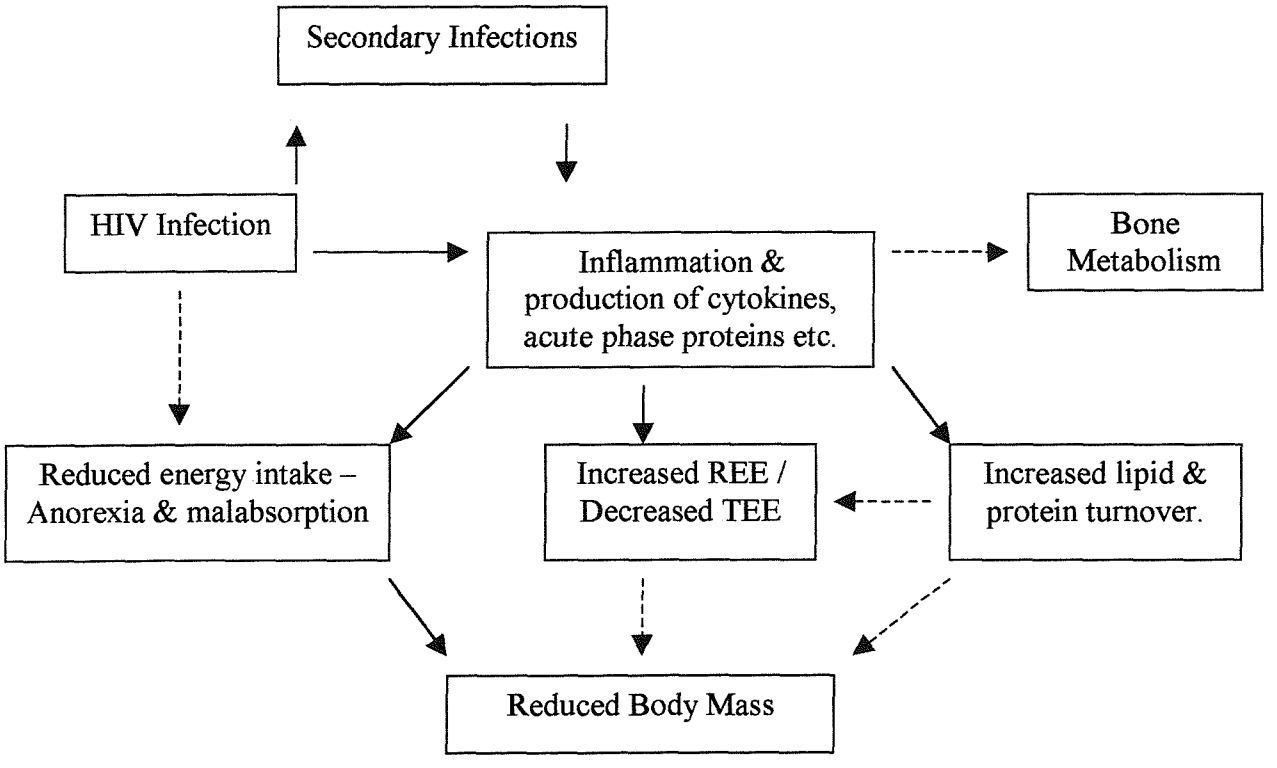


Table 2.3 Cytokine sources and actions

Cytokine	Source	Action and Target
IL-1	Macrophages	Growth of activated T and B cells
IL-2	Activated T Cells	Growth of activated T, B and NK cells
IL-3	Activated T Cells	Growth and differentiation of haematopoietic precursors
IL-4	Activated T Cells	Growth of activated T and B cells
IL-5	Activated T Cells	Growth of activated B cells
IL-6	Activated T Cells and macrophages	Growth and maturation of activated B and T cells
IL-10	Activated T Cells	Inhibits IFN-γ secretion
IFN-γ	Activated T Cells	Activates macrophages and increases their expression of MHC I and II
TNF	Activated T and NK Cells and macrophages	Helps activate cytolytic T cells and phagocytic cells; cytotoxic to tumours

2.5.2 Host metabolic response

Having discussed the host immune response to viral infection, it is not surprising that the energy and nutrient demands of the host are altered during this infective burden. In addition, HIV infection has been shown to produce alterations in body composition, gut function, the endocrine system, protein turnover and lipid and glucose homeostasis. In a recent review by Salas-Salvado & Garcia-Lorda (2001), the abnormalities pre- and post-HAART were discussed. This review and the work of other authors would suggest that the relationship between the observed pre-HAART abnormalities may be similar to the schema shown below, the broken lines indicate where the literature appears inconclusive.



2.5.2.1 HIV infection and body composition

The importance of weight maintenance in HIV patients has been highlighted by Wheeler et al. [1995 & 1996] where a weight loss of just 3 to 5% over four months was associated with subsequent increased risk of mortality and opportunistic infection. Weight loss in HIV can be acute, usually associated with the presence of opportunistic infections, or chronic, frequently associated with gastrointestinal disease and chronic diarrhoea [Macallan et al. 1993]. In addition to the timing of weight loss, there is a suggestion that weight loss may follow one of two different patterns [Sharpstone *et al.* 2000]. The first is a starvation model, where adipose tissue is mobilised to preserve lean body mass such as in cryptosporidiosis infection where anorexia and malabsorption are present. The second model of weight loss is the cachexia model where there is a preferential loss of lean body mass, possibly caused by cytokines and seen in *Mycobacterium avium-intracellulare* infection. In comparison to healthy controls, a reduction in fat mass is observed in HIV infection, possibly due to anorexia experienced at seroconversion [Gazzard 1999]. Although during symptomatic AIDS, body fat content may not be a reliable marker of wasting as some patients exhibit the cachexic model of weight loss described above, where there is a reduction in weight with little loss of fat mass [Grunfield & Feingold 1992]. Kotler et al. (1989) have suggested that it is the loss of lean body mass that is associated with a reduction in survival time. The method used to measure fat and fat free mass compartments in HIV-positive patients may influence these findings. Paton et al. (1997) found that FFM measurement in HIV was comparable using skinfold thickness, dual-energy x-ray absorptiometry (DEXA), total body water (TBW), and bioelectrical impedance (BIA) using the equation of the manufacturer of the equipment (EZComp) but higher using a published prediction equation for BIA (Segal), suggesting the results of BIA vary with the prediction equation used.

However, most individuals with HIV infection do not exhibit a continuous and progressive reduction in weight, but acute periods of weight loss associated with opportunistic infections, which, when treated, resulted in weight gain and periods of weight maintenance [Macallan 1998].

2.5.2.2 HIV infection and energy expenditure

Hommes *et al.* (1991) found REE to be 8% higher in HIV infected patients than healthy controls of similar body composition (7116 ± 173 kJ/d in HIV+ compared to 7058 ± 205 kJ/d in controls) (mean ± standard deviation). REE is also elevated in malnourished, HIV infected patients compared to healthy controls [Melchior *et al.* 1991] suggesting that the elevated energy expenditure may contribute to the weight loss observed in AIDS. When REE is expressed relative to body weight or corrected for lean body mass, the elevated REE is still observed in HIV patients [Grunfeld *et al.* 1992; Macallan *et al.* 1995a]. However, other studies report that REE is reduced in HIV infection [Kotler *et al.* 1990]. The reason for this inconsistency may be that alterations in energy expenditure are not consistent in HIV infection and alter with the progression of the disease. In addition, some authors have estimated REE using predictive equations that use the variables of age, sex, height and weight [Harris-Benedict 1919] in order to obtain comparative data. Both body size and composition are major determinants of REE [Garrow & James 1993]. In an individual with HIV or AIDS associated wasting, body composition may be significantly different. Therefore, comparing predictive REE values for healthy and HIV infected individuals based on weight alone may not be appropriate. Suttman *et al* (1993) compared predictive REE values using the Harris-Benedict equation to indirect calorimetry measurements of REE in 60 HIV-positive patients and found that the measured REE's differed significantly from predicted values, with 40% of patients showing a measured value more or less than 10% of the predicted value. In a cohort of patients over 319 days, 40% of patients lost more than 5% of their initial body weight, this group were found to have an elevated REE compared to their previous measured value. They concluded that elevated REE is not a constant feature of HIV infection but may occur during weight loss, contributing to wasting.

In weight stable HIV infected patients with increased REE, the caloric intake is not increased and it is thought that patients remain weight stable by limiting total energy expenditure through the lethargy and fatigue that accompany infection [Grunfeld & Feingold 1991]. In a study of 27 HIV-positive men, REE measured by indirect calorimetry, TEE measured by doubly-labelled water and energy intake were examined during periods of rapid and slow weight loss. While REE was elevated in the HIV positive males compared to healthy controls, TEE did not change due to compensatory reductions in the non-basal components of energy expenditure. The authors concluded that the primary

determinant of weight loss in this population was a reduction in energy intake [Macallan *et al.* 1995a]. Linear structural models of these variables would also indicate that the correlation between energy intake and energy balance is much stronger than the correlation between TE and energy balance [Sheehan *et al.* 1998]. Energy intake in HIV is related to both activity level and appetite [Sheehan & Macallan 2000].

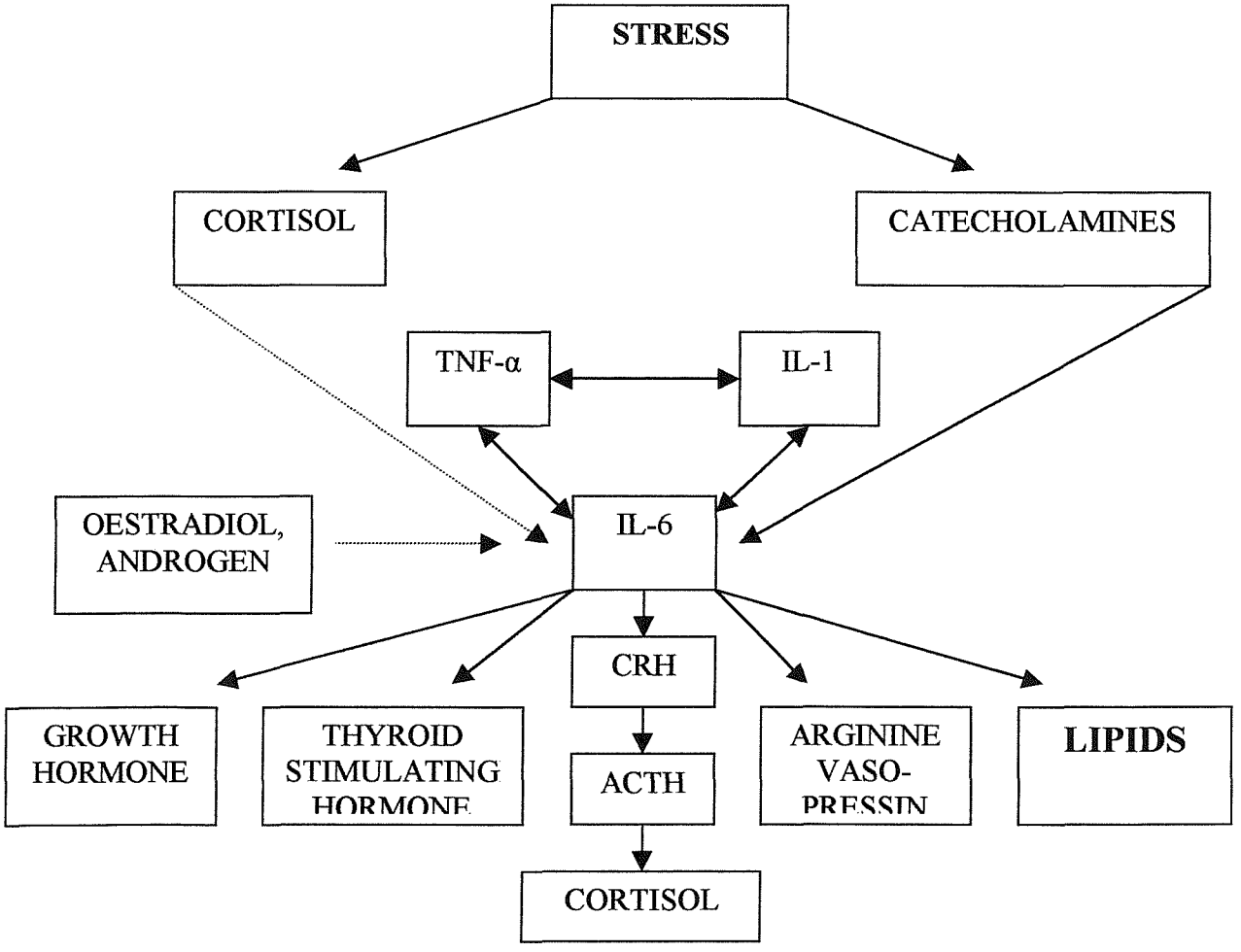
In order to assess the influence of infection, several studies have examined the relationship between REE and viral load. While one study reported a linear relationship [Mulligan *et al.* 1997], other studies have failed to find this relationship between viral load and energy expenditure [Grinspoon *et al.* 1998] or CD4 count and energy expenditure [Suttman *et al.* 1993]. This would indicate that either the elevated REE is not a result of the magnitude of the host immune response to the viral burden, or that viral load or CD4 counts are not good indicators of the magnitude of this response.

2.5.2.3 HIV infection and gut function

Malabsorption in AIDS is often associated with protozoal infection in the gut and may significantly contribute to the weight loss observed in symptomatic AIDS [Sharpstone *et al.* 1996]. However, malabsorption does not appear to be prevalent in asymptomatic, HIV infected patients [Keating *et al.* 1995]. Where malabsorption is present in the early clinical stages of HIV disease and not related to the presence of an enteropathogen, it is associated with atrophy of jejunal villi and manifests as diarrhoea [Miller *et al.* 1988]. In addition, mild pancreatic insufficiency has been reported in AIDS patients [Kapembwa *et al.* 1990] and HIV-infected children [Carrocio *et al.* 1998] contributing to fat malabsorption and steatorrhea. Duodenal biopsies obtained from HIV-infected adults with diarrhoea and malabsorption showed an accumulation of lipid droplets within the enterocyte in 16% of patients [Benhamou *et al.* 1994]. Lipid accumulation in the small intestinal mucosa was significantly associated with higher faecal fat content and lower plasma TAG concentration. This evidence would suggest that HIV infection in the gut could alter both the structure and function of the intestinal epithelium to influence the degree of substrate absorption independently of other enteropathogen infections, although this does appear to be associated with concurrent diarrhoea.

2.5.2.4 HIV infection, the HPA axis and endocrine disturbances

Several glucocorticoid response elements (GRE) have been identified within the genome of HIV. The HIV-1 viral protein R (VPR) can interact with the corticosteriod receptors and cause glucocorticoid resistance or sensitivity, depending on the nature of the tissue [Chrousos & Kino 1997]. Another viral protein, viral infectivity factor (vif) has been reported to induce glucocorticoid activity [Soudeyns *et al.* 1993] and in the HIV gag region, homology has been found to the corticotropin releasing hormone response element binding protein (CRH REB) [Licinio *et al.* 1995]. In addition, the presence of CD4 receptors within the limbic region may interfere with hypothalamic-pituitary-adrenal (HPA) axis activity and cytokines produced during infection may stimulate CRH activity and interact with cortisol [Kumar *et al.* 2000]. These authors propose the model below where HIV infection interacts with the HPA axis through the molecular mechanisms described, the production of proinflammatory cytokines and stress to influence endocrine activity*.



* For a more detailed discussion of the role of androgens in lipodystrophy, see Appendix 5.5

IL-6 is a potent stimulator of the HPA axis, and a 7-day administration of IL-6 can result in enlargement in adrenal size similar to that observed in Cushing's disease [Mastorakos et al. 1993].

The clinical significance of this altered HPA axis in HIV infection is twofold. Firstly patients may exhibit increased serum cortisol with reduced levels of dehydroepiandrosterone (DHEA), resulting in an altered cortisol:DHEA ratio. This imbalance has been related to a shift from a Th1 to a Th2 type cytokine response and a more rapid disease progression [Mayo *et al.* 2002]. Secondly, elevated cortisol may stimulate catabolism contributing to the wasting observed in HIV disease.

Disturbances in endocrine homeostasis may be due directly to HIV infection, the presence of opportunistic infections or the drugs used in the treatment of HIV and related infections. Examples of drug toxicity include pentamidine, which can cause nephrotoxicity and pancreatic toxicity resulting in altered potassium and glucose homeostasis; and ketoconazole, which inhibits adrenal and gonadal steroid synthesis producing a reduction in testosterone. Both the thyroid and adrenal glands have been found at autopsy, to be infected with CMV [Sellmeyer & Grunfeld 1996]. HIV DNA has also been found in the testes and reduced testosterone concentrations are common in AIDS [Dobs et al. 1988]. Direct destruction of endocrine organs by invasive HIV or opportunistic infection resulting in a loss of function is rare [Sellmeyer & Grunfeld 1996].

2.5.2.5 HIV infection and protein turnover

Protein turnover is increased in HIV infection and increases progressively with the development of more advanced disease stages so that by stage IV HIV infection, rates are about 25% higher than in controls [Macallan *et al.* 1995b] possibly as a result of the increased lymphocyte protein fractional synthetic rate [Caso *et al.* 2001]. However, the provision of protein in the diet results in increased protein synthesis and a normal anabolic response. This is not the case in patients with TB or severe septicaemia where provision of protein does not result in an anabolic response, suggesting an anabolic block modulated by specific patterns of cytokines released in response to the infection or insult [Macallan 1998]. Therefore, in HIV the provision of nutrients to overcome protein catabolism may only be effective in the absence of secondary infection [Macallan 1999b].

2.5.2.6 HIV infection and glucose homeostasis

HIV infection is associated with higher rates of insulin clearance and increased peripheral insulin sensitivity as shown by euglycaemic insulin clamp studies [Hommes *et al.* 1991]. This is surprising as elevated TNF-α concentration, as reported in HIV infection, is known to be a potent inhibitor of insulin-stimulated tyrosine phosphorylation of the insulin receptor and insulin receptor substrate 1 (IRS-1) resulting in a reduced peripheral insulin sensitivity [Hotamisligil & Spiegelman 1994]. An increased non-insulin mediated glucose uptake is also common [Heyligenberg *et al.* 1993] possibly as a result of HIV stimulated upregulation of host cell GLUT3 expression [Bell *et al.* 1993]. Hepatic gluconeogenesis is also elevated, possibly to maintain plasma glucose concentrations during elevated glucose disposal [Hommes *et al.* 1991; Mulligan *et al.* 1993]. In asymptomatic HIV infection, increases in circulating glycerol are observed [Christeff et al. 1991] possibly to provide substrates for gluconeogenesis or as a marker of increased adipocyte lipolysis.

2.5.2.7 HIV infection and lipid metabolism

Hypertriacylglycerolaemia, defined as a fasting plasma triacylglycerol (TAG) of >2.3 mmol/l [Tikkanen 1990] is prevalent in AIDS, predominantly due to increased very low density lipoproteins (VLDL), and is positively correlated with the cytokine, interferon-α [Grunfeld *et al.* 1991, 1992; Constans *et al.* 1994]. Interferon-α also shows a positive correlation with decreased TAG clearance [Grunfeld *et al.* 1991] and increased fasting hepatic synthesis of fatty acids [Hellerstein *et al.* 1993]. However, it is not known if interferon-α directly induces hypertriacylglycerolaemia or is a marker for the immune system and possibly other cytokines [Grunfeld & Feingold 1991]. Some researchers state that hypertriacylglycerolaemia is only present in the advanced symptomatic stages of infection (AIDS) and has an inverse correlation with CD4 T cell count [Gomez-Sirvent *et al.* 1994; Christeff *et al.* 1995] and a positive correlation with β2-microglobulin and TNF-α [Fernandez-Miranda *et al.* 1998]. Dietary fish oil has been shown to alter the cytokines released by lymphocytes during HIV infection [Bell *et al.* 1993], although the implications of this on lipid metabolism are unknown.

Other groups have shown that a lesser hypertriacylglycerolaemia already exists in asymptomatic HIV infection in comparison to healthy controls [Hellerstein *et al.* 1993]. In

vitro studies show that HIV destroys host T cells via a reduced host phospholipid synthesis and an increased neutral lipid synthesis producing changes in host-cell membrane permeability, similar to the action of paramyxoviruses [Cloyd *et al.* 1991]. However, the relative contribution of this to the observed elevations in plasma TAG is unknown. Marked increases in plasma TAG concentration appear to be primarily due to a combination of; (a) a reduced clearance time of TAG from plasma due to a reduction in endothelial lipoprotein lipase (LPL) enzyme activity [Grunfeld *et al.* 1991]; (b) an increase in hepatic fatty acid synthesis (lipogenesis) [Hellerstein *et al.* 1993]; (c) an increased mobilisation of free fatty acids from peripheral adipocytes by lipolysis [Feingold *et al.* 1990; Mulligan *et al.* 1993]. The observed increase in lipid synthesis may further influence protein synthesis, through the diversion of amino acid substrates from anabolism to lipogenesis [Macallan 1998].

Fatty acid oxidation is also increased in HIV infection [Mulligan *et al.* 1993; Hommes *et al.* 1991] although whether this is due to the virus or the elevated plasma TAG concentration is unclear. Plasma non-esterified fatty acid concentration may be elevated or reduced compared to controls depending on the stage of infection and the individual's nutritional status [Nunez & Christeff 1994]. While plasma cholesterol [Grunfeld *et al.* 1992] and phospholipid [Klein *et al.* 1992] concentration tend to be lower in HIV-infected subjects compared to healthy controls. Polyunsaturated fatty acids (PUFA) were found to decrease in red blood cell membranes of AIDS patients and a correlation was found with both elevated malondialdehyde concentration (the end-product of lipid peroxidation) and a CD4 count of less than 50 cells/mm^3 [Constans et al. 1995]. This suggests that disease progression is related to oxidative stress. Reduced concentrations of antioxidants such as selenium, vitamin A [Sappey et al. 1994; Constans et al. 1995], glutathione [Jahoor *et al.* 1999] and vitamin E [Pacht *et al.* 1997] have also been found in plasma of HIV infected patients compared to healthy controls.

The mechanism by which HIV infection induces these alterations in lipid metabolism remains unknown. In addition, the further elevations of plasma TAG with the progression of HIV infection to symptomatic AIDS would suggest a positive relationship between the degree of viral burden and plasma TAG. However, in HIV infected individuals with elevated viral load, effective and rapid reduction in viraemia through HAART administration has been shown, in some cases, to increase plasma TAG [Carr *et al.* 1998] and not to reduce lipids within the circulation as would be expected.

2.5.2.8 Summary of the metabolic effects of HIV infection in the Pre-HAART era

The literature would indicate that the presence and duration of HIV infection, in addition to the presence of opportunistic infection has a profound influence on the regulation and metabolism of macronutrients, possibly as a result of altered nutrient requirements during the cycle of infection and immune response. Against a background of reduced energy intake and elevated basal energy requirements, a reduction in the patients nutritional status is often observed resulting in an energy imbalance and weight loss. As weight loss is associated with an increased risk of further infection and mortality, the process can be seen to be self-potentiating. It would therefore be expected that a reduction in viral burden through the use of HAART would result in a down-regulation of this infection and immune response cycle with an associated theoretical reduction in nutrient and energy demand, allowing these metabolic processes to recover.

2.6 Current antiretroviral therapies

Using viral load and CD4 count as indicators of AIDS progression and associated infections, it becomes apparent that maintaining a low viral load and subsequent high CD4 count may delay the progression of the disease. Currently many drugs exist for this purpose and are most commonly used in combination, as this has been shown to be more effective than monotherapies (O'Brien 1998; Gulick *et al.* 1997; Gulick *et al.* 1998; Hammer *et al.* 1996; Autran *et al.* 1997). The standard combination therapy consists of either two nucleoside reverse transcriptase inhibitors (NRTI) (zidovudine, didanosine, zalcitibane, stavudine or lamivudine) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) (nevirapine) or two NRTI's and a protease inhibitor (PI) (saquinavir, indinavir, ritonavir or nelfinavir) [Gazzard 1999]. The combination used is widely known as highly active anti-retroviral therapy (HAART). NRTI's inhibit the viral reverse transcriptase enzyme by stopping the elongation of the DNA chain being created from the viral RNA template. NNRTI's are diverse in structure but all appear to act near the active site of reverse transcriptase to inhibit HIV-1 replication. Protease inhibitors bind to the viral enzyme protease which catalyses the cleavage of large viral proteins to smaller components for particle assembly, resulting in fewer virus particles with a reduced infectious capacity.

Most of these drugs have reported side effects. For the NRTI's, these include peripheral neuropathy, pancreatitis, anaemia, nausea and diarrhoea. Some NNRTI's are associated with the development of a skin rash. Protease inhibitor associated side effects include nausea, diarrhoea, abdominal and back pain and the symptoms associated with lipodystrophy. However, recent reports suggest that lipodystrophy is not exclusive to PI use and may be observed in NRTI and NNRTI therapy also [Saint-Marc *et al.* 1999; Aldeen *et al.* 1999]. While the association of specific drug classes with lipodystrophy has proved difficult to assess due to the combinations used, it does appear extremely complex. For example, drug efficacy is assessed by reductions in viral load and elevations in CD4 count while initial Zidovudine efficacy was assessed by decreases in plasma triglycerides [Mildvan *et al.* 1992].

2.7 Lipodystrophy

2.7.1 Features of Lipodystrophy

Features of lipodystrophy include hypertriacylglycerolaemia, hyperglycaemia, elevated plasma insulin and insulin-resistance with a reduction in weight and an altered pattern of adipose tissue distribution [Carr *et al.* 1998; Walli *et al.* 1998]. In some cases, non-insulin dependent type diabetes has developed involving peripheral insulin-resistance, insulin deficiency and hyperglucagonaemia [Yarasheski *et al.* 1999]. A loss of peripheral subcutaneous adipose tissue, specifically on the limbs, buttocks and face, occurs with a concurrent accumulation of visceral adipose tissue within the abdomen. The resulting increase in abdominal girth has been referred to as 'Crixbelly' due to the association with Crixivan, the trade name for the protease inhibitor indinavir [Kotler 1998]. The ratio of visceral to total adipose tissue (VAT:TAT), as measured by abdominal computed tomography, has been found to increase in patients taking indinavir and is positively correlated with time on therapy and increased plasma TAG [Miller *et al.* 1998]. In addition, some patients develop an increased fat accumulation in the dorsocervical fat pad (termed 'buffalo hump'), although this appears to occur less frequently than the other reported symptoms. The development of this 'hump' is not restricted to HIV patients on protease inhibitor therapy and measurements of urinary 24 hour free cortisol and dexamethasone suppression tests have indicated this is not linked to elevated cortisol as in Cushing's Syndrome [Lo *et al.* 1998; Yanovski *et al.* 1999]. However, patients on HAART

with an increased visceral adipose mass excrete higher 24-hour urinary free cortisol and catecholamine levels and have an elevated RMR [Renard et al. 1999], although the implications of this in the development of lipodystrophy are unclear. Pancreatitis has also been reported in rare cases where plasma TAG is markedly elevated (> 60 mmol/l) [Perry *et al.* 1999]. Osteonecrosis has also been associated with lipodystrophy [Roudiere *et al.* 2000], although some authors suggest a beneficial effect of HAART on bone health [Aukrust *et al.* 1999].

2.7.2 The prevalence of Lipodystrophy

Carr *et al.* (1998) have reported a 64% occurrence of lipodystrophy in HIV infected individuals following a mean 13.9 months on PI therapy. The prevalence of lipodystrophy in PI treated patients appears to be in dispute. A group from Charing Cross Hospital reported 12.5% of patients on protease inhibitor therapy developed lipodystrophy [Shaw *et al.* 1998]. These patients showed a fat loss in the limbs with an increase in the prominence of peripheral veins, an increase in abdominal size, facial weight loss, decreased buttock size and elevated plasma TAG (2.6 to 6.3 mmol/l). Reports of the prevalence of lipodystrophy range from 1% to 80% in HAART treated HIV-positive patients but it does appear that prevalence is increasing in this population [Mauss 2000]. These differences in the reported incidence of lipodystrophy may be due to the lack of agreement between centres on the exact classification of lipodystrophy and how it should be objectively identified within a patient, as well as different patient groups, methods or drugs used as elevated plasma TAG is seen more commonly with ritonavir use [Sullivan *et al.* 1998; Paparizos *et al.* 2000].

More recently, lipodystrophy has been reported during PI sparing treatment regimens. On a nevirapine (NNRTI) containing therapy (mean duration of therapy 12.9 months), the reported incidence of lipodystrophy, assessed by self-reported changes in body shape, was 16% [Aldeen *et al.* 1999]. Lipodystrophy has also been reported in HIV patients following a mean 14 months of NRTI therapy, with a prevalence of 63% in a stavudine treated group and 18.8% in a zidovudine treated group [Saint-Marc *et al.* 1999]. In the NRTI groups, lipodystrophy was characterised by elevated plasma TAG and an increased VAT:TAT ratio.

Factors that have been associated with lipodystrophy development are; 1) the duration of HAART, 2) the nadir of CD4 cells, 3) elevated C-peptide levels, 4) elevated

plasma TAG [Carr *et al.* 1999], 5) higher age [Safrin & Grunfeld 1999] and gender (an increased risk in women) [Martinez *et al.* 2001].

2.7.3 Clinical outcomes of Lipodystrophy

Prior to the advent of HAART, wasting was a major cause of morbidity and mortality in AIDS. HAART has undoubtedly reduced HIV related morbidity and mortality and can maintain low viral loads and elevated CD4 counts in HIV patients for potentially many years. However, the hypertriacylglycerolaemia associated with lipodystrophy development and HAART use has led to an increasing number of reports of cardiovascular disease (CVD) in patients previously at low risk of CVD development [Karmochkine & Raguin 1998; Henry *et al.* 1998; Behrens *et al.* 1998; Vittecoq *et al.* 1998]. Higher plasma lipid concentrations are also exhibited in patients with non-insulin dependent diabetes and familial hypercholesterolaemia (FH) and in these groups, a higher rate of CVD is observed. The risk of CVD is even greater when hypertriacylglycerolaemia is combined with increased visceral adiposity, hyperinsulinaemia and hypertension [Kaplan 1989].

Of more immediate concern than the increased risk of CVD is the reduction in compliance with the therapy. Patients taking HAART may stop treatment due to the pronounced changes in their appearance [Silversides 1999], particularly on the face where the fat pads on the cheeks disappear and the patient appears gaunt and starved. If the drug combination is not taken as recommended, viral replication may increase with an elevated risk of developing a viral strain that is resistant to the drugs. This could minimise the future treatment options available to the patient.

2.7.4 Clinical assessment of Lipodystrophy

Following a meeting of the International Society for Antiviral Research, in San Diego, USA (June, 1999) a case definition for lipodystrophy was proposed. Case definition criteria were suggested for main symptoms, related symptoms and possible related symptoms (Table 2.4). However, not all of these features of the syndrome are apparent in patients exhibiting lipodystrophy and therefore, it is difficult to clinically determine if and when lipodystrophy is present. In a recent review of lipodystrophy, it was concluded that there is currently no diagnostic tool available that allows diagnosis of HIV-associated lipodystrophy syndrome with measurement of a single parameter [Mauss 2000].

An additional complication in the diagnosis of lipodystrophy is the wasting observed in AIDS patients caused by the presence of HIV infection and the related incidence of opportunistic infection. Some studies have characterised lipodystrophy as changes in body composition. These can be self reported or measured by simple measures such as waist to hip ratio, bioelectrical impedance [Schwenk *et al.* 1999], and sonography [Martinez *et al.* 2000] or more complicated measures such as DEXA [Gervasoni *et al.* 1999], computed tomography scanning or magnetic resonance imaging [Engelson *et al.* 1999]. Others studies involve the measurement of fasting plasma TAG, glucose and insulin concentrations as indicators of metabolic competence*.

* For a comparison of LD case definitions with the patients recruited in these trials, see Appendix 5.1.

Table 2.4 Preliminary classification of symptoms of the HIV-associated lipodystrophy syndrome

Main Symptoms

Fat atrophy

- Face (sunken cheeks and eyes, hollow temples, prominent zygomatic arch)
- Arms and legs (prominent veins, skinny appearance)
- Buttocks (loss of contour, loose skin folds)

Fat accumulation

- Abdomen (increased abdominal girth due to visceral fat accumulation)
- Dorsocervical ('buffalo hump')

Related symptoms

- Breast enlargement
- Hypertriglyceridaemia
- Hypercholesterolaemia
- Abnormal insulin resistance or glucose tolerance or new onset diabetes mellitus
- Elevated C-peptide

Possible related symptoms

- Dry skin/ lips
- Hair loss/ brittleness
- Impotence
- Liver steatosis
- Nail dystrophy
- Dysmenorrhea
- Avascular osteonecrosis
- Cardiovascular disease

2.7.5 Possible therapies to treat Lipodystrophy

Where lipodystrophy has thought to be associated with PI use, some patients have switched to an NNRTI in combination with the NRTI base, with a small improvement in visceral fat accumulation [Martinez *et al.* 1999]. However, it now appears that the NRTI therapies may be another risk factor for lipodystrophy development. One NRTI-associated lipodystrophy case was shown to reverse completely 9 months after antiretroviral therapy cessation, although CD4 count also fell with a concurrent rise in viral load [Strobel *et al.* 1999]. Brief interruptions in therapy (7 weeks) may improve dyslipidaemia [Hatano *et al.* 2000] but may also lead to drug resistance. Rigorous exercise programmes have been employed to reduce visceral adipose tissue in lipodystrophy patients [Roubenoff *et al.* 1999]. A reduction in visceral adipose tissue of 1.1 kg was achieved over a sixteen-week period. Resistance exercise training has been shown to reduce plasma TAG in lipodystrophy, probably through muscle hypertrophy and increased TAG clearance by muscle [Yarasheski *et al.* 2001]. The post-trial compliance to these exercise programmes was not assessed. Liposuction and plastic surgery have also been used to remodel areas with abnormal fat depots [Ponce-de Leon *et al. 1999*]. A variety of drugs have been tested in the treatment of lipodystrophy. These include; nandrolone decanoate, an injectable anabolic steroid which following an 8 week administration of 100 mg/week produced significant increases in weight, fat free mass and arm and leg circumferences, although there has been concern that the use of anabolic steroids could further increase the hypertriacylglycerolaemia and hyperinsulinaemia observed in lipodystrophy, this was not seen in this study [Gold & Batterham, 1999]; atorvastatin, given in conjunction with HAART at a dose of either 10 or 20 mg/day for 12 weeks reduced plasma TAG and cholesterol, although, as ritonavir is an inhibitor for atorvastatin metabolism there is a potential for toxicity but no adverse effects were observed in this study [Murillas *et al.* 1999], similar results have been observed for pravastatin, simvastatin and lovastatin although lovastatin appears to have an increased risk of toxicity [Penzak *et al.* 2000] and statins may reduce lymphocyte proliferation [Wierzbicki *et al.* 1998]; gemfibrozil, given in conjunction with HAART produced an 83% reduction in plasma TAG after a median of 175 days and was believed to work via stimulation of PPAR type alpha (peroxisome proliferator-activated receptor) which increases fatty acid oxidation [Ross *et al.* 1999]; fenofibrate produced a mean 80% reduction in plasma TAG after 10 months in 2 lipodystrophy patients [Thomas *et al.* 2000]; metformin administered for 8 weeks to

patients with insulin resistance and visceral fat accumulation produced a reduction in visceral fat of more than 30%, but 14% of patients stopped therapy due to gastrointestinal side effects [Saint-Marc *et al.* 1999], lower doses of metformin have reduced VAT and insulin AUC following OGTT without these side effects [Hadigan *et al.* 2000]; recombinant human growth hormone (rhGH) at a dose of 6 mg per day for 12 weeks produced significant increases in weight and mid-thigh circumference with a significant reduction in waist:hip ratio. A reduced mean overnight growth hormone concentration has been observed in lipodystrophy patients [Rietschel et al. 2001] but it is unclear if this plays a role in the aetiology of the syndrome. There is also a suggestion that longer courses of rhGH may not be as effective and may induce rapid growth of Kaposi's sarcoma [Gazzard, 1999].

All of these drugs are aimed at either reducing the elevated lipids seen in lipodystrophy or changing the altered distribution of body fat. None of these therapies address the syndrome as a whole. In order to do this, the mechanisms underlying the development of lipodystrophy need to be understood.

2.8 Summary of Literature and Hypothesis

While the review of literature has attempted to give an overview of lipid metabolism within a healthy population prior to discussing abnormalities associated with the presence of infection and specific drug therapies, it has highlighted the fact that in a healthy population there are still some areas of lipid metabolism that present important questions. The extent to which an individual is able to maintain healthy plasma levels of lipids against a background of diet, genotype and lifestyle is unknown. The specific way in which the molecular, cellular and endocrine systems regulate the processes associated with uptake of lipid from the gut and metabolism or storage are also unclear and may be modulated by the environmental factors and genotype.

It would appear then that discerning specific effects associated with the presence of infection and the host immune response may be oversimplified, especially when an additional confounding factor is added with the use of HAART and it's associated unknown effects. However, a striking distinction between patients with lipodystrophy and other observed abnormalities in lipid metabolism is the concurrent presence of both fat accumulation and fat wasting, seemingly specific to particular fat depots.

While it may be impossible to determine the exact cause of this probably multifactorial syndrome, it does appear that there is a specific dysregulation of fatty acid uptake and metabolism within subcutaneous adipose tissue.

Hypothesis

Using HAART to suppress viral replication in HIV infection in a subset of patients, results in a reduced capacity of subcutaneous adipose tissue to hydrolyse TAG-rich lipoproteins with a concurrent failure to entrap the resulting fatty acids, resulting in a wasting of subcutaneous adipose tissue with a concurrent elevation in plasma lipid concentrations.

The aims of this programme of research were to firstly examine audit data from 545 HIV-positive patients treated at the Chelsea and Westminster Hospital to determine the prevalence of metabolic abnormalities in HAART treated patients and whether a particular therapy, the age or gender of the patient, or the time on therapy is associated with alterations in lipid and glucose metabolism. Study groups were then drawn from this population, an additional non-treated HIV+ population and a healthy non-HIV population. Metabolic trials were conducted within and between these groups using stable isotope labelled fatty acid in order to determine;

1) the influence of HIV infection *per se* on postprandial lipid metabolism in a study comparing HIV negative males and HIV positive males of similar age.

2) the influence of short term exposure (12 weeks) to HAART on the handling of dietary lipid in an intervention trial where antiretroviral therapy naïve subjects were given a protease inhibitor containing combination therapy and postprandial lipid metabolism was examined following both one and three months of protease inhibitor exposure.

3) whether differences in lipid metabolism exist in our population between patients with lipodystrophy associated exclusively with NRTI use to those with lipodystrophy associated with combination therapy including a protease inhibitor.

Chapter 3 General Methods

3.1 Introduction

The methods presented here were used consistently throughout all trials. Following a description of the subject groups studied and the general study protocol used, there is a brief explanation of the units of notation employed in stable isotope research and the theory underlying the use of stable isotopes as tracers *in vivo*. The isotopic enrichment of unlabelled emulsion and test meal is then discussed and the methods used for the collection, preparation and analysis of breath and blood are presented with relevant validation data. This is followed by a description of the techniques employed in GC-C-IRMS, indirect calorimetry, anthropometry, bowel habit questionnaire data collection and data analysis. There is then a section describing the variability in the anthropometric measures used.

3.2 Subjects

HIV-positive subjects were recruited from the St. Stephen's Centre for HIV research and treatment at the Chelsea and Westminster Hospital by poster or invitation at routine examination. Exclusion criteria for the study were presence of diarrhoea, presence of infection or illness other than HIV infection. Inclusion criteria for the lipodystrophy groups were self reported changes in body habitus, a loss of adipose tissue on the arms, legs, face or buttocks with or without a concurrent gain in central adiposity, and elevated fasting plasma TAG. The inclusion criteria for the protease inhibitor naïve (PIN) group were no prior exposure to protease inhibitor therapy, fasting plasma TAG within the normal range (0.5 – 2.0 mmol/l) and a requirement to start protease inhibitors following the initial study day. Healthy control subjects of similar age were recruited at the Institute of Human Nutrition in Southampton. All subjects were male in order to avoid the influence of; (a) elevated oestradiol at the time of ovulation on plasma lipoproteins [Kim & Kalkhoff 1979] and (b) synthetic oestrogens, such as the contraceptive pill, on plasma TAG [Knopp *et al.* 1981]. An age range was not defined in this study due to the low availability of asymptomatic HIV-positive patients meeting the criteria for the PIN group, as most patients were already taking or had previous experience of PI drugs. In addition, it was extremely difficult to find patients that were willing to take part in the study or who met

the inclusion and none of the exclusion criteria. As discussed in later chapters, several of the patients recruited into the intervention study were withdrawn during the study due to the appearance of secondary opportunistic infections, not known to be present during the initial recruitment phase. The number of concurrent, predominantly pharmaceutical trials taking place within this population also made recruitment into these studies very difficult. As a result of these difficulties, the groups entering the studies described in this thesis may not be as homogeneous as we would have wished in terms of age and body composition. Also, due to the personalised nature of prescribing HAART, not all patients with prior HAART experience had been exposed to the same drugs and not all patients being prescribed HAART for the first time, went on to the same combination. While this would be a more tightly controlled study group, the availability of the patients and the individual drug requirements of the patient prevented this.

3.3 Study Protocol

Subjects were asked to arrive at the day care ward of the HIV centre within the Chelsea and Westminster Hospital at 08:00 hours, following an approximate eight hour overnight fast (control subject studies were conducted to the same protocol but at the Institute of Human Nutrition in Southampton). The subjects were then allowed to rest for approximately 15 minutes while checking their name, age, date of birth and asking them to sign a consent form (see Appendix). A breath sample was then collected and indirect calorimetry was performed for 20 minutes. Following removal of the indirect calorimeter hood, an indwelling cannula was inserted into a deep forearm vein and a postabsorptive 20 ml blood sample drawn. The subject was then fed a breakfast consisting of an emulsion [11.0g double cream, 1.8g extra virgin olive oil, 1.5g sunflower oil, 6.0g casein, 4.5g glucose, 2.3g beet sugar, 5.0g chocolate Nesquik, 700 mg [1-^{13}C] palmitic acid], 40.0g Rice Krispies, 200.0g whole milk, 72.0g white bread, 14.0g Flora margarine and 50.0g cheddar cheese. This meal provided 3720 kJ energy, 33.1g protein, 45.3g fat; of which 49% saturated fatty acids, 27% monounsaturated fatty acids, 15% polyunsaturated fatty acids and 4% trans-fatty acids, and 92.9g carbohydrate; of which 69% starch, 29% sugar and 2% non-starch polysaccharides. The test meal and emulsion were designed to provide one third of the average daily energy intake within the UK and the typical macronutrient composition consumed in a UK diet [Gregory *et al.* 1990].

Following breakfast, 15 ml blood samples were collected every thirty minutes for the first three hours, then at hourly intervals until seven hours after the meal. Breath samples and indirect calorimeter measurements of oxygen consumption and carbon dioxide excretion on breath were obtained at hourly intervals up to seven hours following the meal. The blood samples were collected into lithium heparin evacuated glass tubes for lipid and insulin analysis and flouride oxalate evacuated glass tubes for glucose analysis (see Appendix). All samples were centrifuged immediately to prevent haemolysis. The plasma was then separated, aliquoted and kept refrigerated until frozen at the end of the study day. Following the seven-hour period, subjects had the indwelling cannula removed and anthropometrical measurements were taken. Prior to leaving the ward, the subjects were fed a meal of their choice together with tea or coffee and soft drinks*.

3.4 Stable isotopes as *in vivo* tracers

3.4.1 Units of Notation

As discussed in Section 2.7, chemical elements can exist as one or more species or isotopes that have the same atomic number but a different atomic weight. Generally, there is a stable, commonly found isotope of the element i.e. [^{12}C], a stable, less commonly found isotope of the element i.e. [^{13}C] and an unstable isotope i.e. [^{14}C] which decays emitting radioactivity. While [^{13}C] is much less common, there is a background [^{13}C] level of 1.11 atoms % (that is 1.11 atoms out of 100 carbon atoms are [^{13}C]). This background [^{13}C] is termed natural abundance and whenever [^{13}C] appears at a level higher than natural abundance the term enrichment is used. The unit of notation for enrichment is atoms percent excess (APE), this is the number of carbon atoms out of 100 that are [^{13}C] over and above the 1.11% natural abundance.

In practice, [^{13}C] is measured as [^{12}C]:[^{13}C] ratio by an isotope ratio mass spectrometer and delta values are often used instead of APE to describe the concentration of [^{13}C] within a sample. The delta (δ) value (expressed as $^{0}/_{00}$) is the concentration of [^{13}C] in the sample against a ratio of a laboratory's reference material or working standard. This allows the measurement and representation of much smaller quantities of [^{13}C]. If the isotopic composition of the standard is known, the ratio of sample [^{13}C] to standard [^{13}C]

* Appendix 2.1 shows the study timeline.

can be calculated [Murphy, personal communication, 1995]. The international reference standard is Pee Dee Belemnite (PDB), a carbonate, which has an abundance of 1.1112328 atoms % [^{13}C] and a [^{13}C]:[^{12}C] ratio of 0.0112372 [Craig, 1957]. However, PDB has become scarce, so laboratory working standards tend to be standards calibrated to PDB rather than PDB itself [Coplen *et al.* 1983]. Most natural foodstuffs and biological samples contain less ^{13}C than the PDB standard. Therefore, δ values for these products tend to be negative according to the formula:

$$\delta\ ^{13}C = \frac{^{13}C/^{12}Cu - ^{13}C/^{12}Cs}{^{13}C/^{12}Cs}$$

u - unknown [Schoeller, 1980]

s - standard (PDB)

3.4.2 Theory

The fatty acid, [1-^{13}C]palmitic acid, was produced (Mass Trace, Cambridge, USA) so that the first carbon was [^{13}C] (99 APE). Palmitic acid was used as it is the predominant saturated fatty acid within the UK diet [Gregory *et al.* 1990] and it is in the middle of the range of fatty acid carbon chain lengths. Palmitic acid is also written as C16:0 i.e. a chain length of 16 carbons with zero double bonds, hence it is a saturated fatty acid. Following administration of the [1-^{13}C] palmitic acid tracer, enrichment on breath, in blood lipids, in stool and other accessible pools can be measured. Assuming the tracer fatty acid is digested, absorbed and metabolised in the same way as other dietary fatty acids, distribution of the tracer within the body reflects the distribution of lipid from the diet within that individual.

Measurement of label within stool allows an accurate measure of absorption within the gastrointestinal tract. However, this was not done in these studies due to a lack of facilities for handling faecal samples from HIV positive subjects. Measurement of label on breath (as $^{13}CO_2$) indicates the proportion of the dietary fatty acid that is oxidised (Figure 2.6). Measurements of label within plasma TAG and NEFA fractions as measured by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) allow the metabolic fate of the dietary lipid within the body to be traced over time. Prolonged retention of labelled fatty acid as TAG in the circulation is assumed to reflect an impaired

clearance of lipid from the circulation by peripheral tissues. An elevation of label in circulating fatty acids as NEFA is taken to reflect impaired entrapment of fatty acids from hydrolysed TAG by peripheral tissues.

3.5 [1-^{13}C] Palmitic acid in emulsion

[1-^{13}C] Palmitic acid (700 mg) was administered orally as a single dose in an emulsion consisting of sucrose, glucose, casein, double cream, sunflower oil, olive oil and Nesquick chocolate flavouring as previously described in section 3.3 and based upon work by Emken *et al.* 1993. Palmitic acid was used instead of tripalmitin (the triacylglycerol of palmitic acid) so that the label did not have to first be digested before being absorbed. During the making of the lipid emulsion, it was important that the temperature of the emulsion remained higher than the melting temperature of the [1-^{13}C] palmitic acid (85^{0}C), which, at room temperature, was in crystalline form. This enabled the emulsification of the labelled fatty acid and thus the even presentation of the labelled fatty acid to the gut in small, emulsified particles, promoting absorption of the tracer from the small intestine. The presentation of [1-^{13}C] palmitic acid within an emulsion has been shown to increase the proportion of label absorbed from the gut and to greatly reduce the proportion of label excreted in stool in comparison to previous studies that have added the [1-^{13}C] palmitic acid to fat within the meal [Jones *et al.* 1999]. In addition, the emulsion without the label was analysed to ensure that it did not contribute to the enrichment of breath or plasma. The delta value of the emulsion was –26.97 $^{0}/_{00}$, equivalent to values observed in palmitic acid in plasma lipids in free-living individuals (baseline values) [Bennoson 2000].

3.6 The test meal

The test meal, as described in section 3.3, was developed to represent a standard pattern of macronutrient intake in combination with the emulsion (15% energy from protein, 45% energy from lipid (22% from saturated fat) and 42% energy from carbohydrate), with one third of the average daily energy intake for adults within the UK [Gregory *et al.* 1990]. In addition, a standard portion of the meal was homogenised and analysed for [^{13}C] enrichment, where it was shown to have a delta value of –26.09 $^{0}/_{00}$, equivalent to natural abundance. Therefore, administration of the test meal itself would not produce an increase in [^{13}C] in any of the pools measured [Bennoson 2000].

3.7 Breath sampling

3.7.1 Breath collection and preparation

End tidal breath samples were collected using a 750 ml alveolar breath bag (Quintron, Milwaukee) at baseline, then at hourly intervals for 7 hours following the administration of the test meal and emulsion containing the label. Three 10 ml samples were transferred using a syringe and needle, to evacuated glass 10 ml tubes (Isochem, Finchampstead, UK).

3.7.2 Breath analysis

Two of the three gas evacuated tubes containing the breath samples were analysed by continuous flow-isotope ratio mass spectrometry (CF-IRMS) with GSL (gas, solid, liquid) interface (ABCA system, Europa Scientific Ltd., Crewe) to determine the [^{13}C] enrichment of the CO_2. The third sample was stored for later analysis if the initial two tubes showed variation greater than could be explained by either the precision of the CF-IRMS or differences within samples within and between runs. According to previous studies [Jones, PhD Thesis, 1996], the coefficient of variation for repeated runs of laboratory reference standard (5% CO_2, 95% N_2 mix, δ -32.18 $^0/_{00}$ BOC gases, Manchester, UK) was 0.3% and the coefficient of variation within duplicate breath samples was 1-2 %. The within and between run variability of five fasted and five postprandial breath samples is shown in table 3.1. The coefficient of variation within run 1 and run 2 for five fasted samples (0 h) was 0.25% and 0.28% respectively, and for five postprandial samples (3 h) was 0.16% and 0.46%, similar to the 0.3% observed by Jones for the repeated runs of laboratory reference standard gas. The mean coefficient of variation between run 1 and run 2 for five fasted samples (0 h) was 1.26%, and for five postprandial samples (3 h) was 1.61%. The [^{13}C] enrichment within the sample was then combined with the volume of CO_2 excreted on breath at that time point, as measured by indirect calorimetry, using the equations of Watkins *et al.* 1982 (Figure 3.1) to calculate the percentage of administered dose per hour that is excreted on breath as $^{13}CO_2$. The area under the curve over the seven-hour period was then calculated by integration and expressed as the percentage of the dose administered/7 hours.

Table 3.1 **A test of within and between run variability in breath delta value measured by a 20/20 IRMS (GSL) system (Europa PDZ, Crewe,UK). Data is presented as means, standard deviations and coefficient of variation (CoV).**

	Run 1 (δ)		Run 2 (δ)		0 h	3 h
No.	**0 h**	**3 h**	**0 h**	**3 h**	**Mean**	**Mean**
1	-27.64	-21.14	-27.11	-20.58	-27.38	-20.86
2	-27.69	-21.16	-27.27	-20.71	-27.48	-20.94
3	-27.71	-21.09	-27.27	-20.73	-27.49	-20.91
4	-27.82	-21.09	-27.30	-20.72	-27.56	-20.91
5	-27.77	-21.15	-27.22	-20.52	-27.50	-20.84
Mean	**-27.73**	**-21.13**	**-27.23**	**-20.65**		
SD	**0.07**	**0.03**	**0.08**	**0.10**		
CoV %	**0.25**	**0.16**	**0.28**	**0.46**		

Figure 3.1 **The equations of Watkins *et al.* 1982 to calculate the percentage of administered dose per hour that is excreted on breath as $^{13}CO_2$, combining the change in [^{13}C] enrichment within the breath sample, following label administration compared to the baseline enrichment in breath, with the volume of CO_2 excreted on breath at that time.**

$$\% \text{ administered label excreted/hour} = \frac{\text{mmol excess } ^{13}C/\text{mmol } CO_2 * CO_2 * 100}{\text{mmol } ^{13}C \text{ administered}}$$

Where;

$$\text{mmol excess } ^{13}C/\text{mmol } CO_2 = (\delta\ ^{13}C_t - \delta\ ^{13}C_{t=0}) * R_{PDB} * 1/1000$$

CO_2 (mmol/h) is measured by indirect calorimetry

$$\text{mmol } ^{13}C \text{ administered} = \frac{\text{mg substrate}}{M} * (P*n/100)$$

CO_2 = whole body breath CO_2 excretion

$\delta\ ^{13}C_{t=0}$ = breath delta value at baseline

$\delta\ ^{13}C_t$ = breath delta value from CF-IRMS at time t following administration of the label

R_{PDB} = the ^{13}C:^{12}C ratio of PDB (0.0112372)

P = purity of isotope e.g. [1-^{13}C]palmitic acid is 99% APE

n = the number of carbon atoms in a molecule of labelled compound that are [^{13}C]

M = molecular weight of labelled compound

3.8 Blood sampling

3.8.1 Blood collection and preparation

From an indwelling venous cannula, approximately 3mls of blood was collected into Fluoride Oxalate tubes for analysis of glucose concentration and approximately 10mls of blood was collected into Lithium Heparin tubes for analysis of plasma NEFA and TAG concentrations and [^{13}C]-enrichment by GC-C-IRMS, and insulin concentration by ELISA. All samples were centrifuged immediately at 2500 rpm, 4^0 C for 15 minutes to minimise haemolysis. The plasma was removed and placed into pre-labelled 5ml glass tubes containing the protease inhibitors phenylmethylsulfonyl flouride (1µl PMSF (10mM dissolved in 2-propanol)/ml plasma), trasylol (5µl (10,000 KIE/ml)/ml plasma) and ethylenediaminetetraacetic acid (2µl EDTA (0.5 M pH 7.4)/ml plasma) for later lipid analysis.

Plasma for insulin and glucose determination was placed into pre-labelled eppendorfs. All aliquoting of HIV-positive plasma was performed within a Class II biosafety cabinet to reduce exposure to the HIV virus. The specimens were then kept refrigerated at 4°C for up to 12 hours before freezing at –20°C for up to seven months prior to analysis.

3.8.2 Plasma lipid analysis

The lipid was extracted from the plasma samples and the TAG and NEFA fractions were then isolated from the lipid extract. Following the addition of internal and external standards and the methylation of the fatty acids to fatty acid methyl esters, TAG and NEFA samples were analysed by GC-C-IRMS to examine the changes in both the concentration and enrichment of the TAG and NEFA fractions over time.

3.8.2.1 Lipid extraction from plasma

Standards for TAG (C17:0, triheptadecanoic acid) and for NEFA (C21:0, heneicosanoic acid) were made up in chloroform methanol 2:1 (1 mg/ml) and added to 1ml of plasma. The mass of standard used was chosen to reflect the approximate

concentrations of TAG and NEFA within the plasma sample. Where the plasma was normotriglyceridaemic 60µl of C17:0 standard and 30µl of C21:0 NEFA standard were used, for hypertriglyceridaemic samples 120µl of C17:0 standard was used. Chloroform: methanol (2:1, w/v) containing 50mg/l butylated hydroxytoluene (BHT) (5ml) was added and the sample mixed by inversion. At this point, HIV-positive samples were removed from the biosafety cabinet (Category II) as, following the advice of Professor P.J. Watt (Head of the Virology Department, Southampton General Hospital) it was assumed that the virus was sufficiently inactivated by the addition of solvent to be handled using normal bench top (Category I) procedures. The sample was mixed by vortexing and shaken for 15 minutes. Sodium chloride (1M NaCl) (1 ml) was added, the sample vortexed and centrifuged for 10 minutes at 2000 rpm and 14^0C. The aqueous phase was then discarded into Virkon (Antec International, Sudbury, U.K.; containing potassium monopersulphate, 1% solution made with water) and the solvent phase containing lipid was removed to a new tube. The remaining solid material within the sample was then re-extracted following the addition of 0.9% (w/v) NaCl (1 ml), to mimic the plasma in which the material was originally suspended. The resulting combined solvent phases were dried under nitrogen at 40^0C. The method was based on work by Folch *et al.* 1957. The addition of 0.9% (w/v) NaCl (1 ml) in the re-extraction step was shown in a validation study to increase the recovery of NEFA from the sample from a mean and standard deviation of 32% $\pm$ 11 to 77% $\pm$ 11.

3.8.2.2 Isolation of TAG and NEFA fractions by solid phase extraction

The method of solid phase extraction (SPE) for separation of the lipid classes, TAG and NEFA, was developed within the laboratory at the Clinical Nutrition and Metabolism Unit [Burdge *et al.* 2000] (see Appendix). The dried solvent phase from the lipid extraction was resuspended in chloroform and applied to Bond Elut cartridges (1 ml capacity, aminopropylsilica cartridges) (Varian, California, USA). This mixture was allowed to drip through under gravity into new glass tubes below. The cartridge was then washed with a further two 1 ml chloroform washes drawn through the cartridge using a vacuum system. This glass tube was removed, dried down under nitrogen and kept for later isolation of TAG. The cartridge was then washed with chloroform:methanol (60:40 (v/v)) (1 ml) which eluted the phosphatidylcholine fraction, the glass tube again changed , washed with

methanol and finally with a new tube in place, washed with chloroform:methanol:acetic acid (100:2:2 (v/v/v)) to elute the NEFA fraction. The dried chloroform wash was resuspended in hexane and applied to fresh Bond Elut cartridges preconditioned with hexane. The cartridges were washed further with hexane (1ml) and finally with hexane:chloroform:ethyl acetate (100:5:5 (v/v/v)) (1ml). This eluted the TAG fraction. Both the NEFA and TAG fractions were dried under nitrogen at 40^0C to remove the solvent.

3.8.2.3 Methylation to fatty acid methyl esters

In order to analyse the sample by GC-C-IRMS, the isolated fatty acid and TAG fractions need to be methylated. The reason for this methylation is twofold. Firstly, the methylation of free fatty acids reduces their boiling point to a temperature within the capable range of the GC-C-IRMS and secondly, TAG is a large molecule with more than one fatty acid and therefore, has a high boiling point and so removal of the fatty acids from the glycerol backbone reduces the boiling point of the molecule. In addition, resolution and identification of molecules with multiple fatty acids would be difficult, especially when trying to identify the isotopic enrichment in one of those fatty acids.

To achieve this methylation, samples were heated overnight at 50^0C with toluene (1 ml) and 2% (v/v) sulphuric acid in methanol (2 ml). The samples were then removed and allowed to cool before neutralising agent (25 g $KHCO_3$ + 34.55 g K_2CO_3 + distilled water (500 ml)) (2 ml) and hexane (2 ml) were added. The samples were vortexed, shaken for 15 minutes and centrifuged for 10 minutes at 2000 rpm and 14^0C. The solvent layer was removed and hexane (2 ml) added to the methylated tube for a further solvent extraction. The solvent phase was transferred to 5 ml round bottom tubes, which were dried under nitrogen. The dried round bottom tubes were washed four times with hexane (200 µl), allowing the lipid to be suspended in smaller volumes of solvent for transfer into the mini vials required for GC-IRMS analysis.

3.8.2.4 Gas chromatography combustion isotope ratio mass spectrometry

The GC-C-IRMS equipment consists of a Hewlett Packard 7683 auto sampler, which injects the sample via a self-sealing injection port into a gas chromatography

column. Upon injection the sample is heated to 300°C within the injection port, which causes the sample to become volatile, allowing it to sit in a gas phase. A flow of gas through the system takes the volatile sample into the GC fused silica capillary column, which is kept at 140°C. The increasing temperature programme (the temperature programme and settings are shown in table 3.2) inside the GC oven (Hewlett Packard 6890 GC with Orchid interface) allows the fatty acids to elute from the column entrance and travel down the column at different times, thus separating the individual fatty acids so that they reach the end of the column at different times. The solvent in which the fatty acids were dissolved then goes to the flame ionisation detector (FID) to be burnt off. After the solvent phase, the valve to the FID closes and the fatty acids are redirected into the combustion tube, which is maintained at 860^{0}C and consists of an aluminar tube with 0.2 to 0.5 mm platinized copper oxide granules. The addition of the volatile fatty acid produces the formation of CO_2 and H_2O. Before entering the IRMS, the water must be removed by a nafion membrane water trap where the nafion is dried by a Helium gas counterflow. The CO_2 then enters the IRMS flight tube at the source and is ionised, removing an electron to charge the molecule. A vacuum draws the gas along the flight tube and a magnet that sits halfway along the curved flight tube deflects the particles. At the end of the tube, there are detectors. Where the molecule hits the detectors is dependent on the arc of its path through the flight tube. This, in turn, is dependent on the molecular weight of the molecule, the strength of the magnet and the charge that the molecule carries. Therefore, the IRMS can differentiate between the commonly found isotope of carbon and the heavier, less commonly found carbon isotopes within a fatty acid. Both a table of $[^{12}C]$:$[^{13}C]$ ratio and a chromatogram are then printed. The size of each peak on the chromatogram is directly proportional to the relative abundance of a specific fatty acid within the sample and by using a standard of known concentration; peak sizes can be compared to give the concentration of the measured fatty acids within the sample.

Table 3.2 The temperature programme and settings for the GC-C-IRMS.

	Temperature (^{0}C)	Time (mins)
GC Programme		
Initial	140	-
Iso 1	140	2.00
Ramp 1	6/min	-
Final 1	175	-
Iso 2	175	2.00
Ramp 2	20/min	-
Final 2	240	-
Iso 3	240	1.00
Ramp 3	6/min	-
Final 3	250	-
Iso 4	250	5.00
Injection Port	300	-
Flame ionisation detector	300	-
Combustion Tube	860	-

3.8.2.5 Determination of TAG and NEFA recovery from plasma

Prior to injecting into the GC-C-IRMS, an internal FAME standard (C23:0, tricosanoic acid methyl ester) was added to the sample in the same mass as the original standards i.e. 120 or 60 μg of C17:0 for TAG samples and 30 μg of C21:0 for NEFA samples. The ratio of the FAME standard to the original standard within the sample was used to assess the recovery of lipid from the sample. In order to assess the experimental variability in TAG and NEFA recovery, 10 ml of plasma, collected into lithium heparin evacuated tubes split into 10 fractions. TAG and NEFA fractions were isolated as described above. This was repeated at 2.5 h postprandially and the results are shown in tables 3.2 and 3.3. In the baseline plasma specimen, the recovery of TAG was 89.1 $\pm$ 5.7 % (mean $\pm$ standard deviation) and the recovery of NEFA was 104.3 $\pm$ 6.9 %. In the 2.5h postprandial plasma specimen, the recovery of TAG was 86.7 $\pm$ 4.9 % and the recovery of NEFA was 101.1 $\pm$ 5.3 %. NEFA recoveries above 100% represent compound errors in the method due to dissolving and pipetting standards, and peak integration following GC-C-IRMS analysis.

3.8.2.6 Calculation of NEFA Concentration

The concentration of NEFA within the sample was calculated from the chromatogram using the peak area for the fatty acids and standards in the sample, according to the following equation:

((fatty acid area/C21:0 standard area) * (μg C21:0 added/C21:0 Mr)) / volume (ml)

The coefficient of variation for NEFA concentration in a given specimen was 4.2% (mean sd; 0.39 $\pm$ 0.02 mmol/l) in a fasted specimen (table 3.3) and 6.72% (0.09 $\pm$ 0.006 mmol/l) in the postprandial specimen (table 3.4) for repeated analysis of same specimen.

3.8.2.7 Calculation of TAG Concentration

The concentration of TAG in the plasma sample was calculated from the chromatogram. The individual concentration of fatty acids was calculated as for the NEFA

samples but the molecular weight of the standard (C17:0) was not included in the formula so that the concentration of each fatty acid was expressed as μg/ml. The total fatty acid concentration was then calculated from the sum of the fatty acid concentrations and again expressed as μg/ml total fatty acid. Each triacylglycerol (TAG) consists of three fatty acids plus a glycerol backbone and TAGs are generally a mixture of fatty acids. However, in order to calculate the concentration of TAG in the sample, as mmol/l, the total fatty acid concentration in μg/ml or mg/l was divided by the molecular weight of palmitic acid (256) and then by the number of fatty acid molecules within a TAG molecule. The molecular weight of palmitic acid was used because C16:0 is in the middle of the range of commonly seen fatty acid chain lengths (C14 to C22) and it is abundant in biological samples. The coefficient of variation for TAG concentration was 0.54% (0.46 $\pm$ 0.00 mmol/l) in a given fasted specimen and 2.39% (0.65 $\pm$ 0.02 mmol/l) in a given postprandial specimen (repeated analysis of same specimen).

3.8.2.8 Calculation of the [^{13}C] enrichment of palmitic acid in TAG and NEFA

From the above calculations, the total concentration of palmitic acid (μg/ml) within the sample of interest was determined. In order to establish a relationship between the percentage of [^{13}C] in palmitic acid and the delta value, [^{13}C]-palmitic acid was mixed in varying concentrations with natural [^{13}C] abundance palmitic acid and analysed by the IRMS to obtain a calibration curve. As this is a linear relationship, a regression equation ($x = (y + c) / m$) can be determined. For palmitic acid, this was $x = (\delta\ ^{0}/_{00} + 31.57) / 53.80$.

