Correlation between C-reactive protein, cholesterol profile and statin use

by

MADZIVHE RUDZANI
(200602424)

Research Dissertation

Submitted in fulfillment of the requirements for the degree of

MASTERS OF SCIENCE

in

PHARMACOLOGY

in the

DEPARTMENT OF PHARMACOLOGY THERAPEUTICS

(SCHOOL OF MEDICINE)

at the

UNIVERSITY OF LIMPOPO (MEDUNSA CAMPUS)

SUPERVISOR: Prof Elzbieta Osuch

2013
Declaration

“I Madzivhe Rudzani hereby declare that the work on which this thesis is based, is original (except where acknowledgement indicate otherwise) and that neither the whole work nor any part of it has been, is being, or shall be submitted for another degree at this or any other university, institution for tertiary education or examining body”.

___________________________ ___________________ Signature

Date

Student Number: 200602424
ACKNOWLEDGEMENTS

This thesis is the end of my journey in obtaining my MSc. I have not travelled in a vacuum in this journey; this thesis has been kept on by the Almighty GOD.

This work is dedicated to my son Kevin Wayhudi Makaringe. I really thank you my son for sharing your time with my studies. I know you will be proud of your mom.

Foremost, I would like to express my sincere gratitude to my advisor Prof E. Osuch for the continuous support of my MSc study and research, for her patience, understanding, motivation, enthusiasm, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my MSc study.

I would like to gratefully and sincerely thank Mrs L. Mathibe and Mrs S. Skele, the professional nurses in the pharmacology clinic, for their help and involvement in this thesis.

I would also like to thank Prof Joubert and Mr T Phohu and the team workers at the Natioanla Health Laboratory (NHLS) for the laboratory assistance.

Department of Pharmacology staff Mrs P. Tsipa, Mrs. R. Sebothuma, Mr M. Fata, Mrs T., Mamabolo for their encouragement, thank you.

My warm thanks are due to Ms A. Managa, for guidance assistance with the statistical analysis.

My sincere thanks go to all volunteers at the University of Limpopo (Medunsa Campus) who participated in this study.

I owe my loving thanks to my Parents (Mr Sebastian, Mrs Roselina and Mrs Sara Madzivhe) for their unconditional love, support and guidance have been of great value in this study. Without their encouragement and understanding it would have been impossible for me to finish this work.
My special gratitude is due to my brothers, and my sisters for their loving support. My loving thanks go to my lovely sister Dr. Judith Madzivhe for financial support during this study.

I warmly thank my friends Reason Mathokwane and Emmanuel Mashavhela for their friendly help, and advice in this study.

This study was not going to be fulfilled; I deeply owe my most sincere gratitude to MRC for funding this study.

Pretoria, March 2013

Rudzani Madzivhe
“Thy word is a lamp unto my feet
And a light unto my path
PSALM 119:105”
Table of contents

List of figures ........................................................................................................ IX
List of tables ........................................................................................................ XII
Abbreviation ......................................................................................................... XIII
Summary ................................................................................................................ XV
Appendices ........................................................................................................... 100
Appendix Ia Consent form (English version) ....................................................... 100
Appendix Ib Consent form (Tshwane version) .................................................... 102
Appendix II Study Approval form ....................................................................... 104
Appendix III Patients questionnaire .................................................................. 105
Appendix IV Raw Healthy Volunteers and Patients data collection ............... 117
Chapter 1: Introduction ....................................................................................... 1
  1.1 Back ground ................................................................................................. 1
  1.1.1 Biochemistry of statin .............................................................................. 1
  1.1.2 Pharmacological correlation between statin and cholesterol profile ..... 2
  1.1.3 Pharmacological correlation between statin and C-reactive protein .... 3
  1.2 Aim and objectives ....................................................................................... 3
  1.3 Significance of the study ............................................................................. 4
Chapter 2: Cholesterol profile............................................................................. 6
2.1 Introduction ................................................................. 6
2.1.1 The liver and intestine: sources of cholesterol ................. 7
2.1.2 Physiology of cholesterol ............................................. 7
2.2 Hypercholesterolemia .................................................... 8
2.2.1 Hypercholesterolemia is divided into two categories .......... 9
2.2.1.1 Homozygous familial hypercholesterolemia (HoFH) .... 9
2.2.1.2 Heterozygous familial hypercholesterolemia (HeFH) .... 9
2.2.2 Physiology of hypercholesterolemia ............................. 10
2.2.2.1 Methods of measuring cholesterol absorption and synthesis ...... 10
2.2.2.1.1 Cholesterol absorption assessment methods ............... 10
2.2.2.1.1.1 Sterol balance approach .................................. 10
2.2.2.1.1.2 Plasma isotope ratio technique ......................... 10
2.2.2.1.2 Cholesterol synthesis assessment methods ............... 11
2.2.2.1.2.1 Deuterium incorporation approach .................... 11
2.2.2.1.2.2 Cholesterol synthesis precursor quantification ....... 12
2.2.2.1.3 Physiology factors affecting cholesterol absorption and synthesis ... 12
2.2.2.1.3.1 Genetic factors ............................................ 13
2.2.2.1.3.2 Body weight [Body mass index BMI] ................... 13
2.2.2.1.4 Therapeutic factors affecting cholesterol absorption and synthesis ........................................ 14
2.2.2.1.4.1 Weight loss .............................................. 14
2.2.2.1.4.2 Statin therapy .......................................... 15
2.2.2.1.4.3 Ezetimibe therapy ...................................... 15
2.2.3 Treating two sources of cholesterol-cholesterol production and absorption .......................... 16
2.2.4 Maintaining very low cholesterol levels .................................................. 17