Therefore, if the total palmitic acid concentration (μg/ml) in the sample is known and the percentage of the palmitic acid that is [^{13}C]-palmitic acid is known, the concentration of [^{13}C]-palmitic acid within the sample (μg/ml) can be calculated. The coefficient of variation of the delta value (tables 3.3 and 3.4) obtained for TAG was 6.4% (31.08 $\pm$ 0.29 $^{0}/_{00}$) in the fasted specimen and 5.68% (–31.31 $\pm$ 0.31$^{0}/_{00}$) in the postprandial specimen and for NEFA was 6.58% (–30.86 $\pm$ 0.52 $^{0}/_{00}$) in the fasted specimen and 5.2% (–28.24 $\pm$ 0.79 $^{0}/_{00}$) in the postprandial specimen (repeated analysis of same specimen).

Table 3.3 **A test of within sample variability in plasma TAG and NEFA recovery, concentration and enrichment using ten aliquots of one ten ml fasted plasma specimen. Data is presented as means, standard deviations and coefficient of variation (CoV).**

	Recovery (%)		Concentration (mmol/l)		Enrichment (δ)	
No.	**TAG**	**NEFA**	**TAG**	**NEFA**	**TAG**	**NEFA**
1	92.45	120.98	0.46	0.43	-31.16	-30.77
2	93.85	107.59	0.46	0.39	-31.28	-31.92
3	92.90	104.67	0.46	0.39	-31.11	-30.79
4	92.64	104.89	0.46	0.39	-30.99	-30.11
5	90.50	102.68	0.46	0.40	-31.54	-30.98
6	90.28	95.97	0.46	0.37	-30.58	-30.71
7	74.09	98.23	0.47	0.39	-31.18	-30.83
8	88.89	99.65	0.46	0.39	-30.64	-31.44
9	88.01	102.82	0.46	0.39	-31.25	-30.25
10	87.68	105.91	0.46	0.38	-31.09	-30.81
MEAN	**89.13**	**104.34**	**0.46**	**0.39**	**-31.08**	**-30.86**
SD	**5.70**	**6.87**	**0.00**	**0.02**	**0.29**	**0.52**
CoV %	**6.40**	**6.58**	**0.54**	**4.20**	**0.93**	**1.69**

Table 3.4 **A test of within sample variability in plasma TAG and NEFA recovery, concentration and enrichment using ten aliquots of one ten ml plasma specimen collected 2.5hr postprandially. Data is presented as means, standard deviations and coefficient of variation (CoV).**

	Recovery (%)		Concentration (mmol/l)		Enrichment (δ)	
No.	**TAG**	**NEFA**	**TAG**	**NEFA**	**TAG**	**NEFA**
1	88.50	102.86	0.68	0.09	-31.10	-27.87
2	92.02	104.47	0.65	0.09	-30.98	-27.94
3	89.43	105.13	0.65	0.10	-31.73	-28.28
4	88.50	103.50	0.65	0.09	-31.30	-29.99
5	86.58	102.08	0.65	0.08	-31.54	-27.56
6	86.32	103.90	0.65	0.09	-30.95	-29.12
7	87.18	100.82	0.64	0.09	-31.56	-28.51
8	74.04	86.92	0.64	0.09	-30.92	-27.49
9	89.90	99.69	0.62	0.08	-31.34	-28.04
10	84.38	100.69	0.66	0.08	-31.70	-27.61
MEAN	**86.69**	**101.01**	**0.65**	**0.09**	**-31.31**	**-28.24**
SD	**4.93**	**5.26**	**0.02**	**0.006**	**0.31**	**0.79**
CoV %	**5.68**	**5.20**	**2.39**	**6.72**	**1.00**	**2.79**

3.8.3 Plasma insulin and glucose analysis

Dr. J. Morlese at the Chelsea and Westminster Hospital analysed plasma insulin and glucose concentrations from all collected plasma samples. Plasma glucose concentration was measured using an automated glucose analyser AU600 (Olympus Diagnostics, Southall, UK). Plasma insulin concentration was measured using an automated ELISA assay (ES700; Roche Diagnostics, Lewes, UK). The method has a 40% cross-reactivity with pro-insulin. The between-assay coefficient of variation was 10.5% (10.5 mU/L).

3.9 Indirect calorimetry

A Deltatrac indirect calorimeter (Datex Instrumentarium Corp, Helsinki, Finland) was used. The patient was supine on a bed with a ventilated hood placed over their head, through which room air was drawn at a fixed flow rate of 40 l/minute. A sampling line attached to the Deltatrac and hanging free or attached to the inflow of the hood allowed analysis of the CO_2 and O_2 content in inspired air. Expired air was collected from the hood and analysed for CO_2 and O_2 content. Calculation of energy expenditure was based on the assumption that all O_2 consumed was used to oxidise substrates and all CO_2 produced through oxidation was excreted on breath [Ferrannini 1988]. VCO_2 values provided information on total CO_2 excretion so that [^{13}C] enriched CO_2 could be calculated as a fraction of the total CO_2 expired. The differences in CO_2 and O_2 content between inspired and expired air allowed the calculation of the volume of oxygen consumed (VO_2 ml/min) and the volume of CO_2 excreted (VCO_2 ml/min). Using this information, energy expenditure and respiratory exchange ratio (RER) were calculated according to the Weir equation [Weir 1949]. The RER is a ratio of the oxygen consumption and carbon dioxide production and can be used as an indicator of the substrate being oxidised, for example, for glucose:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O - \Delta H$$

In order to oxidise one molecule of glucose, 6 molecules of oxygen are required and 6 molecules of carbon dioxide are produced. Therefore, the oxidation of carbohydrate

produces a RER of 6/6 or 1. Lipid produces an RER of 0.7. However, the metabolic fuel being oxidised at any one time consists of a mixture of substrates with the RER indicating the primary substrate being oxidised at that time, for example, a RER of 0.98 is predominantly carbohydrate while an RER of 0.75 is predominantly lipid.

The stoichometric equations of Frayn (1983) allow the measurement of both carbohydrate and fat utilisation from the measurement of CO_2 excretion (VCO_2) and O_2 consumption (VO_2) in litres per minute and urinary nitrogen excretion (n) in grams per minute;

$$\text{Carbohydrate utilisation (g/min)} = 4.55\ VCO_2 - 3.21\ VO_2 - 6.04\ n$$

$$\text{Fat utilisation (g/min)} = 1.67\ VO_2 - 1.67\ VCO_2 - 1.92\ n$$

The values obtained for carbohydrate utilisation represent carbohydrate oxidation plus conversion to fat minus carbohydrate synthesis and the values for fat utilisation represent the balance between fat oxidation and fat synthesis. Where negative fat utilisation values are obtained with RER values of more than one, there is a net fat accretion due to increased lipogenesis. Where RER values near 0.7 are achieved, this may represent elevated gluconeogenesis, possibly from alanine and fat oxidation may be underestimated. These equations are rearranged from the initial equations;

$$VO2\ (l/min) = 0.746c + 2.03f + 6.04n$$
$$VCO2\ (l/min) = 0.746c + 1.43f + 4.89n$$

The values for carbohydrate (c), fat (f) and nitrogen (n) utilisation are based on the values in table 3.5. An estimate of urinary nitrogen excretion of 0.0093 g/min was used to correct for protein oxidation. This value allows for a daily urinary nitrogen excretion in the order of 14g N/d (common laboratory urea nitrogen reference range 6-17g/d). Calculation of carbohydrate and fat utilisation using these equations assumes that; a). all oxygen inspired and all carbon dioxide expired are used for substrate oxidation and, b). the conversion of fuels to intermediates results in intermediate oxidation i.e. glucose conversion to lactate and subsequent lactate oxidation. These assumptions may not hold true if intermediates are formed, or the processes of gluconeogenesis or lipogenesis occur without subsequent oxidation i.e. storage or excretion without CO_2 production. Such

situations are observed in patients suffering from sepsis following injury or surgery where glucose production continues despite hyperglycaemia [Gump *et al.* 1974]. It is unclear how chronic infection such as HIV or the presence of lipodystrophy may influence the assumptions underlying the use of these equations. Elevated plasma lactate concentrations within the lipodystrophic HIV population have been reported [Kotler & Engelson 2000]. By estimating the size of the plasma lactate compartment (plasma lactate concentration x plasma volume), the VO_2 and VCO_2 used in lactate synthesis can be calculated and the measured VO_2 and VCO_2 values can be corrected. The H^+ ions generated in lactate formation may also displace CO_2 from bicarbonate and have an effect on CO_2 excretion on breath, although the correction required for this is thought to be very small unless there is a rapid increase in lactate concentration [Frayn 1983]. Frayn states that "it is not strictly possible, from indirect calorimetry alone, to calculate true rates of oxidation of either carbohydrate or fat" and "without other information, e.g., from isotopic studies, it is not possible to put any more detailed interpretation on the calculated rates". The values presented in the following chapters are not corrected for lactate and can only be used to discuss substrate utilisation in mind of the assumptions discussed above. The stable isotope data indicates the recovery of ^{13}C label on breath over the 7 hour period. While plasma lactate was not measured in the groups studied, the recovery of label on breath in the HIV groups with and without lipodystrophy is similar to those observed in the controls.

Table 3.5 **Volumes of O_2 consumed and CO_2 produced in oxidation of various fuels [Frayn 1983]. * One gram of urinary nitrogen is assumed to arise from 6.25 g protein and volumes are expressed per g urinary nitrogen.**

Fuel	O_2 (l/g fuel)	CO_2 (l/g fuel)
Glucose	0.746	0.746
Fat	2.03	1.43
Protein	0.966 6.04*	0.782 4.89*

3.10 Anthropometry

On each study day, patients were weighed using digital scales following removal of shoes, jackets or heavy items of clothing and contents of pockets. Height was measured using a wall-mounted stadiometer and body mass index (BMI) was then calculated (weight kg/height m^2). Using a non-metal anthropometric tape measure, the waist circumference was measured at the midpoint between the lower rib margin and the iliac crest on bare skin. The hip measurement was taken at the widest point around the buttocks over the subject's clothes with removal of items from pockets. The waist circumference, hip circumference and waist to hip ratio were used as crude measures of changes in abdominal fat storage or gluteal wasting over time. While waist circumference has been shown to be comparable to methods such as computed tomography and magnetic resonance imaging scanning for the prediction of abdominal visceral fat mass, waist: hip ratio is a weak predictor of both total and visceral abdominal fat [Clasey *et al.* 1999].

A skinfold measurement at the triceps site was also taken using Harpenden callipers, which measure a double layer of fat, and used to assess the amount of subcutaneous adipose tissue at this site [Durnin & Rahaman 1967]. Three measurements were made halfway between the acromium process and the olecronon, at the centre of the back of the arm and the mid upper arm circumference (MUAC) was measured. Mid upper arm muscle circumference (MUAMC) was then estimated by deducting the subcutaneous adipose tissue, indicated by triceps skinfold (TSF) measurement, from the MUAC [Heymsfield *et al.* 1982]. MUAMC was calculated using the equation; MUAMC (mm) = MUAC (mm) - 4.18 TSF (mm). These values were not used to estimate whole body fat and fat free mass as the skinfold was only measured at one site and not the four sites recommended by Durnin and Womersley (1974) for whole body estimates. In addition, the calculation of whole body fat mass from subcutaneous skinfold measurement assumes a constant relationship between subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT). In the lipodystrophy group, there already existed an imbalance in the adipose patterning between subcutaneous and visceral stores, so that it was inappropriate to use this equation without including the variable SAT: VAT ratio. Also, while the equation factors age and gender in order to compensate for age and gender related differences in adipose tissue patterning, it was not known whether HIV infection alone might alter the relationship between visceral and subcutaneous adipose tissue.

Monofrequency bioelectrical impedance (BIA) was used to estimate body fat using the Bodystat 1500 (Bodystat, Isle of Man, UK) to measure impedance. Electrodes were attached to the subject's right hand, behind the knuckle of the middle finger and on the wrist next to the ulnar head, and to the right foot, behind the second toe and on the ankle between the medial and lateral malleoli. A current of 800 μA was passed from one set of electrodes at a frequency of 50 MHz. The voltage drop, measured at the proximal electrodes, allowed calculation of the impedance by the tissues through which the alternating current passed. Impedance was defined by the equation: $Z^2 = R^2 + X_C^2$. Where Z is impedance (ohms), R is resistance (ohms) and X_C is reactance (ohms). The values, measured by BIA, for resistance and reactance (capacitance) were based on a circuit containing a resistor (extracellular water) and capacitor (the lipid component of the membranes in the body cell mass). Fat and fat free mass were then calculated using a predictive equation based on the impedance value together with height, weight, age and gender [Lukaski *et al.* 1985]. BIA was carried out prior to the evening meal, after emptying the bladder and following removal of the indwelling cannula and intravenous saline infusion to reduce the measured impedance to that produced by the tissues only.

3.10.1 Test of measurement variability within an individual

A 52-year-old male had ten consecutive measures within a 2-hour period, using the anthropometric methods used in this study, to assess the coefficient of variation (CV) produced by operator error. While weight and height were repeatable measures, as would be expected using precise equipment, with a CV close to zero, triceps skinfold and measurements of circumference at the mid upper arm, waist and hips had a higher CV. The triceps skinfold measurement was most variable as it was possible for to either place the callipers at a different point over the triceps or take a different skin fold at consecutive measures. The measurement of waist circumference was also variable, more so than the hips but together providing a higher variability in the waist: hip ratio. The CV for all these measures was less than 5 % (see Table 3.6). The CV was less than 1 % for all BIA repeated measures (see Table 3.7), in part, possibly due to the close to zero variability in height and weight measures. The electrodes were removed and replaced each time and as placement of the electrodes is known to influence the results, this suggested that placing of the electrodes was repeatable.

3.10.2 Test of biological variability within an individual

The same 52-year-old male returned to the Clinical Nutrition and Metabolism Unit on five separate occasions to repeat the anthropometric measures and, taking into account the precision of the measures, the biological variability observed over the period was calculated. Measurements were made between one and seven days apart over a thirteen-day period (see Table 3.8). Unlike the data presented above, there was a small variability in height (CV 0.27%). The subject was measured at approximately the same time each day to reduce the influence of compression of the spine throughout the day and shoes were removed before each measurement, the neck was also stretched to again alleviate compression of the spine. Weight varied between measurements but the CV was less than 1 %. The variability seen in triceps skinfold measurements over this thirteen day period was more than twice that observed during repeated measures on the same day, suggesting a biological variability over this period of almost 5 %. The MUAC and hip circumference measurements showed a variability of more than twice that shown during repeated measures on the same day, again suggesting a biological variability over this period. While BIA measures repeated on the same day produced a CV of less than 1 %, repeated measures over the thirteen day period have much higher CV's (see Table 3.9) possibly due to differences in the state of hydration at the time of measurement. All measurements were made in the fasted state, with the subject having eaten or drunk nothing except water for at least 10 hours prior to the measurement.

The results suggest that using the anthropometric measures, a biological variability within an individual of up to approximately 10 % can be expected with up to 5 % being explained by error in the precision of the repeated measures incurred by the operator. For BIA, the precision was better (< 1 %) but the biological variability within an individual over time could be up to approximately 5 %, possibly related to differences in the state of hydration in the individual over time. It is important to know the extent to which variability in the results, both within and between subjects, can be attributed to either operator error or biological variation over short periods of time. This enables the researcher to assess whether differences within and between individuals over time are true differences.

Table 3.6 **A test of within individual variability using ten measurements made within two hours of height, weight, tricep skinfold, mid upper arm circumference (MUAC), waist circumference, hip circumference and the calculated waist:hip ratio. Data is presented as means and standard deviations.**

TEST OF MEASUREMENT ERROR WITHIN AN INDIVIDUAL							
No.	**Ht (m)**	**Wt (kg)**	**Tricep Skinfold (mm)**	**MUAC (mm)**	**Waist Circ (cm)**	**Hip Circ. (cm)**	**W:H Ratio**
1	1.71	84.4	15.0	35.3	100	116	0.86
2	1.71	84.4	15.2	35.1	102	127	0.80
3	1.71	84.4	15.2	34.9	102	121	0.84
4	1.71	84.4	15.2	34.7	108	120	0.90
5	1.71	84.4	15.4	34.1	106	115	0.92
6	1.71	84.4	16.6	34.5	96	117	0.82
7	1.71	84.4	16.4	34.5	102	118	0.86
8	1.71	84.4	15.8	34.5	101	119	0.85
9	1.71	84.4	16.2	34.7	105	118	0.89
10	1.71	84.4	15.6	34.9	102	118	0.86
MEAN	1.71	84.4	15.7	34.7	102	119	0.86
SD	0.00	0.00	0.57	0.35	3.34	3.35	0.04
CV %	0.00	0.00	3.61	1.00	3.26	2.82	4.21

Table 3.7 **A test of within individual variability using ten measurements made within two hours using Bioelectrical impedance (Bodystat 1500) to measure impedance and to predict the percentage of fat mass and fat free mass and the weight of fat and fat free mass in kilograms. Data is presented as means and standard deviations.**

BIOELECTRICAL IMPEDANCE ANALYSIS					
No.	**Fat (kg)**	**Fat (%)**	**LBM (kg)**	**LBM (%)**	**Impedance (Ω)**
1	20.4	24.2	60.2	75.8	432
2	20.3	24.1	60.3	75.9	431
3	20.3	24.1	60.3	75.9	431
4	20.3	24.1	60.3	75.9	431
5	20.3	24.1	60.3	75.9	431
6	20.3	24.1	60.3	75.9	431
7	20.3	24.1	60.3	75.9	431
8	20.3	24.1	60.3	75.9	431
9	20.3	24.1	60.3	75.9	431
10	20.3	24.1	60.3	75.9	431
MEAN	20.3	24.1	60.3	75.9	431
SD	0.03	0.03	0.03	0.03	0.32
CV %	0.16	0.13	0.05	0.04	0.07

Table 3.8 **A test of within individual biological variability using five measurements made over a thirteen day period of height, weight, tricep skinfold, mid upper arm circumference (MUAC), waist circumference, hip circumference and the calculated waist:hip ratio. Data is presented as means and standard deviations.**

TEST OF BIOLOGICAL VARIABILITY WITHIN AN INDIVIDUAL							
No.	**Ht (m)**	**Wt (kg)**	**Tricep Skinfold (mm)**	**MUAC (mm)**	**Waist Circ (cm)**	**Hip Circ. (cm)**	**W:H Ratio**
1	1.71	84.4	15.7	34.7	102	119	0.86
2	1.71	84.7	16.5	37.0	99	101	0.98
3	1.71	84.0	18.8	36.0	100	104	0.96
4	1.72	84.5	15.1	35.1	99	101	0.97
5	1.71	84.3	16.5	35.2	99	102	0.97
MEAN	1.71	84.4	16.5	35.6	100	105	0.95
SD	0.46	0.26	14.04	0.91	14.88	76.16	0.05
CV %	0.27	0.31	8.50	2.57	1.49	7.22	5.24

Table 3.9 **A test of within individual biological variability using five measurements made over a thirteen day period using Bioelectrical impedance (Bodystat 1500) to measure impedance and to predict the percentage of fat mass and fat free mass and the weight of fat and fat free mass in kilograms. Data is presented as means and standard deviations.**

BIOELECTRICAL IMPEDANCE ANALYSIS					
No.	**Fat (kg)**	**Fat (%)**	**LBM (kg)**	**LBM (%)**	**Impedance (Ω)**
1	20.3	24.1	60.3	75.9	431
2	19.1	22.6	65.6	77.4	407
3	19.3	23.0	64.7	77.0	417
4	18.5	21.9	66.0	78.1	406
5	20.3	24.1	64.0	75.9	431
MEAN	19.5	23.1	64.1	76.9	418
SD	0.79	0.96	2.27	0.96	12.28
CV %	4.04	4.15	3.55	1.25	2.94

3.11 Data presentation and statistical analysis

The results are reported as medians and ranges. Data was treated as non-parametric as the normal distribution for these parameters within an HIV infected population was unknown and therefore, confirmation that the results fall within a normal distribution (normality testing) and were not skewed was unavailable. The Mann-Whitney U Test (the non-parametric equivalent of an unpaired t test) was performed to determine if significant differences existed between the groups at each time point and the Friedman test (the non-parametric equivalent of a one way repeated measures ANOVA) was used to determine if statistical significance was achieved between repeated measures within a group in the intervention study. Significance was assumed if $p<0.05$.

Comparison of two groups using a two-sample test at each time point, such as the Mann-Whitney U Test, is associated with flaws and does not take into account that successive observations on a given subject are likely to be correlated [Matthews *et al.* 1990]. In addition, the observation that significance is achieved in one time point and not the next, when clearly the two measures are correlated, may not be a correct biological interpretation of the data. For these reasons, the results of Mann-Whitney U Tests are displayed and discussed, but for more appropriate biological analysis of the data, summary measures such as area under the curve are employed to describe the response of individuals within groups. Area under the curve is calculated using the trapezium rule where the area under the curve between each time point is the product of the time difference and the average of the two measurements.

To examine the audit data collected (chapter 4.0) the data was divided by the exposure to different drug classes or combinations (4 groups) and the Kruskall Wallis Test with Chi square was used to test significance between the four treatment groups. Multivariate analysis was also performed on this data using the Wilks Lambda test to determine multiple correlation coefficients, followed by the calculation of partial correlation coefficients.

Prospective power analysis could not be conducted before embarking on these studies as the effect size in the primary outcome variable was unknown. Power analysis is employed to determine either the sample size for a given power or vice versa, where the effect size of the variable of interest is known and the power refers to the power to reject the null hypothesis. The primary outcome measure in this study was the postprandial kinetic measurement of label within plasma TAG and the null hypothesis would be that

there was no difference between the groups in this measure. At the time of designing this study, no data was available to show the size and variability of this measure in HIV-positive males. Therefore, it was not possible to accurately calculate the expected effect size between the groups.

A crude estimate of sample size and power can be produced using approximate fasting plasma TAG concentrations for healthy males of this age range (1.0 ± 0.3 mmol/l) and assuming equal variance between groups. At a significance level (α) of 0.05, estimates of sample size for a given power can be produced for a range of effect sizes (difference between the mean of the groups).

Effect size (mmol/l)	Sample size required for a power of 0.95	Sample size required for a power of 0.90	Sample size required for a power of 0.85
0.5	9	8	6
0.6-0.7	6	6	5
0.8	5	3	3

While this data would suggest that the sample sizes (n=5 to n=8) used in these studies have sufficient power to reject the null hypothesis that there is no difference between the groups in fasting plasma TAG if these effect sizes are observed, these calculations assume an equal variance in the study groups and a normal distribution of data. As discussed previously, these assumptions may not be valid for these groups.

Chapter 4.0 Possible factors associated with and the prevalence of elevated fasting plasma triglyceride and glucose concentration in an audit of HAART treated patients.

4.1 Introduction

An audit of 545 patients treated at the Chelsea and Westminster Hospital was undertaken to attempt to identify factors that may be associated with these metabolic perturbations. In addition, the prevalence of elevated plasma TAG and glucose was examined. As plasma TAG and glucose measurements are more routinely taken on patients that have started therapy, patients that were on their first therapy combination and had been for more than six months were selected in order to obtain a more homogeneous population. The audit included data on the type of therapy, duration of therapy, age and gender of the patient, viral load, plasma TAG and glucose. Several of these factors have previously been associated with the development of the lipodystrophy syndrome. These are; 1) the duration of HAART, 2) elevated plasma TAG [Carr *et al.* 1999], 3) higher age [Safrin & Grunfeld 1999] and 4) gender (an increased risk in women) [Martinez et al. 2001].

While this data does not provide enough information to assess whether each patient does or does not have lipodystrophy, the incidence of hypertriglyceridaemia with or without concurrent hyperglycaemia may be suggestive of the percentage of this population that have developed or are likely to develop the metabolic syndrome. This data can then be compared to previous data on the prevalence of lipodystrophy in HAART treated populations to identify if prevalence is similar in different treatment centres. Carr et *al.* (1998) have reported a 64% occurrence of lipodystrophy in HIV infected individuals following a mean 13.9 months on PI therapy, while Charing Cross Hospital reported 12.5% of patients on protease inhibitor therapy developed lipodystrophy with an elevated plasma TAG of 2.6 to 6.3 mmol/l [Shaw *et al.* 1998]. Other centres too have reported that elevated plasma TAG is seen more commonly with the protease inhibitor ritonavir [Sullivan *et al.* 1998; Paparizos *et al.* 2000].

Following 12.9 months of nevirapine (NNRTI), the reported incidence of lipodystrophy, defined by changes in body shape, was 16% [Aldeen *et al.* 1999]. The prevalence of lipodystrophy, characterised by elevated plasma TAG and an increased VAT:TAT ratio, on exclusively NRTI therapy has been reported to be 63% in a stavudine

treated group and 18.8% in a zidovudine treated group [Saint-Marc *et al.* 1999]. While the group treated with NRTI's in isolation is small (n=29), some comparisons may be made.

In addition to comparing this population to the epidemiological data reported by other centres, it is important to verify that the groups that were recruited from the population treated at this centre for the subsequent studies reported in this thesis, were representative of the population in terms of age, ethnicity and where applicable, the combination of HAART used. As discussed previously, females were not selected to take part in these studies due to the influence of the menstrual cycle on lipid metabolism and also the lesser availability of female volunteers.

4.2 Aims

The aims of this audit were;

1. To determine the prevalence of hypertriglyceridaemia and potential metabolic syndrome in this larger population in comparison to figures reported by other authors

2. To examine if elevated plasma lipid or glucose in this population was more prevalent with particular therapy exposure or duration

3. To verify the groups selected for subsequent studies were representative of the population in terms of age and ethnicity and where applicable, HAART combination used.

4.3 Methods

4.3.1 Data collection and analysis

The audit was undertaken from a database of patients held at the Chelsea and Westminster Hospital by Dr John Morlese. The data reported here was based on an audit of 545 patients for which plasma TAG and glucose data were obtained while on their first therapy combination. Plasma TAG and glucose in the population were tested for normal distribution and were found to be non-parametric. The Mann Whitney Test was used to test significance between males and females, and the Kruskall Wallis Test with Chi square was used to test significance between the four treatment groups. Multivariate analysis was also performed using the Wilks Lambda test to determine multiple correlation coefficients, followed by the calculation of partial correlation coefficients.

4.4 Results

4.4.1 Patient characterstics

Figure 4.1 shows the distribution of age within the audit population. The majority of patients (79%) are between 25 and 44 years of age. Only 7% of this population are more than 55 or less than 20 years old. The ethnic background of the patients is shown in figure 4.2, 65% of the population are white Caucasian, 16% are black Afro-Caribbean, 1% are Indian and 1% are of Chinese origin. Of the 545 patients, 347 (63.7%) are treated with a PI/NRTI combination, 3 (0.6%) are treated with PI drugs alone, 29 (5.3%) are treated with NRTI drugs alone and 166 (30.5%) are treated with an NRTI/NNRTI combination (table 4.1). The influence of ethnicity on plasma glucose and TAG concentration has not been examined here due to the small numbers in some of the treatment groups.

4.4.2 Patient age and plasma TAG and glucose concentration

There is no obvious relationship between age and plasma TAG concentration (figure 4.3) in this population, as indicated by the trend line. However, there does appear to be a trend towards increased plasma glucose as age increases (figure 4.4), although both the TAG and glucose data may be influenced by a survival bias.

4.4.3 Patient gender and plasma TAG and glucose concentration

Plasma TAG concentration (figure 4.5) is higher in the males (median 1.8 mmol/l, 0.4-15.3) than in the females in this group (1.2 mmol/l, 0.4-7.3; $p<0.05$). Plasma glucose (figure 4.6) is also higher in males (5.1 mmol/l, 3-14.4) compared to females (5 mmol/l, 3,7-10.7; $p<0.05$). However, there is not an even distribution of gender within this group as 89% of this population are male.

4.4.4 Duration of therapy and plasma TAG and glucose concentration

While Univariate linear regression analysis does not show an association between duration of therapy and plasma TAG concentration (figure 4.7), there is a non-significant trend towards an increase in plasma glucose as the time spent on therapy increases (figure 4.8). However, multivariate analysis (see section 4.4.8) shows the duration of therapy to be the strongest predictor of plasma TAG concentration.

4.4.5 Type of therapy and plasma TAG and glucose concentration

The audit data would suggest that hypertriglyceridaemia (plasma TAG >2.3 mmol/l) is more prevalent with a combination of PI and NRTI therapy (figure 4.9). However, this is due to the greater number of patients treated with this combination. When plasma TAG is divided into quartiles and each quartile expressed as a percentage of the total number of patients in that therapy group, 33% of PI/NRTI combination patients, 44% of NRTI patients and 31% of NRTI/NNRTI combination patients are hypertriglyceridaemic (plasma TAG >2.3 mmol/l, table 4.1).

The data in figure 4.10 also suggests that elevated plasma glucose is more prevalent in PI/NRTI combination treated patients. However, when glucose is expressed in quartiles relative to the number of patients in each group (table 4.2), 17% of the PI/NRTI combination patients, 67% of the PI patients, 28% of the NRTI patients and 17% of the NRTI/NNRTI combination patients have elevated plasma glucose (>6.1 mmol/l). While this suggests a higher prevalence for the PI alone treated group, there are only 3 patients within this group.

The individual drugs or combinations of drugs used to treat individuals have not been analysed as there were a possible 91 different regimens, providing too small groups to draw any inference from the data.

4.4.6 Viral load and plasma TAG concentration

Univariate linear regression analysis showed no obvious association in this group between viral load and plasma TAG concentration (figure 4.11). However, when viral load was analysed for each treatment group (table 4.4), the NRTI group showed a threefold higher percentage of patients (62%) with a viral load of more than 100 copies/ml plasma in comparison to either of the combination groups (PI/NRTI 17%; NRTI/NNRTI 23%).

4.4.7 Plasma glucose and TAG concentration

As the development of the metabolic syndrome is often associated with hypertriglyceridaemia and concurrent hyperglycaemia, the association between plasma TAG and glucose was examined in this group (figure 4.12). While there does appear to be a trend towards an increase in blood glucose with increasing plasma TAG concentration, the trend is weak (r^2= 0.0129). Further analysis of this data shows that 56% of patients have both plasma glucose and TAG concentrations within the normal range. However, 10% of patients are hyperglycaemic with normal TAG levels (table 4.3), 25% are hypertriglyceridaemic with normal blood glucose and only 9% have both hypertriglyceridaemia with concurrent hyperglycaemia. When the data is analysed by treatment group, there appears to be an increased incidence of both hypertriglyceridaemia and hyperglycaemia with the exclusively NRTI treated group, although this was not significant (figure 4.13, table 4.4). A further analysis of the data (table 4.5) shows that while age is not different between the treatment groups, the NRTI group have a significantly longer duration on therapy (median 1238 days, 219-2628) than both the combination groups (PI/NRTI 561 days, 181-1751; NRTI/NNRTI 547 days, 184-1753; $p<0.05$).

4.4.8 Multivariate analysis

The significance of the multiple correlation coefficients determined by the Wilks Lambda test for plasma TAG was tested for the variables of therapy type, duration, CD4 count and viral load. Both therapy duration ($R^2 = 0.933$) and viral load ($R^2 = 0.598$) were found to correlate significantly ($p<0.05$) with plasma TAG concentration. As therapy duration may independently correlate with viral load, a partial correlation coefficient was calculated to determine the influence of therapy duration on plasma TAG concentration, controlling for viral load. Therapy duration remained positively correlated with plasma TAG concentration at $p<0.05$. No significant correlation was found between these variables and plasma glucose concentration.

Table 4.1 Distribution of HAART therapy types and plasma TAG concentration - an audit of 545 HIV-positive patients on the first line therapy (no significant differences were found between groups).

Therapy Type	No. on each Tx	% on each Tx	Plasma TAG < 2.3 mmol/l		Plasma TAG 2.3-5.0 mmol/l		Plasma TAG 5.1-7.5 mmol/l		Plasma TAG > 7.6 mmol/l	
			N	%	N	%	N	%	N	%
PI+NRTI	347	63.7	231	67	98	28	14	4	4	1
PI	3	0.6	3	100	0	-	0	-	0	-
NRTI	29	5.3	16	55	12	41	1	3	0	-
NRTI+ NNRTI	166	30.5	115	69	45	27	5	3	1	0.6
Total	545	100	365	67	155	28	20	4	5	0.9

Table 4.2 **Distribution of HAART therapy types and plasma glucose concentration - an audit of 545 HIV-positive patients on the first line therapy (537 patients had recorded plasma glucose measurements, no significant differences were found between groups).**

Therapy Type	No. on each Tx	% on each Tx	Plasma glucose 3.0-6.1 mmol/l		Plasma glucose 6.2-7.1 mmol/l		Plasma glucose 7.2-8.1 mmol/l		Plasma glucose >8.2 mmol/l	
			N	%	N	%	N	%	N	%
PI+NRTI	341	64	284	83	44	13	9	3	4	1
PI	3	1	1	33	2	67	0	-	0	-
NRTI	29	5	21	72	4	14	2	7	2	7
NRTI+ NNRTI	164	30	146	89	13	8	3	2	2	1
Total	537	100	452	84	63	12	14	3	8	1

Table 4.3 **Distribution of plasma TAG and glucose concentrations from 405 HIV-positive patients (no significant differences were found between groups).**

Plasma TAG concentration (mmol/l)	Plasma glucose 3.0-6.1 mmol/l		Plasma glucose 6.2-7.1 mmol/l		Plasma glucose 7.2-8.1 mmol/l		Plasma glucose >8.2 mmol/l	
	N	%	N	%	N	%	N	%
≤2.3	226	84	33	12	7	3	3	1
2.4-5.0	89	75	24	20	4	3	3	3
5.1-7.5	11	73	3	20	0	-	1	7
>7.5	2	67	0	-	1	33	0	-

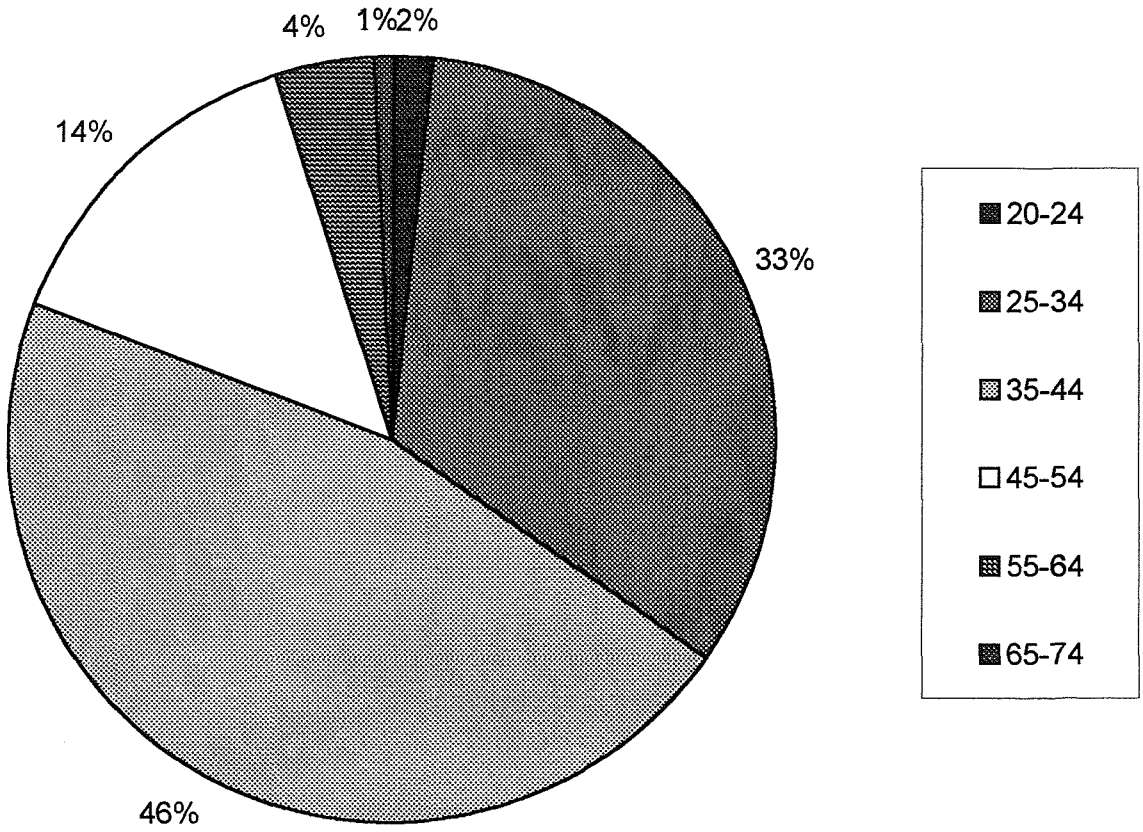
Table 4.4 **Distribution of hypertriglyceridaemia with and without concurrent hyperglycaemia and analysis of viral load in each treatment group from 405 HIV-positive patients by drug class combination (no significant differences were found between groups).**

Plasma concentration	PI/NRTI		PI only		NRTI only		NRTI/ NNRTI	
(mmol/l)	N	%	N	%	N	%	N	%
Total N	347	-	3	-	29	-	166	-
TAG>2.3	116	33	0	-	13	45	51	31
Glucose>6.1	63	18	2	67	8	28	20	12
Elevated TAG & Glucose	26	8	0	-	3	10	8	5
Viral load >100 copies/ml plasma	60	17	0	-	18	62	39	23

Table 4.5 **Patient age and duration of therapy within the four treatment groups, data taken from the 410 of the 545 patients in the audit in which therapy had been initiated for more than 180 days. *Statistically significant difference between the NRTI and other treatment groups using the Kruskall Wallis test ($p<0.05$).**

	PI/NRTI		PI only		NRTI only		NRTI/NNRTI	
	Med	**Range**	**Med**	**Range**	**Med**	**Range**	**Med**	**Range**
Age (y)	37	22-69	40	39-41	38	26-64	38	24-69
Duration of therapy (d)	561	181-1751	1140	191-1328	*1238	219-2628	547	184-1753

Figure 4.1 Distribution of patient ages in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital.



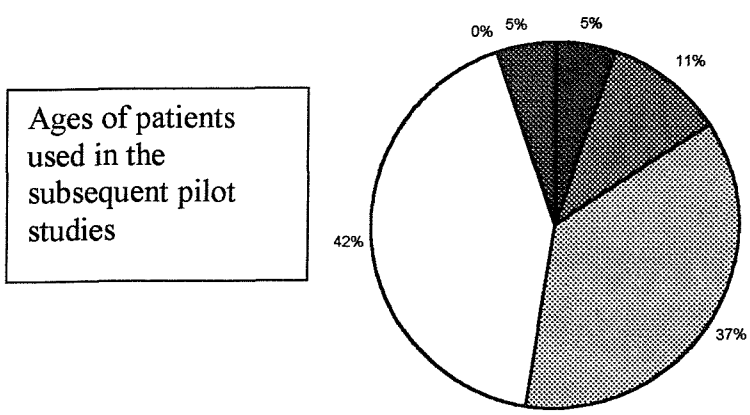


Figure 4.2 Ethnic background of an HIV+ population taken from an audit of 545 patients at the Chelsea and Westminster Hospital.

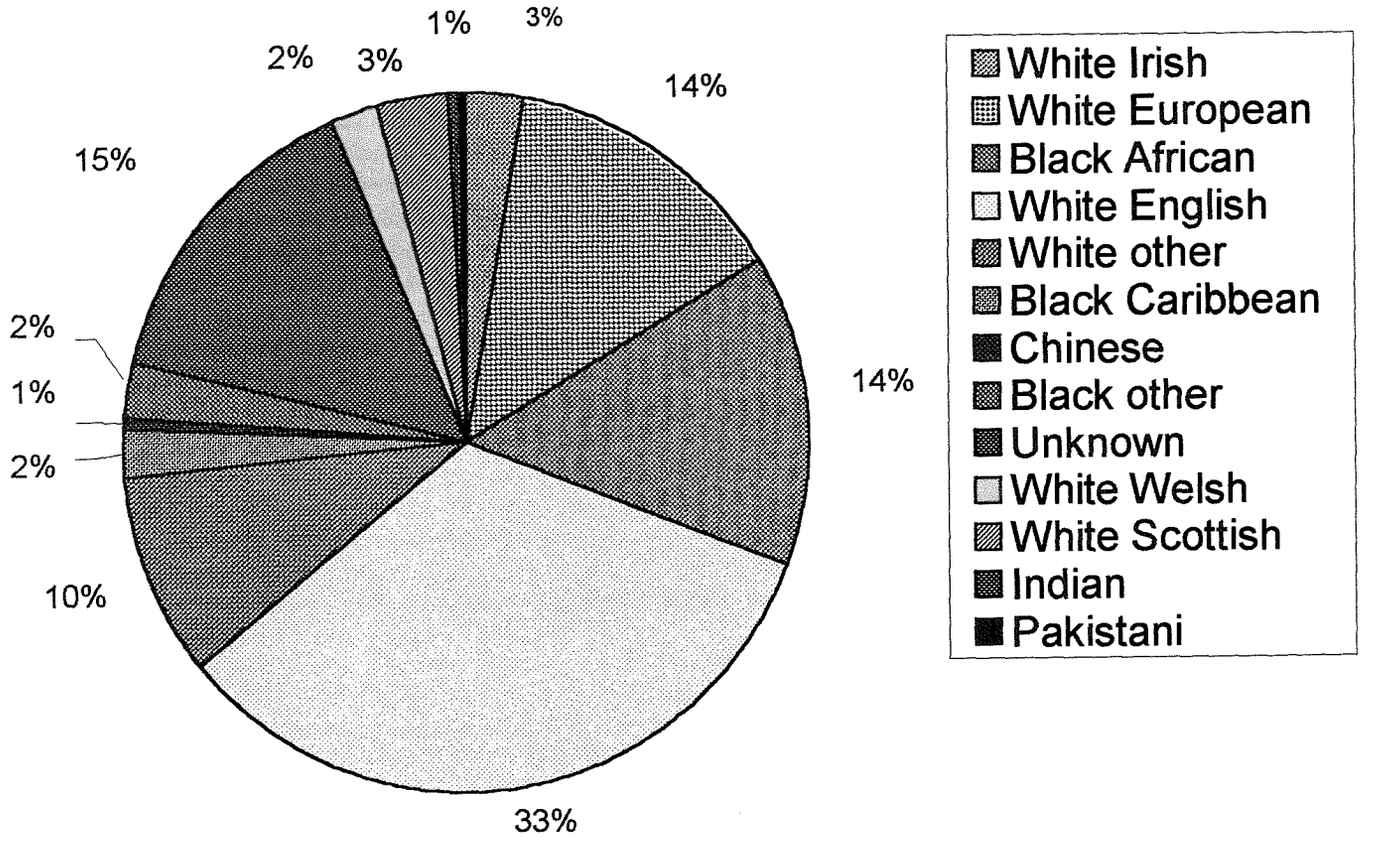


Figure 4.3 Patient age and plasma TAG concentration (mmol/l) at routine examination in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital.

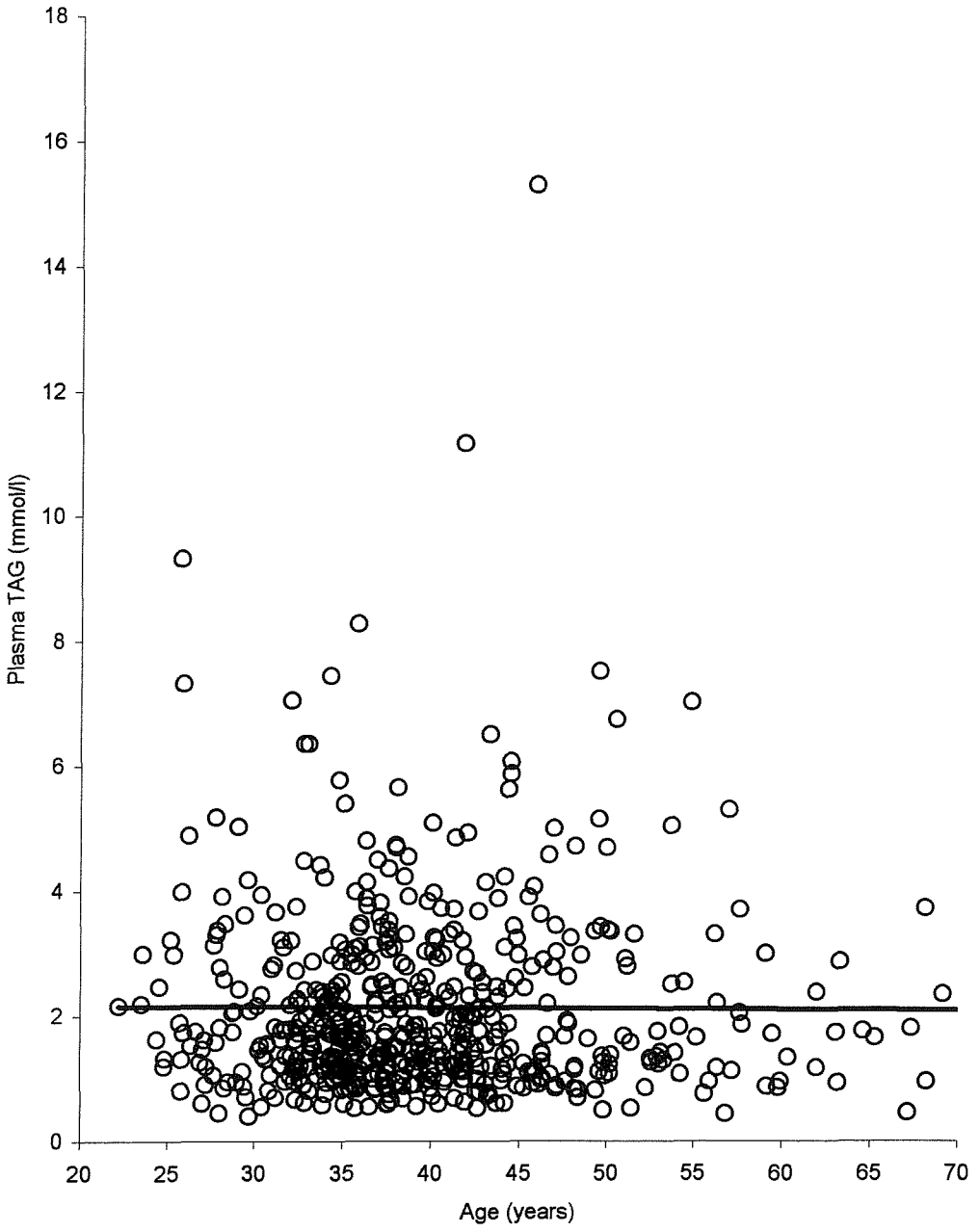


Figure 4.4 Patient age and plasma glucose concentration (mmol/l) at routine examination in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital.

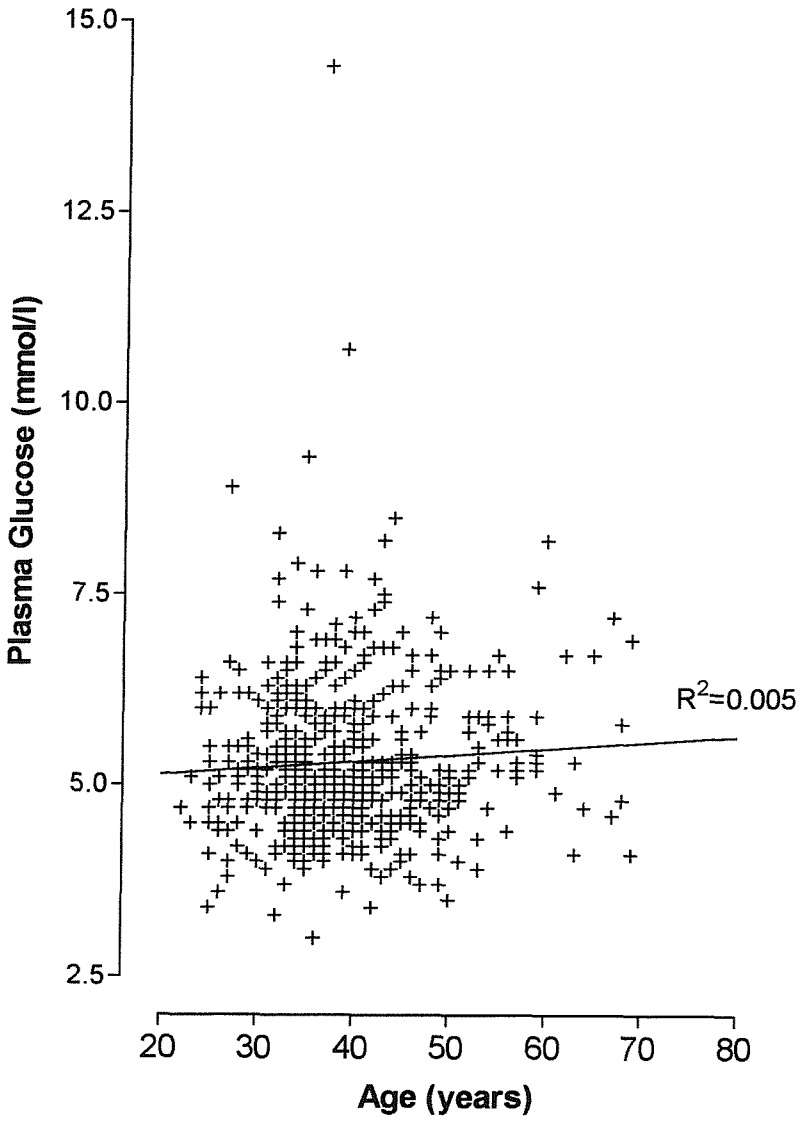


Figure 4.5 Patient gender and plasma TAG concentration (mmol/l) at routine examination in an HIV+ population. Results taken from an audit of 545 patients (n=77 female and n= 468 male) at the Chelsea and Westminster Hospital. There is a significant difference between males and females using the Mann Whitney test ($p<0.05$).

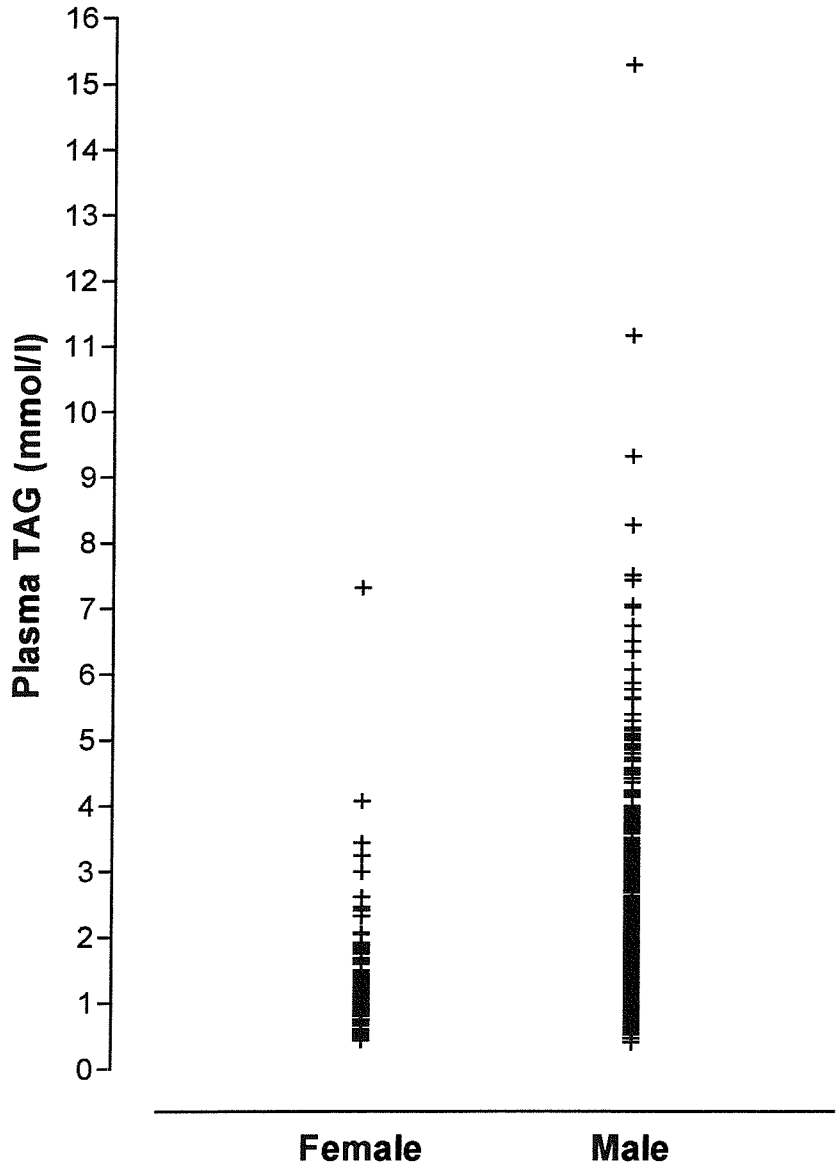


Figure 4.6 Patient gender and plasma glucose concentration (mmol/l) at routine examination in an HIV+ population. Results taken from an audit of 545 patients (n=77 female and n= 468 male) at the Chelsea and Westminster Hospital. There is a significant difference between males and females using the Mann Whitney test ($p<0.05$).

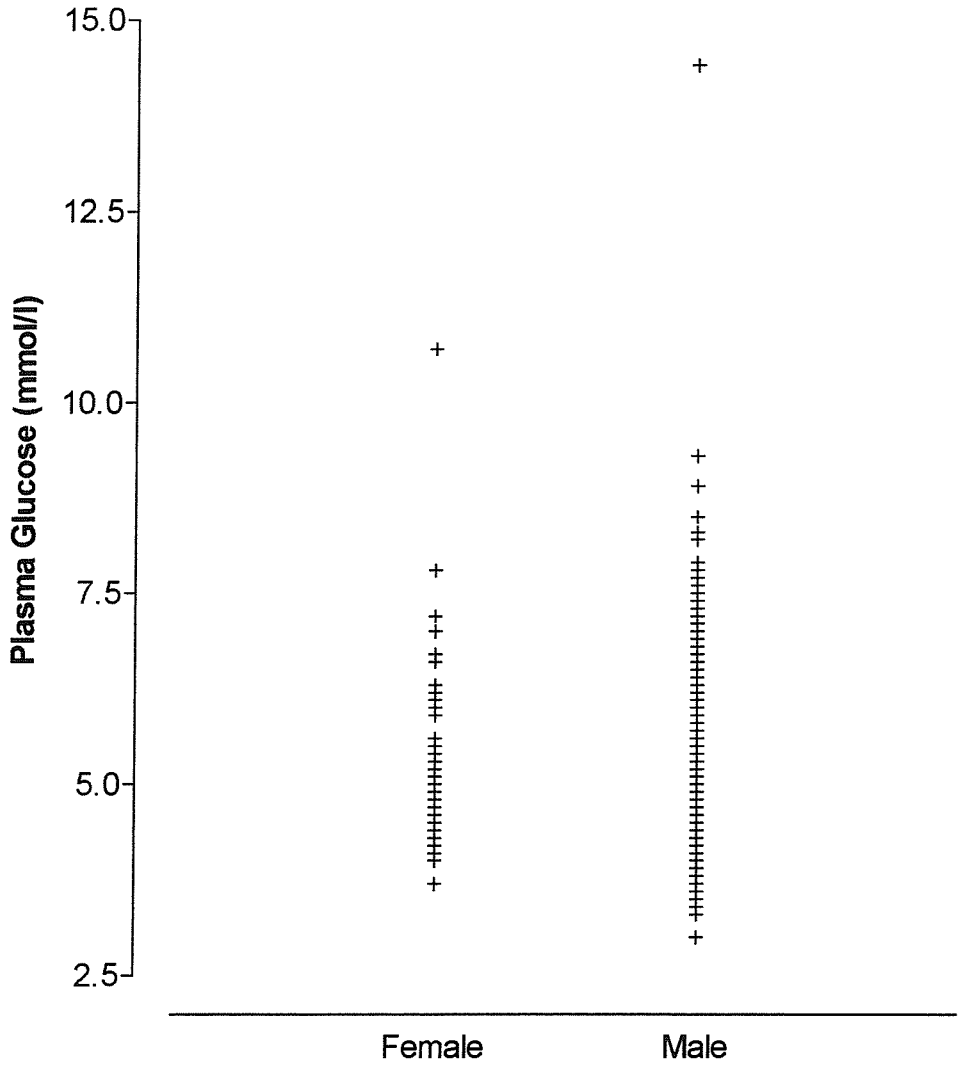


Figure 4.7 Duration on therapy and plasma TAG concentration (mmol/l) in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital.

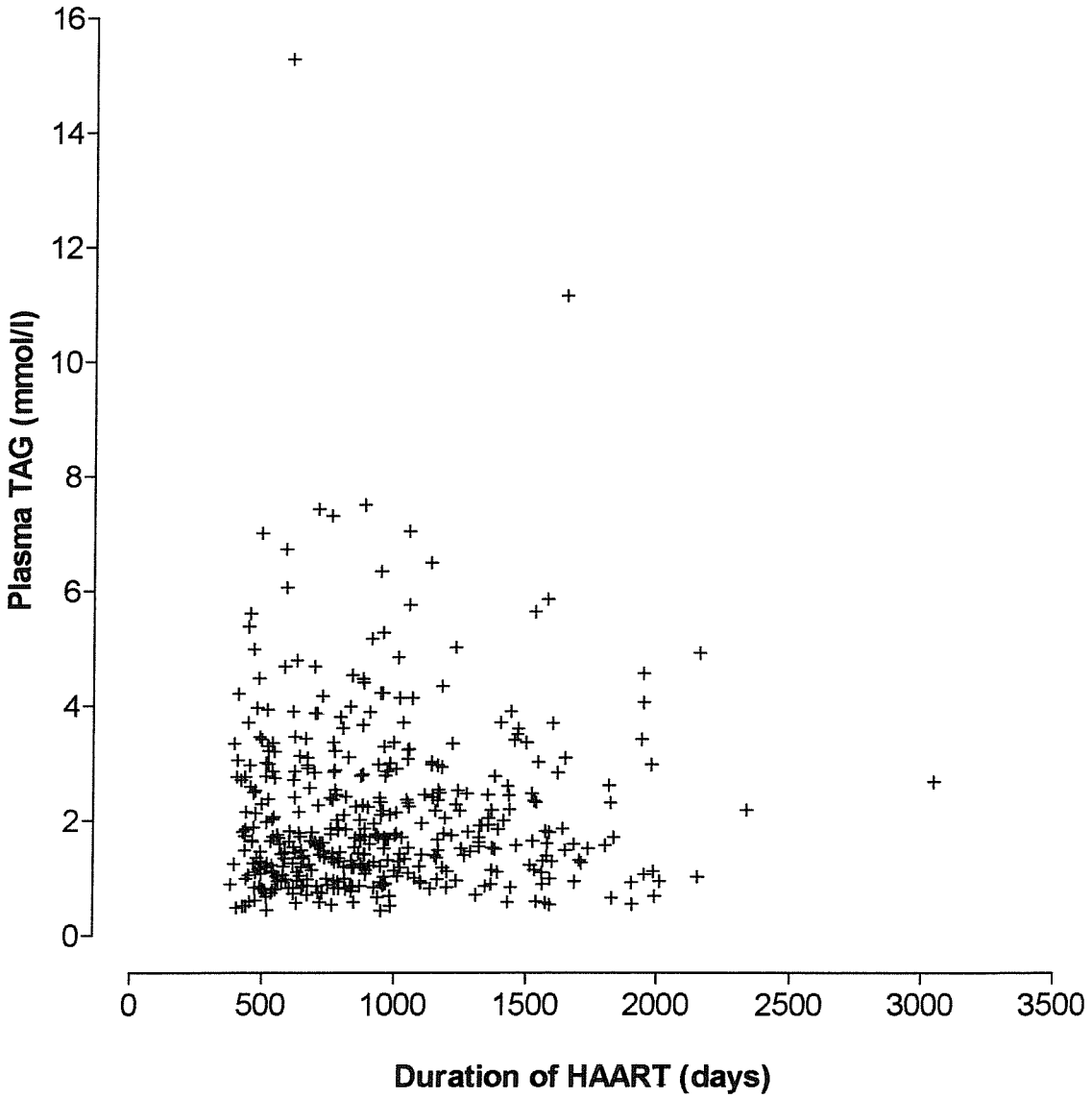


Figure 4.8 Plasma glucose concentration (mmol/l) and duration of therapy in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (410 patients had plasma glucose measurements recorded following more than 6 months of therapy).

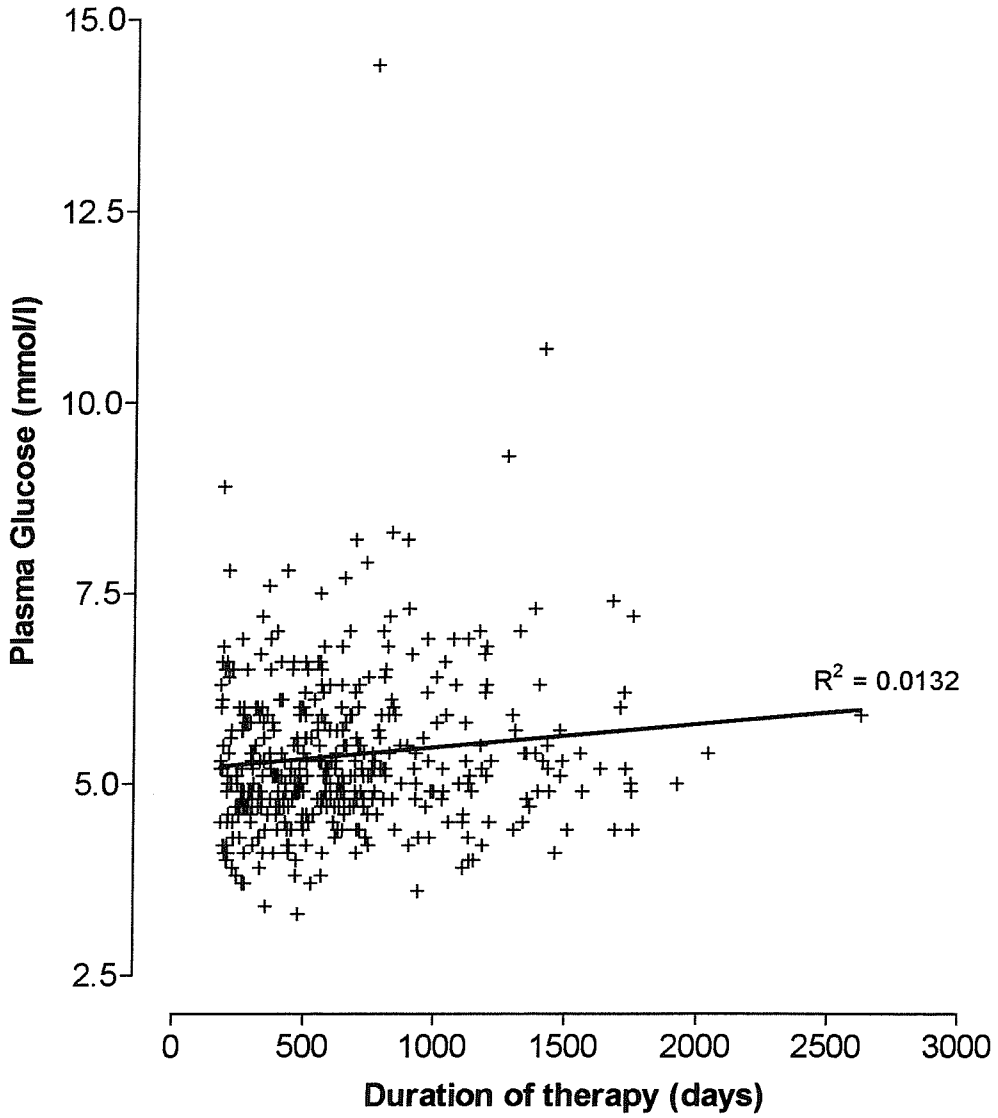


Figure 4.9 Type of therapy and plasma TAG concentration (mmol/l) in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (PI+NRTI n=347, 64%; PI n=3, <1%; NRTI n=29, 5%; NRTI+NNRTI n=166, 31%).