Chapter 3: C-reactive protein ................................................................. 18
3.1 Introduction ..................................................................................... 18
3.1.1 History of CRP: fundamentals .................................................. 18
3.2 The source of the C-reactive protein ............................................. 19
3.3 Structure and Binding sites of CRP .............................................. 20
3.4 Determination of C-reactive protein .......................................... 21
3.5 Biosynthesis and kinetics of CRP ................................................. 22
3.6 Lowering CRP levels: a new therapeutic goal ............................. 23
3.6.1 Statins and CRP ................................................................. 23
3.6.2 Aspirin and CRP ............................................................... 24
3.6.3 Antibiotics and CRP ........................................................... 24
3.6.4 Antioxidants and CRP ......................................................... 24
3.7 Potential Clinical recommendations of C-reactive protein .......... 25
3.8 C-reactive protein levels after statin therapy ............................. 26

Chapter 4: Statin ................................................................. 27
4.1 Introduction ..................................................................................... 27
4.2 Classification .................................................................................. 27
4.3 Mechanism of action of statin .................................................. 28
4.4 Statin and Cholesterol level ..................................................... 29
4.5 Statin and C-reactive protein level ........................................... 30
4.6 Statin and cardiovascular disease .............................................. 30
4.7 Adverse effects of statin use .................................................... 31

Chapter 5: Atorvastatin ................................................................. 33
5.1 Introduction .......................................................... 33
5.2 Chemical structure for atorvastatin ......................................... 34
5.3 Pharmacotherapy uses of atorvastatin ...................................... 34

Chapter 6: Method ................................................................. 36
6.1 Volunteers and patients ...................................................... 36
6.1.1 Healthy volunteer ..................................................... 36
6.1.1 The inclusion criteria were ................................................. 36
6.1.2 Patients ............................................................ 36
6.1.2.1 Measurements .................................................. 38
6.1.2.2 Statistical analysis ............................................... 38
6.2 Apparatus and methods .................................................. 38
6.2.1 Determination of C-reactive protein ................................... 38
6.2.1.1 Immunoradiometric assay (IRMA) ............................. 38
6.2.1.2 Immunonephelometric (Dade Behring N Latex High Sensitivity
CRP™ mono assay……………………………………………………………………………… 39

6.2.1.3 Immunoturbidimetric (Tina-quant CRP detection method; Roche Diagnostics)……………………………………………………………………………… 39

6.3 Blood analyses of cholesterol profile using a Cardio-check test …. 39

6.4 Blood analysis of the Cholesterol profile using the DxC800 system……………………………………………………………………………………………… 40

6.5 Methodology of total cholesterol (TC) determination .......................... 40

6.5.1 Chemical reaction scheme ................................................................. 41

6.5.2 Reagents .......................................................... 41

6.5.2.1 Reagent storage and stability ........................................................ 41

6.5.3 Performance characteristics ............................................................... 41

6.5.3.1 Analytical Range ................................................................. 41

6.5.3.2 Sensitivity ........................................................... 42

6.5.3.3 Equivalency ........................................................... 42

6.5.3.4 Precision ............................................................... 43

6.6 Methodology of low density lipoprotein (LDL) determination ....... 43

6.6.1 Chemical reaction scheme ................................................................. 44

6.6.2 Specimen .......................................................... 44

6.6.2.1 Specimen storage and stability ........................................................ 44

6.6.2.2 Reagents ............................................................... 44

6.6.2.2.1 Volumes per test ......................................................... 45

6.6.2.2.2 Reagent storage and stability ........................................................ 45

6.6.2.3 Performance characteristics of LDL determination ..................... 45
6.6.2.3.1 Analytic range ......................................................... 45
6.6.2.3.2 Sensitivity .......................................................... 45
6.6.2.3.3 Equivalency ......................................................... 46
6.6.2.3.4 Precision .......................................................... 46
6.6.4 Methodology of high density lipoprotein (HDL) determination ... 46
6.6.4.1 Chemical reaction scheme ........................................... 47
6.6.4.2 Specimen ........................................................... 47
6.6.4.2.1 Sample preparation .............................................. 47
6.6.4.3 Reagents .......................................................... 48
6.6.4.3.1 Volumes per test ............................................... 48
6.6.4.3.2 Reactive ingredient ............................................ 48
6.6.4.4 Performance characteristics ....................................... 48
6.6.4.4.1 Analytic range ................................................... 48
6.6.4.4.2 Sensitivity ......................................................... 49
6.6.4.4.3 Equivalence ...................................................... 49
6.6.4.4.4 Precision ........................................................ 49
6.6.5 Methodology of triglyceride (TG) determination ................. 50
6.6.5.1 Chemical reaction scheme ........................................ 50
6.6.5.2 Reagents .......................................................... 51
6.6.5.2.1 Volumes per test ............................................... 51
6.6.5.3 Performance characteristic ....................................... 51
6.6.5.3.1 Analytic range ................................................... 51
6.6.5.3.2 Sensitivity ........................................................ 51
6.6.5.3.3 Equivalency ................................................................. 52
6.6.5.3.4 Precision ......................................................................... 52
6.6.5.1.1.5 Performance characteristics of TG ................................. 52
6.6.5.1.1.5.1 Analytical range ....................................................... 52
6.6.5.1.1.5.2 Analytical sensitivity ................................................ 53
6.6.5.1.1.5.3 Functional sensitivity ................................................ 53
6.6.5.1.1.5.4 Equivalence .............................................................. 53
6.6.5.1.1.5.5 Precision .................................................................. 53
6.7 Blood analysis of C-reactive protein using DxC800 system ........ 54
6.7.1 Methodology of C-reactive protein (CRP) determination ........ 54
6.7.1.1 Chemical scheme reaction ............................................... 54
6.7.1.2 Specimen ........................................................................... 55
6.7.1.2.1 Type of specimen ......................................................... 55
6.7.1.2.2 Specimen storage and stability ....................................... 55
6.7.2.2 Reagents ........................................................................... 55
6.7.2.2.1 Sample volume ............................................................. 56
6.7.2.2.2 Volume per test ............................................................ 56
6.7.2.2.3 Reagent preparation ...................................................... 56
6.8 Blood analysis of CRP using Enzyme-linked immunosorbent assay (ELISA test) ................................................................. 56
6.9 Statistical analysis ..................................................................... 57
Chapter 7: Results ......................................................................... 58
7.1 Baseline .................................................................................. 58
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1.1</td>
<td>Description of study population</td>
</tr>
<tr>
<td>7.1.2</td>
<td>Cardiovascular risk stratification and cholesterol targets</td>
</tr>
<tr>
<td>7.1.2.1</td>
<td>Category 1: High risk</td>
</tr>
<tr>
<td>7.1.2.1</td>
<td>Category 2: Primary prevention of healthy volunteer dyslipidaemia (from NHLS)</td>
</tr>
<tr>
<td>7.1.3</td>
<td>Correlation between cholesterol profile [Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL) and Creactive protein (CRP)] of healthy volunteers (n=33)</td>
</tr>
<tr>
<td>7.1.4</td>
<td>Correlation between patients with normal cholesterol profile and high C-reactive protein (not on Atorvastatin) n=10</td>
</tr>
<tr>
<td>7.1.5</td>
<td>Correlation between cholesterol profiles and CRP of patients on atorvastatin (n=32)</td>
</tr>
<tr>
<td>7.1.6</td>
<td>Correlation between two systems (DxC800 and Cardio-check)</td>
</tr>
<tr>
<td>7.1.6 (a)</td>
<td>Descriptive statistics of volunteers with normal Cholesterol using Cardio-check technique (n=36)</td>
</tr>
<tr>
<td>7.1.6 (b)</td>
<td>Descriptive Statistics of volunteers with high Cholesterol using Cardio-check technique (n=25)</td>
</tr>
<tr>
<td>7.1.7</td>
<td>Correlation between DxC800 and Microtiter plate reader techniques measuring C-reactive protein (n=78)</td>
</tr>
<tr>
<td>7.2</td>
<td>Summary of the results</td>
</tr>
</tbody>
</table>