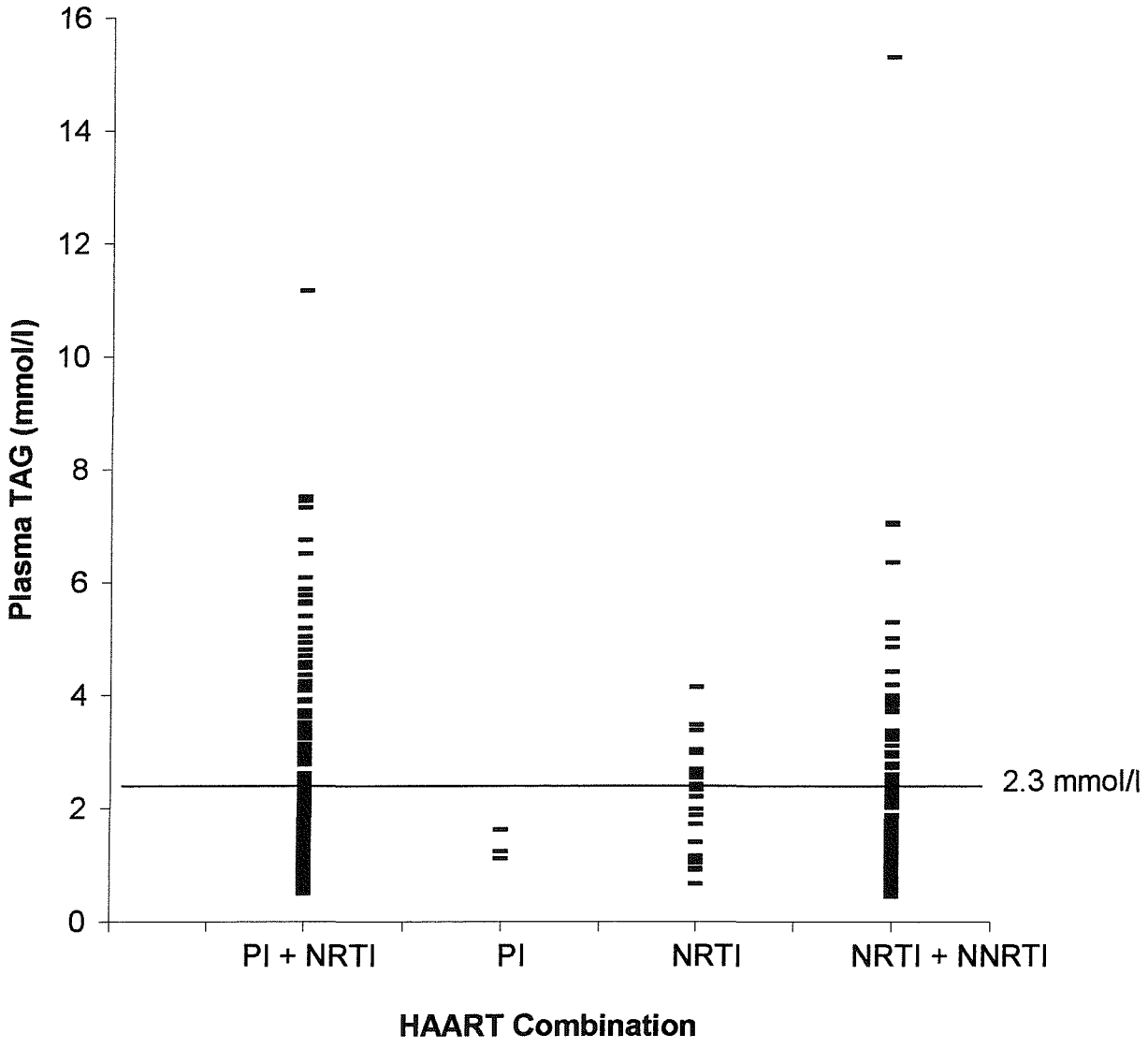


Figure 4.10 Plasma glucose concentration (mmol/l) and therapy type in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (405 patients had plasma glucose and TAG measurements taken at the same time, no significant differences were found between groups).

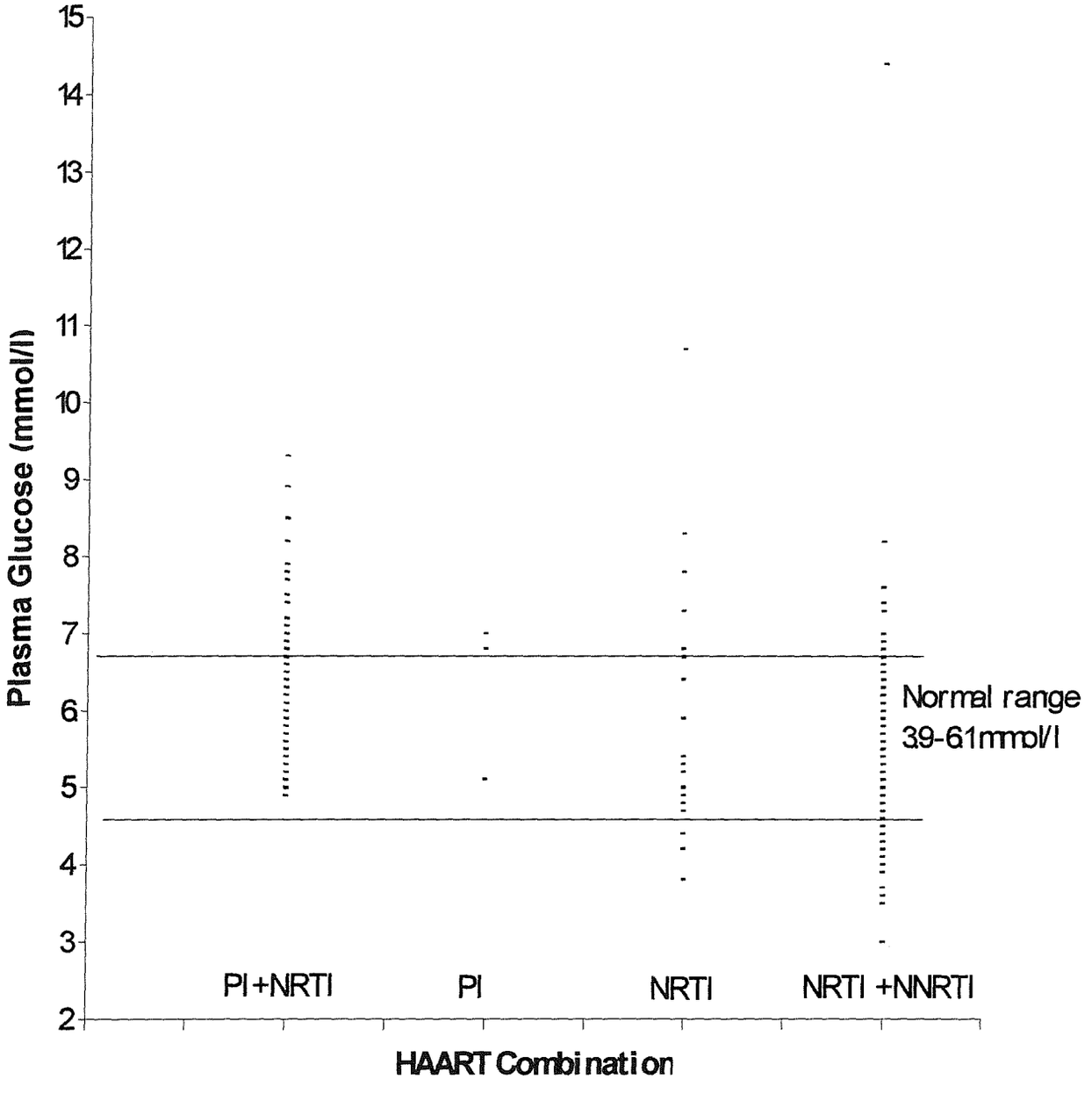


Figure 4.11 Plasma TAG concentration (mmol/l) and viral load in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (406 patients had viral load and plasma TAG measurements taken at the same time).

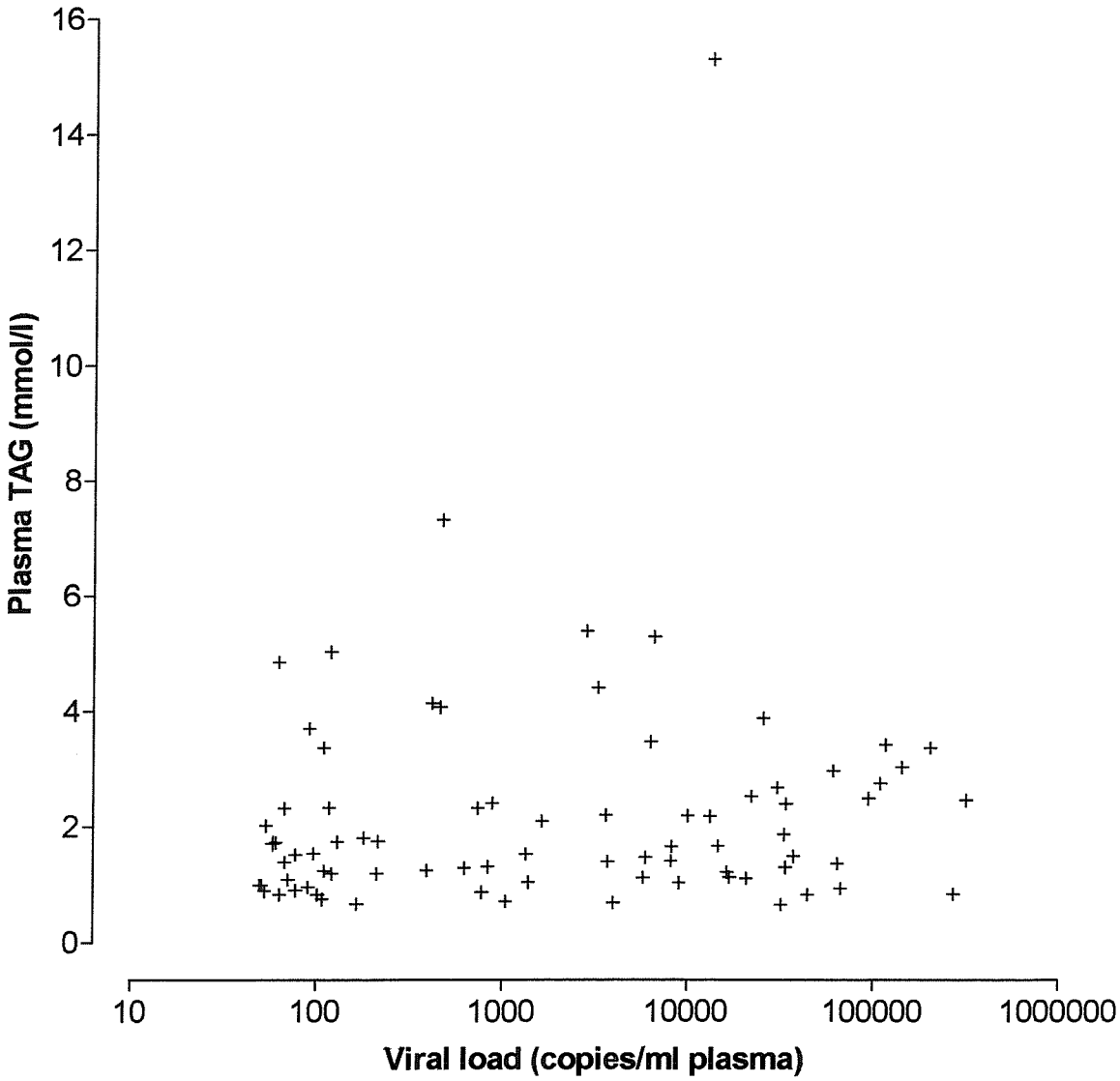
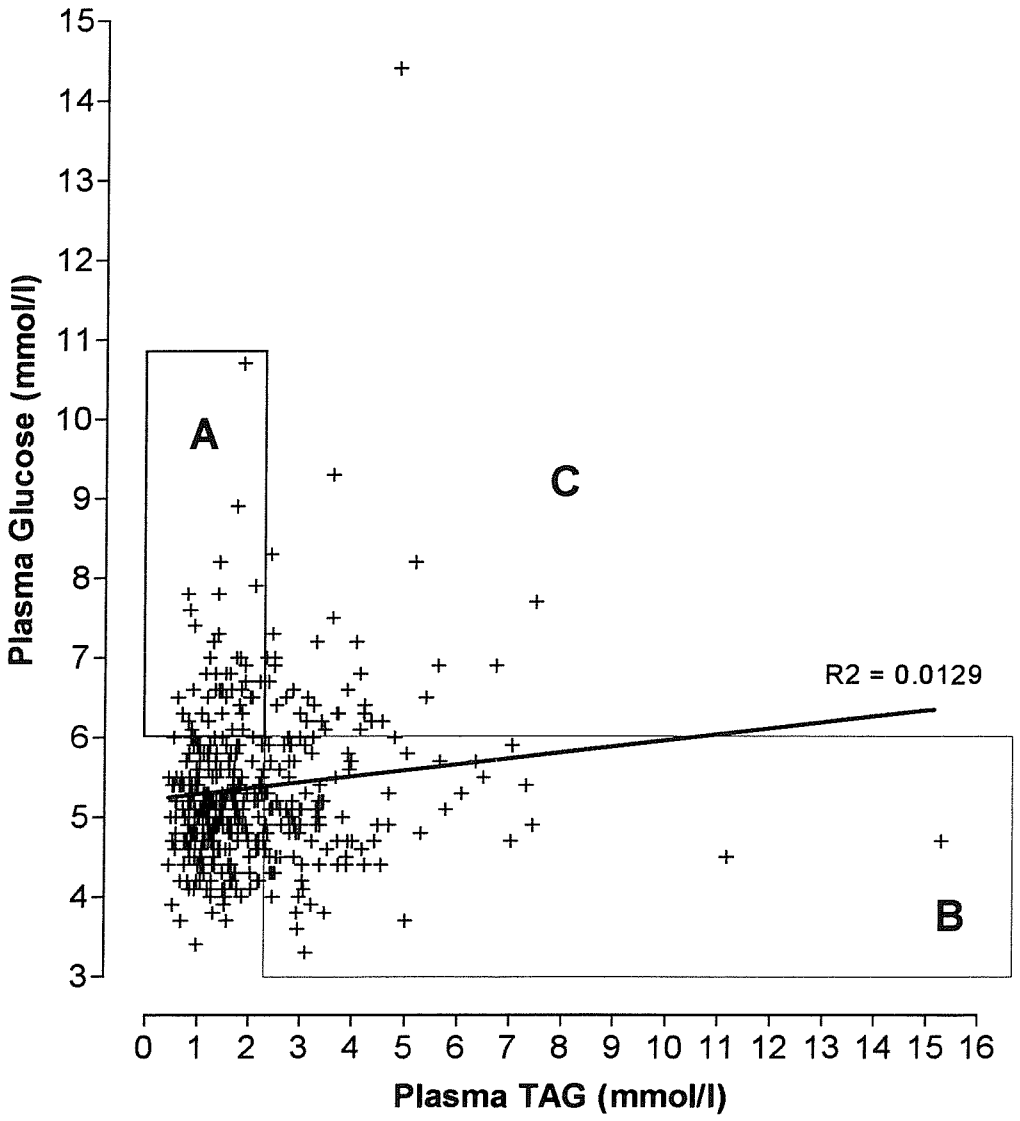


Figure 4.12 Plasma TAG concentration (mmol/l) and glucose concentration (mmol/l) in an HIV+ population. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (405 patients had plasma glucose and TAG measurements taken at the same time).

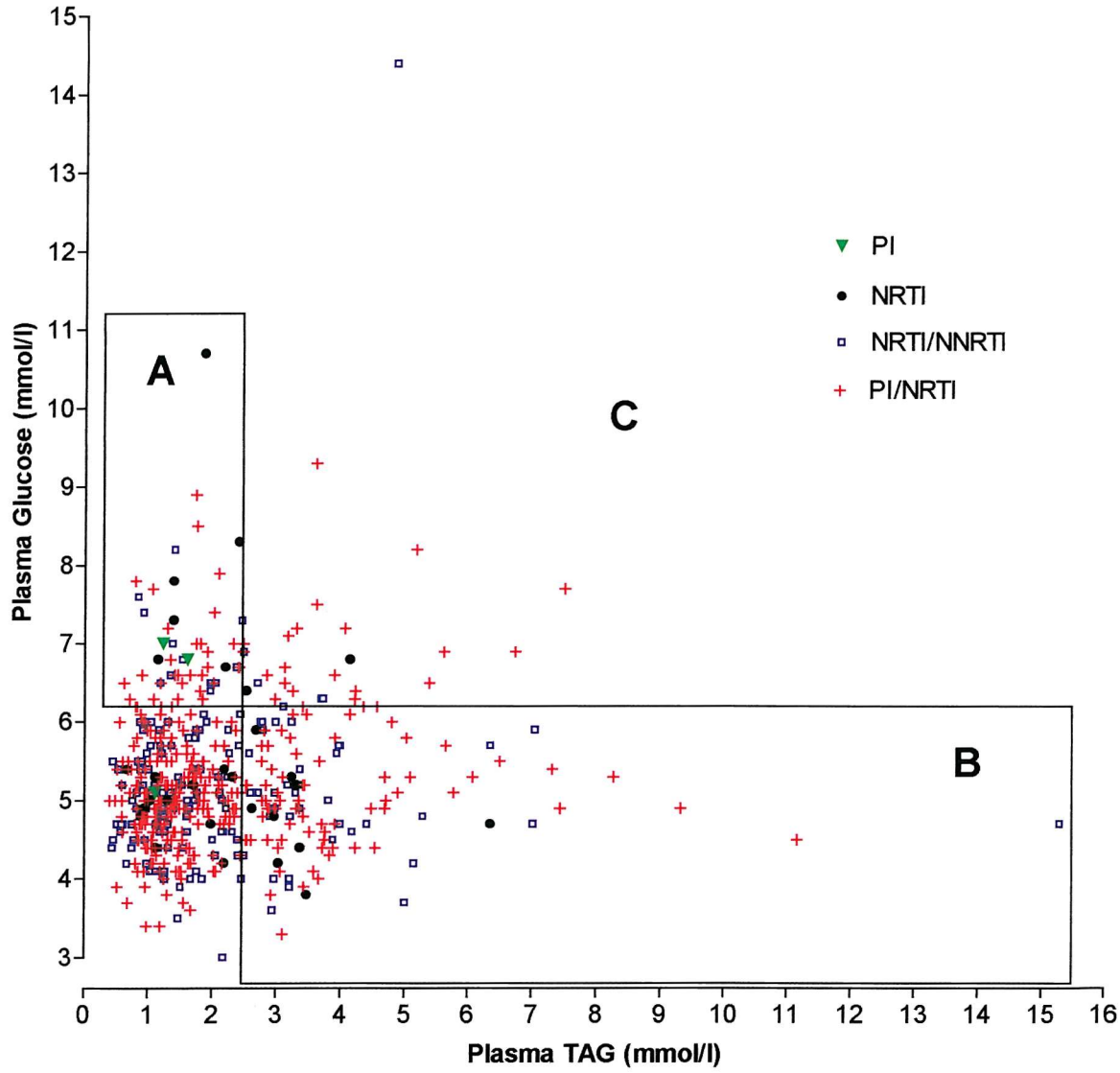


A – Hyperglycaemic (10%)

B – Hypertriglyceridaemic (25%)

C – Hypertriglyceridaemic and hyperglycaemic (9%)

Figure 4.13 Plasma TAG concentration (mmol/l) and glucose concentration (mmol/l) in an HIV+ population by drug class combination. Results taken from an audit of 545 patients at the Chelsea and Westminster Hospital (405 patients had plasma glucose and TAG measurements taken at the same time, no significant differences were found between the groups).



A – Hyperglycaemic

B – Hypertriglyceridaemic

C – Hypertriglyceridaemic and hyperglycaemic

4.5 Discussion

This population was examined in order to determine whether factors such as age, gender, therapy type, duration and degree of viral burden were associated with metabolic perturbations as previously reported by other authors [Carr *et al.* 1999; Safrin & Grunfeld 1999; Martinez et al. 2001]. However, associations do not imply cause and effect and more detailed randomised controlled trials are required in order to determine whether causal relationships exist. In a non-HIV infected population, factors such as smoking, obesity, diet and exercise have been related to the development of hyperlipidaemia [Wilson *et al.* 1996]. Examining these possible lifestyle and anthropometric factors relative to metabolic perturbations is without the scope of this audit.

4.5.1 Patient age and plasma TAG and glucose concentration

While Safrin & Grunfeld (1999) reported that age was associated with the development of lipodystrophy, there does not appear to be a relationship between age and plasma TAG concentration in this population, although, there does appear to be a trend towards increased plasma glucose as age increases. The degree to which these measures may be influenced by a survival bias within the two populations is unknown. In the data obtained here, only 5% of the population are above 55 years old with 1% aged 65 and over. This does not reflect the general population as, in 1999, the proportion of the population aged 65 and over in the United Kingdom was 16 per cent [Percentage of the population aged 65 and over: EU Comparison 1960-1999: Social Trends Dataset]. Therefore, comparisons between the HIV-infected and healthy populations in terms of the influence of age on plasma glucose and lipids may not be appropriate.

4.5.2 Patient gender and plasma TAG and glucose concentration

While the data would suggest that both plasma TAG and glucose concentrations are higher in HIV-positive males than in HIV-positive females, 89% of this population are male and therefore, the female group may not be sufficiently represented for a valid comparison. The data reported by Martinez et al. (2001) would suggest the opposite of this were true, with females more likely to develop lipodystropy. While this audit data does not contain enough information to assess whether or not the patients have lipodystrophy, if an

elevated plasma TAG and glucose are associated with lipodystrophy development, as reported by Carr et al. (1999) in a large epidemiological study, this data may suggest gender differences in either the rate of progression from metabolic to morphologic symptoms or aetiology. However, it could highlight possible differences between centres in the classification and diagnosis of this complex syndrome.

4.5.3 Duration and type of therapy and plasma TAG and glucose concentration

When the data is analysed as a whole, there does not appear to be an association between duration of therapy and plasma TAG concentration, although there is a non-significant trend towards an increase in plasma glucose as the time spent on therapy increases. The duration of HAART has previously been reported to be associated with the development of lipodystrophy [Carr et al. 1999], although this may be drug class related and therefore, masked by the different therapies in this type of analysis. It was not possible to examine individual drug exposure or combinations, as the population was too heterogeneous with a total of 91 possible combinations used. However, when the drug combinations are sorted into four main categories; 1) PI/NRTI, 2) PI only, 3) NRTI only and 4) NRTI/NNRTI, the data shows that 33% of PI/NRTI combination patients, 44% of NRTI patients and 31% of NRTI/NNRTI combination patients are hypertriglyceridaemic (plasma TAG >2.3 mmol/l). It is interesting to note that all these combinations contain at least one NRTI, although when this drug class is used exclusively there appears to be more of an effect.

This is not the case for plasma glucose as 2/3 of the PI only group had elevated glucose. Although, this group is too small to draw conclusions, this may indicate different roles of the two primary drug classes in the development of lipodystrophy. However, larger scale studies would be difficult as PI monotherapies are not routinely used. Also, as the prevalence of lipodystrophy on PI containing regimens has been reported as being higher [Carr et al. 1999], patients are less often given PI containing combinations as the first therapy. Of the remaining treatment groups, 17% of the PI/NRTI combination patients, 28% of the NRTI patients and 16% of the NRTI/NNRTI combination patients have elevated plasma glucose (>6.1 mmol/l), exhibiting a similar pattern to the plasma TAG data. The percentage of patients in each group that have both hypertriglyceridaemia and hyperglycaemia was 8% of the PI/NRTI combination patients, 10% of the NRTI patients and 5% of the NRTI/NNRTI combination patients. A similar pattern is emerging whereby

it appears that NRTI use exclusively is associated with a higher prevalence of elevated plasma lipids, glucose and both. If a combination of hypertriglyceridaemia and hyperglycaemia is indicative of the metabolic syndrome observed with lipodystrophy, then it appears in this group that NRTI use has a higher risk of lipodystrophy development.

For the PI/NRTI combination group, these figures are much lower than those reported by Carr et *al.* (1998) (64% occurrence of lipodystrophy with a combination PI therapy), but are comparable with those from Shaw *et al.* (1998) (12.5% occurrence of lipodystrophy with a combination PI therapy). The suggestion that elevated plasma TAG is seen more often with PI therapy [Sullivan *et al.* 1998; Paparizos *et al.* 2000] does not appear to be supported by this data. However, there may be differences in therapy duration, specific drugs analysed and patient characteristics between the two study populations.

For the NRTI/NNRTI group, the prevalence of hyperglycaemia within the group (17%) is similar to the reported incidence of lipodystrophy by Aldeen *et al.* (1999), defined by changes in body shape (16%).

For an exclusively NRTI treated group, the prevalence of lipodystrophy, characterised by elevated plasma TAG and an increased VAT:TAT ratio, has been reported to be between 18.8% and 63% depending on drug used [Saint-Marc *et al.* 1999]. The data analysed here falls into this range for the prevalence of hypertriglyceridaemia (44%) or hyperglycaemia (28%) independently, although only 10% of this group exhibit both of these traits concurrently.

In order to explain these differences in prevalence of these metabolic parameters between the different treatment groups, the median duration of therapy within each treatment group was examined. The NRTI group shows a significantly longer duration on therapy (55% >1000 days; $p<0.05$) than either of the combination groups (PI/NRTI 12% >1000 days or NRTI/NNRTI 8% >1000 days). The PI group also show a longer median time on therapy (67% >1000 days), although this is not significant due to the small sample size.

In addition, very limited data has been collected on these individuals and it is unclear if the NRTI treated group have other factors, such as lifestyle factors that would predispose them to a higher prevalence of metabolic perturbation than the other treatment groups.

4.5.4 Viral load and plasma TAG concentration

There was no obvious association in this group between viral load and plasma TAG concentration. This would suggest that in HAART treated patients the plasma TAG concentration is independent of the degree of viral replication. However, when the data is analysed according to the different treatment groups, it appears that the NRTI group have a higher prevalence of patients (62%) with an increased viral load (more than 100 copies/ml plasma) in comparison to either the PI/NRTI group (17%) or the NRTI/NNRTI group (23%). This again may help to explain the apparent higher incidences of elevated plasma TAG and glucose within this group, as elevated viral load is used as a marker for both treatment failure and disease severity. The higher the infective burden, the more metabolic perturbations would be expected to be seen as reported by authors in patients prior to the HAART era [Gomez-Sirvent *et al.* 1994; Christeff *et al.* 1995; Fernandez-Miranda *et al.* 1998].

4.5.5 Comparison of the audit population to the study groups

It is important to verify that the groups that we have selected for study from the larger population treated at this centre, are representative of the population. The insert in figure 4.1 shows that in the groups selected for study there is an over-representation of patients aged 45 to 54 years and a comparative under-representation of patients aged 25 to 34 years. This may be due to the inclusion of two treatment groups with lipodystrophy (chapter 7.0) and may reflect the influence of age on the development of lipodystrophy in more homogeneous populations. The groups were selected in chapter 7.0 on the basis of elevated plasma triglyceride and self-reported changes in body shape. While the audit did not include anthropometry measures, the incidence of hypertriglyceridaemia based on the audit data is almost half of the exclusively NRTI treated patients and one third of the PI/NRTI combination treated patients. This would suggest that the groups selected for study may represent a reasonable percentage of the patients treated with the same drug classes. While the study subjects did not represent all of the ethnic backgrounds observed in the audit, the two largest groups of white Caucasian and black Afro-Caribbean were represented.

4.6 Summary

The aims of this study were to assess and compare the prevalence of elevated plasma TAG and glucose concentration in this audit population to values reported by other authors, to look for factors that may be associated with these metabolic perturbations and to examine whether the populations selected for study were representative of a larger population of patients from the same treatment centre. The results demonstrate that:

1. A comparison of the prevalence of hypertriglyceridaemia and hyperglycaemia with the prevalence of lipodystrophy reported in the literature;

    a. In the NRTI treated group, the prevalence of hypertriglyceridaemia or hyperglycaemia independently agrees with the rates of lipodystrophy reported in this group by Saint-Marc *et al.* (1999), although the prevalence of these factors combined is much lower.

    b. In the NRTI/NNRTI combination group, the prevalence of hyperglycaemia is the same as the prevalence of body shape changes reported in this group by Aldeen *et al.* (1999) suggesting the possibility that, in this group, the metabolic and morphologic alterations may be more closely associated.

    c. In the PI/NRTI combination group, the prevalence of both hypertriglyceridaemia and hyperglycaemia is lower than the rates reported by Carr *et al* (1998) but in agreement with those reported by Shaw *et al.* (1998), possibly suggesting differences in sampling time or analysis, or inherent differences in the populations studied.

2. Factors associated with plasma TAG and glucose concentrations;

    a. Age was not associated with elevated plasma TAG concentration although there was a trend towards an increased glucose concentration with increasing age.

b. Male gender was associated with significantly higher levels of TAG and glucose, although the audit population contained nine times more males than females and may therefore, not allow a valid comparison.

c. Patients exclusively treated with NRTI drugs had approximately 30% higher prevalence of hypertriglyceridaemia, a 50% higher prevalence of hyperglycaemia and a 25% higher prevalence of both when compared to those patients on combination therapies i.e. PI/NRTI or NRTI/NNRTI. However, the NRTI group also exhibited a higher median viral load which may reflect the efficacy of this treatment and contribute to the higher incidence of metabolic perturbations found here.

d. Two of the three patients treated exclusively with PI drugs had hyperglycaemia suggesting an interesting area for further study, although patients treated with this therapy are very rare.

In comparing the audit data to the groups selected for inclusion in these pilot studies, it appears that among the study groups there is an over-representation of older HIV+ males. This may be a result of the inclusion of two groups selected for the presence of lipodystrophy indicating an influence of age, although no association between the metabolic perturbations studied and age was found in the audit population. Alternatively, the audit population consists of patients on their first HAART combination and may contain a relatively larger proportion of younger males depending of course, on the duration of infection and age at diagnosis.

This may have implications for the extrapolation of the data from the subsequent metabolic studies to the larger HIV population, although the sample sizes of the groups used in the metabolic studies were small and therefore, extrapolating this data to a larger population should be done with caution. However, the incidence of hypertriglyceridaemia found in both the exclusively NRTI treated patients and the PI/NRTI combination treated patients from the audit would suggest that the groups selected for study may represent a reasonable percentage of the patients treated with the same drug classes.

Chapter 5.0 A comparison of postprandial lipid metabolism in HIV-positive therapy naïve males and HIV-negative males.

5.1 Introduction

HIV infection per se is known to cause alterations in lipid metabolism and elevated plasma TAG (>2.3 mmol/l) is prevalent in AIDS [Tikkanen 1990]. Although, it is unclear if perturbed lipid metabolism is only present in the advanced symptomatic stages of infection [Gomez-Sirvent *et al.* 1994; Christeff *et al.* 1995] or already exists in the earlier asymptomatic stages of infection. Hellerstein *et al.* (1993) have reported elevated de novo lipogenesis in asymptomatic HIV-positive subjects in comparison to healthy controls using the infusion of labelled acetate, but it is unknown whether this results in increases in plasma TAG concentration. Other authors have suggested that there is a reduced clearance of TAG from plasma [Grunfeld *et al.* 1991], an increased mobilisation of free fatty acids from peripheral adipocytes by lipolysis [Feingold *et al.* 1990; Mulligan *et al.* 1993] and that the oxidation of fatty acids may also increase in HIV infection [Mulligan *et al.* 1993; Hommes *et al.* 1991]. Plasma NEFA concentration may also be elevated or reduced compared to controls depending on the stage of infection [Nunez & Christeff 1994]. However, it appears that the characterisation of asymptomatic patients with a high viral burden requiring but prior to taking HAART has not been reported. While HAART is reported to result in elevated plasma TAG and glucose in comparison to data from a healthy population, it is not known what the patients metabolic characteristics are prior to taking this therapy and the extent to which this may alter their metabolic response to therapy.

5.2 Aims

This study was undertaken to examine postprandial lipaemia in HIV-positive males prior to taking HAART in comparison to healthy HIV-negative males of similar age in order to determine the influence of HIV infection without opportunistic infections or AIDS defining illness on lipid metabolism. Using stable isotope labelled fatty acids administered as part of a test meal would further elucidate the influence of dietary lipid intake on plasma TAG and NEFA concentrations in these groups by examining the residence time of the label within plasma TAG and NEFA fractions. A group of HIV-positive males were

selected on the basis of being therapy-naïve with a high viral load and therefore, both a high infective burden while being asymptomatic and a following requirement for HAART.

5.3 Methods

The study protocol and methods used have previously been described in the General Methods Chapter.

5.3.1 Subjects

All HIV-positive males (HIV+, n=6) were recruited at the Chelsea & Westminster Hospital and the HIV-negative males (HIV-, n=8) were recruited at the Institute of Human Nutrition, Southampton. The criteria for the HIV-positive group were; male, HIV-positive with high viral load, asymptomatic with no presence of infection (other than HIV) and therapy-naive. The HIV-negative group criteria were; male, similar age to the HIV positive group and healthy. Of the HIV-positive group, one subject was found to have a malignant tumour within his colon and this set of data was removed from the analysis, leaving n=5. All controls were Caucasian, while one of the HIV-positive group was African. Both groups consisted of smokers and non-smokers and smokers were asked to abstain from smoking for the duration of the trial. However, most had smoked in the morning prior to attending the study and insisted that abstaining from smoking for the 7-hour trial period was not possible.

5.4 Results

5.4.1 Subject characteristics

All subjects in the HIV+ group had high viral loads (table 5.1) with a concurrent low CD4 count (healthy CD4 count is approximately 1000 x 10^6 cells/litre). All subjects were aged between 20 and 55 years old (table 5.2) and the HIV- group (median age 42 years, 32-55) were selected for as similar age as possible to the HIV+ group (median age 45 years, 20-47). The HIV+ group were of similar height to the HIV- group but had significantly lower body weight (HIV+ 64.3 kg, 56.6-78.0; HIV- 85.1 kg, 68.3-95.7; $p<0.05$) and therefore, a lower body mass index (BMI) (HIV+ 20.8 kg/m^2, 19.6-22.3; HIV- 26.6 kg/m^2, 23.4-30.5; $p<0.05$). Three of the five HIV+ subjects and two of the eight HIV- subjects were smokers.

5.4.2 Body composition

The HIV+ group showed significantly lower absolute amounts of both fat mass (HIV+ 10.4 kg, 9.0-12.5; HIV- 17.5 kg, 11.4-22.8; $p<0.05$) and fat-free mass (HIV+ 53.9 kg, 47.6-65.5; HIV- 66.8 kg, 56.9-73.0; $p<0.05$; table 5.3) as measured by BIA. In terms of the relative proportions of fat and fat-free mass to overall body weight, the HIV+ group had a significantly lower percentage body fat (16.0%, 13.5-17.7) compared the HIV- group (21.1%, 15.5-24.5; $p<0.05$) and therefore, a higher percentage of fat-free mass (HIV+ 84.0%, 82.3-86.5; HIV- 79.0%, 75.5-84.5; $p<0.05$). Both waist and hip circumference were higher in the HIV- group compared to the HIV+ group (HIV+ waist 836 mm, 715-847, hip 950 mm, 867-980; HIV- waist 943 mm, 799-1022; hip 1067mm, 945-1120; $p<0.05$; table 5.4). However, as both these parameters were significantly higher, when expressed as waist to hip ratio both groups were similar (HIV+ 0.86, 0.82-0.88; HIV- 0.89, 0.78-0.92). The triceps skinfold measurement was lower in the HIV+ group than in the HIV- group (HIV+ 8.0 mm, 5.1-11.4; HIV- 14.5 m, 7.1-20.9; $p<0.05$). The mid-upper arm circumference (MUAC) was also lower in the HIV+ group (HIV+ 265 mm, 253-320; HIV- 341 mm, 315-379; $p<0.05$). This resulted in the estimated mid-upper arm muscle circumference being significantly lower in the HIV+ group (HIV+ 241 mm, 237-284; HIV- 298 mm, 271-320; $p<0.05$). The differences observed between the two groups in these anthropometric measures were greater than the 5% variability associated with the measures.

5.4.3 Energy expenditure

Table 5.5 shows the resting energy expenditure (REE) calculated from indirect calorimetry measures of VO_2 and VCO_2 expressed as kJ/day, kJ/kg fat free mass (FFM) and as the increase in energy expenditure over the 7 hour period following the meal. This was calculated as incremental area under the curve (AUC_i). REE (kJ/d) was similar between the two groups (HIV+ 7190 kJ/ d, 6312-8485; HIV- 7244 kJ/ d, 6157-8849; ns). However, when the REE was expressed per kilogram of FFM to compensate for differences in size, the HIV+ group had a higher REE per kg than the HIV- group (HIV+ 130 kJ/kg FFM, 119-140; HIV- 108 kJ/kg FFM, 100-123; $p<0.05$). There was no difference in the magnitude of increase in energy expenditure following the meal (diet-induced thermogenesis measured as incremental area under the curve) between the two groups.

5.4.4 Substrate oxidation

Both groups had similar baseline respiratory exchange ratio (RER) values (HIV+ 0.81, 0.79-0.92; HIV- 0.86, 0.78-0.95; table 5.6) and there was no significant difference in the amount of either lipid or carbohydrate oxidised, expressed as grams/hour, in the postabsorptive phase between the groups, although baseline lipid oxidation tended to be higher in the HIV+ group (HIV+ 3.23 g/h, 0.63-5.14; HIV- 2.58 g/h, 0.23-3.63; ns).

During the postprandial period, the total lipid and carbohydrate oxidation, calculated by area under the curve was similar between the two groups (table 5.7). However, the increase in both lipid and carbohydrate oxidation from baseline following the meal (AUC_i) tended to be lower in the HIV+ group (HIV+ 5.2 g lipid/7h, 2.5-10.4, 4.6 g carbohydrate/7h, -3.5-13.9; HIV- 10.4 g lipid/7h, 3.2-18.4, 16.2 g carbohydrate/7h, 0-78.2; ns). The patterns of both carbohydrate and lipid oxidation over the 7 hour period (figure 5.1 and 5.2) are not different between the two groups.

5.4.4.1 Endogenous and exogenous lipid oxidation

The percentage of tracer recovered on breath over 7 hours was used to calculate exogenous lipid oxidation and by difference, endogenous lipid oxidation (table 5.8). The percentage of the administered dose of [1-^{13}C] palmitic acid recovered on breath as $^{13}CO_2$

at 7 hours tended to be higher in the HIV- group (HIV- 12.5%, 6.8-18.3; HIV+ 6.7%, 4.8-15.2; ns). As the amount of total lipid oxidised during the postprandial period was similar between the groups, there were no significant differences in either the amount of endogenous or exogenous lipid oxidised over this period. However, when the pattern of exogenous lipid oxidation over the 7 hours is presented as grams per hour (figure 5.4), the HIV- group show a significantly higher exogenous or dietary lipid oxidation at 2 hours after the meal (HIV+ 0.48 g/h, 0.39-0.62; HIV- 0.85 g/h, 0.57-1.16; $p<0.05$).

5.4.5 Energy derived from substrates in the postprandial period

The relative contributions from carbohydrate, endogenous and exogenous lipids (table 5.9) were not significantly different between the two groups. Protein oxidation was not measured in this study.

5.4.6 Plasma triacylglycerol concentration

There were no significant differences between the groups in fasting plasma triacylglycerol (TAG) concentration, area under the curve (AUC) or the incremental area under the curve (AUC_i) for plasma TAG following the meal. However, one subject in the HIV- group had a fasting plasma TAG concentration of 6.8 mmol/l, and two subjects in this group had elevated plasma TAG AUC (mmol/l/7h) and incremental AUC (mmol/l/7h) at least twofold higher than either the rest of the HIV- group or any subjects in the HIV+ group (table 5.10). As a result, the pattern of plasma TAG concentration was not significantly different between the two groups (figure 5.5).

5.4.7 Plasma [^{13}C] triacylglycerol concentration

The HIV+ group showed a higher [^{13}C] palmitic acid concentration in plasma TAG (figure 5.6) than the HIV- group prior to the meal (HIV+ 0.55 μg/ml, 0.23-2.17; HIV- 0.10 μg/ml, 0.05-0.60; $p<0.05$) and a lower concentration at both 1 hour after the meal (HIV+ 0.64 μg/ml, 0.32-10.06; HIV- 6.70 μg/ml, 3.01-18.41; $p<0.05$) and 1.5 hours after the meal (HIV+ 3.41 μg/ml, 2.68-7.07; HIV- 12.00 μg/ml, 6.99-37.59; $p<0.05$). The total amount of [^{13}C] palmitic acid measured in the plasma TAG fraction over the 7 hours (AUC) was also

lower (table 5.12) in the HIV+ group (HIV+ 13.4 μg/ml/7h, 5.9-16.3; HIV- 44.9 μg/ml/7h, 29.4-117.3; $p<0.05$).

5.4.8 Plasma non-esterified fatty acid concentration

The HIV+ group tended to have a higher fasting NEFA concentration than the HIV- group (HIV+ 0.45 mmol/l, 0.13-0.51; HIV- 0.28 mmol/l, 0.08-0.39; ns). There were no significant differences in NEFA AUC, AUC_i (table 5.11) or pattern over the 7-hour period (figure 5.7).

5.4.9 Plasma [^{13}C]-non-esterified fatty acid concentration

The HIV+ group had a lower concentration of [^{13}C] palmitic acid in the plasma non-esterified fatty acid (NEFA) fraction following the administration of the meal (table 5.12), both as AUC over the 7-hours (HIV+ 1.8 μg/ml/7h, 0.3-5.2; HIV- 3.1 μg/ml/7h, 1.7-3.8; ns) and at 1.5 hours (HIV+ 0.23 μg/ml/7h, 0.12-0.53; HIV- 0.86 μg/ml/7h, 0.51-1.51; $p<0.05$) and 2 hours (HIV+ 0.38 μg/ml/7h, 0.12-0.81; HIV- 0.87 μg/ml/7h, 0.52-1.38; $p<0.05$) after the meal (figure 5.8).

5.4.10 Plasma insulin and glucose concentration

While there were no significant differences in fasting plasma insulin concentration and insulin expressed as AUC between the two groups (table 5.13), in the late postprandial period the HIV+ group had a higher plasma insulin concentration (6h HIV+ 7.9 mU/l, 4.1-12.7; HIV- 4.9 mU/l, 1.5-9.9; 7h HIV+ 7.6 mU/l, 4.8-17.9; HIV- 2.8 mU/l, 1.2-6.7; $p<0.05$, figure 5.11). The HIV+ group also had a lower plasma glucose concentration, both before the test meal (HIV+ 4.8 mmol/l, 4.4-5.0; HIV- 5.2 mmol/l, 4.9-7.; $p<0.05$) and as AUC over the 7 hours (HIV+ 35.9 mmol/l, 32.0-38.8; HIV- 38.6 mmol/l, 37.3-52.0; $p<0.05$).

Table 5.1 Viral load, CD4 and CD8 counts of subjects in the HIV-positive, therapy naive group (HIV+).

SUBJECT CODE	Viral Load (copies/ml plasma)	CD4 (X 10^6 cells/L plasma)	CD8 (X 10^6 cells/L plasma)
1	1987172	0	96
2	168312	65	953
3	84367	362	661
4	36783	145	1157
5	500000	55	877
Median	168312	65	877

Table 5.2 **Characteristics of subjects in both the HIV-positive group (HIV+) and HIV-negative group (HIV-) including age, height, weight, body mass index (BMI) and smoking status. *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test.**

SUBJECT CODE	AGE (Yrs)	HEIGHT (cm)	WEIGHT (kg)	BMI (kg/m^2)	SMOKER
HIV+ GROUP					
1	45	183	69.8	20.8	YES
2	45	176	63.8	20.6	YES
3	20	165	56.6	20.8	YES
4	44	187	78.0	22.3	NO
5	47	181	64.3	19.6	NO
Median (range)	45 (20-47)	181 (165-187)	64.3 (56.6-78.0)	20.8 (19.6-22.3)	
HIV- GROUP					
1	41	171	68.3	23.4	NO
2	32	180	86.0	26.5	YES
3	47	183	84.2	25.1	NO
4	43	177	95.7	30.5	YES
5	36	176	81.6	26.6	NO
6	36	181	86.8	26.5	NO
7	55	180	93.1	29.4	NO
8	45	175	81.7	26.7	NO
Median (range)	42 (32-55)	179 (171-183)	85.1* (68.3-95.7)	26.6* (23.4-30.5)	

*** $P<0.05$**

Table 5.3 **Results of the bioelectrical impedance analysis (BIA) for fat mass (kg) and fat free mass (FFM) (kg), and fat and fat free mass as a percentage of total body weight in the HIV-positive (HIV+) and HIV-negative (HIV-) groups. *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test.**

SUBJECT CODE	FAT (kg)	FFM (kg)	FAT (%)	FFM (%)
HIV+ GROUP				
1	9.4	60.4	13.5	86.5
2	11.3	52.5	17.7	82.3
3	9.0	47.6	15.9	84.1
4	12.5	65.5	16.0	84.0
5	10.4	53.9	16.2	83.8
Median (range)	10.4 (9.0-12.5)	53.9 (47.6-65.5)	16.0 (13.5-17.7)	84.0 (82.3-86.5)
HIV- GROUP				
1	11.4	56.9	16.7	83.3
2	13.3	72.7	15.5	84.5
3	19.0	65.2	22.6	77.4
4	22.7	73.0	23.7	76.3
5	15.4	66.2	18.9	81.1
6	19.5	67.3	22.5	77.5
7	22.8	70.3	24.5	75.5
8	16.0	65.7	19.6	80.4
Median (range)	17.5* (11.4-22.8)	66.8* (56.9-73.0)	21.1* (15.5-24.5)	79.0* (75.5-84.5)

* $P<0.05$

Table 5.4 **Results of anthropometric measures of waist circumference, hip circumference, waist to hip ratio, triceps skinfold, mid upper arm circumference (MUAC) and mid upper arm muscle circumference (MUAMC) in the HIV-positive (HIV+) and HIV-negative (HIV-) groups. *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test.**

SUBJECT CODE	WAIST (mm)	HIP (mm)	WAIST: HIP RATIO	TRICEP SKIN FOLD (mm)	MUAC (mm)	MUAMC (mm)
HIV+ GROUP						
1	847	975	0.87	5.1	253	237
2	840	980	0.86	8.0	265	240
3	715	867	0.82	6.2	260	241
4	836	950	0.88	11.4	320	284
5	798	948	0.84	8.0	270	245
Median (range)	836 (715-847)	950 (867-980)	0.86 (0.82-0.88)	8.0 (5.1-11.4)	265 (253-320)	241 (237-284)
HIV- GROUP						
1	799	945	0.85	7.1	332	310
2	880	1073	0.82	17.9	355	299
3	937	1053	0.89	20.9	337	271
4	996	1085	0.92	18.9	379	320
5	824	1060	0.78	10.1	315	283
6	964	1096	0.88	13.8	327	284
7	1022	1120	0.91	13.4	356	314
8	948	1042	0.91	15.2	345	297
Median (range)	943* (799-1022	1067* (945-1120)	0.89 (0.78-0.92)	14.5* (7.1-20.9)	341* (315-379)	298* (271-320)

*** $P<0.05$**

Table 5.5 **Resting energy expenditure (REE) measured by indirect calorimetry expressed as kilojoules per day (kJ/day) and as kilojoules per kilogram of fat free mass (kJ/kg FFM), and energy expenditure over the 7-hour postprandial study period calculated by area under the curve in the HIV+ and HIV- groups. Fat free mass was measured using bioelectrical impedance analysis. *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test.**

SUBJECT CODE	REE (kJ/d)	REE (kJ/kg FFM/d)	EE (kJ/7h) (AUC_i)
HIV+ GROUP			
1	7190	119	466
2	6312	120	343
3	6604	139	178
4	8485	130	264
5	7524	140	225
Median (range)	7190 (6312-8485)	130 (119-140)	264 (178-466)
HIV- GROUP			
1	6157	108	249
2	7800	107	216
3	6642	102	845
4	8849	121	406
5	7244	109	163
6	6926	103	253
7	7010	100	330
8	8097	123	168
Median (range)	7244 (6157-8849)	108* (100-123)	251 (163-845)

* $P<0.05$

Table 5.6 **Respiratory exchange ratio (RER) and postabsorptive substrate oxidation in the HIV+ and HIV- groups, calculated from VCO_2 and VO_2 measured by indirect calorimetry. Lipid and carbohydrate oxidation is expressed as grams per hour (g/h) prior to the administration of the test meal and emulsion.**

SUBJECT CODE	RER 0h	LIPID OXIDATION (g/h) 0h	CHO OXIDATION (g/h) 0h
HIV+ GROUP			
1	0.80	3.94	5.08
2	0.81	3.23	4.50
3	0.92	0.63	11.84
4	0.79	5.14	5.43
5	0.92	1.13	14.10
Median (range)	0.81 (0.79-0.92)	3.23 (0.63-5.14)	5.43 (4.50-14.10)
HIV- GROUP			
1	0.78	3.63	2.85
2	0.87	2.53	10.03
3	0.85	2.33	7.44
4	0.88	2.63	12.73
5	0.85	2.63	8.31
6	0.88	1.73	9.72
7	0.95	0.23	13.82
8	0.85	3.03	9.55
Median (range)	0.86 (0.78-0.95)	2.58 (0.23-3.63)	9.64 (2.85-13.82)

Table 5.7 **Lipid and carbohydrate oxidation over the 7-hour period following administration of the emulsion and test meal, expressed as grams per 7-hours (g/7h), calculated by area under the curve (AUC) and also expressed as the incremental area under the curve (AUCi), showing the change from baseline in the postprandial period.**

SUBJECT CODE	LIPID OXIDATION AUC (g/7h)	CHO OXIDATION AUC (g/7h)	LIPID OXIDATION AUC_i (g/7h)	CHO OXIDATION AUC_i (g/7h)
HIV+ GROUP				
1	37.3	39.3	10.4	4.6
2	25.4	42.6	3.3	13.9
3	9.9	79.6	5.7	-2.1
4	37.7	48.6	2.5	11.5
5	12.8	93.8	5.2	-3.5
Median (range)	25.4 (9.9-37.7)	48.6 (39.3-93.8)	5.2 (2.5-10.4)	4.6 (-3.5-13.9)
HIV- GROUP				
1	32.6	18.3	7.1	3.3
2	30.5	50.9	12.7	0.5
3	30.4	60.8	18.4	17.8
4	35.7	71.6	17.2	0
5	30.7	74.8	12.3	24.7
6	20.0	64.3	8.4	17.2
7	7.3	103.5	5.9	15.2
8	20.0	19.0	3.2	78.2
Median (range)	30.5 (7.3-35.7)	62.6 (18.3-103.5)	10.4 (3.2-18.4)	16.2 (0-78.2)

Table 5.8 **The calculation of the endogenous and exogenous sources of lipid oxidised over the 7-hour period (g/7h) using the percentage of ^{13}C label recovered on breath over 7-hours, together with the amount of lipid in the test meal and the total lipid oxidised over the 7-hour period, calculated from the area under the curve (AUC).**

SUBJECT CODE	% Administered ^{13}C Dose Excreted on Breath over 7 h	Lipid (g) in Test meal	TOTAL LIPID OX. (AUC) (g/7 h)	EXOGENOUS LIPID OX. (g/7 h)	ENDOGENOUS LIPID OX. (g/7 h)
HIV+ GROUP					
1	15.2	45.3	37.3	6.7	30.4
2	4.8	45.3	25.4	2.2	23.2
3	6.7	45.3	9.9	3.1	6.9
4	10.7	45.3	37.7	4.8	32.8
5	6.7	45.3	12.8	3.0	9.8
Median (range)	6.7 (4.8-15.2)	45.3	25.4 (9.9-37.7)	3.1 (2.2-6.9)	23.2 (6.9-32.8)
HIV- GROUP					
1	12.2	45.3	32.6	5.5	27.1
2	11.8	45.3	30.5	5.3	25.1
3	18.3	45.3	30.4	8.3	22.1
4	13.2	45.3	35.7	6.0	29.7
5	12.8	45.3	30.7	5.8	24.9
6	8.4	45.3	20.0	3.8	16.2
7	6.7	45.3	7.3	3.0	4.3
8	12.9	45.3	20.0	5.9	14.1
Median (range)	12.5 (6.7-18.3)	45.3	30.5 (7.3-35.7)	5.7 (3.0-8.3)	23.5 (4.3-29.7)

Table 5.9 **Calculation of the proportion of energy expended over the 7-hour period derived from carbohydrate (CHO), endogenous lipid and exogenous lipid.**

SUBJECT CODE	EXOG LIPID (kJ/7h)	ENDOG LIPID (kJ/7h)	CHO (kJ/7h)	ENERGY EXPENDED (kJ/7h) (AUC_0)	EXOG LIPID (% EE)	ENDOG LIPID (% EE)	CHO (% EE)
HIV+ GROUP							
1	261	1155	629	2540	10	45	25
2	83	880	681	2148	4	41	32
3	116	260	1274	2083	6	12	61
4	184	1248	778	2707	7	46	29
5	114	371	1501	2389	5	16	63
Median (range)	116 (83-261)	880 (260-1248)	778 (629-1501)	2389 (2083-2707)	6 (4-10)	41 (12-46)	32 (25-63)
HIV- GROUP							
1	204	1002	293	2048	10	49	14
2	198	929	814	2478	8	37	33
3	309	976	972	2782	5	15	35
4	223	1096	1146	2987	3	16	38
5	215	922	1197	2627	8	35	46
6	140	601	1028	2273	6	26	45
7	140	172	1656	2374	6	7	70
8	140	209	304	2488	6	8	12
Median (range)	209 (140-309)	926 (172-1096)	1000 (293-1656)	2483 (2048-2987)	6 (3-10)	21 (7-49)	37 (12-70)

Table 5.10 **Plasma triacylglycerol (TAG) concentration expressed as mmol/litre before the meal (fasting), in the 7 hours following the meal (AUC) and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the incremental area under the curve (AUC_i).**

SUBJECT CODE	FASTING TAG (mmol/l)	POSTPRANDIAL PLASMA TAG AUC (mmol/l/7h)	PEAK PLASMA TAG VALUE (mmol/l)	POSTPRANDIAL TAG RESPONSE AUC_i (mmol/l over 7h)
HIV+ GROUP				
1	1.6	11.7	2.0	1.0
2	1.0	9.7	1.8	2.9
3	1.4	6.1	1.4	0
4	1.2	9.4	2.0	1.2
5	0.9	8.2	1.5	2.2
Median (range)	1.2 (0.9-1.6)	8.9 (6.1-11.7)	1.8 (1.4-2.0)	1.2 (0-2.9)
HIV- GROUP				
1	0.4	4.4	0.8	1.6
2	0.5	5.2	1.0	1.7
3	2.0	24.7	5.2	10.6
4	6.8	57.5	9.0	10.2
5	1.1	9.2	1.5	1.5
6	0.9	8.5	1.5	2.4
7	0.6	7.4	1.2	3.1
8	0.9	8.5	1.6	2.4
Median (range)	0.9 (0.4-6.8)	8.5 (4.4-57.5)	1.5 (0.8-9.0)	2.4 (1.5-10.6)

Table 5.11 **Plasma non-esterified fatty acid (NEFA) concentration expressed as mmol/litre before the meal (fasting), in the 7 hours following the meal (AUC) and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the incremental area under the curve (AUC_i).**

SUBJECT CODE	FASTING NEFA (mmol/l)	POSTPRANDIAL PLASMA NEFA AUC (mmol/l/7h)	POSTPRANDIAL NEFA RESPONSE AUC_i (mmol/l over 7h)
HIV+ GROUP			
1	0.45	2.92	0.57
2	0.39	0.71	0.00
3	0.13	1.38	0.55
4	0.51	1.66	0.00
5	0.47	1.15	0.00
Median (range)	0.45 (0.13-0.51)	1.58 (0.71-2.92)	0.00 (0.00-0.57)
HIV- GROUP			
1	0.30	1.43	0.10
2	0.14	1.23	0.29
3	0.32	2.26	0.49
4	0.39	1.93	0.01
5	0.30	1.45	0.18
6	0.22	1.26	0.04
7	0.08	0.85	0.30
8	0.26	1.69	0.20
Median (range)	0.28 (0.08-0.39)	1.44 (0.85-2.26)	0.19 (0.01-0.49)

Table 5.12 **Plasma [^{13}C] palmitic acid within triacylglycerol (^{13}C-TAG) and within non-esterified fatty acid (^{13}C-NEFA) expressed as μg/ml following administration of the emulsion containing 700 mg [1-^{13}C] palmitic acid and test meal, calculated as the incremental area under the curve (AUC_i). *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test.**

SUBJECT CODE	[^{13}C]-TAG (μg/ml/7h)	[^{13}C]-NEFA (μg/ml/7h)
HIV+ GROUP		
1	16.3	5.2
2	5.9	0.3
3	15.0	1.6
4	10.2	2.1
5	12.1	0.5
Median (range)	13.4 (5.9-16.3)	1.8 (0.3-5.2)
HIV- GROUP		
1	29.4	3.3
2	33.5	2.5
3	117.3	3.8
4	115.2	3.0
5	47.1	2.3
6	38.1	3.4
7	42.7	1.7
8	47.7	3.6
Median (range)	44.9* (29.4-117.3)	3.1 (1.7-3.8)

*** P=0.002**

Table 5.13 **Fasting and postprandial plasma insulin and glucose concentrations calculated as the area under the curve from zero (AUC_0) and incremental area under the curve (AUC_i). *Statistically significant difference between the HIV+ and HIV- group using the Mann Whitney test ($p<0.05$).**

SUBJECT CODE	FASTING PLASMA INSULIN (mU/l)	PLASMA INSULIN AUC (mU/l/7h)	PLASMA INSULIN AUC_i (mU/l/7h)	FASTING PLASMA GLUCOSE (mmol/l)	PLASMA GLUCOSE AUC (mmol/l/7h)	PLASMA GLUCOSE AUC_i (mmol/l/7h)
HIV+ GROUP						
1	4.3	86.3	56.1	4.4	32.0	2.3
2	7.7	133.6	79.6	4.8	38.8	5.3
3	13.7	135.1	78.6	4.7	33.5	2.4
4	12.3	266.2	180.7	5.0	37.5	7.0
5	10.4	106.6	40.7	5.0	35.9	1.8
Median (range)	10.4 (4.3-13.7)	133.6 (86.3-266.2)	78.6 (40.7-180.7)	4.8 (4.4-5.0)	35.9 (32.0-38.8)	2.4 (1.8-7.0)
HIV- GROUP						
1	0.9	87.1	80.8	5.2	38.6	5.0
2	1.0	43.2	36.2	5.8	40.2	3.3
3	7.8	178.1	142.1	4.9	37.3	5.5
4	10.5	219.1	150.1	7.5	52.0	8.7
5	7.4	170.3	124.1	5.1	37.4	2.2
6	7.7	183.6	142.5	5.2	39.9	5.3
7	7.7	140.7	98.4	6.9	38.2	10.1
8	5.5	151.3	118.3	5.7	42.3	3.3
Median (range)	7.4 (0.9-10.5)	170.3 (43.2-219.1)	124.1 (36.2-150.1)	5.2* (4.9-7.5)	38.6* (37.3-52.0)	5.0 (2.2-10.1)

Figure 5.1 **Median carbohydrate oxidation during the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the HIV- and HIV+ groups. The error bars show the minimum and maximum values or range.**

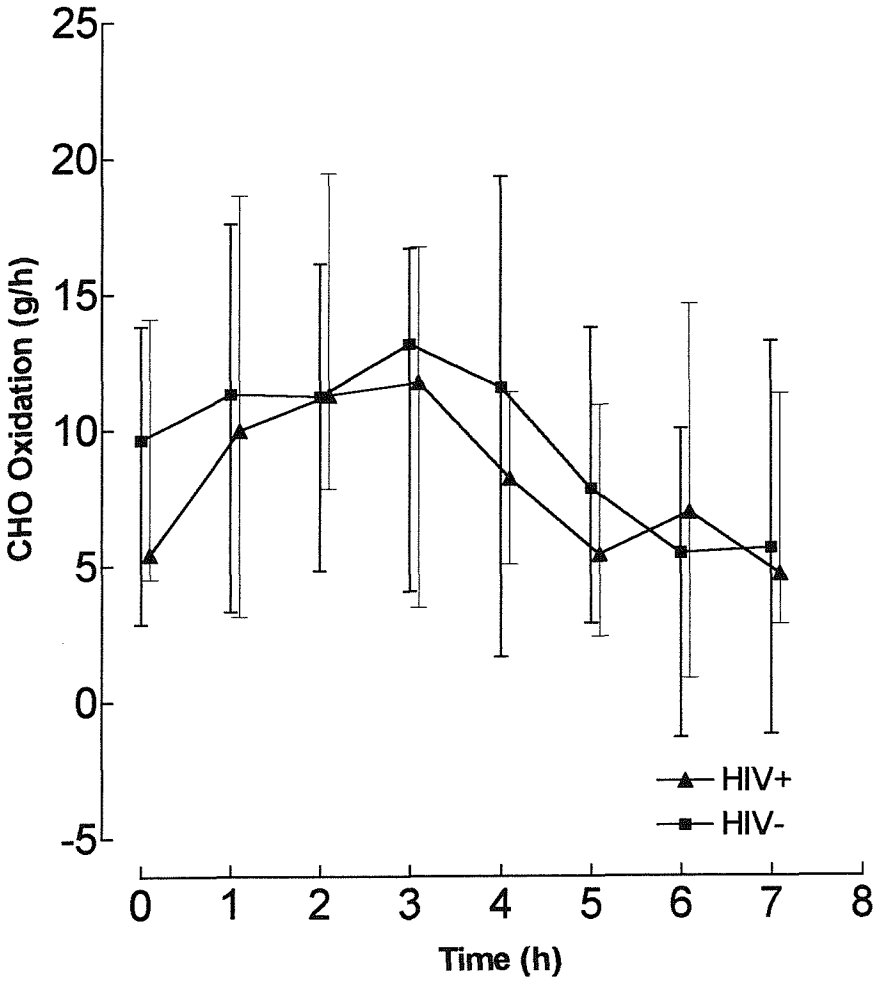


Figure 5.2 **Median lipid oxidation during the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the HIV- and HIV+ groups.**

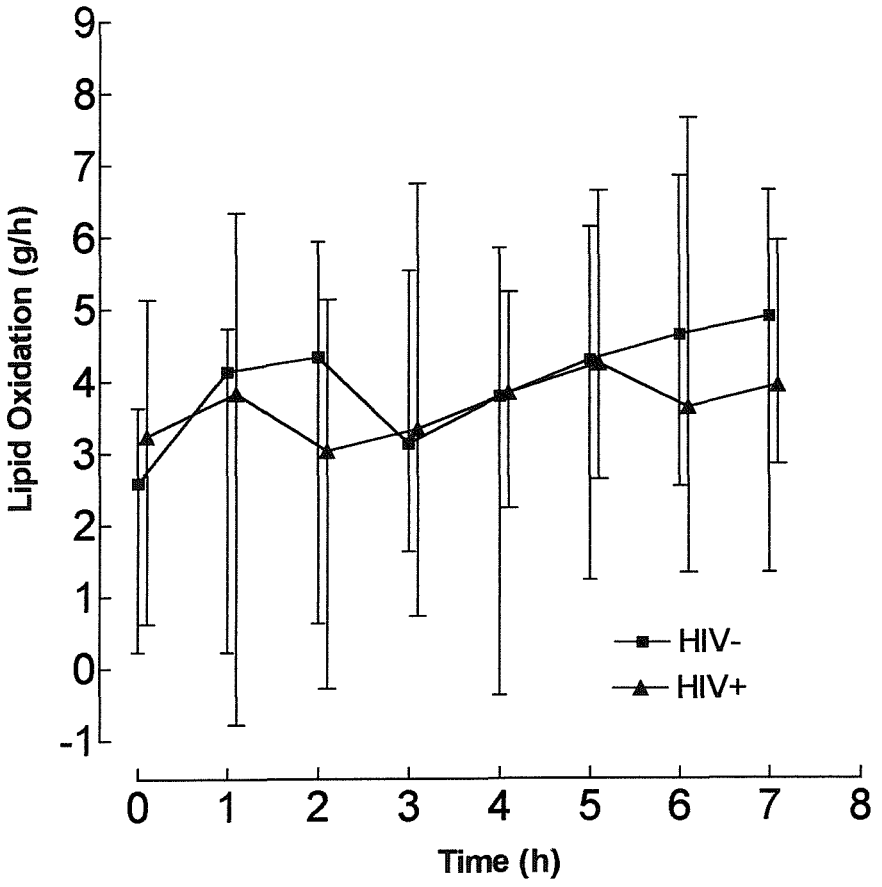


Figure 5.3 **Endogenous lipid oxidation over the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the HIV- and HIV+ groups.**

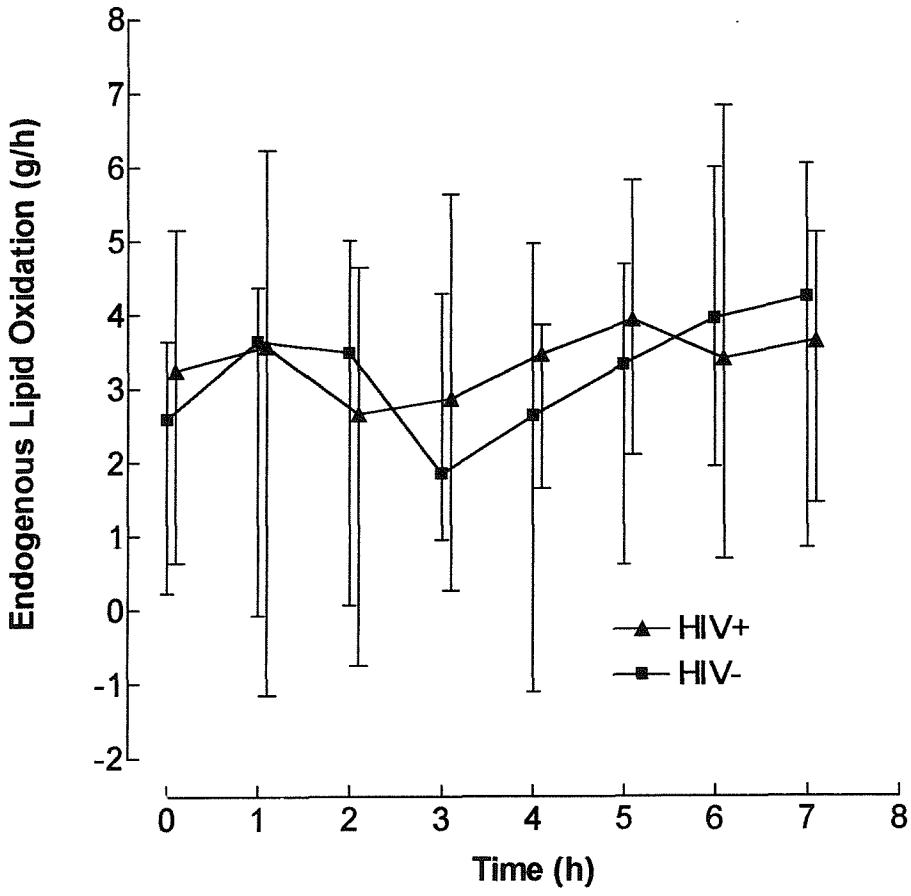


Figure 5.4 **Exogenous lipid oxidation over the 7-hour period following administration of the emulsion and test meal, calculated from the recovery of label on breath as $^{13}CO_2$, expressed as grams per hour (g/h) for the HIV- and HIV+ groups. *Significantly different from the HIV+ group using the Mann Whitney test ($P<0.05$).**