**Chapter 8:** Discussion and Conclusion | 77

**Chapter 9:** References | 82
# List of figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Structure of cholesterol</td>
</tr>
<tr>
<td>3.3</td>
<td>Schematic illustration of pentameric CRP with possible binding sites</td>
</tr>
<tr>
<td>4.2.2</td>
<td>The endogenous mevalonate pathway leading to cholesterol Biosynthesis</td>
</tr>
<tr>
<td>5.2</td>
<td>Chemical Structure of atorvastatin</td>
</tr>
<tr>
<td>6.1.2</td>
<td>Flow diagram of the study</td>
</tr>
<tr>
<td>7.1.2.1</td>
<td>Body mass index (BMI) of healthy volunteers</td>
</tr>
<tr>
<td>7.1.2.2</td>
<td>Body mass index (BMI) of patients</td>
</tr>
<tr>
<td>7.1.3.1</td>
<td>Correlation between TC and CRP of healthy volunteers (n=33)</td>
</tr>
<tr>
<td>7.1.3.2</td>
<td>Correlation between TG and CRP of healthy volunteers (n=33)</td>
</tr>
<tr>
<td>7.1.3.3</td>
<td>Correlation between HDL and CRP of healthy volunteers (n=33)</td>
</tr>
<tr>
<td>7.1.3.4</td>
<td>Correlation between LDL and CRP of healthy volunteers (n=33)</td>
</tr>
<tr>
<td>7.1.4.1</td>
<td>Correlation between TC and CRP (patients not on atorvastatin) (n=10)</td>
</tr>
<tr>
<td>7.1.4.2</td>
<td>Correlation between TG and CRP (patients not on atorvastatin) (n=10)</td>
</tr>
</tbody>
</table>
Figure 7.1.4.3  Correlation between HDL and CRP
(patients not on atorvastatin) (n=10) ............................ 66

Figure 7.1.4.4  Correlation between LDL and CRP
(Patients not on atorvastatin) (n=10) ............................ 67

Figure 7.1.5.1  Correlation between TC and CRP in patients on
atorvastatin after three months of treatment (n=32) ........ 68

Figure 7.1.5.2  Correlations between TG and CRP in patients on
atorvastatin after three months of treatment (n=32) ........ 68

Figure 7.1.5.3  Correlations between HDL and CRP in patients on
atorvastatin after three months of treatment (n=32) ........ 69

Figure 7.1.5.4  Correlations between LDL and CRP in patients on
atorvastatin after three months of treatment (n=32) ........ 69

Figure 7.16.1  Correlation between the TG using DxC800 and
Cardio-chek techniques ............................................. 70

Figure 7.1.6.2  Correlation between the TC using DxC800 and
Cardio-chek techniques ............................................. 71

Figure 7.1.6.3  Correlation between the HDL using DxC800 and
Cardio-chek techniques ............................................. 71

Figure 7.1.6.4  Correlation between the LDL using DxC800 and
Cardio-chek techniques ............................................. 72
Figure 7.1.7.1  Correlation between DxC800 and microtiter plate reader techniques measuring CRP of healthy volunteers (n=33)........ 73

Figure 7.1.7.2  Correlation between DxC800 and microtiter plate reader techniques measuring CRP of patients with normal cholesterol and high C-reactive protein (n=10) .................. 74

Figure 7.1.7.3  Correlation between DxC800 and microtiter plate reader techniques measuring CRP of patients with high cholesterol and normal C-reactive protein (n=26) ......................... 74

Figure 7.1.7.4  Correlation between DxC800 and microtiter plate reader techniques measuring CRP with both high cholesterol and high C-reactive protein (n=6) ............................ 75

Figure 7.1.7.5  Correlation between DxC800 and microtiter plate reader techniques measuring C-reactive protein of patients after three months of 20mg atorvastatin (n=6) ....................... 75
List of Tables

Table 6.5.3.1 Analytic range of the SYNCHRON® system ......................... 42
Table 7.1.1 Subject characteristics of volunteers groups ......................... 58
Table 7.1.2 Subject characteristics of patients groups ......................... 58
Table 7.1.2 Cardiovascular risk stratification and cholesterol targets ........... 59
Table 7.2 The relationship between atorvastatin, C-reactive protein
and lipid profile in patients with moderate hyperlipidaemia ........ 76
Abbreviations

4-AAP: 4-aminoantipyrine
ACS: Acute coronary syndromes
AFCAPS/TexCAPS: Air Force/Texas Coronary Atherosclerosis Prevention Study
BMI: Body mass index
CARE: Cholesterol and Recurrent Events
RES: Recurrent Events study
CDC/AHA: Centers for Disease Control and Prevention and the American Heart Association
CE: Cholesterol esterase
CHD: Coronary heart disease
CO: Cholesterol oxidase
CRP: C-reactive protein
CTT: Cholesterol Treatment Trialists' Collaborators
DHBS: 3, 5-dichloro-2-hydroxybenzenesulfonic acid
EDTA: Ethylenediaminetetraacetic acid
FSR: Fractional cholesterol synthesis rate
GK: Glycerol kinase
GPO: Glycerophosphate oxidase
GTP: Guanosine 5’- Triphosphate
HDL: High density lipoprotein
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeFH:</td>
<td>Heterozygous familial hypercholesterolemia</td>
</tr>
<tr>
<td>HMG-CoA:</td>
<td>3-hydroxy 3-methylglutaryl CoA reductase</td>
</tr>
<tr>
<td>HoFH:</td>
<td>Homozygous familial hypercholesterolemia</td>
</tr>
<tr>
<td>HPO:</td>
<td>Horseradish peroxidase</td>
</tr>
<tr>
<td>HPO:</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>IL-1 β:</td>
<td>Interleukin-1 β</td>
</tr>
<tr>
<td>IL-6:</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>IRMA:</td>
<td>Immunoradiometric assay</td>
</tr>
<tr>
<td>LDL:</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LIF:</td>
<td>Oncostatin M and leukemia inhibitory factor</td>
</tr>
<tr>
<td>LIPID:</td>
<td>Long-Term Intervention with Pravastatin in Ischaemic Disease</td>
</tr>
<tr>
<td>LPS:</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>MRC/BHF:</td>
<td>Medical Research Council/British Heart Foundation</td>
</tr>
<tr>
<td>NCEP:</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>NHLS:</td>
<td>National health laboratory service</td>
</tr>
<tr>
<td>NO:</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>ROS:</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SD:</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SnRNPs:</td>
<td>Small nuclear ribonucleoproteins</td>
</tr>
<tr>
<td>SPARCL:</td>
<td>Stroke Prevention by Aggressive Reduction in Cholesterol level</td>
</tr>
<tr>
<td>SREBP’s:</td>
<td>Sterol regulatory element-binding proteins</td>
</tr>
<tr>
<td>TG:</td>
<td>Triglyceride</td>
</tr>
<tr>
<td>TMB:</td>
<td>3, 3’,5, 5’ tetramethyl-benzidine</td>
</tr>
</tbody>
</table>
Summary

Correlation between C-reactive protein, cholesterol profile and statin use

HMG-CoA reductase inhibitors, more commonly known as statins are a widely used group of cholesterol-lowering agents that act by inhibiting the enzyme (HMG CoA) reductase, which catalyses the rate-limiting step in cholesterol biosynthesis. Statins has been shown to reduce total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), and C-reactive protein (CRP) levels and increase high density lipoprotein (HDL) levels of patients.