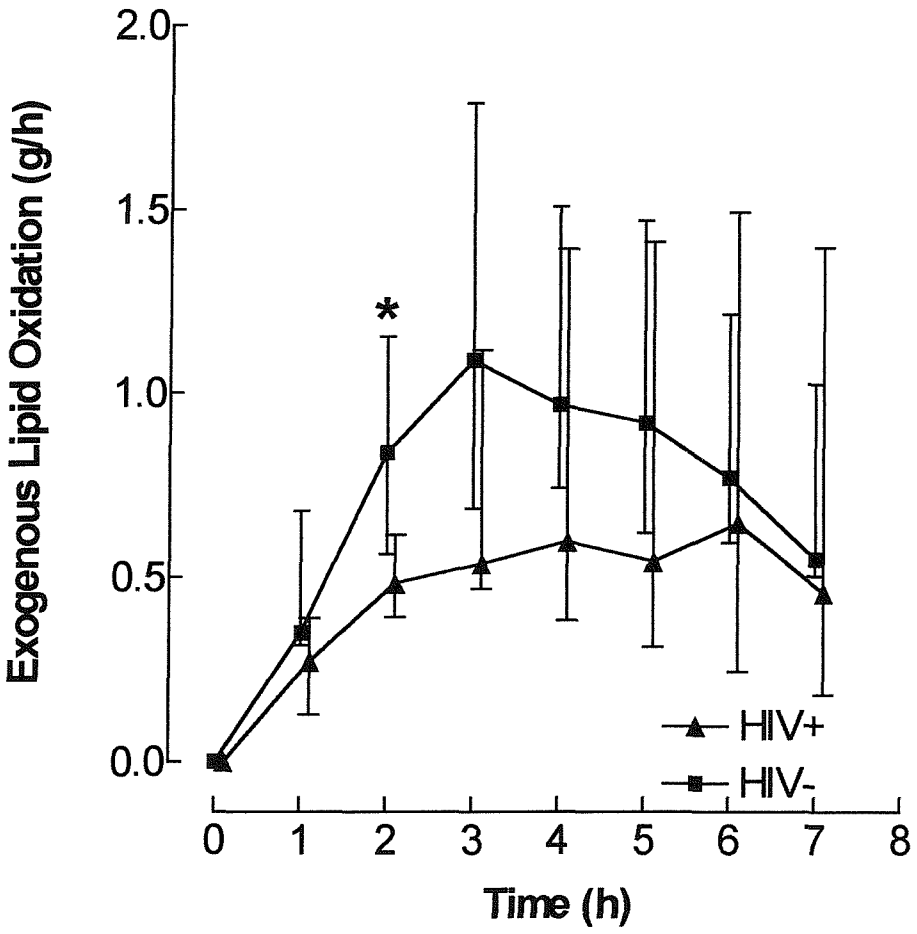


Figure 5.5 **Plasma triacylglycerol concentrations (mmol/l) during the 7-hour period following administration of the emulsion and test meal, for the HIV- and HIV+ groups.**

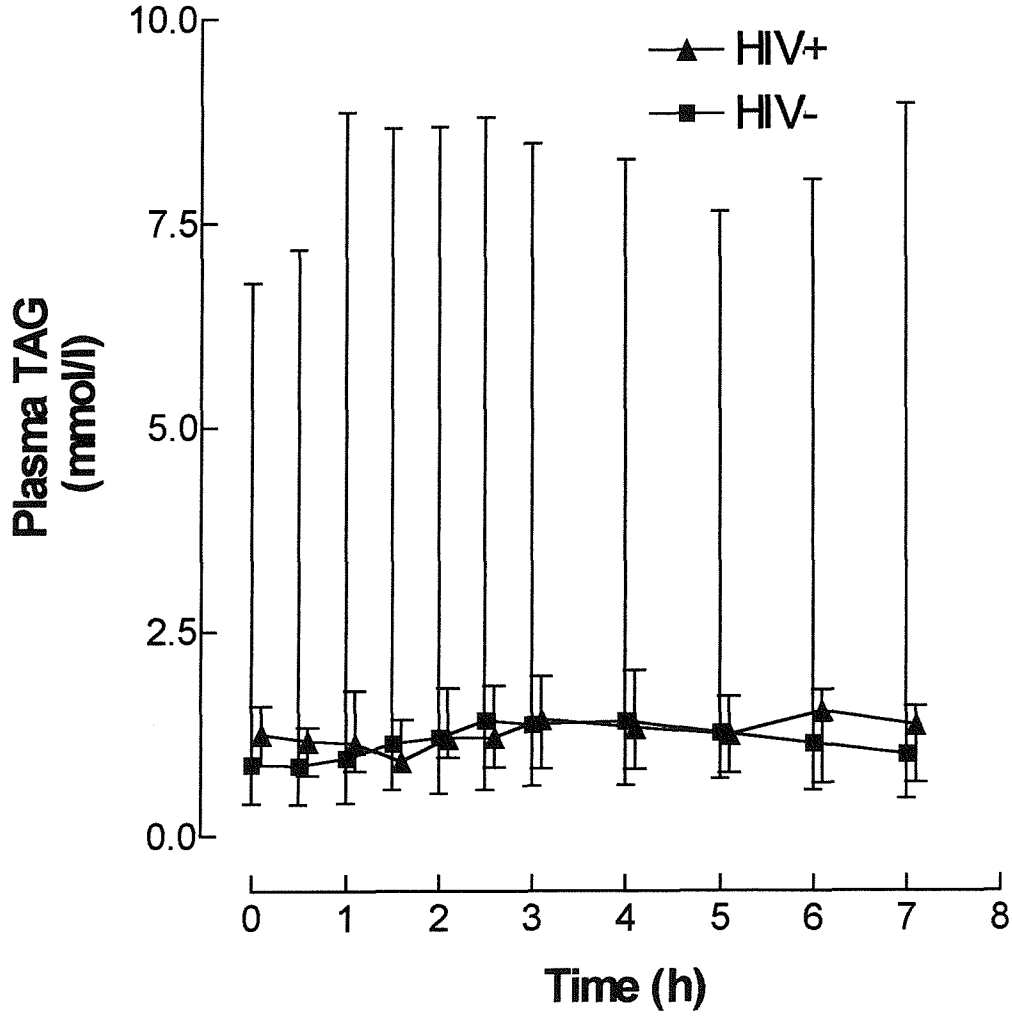


Figure 5.6 **Plasma [^{13}C] -triacylglycerol concentrations (μg/ml) during the 7-hour period following administration of the emulsion and test meal, for the HIV- and HIV+ groups. *Significantly different from the HIV+ group using the Mann Whitney test (P<0.05).**

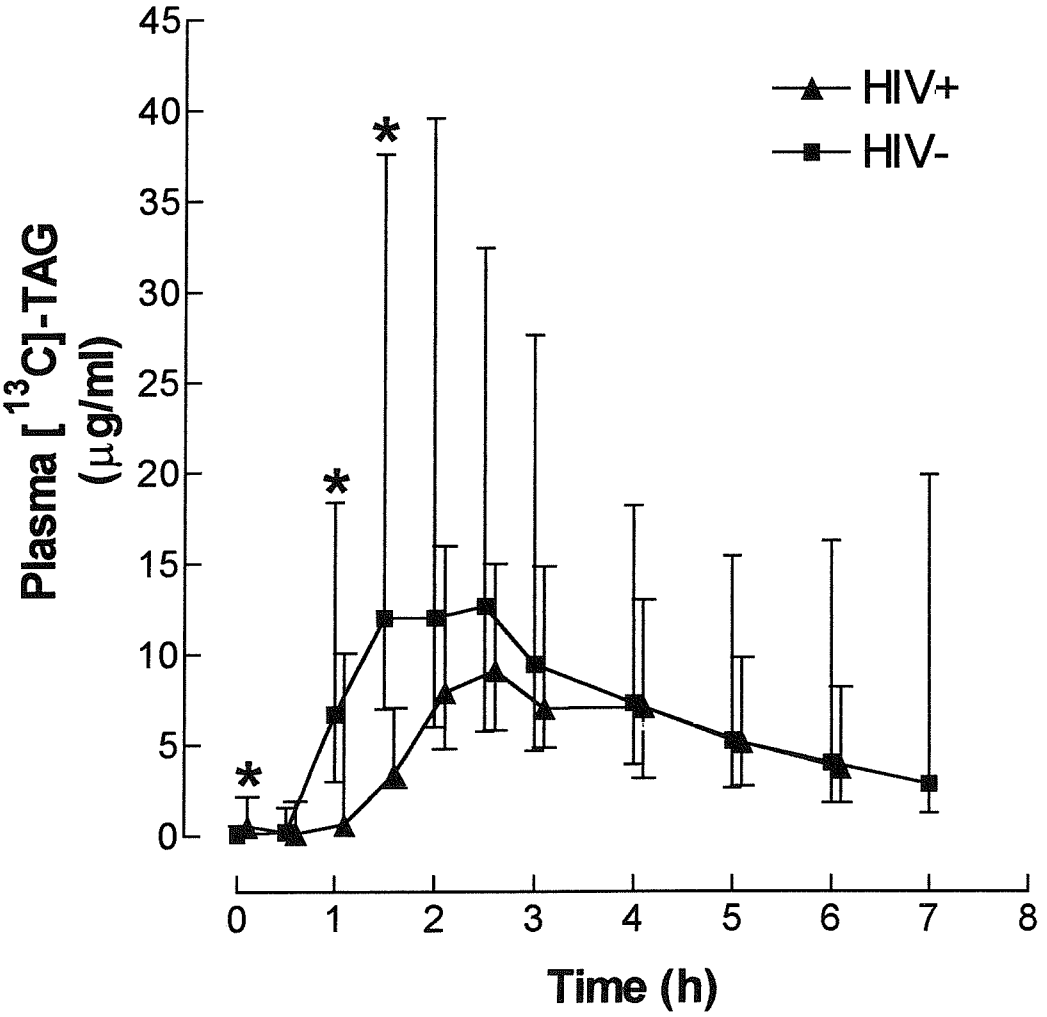


Figure 5.7 **Plasma non-esterified fatty acid (NEFA) concentrations (mmol/l) during the 7-hour period following administration of the emulsion and test meal, for the HIV- and HIV+ groups.**

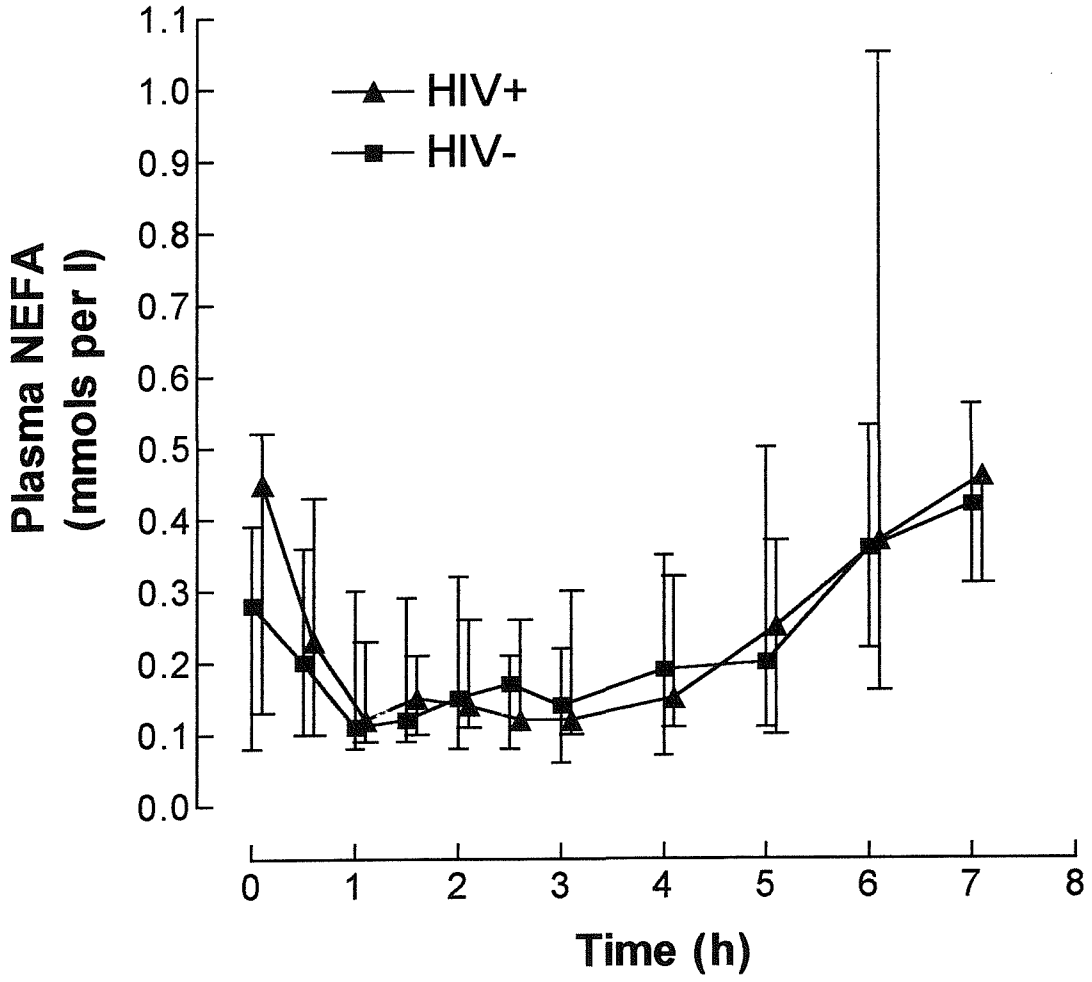


Figure 5.8 **Plasma [13C]-non-esterified fatty acid (NEFA) concentrations (μg/ml) during the 7-hour period following administration of the emulsion containing 700mg [1-13C] palmitic acid and test meal, for the HIV- and HIV+ groups. *Significantly different from the HIV+ group using the Mann Whitney test (P<0.05).**

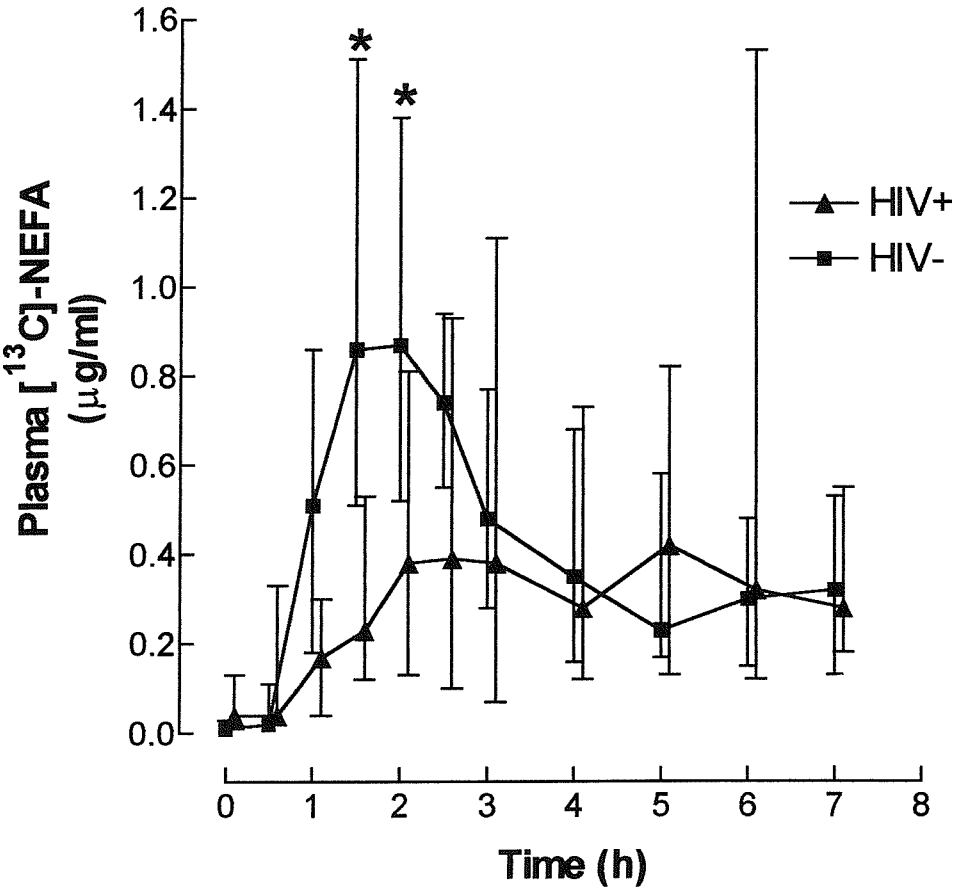


Figure 5.9 **Fasting plasma TAG concentrations (mmol/l) compared to the postprandial plasma TAG AUC during the 7-hour period following administration of the emulsion and test meal (mmol/l/7h) in the HIV+ and HIV- groups.**

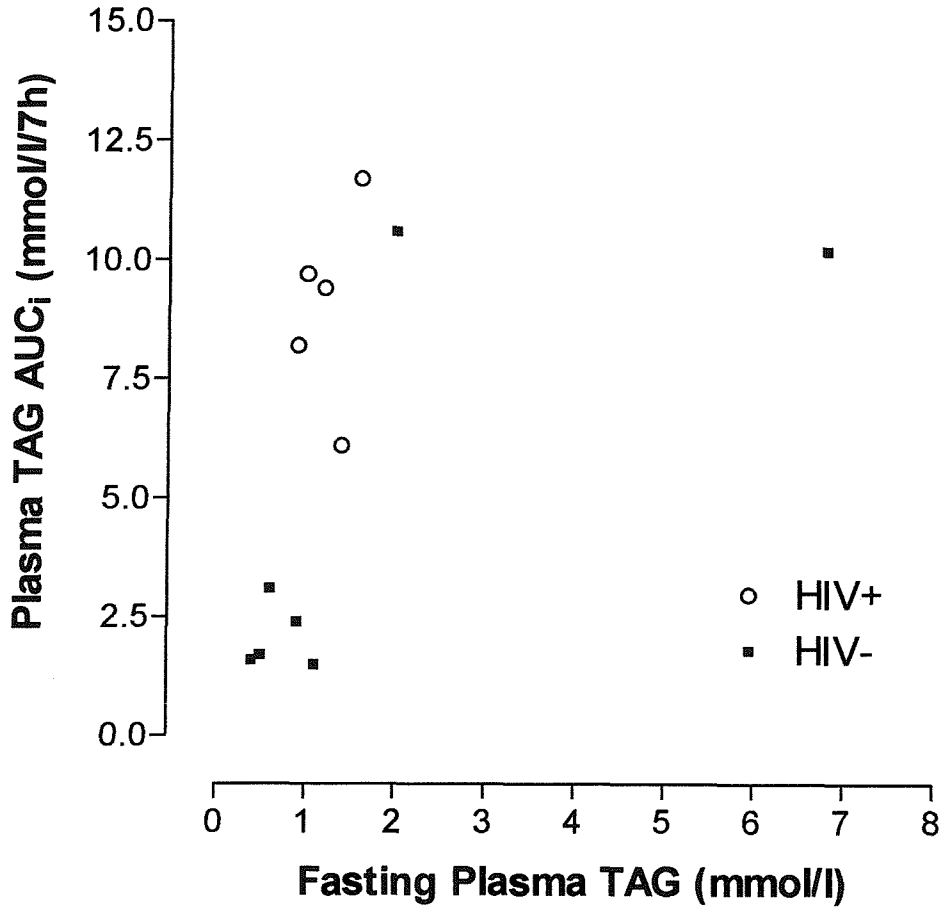


Figure 5.10 **Association between viral load (copies/ml plasma), CD4 count (x 10^6 cells/litre plasma) and body mass index (BMI, kg/m^2) in the HIV+ group (n=5).**

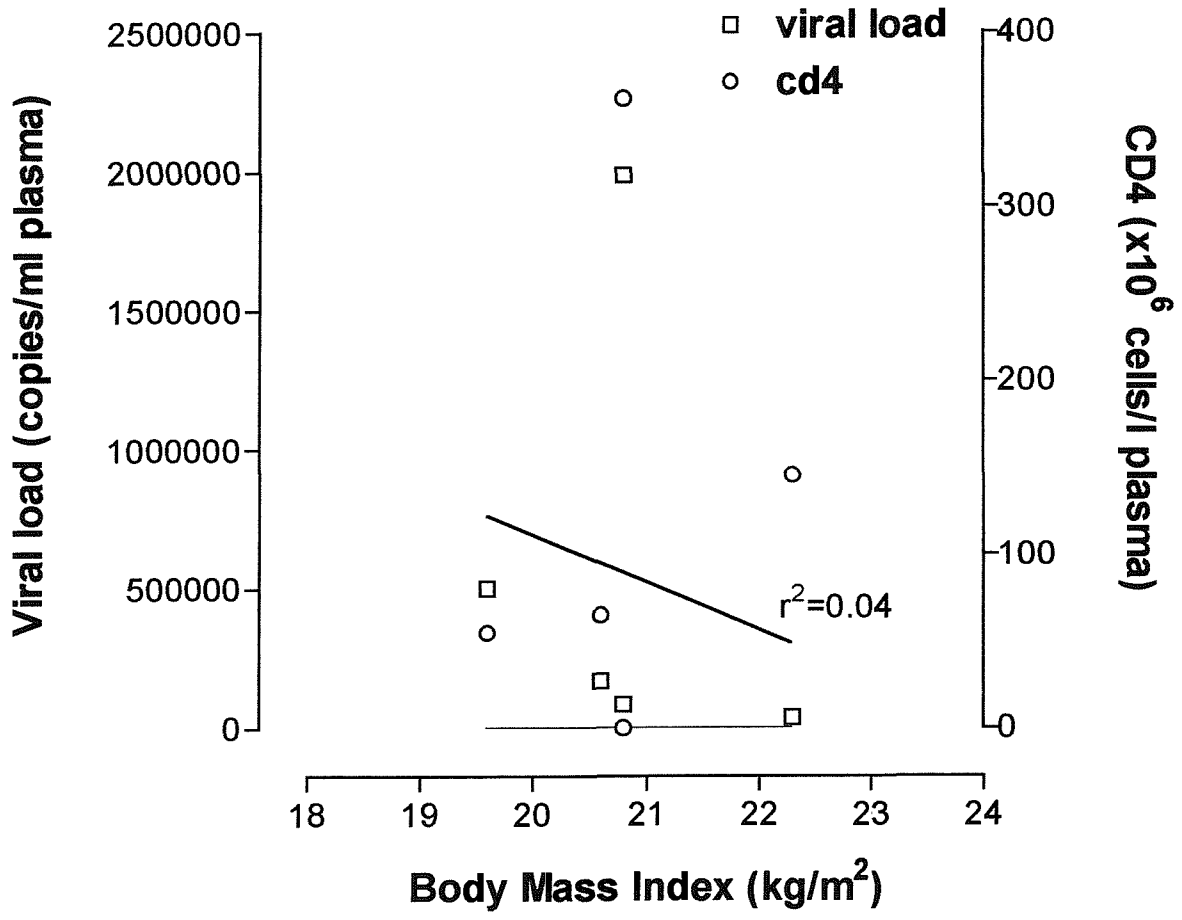


Figure 5.11 **The change in plasma insulin concentration (mU/l) following administration of the test meal and for the HIV- and HIV+ groups. *Significantly different from the HIV+ group using the Mann Whitney test ($P<0.05$).**

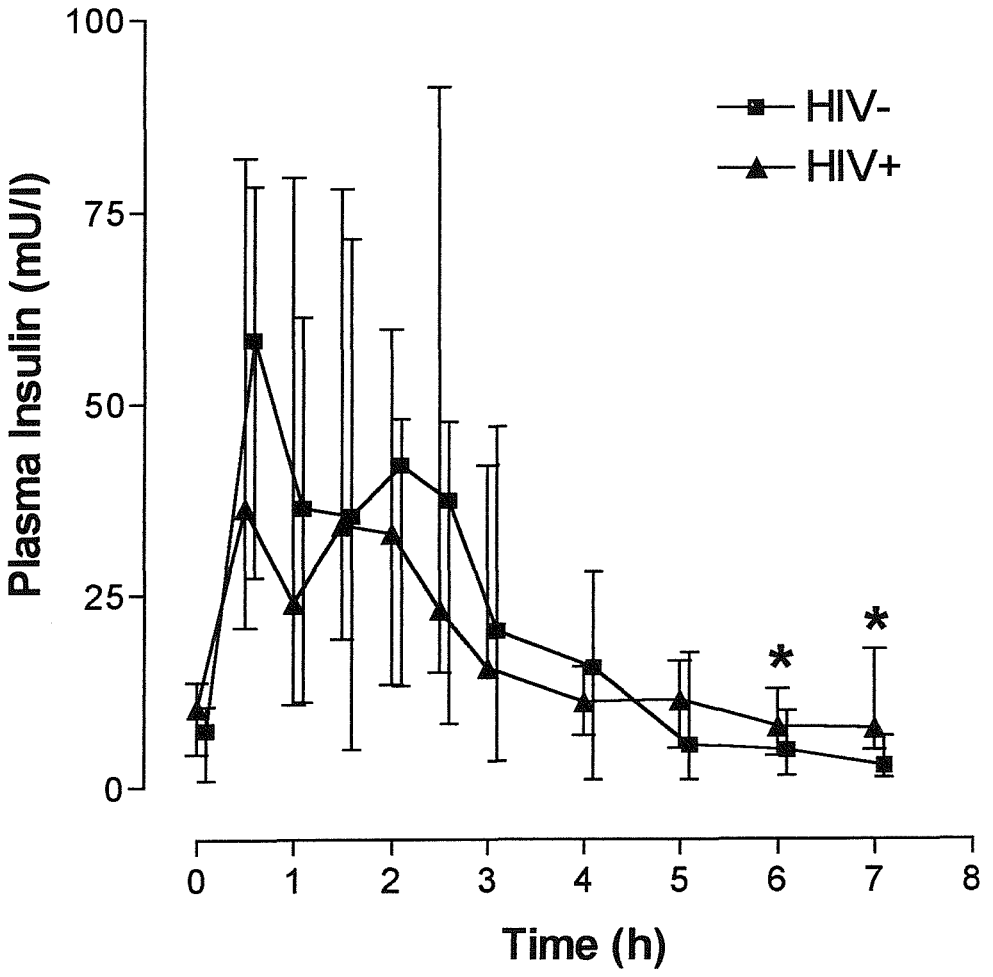
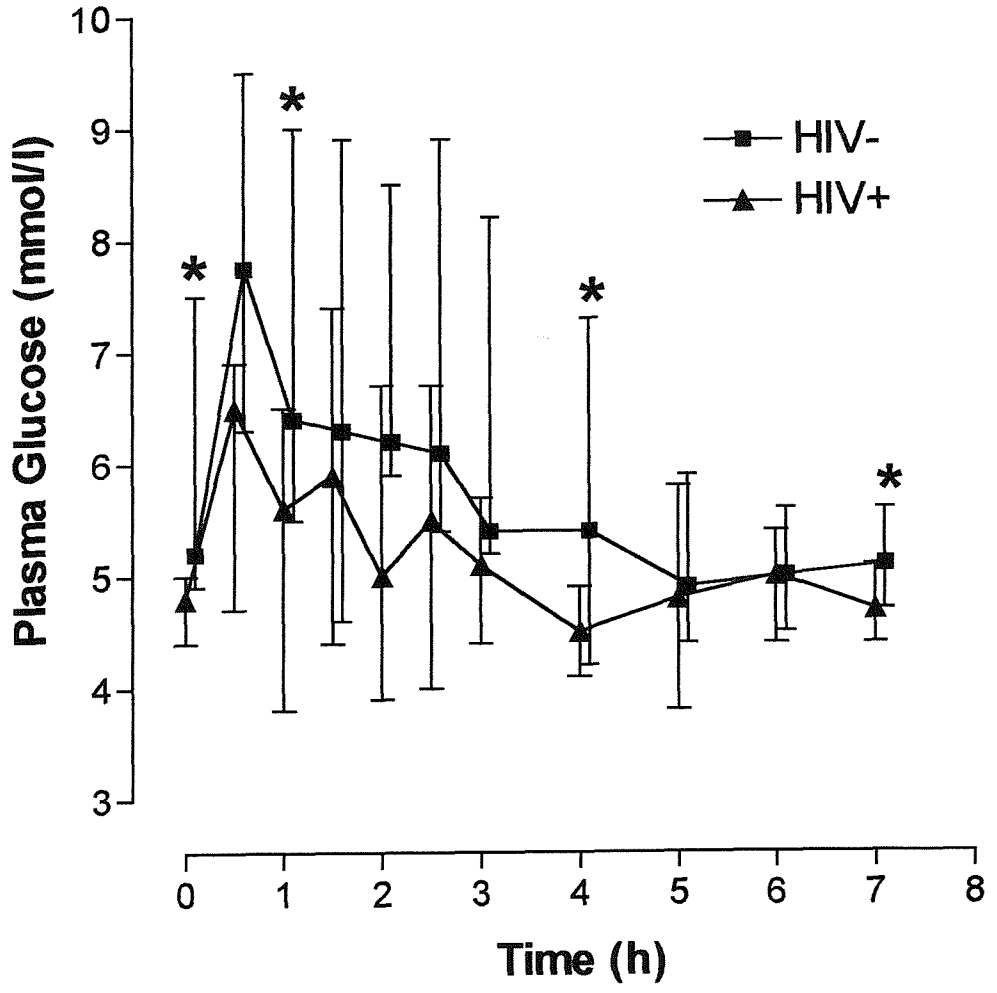


Figure 5.12 **The change in plasma glucose concentration (mmol/l) following administration of the test meal and for the HIV- and HIV+ groups. *Significantly different from the HIV+ group using the Mann Whitney test ($P<0.05$).**



5.5 Discussion

The study reported in this chapter was the first time that stable isotopes tracers were used to investigate the differences in postprandial lipid metabolism between HIV-positive subjects and HIV-negative subjects. While the results may indicate differences in the partitioning of dietary lipid once absorbed, due to the lack of stool collection and analysis, no statements can be made in this chapter as to the degree of absorption of label from the diet within these groups. The aim of the chapter was to investigate differences in the handling and partitioning of dietary lipid in the postprandial period in HIV-positive males compared to healthy males of similar age, in order to assess the influence of HIV infection per se on this process.

5.5.1 Subject characteristics and body composition

While all subjects in the HIV+ group had high viral load and a significantly lower weight for height (BMI) than the HIV- controls, there does appear to be a trend towards a lower BMI value with the highest viral loads (figure 5.10). Mulligan *et al.* (1998) found a linear relationship between viral load and energy expenditure suggesting that BMI is associated with the degree of viral burden, through an increase in energy expenditure. Although this relationship does not persist with the CD4 count possibly suggesting inter-individual differences in the capacity to maintain an immune response against the virus. In addition, viral load may not be a true reflection of viral kinetics [Pantaleo *et al.* 1993].

The composition of the lower body mass observed in the HIV+ group was also significantly different to the HIV- group as measured by BIA, with a lower percentage of fat mass. As this data has been taken from a single measurement, no inference can be drawn from the data on the degree or type of weight change incurred by the HIV+ group. However, the literature reports that HIV infection is often associated with a degree of weight loss and if this were the case in these patients, then the body composition would suggest a starvation-type model of weight loss with a preferential reduction in fat mass in order to spare lean tissue.

The lower measured values for hip and waist circumference and triceps skinfold also indicate lower absolute amounts of fat in the HIV+ group, while the lower measured MUAC and calculated MUAMC indicate a lesser amount of fat-free mass in the HIV+ group. While this appears to support the measurements made by the BIA, more sensitive

methods such as DEXA, MRI or CT imaging may be required to verify these results, although these measures were not within the scope of these studies.

5.5.2 Energy expenditure

Resting energy expenditure measured as kilojoules per day by indirect calorimetry was not different between the two groups. However, when REE was expressed per kilogram of fat-free mass, as measured by BIA, the HIV+ group had significantly greater energy expenditure. Elevated REE relative to fat-free mass in HIV+ patients has been shown previously [Grunfeld *et al.* 1992; Salas-Salvado & Garcia-Lorda 2001] and may suggest an energy cost associated with chronic infection. However, inferring from this data that alterations in REE may be associated with the observed differences in body composition may be inappropriate as energy intake relative to total energy expenditure (TEE) determines energy balance and neither of these parameters were measured in this study. In the study by Salas-Salvado and Garcia-Lorda, the variability observed in REE measurements was taken to suggest that hypermetabolism is not a constant feature during the course of HIV infection and may be influenced further by the presence of opportunistic infections and regulated as a compensatory response to malabsorption. As the variability observed in the HIV+ group is no greater than that observed in the HIV- group, it is possible that in terms of factors associated with REE, the HIV+ group studied here is more homogeneous. In addition, while malabsorption was not assessed within this group, the HIV+ subject with the lowest REE/kg FFM also had the highest recovery of label from breath.

5.5.3 Substrate Oxidation

Based on VO_2 and VCO_2 measurements made by indirect calorimetry, in both the postabsorptive and postprandial phase, there were no differences in either the amounts of total lipid or carbohydrate oxidised between the groups. The amount of dietary lipid oxidised, assessed by the recovery of tracer on breath as $^{13}CO_2$, in the postprandial period was lower in the HIV+ group. While the median value for the recovery of the label on breath in the HIV+ group was only approximately 5% lower than the HIV- group, this may indicate either a reduced or delayed absorption of label from the gut in the HIV+ group. This is a possibility as reduced gut function is associated with HIV infection, as discussed

in the literature review. However malabsorption is often associated with concurrent diarrhoea [Miller *et al.* 1988] and all patients were asked at the time of study if they were free of diarrhoea as part of the inclusion/exclusion criteria. Without measuring stool losses of the label, aspects of gut function such as digestion or absorption in these groups cannot be assessed. In addition, the degree to which the two groups were similar in terms of the size of their bicarbonate pool and the flux through this pool of CO_2 from substrate oxidation is also unknown. Assuming that recovery of label on breath is indicative of the oxidation of dietary lipid within these groups, dietary lipid oxidation was lower in the HIV+ group. In the absence of differences in gut function between these two groups, the reduced dietary lipid oxidation may be indicative of altered requirements for lipid within the HIV infected group.

5.5.4 Plasma triacylglycerol and non-esterified fatty acid concentration and [^{13}C] enrichment

While there appear to be no differences between the groups in plasma TAG concentration either in the postprandial or the postabsorptive period, some individuals appear to show a markedly elevated fasting plasma TAG concentration and postprandial lipaemic response. While this may have been the expected result for the HIV+ group, following the work of Hellerstein *et al.* (1993) who observed that hypertriacylglycerolaemia exists in asymptomatic HIV infection in comparison to healthy controls, the highest values were seen in two subjects from the HIV- group, suggesting that HIV infection is not the only factor contributing to hypertriglyceridaemia within this group. In neither group, does there appear to be a simple relationship between the fasting plasma TAG concentration and the magnitude of the increase in plasma TAG AUC (AUCi) following the meal (figure 5.9), suggesting that while the fasting plasma TAG concentration may influence the magnitude of the postprandial lipaemic response, it is not an independent factor and can not be used to predict the magnitude of this response.

While the HIV- group show a typical pattern for plasma TAG in the postprandial period i.e. an initial increase, with a peak at around 2 hours, followed by a reduction to near baseline values by 7 hours, the HIV+ group appear to show a different pattern. In this group there is an initial reduction in TAG concentration, which may be explained by a reduction in VLDL synthesis or export by the liver, possibly due to insulin release stimulated by the meal. This response may be more obvious in this group due to higher

levels of VLDL in the fasting state or possibly an elevated insulin secretion or sensitivity within this group. A measure of insulin sensitivity within these groups taken in the immediate postprandial period would help to explain these results.

The concentration of [^{13}C] palmitic acid within TAG was higher in the HIV+ group prior to the meal. This may reflect the degree of dietary manipulation prior to the study, as control subjects recruited at Southampton were requested to abstain from eating foods naturally enriched with ^{13}C, such as sweetcorn, maize and corn products for 3 days prior to the study day. The HIV+ group recruited at the Chelsea and Westminster Hospital were not given this advice. During the postprandial period, the HIV+ group had a lower [^{13}C] palmitic acid concentration within TAG than the HIV- group. The pattern shown by the HIV+ group suggests a reduction in the absorption of label from the gut, an increased clearance of label from plasma, or a combination of these events as the magnitude of this response, as indicated by the AUC for this measure is three-fold lower in the HIV+ group compared to the HIV- group. An alternative explanation for this reduced response could be that the lipid is being retained within the enterocyte until the next meal, as other studies have suggested [Fielding et al, 1996].

Both groups showed an initial reduction in plasma NEFA concentration probably due to the increase in insulin triggered by the meal. Insulin inhibits hormone sensitive lipase (HSL) within adipocytes, resulting in a reduction in the hydrolysis of stored TAG to NEFA for release into the circulation. While there were no differences in plasma NEFA concentration between the two groups, the [^{13}C] palmitic acid concentration within NEFA was lower in the early postprandial period in the HIV+ group. This may be due to the lower concentration of [^{13}C]-TAG within this group as dietary fatty acids appearing in the circulation in the early postprandial period may originate from chylomicron TAG hydrolysis and subsequent fatty acid release.

5.5.5 Plasma insulin and glucose concentration

The data shown here suggests that plasma glucose and insulin are lower in the presence of HIV infection. This is in agreement with previously reported data showing that in HIV infection, subjects generally had lower or normal glucose without insulin resistance [Heijligenberg et al. 1993; Stein et al. 1990]. In addition, the higher levels observed in the control group may suggest that factors other than HIV are related to alterations in the control of plasma glucose levels and insulin sensitivity in this gender and age range.

5.5.6 Summary

The aim of the study described in this chapter was to examine the differences in postprandial lipid metabolism, specifically the metabolic disposal of [1-^{13}C] palmitic acid between HIV positive patients and HIV negative, healthy controls. The results demonstrate that:

1) The HIV-positive group have a higher resting energy expenditure per kilogram of fat-free mass and a lower BMI than the healthy controls, possibly related to the presence of chronic infection, although there appear to be no differences in the amount of lipid or carbohydrate oxidised to meet this elevated energy requirement.

2) While not obviously hypertriglyceridaemic, the HIV-positive group have a higher percentage of subjects with a fasting plasma TAG concentration of 1 mmol/l or above (HIV+ 80%; HIV- 38%). However, the highest plasma lipid concentrations in both the postabsorptive and postprandial periods where observed in two individuals from the HIV- group. This would suggest that while there may be widespread perturbation in lipid metabolism associated with HIV infection, this is not the only contributing factor to elevated lipid in the subjects studied. There was no relationship in the HIV+ group between fasting TAG concentration and either CD4 count or viral load as has been reported in other studies.

3) The HIV+ group showed a lower concentration of [^{13}C] palmitic acid in both plasma TAG and NEFA and a lower recovery of label on breath as $^{13}CO_2$ suggesting either a reduced absorption of label from the gut or a faster clearance of label from the circulation, with lower proportional dietary fatty acid oxidation.

4) The HIV+ group showed lower plasma concentrations of insulin and glucose in both the postabsorptive and postprandial period, suggesting that HIV infection might influence glucose metabolism, insulin secretion and/or clearance, and possibly insulin sensitivity.

Chapter 6.0 A trial comparing postprandial lipaemia in HIV positive patients prior to HAART therapy and at 1 month and 3 months following the initiation of HAART including a protease inhibitor

6.1 Introduction

Following the literature review and the results of chapters 4.0 and 5.0, it is apparent that while HIV infection may influence lipid metabolism, treatment with HAART can result in the dysregulation of this metabolic process in some patients, resulting in markedly elevated plasma lipid levels. While the audit data reported in chapter 4.0 would suggest that NRTI therapy alone is associated with an increased prevalence of hypertriglyceridaemia and hyperglycaemia, previous literature suggests that inclusion of the PI drug class in the HAART combination results in earlier and more exaggerated increases in plasma TAG [Silva et al, 1998; Carr et al, 1999]. In order to examine early changes in lipid metabolism that may occur with HAART initiation including a PI, postprandial lipaemia was investigated using stable isotope tracers in patients with no previous antiretroviral therapy and then at both one month and 3 months following the initiation of HAART including a PI. While the intention was to continue these studies at 6, 12, 18 and 24 months, this was not practicable as by this time, patients had either altered their treatment regimen or did not want to volunteer for further studies. Only two of the initial group of patients both qualified for and consented to do further trials and it was felt that presenting data on two patients would not provide sufficient data on which to draw conclusions about the effects of longer term HAART use on postprandial lipid metabolism.

6.2 Aims

The aim of this intervention trial was to determine if, using fatty acid tracers, early changes that may occur in the handling of dietary fatty acids, could be measured between HIV-positive therapy naive males and the same patients following both one and three months of HAART including a PI to determine if altered handling of dietary lipid could contribute to elevated plasma TAG levels, as assessed in the absence of chronically elevated plasma TAG concentrations.

6.3 Methods

6.3.1 Subjects

The therapy naïve group were male, HIV positive with no previous history of PI or any antiretroviral exposure and normotriglyceridaemic. Normal fasting plasma TAG was confirmed prior to the study by the Chemical Pathology Department of the respective hospitals. Seven males were recruited into the PI-naïve group (PINa-g), of which three were later removed. All remaining subjects were found, by examination, to be asymptomatic at the time of the trial. The criteria for the HIV-positive group were; male, HIV-positive with high viral load, asymptomatic with no presence of infection (other than HIV), therapy-naive and normotriglyceridaemic. Of this group, subject PINe was found to have a malignant tumour within his colon and was removed from the study. Another subject, PINc was found to be hypertriglyceridaemic (TAG >6.8 mmol/l) with a history of AZT use and was also removed from the study. One of the HIV-positive group was African and the remainder were caucasian. Of the five subjects remaining in this group, one subject (PINd) was non-compliant with the HAART regimen and was, therefore, removed from the data set leaving four subjects within the group. The study protocol and methods used have previously been described in the General Methods Chapter. The group consisted of smokers and non-smokers and smokers were asked to abstain from smoking for the duration of the trial.

6.3.2 Statistical Analysis

In this chapter, the Friedman test was used to assess significance between repeated measures over time within the group, as this test is the non-parametric equivalent of the parametric one factor repeated measures ANOVA (analysis of variance) but does not make the assumptions about the underlying distribution of the data. Significance was achieved at $p<0.05$ for a chi square table value of ≥ 6.5.

6.4 Results

6.4.1 Subject Characteristics

There was a significant reduction in viral load (0PIN 334156 copies/ml, 36783-1987172; 3PIN 26 copies/ml, <25-113; $p<0.05$) and increase in CD4 count (0PIN 60 x 10^6 cells/l, 0-145; 3PIN 207 x 10^6 cells/l, 24-242; $p<0.05$) in the group during the three months HAART exposure (table 6.1). Subjects were placed on an appropriate HAART combination following discussion with their physician and the combinations used are shown in table 6.2, all of the combinations consisted of one or two PI's plus two NRTI's. The median age of the group was 45 years (range 44-47). Three of the four subjects gained weight (median weight gain 5.4 kg) over the three month period, and subject PINa remained weight stable.

6.4.2 Body Composition

Using BIA (table 6.4), the group showed a significant gain in fat mass (median gain of 1.6 kg) over the three month HAART period ($p>0.05$), although there was no significant change in the relative proportions of fat and fat-free mass. There were no significant changes in any of the other anthropometric indices used (table 6.5). Only the difference observed in triceps skinfold measurement over the 3-month period achieved a variability of greater than the 10% produced by a combination of operator error and within-individual variability.

6.4.3 Energy expenditure

Resting energy expenditure (REE) increased in three of the four subjects, although this was not significant as PINf showed a reduction in REE over the 3-month period (table 6.6). When REE was expressed per kilogram of fat free mass (FFM), the same pattern was observed with an increase in 3 of the 4 subjects (figure 6.1). There was no change in the increase in energy expenditure over the 7-hour postprandial period (AUC_i), following the three-month period of HAART.

6.4.4 Substrate Oxidation

There was no change in respiratory exchange ratio (RER) or the postabsorptive lipid or carbohydrate oxidation over the three-month period (table 6.7). There was no difference in the postprandial oxidation of either lipid (figure 6.2) or carbohydrate when expressed as both grams per 7-hours (AUC) or the increase from baseline over the 7-hours (AUC_i) following 3 months of HAART. However, the 1-hour measurement of postprandial carbohydrate oxidation (figure 6.3) increased significantly over this period (0PIN 8.39 g/h, 3.12-11.26; 1PIN 11.91 g/h, 8.62-15.95; 3PIN 13.93 g/h, 8.75-17.41; $p<0.04$). The recovery of label on breath did not alter significantly over the 3-month treatment period and therefore, neither endogenous or exogenous lipid oxidation expressed as AUC in both grams (table 6.9) and kilojoules (table 6.10) changed significantly during this time although, there appeared to be a shift towards a higher relative oxidation of carbohydrate for energy at both 1 and 3 months (figure 6.4).

6.4.5 Plasma triacylglycerol concentration

There was no change in fasting plasma TAG concentration over the three-month treatment period (table 6.11). The excursion in plasma TAG over the 7 hours following the meal (AUC) tended to increase after the treatment period, and this was shown by the significant effect of time on the plasma TAG excursion at 3 months (figure 6.5, $p<0.05$). There were no significant differences in the postprandial plasma TAG pattern over the three-month period.

6.4.6 Plasma [^{13}C]-triacylglycerol concentration

Following three months of PI therapy, there was a trend towards a greater accumulation (AUC_i) of [^{13}C] palmitic acid within plasma TAG (table 6.13) over the 7 hours following administration of the label (0PIN 32.71 μg/ml per 7h, 21.75-59.01; 1PIN 32.28 μg/ml per 7h, 14.25-49.84; 3PIN 56.06 μg/ml per 7h, 37.55-88.30; ns). At both 1 and 3 months of therapy, there was a significant increase in plasma [^{13}C]-TAG at 1.5 hours after the test meal and emulsion (figure 6.6) (0PIN 3.09 μg/ml, 2.68-4.34; 1PIN 9.88 μg/ml, 3.75-15.28; 3PIN 9.18 μg/ml, 6.73-13.22; $p<0.04$).

6.4.7 Plasma non-esterified fatty acid concentration and [^{13}C]- non-esterified fatty acid concentration

Following both one and three months therapy, there was a trend towards lower fasting plasma NEFA concentration (table 6.12, figure 6.7), in comparison to the therapy naïve values (0PIN 0.46 mmol/l, 0.39-0.51; 1PIN 0.29 mmol/l, 0.22-0.50; 3PIN 0.28 mmol/l, 0.20-0.62; ns). There was no change in plasma NEFA pattern over the postprandial period or the cumulative AUC or AUCi over the 3 months. There were no differences observed in [^{13}C] palmitic acid concentration within NEFA over the treatment period (table 6.13, figure 6.8).

6.4.8 Plasma insulin and glucose concentrations

There were no significant changes in fasting glucose or insulin concentrations or postprandial AUC over the treatment period (table 6.14). When the patterns of postprandial plasma glucose and insulin were examined (figure 6.9), there was a significant increase in the 7h plasma insulin value (0PIN 12.48 mU/l, 4.84-17.94; 1PIN 6.46 mU/l, 3.97-12.15; 3PIN 15.77 mU/l, 6.28-35.12; $p<0.05$) and a significant fall in the 4h plasma glucose value (0PIN 4.6 mmol/l, 4.1-4.9; 1PIN 5.0 mmol/l, 4.7-5.9; 3PIN 4.3 mmol/l, 3.1-4.6; $p<0.05$) following the 3 month treatment period.

Table 6.1 **Viral load, CD4 and CD8 counts of subjects in the HIV-positive group prior to therapy (0PIN), at one month (1PIN) and three months (3PIN) following HAART initiation. **Undetectable (U) indicates below the level of detection (<25 copies/ml) for the PCR assay used. *Significant change over time within the group using the Friedman test.**

SUBJECT CODE	Viral Load (copies/ml plasma)	CD4 (X 10^6 cells/L plasma)	CD8 (X 10^6 cells/L plasma)
0PINa	1987172	0	96
0PINb	168312	65	953
0PINf	36783	145	1157
0PINg	500000	55	877
Median (range)	334156 (36783-1987172)	60 (0-145)	915 (96-1157)
1PINa	U**	208	107
1PINb	1556	225	2058
1PINf	449	153	883
1PINg	672	152	1089
Median (range)	561 (<25-1556)	181 (152-225)	986 (107-2058)
3PINa	51	24	263
3PINb	113	242	1455
3PINf	U**	220	1021
3PINg	U**	194	1020
Median (range)	26* (<25-113)	207* (24-242)	1021 (263-1455)

***p<0.05**

Table 6.2 **Therapy details of the HAART combination used in the previously therapy naïve group during the three month study period.**

SUBJECT CODE	Combination during the three month study period
0PINa	RTV SQV d4T 3TC
0PINb	d4T 3TC IND
0PINf	AZT 3TC IND
0PINg	RTV SQV AZT 3TC

Abbreviations:

d4T	**Stavudine (NRTI)**	**3TC**	**Lamivudine (NRTI)**
SQV	**Saquinavir (PI)**	**IND**	**Indinavir (PI)**
RTV	**Ritonavir (PI)**	**AZT**	**Zidovudine (NRTI)**

Table 6.3 **Characteristics of subjects in the therapy-naïve (0PIN) group and at one month (1PIN) and three months (3PIN) following the initiation of HAART, including height, weight and body mass index (BMI).**

SUBJECT CODE	HEIGHT (cm)	WEIGHT (kg)	BMI (kg/m^2)
THERAPY NAÏVE GROUP			
0PINa	183	69.8	20.8
0PINb	176	63.8	20.6
0PINf	187	78.0	22.3
0PINg	181	64.3	19.6
Median (range)	182 (176-187)	67.1 (63.8-78.0)	20.7 (19.6-22.3)
1 MONTH HAART			
1PINa	184	69.0	20.4
1PINb	177	63.1	20.1
1PINf	187	82.3	23.5
1PINg	181	64.0	19.5
Median (range)	183 (177-187)	66.5 (63.1-82.3)	20.3 (19.5-23.5)
3 MONTHS HAART			
3PINa	184	69.7	20.6
3PINb	178	69.2	21.8
3PINf	188	84.3	23.9
3PINg	180	69.6	21.5
Median (range)	182 (178-188)	69.7 (69.2-84.3)	21.7 (20.6-23.9)

Table 6.4 **Results of the bioelectrical impedance analysis (BIA) for fat mass (kg) and fat free mass (FFM) (kg), and fat and fat free mass as a percentage of total body weight in the therapy naïve (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART. *Significant change over time within the group using the Friedman test.**

SUBJECT CODE	FAT (kg)	FFM (kg)	FAT (%)	FFM (%)
PROTEASE INHIBITOR NAÏVE GROUP				
0PINa	9.4	60.4	13.5	86.5
0PINb	11.3	52.5	17.7	82.3
0PINf	12.5	65.5	16.0	84.0
0PINg	10.4	53.9	16.2	83.8
Median (range)	10.9 (9.4-12.5)	57.2 (52.5-65.5)	16.1 (13.5-17.7)	83.9 (82.3-86.5)
1 MONTH PI THERAPY				
1PINa	8.4	60.6	12.2	87.8
1PINb	10.1	53.0	16.0	84.0
1PINf	16.0	66.3	19.4	80.6
1PINg	9.8	54.2	15.3	84.7
Median (range)	9.6 (8.4-16.0)	57.4 (53.0-66.3)	15.7 (12.2-19.4)	84.4 (80.6-87.8)
3 MONTHS PI THERAPY				
3PINa	10.9	58.8	15.6	84.4
3PINb	11.8	57.4	17.1	82.9
3PINf	16.2	68.1	19.2	80.8
3PINg	13.1	56.5	18.8	81.2
Median (range)	12.5* (10.9-16.2)	58.1 (56.5-68.1)	18.0 (15.6-19.2)	82.1 (80.8-84.4)

*$p<0.05$

Table 6.5 **Anthropometry measurements including waist and hip circumference, waist to hip ratio, triceps skinfold measured with Harpingdon callipers, mid upper arm circumference (MUAC) and the calculated mid upper arm muscle circumference (MUAMC) in the therapy naïve (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	WAIST (mm)	HIP (mm)	WAIST: HIP RATIO	TRICEP SKIN FOLD (mm)	MUAC (mm)	MUAMC (mm)
THERAPY NAÏVE						
0PINa	847	975	0.87	5.1	253	237
0PINb	840	980	0.86	8.0	265	239
0PINf	836	950	0.88	11.4	320	284
0PINg	798	948	0.84	8.0	270	244
Median (range)	838 (798-847)	963 (948-980)	0.87 (0.84-0.88)	8.0 (5.1-11.4)	268 (253-320)	242 (237-284)
1 MONTH HAART						
1PINa	823	966	0.85	5.9	250	231
1PINb	819	955	0.86	7.9	261	236
1PINf	881	1045	0.84	10.5	310	277
1PINg	820	907	0.90	11.1	262	227
Median (range)	822 (819-881)	961 (907-1045)	0.86 (0.84-0.90)	9.2 (5.9-11.1)	262 (250-310)	234 (227-277)
3 MONTHS HAART						
3PINa	843	982	0.86	4.8	244	228
3PINb	869	966	0.90	9.3	282	252
3PINf	900	1015	0.89	15.1	323	275
3PINg	849	1012	0.84	13.0	290	249
Median (range)	859 (843-900)	997 (966-1015)	0.88 (0.84-0.90)	11.2 (4.8-15.1)	286 (244-323)	251 (228-275)

Table 6.6 **Resting energy expenditure (REE) measured by indirect calorimetry, expressed as kilojoules per day (kJ/d) and as kilojoules per kilogram of fat free mass as measured by bioelectrical impedance (kJ/kg FFM/d), and the increase in energy expenditure following the test meal expressed as kilojoules per 7 hours (kJ/7h) measured as the incremental area under the curve (AUC_i) in the therapy naïve (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	REE (kJ/d)	REE (kJ/kg FFM/d)	EE (kJ/7h) AUC_i
THERAPY NAÏVE			
0PINa	7190	119	466
0PINb	6312	120	343
0PINf	8485	130	264
0PINg	7524	140	225
Median (range)	7357 (6312-8485)	125 (119-140)	304 (225-466)
1 MONTH HAART			
1PINa	7775	128	324
1PINb	6646	125	285
1PINf	7817	118	397
1PINg	7984	147	301
Median (range)	7796 (6646-7984)	127 (118-147)	313 (285-397)
3 MONTHS HAART			
3PINa	8235	140	241
3PINb	7900	138	93
3PINf	7691	113	520
3PINg	8527	151	167
Median (range)	8068 (7691-8527)	139 (113-151)	204 (93-520)

Table 6.7 **Respiratory exchange ratio (RER) and lipid and carbohydrate (CHO) oxidation, expressed as grams per hour, prior to the administration of the test meal in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	RER 0h	LIPID OXIDATION (g/h) 0h	CHO OXIDATION (g/h) 0h
THERAPY NAIVE			
0PINa	0.80	3.94	5.08
0PINb	0.81	3.23	4.50
0PINf	0.79	5.14	5.43
0PINg	0.92	1.13	14.10
Median (range)	0.81 (0.79-0.92)	3.59 (1.13-5.14)	5.26 (4.50-14.10)
1 MONTH HAART			
1PINa	0.84	3.53	7.62
1PINb	0.86	2.13	8.15
1PINf	0.90	1.53	12.84
1PINg	0.85	3.23	8.93
Median (range)	0.86 (0.84-0.90)	2.68 (1.53-3.53)	8.54 (7.62-12.84)
3 MONTHS HAART			
3PINa	0.80	4.74	5.79
3PINb	0.90	1.33	11.38
3PINf	0.91	1.43	12.71
3PINg	0.90	1.83	13.87
Median (range)	0.90 (0.80-0.91)	1.63 (1.33-4.74)	12.05 (5.79-13.87)

Table 6.8 **Lipid and carbohydrate (CHO) oxidation, expressed as grams per 7 hours, following the administration of the test meal, calculated as the area under the curve (AUC_0) and the incremental area under the curve (AUC_i), in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	LIPID OXIDATION (g/7h) AUC_0	CHO OXIDATION (g/7h) AUC_0	LIPID OXIDATION (g/7h) AUC_i	CHO OXIDATION (g/7h) AUC_i
THERAPY NAÏVE				
0PINa	38.3	39.3	10.4	4.6
0PINb	25.4	42.6	3.3	13.9
0PINf	37.7	48.6	2.5	11.5
0PINg	12.8	93.8	5.2	-3.5
Median (range)	31.6 (12.8-38.3)	45.6 (39.3-93.8)	4.3 (2.5-10.4)	8.1 (-3.5-13.9)
1 MONTH HAART				
1PINa	26.9	66.1	2.8	13.8
1PINb	18.1	66.0	3.8	10.2
1PINf	17.3	98.6	6.6	9.8
1PINg	20.2	84.0	-1.5	22.4
Median (range)	19.2 (17.3-26.9)	75.1 (66.0-98.6)	3.3 (-1.5-6.6)	12.0 (9.8-22.4)
3 MONTHS HAART				
3PINa	26.7	70.0	-5.6	30.2
3PINb	14.5	89.1	5.8	10.4
3PINf	20.2	93.6	11.0	5.7
3PINg	23.7	54.9	20.6	-41.0
Median (range)	22.0 (14.5-26.7)	79.6 (54.9-93.6)	8.4 (-5.6-20.6)	8.1 (-41.0-30.2)

Table 6.9 **The calculation of the endogenous and exogenous sources of lipid oxidised over the 7 hour period (g/7h) using the percentage of ^{13}C label recovered on breath over 7 hours, together with the amount of lipid in the test meal and the total lipid oxidised over the 7 hour period, calculated from the area under the curve (AUC), in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	% Administered ^{13}C Dose Excreted on Breath over 7 h	Fat (g) in Test meal	TOTAL LIPID OX. (g/7 h) AUC_0	EXOGENOUS LIPID OX. (g/7 h)	ENDOGENOUS LIPID OX. (g/7 h)
THERAPY NAÏVE					
0PINa	15.16	45.25	37.25	6.86	30.39
0PINb	4.83	45.25	25.35	2.19	23.16
0PINf	10.68	45.25	37.67	4.83	32.84
0PINg	6.65	45.25	12.77	3.01	9.76
Median (range)	8.67 (4.83-15.16)	45.25	31.30 (12.77-37.67)	3.92 (2.19-6.86)	26.78 (9.76-32.84)
1 MONTH HAART					
1PINa	9.23	45.25	26.90	4.18	22.72
1PINb	4.92	45.25	18.07	2.23	15.84
1PINf	6.66	45.25	17.26	3.01	14.25
1PINg	5.53	45.25	20.24	2.50	17.74
Median (range)	6.10 (4.92-9.23)	45.25	19.16 (17.26-26.90)	2.76 (2.23-4.18)	16.79 (14.25-22.72)
3 MONTHS HAART					
3PINa	13.35	45.25	26.68	6.04	20.64
3PINb	8.03	45.25	14.54	3.63	10.91
3PINf	5.47	45.25	20.24	2.48	17.76
3PINg	6.25	45.25	23.73	2.83	20.90
Median (range)	7.14 (5.47-13.35)	45.25	21.99 (14.54-26.68)	3.23 (2.48-6.04)	19.20 (10.91-20.90)

Table 6.10 **Calculation of the proportion of energy expended over the 7-hour period derived from carbohydrate (CHO), endogenous lipid and exogenous lipid, in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	EXOG. LIPID (kJ/7h)	ENDOG LIPID (kJ/7h)	CHO (kJ/7h)	ENERGY EXPENDED (kJ/7h) (AUC_0)	EXOG LIPID (% EE)	ENDOG LIPID (% EE)	CHO (% EE)
THERAPY NAÏVE							
0PINa	261	1155	629	2540	10	45	25
0PINb	83	880	681	2148	4	41	32
0PINf	184	1248	778	2707	7	46	29
0PINg	114	371	1501	2389	5	16	63
Median (range)	149 (83-261)	1017 (371-1248)	730 (629-1501)	2465 (2148-2707)	6 (4-10)	43 (16-46)	30 (25-63)
1 MONTH HAART							
1PINa	159	863	1057	2548	6	34	41
1PINb	85	602	1057	2190	4	27	48
1PINf	114	542	1577	2651	4	20	60
1PINg	95	674	1344	2594	4	26	52
Median (range)	105 (85-159)	638 (542-863)	1200 (1057-1577)	2571 (2190-2651)	4 (4-6)	27 (20-34)	50 (41-60)
3 MONTHS HAART							
3PINa	230	784	1119	2605	9	30	43
3PINb	138	415	1426	2367	6	18	60
3PINf	94	675	1498	2724	3	25	55
3PINg	108	794	878	2610	4	30	34
Median (range)	123 (94-230)	730 (415-794)	1273 (878-1498)	2608 (2367-2724)	5 (3-9)	27 (18-30)	49 (34-60)

Table 6.11 **Fasting plasma triacylglycerol (TAG) expressed as mmol/litre and the magnitude of the postprandial lipaemic response following administration of the test meal and emulsion, calculated as both the area under the curve (AUC) and the incremental area under the curve (AUC_i) in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	FASTING TAG (mmol/l)	POSTPRANDIAL TAG AUC (mmol/l/7h)	POSTPRANDIAL TAG RESPONSE AUCi (mmol/l per 7h)
THERAPY NAÏVE			
0PINa	1.6	11.7	1.0
0PINb	1.0	9.7	2.9
0PINf	1.2	9.4	1.2
0PINg	0.9	8.2	2.2
Median (range)	1.1 (0.9-1.6)	9.6 (8.2-11.7)	1.7 (1.0-2.9)
1 MONTH HAART			
1PINa	1.4	8.5	0.1
1PINb	1.2	12.1	3.8
1PINf	1.0	8.7	2.0
1PINg	0.9	6.7	0.7
Median (range)	1.1 (0.9-1.4)	8.6 (6.7-12.1)	1.3 (0.1-3.8)
3 MONTHS HAART			
3PINa	1.1	8.9	1.0
3PINb	1.2	11.8	3.6
3PINf	1.0	9.8	3.1
3PINg	1.5	11.8	1.5
Median (range)	1.2 (1.0-1.5)	10.8 (8.9-11.8)	2.3 (1.0-3.6)

Table 6.12 **Plasma non-esterified fatty acid (NEFA) concentration expressed as mmol/litre before the meal (fasting), in the 7 hours following the meal (AUC) and the change in plasma NEFA from fasting following administration of the test meal and emulsion, calculated as the incremental area under the curve (AUC_i) in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	FASTING NEFA (mmol/l)	POSTPRANDIAL PLASMA NEFA AUC (mmol/l/7h)	POSTPRANDIAL NEFA RESPONSE AUC_i (mmol/l over 7h)
THERAPY NAIVE GROUP			
0PINa	0.45	2.92	0.57
0PINb	0.39	0.71	0.00
0PINf	0.51	1.66	0.00
0PINg	0.47	1.15	0.00
Median (range)	0.46 (0.39-0.51)	1.41 (0.71-2.92)	0.00 (0.00-0.57)
1 MONTH HAART			
1PINa	0.50	1.19	0.00
1PINb	0.27	1.50	0.11
1PINf	0.31	1.51	0.01
1PINg	0.22	0.91	0.00
Median (range)	0.29 (0.22-0.50)	1.35 (0.91-1.51)	0.01 (0.00-0.11)
3 MONTHS HAART			
3PINa	0.62	1.60	0.00
3PINb	0.20	2.23	1.23
3PINf	0.34	1.71	0.17
3PINg	0.22	1.24	0.18
Median (range)	0.22 (0.20-0.62)	1.66 (1.24-2.23)	0.17 (0.00-1.23)

Table 6.13 **Plasma [^{13}C] palmitic acid within triacylglycerol (^{13}C-TAG) and within non-esterified fatty acid (^{13}C-NEFA) expressed as μg/ml following administration of the emulsion containing 700 mg [1-^{13}C] palmitic acid and test meal, calculated as the incremental area under the curve (AUC_i) in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	[^{13}C]-TAG (μg/ml/7h)	[^{13}C]-NEFA (μg/ml/7h)
THERAPY NAIVE GROUP		
0PINa	59.01	5.18
0PINb	21.75	0.30
0PINf	38.25	2.14
0PINg	27.17	0.50
Median (range)	32.71 (21.75-59.01)	1.32 (0.30-5.18)
1 MONTH HAART		
1PINa	28.15	1.28
1PINb	36.41	1.33
1PINf	49.84	3.08
1PINg	14.25	0.61
Median (range)	32.28 (14.25-49.84)	1.31 (0.61-3.08)
3 MONTHS HAART		
3PINa	37.66	1.76
3PINb	88.30	3.03
3PINf	51.39	2.68
3PINg	60.73	1.54
Median (range)	56.06 (37.66-88.30)	2.22 (1.54-3.03)

Table 6.14 **Plasma insulin and glucose concentration expressed as mU/l and mmol/l respectively prior to the meal and following administration of the emulsion and test meal, calculated as both area under the curve (AUC) and incremental area under the curve (AUC_i) over 7 hours in the therapy naive (0PIN) group and following 1 month (1PIN) and 3 months (3PIN) of HAART.**

SUBJECT CODE	FASTING PLASMA INSULIN (mU/l)	PLASMA INSULIN AUC (mU/l/7h)	PLASMA INSULIN AUC_i (mU/l/7h)	FASTING PLASMA GLUCOSE (mmol/l)	PLASMA GLUCOSE AUC (mmol/l/7h)	PLASMA GLUCOSE AUC_i (mmol/l/7h)
PROTEASE INHIBITOR NAÏVE GROUP						
0PINa	4.3	86.3	56.1	4.4	32.0	1.7
0PINb	7.7	133.6	79.6	4.8	38.8	5.2
0PINf	12.3	266.2	180.3	5.0	37.5	4.7
0PINg	10.4	106.6	37.4	5.0	35.9	1.3
Median (range)	9.0 (4.3-12.3)	120.1 (86.3-266.2)	67.9 (37.4-180.3)	4.9 (4.4-5.0)	36.7 (32.0-38.8)	3.2 (1.3-5.2)
1 MONTH HAART						
1PINa	7.2	95.2	47.7	4.8	35.8	2.5
1PINb	4.8	149.6	116.2	5.6	38.3	1.2
1PINf	11.0	264.5	190.5	4.8	34.8	2.8
1PINg	7.6	112.1	59.4	4.8	38.1	4.5
Median (range)	7.4 (4.8-11.0)	130.9 (95.2-264.5)	87.8 (47.7-190.5)	4.8 (4.8-5.6)	36.9 (34.8-38.3)	2.6 (1.2-4.5)
3 MONTHS HAART						
3PINa	24.0	111.1	1.6	5.0	34.7	1.4
3PINb	11.8	123.0	50.1	5.2	34.9	0.7
3PINf	8.5	407.9	348.4	5.0	30.1	4.1
3PINg	8.5	141.8	82.3	5.1	35.4	1.1
Median (range)	10.1 (8.5-24.0)	132.4 (111.1-407.9)	66.2 (1.6-348.4)	5.1 (5.0-5.2)	34.8 (30.1-35.4)	1.2 (0.7-4.1)

Figure 6.1 **Resting energy expenditure (REE), as measured by indirect calorimetry, expressed as kilojoules per kilogram of fat free mass (FFM, measured by bioelectrical impedance) in the therapy naïve group and following 1 month and 3 months of HAART.**

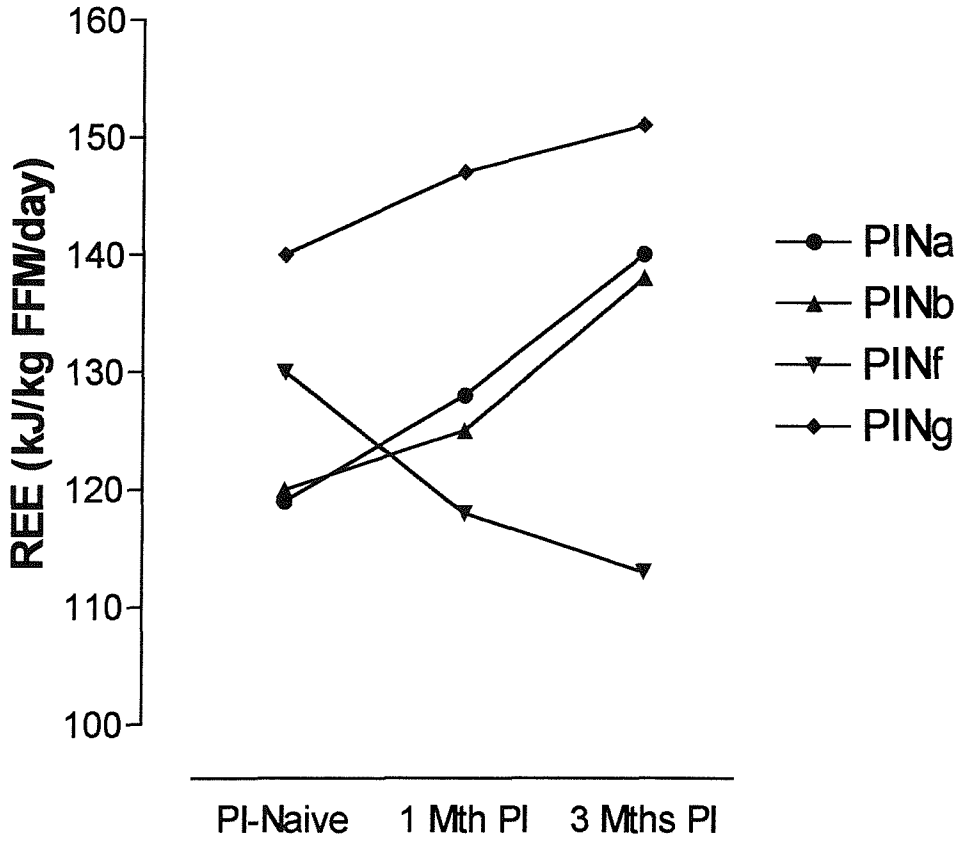


Figure 6.2 **The pattern of lipid oxidation over the 7-hour period following the administration of the test meal, expressed as grams per hour (g/h), in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. Data is presented as medians and the error bars represent the range (minimum and maximum values).**

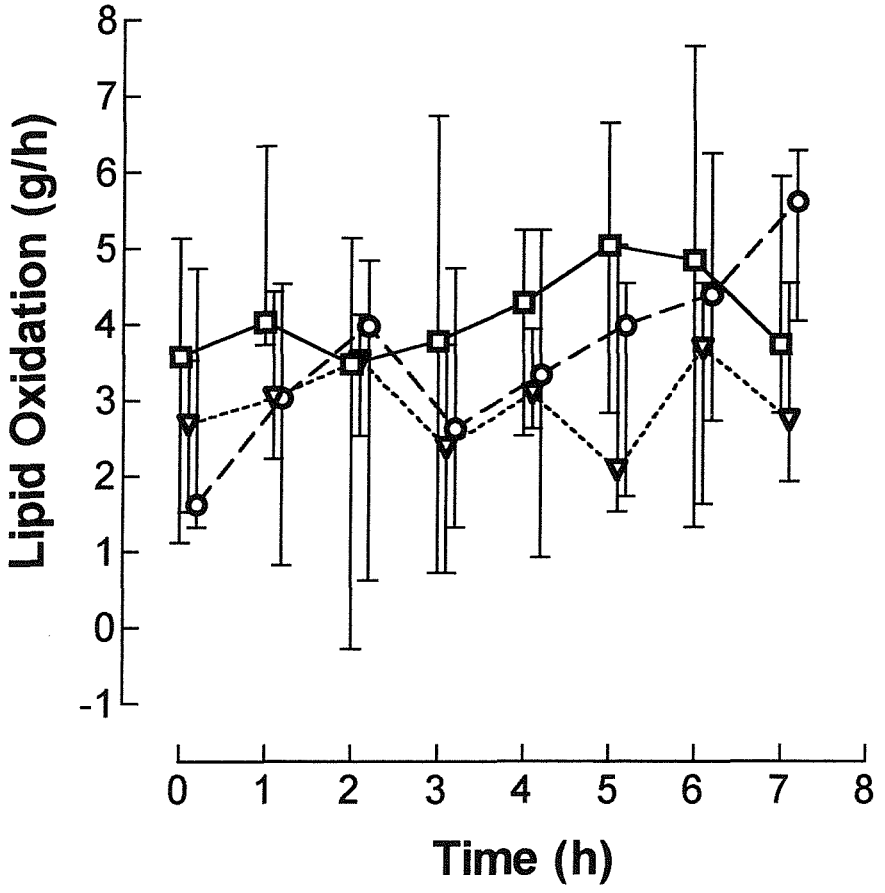
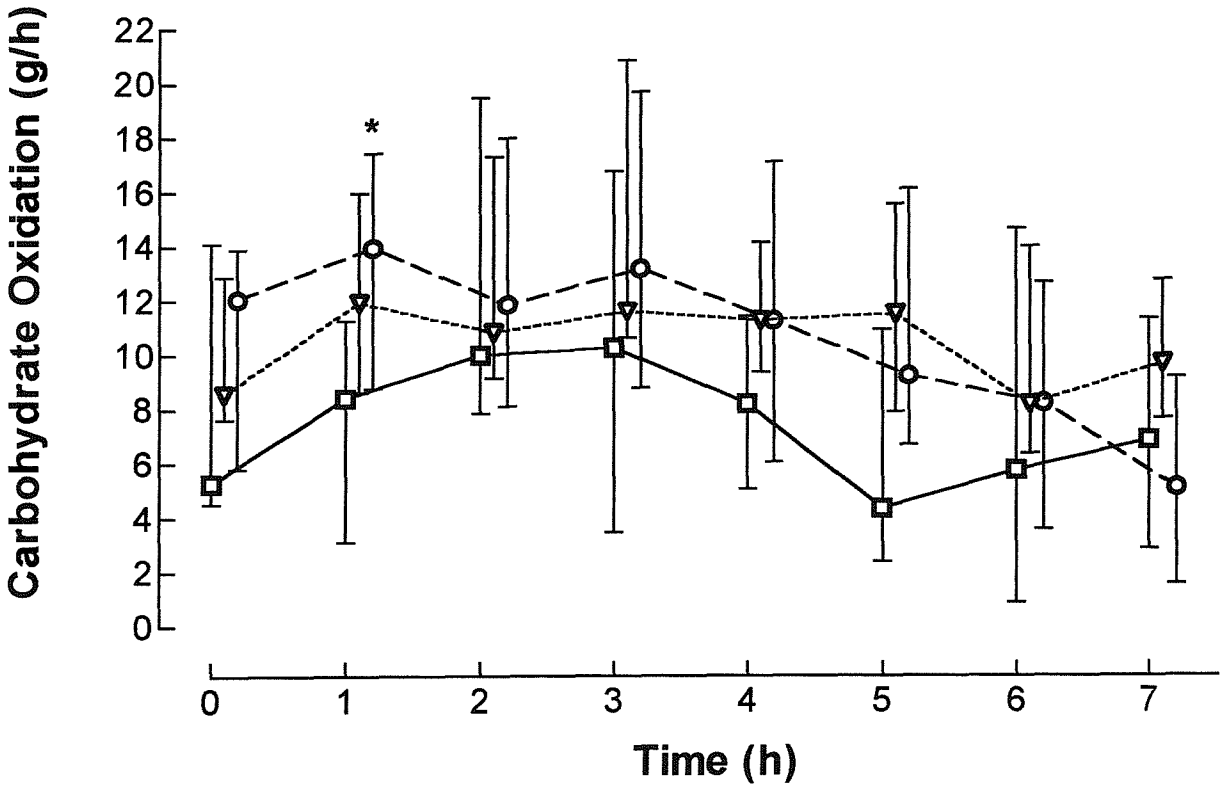


Figure 6.3 **The pattern of carbohydrate oxidation over the 7 hour period following the administration of the test meal, expressed as grams per hour (g/h), in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. *Significant change over the 3 month period within the group using the Friedman test.**



p<0.04

Figure 6.4 **The percentage of energy derived from carbohydrate (CHO), endogenous and exogenous lipid oxidation of the 7 hour period following the administration of the test meal in the therapy naïve group ( ) and following 1 month ( ) and 3 months ( ) of HAART.**

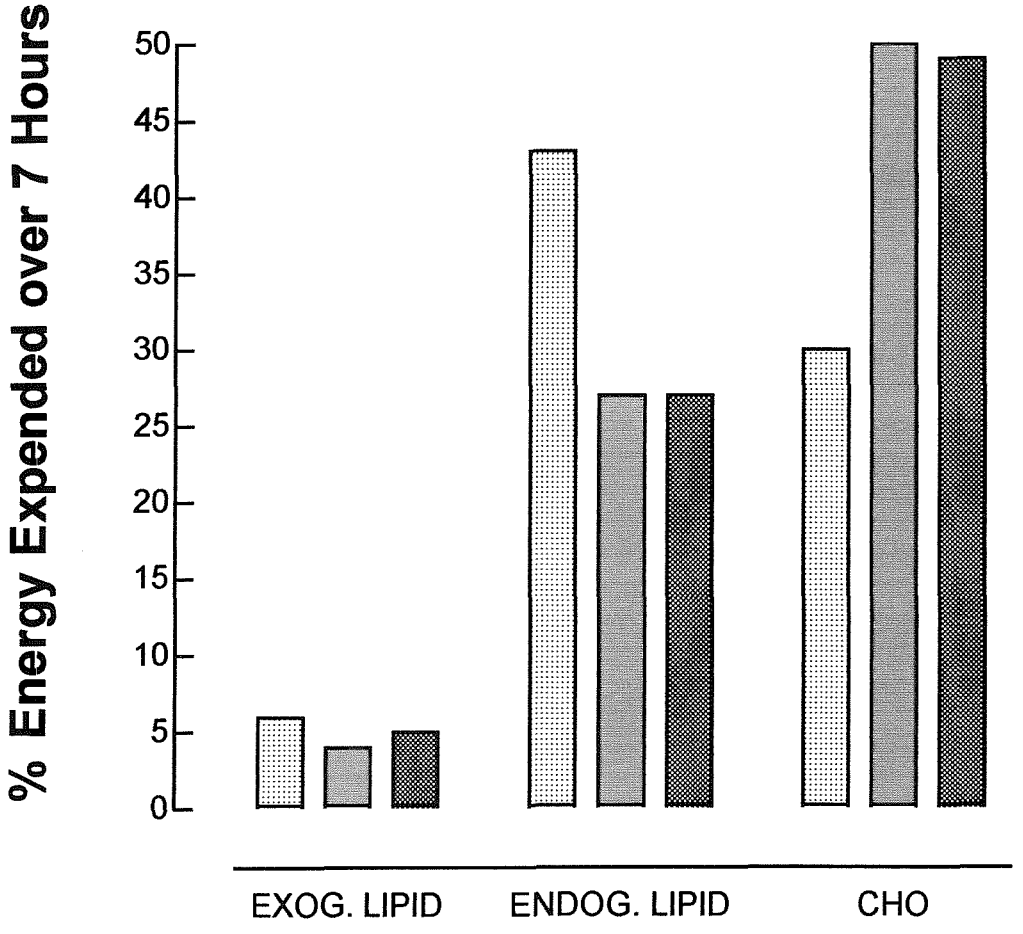


Figure 6.5 The pattern of change in plasma triacylglycerol (TAG) following administration of the test meal and emulsion expressed as mmol/l in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. There is a significant effect of time on the plasma TAG excursion at 3 month using the Friedman test ($p<0.05$).

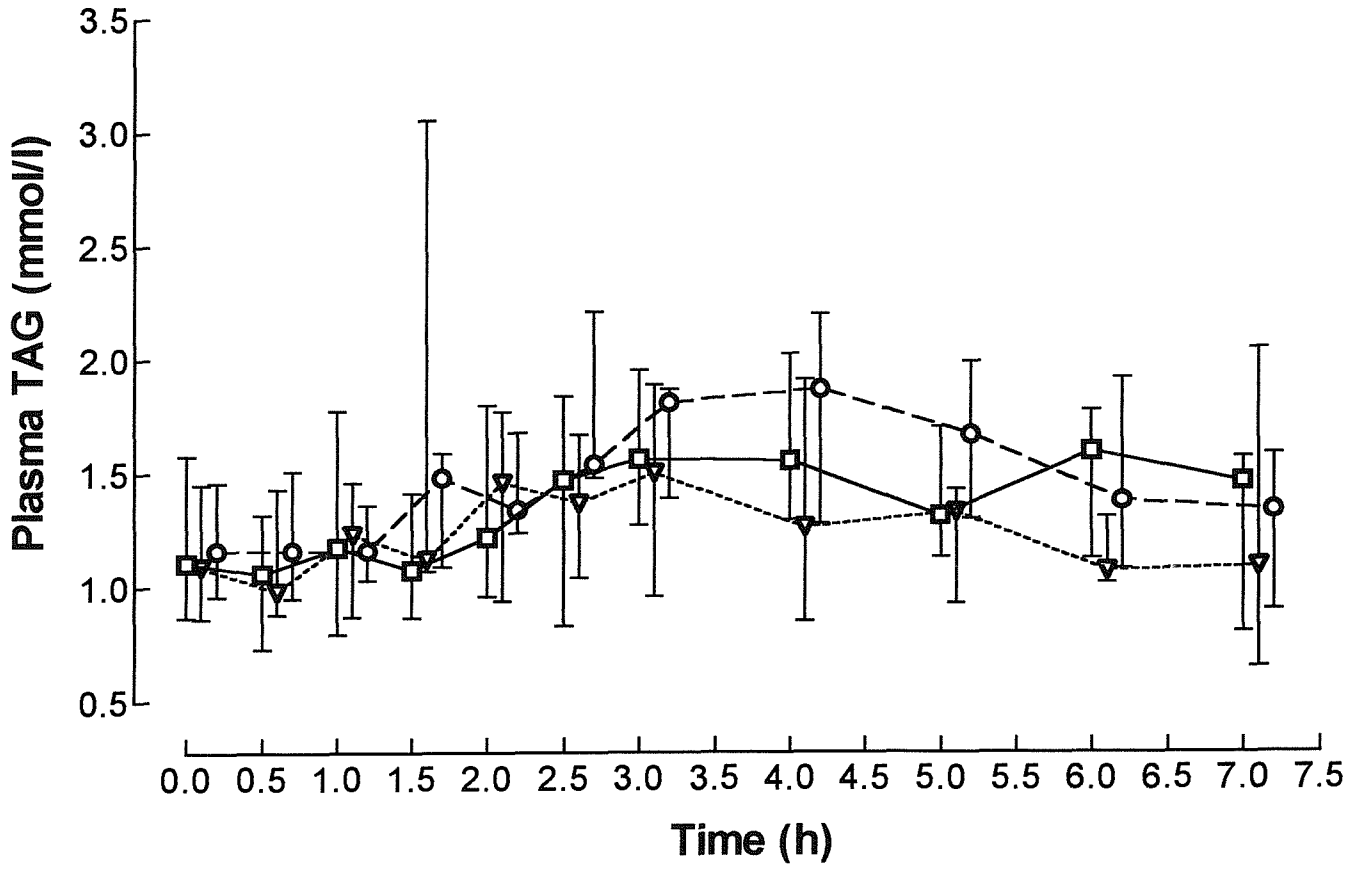
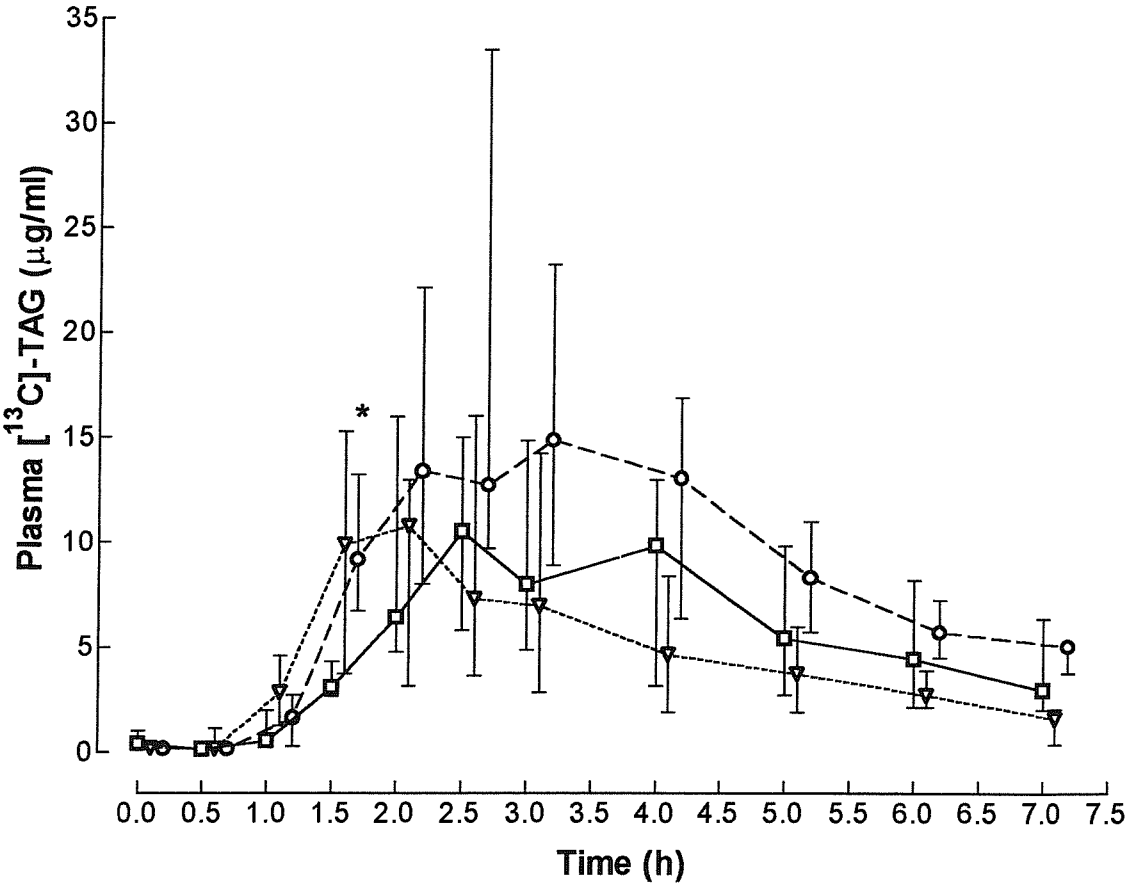


Figure 6.6 **The pattern of change in plasma [^{13}C]-triacylglycerol (TAG) following administration of the test meal and emulsion expressed as μg/ml in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. There is a significant effect of time on the plasma [^{13}C]-TAG excursion at baseline and at 1 and 3 months using the Friedman test ($p<0.05$). *Significant change over the 3 months within the group using the Friedman test.**



*p<0.04

Figure 6.7 **The pattern of change in plasma non-esterified fatty acid (NEFA) concentration following administration of the test meal and emulsion expressed as mmol/l in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. There is a significant effect of time on the plasma NEFA excursion at 3 months only using the Friedman test ($p<0.05$).**

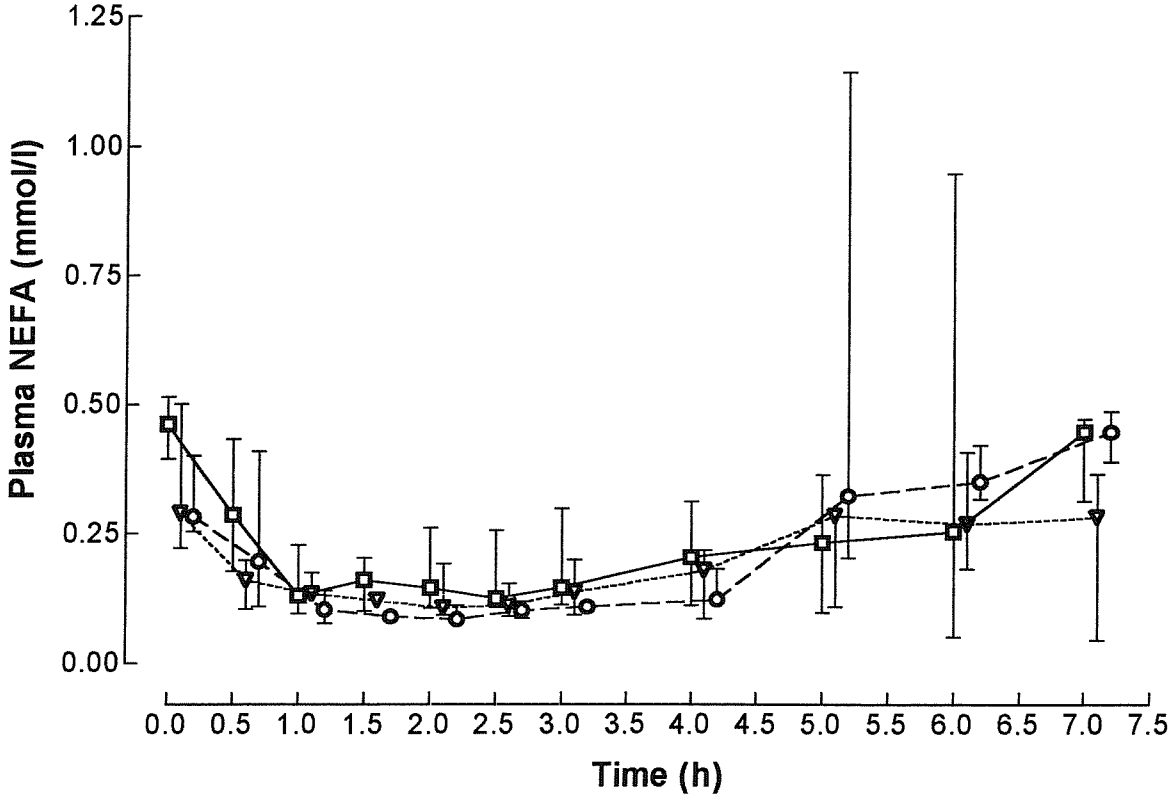


Figure 6.8 The pattern of change in plasma [^{13}C]-non-esterified fatty acid (NEFA) concentration following administration of the test meal and emulsion expressed as μg/ml in the therapy naïve group (□) and following 1 month (▽) and 3 months (○) of HAART. There is a significant effect of time on the plasma [^{13}C]-NEFA excursion at both 1 and 3 months using the Friedman test ($p<0.05$).

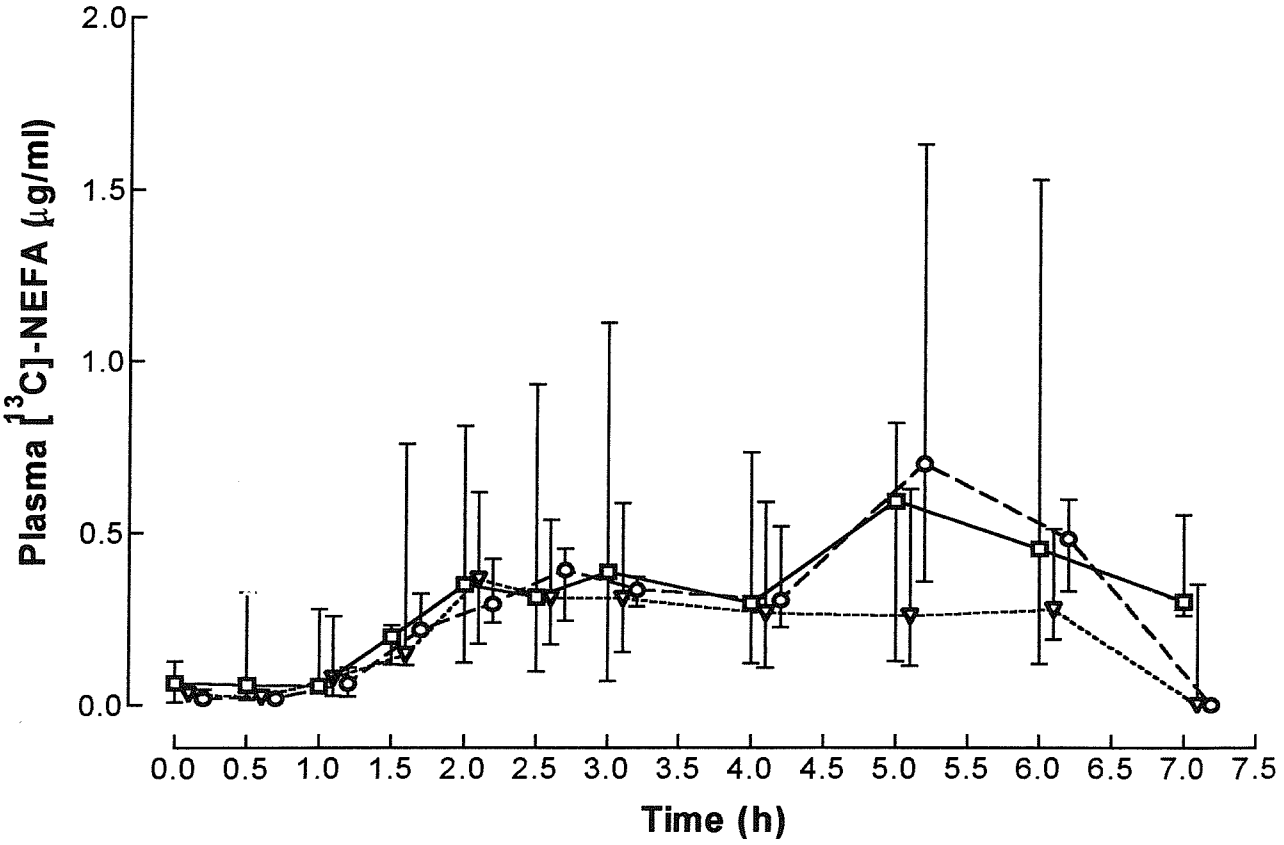
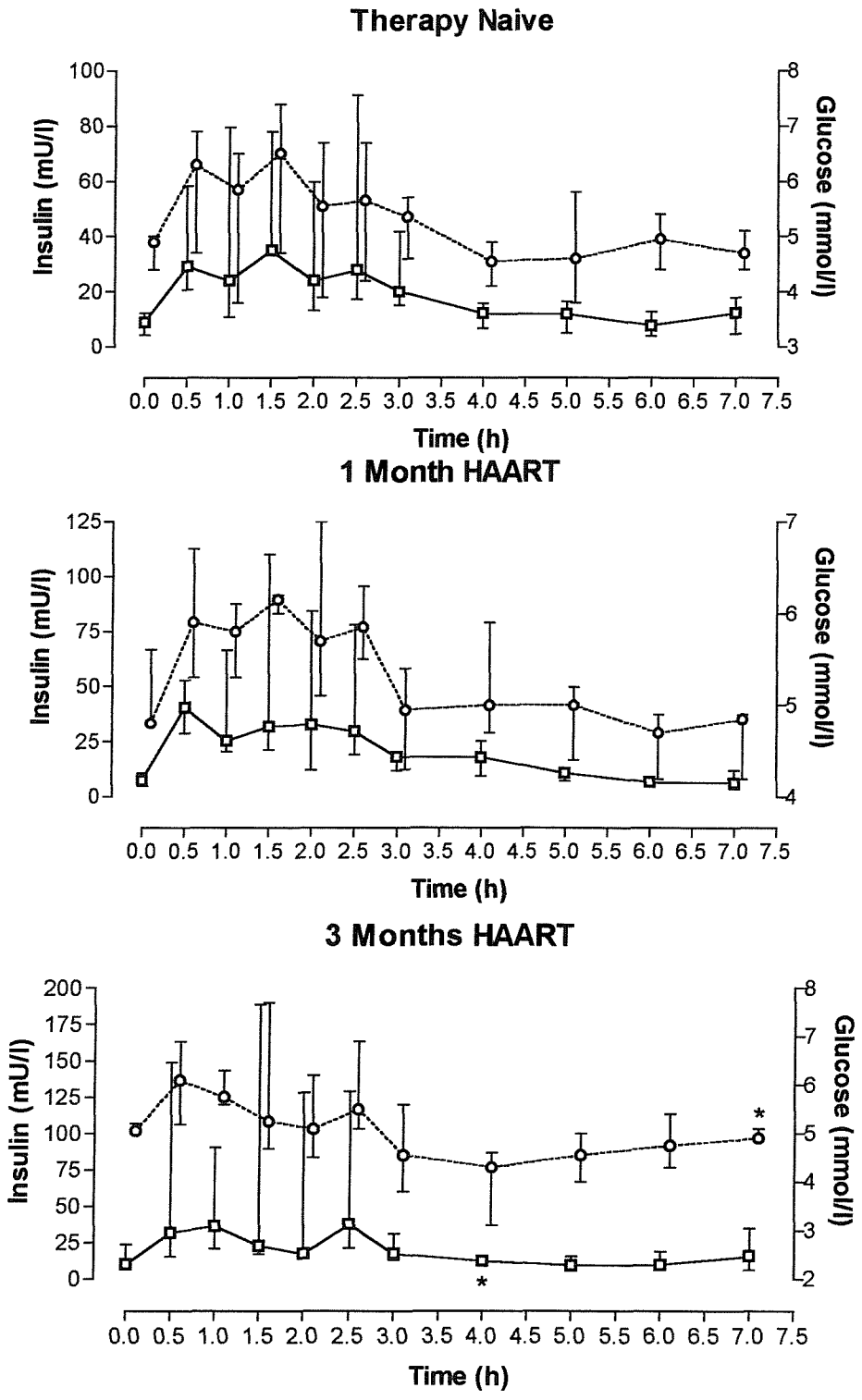


Figure 6.9 **The change in plasma insulin (□) and glucose (▽) concentrations following administration of the test meal and emulsion in the therapy naïve group and following 1 month and 3 months of HAART. *There is a significant change over 3 months in 7h insulin and 4h glucose values within the group using the Friedman test ($p<0.05$).**



6.5 Discussion

This study was undertaken to examine the influence of HAART containing a PI on postprandial lipid metabolism in a group of patients with high viral load, but without elevated background plasma TAG concentrations. As discussed in the introduction, the study was originally planned as a longer term study but factors outside of our control resulted in this study being shortened to a 3 month period. Within this 3 month period, all subjects in the group had a good virological response to the therapy and showed a significant reduction in viral load with an increased CD4 count. With the exception of one subject (PINa), the group gained weight during the treatment period, which according to the results of BIA was predominantly body fat. An increased body fat mass could have been a result of either; a) an effect of HAART on the adipose compartment, or b) the reduction in viral burden allowing the patient to feel better and possibly eat more. From the anthropometry used, there was not any evidence of abnormal fat distribution within the group. Another explanation for the weight gain could be a reduction in energy expenditure associated with the reduced viral burden as a result of HAART. However, during the treatment period, 3 of the 4 subjects in the group show an increased REE, possibly due to changes in FFM.

There appeared to be an increase in carbohydrate oxidation, especially in the early postprandial period following HAART. However, without information on the diet preceding the study day, it is difficult to determine if this is a result of underlying metabolic alterations or a change in the macronutrient content of the meals eaten prior to the study day. During the treatment period, there was no change in the amount of label recovered on breath up to 7 hours after label administration. Recovery of label, as previously discussed, is a function of; digestion of the emulsion, absorption of the tracer, oxidation of the fatty acid, production of $^{13}CO2$, mixing of the $^{13}CO2$ with the body's bicarbonate pool and finally, release of $^{13}CO2$ as breath carbon dioxide excretion. The recovery of $^{13}CO2$ from breath cannot measure what is happening at each stage of this process, only that the process is taking place. Recovery of label on breath cannot be used as a direct measure of absorption of the fatty acid from the gut. Previous data, obtained from healthy 20 to 30 year old males showed the excretion of ^{13}C label in stool following the administration of [1- ^{13}C] palmitic acid in emulsion was 36.4 $\pm$ 9.9% of the administered dose [Jones et al, 1998]. However, it is questionable whether this measurement can be translated to the HIV+ subject group, particularly when the literature

reports reduced gut function and absorption in HIV infection [Sharpstone et al. 1999]. In order to accurately assess the absorption of the label from the gut, stool losses should be measured by isolating lipid from the stool and measuring the enrichment within this lipid. This was not undertaken during this trial, due to the lack of facilities to collect and process stool from HIV infected individuals.

While fasting plasma TAG concentration did not change, there was a trend towards an increased magnitude of TAG excursion in the postprandial period. In addition, there was a significant increase in enrichment within the TAG fraction in the early postprandial period, suggesting a delay in the clearance of dietary lipid from the circulation in the first few hours after the meal. There were no significant differences in plasma NEFA concentration or the enrichment within this fraction following HAART.

Following three months of HAART, there was an increase in plasma insulin concentration in the late postprandial period, and a reduction in glucose concentration at 4 hours after the meal. While this may indicate alterations in insulin sensitivity, this conclusion cannot be reached without measuring sensitivity using methods such as the hyperinsulinaemic, euglycaemic clamp or the oral glucose tolerance test.

6.6 Summary

The aim of this intervention trial was to determine if, using fatty acid tracers, changes in the handling of dietary fatty acids were observed in HIV+ therapy naïve males following 3 months of HAART including a PI, in order to determine if a reduced clearance of dietary lipid occurs with short-term HAART use. The results demonstrate that:

1) Rapid reductions in viral load and increases in CD4 count are observed with three months HAART use including at least one PI and patients gain fat mass during this period, although the reason for this is unclear.

2) There is an increase in postprandial carbohydrate oxidation, although this may be due to changes in diet preceding the study day.

3) While there are no changes in fasting plasma TAG or NEFA concentrations, enrichment within the TAG fraction suggests there is a delayed clearance of dietary lipid from the circulation in the early postprandial period following HAART use.

4) There are subtle changes in postprandial plasma glucose and insulin concentrations although it is unclear whether these represent changes in insulin sensitivity following HAART use.

Chapter 7.0 A study to investigate the influence of combination therapy including a protease inhibitor compared with an antiretroviral drug regimen excluding protease inhibitors on postprandial lipid metabolism in lipodystrophic HIV males.

7.1 Introduction

The study presented in chapter 6.0 was designed to investigate the influence of HAART including a PI on postprandial lipid metabolism and to examine the alterations in lipid metabolism that occur during exposure to HAART that may contribute to the development of lipodystrophy. As discussed in that chapter, the study could not be continued past the three month stage due to reasons beyond the investigators control. Therefore, this study was undertaken to examine the influence of longer term HAART use on postprandial lipid metabolism in patients already having developed lipodystrophy.

While the literature reports a stronger association between the development of lipid abnormalities and protease inhibitors (PI) containing HAART than drug regimens that do not include this drug class, the audit data presented in Chapter 4.0 would suggest that exclusive NRTI use results in a higher prevalence of both hypertriglyceridaemia and hyperglycaemia. While there may be confusion over which drug classes or combinations are associated with higher rates of metabolic abnormalities, it does appear that the metabolic response to HAART may vary with the drug class or classes used. It is unclear whether reported differences between these drug classes are the result of a direct independent effect of either drug class or due to the virological response or host immune reconstitution that occurs in response to these drugs and possibly differences in their efficacy. Indeed, the association between hypertriglyceridaemia, lipodystrophy and PI drugs may be temporal, as the discovery of lipodystrophy and the use of PI drugs have both occurred within the last few years. An independent effect of the PI Indinavir has been reported on insulin resistance in healthy, HIV-negative men [Noor et al, 2001]. However, studies of this nature are rare due to the ethical considerations of administering potentially toxic drugs to non-HIV infected subjects. The presence of lipid abnormalities with the exclusive use of NRTI drugs has been reported elsewhere [Saint-Marc et al. 1999], but the extent to which the severity of these lipid abnormalities is related to the presence of a PI or the exclusive use of NRTI therapy within the population studied was unknown.

In this study postprandial lipid metabolism was investigated again using stable isotope labelled fatty acids in two groups of HIV-positive males with lipodystrophy, classified by self-reported changes in body fat distribution. While one group (PI-lipodystrophy group, PIL n=6) had been treated with a regimen including typically two NRTI drugs plus a PI, the other group had never been exposed to any PI drug and had been treated with NRTI drugs only (dual nucleoside lipodystrophy group, DNL n=7).

7.2 Aims

The aim of this study was to examine the influence of NRTI-associated lipodystrophy on the metabolic fate of dietary lipid compared to the PI-lipodystrophy group where lipodystrophy development is reported to be both more prevalent and severe.

7.3 Methods

7.3.1 Subjects

All subjects were recruited at the Chelsea and Westminster Hospital. Both lipodystrophy groups were initially identified by self-reported changes in body shape and elevated fasting plasma TAG was confirmed by the Chemical Pathology Department of the Chelsea and Westminster Hospital from a blood sample taken during the patient's routine examination. Six males met the criteria for the PI-lipodystrophy group (PILa-f). All were Caucasian and had been exposed to PI therapy for approximately 12 months (see tables 7.1 and 7.2 for viral loads, CD4, CD8 counts and therapy details and duration for both groups). Seven males met the criteria for the dual NRTI-lipodystrophy group (DNLa-g). All were Caucasian and had been exposed to NRTI drug therapy for a median 39 months. All subjects were found, by examination, to be asymptomatic at the time of the trial.

7.4 Results

7.4.1 Subject characteristics

The dual nucleoside lipodystrophy patients tended to have a lower viral load (DNL <25 copies/ml, <25-20763; PIL 421 copies/ml, <25-138514;ns) and a higher CD4 count (DNL 441 x10^6 cells/l, 256-939; PIL 243 x10^6 cells/l; ns) compared to the PI-

lipodystrophy group (table 7.1). Table 7.2 shows the therapy at the time of study and previous therapy exposure of both groups with the duration of each combination taken. In the DNL group, the median duration of NRTI use was 39 months (range 24-128 months). In the PIL group, the median duration of PI use was 18.5 months (range 4-27 months) while the median duration of NRTI use in this group was 43 months (range 26-117 months). Only PILe had been exposed to an NNRTI drug and this was for 4 months prior to the study date. With the exception of DNLb, all subjects were of a similar age (31-54 years old, table 7.3). The DN-lipodystrophy group tended to weigh less than the PI-lipodystrophy group (DNL 66 kg, 62.7-73.9; PIL 71.1 kg, 66.3-78.1; ns) and therefore, have a lower body mass index (BMI) (DNL 22.7 kg/m^2, 21.2-23.1; PIL 23.8 kg/m^2, 22.4-25.3; ns). There were three smokers in either group.

7.4.2 Body composition

From BIA (table 7.4), there were no differences between the groups in the absolute or relative amounts of fat and fat-free mass when measured with this technique. There were no significant differences observed in any of the other anthropometric measurements made (table 7.5).

7.4.3 Energy expenditure

Table 7.6 shows the resting energy expenditure (REE) calculated from indirect calorimetry measures of VO_2 and VCO_2 expressed as kJ/day, kJ/kg fat free mass (FFM) and as the increase in energy expenditure over the 7 hour period following the meal, calculated as incremental area under the curve (AUC_i). REE (kJ/d) tended to be lower in the DN-lipodystrophy group than in the PI-lipodystrophy group (DNL 7984 kJ/ d, 7106-8694; PIL 8694 kJ/ d, 7817-8987; ns). However, when the REE was expressed per kilogram of FFM, as measured by BIA to compensate for differences in size, the groups were not different. There was no difference between the groups in the increase in energy expenditure, often referred to as diet-induced thermogenesis, over the 7-hour postprandial period.

7.4.4 Substrate oxidation

There were no differences between the groups in RER or the amount of either lipid or carbohydrate oxidised, expressed as grams/hour, in the postabsorptive phase (table 7.7) prior to the test meal. During the postprandial period, there were no differences in total lipid or carbohydrate oxidation expressed as AUC, AUC_i (table 7.8) or grams per hour (figures 7.1 and 7.2) between the groups. However, the recovery of the label on breath (figure 7.3) tended to be higher in the DN-lipodystrophy group (DNL 11.7 %, 10.2-19.9; PIL 8.3 %, 2.5-14.9; ns) indicating that the DN-lipodystrophy group tended to oxidise more exogenous lipid (table 7.9, figure 7.5) (DNL 5.29 g/7h, 4.2-9.00; PIL 3.75 g/7h, 1.13-6.73; ns) and less endogenous lipid (DNL 27.25 g/7h, 13.80-34.46; PIL 31.22 g/7h, 24.34-33.77; ns) than the PI-lipodystrophy group over the 7-hour period. Endogenous lipid oxidation (g/h, figure 7.4) was significantly less in the DN-lipodystrophy group at 2 hours after the meal (DNL 3.41 g/h, 2.14-5.11; PIL 4.97 g.h, 3.67-5.60; $p<0.04$).

7.4.5 Plasma triacylglycerol concentration

The DN-lipodystrophy group had a lower fasting plasma triacylglycerol (TAG) than the PI-lipodystrophy group (DNL 1.8 mmol/l, 0.9-2.9; PIL 8.5 mmol/l, 4.5-38.5; $p=0.001$). The AUC for plasma TAG following the meal (table 7.10) indicates the magnitude of the postprandial TAG response, which was significantly lower in the DN-lipodystrophy group (DNL 20.5 mmol/l per 7h, 6.5-26.5; PIL 12.1 mmol/l per 7h, 2.9-128.1; $p=0.001$). Plasma TAG was approximately four times lower in the DN-lipodystrophy group compared to the PI-lipodystrophy group at all time points measured after the meal ($p<0.01$; figure 7.6).

7.4.6 Plasma [^{13}C] triacylglycerol concentration

The PI-lipodystrophy group showed approximately four times more enrichment in [^{13}C] palmitic acid within the plasma TAG fraction than the DN-lipodystrophy group ($p<0.02$) at all time points measured after 2.5 hours (figure 7.7). The AUC_i for plasma [^{13}C]-TAG over the 7-hour period was approximately 3 fold higher (table 7.12) in the PI-

lipodystrophy group than in the DN-lipodystrophy group (DNL 84.2 μg/ml per 7h, 33.6-165.3; PIL 281.5 μg/ml per 7h, 143.1-462.3; p=0.002).

7.4.7 Plasma non-esterified fatty acid concentration

The DN-lipodystrophy group had a significantly lower fasting plasma NEFA concentration (DNL 0.35 mmol/l, 0.15-0.48; PIL 0.67 mmol/l, 0.43-1.02; p<0.01; table 7.11) and a significantly lower plasma NEFA concentration was maintained in this group until 2.5 hours after the meal, then dropped again at 6 hours after the meal (figure 7.8). The AUC was also significantly lower in the DN-lipodystrophy group compared to the PI-lipodystrophy group (DNL 0.36 mmol/l per 7h, 0.00-0.96; PIL 2.90 mmol/l per 7h, 2.25-5.13; p<0.04). Both groups showed similar postprandial plasma NEFA pattern with an initial approximate 50% decrease in concentration, however this appeared to take longer to achieve in the PI-lipodystrophy group (4 hours to nadir compared to 1 hour in the DN-lipodystrophy group).

7.4.8 Plasma [^{13}C]-non-esterified fatty acid concentration

The DN-lipodystrophy group had twice as much enrichment in the plasma non-esterified fatty acid (NEFA) fraction following the administration of [1-^{13}C] palmitic acid (figure 7.9) in the early postprandial period (1-2 hours after the meal, p<0.04) compared to the PI-lipodystrophy group. The AUCi for plasma [^{13}C]-NEFA was also higher in the DN-lipodystrophy group (DNL 4.5 μg/ml per 7h, 3.0-5.2; PIL 2.8 μg/ml per 7h, 1.6-4.7; p<0.04).

7.4.9 Plasma insulin and glucose concentrations

While both fasting insulin and glucose and the magnitude of insulin and glucose response where not different between the two groups (table 7.13), the increase in plasma insulin concentration in response to the meal appeared delayed in the DN-lipodystrophy group (figure 7.10). In addition, there was a non-significant trend towards an increased plasma glucose concentration between 1 and 5 hours after the meal in the DN-lipodystrophy group.

Table 7.1 **Viral load, CD4 and CD8 counts of subjects in the PI-Lipodystrophy group (PIL) compared to the Dual nucleoside-lipodystrophy group (DNL). **Undetectable (U) indicates that plasma viral RNA copies were below the limit of detection for the assay of 25 copies/ml.**

SUBJECT CODE	Viral Load (copies/ml plasma)	CD4 (X 10^6 cells/L plasma)	CD8 (X 10^6 cells/L plasma)
PI-LIPODYSTROPHY GROUP			
PILa	798	555	918
PILb	43	330	771
PILc	138514	152	587
PILd	U**	382	2355
PILe	20	156	941
PILf	8619	121	1687
Median (range)	421 (<25-138514)	243 (121-555)	930 (587-2355)
NRTI-LIPODYSTROPHY GROUP			
DNLa	766	939	1434
DNLb	U**	466	994
DNLc	U**	583	681
DNLd	20763	441	1544
DNLe	U**	256	482
DNLf	937	441	1544
DNLg	U**	360	655
Median (range)	<25 (<25-20763)	441 (256-939)	994 (482-1544)

Table 7.2 **Therapy details at the time of study and previous therapy exposure of the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	Combination at time of study	Duration (months)	Previous therapy exposure	Duration (months)
PI-LIPODYSTROPHY GROUP				
PILa	d4T SQV RTV	4	ZDV ddC	22
PILb	3TC d4T RTV	18	AZT ddC	25
PILc	RTV SQV d4T	6	3TC d4T	13
			3TC AZT SQV	4
			ddC AZT	7
			AZT	19
PILd	RTV SQV d4T	12	3TC d4T SQV	7
	3TC		d4T ddI	6
			ddI	4
			AZT	14
PILe	IND 3TC d4T	19	SQV 3TC AZT	2
			ddI 3TC SQV AZT	2
			3TC NVP ddC	4
			AZT	5
			3TC ddC AZT	3
			AZT ddC	3
PILf	d4T RTV SQV	15	IND 3TC d4T	12
	NVP		AZT 3TC	13
			ddI	30
			AZT	47
NRTI-LIPODYSTROPHY GROUP				
DNLa	ddI d4T	26	NONE	
DNLb	ddI AZT	96	NONE	
DNLc	d4T ddI	39	NONE	
DNLd	3TC d4T	32	NONE	
DNLe	ddI d4T	40	DDI AZT	1
DNLf	3TC AZT	72	AZT	56
DNLg	ddI d4T	24	NONE	

Abbreviations:

d4T	**Stavudine (NRTI)**	**3TC**	**Lamivudine (NRTI)**
SQV	**Saquinavir (PI)**	**IND**	**Indinavir (PI)**
RTV	**Ritonavir (PI)**	**ddI**	**Didanosine (NRTI)**
NVP	**Nevirapine (NNRTI)**	**AZT**	**Zidovudine (NRTI)**
ddC	**Zalcitabine (NRTI)**		

Table 7.3 **Characteristics of subjects in both the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL) including age, height, weight, body mass index (BMI) and whether they smoked.**

SUBJECT CODE	AGE (Yrs)	HEIGHT (cm)	WEIGHT (kg)	BMI (kg/m^2)	SMOKER
PI-LIPODYSTROPHY GROUP					
PILa	40	169	67.0	23.5	YES
PILb	36	178	78.1	24.6	YES
PILc	35	178	71.0	22.4	NO
PILd	44	175	77.4	25.3	NO
PILe	50	172	71.1	24.0	YES
PILf	54	168	66.3	23.5	NO
Median (range)	42 (35-54)	173.5 (168-178)	71.1 (66.3-78.1)	23.8 (22.4-25.3)	
NRTI-LIPODYSTROPHY GROUP					
DNLa	31	168	65.1	23.1	NO
DNLb	67	172	67.2	22.7	NO
DNLc	47	175	66.0	21.2	YES
DNLd	49	178	71.9	22.7	YES
DNLe	53	172	62.7	21.2	NO
DNLf	43	171	65.1	22.3	NO
DNLg	43	172	73.9	25.0	YES
Median (range)	49 (31-67)	172 (168-178)	66 (62.7-73.9)	22.7 (21.2-23.1)	

Table 7.4 **Results of the bioelectrical impedance analysis (BIA) for fat mass (kg) and fat free mass (FFM) (kg), and fat and fat free mass as a percentage of total body weight in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	FAT (kg)	FFM (kg)	FAT (%)	FFM (%)
PI-LIPODYSTROPHY GROUP				
PILa	9.2	57.8	13.7	86.3
PILb	10.5	67.6	13.4	86.6
PILc	8.1	62.9	11.4	88.6
PILd	11.1	66.3	14.3	85.7
PILe	13.9	57.2	19.5	80.5
PILf	12.3	54.0	18.6	81.4
Median (range)	10.8 (8.1-13.9)	60.4 (54.0-67.6)	14.0 (11.4-19.5)	86.0 (80.5-88.6)
NRTI-LIPODYSTROPHY GROUP				
DNLa	4.2	60.9	6.5	93.5
DNLb	15.1	52.1	22.5	77.5
DNLc	11.6	54.5	17.5	82.5
DNLd	10.7	61.2	14.9	85.1
DNLe	10.6	52.1	16.9	83.1
DNLf	11.1	54.0	17.1	82.9
DNLg	8.9	65.0	12.0	88.0
Median (range)	11.2 (4.2-15.1)	54.8 (52.1-65.0)	16.9 (6.5-22.5)	83.1 (77.5-93.5)

Table 7.5 **Results of anthropometric measures of waist circumference, hip circumference, waist to hip ratio, triceps skinfold, mid upper arm circumference (MUAC) and mid upper arm muscle circumference (MUAMC) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	WAIST (mm)	HIP (mm)	WAIST: HIP RATIO	TRICEP SKIN FOLD (mm)	MUAC (mm)	MUAMC (mm)
PI-LIPODYSTROPHY GROUP						
PILa	835	949	0.88	9.4	297	267
PILb	895	996	0.90	5.1	310	294
PILc	832	980	0.85	5.1	291	275
PILd	889	1029	0.86	6.1	284	265
PILe	850	980	0.87	7.0	283	261
PILf	905	930	0.97	5.2	280	264
Median (range)	869.5 (832-905)	980 (930-1029)	0.87 (0.85-0.97)	5.7 (5.1-9.4)	287.5 (280-310)	266 (261-294)
NRTI-LIPODYSTROPHY GROUP						
DNLa	778	930	0.84	2.4	322	314
DNLb	990	930	1.06	7.6	258	234
DNLc	905	956	0.95	10.4	289	256
DNLd	888	976	0.91	6.6	304	283
DNLe	888	937	0.95	5.1	284	268
DNLf	849	1010	0.84	7.1	292	269
DNLg	810	1010	0.80	3.0	328	318
Median (range)	888 (778-990)	937 (930-1010)	0.95 (0.80-1.06)	6.6 (2.4-10.4)	289 (258-328)	268 (234-318)

Table 7.6 **Resting energy expenditure (REE) measured by indirect calorimetry expressed as kilojoules per day (kJ/day) and as kilojoules per kilogram of fat free mass (kJ/kg FFM), and energy expenditure over the 7-hour postprandial study period calculated by area under the curve in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL). Fat free mass was measured using bioelectrical impedance analysis.**

SUBJECT CODE	REE (kJ/d)	REE (kJ/kg FFM/d)	EE (kJ/7h) (AUC_i)
PI-LIPODYSTROPHY GROUP			
PILa	7817	135	336
PILb	8611	127	409
PILc	8903	142	351
PILd	8778	132	303
PILe	8444	148	174
PILf	8987	166	362
Median (range)	8694 (7817-8987)	138 (127-166)	344 (174-409)
NRTI-LIPODYSTROPHY GROUP			
DNLa	8694	143	311
DNLb	7106	136	309
DNLc	7984	149	437
DNLd	8485	139	235
DNLe	7608	92	376
DNLf	7691	142	315
DNLg	8485	131	567
Median (range)	7984 (7106-8694)	139 (92-149)	315 (235-567)

Table 7.7 **Respiratory exchange ratio (RER) and postabsorptive substrate oxidation calculated from VCO_2 and VO_2 measured by indirect calorimetry. Lipid and carbohydrate oxidation is expressed as grams per hour (g/h) prior to the administration of the test meal and emulsion in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	RER 0h	LIPID OXIDATION (g/h) 0h	CHO OXIDATION (g/h) 0h
PI-LIPODYSTROPHY GROUP			
PILa	0.82	3.84	7.05
PILb	0.84	3.73	9.33
PILc	0.80	5.04	6.74
PILd	0.86	3.21	11.09
PILe	0.78	5.64	4.06
PILf	0.84	3.94	9.75
Median (range)	0.83 (0.78-0.86)	3.89 (3.21-5.64)	8.19 (4.06-11.09)
NRTI-LIPODYSTROPHY GROUP			
DNLa	0.81	4.64	7.27
DNLb	0.77	4.64	3.01
DNLc	0.79	4.74	5.15
DNLd	0.86	3.03	10.76
DNLe	0.89	1.83	11.54
DNLf	0.90	1.53	12.44
DNLg	0.83	4.04	8.27
Median (range)	0.83 (0.77-0.90)	4.04 (1.53-4.74)	8.27 (3.01-12.44)

Table 7.8 **Lipid and carbohydrate oxidation over the 7 hour period following administration of the emulsion and test meal, expressed as grams per 7 hours (g/7h), calculated by area under the curve (AUC) and also expressed as the incremental area under the curve (AUCi), showing the change from baseline in the postprandial period in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	LIPID OXIDATION AUC (g/7h)	CHO OXIDATION AUC (g/7h)	LIPID OXIDATION AUC_i (g/7h)	CHO OXIDATION AUC_i (g/7h)
PI-LIPODYSTROPHY GROUP				
PILa	28.2	66.8	3.8	19.5
PILb	34.5	69.6	8.4	7.7
PILc	40.5	56.5	5.3	9.4
PILd	33.3	69.9	10.8	0.0
PILe	37.7	42.9	1.1	16.8
PILf	31.3	82.0	6.1	18.9
Median (range)	33.9 (28.2-40.5)	68.2 (42.9-82.0)	5.7 (1.1-10.8)	13.1 (0.0-19.5)
NRTI-LIPODYSTROPHY GROUP				
DNLa	31.9	72.1	2.8	24.8
DNLb	39.4	23.7	6.9	7.6
DNLc	31.0	69.2	1.1	33.2
DNLd	37.8	48.4	16.5	0.0
DNLe	19.5	87.3	7.1	13.6
DNLf	35.4	44.4	24.7	0.0
DNLg	33.9	79.6	5.6	23.4
Median (range)	33.9 (19.5-39.4)	69.2 (23.7-87.3)	6.9 (1.1-24.7)	13.6 (0.0-33.2)

Table 7.9 **The calculation of the endogenous and exogenous sources of lipid oxidised over the 7 hour period (g/7h) using the percentage of ^{13}C label recovered on breath over 7 hours, together with the amount of lipid in the test meal and the total lipid oxidised over the 7 hour period, calculated from the area under the curve (AUC) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	% Administered ^{13}C Dose Excreted on Breath over 7 h	Lipid (g) in Test meal	TOTAL LIPID OX. (AUC) (g/7 h)	EXOGENOUS LIPID OX. (g/7 h)	ENDOGENOUS LIPID OX. (g/7 h)
PI-LIPODYSTROPHY GROUP					
PILa	8.54	45.25	28.2	3.86	24.34
PILb	2.49	45.25	34.5	1.13	33.37
PILc	14.88	45.25	40.5	6.73	33.77
PILd	6.74	45.25	33.3	3.05	30.25
PILe	12.19	45.25	37.7	5.52	32.18
PILf	8.02	45.25	31.3	3.63	27.67
Median (range)	8.28 (2.49-14.88)	45.25	33.9 (28.2-40.5)	3.75 (1.13-6.73)	31.22 (24.34-33.77)
NRTI-LIPODYSTROPHY GROUP					
DNLa	10.20	45.25	31.9	4.62	27.28
DNLb	10.70	45.25	39.4	4.84	34.56
DNLc	10.70	45.25	31.0	4.84	26.16
DNLd	11.70	45.25	37.8	5.29	32.51
DNLe	12.60	45.25	19.5	5.70	13.80
DNLf	19.90	45.25	35.4	9.00	26.40
DNLg	14.70	45.25	33.9	6.65	27.25
Median (range)	11.70 (10.20-19.90)	45.25	33.9 (19.5-39.4)	5.29 (4.62-9.00)	27.25 (13.80-34.56)

Table 7.10 **Fasting plasma triacylglycerol (TAG) expressed as mmol/litre, in the 7 hours following the meal (AUC) and the change in plasma TAG from fasting following administration of the test meal and emulsion, calculated as the incremental area under the curve (AUC_i) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL). *Statistically significantly different from the PIL group using the Mann Whitney test.**

SUBJECT CODE	FASTING TAG (mmol/l)	POSTPRANDIAL PLASMA TAG AUC (mmol/l/7h)	POSTPRANDIAL TAG RESPONSE AUC_i (mmol/l over 7h)
PI-LIPODYSTROPHY GROUP			
PILa	6.3	46.5	2.9
PILb	10.6	77.7	4.0
PILc	4.5	38.3	6.8
PILd	16.6	244.1	128.1
PILe	6.1	59.6	17.3
PILf	38.5	288.7	33.8
Median (range)	8.5 (4.5-38.5)	68.6 (38.3-288.7)	12.1 (2.9-128.1)
NRTI-LIPODYSTROPHY GROUP			
DNLa	2.94	21.26	1.22
DNLb	2.49	20.50	3.23
DNLc	2.75	21.13	3.19
DNLd	1.06	10.41	3.04
DNLe	1.13	14.02	6.20
DNLf	0.86	6.50	0.94
DNLg	1.83	26.54	13.71
Median (range)	1.83* (0.86-2.94)	20.50* (6.50-26.54)	3.19 (0.94-6.20)

*** p=0.001**

Table 7.11 **Plasma non-esterified fatty acid (NEFA) concentration expressed as mmol/litre before the meal (fasting), in the 7 hours following the meal (AUC) and the change in plasma NEFA from fasting following administration of the test meal and emulsion, calculated as the incremental area under the curve (AUC_i) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL). *Statistically significantly different from the PIL group using the Mann Whitney test.**

SUBJECT CODE	FASTING NEFA (mmol/l)	POSTPRANDIAL PLASMA NEFA AUC (mmol/l/7h)	POSTPRANDIAL NEFA RESPONSE AUC_i (mmol/l over 7h)
PI-LIPODYSTROPHY GROUP			
PILa	0.94	5.13	0.51
PILb	0.60	2.25	0.00
PILc	0.51	2.61	0.09
PILd	0.73	3.19	0.00
PILe	0.43	2.40	0.20
PILf	1.02	5.13	0.00
Median (range)	0.67 (0.43-1.02)	2.90 (2.25-5.13)	0.05 (0.00-0.51)
NRTI-LIPODYSTROPHY GROUP			
DNLa	0.35	0.12	0.03
DNLb	0.48	0.00	0.02
DNLc	0.41	0.01	0.04
DNLd	0.15	0.96	0.01
DNLe	0.19	0.71	0.01
DNLf	0.45	0.36	0.03
DNLg	0.24	0.59	0.02
Median (range)	0.35* (0.15-0.48)	0.36* (0.00-0.96)	0.02 (0.01-0.04)

*** $p<0.01$**

Table 7.12 **Plasma [^{13}C] palmitic acid within triacylglycerol (^{13}C-TAG) and within non-esterified fatty acid (^{13}C-NEFA) expressed as μg/ml following administration of the emulsion containing 700 mg [1-^{13}C] palmitic acid and test meal, calculated as the incremental area under the curve (AUC_i) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL). *Statistically significantly different from the PIL group using the Mann Whitney test.**

SUBJECT CODE	[^{13}C]-TAG (μg/ml/7h)	[^{13}C]-NEFA (μg/ml/7h)
PI-LIPODYSTROPHY GROUP		
PILa	143.1	4.7
PILb	271.9	1.8
PILc	178.3	3.1
PILd	331.1	1.6
PILe	291.1	2.9
PILf	462.3	2.6
Median (range)	281.5 (143.1-462.3)	2.8 (1.6-4.7)
NRTI-LIPODYSTROPHY GROUP		
DNLa	95.9	3.0
DNLb	84.2	3.0
DNLc	69.4	3.5
DNLd	56.4	4.8
DNLe	139.6	5.2
DNLf	33.6	4.8
DNLg	165.3	4.5
Median (range)	84.2* (33.6-165.3)	4.5* (3.0-5.2)

*** $p<0.04$**

Table 7.13 **Fasting and postprandial plasma insulin and glucose concentrations calculated as the area under the curve from zero (AUC_0) and incremental area under the curve (AUC_i) in the PI-Lipodystrophy group (PIL) and the Dual nucleoside-lipodystrophy group (DNL).**

SUBJECT CODE	FASTING PLASMA INSULIN (mU/l)	PLASMA INSULIN AUC (mU/l/7h)	PLASMA INSULIN AUC_i (mU/l/7h)	FASTING PLASMA GLUCOSE (mmol/l)	PLASMA GLUCOSE AUC (mmol/l/7h)	PLASMA GLUCOSE AUC_i (mmol/l/7h)
PI-LIPODYSTROPHY GROUP						
PILa	8.1	181	129	4.1	34.0	5.3
PILb	6.0	146	104	4.7	34.8	2.4
PILc	4.5	119	87	5.4	40.8	3.8
PILd	26.2	400	218	7.0	57.1	10.8
PILe	14.1	172	78	4.8	37.3	4.1
PILf	3.1	384	362	4.8	39.1	6.4
Median (range)	7.0 (3.1-26.2)	177 (119-400)	117 (78-362)	4.8 (4.1-7.0)	38.2 (34.0-57.1)	4.7 (2.4-10.8)
NRTI-LIPODYSTROPHY GROUP						
DNLa	3.8	192	166	4.7	38.0	11.2
DNLb	12.9	458	368	6.3	65.1	28.8
DNLc	10.2	225	156	5.1	43.7	9.6
DNLd	5.3	119	92	4.9	40.0	6.6
DNLe	12.2	235	155	5.3	44.6	9.2
DNLf	13.8	468	371	6.1	67.8	31.5
DNLg	2.3	36	29	4.8	32.5	2.9
Median (range)	10.2 (2.3-13.8)	225 (36-468)	156 (29-371)	5.1 (4.7-6.3)	43.7 (32.5-67.8)	9.6 (2.9-31.5)

Figure 7.1 **Median carbohydrate oxidation during the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. Data is presented as medians and the error bars show minimum and maximum values (range).**

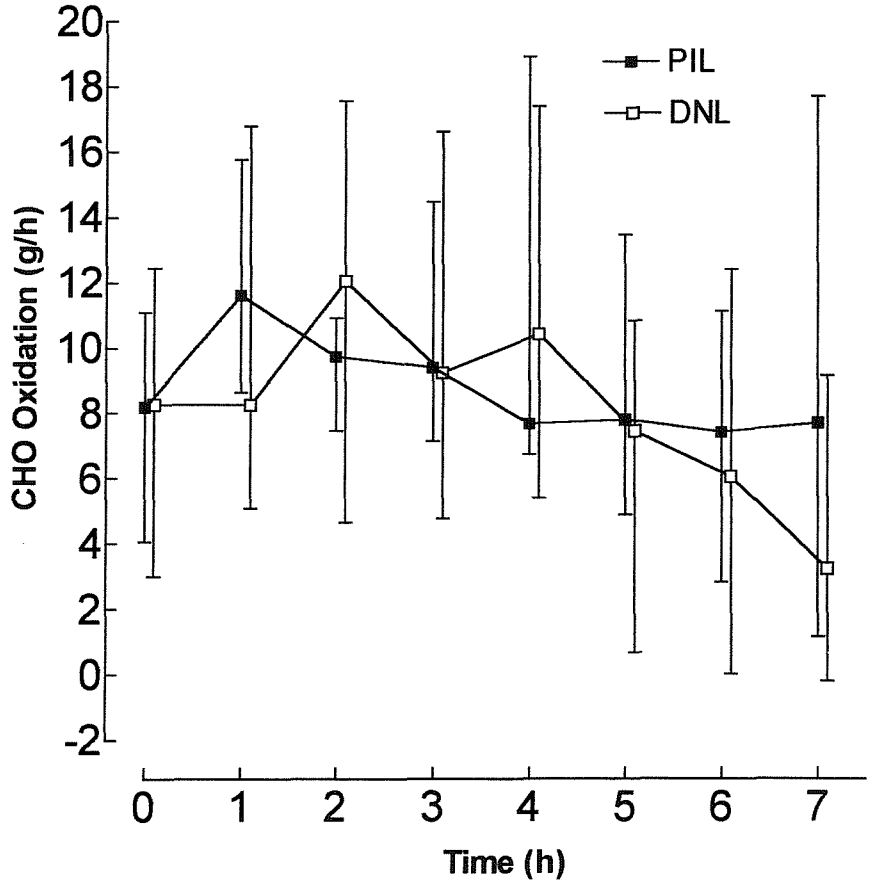


Figure 7.2 **Median lipid oxidation during the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups.**

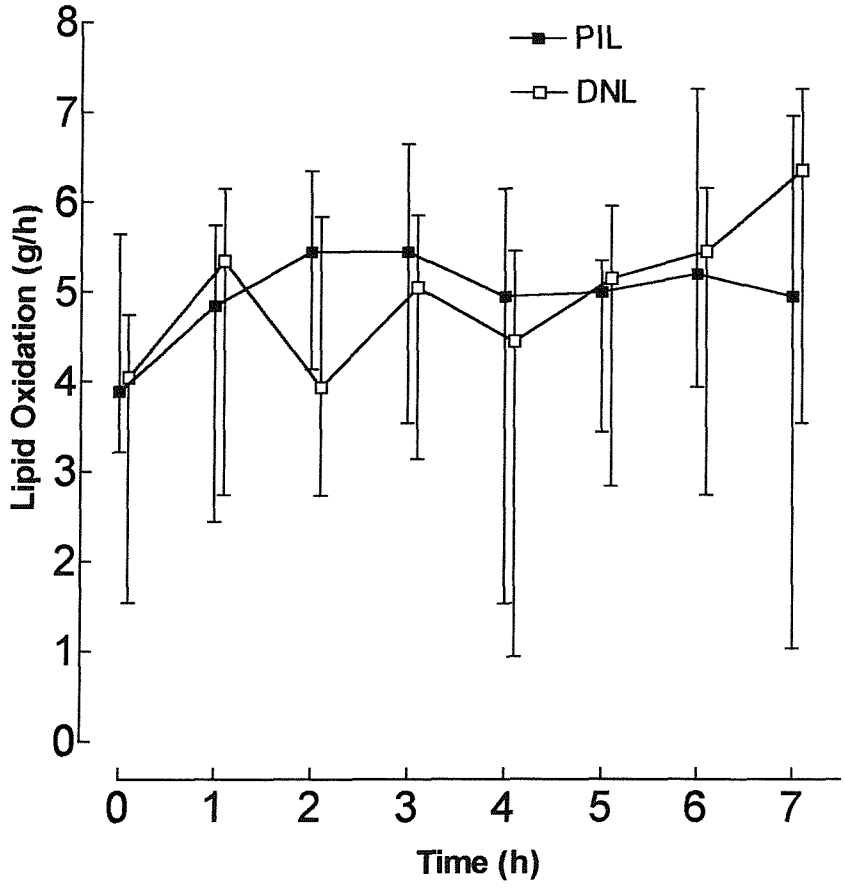


Figure 7.3 **Recovery of the label on breath as 13CO2 over the 7-hour period following administration of the emulsion and test meal, expressed as a percentage of the administered dose for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups.**

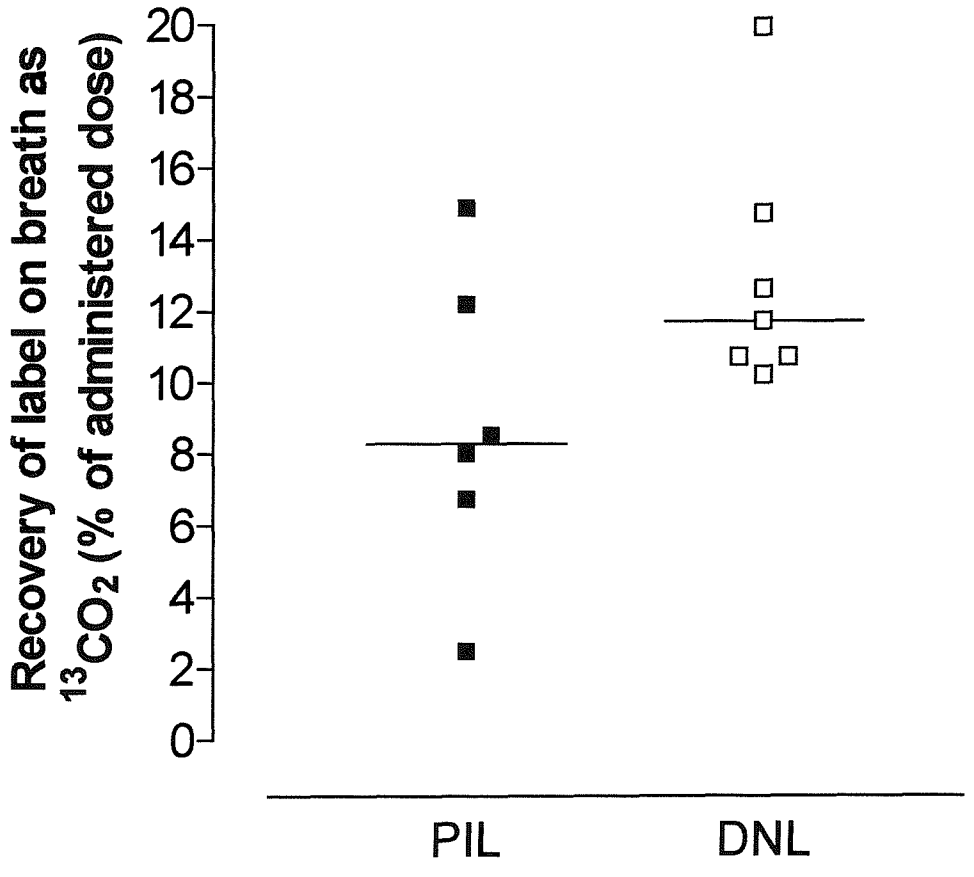
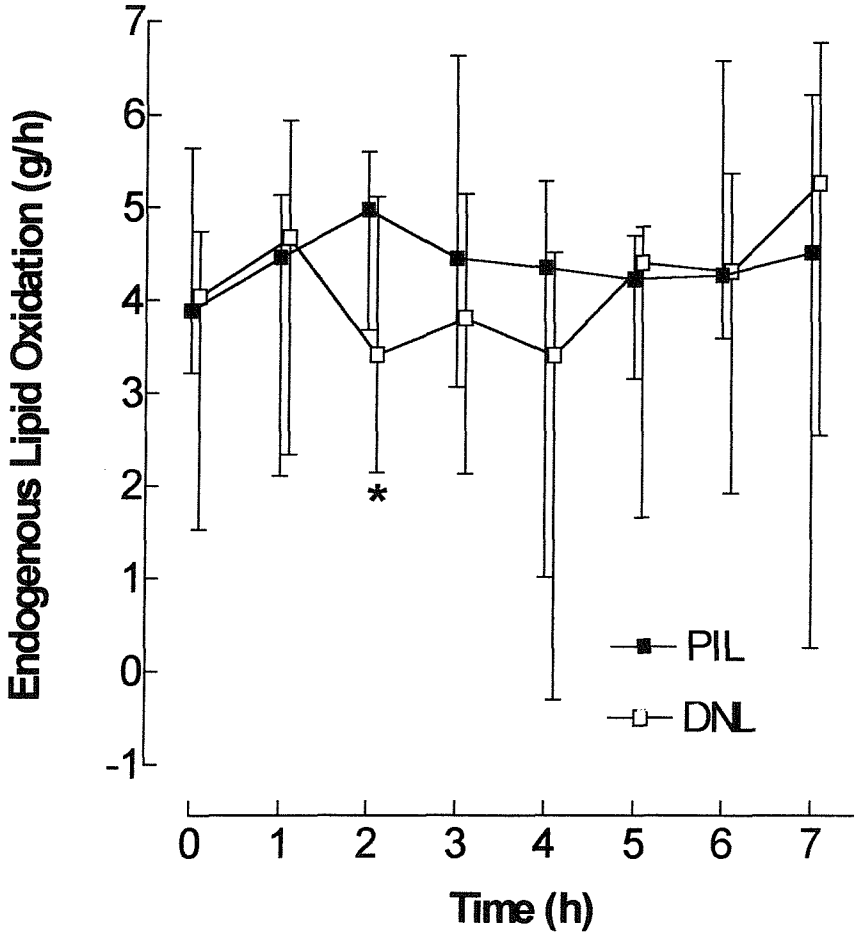


Figure 7.4 **Endogenous lipid oxidation over the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. *Statistically significantly different from the PI-lipodystrophy group using the Mann Whitney Test.**



*p<0.04

Figure 7.5 **Exogenous lipid oxidation over the 7-hour period following administration of the emulsion and test meal, expressed as grams per hour (g/h) for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups.**

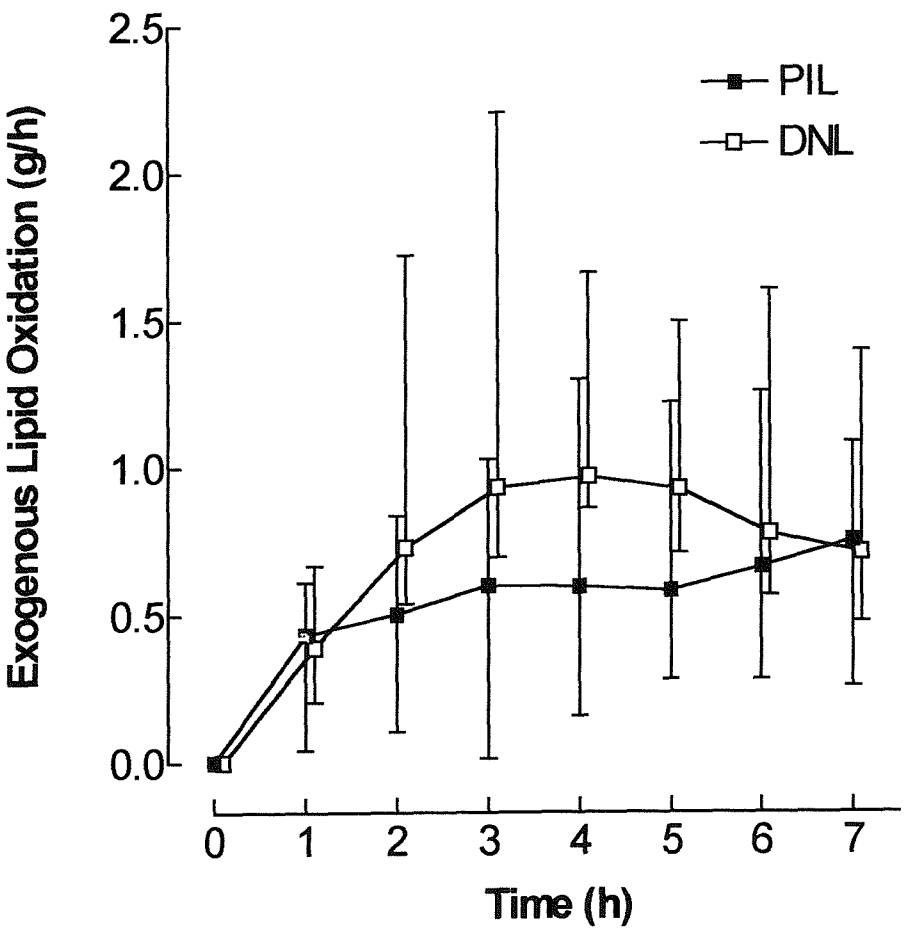
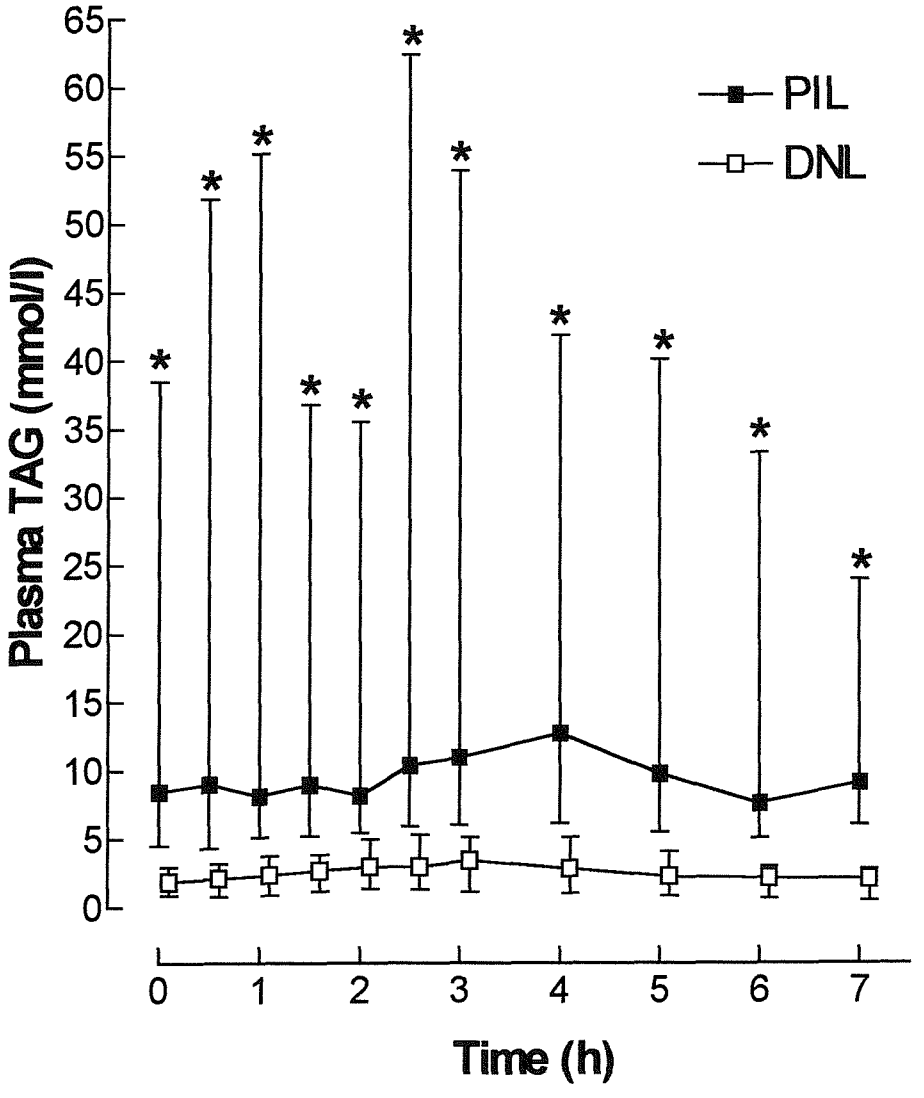
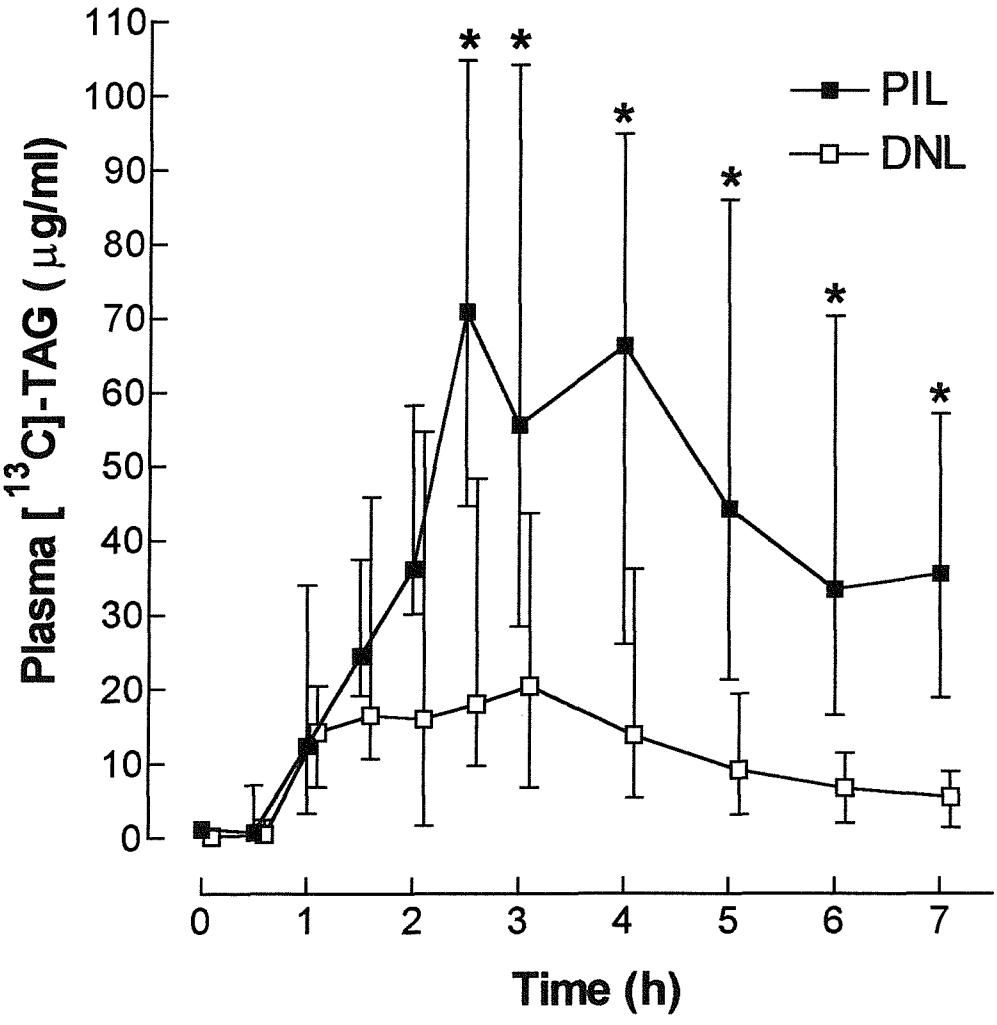


Figure 7.6 **Plasma triacylglycerol concentrations (mmol/l) during the 7-hour period following administration of the emulsion and test meal, for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. * Statistically significantly different from the PI-lipodystrophy group using the Mann Whitney Test.**



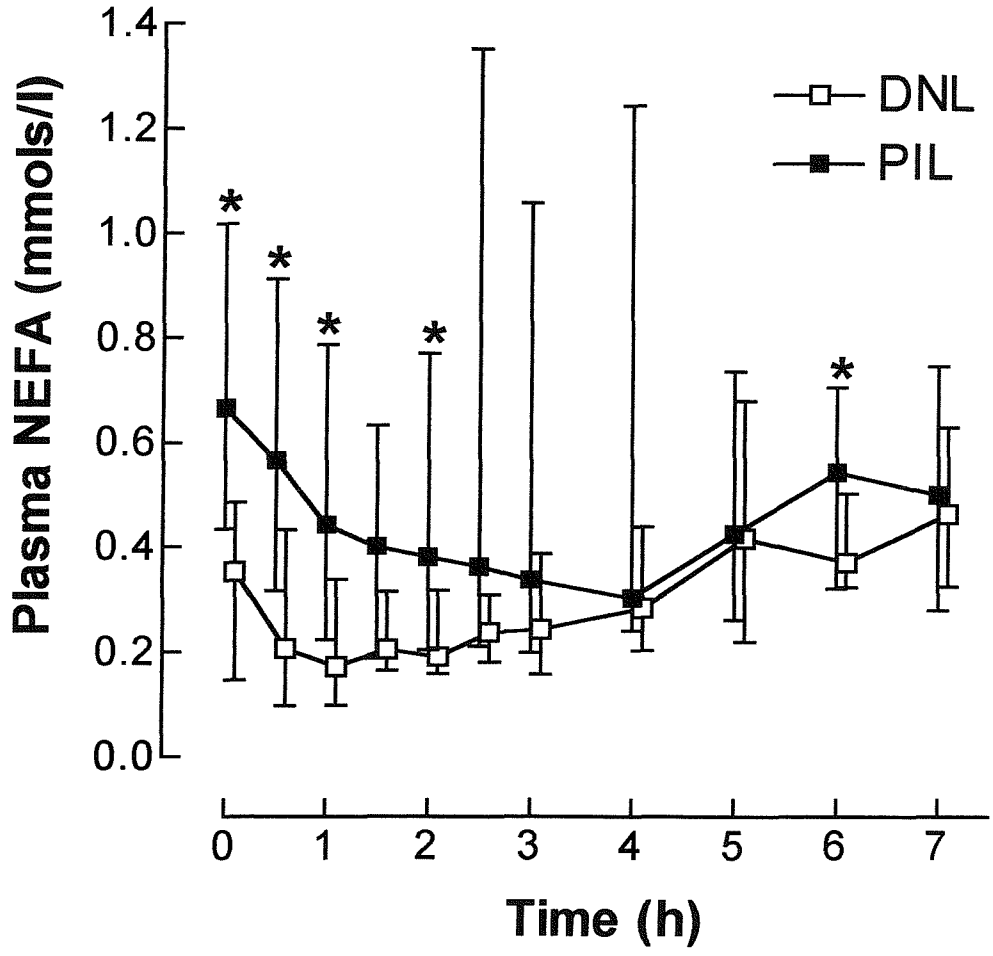
*p<0.01

Figure 7.7 **Plasma [^{13}C] -triacylglycerol concentrations (μg/ml) during the 7-hour period following administration of the emulsion and test meal, for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. *Statistically significantly different from the PI-lipodystrophy group using the Mann Whitney Test.**



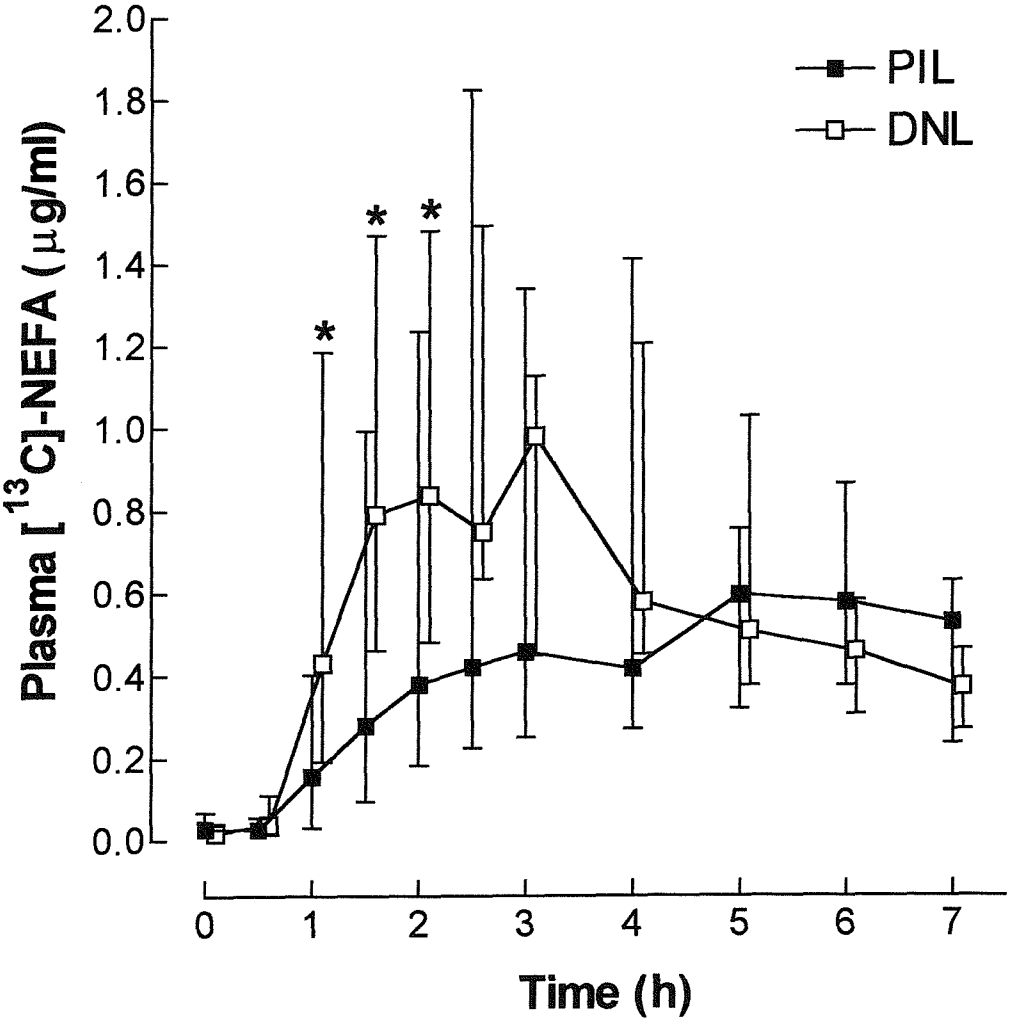
*p<0.04

Figure 7.8 **Plasma non-esterified fatty acid (NEFA) concentration (mmol/l) during the 7-hour period following administration of the emulsion and test meal, for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. *Statistically significantly different from the PI-lipodystrophy group using the Mann Whitney Test.**



*p<0.04

Figure 7.9 **Plasma [13C]-non-esterified fatty acid (NEFA) concentrations (μg/ml) during the 7-hour period following administration of the emulsion containing 700mg [1-13C] palmitic acid and test meal, for the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups. *Statistically significantly different from the PI-lipodystrophy group using the Mann Whitney Test.**



*p<0.04

Figure 7.10 **The change in plasma insulin concentration (mU/l) following administration of the test meal and emulsion in the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups.**

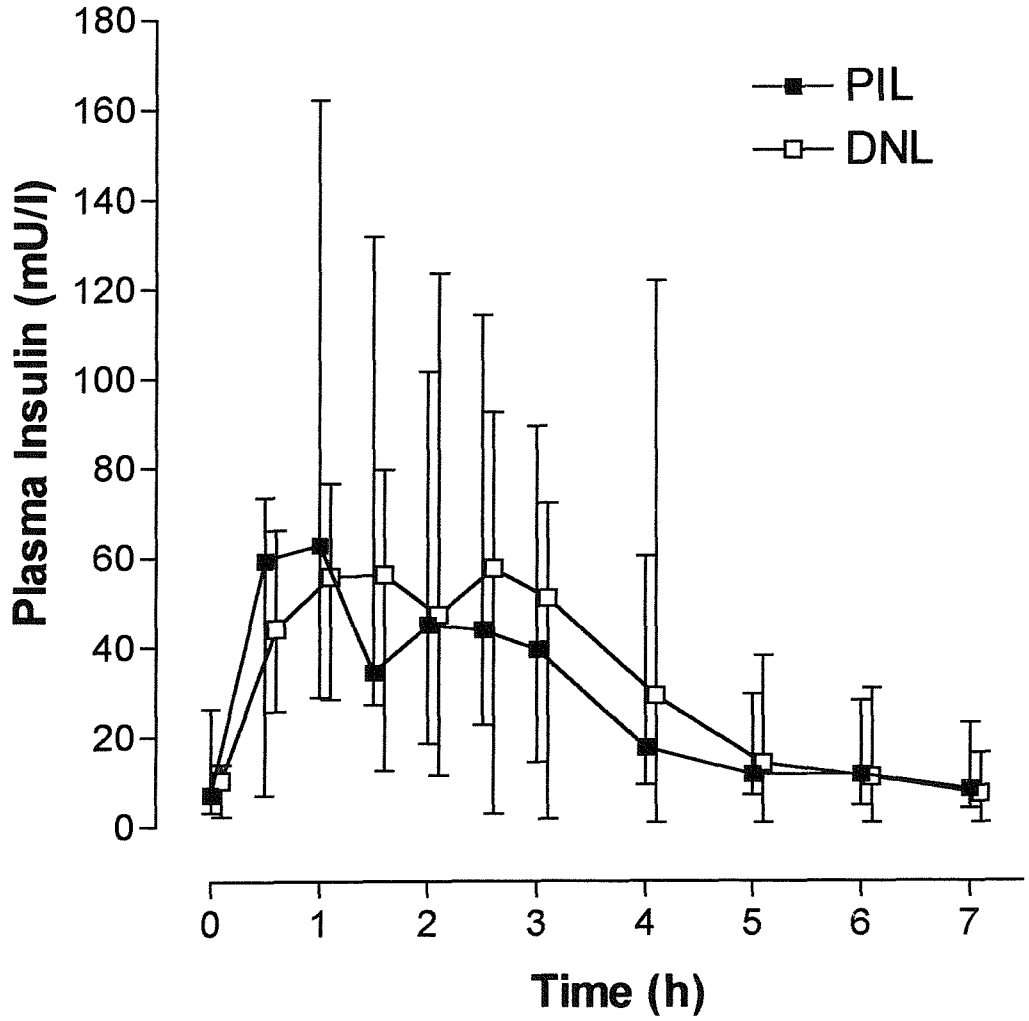
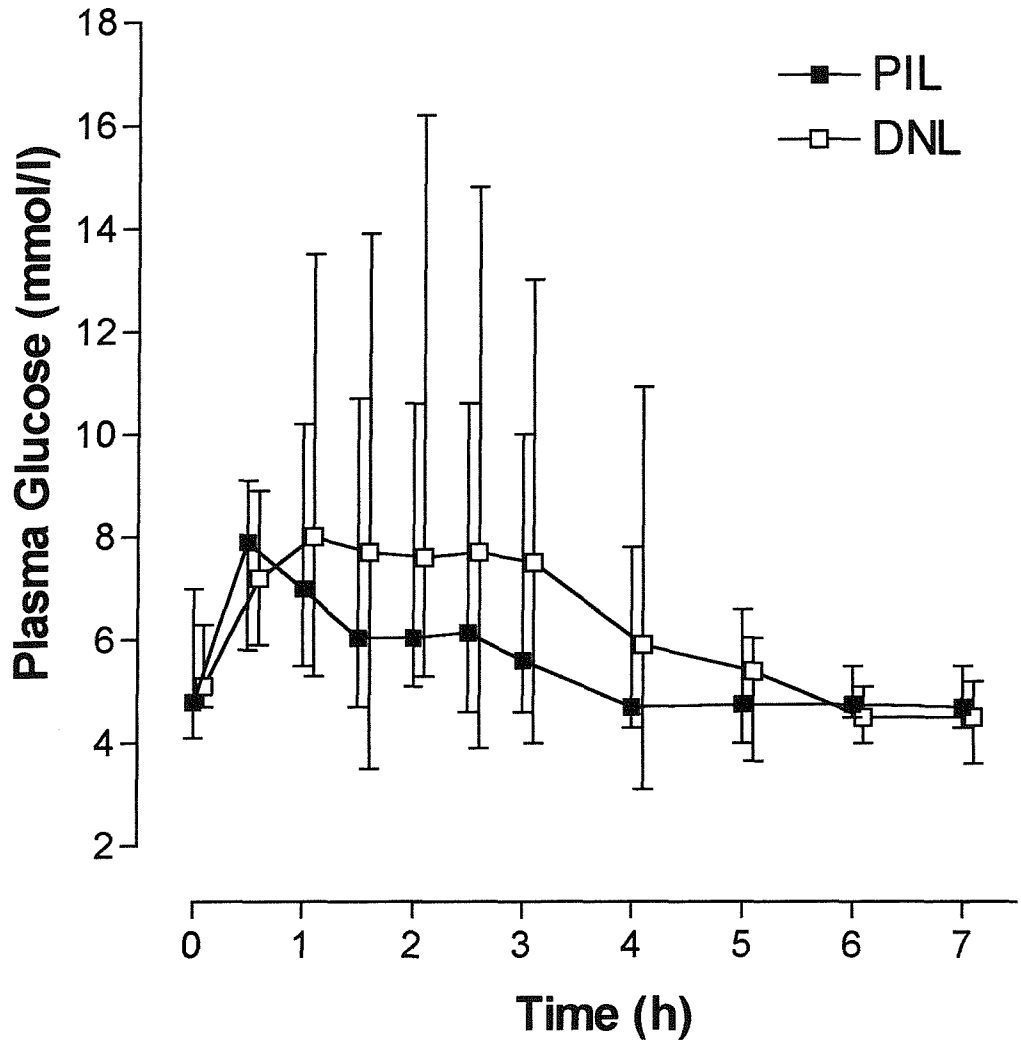


Figure 7.11 **The change in plasma glucose concentration (mmol/l) following administration of the test meal and emulsion in the PI-lipodystrophy (PIL) and DN-lipodystrophy (DNL) groups.**



7.5 Discussion

In this study, stable isotopes tracers were used to investigate postprandial lipid metabolism in HIV-positive patients with lipodystrophy. This chapter was aimed at investigating both the alterations in lipid metabolism that occur with HAART use of more than three months and the differences in postprandial lipid metabolism in HIV-positive patients who have developed lipodystrophy whilst taking NRTI's alone or an NRTI and PI combination.

7.5.1 Subject characteristics and body composition

The dual NRTI-lipodystrophy group, tended to maintain a lower viral load and higher CD4 count than the PI-lipodystrophy group, while the PI-lipodystrophy group tend to have a more complicated history of therapy. The median exposure of PI drugs in this group was 18.5 months. In both groups, the median exposure to NRTI drugs was approximately 40 months. This may emphasize the point that the association between PI drugs and lipodystrophy is merely temporal. The introduction of a PI may also be a marker for a more complicated disease progression, indicating that, unlike in the group exclusively treated with NRTI's, this approach was not sufficient to maintain viral suppression.

BMI tended to be lower in the dual NRTI-lipodystrophy group, although this was not significant. This does not fit with the suggestion that BMI and weight loss may be related to the degree of viral burden. There were no differences between the groups in any of the anthropometric measures made or in the BIA data. However, this was estimated based on the equations employed by the Bodystat 500 analyser using the measurement of impedance through tissues. Impedance, as previously described in Chapter 3.0, is defined by the equation: $Z^2 = R^2 + X_C^2$. Where Z is impedance, R is resistance and X_C is reactance. The values measured by BIA for resistance and reactance (capacitance) are based on a series circuit containing a resistor (extracellular water) and capacitor (the lipid component of the membranes in the body cell mass). Kotler et al (1996) developed equations based on the body as a parallel circuit of resistors and capacitors and validated these equations in both healthy and HIV infected individuals of varied race and gender. The equations based on the parallel circuit were validated against techniques such as hydrodensitometry and total body potassium and were found to be better predictors of FFM and total body water in both healthy and HIV infected subjects. Therefore, the BIA

data presented here was based on equations that have not been validated for use in HIV infected groups and may need evaluating for use within this group [Schwenk et al, 1999]. The trend towards a lower percentage body fat and higher percentage FFM in the lipodystrophy group, estimated using BIA, does agree with the lower triceps skinfold and significantly higher mid upper arm muscle circumference observed in this group. However, as the lipodystrophy group reported an altered distribution of body fat, the subcutaneous adipose tissue at the triceps site cannot be taken as an indicator of the whole body percentage fat mass.

7.5.2 Energy expenditure & substrate oxidation

Resting energy expenditure tended to be higher in the PI-lipodystrophy group, but this did not remain elevated when expressed per kilogram of FFM. However, as discussed previously, FFM was measured using BIA and possibly inappropriate equations for this population. The dual NRTI-lipodystrophy group appeared to oxidise more grams of dietary lipid in the postprandial period in comparison the PI-lipodystrophy group. This may have been an artefact related to preceding diet or the dual NRTI-lipodystrophy group could have absorbed more labelled fatty acids from the meal, although exclusion criteria for both groups included the presence of diarrhoea.

7.5.3 Plasma triacylglycerol and non-esterified fatty acid concentration and [^{13}C] enrichment

The PI-lipodystrophy group showed an approximate four-fold higher plasma TAG concentration in both the postabsorptive and postprandial periods compared to the dual NRTI-lipodystrophy group. While both groups were recruited on the basis of having elevated plasma TAG concentration, it appears that the addition of a PI to an NRTI treatment regimen results in a marked increase in plasma TAG both in the postabsorptive and postprandial periods. The increased retention of the label within the plasma TAG fraction following the meal observed in the PI-lipodystrophy group was not present in the dual NRTI-lipodystrophy group. This would suggest the reduced or delayed clearance of dietary TAG from the circulation observed in the PI-lipodystrophy group does not occur to the same extent within the dual NRTI-lipodystrophy, assuming that the absorption of label from the gut is similar.

Plasma NEFA concentration in both the postabsorptive and early postprandial periods was elevated in the PI-lipodystrophy group in comparison to the dual NRTI-lipodystrophy group. In addition, the time taken to reach plasma NEFA nadir in the postprandial period was prolonged in the PI-lipodystrophy group and may be indicative of the insulin resistance reported to occur with PI use in the absence of infection, although insulin sensitivity was not assessed in these subjects. However, the apparent delayed insulin response to the meal would result in a slower suppression of plasma NEFA concentration in the early postprandial period.

Palmitic acid enrichment within the plasma NEFA fraction was two-fold higher in the dual NRTI-lipodystrophy group in the early postprandial period. This may have been due to more hydrolysis of labelled fatty acids from TAG-rich particles, which is possible as the clearance of dietary TAG from the circulation does appear to be lower in the PI-lipodystrophy group during the early postprandial period. Alternatively, this may represent a less efficient entrapment of fatty acids following hydrolysis in the dual NRTI-lipodystrophy group, or a combination of these events.

7.6 Summary

The aim of the study described in this chapter was to examine the differences in postprandial lipid metabolism, specifically the metabolic disposal of [1-^{13}C] palmitic acid between HIV positive males with lipodystrophy taking either NRTI drugs alone or NRTI drugs in combination with protease inhibitors. The results demonstrate that:

1) The duration of NRTI use at the time of self-reporting the phenotypic changes associated with lipodystrophy group was similar for both groups, suggesting a possible temporal relationship of PI drugs with lipodystrophy.

2) The oxidation of dietary lipid was lower in the PI-lipodystrophy group during the early postprandial period, possibly due to the elevated concentration of lipid within the circulation as both TAG and NEFA in the postabsorptive and postprandial period and a possible reduced capacity to take up fatty acids from the circulation.

3) While the PI-lipodystrophy group showed a prolonged retention of [^{13}C] palmitic acid within TAG in the circulation, the postprandial concentration of [^{13}C] palmitic

acid within NEFA was elevated in the dual NRTI-lipodystrophy group. This would suggest that while both groups show elevated postprandial plasma TAG concentrations compared to levels observed in HIV-negative healthy males of less than 2 mmol/l (Bennoson Thesis 1999), the underlying perturbations that result in this elevated lipid may be different between the two groups.

4) The pattern of plasma non-esterified fatty acid (NEFA) concentration during the 7-hour period following the meal in the PI-lipodystrophy group may be a result of delayed insulin secretion or indicate reduced insulin sensitivity, although a measure of insulin sensitivity is needed to verify this.

Chapter 8.0 Combined summary of data

Before entering into a general discussion, it is important to reiterate the key findings of each chapter and collectively examine the data presented in this thesis in order to better interpret these findings. This chapter presents these key findings together with data from all groups presented in tables and graphs for comparison of differences between all groups studied. This chapter follows the order of the chapters already presented with a discussion of the key findings from chapters 4.0, 5.0, 6.0 and 7.0 and where appropriate, comparisons are made between the groups.

8.1 Audit of HIV-positive population on first HAART regimen

The results of Chapter 4.0 indicate that, in this population age is not correlated with plasma triglyceride concentration. The results of multivariate analysis suggest that the strongest predictor of plasma TAG concentration is the length of time spent on therapy, independent of the type of therapy used. This observation within this population of 545 HIV-positive patients on HAART would support the theory that lipodystrophy results from the prolonged use of therapy [Saint-Marc et al. 1999]. The use of both PI and NRTI appears to amplify the metabolic perturbations that occur with therapy in some individuals, as shown by the results of Chapter 7.0 and the previous work of Carr et al. (1998, 1999). Therefore, it would appear that while there is an increased prevalence of hypertriglyceridaemia and hyperglycaemia in patients using NRTI therapy, addition of a PI in some individuals exacerbates the condition resulting in more severe perturbations that may be more easily and therefore, more commonly observed. Taken together with the fact that the PI/NRTI is more commonly used than NRTI alone due to clinical efficacy, this could result in the false impression that PI drugs used as part of HAART are more commonly associated with metabolic perturbations.

Selection of patients for studies using inclusion criteria weighted towards the metabolic perturbations may therefore result in a bias towards those patients with markedly elevated plasma lipids associated with the addition of PI drugs to the NRTI therapy backbone. This may explain why in this audit population the rate of combined hyperlipidaemia and hyperglycaemia associated with PI/NRTI therapy combination is far less than those reported previously. Interestingly, in the three patients treated exclusively with PI drugs there appeared to be a high incidence of hyperglycaemia.

While it is very rare that patients are treated exclusively with PI's, this finding would agree with the work of Noor *et al.* (2001) that showed an effect of Indinavir on insulin sensitivity in healthy, HIV-negative subjects independent of alterations in plasma TAG or free fatty acids.

The audit data also suggests that overall 10% of the population are hyperglycaemic, 25% are hypertriglyceridaemic, while only 9% exhibit both metabolic perturbations. In this population, the prevalence of hypertriglyceridaemia and hyperglycaemia associated with the use of different drug classes agrees with several studies previously reported and the only apparent factor associated with a higher incidence of metabolic perturbations in NRTI use. However, a comparison of this data to the data collected in the lipodystrophy groups shows that 57% of the NRTI-lipodystrophy group, while having a fasting plasma TAG concentration of <2.3 mmol/l, were clearly hyperlipidaemic in the postprandial period. This may suggest that the 25% prevalence of hyperlipidaemia within the audit population may be nearer to 70%, especially in NRTI users.

8.2 HIV-positive males compared with healthy males of similar age

While the work presented in this thesis was designed to examine the metabolic perturbations that occur with the use of these different therapies, it was important first to examine the influence of HIV infection alone on this process. Previous studies have reported that plasma TAG is elevated in HIV infection and increases as the disease progresses. In our study, we found HIV-positive therapy naïve males to have lower plasma TAG, glucose and insulin in comparison to HIV-negative males of similar age. This was due to two hypertriglyceridaemic males in the HIV-negative group and highlights the role of factors other than HIV infection in the development of hypertriglyceridaemia. The role of genotype, programming in early life, lifestyle, plane of nutrition (diet) and body habitus in the development or susceptibility of individuals to the metabolic alterations associated with lipodystrophy requires further investigation. The data for these two hypertriglyceridaemic HIV-negative subjects was included in chapter 5.0 as it was felt important to highlight that hypertriglyceridaemia occurs in a non-HIV population of similar age. However, it could be argued that this is not a comparison of HIV-positive males to healthy males of similar age, as hypertriglyceridaemia may indicate an underlying pathological process. Therefore, the summary data in this chapter has removed the hypertriglyceridaemic controls from the data set, in order to obtain a better comparison of

HIV-positive subjects to healthy controls. Figure 8.1 shows the postprandial plasma TAG data for the HIV-negative control group (excluding the hypertriglyceridaemic males) in comparison to the other groups studied. The pattern here is as would be expected based on the work of other authors, that HIV infection causes an increase in plasma TAG concentration but the addition of HAART increases this further, with the most marked increase observed in patients taking protease inhibitors as part of HAART.

In addition to the differences in postprandial plasma TAG concentration, the control group showed a lower energy expenditure when compared to all the HIV-positive groups, both as REE (kJ/kg LBM) and as energy expenditure over the 7 hour postprandial period. Total energy expenditure over 24 hours was not measured in this study in order to assess whether differences could be observed and whether these would be related to body mass.

8.3 Short term use of PI/NRTI combination

In this group, it is possible that the increased recovery of label from plasma is a result of a delayed clearance of dietary lipid from the circulation. Chylomicrons remaining in the circulation would compete for LPL binding with chylomicrons that enter the circulation from subsequent meals, resulting in a further elevation of plasma TAG concentration. In addition, reduced peripheral clearance would increase the clearance of chylomicrons and chylomicron remnants by the liver, thus providing components for VLDL synthesis and release, further exacerbating plasma TAG concentration and LPL binding. While this is speculative, it would appear that elevated circulating endogenous lipid, while probably contributing to the process, does not cause the delayed clearance of exogenous lipid from the circulation as these patients did not have elevated endogenous plasma TAG concentrations. What remains unclear is whether this reduction in dietary lipid clearance is a result of the reduced viral burden, a direct effect of PI's, a direct effect of NRTI's or any combination of these. HIV infection is known to be associated with a reduced clearance time of TAG from plasma [Grunfeld et al. 1991], although reductions in viral burden should, in theory, ameliorate this. Therefore, the alterations observed in these patients are likely to be due to the HAART.

In order to investigate the elevated plasma TAG observed following a mean 22 weeks of HAART including a PI, Berthold et al. (1999) isolated the plasma VLDL fraction by ultracentrifugation and measured VLDL TAG and apo-B concentration in a group of 19 HIV-positive patients. They suggested that the increase in plasma TAG observed during

this period was due to increases in both VLDL particle number (as assessed by increases in total and VLDL apo-B) and the amount of TAG in each TAG-rich lipoprotein particle (triglyceride:apo-B ratio). However, they do not say whether their patients were fasted at the time of measurement and it is therefore, difficult to assess if the sample in which they measured VLDL apo-B would be contaminated with chylomicron remnants. In addition, no hypothesis was suggested for why these TAG-rich lipoprotein fractions were elevated.

8.4 NRTI lipodystrophy in comparison to NRTI/PI combination lipodystrophy

The data from Chapter 7.0 shows that while the disturbed metabolic handling of lipid in lipodystrophy can occur following approximately 40 months of exclusively NRTI use, the addition of a PI drug to this combination may exacerbate the degree of this metabolic disturbance, possibly through alterations in insulin sensitivity within this group. In addition, while NRTI-lipodystrophy appears to be associated with mild to moderate elevations of plasma TAG and retention of the label in the plasma NEFA pool, PI-lipodystrophy is typically associated with much higher plasma TAG concentrations with a retention of label within the plasma TAG pool (table 8.3, figures 8.1-8.6). This may indicate that perturbed lipid metabolism occurs by distinct and separate mechanisms with each drug class, but that a combination of the two results in a cumulative and markedly increased disturbance in the metabolic capacity to handle both dietary lipid and lipid already present within the body. Carr *et al.* (1998) have also reported that the addition of a protease inhibitor to an NRTI drug resulted in more marked metabolic perturbations associated with the lipodystrophy.

However, it is unclear if this is a direct effect of the drug as the addition of a PI may also be a marker for a more complicated disease progression, indicating that, unlike in the group exclusively treated with NRTI's, this approach was not sufficient to maintain viral suppression. If this is the case, it could be that the severity of lipodystrophy is associated with the severity of infection or the degree of difficulty experienced in achieving antiretroviral control. There are a number of factors that could influence the severity of infection. The first of these is the strain of HIV as there are many different strains of HIV, some of which may be more virulent or less responsive to certain drug classes. The immune capacity of the host may also influence this process, and this capacity is a result of genetic, nutritional and general health influences. Other factors may include when therapy was initiated relative to the time of infection, patient compliance with

therapy, the initial efficacy of the first drug combination, the ability of the virus to establish resistance to any of the treatments administered and the host's nutritional and general health throughout this process.

A more marked metabolic perturbation in the PI/NRTI combination treated lipodystrophy group was evident by a fourfold higher plasma TAG concentration and higher plasma NEFA concentration both in the postabsorptive and postprandial period. The presence of the label within these two pools was significantly different between the two groups in the postprandial period. However, it is unclear if the differences observed in the metabolic partitioning of the label represent the influence of the drugs on specific part(s) of this process or the effect of the altered TAG and NEFA concentration within the plasma. It is possible that the reason for what appears to be a delayed clearance of dietary lipid from the circulation in the combination treated group, is actually due to the presence of large amounts of endogenous lipid within the circulation competing for the LPL enzyme and uptake mechanisms. A lesser hydrolysis of chylomicron TAG could also explain a lower proportion of the label in the NEFA pool. However, while this may explain the results obtained using the labelled fatty acid, it does not explain how these metabolic perturbations arise. In order for the plasma TAG to become elevated, the processes of secretion into the circulation or uptake from the circulation or both must have altered so that over a period of time, TAG accumulates within the circulation.

The findings using the label may suggest that while NRTI's reduce the capacity of the cell to take up fatty acids, PI's have an additional influence that decreases lipoprotein TAG clearance or increase secretion of TAG-rich lipoproteins from the liver, possibly through reduced insulin sensitivity, as reported with PI use in healthy volunteers [Noor *et al.* 2001].

Table 8.1 Comparison of subject characteristics in all groups. Data is presented as median (min, max). The statistical significance of group effect is determined by the Kruskal-Wallis test where significance is assumed at $p<0.05$. The letters (a,b,c,d) indicate where these differences lie as assessed by the Mann-Whitney U Test ($p<0.05$).

	HIV- (n 6)	HIV+ (n 5)	PIL (n 6)	DNL (n 7)	Significance
Age (years)	39 (32, 55)	45 (20, 47)	42 (35, 54)	49 (31, 67)	ns
Height (m)	1.78 (1.71, 1.81)	1.81 (1.65, 1.87)	1.77 (1.68, 1.80)	1.72 (1.68, 1.78)	ns
Weight (kg)	83.9 (68.3, 93.1)	64.3 (56.6, 78.0)	71.1 (66.3, 78.1)	66.0 (62.7, 73.9)	ns
Body Mass Index (kg/m2)	26.6 (23.4, 29.4)	20.8 (19.6, 22.3)	23.8 (22.4, 25.3)	22.7 (21.2, 23.1)	ns
Triceps Skinfold thickness (mm)	13.6 (7.1, 17.9)	8.0 (5.1, 11.4)	5.7 (5.1, 9.4)	6.6 (2.4, 10.4)	ns
Fat Mass from BIA (%)	17.8 (15.5, 22.5)	17.7 (13.5, 24.5)	14.0 (11.4, 19.5)	16.9 (6.5, 22.5)	ns
Fat Mass from BIA (kg)	16.0 (11.4, 22.8)	10.5 (9.0, 12.5)	10.8 (8.1, 13.9)	11.2 (4.2, 15.1)	ns
Lean Body Mass from BIA (kg)	65.7 (56.9, 72.7)	53.9 (47.6, 66.5)	60.4 (54.0, 67.6)	54.8 (52.1, 65.0)	ns
Mid Upper Arm Muscle Circ (mm)	339 (315, 356)	241 (237, 284)	266 (261, 294)	289 (258, 328)	ns
Waist Circumference (mm)	914 (799, 1022)	836 (715, 847)	870 (832, 905)	888 (778, 990)	ns
Hip Circumference (mm)	1067 (945, 1120)	950 (867, 980)	980 (930, 1030)	937 (930, 1010)	ns
Waist:Hip Ratio	0.86 (0.78, 0.91)	0.86 (0.82, 0.88)	0.87 (0.85, 0.97)	0.95 (0.80, 1.06)	ns
Viral Load (copies/ml plasma)	not determined	168312 (36800 - 1987200) a	412 (0 - 138514) b	<25 (<25 - 20763) c	P<0.01
CD4 Count (cells x 106/l plasma)	not determined	65 (0 - 362) a	243 (121 - 555) b	441 (256 - 939) b	P<0.05
CD8 Count (cells x 106/l plasma)	not determined	877 (96 - 1157)	930 (587 - 2355)	994 (482 - 1544)	ns

Table 8.2 Comparison of energy expenditure, respiratory exchange ratio and oxidation data in the postabsorptive and postprandial period between all groups. Data is presented as median (min, max). The statistical significance of group effect is determined by the Kruskal-Wallis test where significance is assumed at $p<0.05$. The letters (a,b,c,d) indicate where these differences lie as assessed by the Mann-Whitney U Test ($p<0.05$).

	HIV- (n 6)	HIV+ (n 5)	PIL (n 6)	DNL (n 7)	Significance
Post Absorptive State					
REE (kJ/kg LBM/d)	108 (100, 123) a	130 (119, 140) b	138 (127, 166) b	139 (92, 149) b	P< 0.01
RER	0.86 (0.78, 0.95)	0.81 (0.79, 0.92)	0.83 (0.78, 0.86)	0.83 (0.77, 0.90)	ns
Net Lipid oxidation (% REE)	33 (24, 69)	47 (7, 53)	47 (34, 65)	45 (17, 64)	ns
Net CHO oxidation (% REE)	49 (15, 57)	34 (29, 73)	38 (20,52)	40 (17,66)	ns
Post Prandial State					
EE AUC (kJ/kg LBM per 7h)	35.0 (33.8, 40.0) a	42.0 (40.9, 44.8) b	45.6 (43.1, 55.1) b	46.7 (31.2, 51.6) b	P< 0.01
Increase EE AUC (kJ/kgLBM per 7h)	3.40 (2.51, 4.68) a	4.19 (3.75, 7.71) a,b	5.70 (2.92, 6.69) b	5.81 (3.82, 8.69) b	P< 0.05
Breath $^{13}CO_2$ (% dose per 7h)	12.0 (6.7, 12.9)	6.7 (4.8, 15.2)	8.3 (2.5, 14.9)	11.7 (10.2, 19.9)	ns
CHO Oxidation (% EE)	39 (12, 70)	32 (25, 63)	39 (26, 44)	40 (16, 59)	ns
Exogenous Lipid Oxidation (% EE)	7 (6, 10)	6 (4, 10)	5 (1, 9)	8 (6, 13)	ns
Endogenous Lipid Oxidation (% EE)	31 (7, 49)	41 (12, 46)	42 (35,46)	36 (20, 55)	ns

Table 8.3 Comparison of plasma TAG, NEFA, insulin and glucose in the postabsorptive and postprandial period between all groups. Data is presented as median (min, max). The statistical significance of group effect is determined by the Kruskal-Wallis test where significance is assumed at $p<0.05$. The letters (a,b,c,d) indicate where these differences lie as assessed by the Mann-Whitney U Test ($p<0.05$).

	HIV- (n 6)	HIV+ (n 5)	PIL (n 6)	DNL (n 7)	Significance
Post Absorptive State					
Plasma TAG (mmol/l)	0.7 (0.4, 1.1) a	1.20 (0.90, 1.60) b	8.45 (4.5, 38.5) c	1.83 (0.86, 2.94) b	P< 0.001
Plasma NEFA (mmol/l)	0.26 (0.08, 0.30) a	0.45 (0.13, 0.51) a	0.67 (0.43, 1.02) b	0.35 (0.15, 0.48) a	P< 0.01
Plasma Glucose (mmol/l)	5.1 (4.9, 6.8)	4.80 (4.4, 5.0)	4.8 (4.1, 7.0)	5.1 (4.7, 6.3)	ns
Plasma Insulin (mmol/l)	7.4 (0.9, 7.7)	10.4 (4.3, 13.7)	7.0 (3.1, 26.2)	10.2 (2.3, 13.8)	ns
Post Prandial State					
Peak Plasma TAG (mmol/l)	1.21 (0.62, 1.55) a	1.78 (1.25, 2.02) a	13.5 (6.2, 55.0) b	3.49 (1.32, 5.30) c	P< 0.001
Nadir Plasma NEFA (mmol/l)	0.11 (0.08, 0.16) a	0.11 (0.10, 0.20) a	0.28 (0.19, 0.63) b	0.16 (0.10, 0.21) a	P< 0.01
Peak Plasma Glucose (mmol/l)	7.6 (6.2, 8.1)	6.90 (6.10, 7.40)	7.60 (5.8, 10.7)	8.00 (5.9, 16.2)	ns
Peak Plasma Insulin (mmol/l)	66.4 (27.3, 78.3)	53.0 (23.9, 79.5)	68.3 (58.1, 162.0)	64.6 (24.1, 123.4)	ns
Area Under the Curve (per 7h):					
Plasma TAG (mmol/l)	6.6 (4.4, 9.2) a	9.5 (8.0, 14.9) b	67.8 (37.7, 286.0) c	20.5 (6.5, 26.5) d	P< 0.001
Increment in Plasma TAG (mmol/l)	1.9 (1.5, 3.1) a	2.0 (0.7, 2.9) a	11.6 (2.3, 127.0) b	3.2 (0.9, 6.2) a	P< 0.01
Plasma ^{13}C-TAG (ug/ml)	34 (29, 48) a	35 (23, 72) a	229 (151, 472) b	84.2 (33.6, 165.3) c	P< 0.001
Plasma NEFA (mmol/l)	1.34 (0.85, 1.69) a	1.37 (0.74, 2.70) a	2.89 (1.87, 4.76) b	0.80 (0.67, 1.38) a	P< 0.01
Plasma ^{13}C NEFA (ug/ml)	2.8 (1.7, 3.6)	1.71 (0.53, 5.23)	2.96 (1.87, 4.76)	4.5 (3.0, 5.2)	ns
Plasma Glucose (mmol/l)	38.0 (37.3, 40.2)	35.5 (29.6, 38.9)	37.7 (34.1, 56.7)	43.7 (32.5, 67.8)	ns
Plasma Insulin (mmol/l)	128.7 (43.2, 178.1)	119.0 (59.7, 236.0)	167.0 (91.1, 401.1)	224.6 (35.5, 467.6)	ns

Figure 8.1 Plasma triacylglycerol concentrations (mmol/l) during the 7-hour period following administration of the emulsion and test meal, in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.

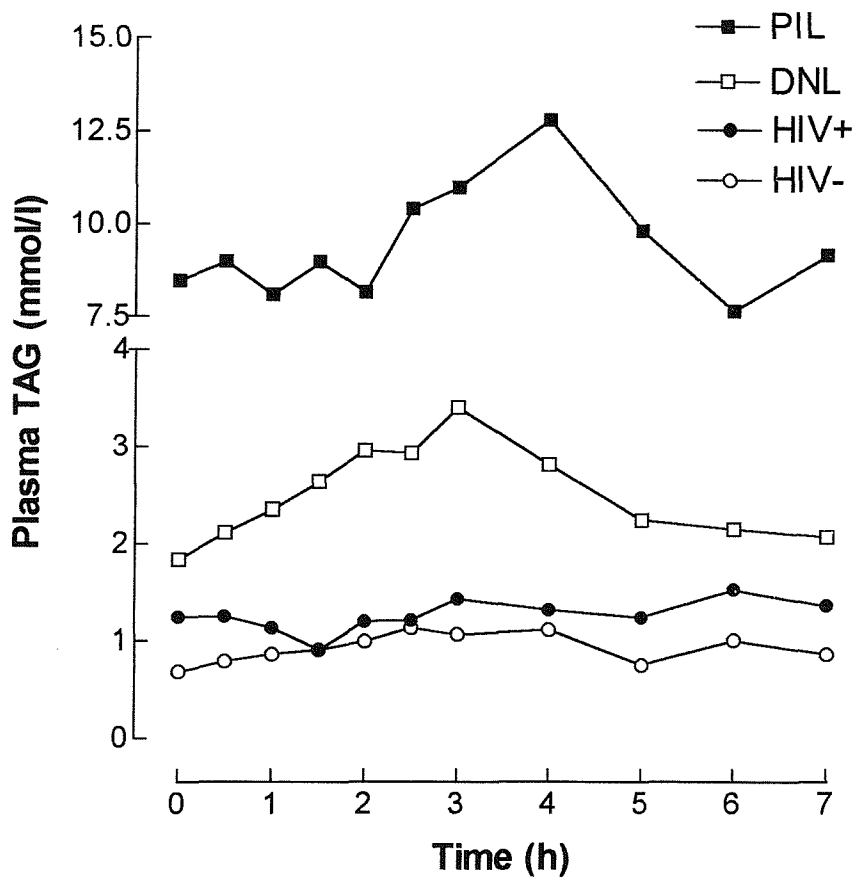


Figure 8.2 Postprandial plasma [^{13}C]-TAG concentration in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.

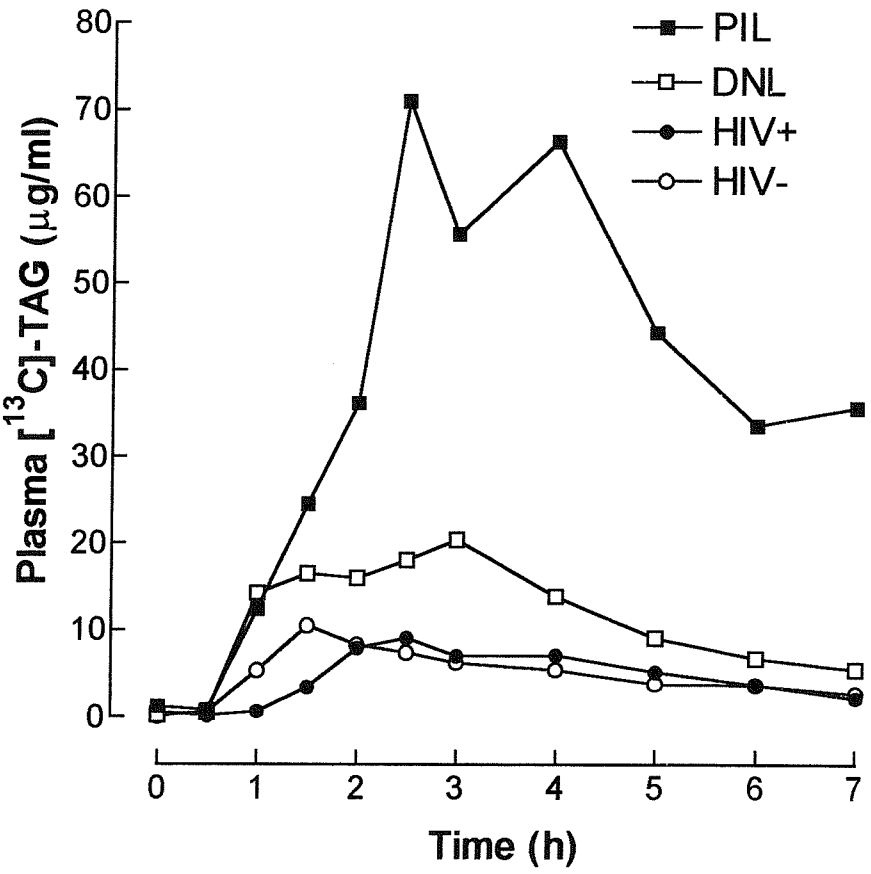


Figure 8.3 Postprandial plasma [^{13}C]-TAG area under the curve over 7 hours in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.

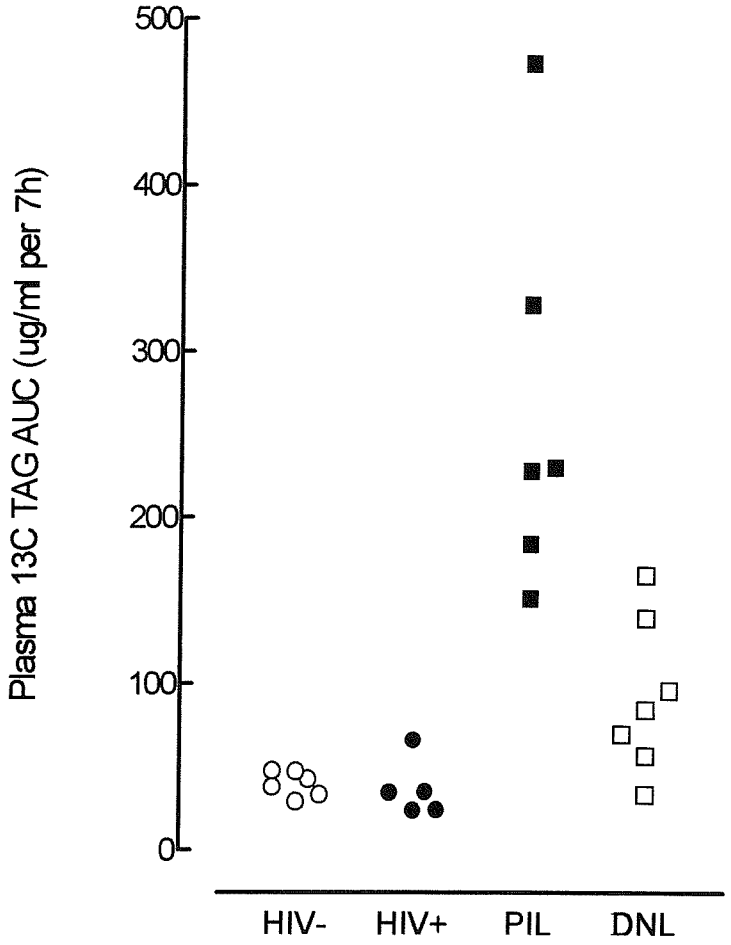


Figure 8.4 Postprandial plasma NEFA concentration in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.

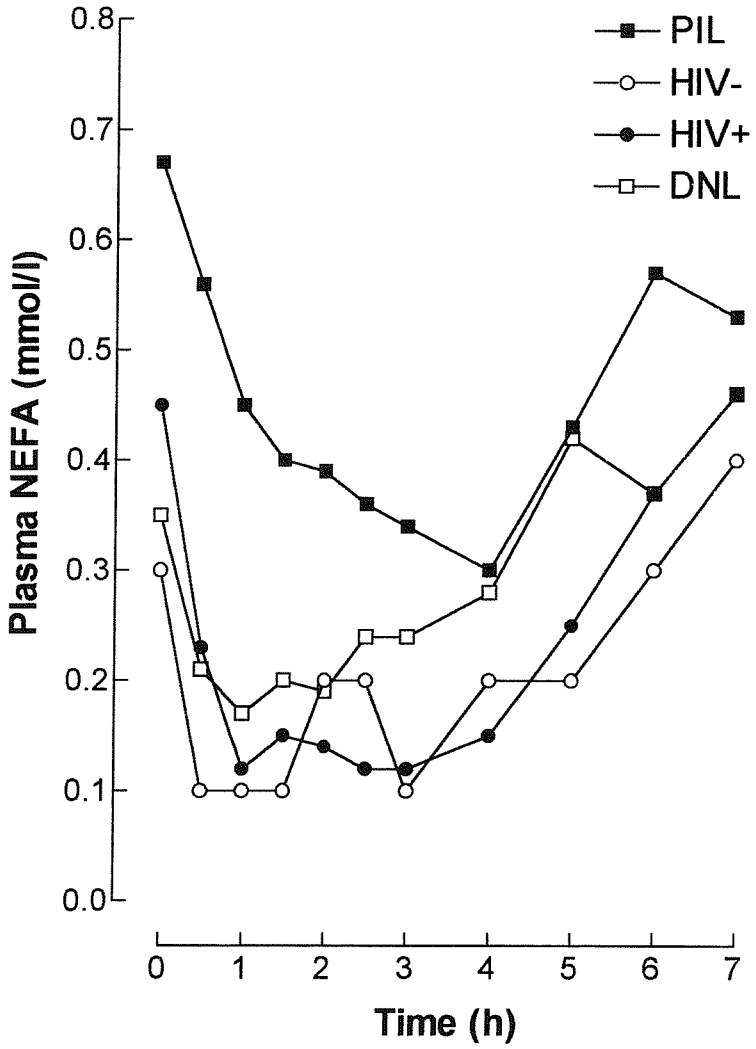


Figure 8.5 Postprandial plasma [^{13}C]-NEFA concentration in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.

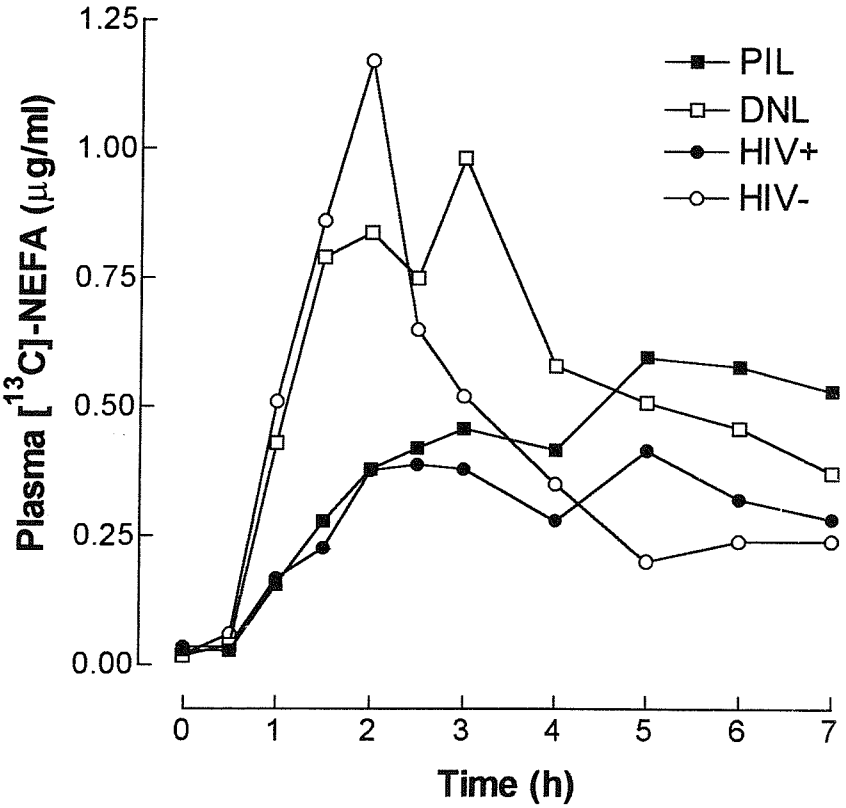
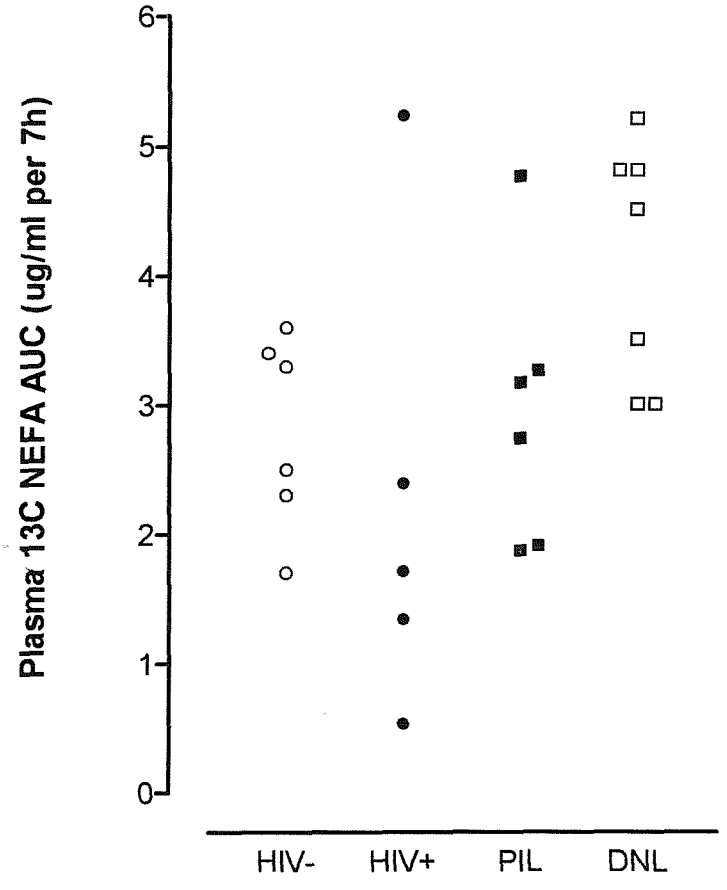


Figure 8.6 Postprandial plasma [^{13}C]-NEFA area under the curve over 7 hours in the HIV-negative (HIV-), HIV-positive therapy naïve (HIV+), HIV-positive with PI-lipodystrophy (PIL) and HIV-positive with NRTI-lipodystrophy (DNL) groups.



Chapter 9.0 General Discussion

The primary purpose of the work described in this thesis was to directly examine whole body macronutrient metabolism in patients on differing HAART regimens and in particular, to elucidate the effects of NRTI's alone and in conjunction with PI in comparison to that seen in therapy-naïve HIV seropositive patients and HIV negative controls. Hyperlipidaemia, one of the most obvious indicators of metabolic dysfunction associated with HAART, reflects an imbalance between the rate at which lipid enters the circulation and its clearance by peripheral tissues. Thus it was necessary to understand the relationship between these processes if the underlying metabolic lesion is to be clarified. Peripheral clearance by muscle, adipose tissue or liver, requires a combination of endothelial lipoprotein lipase to release fatty acids from circulating TAG-rich particles, and then effective 'trapping' of the fatty acids released by hydrolysis [Playford & Watts 1999]. Impaired co-ordinate control of these processes is likely to increase the flow of non-esterified fatty acids (NEFA) from the periphery to the liver, increasing hepatic VLDL secretion and leading to hypertriglyceridaemia, hyperglycaemia and peripheral insulin resistance. In the studies described in this thesis, the relative contributions of HIV infection and each drug class to the metabolic dysregulation, fasting metabolic parameters and the metabolic response to a standard lipid containing meal were assessed. The choice of patient and control populations in these studies have made it possible to evaluate the impact of HIV infection, NRTI therapy and NRTI+PI therapy on lipid handling relative to that seen in HIV negative healthy individuals. Patients treated with PIs alone, to establish whether the observed effects were specifically related to NRTI's rather than cytokines released secondary to immune restoration, were not available to study.

The main events studied were the kinetics and time course of ^{13}C-fatty acid in the TAG and NEFA pools within the circulation and the kinetics and time course of ^{13}C label excretion in breath. Prolonged retention of the labelled fatty acids as TAG in the circulation was assumed to reflect an impaired clearance of lipid from the circulation by peripheral tissues. Any elevation of labelled fatty acids as NEFA was taken to reflect impaired entrapment of fatty acids from hydrolysed TAG by peripheral tissues. The extent of recovery of label in breath as $^{13}CO_2$ reflects oxidation of dietary lipid and any differences between groups interpreted as reflecting differences in the oxidation of labelled fatty acid by peripheral tissues, particularly liver and skeletal muscle.

These studies demonstrate that whereas HIV-infection alone (therapy naïve) does not markedly alter lipid handling over the postprandial period, NRTI therapy alone and in combination with PIs impairs the usual processes whereby circulating TAG are hydrolysed in peripheral tissues, and the resulting fatty acids taken up by cells. This impairment is most evident in those patients receiving PI-based HAART in whom the hypertriglyceridaemia was most marked. Finally, it was not possible to demonstrate any obvious defect in mitochondrial lipid oxidation with HIV infection or NRTI-containing HAART with or without PIs. This would imply that mitochondrial toxicity is not a critical mechanism of dyslipidaemia in persons with lipodystrophy*.

The most likely explanation of the results obtained in this work is that in patients treated with HAART there are significant changes in lipid handling in the periphery, which lead in time to significant hyperlipidaemia. The mechanisms through which the changes in blood lipid concentration are brought about appear to differ for different drug combinations. The most important findings are that NRTI impairs fatty acid entrapment in the periphery. When a PI is added to this regimen there is also impairment in the handling of an oral lipid load, which appears to be related to an impaired effective lipolysis. These changes appear to become more marked the longer the duration of therapy, although it may be that there is a contribution from the underlying progress of the disease process itself. Some caution is needed with this interpretation, because for a clinical study of this kind there are important difficulties in obtaining an ideal experimental design. As a result there are important, but unavoidable, differences in the baseline characteristics between the study groups, for example in terms of viral load, disease progression and the initial lipid metabolic state. Nevertheless, the most likely explanation of the differences in the responses to the test meal described here can be attributed to separate effects upon lipid hydrolysis and fatty acid entrapment in the periphery.

Three further observations add weight to the suggestion that the clearance of TAG and fatty acid turnover may be altered in HIV-associated lipodystrophy. Firstly, Sekhar *et al.* (2002) have shown increased lipid turnover (whole body rates of lipolysis, re-esterification and NEFA oxidation) and decreased TAG clearance using steady state kinetics in HAART-treated HIV patients with central obesity, peripheral fat wasting and elevated CM and VLDL-TAG compared to HIV-negative controls. Despite different methodology our findings are in agreement with these steady state data. Secondly, a

* For a more detailed discussion, see Appendix 5.4

marked decrease in heparin-releasable hepatic lipase and lipoprotein lipase activity has been reported in HIV-infected men with severe hypertriglyceridaemia treated with PI-containing regimens in comparison to healthy controls [Baril *et al.* 2001]. Low LPL activity may result in increased levels of CM and VLDL TAG and the low hepatic lipase activity may result in an accumulation of particles rich in cholesterol and apolipoprotein-E (CM remnants and intermediate density lipoproteins). Finally, a reduced rate of VLDL transfer into denser lipoproteins has been reported by Schmitz et al. (2001), implying lower rates of LPL-mediated delipidation. In addition to these findings, Hadigan *et al.* (2002) have recently reported increased free fatty acid flux in HIV-positive patients associated with the use of the NRTI Stavudine. While this study did not control for the concurrent use of protease inhibitors or the presence or absence of lipodystrophy, it is in agreement with our findings that NRTI use results in alterations in free fatty acid metabolism.

The aim of this chapter is to discuss these research findings within the framework of the mechanisms proposed for the development of lipodystrophy. The potential impact of factors such as the presence of specific genetic polymorphisms, cytokine levels, endocrine function and the progression of HIV infection are discussed, followed by a discussion of the potential mechanisms whereby these specific drug classes could directly or indirectly result in the alterations in lipid metabolism observed within this thesis.

9.1 Currently proposed mechanisms for Lipodystrophy development

This work suggests that there is an influence of the drugs used in HAART on the clearance of dietary lipid from the circulation. However, the data collected from a healthy control group of similar age would indicate that gross metabolic lipid perturbations occur in the absence of infection and treatment with HAART suggesting a role for other factors.

Some underlying genetic polymorphisms have been suggested to predispose an individual to lipodystrophy. Maher et al. (2000) found an increased frequency of polymorphism –238 in the TNF-α gene in patients with lipodystrophy compared to those without. A β-3 adrenergic receptor polymorphism in visceral fat, associated with visceral obesity, elevated TAG and insulin resistance has also been found to be higher in patients with lipodystrophy compared to controls although not significantly [Vonkeman et al. 2000]. The frequency of both these mutations was not high enough to be the sole explanation for lipodystrophy development. In other types of lipodystrophy, such as

Dunnigan's, a mutation in nuclear lamin A/C, a component of the nuclear envelope has been found [Shackleton *et al.* 2000]. This mutation is also found to be associated with hyperinsulinaemia, dyslipidaemia, hypertension and diabetes [Hegele *et al.* 2000a] and is thought to be due to impaired interactions between the mutant lamin in the nuclear envelope and chromatin, transcription factors and/or other nuclear or cytosolic proteins [Hegele *et al.* 2000b]. This mutation has not yet been investigated in HIV lipodystrophy patients. Of more promise are the findings of Perret *et al.* (2001) that apo-CIII polymorphisms may explain up to 43% of the variability in plasma TAG concentration in PI-treated patients. However, none of these proposals have conclusively been identified as the mechanism for lipodystrophy development or can explain the array of symptoms observed in lipodystrophy.

There is also evidence that cytokine concentrations can influence the signalling processes that link the endothelial phase of TAG hydrolysis (LPL) and the sub-endothelial phase of fatty acid uptake and metabolism. This process involves the complement C3 derived acylation stimulating protein (ASP) [Sniderman *et al.* 1997; Scantlebury *et al.* 1998] and recent data have indicated that conversion of C3 to ASP is inhibited by tumour necrosis factor (TNF)α. TNF release into the medium and serum concentration of sTNF receptor type II has been found to be higher and conversion of C3 to ASP lower in fat biopsy samples derived from individuals with clinical lipodystrophy relative to the HIV-positive and HIV negative controls [Ionescu *et al.* 2002]. In addition, measurements of TNF receptor levels during and 3 months after stopping PI therapy showed a significant decrease [Maher et al. 2000] and an increased synthesis of TNF-α has been shown in CD4 and CD8 T cells of lipodystrophy patients [Ledru *et al.* 2000]. The acquired lipodystrophy Barraquer-Simon's syndrome, which has phenotypic similarity to HIV-associated lipodystrophy, is also accompanied by low levels of complement C3 hence low ASP [Meyrier *et al.* 1997]. Recent work from Grimble *et al.* has suggested that genetic polymorphisms in the TNF-α gene and lymphotoxin-a (LT-a) gene may modulate the cytokine response of an individual to infection [Grimble *et al.* 2002; Howell *et al.* 2002] indicating that the metabolic response to infection may be predicted by genotype. Once the molecular mechanisms underlying lipodystrophy and these metabolic perturbations are better characterised, genotyping may provide a useful tool in deciding which HAART combination is least likely to have these effects in an individual.

Interferon-α (IFN-α), administered to treat Hepatitis C co-infection is known to increase plasma TAG levels [Morlese *et al.* 2000] and a significant positive correlation has been found between IFN-α accumulation and increased TAG, cholesterol and ApoB in HIV-positive males with lipodystrophy [Christeff et al. 2002]. In this study, multivariate analysis suggested that IFN-α is the best marker for lipid perturbations associated with lipodystrophy, followed by insulin and cortisol:DHEA ratio.

Some similarities exist between lipodystrophy and the clinical features of Cushing's syndrome, and have led some authors to suggest that lipodystrophy is a Pseudo-Cushing syndrome [Miller *et al.* 1998]. While serum cortisol levels were elevated in HIV-positive patients compared to controls, no differences were found between individuals with and without lipodystrophy [Christeff et al. 1999]. Differences were observed in DHEA concentration in the lipodystrophy group and the authors hypothesised that the altered cortisol:DHEA ratio was due to PI interactions with the cytochrome P450 isoforms involved in steroid metabolism, producing an imbalance between lipolysis and lipogenesis resulting in peripheral fat loss and central fat accumulation. Yanovski et al. (1999) showed normal serum cortisol levels, cortisol response after CRH stimulation, cortisol binding globulin concentration and glucocorticoid number and affinity in lipodystrophy patients, suggesting that alterations to the HPA axis are not a cause of lipodystrophy.

Other groups have looked at the central role of insulin in the development of lipodystrophy. In PI treated subjects following an oral glucose tolerance test (OGTT), elevated levels of insulin, proinsulin and C-peptide have been found indicating a possible Beta-cell dysfunction [Behrens *et al.* 1999]. Martinez and Gatell (1998) have proposed an alternative mechanism of protease inhibitors inhibition of the cellular enzymes insulin protease and glutathione-insulin transhydrogenase, which catalyse the breakdown of insulin once taken up by the cell via the insulin receptor. The reduction in insulin degradation would cause hyperinsulinaemia, which over a period of time, would result in insulin-resistance, leading to an increase in fatty acid release from adipocytes. While these mechanisms have yet to be proven, other protease inhibitor drugs (Camostat) have been shown to cause pancreatic hypertrophy and prevent pancreatitis in a spontaneous pancreatitis rat model [Sugiyama *et al.* 1996].

Other reporters have suggested that lipodystrophy development is not due to the therapy but to either the progression of the infectious process or, in contrast, a rapid reduction in infective burden as a result of therapy [Goujard *et al.* 2001].

Hyperinsulinaemia is positively correlated with low CD4 counts and AIDS progression [Shikuma *et al.* 1999], while REE has been found to be higher in individuals where HAART has achieved a significant reduction in viral burden compared to HAART naïve individuals with high viral loads [Shevitz *et al.* 1999]. However, it is unclear if the increased REE is accompanied by an increase in fat-free mass. Using magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DEXA), increased visceral adiposity was shown in HIV infected patients on HAART and was associated with higher CD4 counts and lower viral burdens [Engelson *et al.* 1999]. As lipodystrophy has developed in HAART treated patients without improvement in viral load [Mezzaroma *et al.* 1999; Panse *et al.* 2000] it would appear that to a certain extent the influence of HAART on these metabolic processes may be independent of the virological response to treatment. An alternate theory is that antiviral agents have allowed patients to survive to the disease stage at which this syndrome would naturally occur [Buss & Duff 1999; Wanke 1999].

The primary perturbation appears to be in lipid metabolism as following HAART initiation including PI drugs, rapid and sustained increases in plasma TAG are observed within 4 weeks [Churchill et al. 1998]. The data presented in this thesis suggests that exclusively NRTI treated patients exhibit retention of the labelled fatty acid within the NEFA pool, while the PI/NRTI combination treated patients show retention of label within the TAG pool, indicating independent effects of these two drug classes on the postprandial uptake and metabolism of fatty acids. While adipose, muscle and liver are the predominant tissues that remove fatty acids from the circulation, the following sections will primarily discuss fatty acid uptake and metabolism within the adipocyte only. The rationale for this is that muscle uptake and metabolism of fatty acids does not appear to be impaired in the patients studied as indicated by the recovery of label on breath, an indirect measure of fatty acid oxidation. In addition, hepatic steatosis is rare in lipodystrophic patients while plasma VLDL-TAG is high [Berthold et al. 1999], indicating that the liver is able to take up and export fatty acids. However, the uptake and storage of fatty acids within adipocytes from subcutaneous adipose tissue is profoundly altered as evidenced by the phenotypic changes associated with lipodystrophy.

9.1.1 Mechanisms for NRTI induced changes in free fatty acid uptake by the adipocyte.

9.1.1.1 Direct effects

Stahl et al (1999) have shown that antisense oligonucleotide treatment of enterocytes produces a reduction in the levels of the human fatty acid transport protein 4 (hsFATP4) resulting in reduced cell fatty acid uptake. NRTI drugs consist of dideoxy analogues of dinucleotide triphosphate (also called ddNTP). Incorporation of these will terminate the elongation of the DNA chain being copied by viral reverse transcriptase from the viral RNA. These ddNTP are known to be potential substrates for the mitochondrial enzyme DNA polymerase γ but it is unclear if ddNTP are substrates for other enzymes involved in DNA replication and transcription. Polymerase γ has been the subject of investigation because mitochondrial DNA replication takes place in the cytosol and ddNTPs are targeted to the cytosol to reduce viral replication. However, the extent to which these ddNTPs can influence nucleic acid processing within other structures, such as the endoplasmic reticulum and nucleus is unclear.

Several bacteria and viruses have been shown to enter the cell via caveolae in the cell membrane, resulting in their transport intact into the endoplasmic reticulum (ER) [Pelkmans et al. 2001]. Therefore, it is possible that ddNTPs associated with virus could also enter the cell by this route, resulting in the delivery of an effective antisense dinucleotide into the endoplasmic reticulum. While the rough endoplasmic reticulum (RER) is involved in the synthesis of proteins for inclusion in the cell membrane or for export from the cell, the smooth endoplasmic reticulum (SER) is the site of fatty acid, phospholipid and steroid synthesis. Therefore, the presence of these synthetic dinucleotides within the ER may influence the production of hsFATP4 and other proteins destined for the cell membrane or export either by influencing the RNA sequence from which the proteins are translated or by entry into the nucleus via the ER cellular transport system for inclusion in the DNA template.

9.1.1.2 Indirect effects

As ddNTPs are known to be substrates for polymerase γ [Brinkman *et al.* 1998] and elevated levels of mitochondrial DNA deletions are reported in spermatozoa from HAART treated patients [St John *et al.* 2000], it is possible that the adipocyte mitochondria has a reduced capacity to generate ATP, resulting in a reduction in ATP dependent membrane transport processes. This could reduce the fatty acid uptake by the cell through ATP dependent protein mediated transport, although fatty acid diffusion across the cell membrane may still occur. In addition, a reduced ATP availability may lead to a reduction in cellular ATP dependent reactions such as esterification of free fatty acids to TAG. However, direct measures of mitochondrial respiration in lymphocytes from NRTI-treated patients have failed to find a defect in mitochondrial β-oxidation [Wachsman *et al.* 1996] and myopathy is not commonly reported associated with lipodystrophy. This may indicate site-specific differences in ddNTP inclusion into mtDNA, possibly related to cell type and rate of replication.

Patients with multiple symmetrical lipomatosis (MSL) show an accumulation of fatty tissue within the subcutaneous regions of the neck and shoulders, accompanied by hypertriacylglycerolaemia and insulin-resistance. One of the main causes of MSL is thought to be mitochondrial dysfunction and it was the similarities between MSL and lipodystrophy that lead the authors to suggest that NRTI's could cause lipodystrophy via mitochondrial dysfunction [Brinkman *et al.* 1999].

Elevated lactate levels observed in NRTI-associated lipodystrophy [Harris *et al.* 1999] have also been taken to indicate mitochondrial dysfunction as lactate is believed to be elevated due to a reduction in glucose oxidation. Direct measures of oxidative and glycolytic muscle capacity during and following exercise in lipodystrophy patients with elevated lactate do not support this theory and may suggest a reduced hepatic clearance of lactate rather than an increased muscle output [Roge *et al.* 2002]. An alternative explanation for these elevated lactate levels may be that during chronic infection, as in the metabolic response to injury, glucose uptake by adipose tissue is inhibited [Fellander *et al.* 1996]. In the postabsorptive state, adipose tissue can take up substantial amounts of glucose, most of which is converted to lactate [Marin *et al.* 1987; Hagstrom *et al.* 1990]. An improvement in HIV status as provided by HAART may remove this inhibition and allow an increased lactate production and secretion by adipose tissue.

However, NRTI related metabolic perturbations may be specific to certain drugs as PI-experienced patients with lipodystrophy show a significant improvement in plasma TAG, glucose and insulin-resistance when switched to the NRTI nevirapine for a mean 8 months [Martinez *et al.* 1999].

9.1.2 Mechanisms for PI induced changes in TAG clearance

9.1.2.1 Direct effects

Studies in non-HIV infected volunteers have shown that Ritonavir increases plasma TAG levels within two weeks of therapy [Purnell *et al.* 2000]. In order to understand the mechanism by which HIV-1 protease inhibitors could cause lipodystrophy, Carr *et al.* compared an amino acid sequence from about the catalytic region of the HIV-1 protease enzyme to the mammalian protein and genome sequences held in Genbank, EMBASE and Swiss protein libraries. A 63% homology was found with the low density lipoprotein receptor related protein (LRP) and a 58% homology was found with the C-terminal region of the cytoplasmic retinoic acid binding protein type 1 (CRABP-1). A mechanism was proposed whereby inhibition of CRABP-1 by PI caused a reduction in differentiation of peripheral adipocytes with an increase in apoptosis via a cascade involving the metabolism of retinoic acid to cis-9-retinoic acid, leading to a reduction in retinoic X receptor (RXR) stimulation. The concurrent inhibition of LRP produces impairment of the function of the LRP-Lipoprotein lipase (LPL) complex so that there is a reduction in the cleavage of circulating TAG with a reduced LRP-mediated hepatic uptake of lipoproteins. While a reduced clearance of lipid from circulating lipoproteins in PI treated subjects would fit with the stable isotope data gathered in this research, subsequent in vitro work using physiological concentrations of PI's has shown that, while PI's do result in a reduced cellular adipogenesis and increased lipolysis, this effect is not dependent on the absence of RXR ligand [Lenhard *et al.* 2000a].

However, subcutaneous adipocyte apoptosis has been shown in adipose tissue biopsy samples from HIV positive patients with PI-associated lipodystrophy [Domingo et al. 1999]. Apoptosis was more prevalent in HIV patients where PI therapy had achieved a greater increase in CD4 and CD8 T cells and a larger fall in viral load. Large cohort studies have failed to find a relationship between the development of lipodystrophy and the improvement in HIV status [Tsiodras et al. 2000]. The audit data collected also did not find

a relationship between hypertriglyceridaemia and viral load or CD4 count as markers of HIV status.

In a study of apolipoprotein profile in lipodystrophy, an elevated Apo-CIII level was found which correlated with the presence of lipodystrophy [Bonnet *et al.* 2001]. Apo-CIII reduces the clearance of TAG-rich lipoproteins via inhibition of the Apo-CII activated lipoprotein lipase and prevention of Apo-E interaction with the LDL receptor or LDL receptor-related protein (LRP). The authors suggest that Apo-CIII is increased by the action of PI's on PPAR [Carr *et al.* 1998], however it is PPAR-α that regulates Apo-CIII synthesis and not PPAR-γ as discussed by Carr and colleagues.

9.1.2.2 Indirect effects

The inactive sterol regulatory element binding protein (SREBP) is attached by protein binding domains to the ER. When cellular cholesterol concentration decreases, site-1 protease (SIP) is activated which cleaves SREBP in the ER lumen, releasing the transcriptionally active NH_2-fragments of SREBP from the membrane into the cytosol [Espenshade et al. 1999]. SREBP exerts its effects via stimulation of the PPARγ-RXR transcription factor to upregulate cellular cholesterol and fatty acid synthesis and uptake via the increased production of proteins such as FATP, FABP and the insulin receptor. Therefore SREBP action is dependent on the ability of this protein to cross the nuclear membrane. Supraphysiological doses of Indinavir have been shown to alter the proteolytic maturation of SREBP within the cytosol resulting in the aggregation of partially processed SREBP at the nuclear membrane [Caron et al. 2000]. The two protease enzymes involved in this process may present two targets for the protease inhibitor drugs in vivo, resulting in reduced SREBP function and ultimately reduced adipocyte fatty acid synthesis and uptake, insulin resistance and increased adipocyte lipolysis. Elevated circulating free fatty acids may stimulate insulin production, exacerbating the situation. This theory would be supported by the in vitro observation that physiological doses of protease inhibitors reduce insulin binding to adipocytes by 50% within 15days of exposure [Germinario *et al.* 2000].

However, the affinity of the viral protease inhibitors for these proteins and the influence of Indinavir on SREBP maturation at physiological doses are unknown. This is further confused by the ability of TNFα to upregulate mature SREBP in both mouse [Diomede *et al.* 2001] and human hepatocytes [Lawler *et al.* 1998]. However, HAART has

been shown to significantly reduce the production of TNFα by activated T cells within three weeks [Lew *et al.* 2001]. Studies in mice have also shown that Ritonavir increases fatty acid synthesis 3 to 4 fold in adipocytes and liver, and increases hepatic VLDL secretion 2-fold, concurrent with increases in activated sterol regulatory element binding protein (SREBP-1) [Riddle et al. 2001]. However, rodent data should be interpreted with caution, as Ritonavir has been found to produce different effects on lipid metabolism in rats compared to humans [Ye et al. 1998].

Overdosage with all-trans retinoic acid (ATRA) may cause similar changes in lipid metabolism to those observed with PI's. In order to investigate this, Lenhard et al. (2000b) exposed stem cells to PI's and high dose ATRA in vitro and found that Indinavir increased the effects of ATRA on lipid accumulation during fat cell differentiation. However, the doses of ATRA used in this study are likely to be much higher than those observed in vivo.

In vitro work with HepG2 liver cells suggests that PI's stimulate hepatic TAG synthesis [Lenhard *et al.* 2000]. Although the PI dose administered may not have reflected the physiological concentrations of these drugs that hepatocytes are exposed to in vivo. Another in vitro study examined the influence of physiological doses of PI's on differentiating adipocytes and found a reduction in TAG accumulation and aP2 (a fatty acid binding protein) mRNA expression, suggesting a reduced differentiation [Zhang et al. 1999]. This does not, however, provide an insight into the influence of these drugs on mature adipocytes.

While much work has focused on the ability of protease inhibitors to bind to host proteins and alter cell function, an alternative explanation could be that the large amounts of redundant viral protein produced in the cytosol as a result of PI inhibition of viral replication alter cell function. In Huntington's disease, an abnormal Huntingtin protein characterised by an extended trinucleotide repeat sequence is produced. While this protein is tagged with ubiquitin, marking the protein for proteasomal degradation, the protein is not degraded [Bence *et al.* 2001]. Instead, the protein is hydrolysed by the enzyme caspase and protein fragments migrate to the nucleus [Wellington *et al.* 2000]. The quantity of these nuclear protein fragments has been associated with the degree of pathology indicating a link between these and the loss of cell function [Kegel *et al.* 2001]. It is possible too that the generation of surplus non-functional proteins within the cytosol in PI-

treated HIV-infected cells could produce a similar result. Although the mechanism for this, as in Huntington's disease, needs further investigation*.

9.2 Summary and conclusion

This programme of research was undertaken to explore the processes that regulate lipid metabolism in HIV infection with and without the use of HAART or development of lipodystrophy, as the most obvious derangements of the lipodystrophy syndrome are reflected by changes in body fat mass and distribution, and circulating lipid concentrations. In HIV-positive subjects identified as having lipodystrophy, fasting plasma TAG is routinely between 9 and 20 mmol/l with values having been reported as high as 120 mmol/l [Morlese, 1998 personal communication]. Although the accuracy of analysis with these extreme values may be questionable, the risk of pancreatitis and other detrimental effects is becoming increasingly apparent.

The aim of the research was to achieve an improved understanding of how these processes are normally controlled and how they may be altered in response to infection and treatment using stable isotope methods to measure the metabolic disposal of [1-^{13}C] palmitic acid, in order to determine the effect of infection and therapy upon; (1) the contribution of dietary lipid to the postprandial plasma triacylglycerol concentration, (2) the contribution of dietary lipid to the postprandial plasma non-esterified fatty acid concentration and (3) the extent to which the lipid from the meal is oxidised. In this respect, this work is not only of value to HIV, but also other metabolic disorders of lipid metabolism that may be mediated through common pathways.

The results of the small studies conducted in this work would suggest that hypertriglyceridaemia is not unique to lipodystrophic or even HIV-positive males. Marked hypertriglyceridaemia was found in a group of healthy males selected on the basis of age, suggesting that factors such as age, gender, genotype and/or environmental factors influence this process. On this basis, elevated plasma lipids would be expected in a number of the HIV-positive population and probably more exaggerated due to the presence of infection. What clearly sets the lipodystrophy patients apart from those individuals with hypertriglyceridaemia is their appearance. While central obesity or the accumulation of lipid within adipose stores surrounding the viscera is commonly associated with elevated

* Drug virus interactions are discussed further in Appendix 5.3 for both PI and NRTI classes.

lipid in both the non-HIV population and patients with lipodystrophy, concurrent lipoatrophy is unique to lipodystrophy patients. This would suggest that the anti-retroviral drugs are either directly or indirectly influencing subcutaneous adipose tissue metabolism and it is this that results in the exaggerated metabolic perturbations.

As discussed in the introduction, metabolism is a demand led process and this demand is satisfied by the availability of nutrients within a circulating pool. The concentration of macronutrients within this pool is maintained within defined levels at all times such that the rate of entry into the pool matches the rate of removal from the pool into the tissues. Therefore, concentration can be kept constant despite marked changes in flux or input. The observation that there is both a marked turnover of lipid through this pool with a mismatch between flow into and out of the pool indicates not only an increased metabolic demand for lipid, but also a loss of the coordinated control of this process.

The multifactorial aetiology of lipodystrophy resulting from interactions between HIV infection and TAG clearance, energy balance and/or HAART; the effects of HAART on insulin sensitivity, glucose metabolism, adipocyte differentiation and enzyme/receptor function; or the altered hormonal and cytokine profiles has been previously suggested [Gharakhanian 2001]. It remains unclear whether lipodystrophy would result from a combination of these or the presence or absence of some factors. However, the investigation of the lipodystrophy syndrome is complicated by its multifactorial nature. Research has to take into account; the presence of HIV infection; the immune response to infection involving cytokines that profoundly influence the metabolism of lipids and glucose; the presence of associated genetic polymorphisms; the patients age, gender, ethnic background and body composition; the therapy type and duration; and the combinations of therapy used along with the patients medical history and the presence of other opportunistic infections. To determine the specific effects of the antiretroviral drugs on the metabolic parameters associated with lipodystrophy, a randomised-controlled trial is required of each drug independently, then in combination in HIV-negative, healthy males. While the study of Noor *et al.* (2000) has reported the effects of Indinavir in such a trial, few other centres are likely to receive ethical approval for such studies. An opportunity for this type of study exists in individuals exposed to HIV who are prescribed post-exposure prophylaxis with antiretrovirals. While these drugs are not given in isolation as this may result in reduced efficacy, there is scope to evaluate different combinations of drugs in a healthy population. The problem lies in recruiting these individuals at a time of emotional distress and the random nature of HIV exposure resulting in poorly defined and

heterogeneous study groups. In vitro cell culture systems provide a way in which the effects of these drugs on cellular fatty acid and glucose uptake and response to insulin may be investigated. However, a large proportion of the studies reporting results using this technique have used large drug doses that are not representative of the physiological concentrations commonly found in HAART treated patients.

In conclusion, this work provides evidence that suggests protease inhibitor drugs contribute to hyperlipidaemia by a reduced capacity for lipoprotein hydrolysis, while nucleoside reverse transcriptase inhibitors contribute to hyperlipidaemia by a reduced efficiency of fatty acid entrapment within the periphery. While NRTI alone may result in the development of lipodystrophy, the combination of both NRTI and PI produces synergistic effects resulting in more marked metabolic perturbations. This work does not support the theory that reduced mitochondrial function is important in the aetiology of lipodystrophy. Furthermore, estimations of the prevalence of lipodystrophy based on fasting plasma TAG measures may underestimate the prevalence of hyperlipidaemia in up to 50% of cases. Further work is required to assess how this process is influenced by other factors and whether specific combinations may be more or less toxic to the HIV-positive population as a whole or to specific individuals.

A Chapter 10.0 Appendices

Appendix 1.0 Patient information sheet

ST STEPHEN'S CENTRE
369 FULHAM ROAD
LONDON
TEL: 0181 846 6161

PATIENT INFORMATION SHEET

Combination anti-retroviral therapy with protease inhibitors has significantly altered the long-term outlook in patients with HIV disease. Not only has it been shown to reduce the incidence of opportunistic infections but it also prolongs patient's life expectancy.

Unfortunately, changes in body fat have emerged as a major side effect of protease inhibitors. This phenomenon is known as lipodystrophy, the main features of which appear to be decreased fat in the limbs and buttocks, facial wasting and sometimes a corresponding increase in fat around the belly. It is believed that protease inhibitors may disturb the way that the body produces, utilises and distributes fat, resulting in abnormal amounts of fat in the blood. The precise mechanism, which causes this, is still unknown.

Lipodystrophy affects a large number of patients taking protease inhibitors and tends to occur within the first six to nine months of treatment. It then seems to reach equilibrium. This side effect may well result in an increased risk of heart disease and pancreatitis as well as being visually distressing to the patient.

It was initially thought to occur only with Indinavir (trade name CRIXIVAN hence the term CRIXBELLY), however it now seems that all protease inhibitors can cause lipodystrophy.

As protease inhibitors have significantly altered the outlook in HIV disease, it is important that patients continue to take these drugs. However we will need to understand more clearly why these abnormalities of fat metabolism occur and monitor patients carefully to try and ascertain which patients will develop this problem and if need be, switch them to other available medications earlier rather than later. There is some evidence that the fat changes do reverse when the patient stops protease inhibitors.

STUDY OUTLINE

6 individuals with lipodystrophy will be studied once.
6 individuals without lipodystrophy and about to commence protease inhibitor therapy will be studied once before starting therapy then one month later and again three months after starting therapy.

Appendix 2.0 Study protocol

1. Each patient will have fasting blood drawn to measure glucose, insulin, triglycerides, cholesterol, and non-esterified fatty acids.
2. Height, weight, waist and hip measurements.
3. Skin fold measurements at four sites, biceps, triceps, subscapular and suprailiac.
4. Resting energy expenditure (REE) will be measured to assess the metabolism of the subject.
5. The subject will then receive a test meal that contains a labelled fat (palmitate) that is present naturally in the body and in foods we consume, and will remain fasted for the next 10 hours. The amount excreted in the faeces will be assessed by collecting the faeces for 3 days after the study – this is a test of fat absorption. The amount oxidised or "burnt up" by the body will be assessed by measuring carbon dioxide in the breath over 10 hours and then at home 15 hours and again after 24 hours. The transport and distribution of fat in the blood will be measured in serial blood samples taken at hourly intervals for 10 hours.

FOLLOW UP

For patients without lipodystrophy, at one month and three months after commencing anti-protease, the same protocol will be undertaken.

USEFULNESS OF RESULTS

This study will identify which elements of fat metabolism are altered in patients receiving protease inhibitors.

This will help in the following;

1. identify treatment measures more rationally
2. identify when the derangements of fat metabolism occur
3. identify the characteristics of those patients most likely to develop lipodystrophy

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

If you have any queries, please don't hesitate to contact me, via the hospital switchboard;

Chelsea & Westminster Hospital Tel: 0181 746 8000

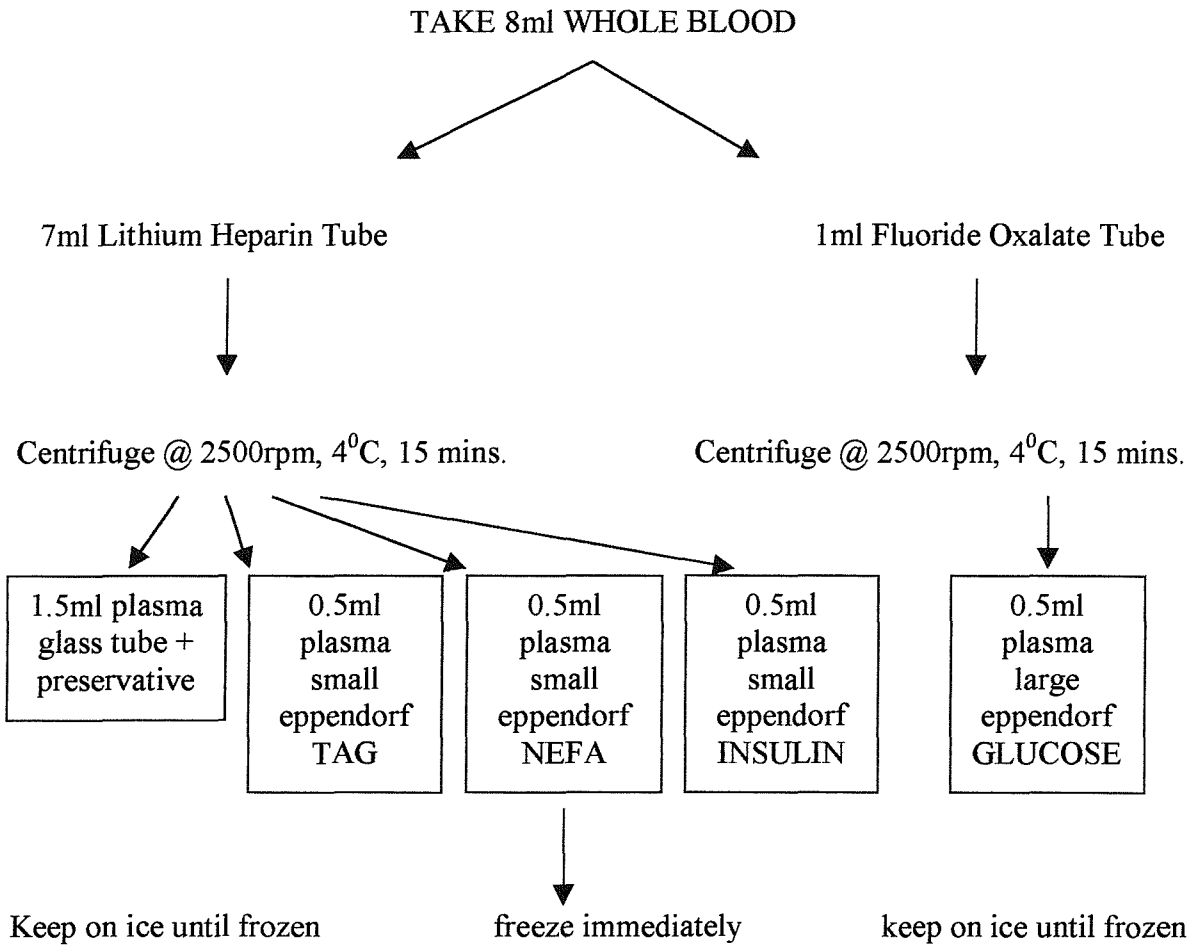
Dr John Morlese Bleep 5615

Appendix 2.1 Study timeline

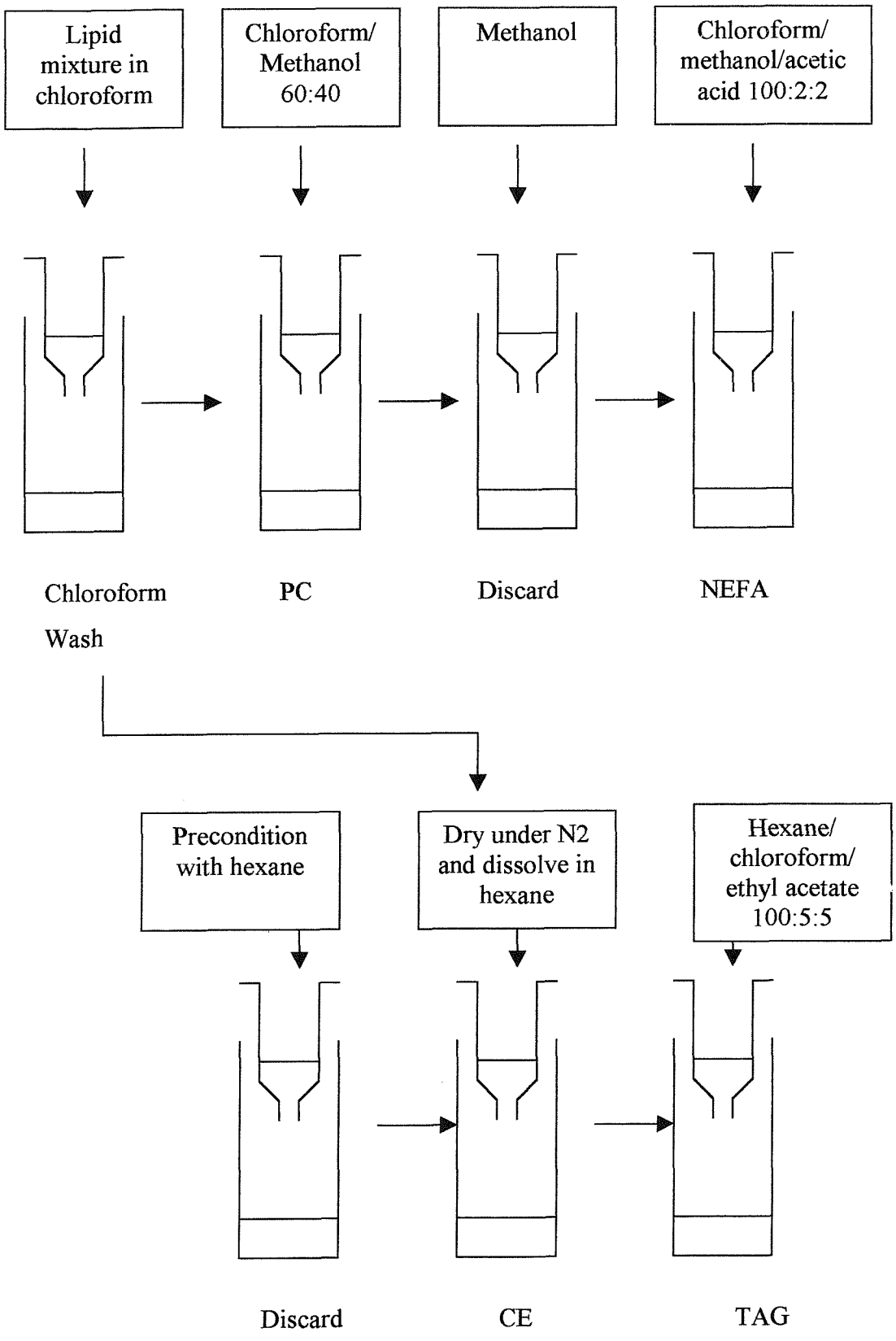
Anthropometry													X
Breath sample	X			X		X		X	X	X	X		X
Blood sample	X		X	X	X	X	X	X	X	X	X		X
IC	___			___		___		___	___	___	___		___
Time (hours)	-0.5	0	0.5	1	1.5	2	2.5	3	4	5	6		7

Test meal & labelled emulsion

Appendix 3.0 Blood preparation schema



Appendix 4.0 Bond elut schema



Appendix 5.0 Addendum following oral examination

Appendix 5.1 How do current case definitions of lipodystrophy compare with the patients recruited in these studies?

The first relatively widely adopted definition of lipodystrophy was presented at the European Symposium on Lipodystrophy and HIV infection in Marrakech (2000) and was referred to as the Marrakech classification of Lipodystrophy. This classification was based on subjective measures for the most part whereby both the patient and physician had to agree on the lipodystrophy classification i.e. lipoatrophy alone, lipohypertrophy alone, mixed morphology or metabolic changes alone. Only the metabolic changes employed objective measures. Prior to this, most studies employed the subjective measure alone of patient and physician agreement on either the presence or severity of lipodystrophy. The patients recruited for the work reported in this thesis were approached between 1998 and 2000, prior to the availability of the Marrakech classification.

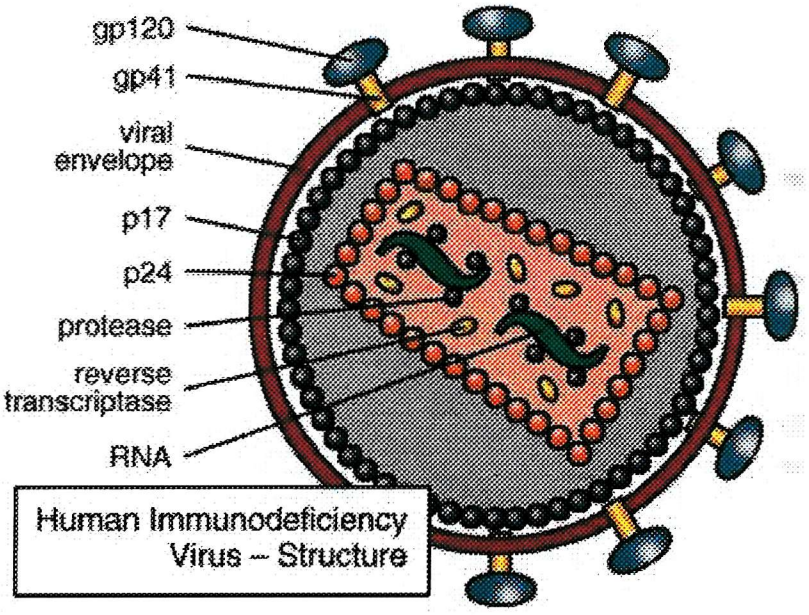
Since this initial classification system, there have been several reported case definitions of the lipodystrophy syndrome. Carr, on behalf of the HIV Lipodystrophy Case Definition Study Group, presented a definition at the 9th Conference on Retroviruses and Opportunistic Infections (2002). Over one thousand patients were recruited to this study from sites in the United States, Europe and Australasia. From these patients, those with lipodystrophy were identified again by subjective assessment of both patient and physician with the severity of lipoatrophy defined as moderate (readily noticeable by patient or physician) or severe (readily noticeable by a casual observer). Controls were also identified within this group, i.e. those with no patient or physician observed lipodystrophy. Where lipohypertrophy was present alone or where the patient and physician could not agree, these patients were non-assigned. The cases and controls then underwent assessment of demographics, antiretroviral and metabolic drug history, HIV disease, body composition and metabolic status. A model which included low trunk:peripheral fat ratio (DEXA), report of abdominal bloating, low leg fat % (DEXA), lower alcohol consumption, higher waist:hip ratio, higher total:HDL cholesterol ratio, higher anion gap, report of increased bleeding tendency, higher VAT:SAT ratio (CT), and greater age had a sensitivity of 84.4% and specificity of 81.4%. This would suggest that even with the use of expensive and complicated analytical tools such as CT and DEXA, this model fails to correlate with the subjective identification of lipodystrophy in 20% of patients.

A further report from Grunfeld at the XIV International AIDS Conference (2002) would suggest that in a US population, increased visceral adiposity is more prevalent in a non-HIV population than in an HIV-lipodystrophy group, suggesting that abdominal fat accumulation may not be part of the lipodystrophy syndrome as once thought. Therefore, there appears to be some difficulty in obtaining a case definition for HIV-lipodystrophy. It is possible that as previously discussed this syndrome is multifactorial in aetiology and that the presence or absence of some of these factors may result in a different presentation within a spectrum of symptoms associated with the lipodystrophy syndrome. If this were the case and, for example, PI drugs produced one set of symptoms, NRTI produced a distinctly different set of symptoms, and the combination of the two resulted in a mixed presentation of these, then it would be apparent that a single case definition would be extremely difficult to define.

When recruiting patients for the studies reported in this thesis, there was and still remains no case definition that can be applied to the patient population in order to easily identify those with lipodystrophy. At the time, DEXA and CT scans were not available due to a lack of funding. However, in order to avoid employing subjective measures alone to identify patients with lipodystrophy, fasting plasma triglyceride was measured in all patients prior to entry into the study. Previously, Christeff *et al* (1999) had shown a correlation between those patients identified with lipodystrophy by subjective clinical score and elevated plasma triglyceride.

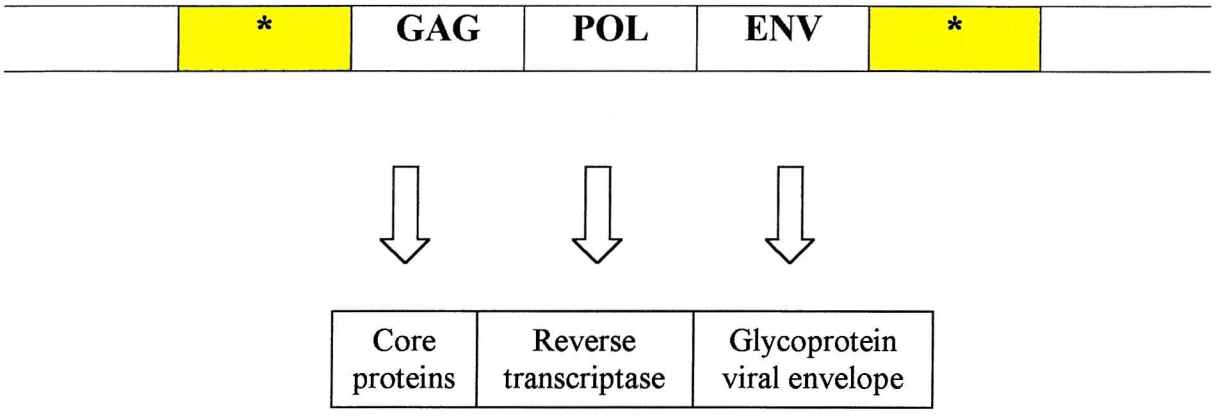
In order to avoid misclassification of lipodystrophy patients, only those patients with markedly elevated plasma triglyceride were included in the study. While it could be argued that these patients may not represent the lipodystrophy population as a whole, the use of patients with severe metabolic perturbation in addition to the normal subjective clinical assessment of lipodystrophy phenotype was the most accurate method of lipodystrophy diagnosis available at the time of recruitment.

Appendix 5.2 **How does the HIV virus enter the host cell and replicate?**



The above diagram shows the structure of HIV. The gp120 and gp41 Env proteins aid viral entry into the host cell, while the p17 and p24 Gag proteins are structural components of the virion. The virus has a 9000 bp genome (shown below) that may incorporate mutations each time it replicates due to a lack of proofreading capacity.

Retrovirus genome:



*initial short repeats – expanded in DNA copy to form long tandem repeats (LTR), non-coding regions containing enhancer and promoter regions that facilitate expression of the viral genome by host machinery.

The protein that is responsible for viral entry into the host cell is the Env protein. This protein is a trimer consisting of 3 gp120 subunits that are attached to 3 gp41 subunits. While it appears that the host CD4 receptor and an appropriate coreceptor are prerequisites for viral entry into the cell, the virus attachment to the cell can be independent of these. HIV-1 can bind efficiently to dendritic cells via the surface protein DC-SIGN [Geijtenbeek *et al.* 2000]. Once bound the virus can either infect that cell more efficiently or be presented to an adjoining cell that expresses CD4 and a coreceptor, such as a T cell. Therefore, dendritic cells through the normal antigen presenting process can result in more efficient infection of circulating T cells.

Once attached to the cell surface, the gp120 subunit of Env binds to the host CD4 receptor [Kwong *et al.* 1998]. This process results in structural changes in gp120 whereby variable regions or V-loops on gp120 are repositioned to expose a highly conserved area of this protein, termed the bridging sheet [Rizzuto *et al.* 1998]. These bridging sheets bind to the host coreceptors CCR5 or CXCR4 and HIV-1 strains are designated as R5 (those that bind CCR5), X4 (those that bind CXCR4) or R5X4 (those that bind both) [Berger *et al.* 1998]. While R5 viruses appear to be associated with newly infected patients, X4 viruses may take longer to emerge but their presence is associated with accelerated disease progression [Connor *et al.* 1997].

Once receptor and coreceptor binding has occurred, the Env protein causes membrane fusion of the virus and host cell similar to that observed with both the Influenza and Ebola viruses. Binding triggers a formational change in gp41 to expose a fusion peptide, a hydrophobic stretch of amino acids that inserts into the cellular membrane [Doms & Moore 2000]. At this point, gp41 is an integral component of both viral and host membranes and a further conformational change in this protein brings the host and viral membrane into close proximity [Chan *et al.* 1997; Weissenhorn *et al.* 1997]. This protein structural transition is thought to generate free energy, which is sufficient to generate lipid mixing and membrane fusion [Melikyan *et al.* 2000].

Once inside the cell, the particle is uncoated down to viral core consisting of a protein shell composed of viral capsid protein (p24) surrounding an internal ribonucleoprotein complex i.e. viral RNA and associated proteins such as reverse transcriptase, integrase and proteins associated with the core particle. While the mechanism for the disassembly of this core is unknown, it appears that a stable core structure is crucial for viral replication [Forshey et al. 2002]. However, disassembly of the core must occur in such a way that the genetic information and associated enzymes remain intact and these

components are directed to the appropriate cellular compartment. For retroviruses this is the nucleus. Entry into the nucleus is dependent on the presence of nuclear localisation signals (NLS), which allow subviral particles to cross the nuclear pore. Within the viral core or viral preintegration complex (PIC), several proteins contain this NLS sequence, including the viral integrase, Vpr and matrix proteins [Kawano & Koyanagi, 2002; Sherman et al. 2002]. In addition, nuclear import of the PIC is enhanced by a cDNA intermediate of reverse transcription known as the "central DNA flap", although the mechanism for this interaction is unknown.

In order to incorporate the viral genetic material into the host genome, the viral single stranded RNA must then be transcribed by the viral reverse transcriptase enzyme to form a double stranded DNA intermediate. This dsDNA intermediate is longer than the viral genome length and is often referred to as the provirus. A host transfer RNA (tRNA) primer begins synthesis by binding to the viral genome at a 3' long terminal repeat (LTR) and partially unfolding to base pair with a complimentary segment of the viral RNA [Misra & Knox 1998; Doublie et al. 1998]. Reverse transcriptase then uses this primer to form a complimentary strand of DNA, forming a DNA-RNA hybrid. Next the viral ribonuclease H (RNAase H, a subunit of the viral reverse transcriptase enzyme) digests the original RNA strand, leaving only a few purines to act as a primer for the second DNA strand. Once synthesis is complete, RNAase H removes the original tRNA primer [Misra & Knox 1998]. The reverse transcriptase enzyme consists of a "fingers" and a "palm" domain and upon binding free nucleotides the fingers close down on the palm, trapping the template strand and nucleotide. When these finger domains open again, the pyrophosphate molecule generated when the nucleotide is added to the template is released and the fingers can take up the next nucleotide [Huang et al. 1998]. Reverse transcriptase can incorporate twenty nucleotides per second and each nucleotide is added by a reaction between the 3' hydroxyl group of the growing DNA strand and the alpha-phosphate group of the nucleotide [Doublie et al. 1998]. As the HIV I reverse transcriptase is both tolerant of non-standard base pairs and has no exonuclease or "proofreading" capacity, there is a high rate of mutation within the viral genome per replication cycle. The viral protein Vif has also been shown to be important in this reverse transcription process, although the role of Vif in the process remains unknown [Goncalves et al. 2002].

Once generated, the dsDNA provirus must integrate into the host genome. This process involves DNA circularisation, modulated by the host DNA-binding component Ku [Jeanson et al. 2002]. Integration of the viral DNA into the host chromosome is catalysed

by the Mg dependent viral integrase through 3' end processing of the viral DNA and strand transfer [Grobler et al. 2002]. Recent evidence suggests that the provirus integrates into the host genome preferentially in active gene sites, particularly those genes that are activated as a result of HIV-1 infection of the cell [Schroder et al. 2002]. Replication of the viral DNA is switched on by the viral Tat protein, which binds the positive transcription elongation factor complex b (P-TEFb) in the HIV-1 promoter region, P-TEFb then binds nascent HIV-1 trans-activation response region (TAR) RNA, creating a stable ribonucleoprotein complex. This process activates elongation of RNA polymerase II transcription at the HIV-1 promoter [Richter et al. 2002].Viral Tat protein is regulated by the host protein Tip110 [Liu et al. 2002].

The resulting viral mRNA that is produced is exported from the nucleus bound to the viral RNA binding protein Rev [Heguy 1997]. From the RNA transcript, the viral protease and the Gag, Pol and Env polyproteins are translated. The viral protease is an aspartyl endopeptidase that catalyses the cleavage of the gag and gag-pol poly or precursor proteins at Tyr-Pro or Phe-Pro sequences [Tomasselli & Heinrikson 2000]. The Env polyprotein is cleaved by a cellular protease. Protease action produces mature viral proteins that can be incorporated into the newly assembled virus particles (virions) for release from the cell either by lysis of the cell or budding from the cell membrane.

Appendix 5.3 How do the antiretroviral drugs interact with HIV replication and how could toxicity result?

As discussed in the literature review (Chapter 2), the two main classes of drugs used as antiretroviral therapy are reverse transcriptase inhibitors (RTI) and protease inhibitors (PI). The sites of action of these compounds are also discussed in Chapter 2. Of the RTI drugs there are two types, nucleoside (NRTI) and non-nucleoside (NNRTI). NRTI's consist of deoxynucleoside triphosphate analogues of natural nucleotides e.g. thymidine, guanosine, cytosine and adenosine, but without the 3' hydroxyl group necessary for DNA chain elongation. An example of this is AZT (3'azido-2',3'-dideoxythymidine) which has an azido group (N_3) instead of an hydroxyl group. Incorporation of NRTI's into the nascent viral DNA by viral reverse transcriptase terminates chain elongation to prevent the formation of provirus intermediate. While in vitro work has suggested that these NRTI analogues can be incorporated by viral reverse transcriptase with high efficiency relative to the natural substrates [Ray et al. 2002], several mutations have been identified that lead to viral resistance to these analogues [Coffin et al. 1997; Sluis-Cremer et al. 2000]. These mutations in reverse transcriptase are thought to alter the enzyme discrimination between NRTI's and the natural substrates, alter the enzyme primer/template interactions or enhance removal of the chain-terminating residue from the nucleoside analogue.

Non-nucleoside reverse transcriptase inhibitors (NNRTI) bind allosterically, altering the structure, and hence then function of reverse transcriptase. All NNRTI's bind in a hydrophobic pocket near the polymerase active site causing a conformational change that effectively locks the polymerase active site in an inactive conformation [Esnouf et al. 1995]. Similar to the NRTI's, mutations in the reverse transcriptase enzyme may also confer resistance to this drug class. However, NNRTI use may also result in mutations that modulate an AZT-resistant strain to become AZT sensitive [Misra & Knox 1998].

Protease inhibitors target the viral protease enzyme and act a reversible and selective inhibitor by binding in the protease active site. PI's such as Saquinavir are reported to have a 50,000-fold lower affinity for human proteases than the viral protease. However, there are several host cell aspartate proteases such as Cathepsins D and E. These are lysosomal proteases that are believed to play a role in macrophage function and it is possible that HIV protease inhibitors may inhibit these host proteases exacerbating immunosuppression in AIDS. However, in vitro work using physiological concentrations of PI's does not support this hypothesis [Bugelski et al. 1992]. To date, no further studies

have shown PI inhibition of other host aspartate proteases. However, Carr et al. (1998) have reported the viral protease enzyme active site shares a degree of homology with both the lipoprotein receptor related protein (LRP) and the cis retinoic acid binding protein (CRABP) suggesting that PI's may interact with these proteins. This theory is discussed within the general discussion (Chapter 9).

The NRTI's are known to be substrates for polymerase gamma (Pol γ) and can be incorporated into mitochondrial DNA during replication. The efficiency with which these analogues are incorporated both by the viral reverse transcriptase and the mitochondrial Pol γ enzyme in comparison to their natural nucleoside counterparts appears to be related to stereochemical selectivity resulting in differential toxicity between compounds [Anderson 2002]. Yamaguchi et al. (2002) have shown the incorporation of AZT into mitochondrial DNA resulting in reduced ATP generation and oxidative stress. However, as with many of the reported in vitro studies, a supraphysiological concentration of drug was used (approximately 2500 times higher than that observed in vivo). While the viral reverse transcriptase and Pol γ do appear to incorporate nucleoside analogues, the host nuclear polymerases (α and β) are able to differentiate between the analogues and their natural counterparts, showing a high specificity for the naturally occurring nucleosides [Yamaguchi et al. 1994], even when NRTI are provided at 150 times the normal in vivo concentration [Huang et al. 1990]. This would suggest that while NRTI's may result in cellular toxicity through incorporation into mitochondrial DNA, toxicity may not be a result also of incorporation into host nuclear DNA.

Appendix 5.4 Does this work support or refute the mitochondrial toxicity hypothesis as suggested by Brinkman et al. (1999)?

The mitochondrial or Pol γ hypothesis for NRTI toxicity was first reported by Brinkman et al. (1998). This paper presented evidence showing clearly that NRTI incorporation into mitochondrial DNA was possible and this in turn could result in mitochondrial mutations. Mitochondrial toxicity was observed in a trial of Fialuridine, a nucleoside analogue used to treat Hepatitis B, where a reduction in mitochondrial DNA was observed with the highest impact being in the most metabolically active tissues (muscle > liver > kidney > heart) [Lewis et al. 1997]. In the Brinkman paper, further evidence was cited for the toxic influence of HIV NRTI's on mitochondria in myocytes, neurones, pancreatic tissue, bone marrow and hepatocytes and these toxic events linked to the side effects reported with NRTI use such as myopathy, pancreatitis, liver failure and lactic acidosis. This relationship between mitochondrial DNA reduction and symptoms of nucleoside analogue associated toxicity is further supported by the high incidence of adverse events, similar to those described in HIV NRTI toxicity, in the Fialuridine trial, resulting in the trial terminating at 13 weeks.

In a subsequent paper, Brinkman et al. (1999) described how this mitochondrial toxicity theory could be applied to explain the lipoatrophy observed in HIV-lipodystrophy. This subsequent theory was based on the similarities between lipodystrophy and multiple symmetrical lipomatosis (MSL) and the suggested link between MSL and mitochondrial toxicity. However, since the publication of this article, it has been shown that MSL does not appear to be associated with mitochondrial cytopathy, either in the form of mitochondrial morphological alterations, the mitochondrial DNA content or the biochemistry of the mitochondrial respiratory chain [Munoz-Fernandez et al. 2001]. The authors conclude that MSL may occur as a result of disordered lipid metabolism. Given this finding, the underlying basis for the NRTI mitochondrial toxicity theory for lipoatrophy should be questioned. In addition to this, it is unclear why mitochondrial toxicity would result in apoptosis only in subcutaneous adipocytes given that the major mitochondrial toxic effects would be expected in muscle, but muscle wasting is not reported commonly as a symptom of HIV-lipodystrophy. In the work reported in this thesis, there were no significant differences in lean body mass between either the NRTI treated or untreated HIV positive subjects.

In the general discussion, I have made the statement; "this work does not support the theory that reduced mitochondrial function is important in the aetiology of lipodystrophy". This statement is based both on the findings for lean body mass stated above, but also on the finding that the oxidation of labelled fatty acid was not significantly different between HIV-positive subjects treated with NRTI's, HIV-positive subjects not treated with NRTI's and HIV-negative healthy controls, despite the presence of marked lipoatrophy in the NRTI-lipodystrophy and PI/NRTI-lipodystrophy groups. The statement therefore, is based on the assumption that the recovery of label on breath is an indirect measure of mitochondrial function. The largest contribution to this oxidation of substrate would be expected to be from those tissues that are both most metabolically active and relatively quantitatively important, such as muscle and liver, the two tissues reported to be most susceptible to mitochondrial DNA depletion with the use of nucleoside analogues.

The use of the recovery of a labelled substrate to indicate altered mitochondrial function is not unique and several investigators have validated this technique using ^{13}C-labelled ketoisocaproate to measure hepatic mitochondrial function [Michaletz et al. 1989; Lauterburg et al. 1995]. Recovery of the label on breath relies of the oxidative decarboxylation and dehydrogenation of the compound by mitochondria in a process similar to that of fatty acid oxidation.

Therefore, based on the assumption that the recovery of label reflects the mitochondrial oxidation of labelled substrate in the tissues most likely to obtain reduced mitochondrial function as a result of the NRTI mitochondrial toxicity, it appears that this work does not support a theory proposing reduced mitochondrial function in lipodystrophy aetiology. However, it is likely that NRTI can alter mitochondrial DNA content and the degree of DNA mutation in specific tissues, resulting in NRTI toxicity symptoms such as myopathy in some patients. What remains unclear is how this relates to the aetiology of lipodystrophy and there appear to be some key outstanding questions in this area. For example, if NRTI's can cause mitochondrial mutations in muscle, liver, heart, pancreas and nerves, then why does apoptosis appear to be isolated to subcutaneous adipocytes? Are lipoatrophy and "classical" NRTI toxicity symptoms such as myopathy and lactic acidosis related? In chronic mitochondrial disease such as MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke) progressive worsening of symptoms is observed. Given the estimate that 80% of mitochondrial DNA must be mutated to result in a loss of function, how and why do patients with lipodystrophy have improved symptoms on stopping HAART? Are there other direct or indirect interactions between NRTI and

mitochondria that do not involve the inclusion of ddNTP by Pol γ i.e. NRTI interactions with the mitochondrial membrane [Hobbs et al. 1995; Barile et al. 1998; Masini et al. 1999], increases in reactive oxygen species [Nulton-Persson & Szweda 2001] or alterations in fatty acid [Sreekumar et al. 2002] or phospholipid concentration [Seidman et al. 2002]?

This thesis was not designed to address these questions. In addition, no comment can be made as to the extent of mitochondrial DNA mutations or deletions in specific tissues of the patients studied, as this was not assessed. However, it does appear that at a whole body level, substrate oxidation by mitochondria is not impaired.

Appendix 5.5 Do alterations in androgen levels play a role in the aetiology of lipodystrophy?

Reduced free testosterone levels have been observed in HIV-positive females and males with AIDS wasting syndrome (AWS) and correlated with reduced muscle mass [Grinspoon et al. 1997, 1996]. The authors suggest this relationship may be mediated by both androgen deficiency and growth hormone (GH) resistance, as GH was found to be elevated, while IGF-1 was reduced in males with AWS. Hypoandrogenaemia in AWS was thought to be primarily due to the secondary effects of severe illness, possibly mediated through cytokines [Grinspoon et al. 1994].

With the increasing prevalence of HAART use, the incidence of AWS has reduced. However, it is unclear if these previously reported alterations in androgen levels play a role in the aetiology of the HAART-associated lipodystrophy syndrome. Norbiato et al. (2000) have reported a fat redistribution syndrome in HAART treated women, characterised by a progressive enlargement of breast and abdominal girth with fat loss in the lower limbs associated with reduced production of TNF-alpha, IL-10 and increased IL-12. While this may suggest that these alterations could be a result of changes in androgen levels mediated by cytokine production, no differences in hormonal status were reported between this group and a HAART treated group without fat redistribution.

In contrast to the studies of AWS in the pre-HAART era, Hadigan et al. (2000b) have reported an increase in free testosterone levels in females with lipodystrophy in comparison to both non-lipodystrophic HIV-positive females and healthy controls. The lipodystrophy group also showed reduced sex hormone binding globulin levels and an increased LH:FSH ratio. While it appears that hyperandrogenaemia may be a further feature of the HIV-lipodystrophy syndrome in women, the role of elevated androgen levels concurrent with changes in cytokine production, in the aetiology of lipodystrophy require further investigation.

Chapter 11.0 Reference List

Abrumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. J Biol Chem 1993;268:17665-8.

Aldeen T, Wells C, Hay P, Davidson F, Lau R. Lipodystrophy associated with nevirapine-containing antiretroviral therapies. AIDS 1999;13:865-7.

Amri EZ, Bonino F, Ailhaud G, Abumrad NA, Grimaldi PA. J Biol Chem 1995;270:2367-71.

Anderson KS. Perspectives on the molecular mechanism of inhibition and toxicity of nucleoside analogs that target HIV-1 reverse transcriptase. Biochim Biophys Acta 2002;1587(2-3):296-9.

Aukrust P, Haug CJ, Ueland T. Decreased bone formative and enhanced resorptive markers in human immunodeficiency virus infection: indication of normalisation of the bone remodelling process during highly active antiretroviral therapy. J Clin Endocrin & Metab 1999;84:145-50.

Autran B, et al. Positive effects of combined antiretroviral therapy on CD4 T cell homeostasis and function in advanced HIV disease. Science 1997; 277: 112.

Balasse EO, Ooms HA. Role of plasma free fatty acids in the control of insulin secretion in man. Diabetol 1973; 9: 145-51.

Baril L, Beucler I, Valantin M, et al. Low lipolytic enzyme activity in patients with severe hypertriglyceridaemia on highly active antiretroviral therapy. AIDS 2001;5:415-7.

Barile M, Valenti D, Quagliarello E, et al. Mitochondria as cell targets of AZT (zidovudine). Gen Pharmacol 1998;31:531-8.

Behrens G, Schmidt H, Meyer D, Stoll M, Schmidt R. Vascular complications associated with use of protease inhibitors. Lancet 1998;351:1958-60.

Behrens G, Dejam A, Schmidt H, Balks HJ, Brabant G, Korner T, Stoll M, Schmidt RE. Impaired glucose tolerance, beta cell function, and lipid metabolism in HIV patients under treatment with protease inhibitors. AIDS 1999;13:F63-F70.

Beil SJ, Mascioli EA, Forse RA, et al. Nutritional support and HIV. Parasitol 1993; 107: S53-67.

Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science 2001;292(5521):1552-5.

Benhamou Y, Hilmarsdottir I, et al. Association of lipid accumulation in small intestinal mucosa with decreased serum triglyceride and cholesterol levels in AIDS. Dig Dis Sci 1994; 39(10): 2163-9.

Bennoson J Thesis: The impact of manipulating the macronutrient composition of meals on postprandial lipid metabolism, March 2000.

Berger EA, Doms RW, Fenyo EM, et al. A new classification for HIV-1. Nature 1998; 391:240.

Berk PD, Potter BJ, Sorrentino D, Stump D, Kiang CL, Zhou SL, Horio Y, Wada H. Ann NY Acad Sci 1990;585:379-85.

Berk PD, Wada H, Horio Y, Potter BJ, Sorrentino D, Zhou SL, Isola LM, Syump D, Kiang CL, Thung S. Proc Natl Acad Sci USA 1990;87:3484-8.

Berthold HK, Parhofer KG, Ritter MM. Influence of protease inhibitor therapy on lipoprotein metabolism. J Intern Med 1999;246:567-75.

Boden G. Free fatty acids, insulin resistance and type 2 diabetes mellitus. Proc Assco Am Physicians 1999; 111(3): 241-8.

Bonen A, Luken J, Liu S, Dyck D, Kiens B, et al. Palmitate transport and fatty acid transporters in red and white muscles. Am J Physiol 1998;275:E471-E478.

Bonnet E, Ruidavets JB, Tuech J. Apoprotein C-III and E-containing lipoparticles are markedly increased in HIV-infected patients treated with protease inhibitors: association with the development of lipodystrophy. J Clin Endocrin & Metab 2001;86:296-302.

Brenna J. Use of stable isotopes to study fatty acid and lipoprotein metabolism in man. Prostaglandins Leukot Essent Fatty Acids 1997;57:467-72.

Brinkman K, Hofstede H, Burger D, Smeitinik J, Koopmans P. Adverse effects of reverse transcriptase inhibitors: mitochondrial toxicity as common pathway. AIDS 1998;12:1735-44 (abstr).

Brinkman K, Smeitinik J, Romjin J, Reiss P. Mitochondrial toxicity induced by nucleoside-analogue reverse transcriptase inhibitors is a key factor in the pathogenesis of antiretroviral-therapy-related lipodystrophy. Lancet 1999;354:1112-5.

Bucki R, Zendzian-Piotrowska M, Nawrocki A, Gorski J. Effect of increased uptake of plasma fatty acids by the liver on lipid metabolism in the hepatocell nuclei. Prostaglandin Leukot Essent Fatty Acids 1997; 57(1): 27-31.

Bugelski PJ, Kaplan JM, Hart TK, et al. Effect of HIV protease inhibitor on human monocyte function. AIDS Res Hum Retroviruses 1992;8(12):1951-8.

Burdge G, Wright P, Jones AE, Wootton SA. A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid phase extraction. Br J Nutr 2000;84:1-8.

Buss N, Duff F. Protease inhibitors in HIV infection. Lipodystrophy may be a consequence of prolonged survival [letter]. BMJ 1999;318:122.

Calles-Escandon J, Sweet L, Ljungqvist O, Hirshman MF. Life Sci 1996;58:19-28.

Campbell L, Gumbleton M, Ritchie K. Caveolae and the caveolins in human disease. Adv Drug Deliv Rev 2001;49(3):325-35.

Caron M, Auclair M, Vigouroux C, Glorian M, Forest C, Capeau J. The HIV protease inhibitor Indinavir impairs adipocyte differentiation and induces insulin resistance by probably altering ADD-1/SREBP-1 maturation. Antiviral Therapy 2000;5(suppl.5):4. Abstract 03.

Carr, A, Samaras, K, Chisholm, D, and Cooper, D. Pathogenesis of HIV-1 protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. Lancet 1998; 352: 1881-1883.

Carr, A, Samaras, K, Burton, S, Law, M, Freund, J, Chisholm, D, and Cooper, D. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998;12: F51-F58.

Carr, A, Samaras, K, Thorisdottir, A, Kauffman, G, Chisholm, D, and Cooper, D. Diagnosis, prediction and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet 1999; 353: 2093-2099.

Carr, A. HIV protease inhibitor-induced lipodystrophy syndrome. AIDS Rev 1999; 1: 29-36.

Caso G, Garlick PJ, Gelato MC, McNurlan MA. Lymphocyte protein synthesis is increased with progression of HIV disease to AIDS. Clin Sci 2001; 101:583-589.

Chan DC, Fass D Berger JM, Kim PS. Core structure of gp41 from the HIV envelope glycoprotein. Cell 1997; 89:263-273.

Christeff, N, Lortholary, O, Casassus, P, Thobie, N, Dalle, M, Veyssier, P, Guillevin, L, and Nunez, E. Serum lipid concentration with reference to the clinical and immunological status of HIV infected men. A..Med Interne 1995; 146(7): 490-495.

Christeff N, Melchior JC, de Truschis P, Perronne C, Nunez EA, Gougeon ML. Lipodystrophy defined by a clinical score in HIV-infected men on HAART: correlation between dyslipidaemia and steroid hormone interactions. AIDS 1999; 13: 2251-60.

Christeff N, Melchior JC, de Truschis P, Perronne C, Gougeon ML. Increased serum interferon alpha in HIV-1 associated lipodystrophy syndrome. Eur J Clin Inv 2002; 32: 43-50.

Chrousos GP, Kino T. The HIV Vpr gene product can cause glucocorticoid hypersensitivity or resistance: implications for the pathogenesis and clinical presentation of AIDS. Psychoneuroendocrinology 1997; 22:S160.

Churchill DR, Pym AS, Babiker AG, Back DJ, Weber J. Hyperlipidaemia following treatment with protease inhibitors in patients with HIV-1 infection. Br J Clin Pharmacol 1998;46:518-9.

Clasey, J, Bouchard, C, Teates, C, Riblett, J, Thorner, M, Hartman, M, and Weltman, A. The use of anthropometric and DEXA measures to estimate total abdominal and abdominal visceral fat in men and women. Obesity Res 1999; 7(3): 256-264.

Cloyd MW, Lynn WS. Perturbation of host cell membrane is the primary mechanism of HIV cytopathology. Virology 1991; 181(2): 500-11.

Connor RI, Sheridan KE, Ceradini D, Choe S, Landau NR. Change in coreceptor use correlates with disease progression in HIV-1 infected individuals. J Exp Med 1997;185:621-628.

Constans J, Peuchant E, Pellegrin JL, et al. Fatty acids and plasma antioxidants in HIV patients: correlation with nutritional and immunological status. Clin Biochem 1995; 28(4): 421-6.

Coplen T, Kendall C, Hopple J. Comparison of stable isotope reference samples. Nature 1983; 302: 236-8.

Craig H. Isotopic standards for carbon and oxygen and corrective factors for mass-spectrometric analysis of carbon dioxide. Geochem Cosmochim Acta 1957; 12: 133-49.

Diomede L, Albani D, Bianchi M, Salmona M. Endotoxin regulates the maturation of sterol regulatory element binding protein-1 through the induction of cytokines. Eur Cytokine Netw 2001;12(4):625-30.

Dobs AS, Dempsey MA, Ladenson PW et al. Endocrine disorders in men infected with HIV. Am J Med 1988; 84(3): 611-6.

Domingo P, Matias-Guiu X, Pujol RM, Francia E, Lagarda E, Sambeat MA, Vazquez G. Subcutaneous adipocyte apoptosis in HIV-1 protease inhibitor-associated lipodystrophy. AIDS 1999;13:2261-7.

Doms RW, Moore JP. HIV-1 membrane fusion: targets of opportunity. J Cell Biol 2000; 151:F9-F13.

Doublie S, Ellenberger T. The mechanism of action of T7 DNA polymerase. Curr Opin Struct Biol 1998;9(6):704-12.

Durnin J, Rahaman M. The assessment of the amount of fat in the human body from measurements of skinfold thickness. Br J Nutr 1967;21:681-9.

Durnin J, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77-97.

Emken E, Adolf R, Rohwedder W, Gulley R. Influence of linleic acid on desaturation and uptake of deuterium-labeled palmitic and stearic acids in humans. Bioclinica et Biophysica Acta 1993;1170:173-81.

Engelson E, Kotler D, Tan Y, Agin D, Wang J, Pierson RN, Heymsfield SB. Fat distribution in HIV-infected patients reporting truncal enlargement quantified by whole-body magnetic resonance imaging. Am J Clin Nutr 1999;69:1162-9.

Eschwege E *et al.* Horm Metab Res 1995; 17(suppl):41-46.

Esnouf R, Ren J, Ross C, et al. Mechanism of inhibition of HIV-1 reverse transcriptase by non-nucleoside inhibitors. Nat Struct Biol 1995;2(4):303-8.

Espenshade PJ, Cheng D, Goldstein JL, Brown MS. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element binding proteins. J Biol Chem 1999;274(32):795-804.

EU Comparison 1960-1999: Social Trends Dataset: Percentage of the population aged 65 and over: http://www.statistics.gov.uk/statbase/ssdataset.asp?vlnk=3403.

Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Pearce SF, Silverstein RL. J Biol Chem 1999;274:19055-62.

Feingold K, Adi S, Staprins I et al. Diet affects the mechanisms by which TNF stimulates hepatic triglyceride production. Am J Physiol 1990;259:E177-E184.

Fellander G, Bolinder J, Ungerstedt U, et al. Carbohydrate three carbon metabolism of adipose tissue during surgery. Nutr 1996; 12: 589.

Fernandez-Miranda C, Pulido F, Carrillo JL, et al. Lipoprotein alterations in patients with HIV infection: relation to cellular and humoral immune markers. Clin Chim Acta 1998; 274: 63-70.

Ferrannini E. The theoretical bases of indirect calorimetry: A review. Metabolism 1988;37:287-301.

Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn K. Postprandial lipaemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. Am J Clin Nutr 1996;63:36-41.

Fielding CJ. Caveolae and signaling. Curr Opin Lipidol 2001;12(3):281-7.

Fielding CJ, Fielding PE. Cholesterol and caveolae: structural and functional relationships. Biochim Biophys Acta 2000;1529(1-3):210-22.

Fitscher BA, Riedel HD, Young KC, Stremmel W. Biochem Biophys Acta 1998;1443:381-5.

Forshey BM, von Schwedler U, Sundquist WI, Aiken C. Formation of a HIV type 1 core of optimal stability is crucial for viral replication. J Virol 2002;76:5667-77.

Frayn K. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 1983;55:628-34.

Frayn K. Metabolic Regulation A Human Perspective. London: Portland Press, 1997.

Frohnert BI, Hui TY, Bernlohr DA. J Biol Chem 1999;274:3970-7.

Fromm, Hoffman. Lancet 1971;2:621.

Garg A, Grundy SM. Treatment of dyslipidaemia in NIDDM with lovastatin. Am J Cardiol 1988; 62: 44-49J.

Garrow JS, James WPT. Human Nutrition and Dietetics. 9th Ed.1993. Churchill Livingstone, London.

Geijtenbeek TBH, Kwon DS, Torensma R, et al. DC-SIGN a dendritic cell-specific HIV-1 binding protein that enhances trans-infection of T cells. Cell 2000;100:587-597.

Gerber GE, Mangroo D, Trigatti BL. Mol Cell Biochem 1993;123:39-44.

Germinario RJ, Continelli L, Pratt S. Sugar transport regulation: comparative characterization of the effect of NADH CoQ reductase deficiency in two cell culture systems. Proc Soc Exp Biol Med 2000; 225(2): 116-22.

Gervasoni C, Ridolfo A, Trifiro G et al. Redistribution of body fat in HIV-infected women undergoing combined antiretroviral therapy. AIDS 1999;13:465-71.

Gharakhanian S. The HIV-associated lipodystrophy syndrome: research, results, yet more questions. AIDS 2001;15:271-3.

Gill JM, Mees GP, Frayn KN, Hardman AE. Moderate exercise, postprandial lipaemia and triglyceride clearance. Eur J Clin Inv 2001; 31(3):201-7.

Gold J, Batterham M. Nandrolone decanoate; use in HIV-associated lipodystrophy syndrome: a pilot study. Int J STD & AIDS 1999;10:558.

Gomez-Sirvent JL, Alonso-Socas MM, Santolaria-Fernandez FJ, Gonzalez-Reimers CE, Rodriguez-Moreno F, Essardas-Daryanani H. Prognostic value of triglyceride levels in early HIV infection. AIDS 1994;8:1740-1.

Goncalves J, Silva F, Freitas-Vieira A, et al. Functional neutralization of HIV-1 Vif protein by intracellular immunization inhibits reverse transcription and viral replication. J Biol Chem 2002;277:32036-45.

Gotch FM. Immunology of HIV in "Chelsea & Westminster Hospital AIDS Care Handbook", Mediscript Ltd. London 1999.

Goujard C, Boufassa F, Deveau C, Laskri D, Meyer L. Incidence of clinical lipodystrophy in HIV-infected patients treated during primary infection. AIDS 2001;15:282-4.

Greenwalt DE, Scheck SH, Rhinehart-Jones t. J Clin Invest 1995;96:1382-8.

Gregory, J, Foster, K, Tyler, H, and Wiseman, M. The dietary and nutritional survey of British adults. 1990. London, HMSO.

Grimble R, Calder P, Howell M, O'Reilly G, Turner S, Markovic O, East M. Polymorphisms of lymphotoxin-a (LT-a) but not TNF-α gene influence the ability of fish oil supplementation to suppress TNF-α production by peripheral blood mononuclear cells in healthy men. AJCN 2002; In Press.

Grinspoon S, Donovan DS, Bilezikian JP. Aetiology and pathogenesis of hormonal and metabolic disorders in HIV infection. Ballieres Clin Endocrinol Metab 1994;8(4):735-55.

Grinspoon S, Corcoran C, Lee K, et al. Loss of lean body mass correlates with androgen levels in hypogonadal men with acquired immunodeficiency syndrome and wasting. J Clin Endocrinol Metab 1996;81(11):4051-8.

Grinspoon S, Corcoran C, Miller K, et al. Body composition and endocrine function in women with acquired immunodeficiency syndrome wasting. J Clin Endocrinol Metab 1997;82(5):1332-7.

Grinspoon S, Corcoran C, Miller K, Wang E, Hubbard J, Schoenfield J, Anderson E, Basgoz N, Klibanski A. Determinants of increased energy expenditure in HIV-infected women. AJCN 1998; 68:720-725.

Grobler JA, Stillmock K, Hu B, et al. Diketo acid inhibitor mechanism and HIV-1 integrase: implications for metal binding in the active site of phosphotransferase enzymes. Proc Natl Acad Sci USA 2002;99:6661-6.

Grunfield C, Kotler D, Shigenaga J. Circulating IFN-alpha and hypertriglyceridaemia in AIDS. Am J Med 1991;90:154-62.

Grunfield C, Feingold KR. Metabolic disturbances and wasting in AIDS. NEJM 1992; 327: 329-37.

Grunfield, C, Pang, M, Doerrler, W, Shigenaga, J, Jensen, P, and Feingold, K. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. J Clin Endocrin & Metab 1992; 74: 1045-1052.

Gulick RM, Mellor JW, Havlir D, et al. Simultaneous versus sequential initiation of treatment with Indinavir, zidovudine and lamivudine for HIV-1 infection: 100 week follow up. JAMA 1998; 280(1): 35-41.

Gumbleton M, Abulrob AG, Campbell L. Caveolae: an alternative membrane transport compartment. Pharm Res 2000;17(9):1035-48.

Gump FE, Long C, Killian P, et al. Studies of glucose intolerance in septic injured patients. J Trauma 1974; 14(5): 378-88.

Hadigan C, Corcoran C, Basgoz N, Davis B, Sax P, Grinspoon S. Metformin in the treatment of HIV Lipodystrophy Syndrome. JAMA 2000a;284:472-7.

Hadigan C, Corcoran C, Pieucuch S, et al. Hyperandrogenemia in human immunodeficiency virus infected women with the lipodystrophy syndrome. J Clin Endocrinol Metab 2000b;85(10):3544-50.

Hadigan C, Sudhir B, Rabe J, Young V, Grinspoon S. Increased rates of lipolysis among HIV-infected men receiving HAART. Metabol 2002;51:1143-1147.

Hagstrom E, Arner P, Ungerstedt U, et al. Subcutaneous adipose tissue: a source of lactate production after glucose ingestion in man. Am J Physiol 1990; 258: E888.

Hammer SM, Katzenstein DA, Hughes MD et al. A trial comparing nucleoside montherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic mm. NEJM 1996; 335: 1081-90.

Han J, Hajjar DP, Tauras JM, Nicholson AC. J Lipid Res 1999;40:830-8.

Hardie D, Carling D. The AMP-activated protein kinase: fuel gauge of the mammalian cell? Eur J Biochem 1997;246:259-73.

Harmon CM, Luce P, Beth AH, Abrumrad NA. J Membr Biol 1991;121:261-8.

Harmon CM, Luce P, Abrumrad NA. Biochem Soc Trans 1992;20:811-3.

Harris JA, Benedict FG. A biometric study of basal metabolism in man. Washington DC: Carnegie Institute Publication No. 279, 1919.

Harris M, Tesiorowski A, Chan K, Hogg R, Rosenberg F, Chan Yan C, Thompson C, O'Shaughnessy M, Montaner J. Lactic acidosis complicating antiretroviral therapy: frequency and correlates. Antiviral Ther 1999; 5: 31.

Hatano H, Miller K, Yoder CP, Yanovski JA, Sebring NG, Jones EC, Davey RT. Metabolic and anthropometric consequences of interruption of highly active antiretroviral therapy. AIDS 2000;14:1935-42.

Hegele RA, Anderson CM, Wang J, Jones DC, Cao H. Association between nuclear lamin A/C R482Q mutation and partial lipodystrophy with hyperinsulinaemia, dyslipidaemia, hypertension and diabetes. Genome Res 2000;10:652-8.

Hegele RA. The envelope, please: Nuclear lamins and disease. Nat Med 2001;6:136-7.

Heguy A. Inhibition of the HIV Rev transactivator: A new target for therapeutic intervention. Front Biosci 1997;2:d283-97.

Heijligenberg R, Romjin JA, Hommes MJT, Endert E, Eeftinck-Schattenkerk JKM, Sauerwein HP. Non-insulin mediated glucose uptake in HIV-infected men. Clin Sci 1993; 84: 209-16.

Hellerstein, M, Grunfield, C, Wu, K, Christiansen, M, Kaempfer, S, Kletke, C, and Shackleton, C. Increased de novo hepatic lipogenesis in HIV infection. J Clin Endocrin & Metab 1993; 76: 559-565.

Hennes MM, Dua A, Kissebah AH. Effects of free fatty acids and glucose on splanchnic insulin dynamics. Diab 1997; 46: 57-62.

Henry K, Melroe H, Huebsch J et al. Severe premature coronary artery disease with protease inhibitors. Lancet 1998;351:1328.

Heymsfield SB et al. Anthropometric measurements of muscle mass : revised equations for calculating bone-free arm muscle area. AJCN 1982;36:680-690.

Hirsch D, Stahl A, Lodish HF. Proc Natl Acad Sci USA 1998;95:8625-9.

Hobbs GA, Keilbaugh SA, Rief PM, et al. Cellular targets of AZT: an early (non-delayed) effect on oxidative phosphorylation. Biochem Pharmacol 1995;50:381-90.

Hommes M, Romjin J, Endert E, Sauerwein H. Resting energy expenditure and substrate oxidation in HIV-infected asymptomatic men: HIV affects host metabolism in the early asymptomatic stage. Am J Clin Nutr 1991;54:311-5.

Hotamisligil GS, Spiegelman BM. Tumour necrosis factor-alpha: a key component of the obesity-diabetes link. Diabetes 1994; 43(11): 1271-8.

Howell WM, Calder PC, Grimble RF. Gene polymorphisms, inflammatory disease and cancer. Proc Nut Soc 2002; In Press.

Huang H, Farquhar D, Plunkett W. Selective action of 3'-azido-3'-deoxythymidine 5'-triphosphate on viral reverse transcriptases and human DNA polymerases. J Biol Chem 1990;265(20):11914-8.

Huang H, Chopra R, Verdine G, Harrison S. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: Implications for drug resistance. Science 1998;282:1669-75.

Huang H, Starodub O, McIntosh A, Kier AB, Schroeder F. Liver fatty acid binding protein targets fatty acids to the nucleus. J Biol Chem 2002; 22:S0021-9258.

Hussain MM, Goldberg IJ,Weisgraber KH, Mahley RW, Innerarity TL. Uptake of chylomicron by the liver but not by bone marrow is modulated by LPL activity. Arterioscler Thromb Vasc Biol 1997; 17(7): 1407-13.

Hutber C, Rasmussen B, Winder W. Endurance training attenuates the decrease in skeletal muscle malonyl-CoA with exercise. J Appl Physiol 1997;83:1917-22.

Ibrahimi A, Bonen A, Blinn WD, Hajri T, Li X, Zhong K, Cameron R, Abumrad NA. J Biol Chem 1999:274:26761-6.

Ionescu G, He Q, Engelson ES, et al. Acylation stimulating protein (ASP) and tumor necrosis factor (TNF) production in subcutaneous adipose tissue of HIV-infected patients with and without lipodystrophy. World AIDS Conference, Barcelona, July 7-12, 2002, abstract WePeA5794.

Isola LM, Zhou SL, Kiang CL, Stump D, Bradbury MW, Berk PD. Proc Natl Acad Sci USA 1995;92:9866-70.

Issekutz B, Paul P, Miller H, Bortz W. Oxidation of plasma free fatty acids in lean and obese humans. Metabolism 1968;17:62-73.

Jahoor F, Jackson A, Gazzard B, Phillips G, Sharpstone D, et al. Erythrocyte glutathione deficiency in symptom-free HIV infection is associated with reduced synthesis rate. Am J Physiol 1999; 276: E205-11.

Jeanson L, Subra F, Vaganay S et al. Effect of Ku80 depletion on the preintegrative steps of HIV-1 replication in human cells. Virology 2002;300:100-8.

Jones AE, Stolinski M, Smith R, Murphy JL, Wootton SA. Effect of fatty acid chain length and saturation on the gastrointestinal handling and metabolic disposal of dietary fatty acids in women. Br J Nutr 1999;81:37-43.

Kapembwa MS, Fleming SC, Griffen GE, et al. Fat absorption and exocrine pancreatic function in HIV infection. Q J Med 1990; 74: 79.

Kaplan N. The deadly quartet; upper-body obesity, glucose intolerance, hypertriglyceridaemia, and hypertension. Arch Intern Med 1989;149:1514-20.

Karmochkine, M and Raguin, G. Severe coronary artery disease in a young HIV-infected man with no cardiovascular risk factor who was treated with indinavir. AIDS 1998; 12: 2499-2514.

Keating J, Bjarnason I, Somasundaram S, et al. Intestinal absorptive capacity, intestinal permeability and jejunal histology in HIV and their relation to diarrhoea. Gut 1995; 37: 623.

Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, Sapp E, Wang Y, Qin ZH, Chen JD, Nevins JR, Aronin N, Di Figlia M. Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J Biol Chem 2001 5th Dec (epublished on medline ahead of print).

Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2001;2(4):282-6

Kim H, Kalkhoff R. Changes in lipoprotein composition during the menstrual cycle. Metabolism 1979;28:663-8.

Klein A, Bruser B, Bast M, et al. Progress of HIV infection and changes in lipid membrane structure of CD4+ T cells. AIDS 1992; 6(3): 332-3.

Knopp R, Walden C, Wahl P et al. Oral-contraceptive and post-menopausal estrogen effects on lipoprotein triglyceride and cholesterol in an adult female population - relationship to estrogen and progestin potency. J Clin Endocrin 1981;53:1123-32.

Kotler DP. HIV-related wasting: malabsorption syndromes. Semin Oncol 1998; 25: 70-5.

Kotler D, Burastero S, Wang J, Pierson R. Prediction of body cell mass, fat-free mass, and total body water with bioelectrical impedance analysis: effects of race, sex and disease. Am J Clin Nutr 1996;64:489S-97S.

Kotler, D and Engelson, E. Lipodystrophy, liver disease, and endocrine function. http://hiv.medscape.com/Medscape/HIV/journal/1999/v05.n04/mha0712.kotl/mha072.kotl-01.html. 15-7-2000. Medscape Inc.

Kotler DP, Tierney AR, Wang J, Pierson RN. Magnitude of body cell mass depletion and the timing of death from wasting in AIDS. AJCN 1989; 50:444-447.

Kowano Y, Koyanagi Y. Molecular mechanism of HIV-1 replication. Nippon Rinsho 2002; 60(4):662-8.

Kumar M, Goodkin K, Kumar AM, Baldewicz TT, Morgan R, Eisdorfer C. HIV-1 infection, neuroendocrine abnormalities, and clinical outcomes. CNS Spectrums 2000; 5(5): 55-65.

Kwong PD, Wyatt R, Robinson J, Sweet RW, et al. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998;393:648-659.

Lauterburg B, Bircher J. Expiratory measurement of maximal aminopyrine demethylation in vivo: Effect of phenobarbital, partial hepatectomy, portacaval shunt and bile duct ligation in the rat. J Pharmacol Exp Ther 1976;196:501.

Lauterburg BH, Grattagliano I, Gmur R, Stalder M, Hilderbrand P. Noninvasive assessment of the effect of xenobiotics on mitochondrial function in human beings: studies with acetylsalicylic acid and ethanol with the use of the carbon 13-labeled ketoisocaproate breath test. J Lab Clin Med 1995;125(3):378-83.

Lawler JF, Yin M, Diehl AM, Roberts E, Chatterjee S. TNFα stimulates the maturation of SREMP-1 in human hepatocytes through the action of neutral sphingomyelinase. J Biol Chem 1998;273(9):5053-9.

Ledru E, Christeff N, Patey O, de Truchis P, Melchior JC, Gougeon ML. Alteration of tumor necrosis factor-alpha T-cell homeostasis following potent antiretroviral therapy: contribution to the development of human immunodeficiency virus-associated lipodystrophy syndrome. Blood 2000;95:3191-8.

Lenhard JM, Croom DK, Weiel JE, Winegar DA. HIV protease inhibitors stimulate hepatic triglyceride synthesis. Aterioscler Throm Vasc Biol 2000a;20:2625-9.

Lenhard JM, Weiel JE, Paulik MA, Furfine ES. Stimulation of vitamin A1 acid signaling by the HIV protease inhibitor Indinavir. Biochem Pharmacol 2000b; 59:1063-8.

Lew E, Gallagher L, Kuehnert M, Rimland D, Hubbard M, Parekh B, Zell E, Jarvis W, Jason J. Intracellular cytokines in the acute response to HAART. AIDS 2001;15(13):1665-70.

Lewis W, Griniuviene B, Tankersley KO, et al. Depletion of mitochondrial DNA, destruction of mitochondria, and accumulation of lipid droplets result from fialuridine treatment in woodchucks. Lab Invest 1997;76:77-87.

Licinio J, Gold PW, Wong ML. A molecular mechanism for stress-induced alterations in susceptibility to disease. Lancet 1995; 346: 104-6.

Liu Y, Li J, Kim BO, Pace BS, He JJ. HIV-1 Tat protein mediated transactivation of the HIV-1 long terminal repeat promoter is potentiated by a novel nuclear Tat-interacting protein of 110kDa, Tip110. J Biol Chem 2002;277:23854-63.

Lo, J, Mulligan, K, Tai, V, Algren, H, and Schambelan, M. Buffalo hump in men with HIV infection. Lancet 1998; 351: 867-870.

Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. Body shape changes in HIV-infected patients [letter]. J Acquir.Immune.Defic.Syndr.Hum.Retrovirol. 1998;19:307-8.

Luiken JJ, Turcotte LP, Bonen A. J Lipid Res 1999;40:1007-16.

Lukaski H, Johnson P, Bolonchuk W, Lykken G. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985;41:810-7.

Macallan DC, Noble C, Baldwin C, Foskett M, MacManus T, Griffen GE. Prospective analysis of patterns of weight change in stage IV HIV infection. J Clin Nutr 1993; 58: 417-424.

Macallan DC, Noble C, Baldwin C, Jebb SA, Prentice AM, Coward A, Sawyer MB, McManus TJ, Griffin GE. Energy expenditure and wasting in human immunodeficiency virus infection. NEJM 1995a; 333(2): 83-8.

Macallan DC, McNurlan MA, Milne E, et al. Whole body protein turnover from leucine kinetics and the response to nutrition in human immunodeficiency virus infection. AJCN 1995; 61: 818.

Macallan DC. Metabolic abnormalities and wasting in human immunodeficiency virus infection. Proc Nut Soc 1998; 57: 373-380.

Macallan DC. Malnutrition in Tuberculosis. Diagn Microbiol Infect Dis 1999a; 34: 153-157.

Macallan DC. Wasting in HIV infection and AIDS. J Nutr 1999b;129:238S-242S.

Macallan DC, McNurlan MA, Milne E, Calder AG, Garlick PJ, Griffin GE. Whole body protein turnover from leucine kinetics and the response to nutrition in Human Immunodeficiency Virus infection. AJCN 1995b; 61: 818-826.

Macallan DC, Noble C, Baldwin C, Jebb SA, Prentice AM, Coward WA, Sawyer MB, McManus TJ, Griffin GE. Energy expenditure and wasting in Human Immunodeficiency Virus infection. NEJM 1995a; 333(2): 83-8.

Maher B, Lloyd J, Wilkins EGL et al. Lipodystrophy in patients with HIV-1 infection: effect of stopping protease inhibitors on TNF-receptor levels, insulin resistance, and lipid profile. AIDS 2000;14:S56 (abstr).

Man MZ, Hui TY, Schaffer JE, Lodish HF, Bernlohr DA. Mol Endocrinol 1996;10:1021-8.

Mann J, Chisholm A. Nutritional determinants of serum triglycerides. Nutr Metab Cardiovasc Dis 1999;9:1-8.

Marrakech classification of HIV Lipodystrophy. Presented at: European Symposium on Lipodystrophy and HIV Infection, April 24-25, 2000; Marrakech, Morocco.

Marin P, Rebuffe-Scrive M, Smith U, et al. Glucose uptake in human adipose tissue. Metabol 1987; 36: 1154.

Martin G, Schoonjans K, Lefebvre Am, Staels B, Auwerx J. J Biol Chem 1997;272:28210-7.

Martinez, E. and Gatell, J. M. Metabolic abnormalities and use of HIV-1 protease inhibitors. Lancet 1998; 352: 821-822.

Martinez E, Conget I, Lozano L, Casamitjana R, Gatell JM. Reversion of metabolic abnormalities after switching from HIV-1 protease inhibitors to nevirapine. AIDS 1999;13:805-10.

Martinez E, Bianchi L, Garcia-Viejo MA, Bru C, Gatell JM. Sonographic assessment of regional fat in HIV-1-infected people [letter] [In Process Citation]. Lancet 2000;356:1412-3.

Martinez E, Mocroft A, Garcia-Viejo MA et al. Risk of lipodystrophy in HIV-1 infected patients treated with protease inhibitors: a prospective cohort study. Lancet 2001;357:592-8.

Masini A, Scotti C, Calligaro A, et al. Zidovudine induced experimental myopathy: dual mechanism of mitochondrial damage. J Neurol Sci 1999;166:131-40.

Mastorakos G, Chrousos GP, Weber JS. Recombinant interleukins-6 activates the hypothalamic-pituatary-adrenal axis in humans. J Clin Endocrinol Metab. 1993; 77: 1690-4.

Matthews JNS, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. BMJ 1990; 300:230-5.

Mauss S. HIV-associated lipodystrophy syndrome. AIDS 2000;14:S197-S208.

Mayo J, Collazos J, Martinez E, Ibarra S. Adrenal function in the HIV-infected patient. Arch Intern Med 2002; 162(10): 1095-8.

McGarry J, Mills S, Long C, Foster D. Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase. Biochem J 1983;214:21-8 (abstr).

Melchior JC, Salmon D, Rigaud D, Leport C, Bouvet E, Detruchis P, Vilde J, Vachon F, Couland J, Apfelbaum M. Resting energy expenditure is increased in stable, malnourished HIV-infected patients. AJCN 1991; 53: 437-441.

Melikyan GB, Markosyan RM, Hemmati H, Delmedico MK, Lambert DM, Cohen FS. Evidence that the transition of HIV-1 gp41 into a six-helix bundle, not the bundle configuration, induces membrane fusion. J Cell Biol 2000; 151:413-423.

Memon RA, Feingold KR, Moser AH, Fuller J, Grunfield C. Am J Physiol 1998;274:E210-E217.

Mero N, Syvanne M, Eliasson B, Smith U, Taskinen MR. Postprandial elevation of ApoB-48-containing triglyceride-rich particles and retinyl esters in normolipemic males who smoke. Arterioscler Thromb Vasc Biol 1997;17(10):2096-102.

Meyrier A. The patient with glomerulonephritis and lipodystrophy. Nephrol Dial Transplant 1997;12:226-227.

Mezzaroma I, Carlesimo M, Pinter E, Muratori DS, Di Sora F, Chiarotti F, Cunsolo MG, Sacco G, Aiuti F. Clinical and immunologic response without decrease in virus load in patients with AIDS after 24 months of highly active antiretroviral therapy [see comments]. Clin Infect.Dis 1999;29:1423-30.

Michaletz PA, Cap L, Alpert E, Lauterburg BH. Assessment of mitochondrial function in vivo with a breath test utilizing alpha-ketaisocaproic acid. Hepatol 1989;10(5):829-32.

Mildvan D, Machado SG, Wilets I, Grossberg SE. Endogenous interferon and triglyceride concentrations to assess response to Zidovudine in AIDS and advanced AIDS-related complex. Lancet 1992; 339: 453-6.

Miller AR, Griffen GE, Batman P. Jejunal mucosal architecture and fat absorption in male homosexuals infected with human immunodeficiency virus. Q J Med 1988;69:1009-19.

Miller KK, Daly PA, Sentochnik D, et al. Pseudo-Cushing's Syndrome in HIV-infected patients. Clin Infect Dis 1998; 27: 68-72.

Miller K, Jones E, Yanovski J, Shankar R, Feuerstein I, Falloon J. Visceral abdominal fat accumulation associated with the use of indinavir. Lancet 1998; 351: 871-875.

Misra D, Knox W. Emory University. Structure of HIV-1 reverse transcriptase complex (Website) 1998.

Montague CT, Prins JB, Sanders L et al. Depot-related gene expression in subcutaneous and omental adipocytes. Diabetes 1998; 47: 1384-1391.

Morlese JM, Qazi NA, Asboe D, Gazzard B, Nelson M. Interferon-α therapy increases plasma triglyceride concentrations to HIV-1 seropositive patients. AIDS 2000;14:S56 (abstr).

Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. J Biol Chem 1998;273:16710-4.

Mulligan K, Grunfeld C, Hellerstein M, Neese R, Schambelan M. Anabolic effects of recombinant human growth hormone in patients with wasting associated with HIV infection. J Clin Endocrin & Metab 1993;77:956-62.

Mulligan K, Tai VW, Schambelan M. Energy expenditure in HIV infection. NEJM 1997; 336: 70-1.

Munoz-Fernandez C, Aladro Y, Conde, Campos Y, Arenas J. Multiple symmetrical lipomatosis with familial polineuropathy. Rev Neurol 2001;32(12):1107-11.

Murillas J, Martin T, Ramos A, Portero J. Atorvastatin for protease inhibitor-related hyperlipidaemia. AIDS 1999;13:1424-5.

Murray I; Kohl J; Cianflone K. Acylation-stimulating protein (ASP): structure-function determinants of cell surface binding and triacylglycerol synthetic activity. Biochem J 1999;342(1):41-8.

Murray I; Sniderman AD; Cianflone K. Mice lacking acylation stimulating protein (ASP) have delayed postprandial triglyceride clearance. J Lipid Res 1999b; 40(9):1671-6.

Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Cell 1998;93:229-40.

Noor M, et al. Absent HIV infection, Indinavir causes insulin resistance. AIDS 2001;15:11-8.

Norbiato G, Trifiro G, Galli M, Gervasoni C, Clerici M. Fat redistribution in HIV-infected patients. A new hormonal immune disorder? Ann N Y Acad Sci 2000;917:951-5.

Norkin LC. Caveolae in the uptake and targeting of infectious agents and secreted toxins. Adv Drug Deliv Rev 2001;49:301-15.

Nulton-Persson AC, Swzeda LI. Modulation of mitochondrial function by hydrogen peroxide. J Biol Chem 2001;276(26):23357-61.

Nunez E, Christeff N. Steriod hormone, cytokine, lipid and metabolic disturbances in HIV infection. Ballieres Clin Endocrinol Metab 1994;8:803-24.

O'Brien TR, Rosenberg PS, Yellin F, Goedert JJ. Longitudinal HIV-1 RNA levels in a cohort of homosexual men. J AIDS Hum Retrovir 1998; 18(2): 155-61.

Pacht ER, Diaz P, Clanton T, et al. Serum vitamin E decreases in HIV-seropositive subjects over time. J Lab Clin Med 1997; 130(3): 293-6.

Pantaleo G, Graziosi C, Demarest JF et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 1993; 362: 355-8.

Panse I, Vasseur E, Raffin-Sanson ML, Staroz F, Rouviex E, Saiag P. Lipodystrophy associated with protease inhibitors. Br J Dermatol 2000;142:496-500.

Paparizos VA, Kyriakis KP, Botsis C, Papastamopoulos V, Hadjivassiliou M, Stavrianeas NG. Protease inhibitor therapy-associated lipodystrophy, hypertriglyceridaemia and diabetes mellitus. AIDS 2000;14:903-5.

Parpal S, Karlsson M, Thron H, Stralfors P. Cholesterol depletion disrupts caveolae and insulin receptor signaling for metabolic control via insulin receptor substrate-1, but not for mitogen-activated protein kinase control. J Biol Chem 2001;276(13):9670-8.

Paton NI, Macallan DC, Jebb SA, Noble C, Baldwin C, Pazianas M, Griffin GE. Longitudinal changes in body composition measured with a variety of methods in patients with AIDS. J Acquir Immune Defic Syndr Hum Retrovirol 1997;14(2):119-27.

Pelkmans L, Kartenbeck J, Helenius A. Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat Cell Biol 2001;3(5):473-83.

Penzak SR, Chuck SK, Stajich GV. Safety and efficacy of HMG-CoA reductase inhibitors for treatment of hyperlipidaemia in patients with HIV infection. Pharmacotherapy 2000;20:1066-71.

Perret et al. AIDS 2001;15:2397-2406.

Perry RC, Cushing H, Deeg MA. Ritonavir, triglycerides and pancreatitis. Clin Inf Dis 1999;28:161-2.

Playford D, Watts GF. Endothelial dysfunction, insulin resistance and diabetes: exploring the web of causality. Aust NZ J Med 1999;29:523-534.

Poirier H, Degrace P, Niot I, Bernard A, Besnard P. Eur J Biochem 1996;238:368-73.

Ponce-de-Leon S, Iglesias M, Ceballos J, Ostrosky-Zeichner L. Liposuction for protease-inhibitor-associated lipodystrophy [letter]. Lancet 1999;353:1244.

Prows DR, Murphy EJ, Schroeder F. Intestinal and liver fatty acid binding proteins differentially affect uptake and esterification in L-cells. Lipids 1995; 30(10): 907-10.

Purnell JQ, Zambon A, Knopp RH, Pizzuti DJ, Achari R, Leonard JM, Locke C, Brunzell JD. Effect of Ritonavir on lipids and post-heparin lipase activities in normal subjects. AIDS 2000; 14: 51-57.

Randle P, Garland P, Hales C, Newsholme E. The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1963;1:785-9.

Rasmussen B, Wolfe R. Regulation of fatty acid oxidation in skeletal muscle. Ann Rev Nutr 1999;19:463-84.

Ray AS, Yang Z, Shi J, et al. Insights into the molecular mechanism of inhibition and drug resistance for HIV-1 RT with carbovir triphosphate. Biochemistry 2002;41:5150-62.

Reaven M. Insulin resistance and human disease: a short story. J Basic Clin Physiol Pharmacol 1998; 9: 387-406.

Renard E. Syndrome of body fat distribution in HIV-1 infected patients: relationships to cortisol and catecholamines. Clin Endocrinol 1999;51:223.

Richter S, Ping YH, Rana TM. TAR RNA loop: a scaffold for the assembly of a regulatory switch in HIV replication. Proc Natl Acad Sci USA 2002; 99(12):7928-33.

Riddle, T. M., Kuhel, D. G., Woollett, L. A., Fichtenbaum, C., and Hui, D. Y. HIV protease inhibitor therapy increases hepatic lipoprotein production via stabilisation of activated sterol regulatory element binding protein-1 (SREBP-1) in the nucleus. 242. 2001. 8th Conference on Retroviruses and Opportunistic Infection.

Rietschel P, Hadigan C, Corcoran C et al. Assessment of growth hormone dynamics in HIV-related lipodystrophy. J Clin Endocrin & Metab 2001;86:504-10.

Rizzuto CD, Wyatt R, Hernandez-Ramos N, et al. A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding. Science 1998:280; 1949-53.

Roden M, Price TB, Perseghin G, et al. Mechanism of free fatty acid induced insulin resistance in humans. JCI 1996; 97(12): 2858-65.

Roge BT, Calbert JA, Moller K et al. Skeletal muscle mitochondrial function and activity in HIV-infected patients with lipodystrophy and elevated p-lactate levels. AIDS 2002; 16(7): 973-82.

Ross L. Antiviral Therapy 1999;4:79.

Roubenouff R, Weiss L, McDermott A et al. A pilot study of exercise training to reduce trunk fat in adults with HIV-associated fat redistribution. AIDS 1999;13:1373-5.

Roudiere L, Viard J-P. Osteonecrosis of the hip, lipodystrophy and antiretroviral treatment. AIDS 2001;14:2056.

Safrin S, Grunfield C. Fat distribution and metabolic changes in patients with HIV infection. AIDS 1999;13:2493-505.

Saha A, Vavvas D, Kurowski T et al. Malonly-CoA regulation in skeletal muscle: its link to cell citrate and the glucose-fatty acid cycle. Am J Physiol 1997;272:E641-E648.

Saint-Marc T, Partisani M, Poizot-Martin I et al. A syndrome of peripheral fat wasting (lipodystrophy) in patients receiving long-term nucleoside analogue therapy. AIDS 1999;13:1659-67.

Saint-Marc, T, Poizot-Martin, I, Partisani, M, Fabre, J, and Touraine, J. A syndrome of lipodystrophy in patients receiving a stable nucleoside-analogue therapy. 1999. Conf Retroviruses Opportunistic Infect. 31-1-1999.

Saint-Marc T, Touraine JL. The effects of discontinuing stavudine therapy on clinical and metabolic abnormalities in patients suffering from lipodystrophy [letter]. AIDS 1999;13:2188-9.

Salas-Salvado J, Garcia-Lorda P. The metabolic puzzle during the evolution of HIV infection. Clin Nutr 2001; 20(5): 379-391.

Saleh J, Blevins JE, Havel PJ, Barrett JA, Gietzen DW, Cianflone K. Acylation stimulating protein (ASP) acute effects on postprandial lipemia and food intake in rodents. Int J Obes Relat Metab Disord 2001;25(5):705-13.

Sappey C, Leclercq P, Coudray C, et al. Vitamin, trace element and peroxide status in HIV seropositive patients: asymptomatic patients present severe beta-carotene deficiency. Clin Chim Acta 1994; 230: 35-42.

Sartor H, Loose R, Tucha O, Klein HE, Lange KW. MELAS: a neuropsychological and radiological follow-up study. Mitochondrial encephalomyopathy, lactic acidosis and stroke. Acta Neurol Scand 2002;106(5):309-13.

Sasaki Y. J Lab Clin Med 1970;76:824.

Scantlebury T, Maslowska M, Cianflone K. Chylomicron-specific enhancement of acylation stimulating protein and precursor protein C3 production in differentiated human preadipocytes. J Biol Chem 1998;273:20903-20909.

Schlegel A, Lisanti MP. The caveolin triad: caveolae biogenesis, cholesterol trafficking, and signal transduction. Cytokine Growth Factor Rev 2001;12(1):41-51.

Schmitz M, Michl GM, Walli R et al. Alterations in apolipoprotein B metabolism in HIV-infected patients with antiretroviral combination therapy. J Acquir Immune Defic Syndrome 2001;26:225-235.

Schoeller D, Schneider J, Solomons N, Watkins J, Klein P. Clinical diagnosis with the stable isotope 13C in CO2 breath tests: methodology and fundamental considerations. J Lab Clin Med 1977;90:412-21.

Schroder AR, Shinn P, Chen H, et al. HIV-1 integration in the human genome favors active genes and local hotspots. Cell 2002; 110:521-9.

Schwabe A, Cozzetto F, Bennett L, Mellinkoff S. Estimation of fat absorption by monitoring of expired radioactive carbon dioxide after feeding a radioactive fat. Gastroenterol 1962;42:285-91.

Schwenk A, Beisenherz A, Kremer G, Diehl V, Salzberger B, Fatkenheuer G. Bioelectrical impedance analysis in HIV-infected patients treated with triple antiretrovial treatment. Am J Clin Nutr 1999;70:867-73.

Schwieterman W, Sorrentino D, Potter BJ, Rand J, Kiang CL, Stump D, Berk PD. Proc Natl Acad Sci USA 1988;85:359-63.

Seidman MD, Khan MJ, Tang WX, Quirk WS. Influence of lecithin on mitochondrial DNA and age-related hearing loss. Otolaryngol Head Neck Surg 2002;127(3):138-44.

Sekhar RV, White AC, Jahoor F, Visnegarwala F, Reeds PJ, Balasubramanyam B. HIV-associated fat redistribution syndrome is associated with marked acceleration of lipid turnover. 242. 2001. 8th Conference on Retroviruses and Opportunistic Infections.

Sekhar RV, Jahoor F, White AC, et al. Metabolic basis of HIV-lipodystrophy syndrome. Am J Physiol Endocrinol Metab. 2002;283(2):E332-7.

Sellmeyer DE, Grunfeld C. Endocrine and metabolic disturbances in HIV infection and AIDS. Endocr Rev 1996; 17: 518-32.

Serwadda D, Sewankambo NK, Carswell JW, Bayley AC, Tedder RS, Weiss RA, Mugerwa RD, Lwegaba A, Kirya GB, Downing RG, Clayden SG, Dalgleish AG. Slim disease: a new disease in Uganda and it's association with HTLV-III infection. Lancet 1985; ii: 849-852.

Shackleton S, Lloyd DJ, Jackson SNJ, Evans R, Niermeijer MF, Singh BM, Schmidt H, Brabant G, Kumar S, Durrington PN, Gregory S, O'Rahilly S, Trembath RC. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. Nat Genet 2000;24:153-6.

Sharpstone DR, Murray CP, Ross HM et al. Energy balance in asymptomatic HIV infection. AIDS 1996; 10: 1377.

Sharpstone D, Phelan M, Gazzard B. Differential metabolic response in AIDS-related chronic protozoal diarrhoea. HIV Med 2000; 1(2): 102-6.

Shaw, A, Mclean, K, and Evans, B. Disorders of fat distributiom in HIV infection. Int J STD & AIDS 1998; 9: 595-599.

Sheehan LA, Macallan DC, Griffen GE. Determinants of energy intake and expenditure in HIV and AIDS. J AIDS 1998

Sheehan LA, Macallan DC. Determinants of energy intake and energy expenditure in HIV and AIDS. Nutrition 2000;16(2):101-6.

Sherman MP, De Noronha CM, Williams SA, Greene WC. Insights into the biology of HIV-1 viral protein R. DNA Cell Biol 2002;21(9):679-88.

Shevitz A, Knox T, Spiegalman D, Roubenouff R, Gorbach S, Skolnik P. Elevated resting energy expenditure among HIV-seropositive persons receiving HAART. AIDS 1999;13:1351-7.

Shikuma C, Waslien C, McKeague J, Baker N, Arakaki M, Cui XW, Souza S, Imrie A, Arakaki R. Fasting hyperinsulinaemia and increased WHR in non-wasting individuals with AIDS. AIDS 1999;13:1359-65.

Silva M, Skolnik P, Gorbach S et al. The effect of protease inhibitors on weight and body composition in HIV-infected patients. AIDS 1998;12:1645-51.

Silversides A. Protease inhibitors raising quality-of-life issues for HIV patients. CMAJ. 1999;160:894.

Sluis-Cremer N, Arion D, Parniak MA. Molecular mechanisms of HIV-1 resistance to NRTI's. Cell Mol Life Sci 2000;57:1408-22.

Smith A. Lecture notes on clinical biochemistry. Oxford: Blackwell Science, 1998.

Sniderman AD, Cianflone K, Summers L, Fielding B, Frayn K. The acylation-stimulating protein pathway and regulation of postprandial metabolism. Proc Nutr Soc 1997;56(2):703-12.

Sorrentino D, Stump D, Potter BJ, Robinson RB, White R, Kiang CL, Berk PD. J Clin Invest 1988;82:928-35.

Soudeyns H, Geleziunas R, Shyamala G, Hiscott J, Wainberg MA. Identification of a novel glucocorticoid response element within the genome of HIV type 1. Virology 1993; 194: 758-68.

Spady DK, Willard MN, Meidell RS. Role of aceyl CoA: cholesterol acyl transferase in the control of hepatic VLDL secretion and LDL receptor expression in the mouse and hamster. J Biol Chem 2000; 275(35): 27005-12.

Sreekumar R, Unnikrishnan J, Fu A, Nygren J, et al. Impact of high-fat diet and antioxidant supplementation on mitochondrial functions and gene transcripts in rat muscle. Am J Physiol Endocrinol Metab 2002;282(5):E1055-61.

Stahl A, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, Watson N, Patel S, Kotler M, Raimondi A, Tartaglia LA, Lodish HF. Mol Cell 1999;4:299-308.

Stein TP, Nutinsky C, Schluter MD, Leskiw MJ. Protein and energy substrate metabolism in AIDS patients. Metabolism 1990; 39: 876-81.

Steiner G. From an excess of fat, diabetics die. JAMA 1989; 262(3): 398-9.

St John J, Mital D, Taylor S, White DJ. Multiple mtDNA deletions in human spermatozoa from long term HAART. Antiviral Therapy 2000;5(suppl 5):37. Abstract P23.

Stremmel W. J Clin Invest 1988;82:2001-10.

Stremmel W, Lotz G, Strohmeyer G, Berk PD. J Clin Inv 1985a;75:1068-76.

Stremmel W, Strohmeyer G, Berk PD. Proc Natl Acad Sci USA 1986;83:3584-8.

Stremmel W, Strohmayer G, Borchard F, Kochwa B, Berk P. Isolation and partial characterisation of a fatty acid binding protein in rat liver plasma membranes. Proc Natl Acad Sci USA 1985b;82:4-8.

Strobel M, Muller P, Claudel P. Complete reversibility of severe nucleoside-induced lipodystrophy [letter]. AIDS 1999;13:2606-7.

Sugiyama M, Kobori O, Atomi Y, Wada N, Kuroda A, Muto T. Effect of oral administration of protease inhibitor on pancreatic exocrine function in WBN/Kob rats with chronic pancreatitis. Pancreas 1996;13(1):71-9.

Sullivan, A, Feher, M, Nelson, M, and Gazzard, B. Marked hypertriglyceridaemia associated with ritonavir therapy. AIDS 1998; 12(11): 1393-1395

Suttman U, Ockenga J, Hoogestraat L, Selberg O, Schedel I, Deicher H, Muller MJ. Resting energy expenditure and weight loss in HIV-infected patients. Metabolism 1993; 42: 1173-9.

The HIV Lipodystrophy Case Definition Study Group. A case definition study of lipodystrophy in HIV-infected adults. JAMA. In Press.

Thomas JC, Lopes-Virella MF, Del Bene VE, Cerveny JD, Taylor KB, McWhorter LS, Bultemeier NC. Use of fenofibrate in the management of protease inhibitor-associated lipid abnormalities. Pharmacotherapy 2000;20:727-34.

Tikkanen M, Ojala J, Helve E. Long term use of Lovastatin in different types of hyperlipidaemia. Atherosclerosis 1992;97:S27-S32.

Trigatti BL, Mangroo D, Gerber GE. J Biol Chem 1991;266:22621-5.

Tsiodras S, Mantzoros C, Hammer S, Samore M. Effects of protease inhibitors on hyperglycemia, hyperlipidemia, and lipodystrophy: a 5-year cohort study. Arch Intern Med 2000;160:2050-6.

Tso, P.Gastrointestinal digestion and absorption of lipid. Adv in Lipid Res 1985; 21:143-186.

Turcotte L, Srivastava A, Kiens B. Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. Am J Physiol 1992;262:E791-E799.

Turcotte L, Srivastava A, Chiasson J. Fasting increases plasma membrane fatty acid-binding protein in red skeletal muscle. Mol Cell Biochem 1997;166:153-8.

Turcotte LP, Srivastava AK, Chiasson JL. Mol Cell Biochem 1997;166:153-8.

Van Berkel TJ, Fluiter K, van Velzen AG, Vegelezang CJ, Ziere GJ. LDL receptor independent and dependent uptake of lipoproteins. Atherosclerosis 1995; 118: S43-50.

Vander AJ, Sherman JH, Luciano DS. Human Physiology 6th Ed. McGraw Hill Inc. London 1994.

Vittecoq D, Escaut L, Monsuez J. Lancet 1998;351:1960.

Vonkemann HE, ten Napel CHH, van Oeveren-Dybicz AM, Vermes I. Beta sub(3) adrenergic receptor polymorphism and the antiretroviral therapy-related lipodystrophy syndrome. AIDS 2000;14:1463-4.

Wachsman M, Barditch-Crovo P, Lietman PS, Trapnell CB. Lack of beta-oxidation defects in HIV-positive subjects with and without chronic zidovudine exposure. Blood 1996;88:3243-4.

Walli R, Herfort O, Michl G, Demant T, Jager H, Dieterle C, Bognor JR, Landgraf R, Goebel FD. Treatment with protease inhibitors associated with peripheral insulin resistance and impaired oral glucose tolerance in HIV-1 infected patients. AIDS 1998; 12: F167-73.

Wanke C. Epidemiological and clinical aspects of the metabolic complications of HIV infection The fat redistribution syndrome. AIDS 1999;13:1287-93.

Watkins J, Klein P, Schoeller D, Kirschner B, Park R, Perman J. Diagnosis and differentiation of fat malabsorption in children using 13C labelled lipids - trioctanoin, triolein and palmitic acid breath tests. Gastroenterol 1982;82:911-7.

Weir J B de V. New methods for calculating metabolic rate , with special reference to protein metabolism. J Physiol 1949;109:1-9.

Weiss RA.Novel HIV systems. Nature 1988; 335: 591.

Weissenhorn W, Dessen A, Harrison SC, Skehel JJ, Wiley DC. Atomic structure of the ectodomain from HIV-1 gp41. Nature 1997; 387:426-430.

Wellington CL, Leavitt BR, Hayden MR. Huntington disease: new insights on the role of Huntingtin cleavage. J Neural Transm Suppl 2000;58:1-17.

Wheeler DA, Launer C, Bartsch G, Muurahainen N, Besch L, Abrams D, Gibert C. Change in obdy weight as a predictor of opportunistic complications in HIV. Proceedings of 35th Interscience Conference on Antimicrobial Agents and Chemotherapy 1995; 1:215.

Wheeler DA, Muurahainen N, Elion R, Launer C, Gilbert C, Bartsch G. Change in body weight as a predictor of death and opportunistic complications (OC) in HIV by history of prior OC. Proceedings of XI International Conference on AIDS 1996; 1:332.

Wierzbicki AS, Reynolds TM, Crook MA, et al. Lipid lowering therapy in patients with HIV infection. Lancet 1998; 352: 1782.

Wilson LA, Barber MC, Travers MT, et al. Effect of plane of nutrition and growth hormone treatment on insulin receptor gene expression and kinase activity of sheep muscle and adipose tissue. Biochem Soc Trnas 1996; 24(2): 222S.

Winder W, Arogyasami J, Barton R, Elayan I, Vehrs P. Muscle malonyl-CoA decreases during exercise. J Appl Physiol 1989;67:2230-3.

Wolfe R, Jahoor F. Recovery of labelled CO2 during the infusion of C-1 verses C-2 labelled acetate: implications for tracer studies of substate oxidation. Am J Clin Nutr 1990;51:248-52.

Wolfram C, Buhlmann C, Rolf B, Borchers T, Spencer F. Variation of liver type fatty acid binding protein content in the human hepatoma cell line HepG2 by peroxisomal proliferators and antisense RNA affects the rate of fatty acid uptake. Biochim Biophys Acta 1999; 1437(2): 194-201.

Xiang S, Cianflone K, Kalant D, Sniderman A. Differential binding of triglyceride-rich lipoproteins to lipoprotein lipase. J Lipid Res 1999;40:1655-62.

Yamaguchi T, Iwanami N, Shudo K, Saneyoshi M. Chiral discrimination of enantiomeric 2'-deoxythymidine 5'-triphosphate by HIV-1 reverse transcriptase and eukaryotic DNA polymerases. Biochem Biophys Res Commun 1994; 200(2):1023-7.

Yamaguchi T, Katoh I, Kurato S. Azidothymidine causes functional and structural destruction of mitochondria, glutathione deficiency and HIV-1 promoter sensitization. Eur J Biochem 2002;269:2782-8.

Yanovski J, Miller K, Kino T et al. Endocrine and metabolic evaluation of HIV-1 infected patients with evidence of protease inhibitor-associated lipodystrophy. J Clin Endocrin & Metab 1999;84:1925-31.

Yarasheski KE, Tebas P, Sigmund C, Dagog-Jack S, Bohrer A, Turk J, Halban PA, Cryer PE, Powderley WG. Insulin-resistance in HIV protease inhbitor-associated diabetes. J AIDS 1999; 21: 209-16.

Yarasheski K, Tebas P, Stanerson B. Resistance exercise training reduces hypertriglyceridaemia in HIV-infected men treated with antiviral therapy. J Appl Physiol 2001;90:133-8.

Ye J, Samaras K, Bonner KM, Cooney GJ, Chisholm D, Kraegen EW. Ritonavir has paradoxical effects on lipid metabolism and insulin sensitivity in rats compared with humans. AIDS 1998;12:2236-7.

Zhang B, MacNaul K, Szalkowski D, Li Z, Berger J, Moller DE. Inhibition of adipocyte differentiation by HIV protease inhibitors. J Clin Endocrin & Metab 2000;84:4274-7.

Zhou SL, Stump D, Isola LM, Berk PD. Biochem J 1994;297:315-9.

Zhou SL, Stump D, Kiang CL, Isola LM, Berk PD. Proc Soc Exp Biol Med 1995;52:121-7.

Zhou SL, Gordon RE, Bradbury M, Stump D, Kiang CL, Berk PD. Hepatology 1998;27:1064-74.