The main objective of this study was to investigate the short term effect of 20mg of atorvastatin on high sensitivity CRP concentration and the correlation between changes in total cholesterol, LDL-C and high sensitivity CRP concentrations in a group of patients with moderate hyperlipidaemia.

The DxC800 system was used in this study to measure cholesterol profile and C-reactive protein and statin use in the blood serum of patients, a Cardio-chek PA electronic hand model was used to measure cholesterol profile in the blood plasma of patients, and Elisa test used to determine the C-reactive protein in the blood serum of patients. This could provide to identify individuals with high risk for CRP level and cholesterol profile.
High-sensitivity C-reactive protein (hs-CRP) is a non-specific acute-phase protein produced by the liver in response to tissue injury, infection, inflammation and has recently emerged as a valuable marker of cardiovascular risk for various cardiovascular-related disorders.

Evidence suggests patients with high hs-CRP/normal LDL-C are at greater risk of the cardiovascular complications than those with normal hs-CRP/high LDL-C and elevated hs-CRP levels predict poor prognosis and outcome in patients with cardiovascular disease. The major beneficial effect of statins-reducing the risk for coronary events has primarily been ascribed to reductions in LDL-C but may in part be related to a direct anti-inflammatory action (decreased hs-CRP) concentration. Lowering LDL-cholesterol by statins has been proven to be associated with reduction of pro-inflammatory regulators e.g. activation of the transcription factor NF-κB.

The study could help to identify individuals with high risk for cardiovascular disease and artery coronary disease using determination of CRP and cholesterol profile. The measurement of CRP and cholesterol profile would possibly have immediate clinical applicability. Basic research should actively search for therapeutic markers that may provide important targets in preventing cardiovascular diseases, such markers might include CRP. The identification of patients at risk for developing serious cardiovascular disease, by measuring the CRP and cholesterol profile, may offer best hope reducing what is rapidly becoming a global epidemic. High sensitivity CRP together with other known cardiac risk factors may be used as a predictor of cardiovascular disease and can be used in primary prevention as a cardiovascular risk marker.

The major finding of this study was a significant decrease in hs-CRP level from 8.4 ± 8.19 to 6.66 ± 7.49 mg/L± (p< 0.021). Total cholesterol decreased from 5.93 ± 0.96 to 4.49 ± 0.59 mmol/L (p<0000.3) and LDL-cholesterol decreased from 3.79 ± 0.99 to 2.7 ± 0.57 mmol/L (p< 0000.7).

In this study the output showed that there was no significant correlation between total cholesterol, triglyceride, HDL level and CRP levels of the healthy volunteers.
There was also no significant correlation between the total cholesterol level and hs-CRP level, LDL-cholesterol and hs-CRP, HDL- cholesterol and hs-CRP in patients before and after 3 months of treatment with 20 mg of atorvastatin although the decrease in hs-CRP was fully established after 3 months of treatment and is strongly suggestive that lipids can produce inflammatory changes.

The study has shown that the changes in CRP were independent of lipid changes, so the reason for the anti-inflammatory effect of statins needs to be still elucidated.

The study has shown that atorvastatin is cholesterol-and-CRP levels-lowering drug and that effect can be achieved already after 3 months of treatment with 20mg of atorvastatin.

Decrease of HDL level (instead of increasing HDL level) after three months of 20mg atorvastatin, could be due to other factors and need to be investigated.

Amazingly BMI was not found to be a predictor for changes in cholesterol profile but could be a predictor for changes in CRP level. Volunteers with BMI above 30 kg/m$^2$ had CRP above 7.5mg/l.

In conclusion, atorvastatin has an anti-inflammatory effect as confirm with the lowering of hs-CRP. Since no correlation could be found between LDL-C and hs- CRP it could be concluded that statins lower plasma levels of hs-CRP in a manner largely independent of LDL cholesterol lowering. High sensitivity CRP together with other known cardiac risk factors may be used as a predictor of cardiovascular disease and can be used in primary prevention, although caution should be taken to regard hs-CRP as highly specific cardiovascular risk marker.

Results suggest that hs-CRP could be used to target high-risk patients who may benefit from early statin use. However it should be investigated on a larger group of patient whether hs-CRP reduction, independent of LDL cholesterol reduction, results in a clinical benefit.
The Cardio-chek test is suitable for screening the cholesterol profile of volunteers or patients and the DxC800 system is suitable for screening the C-reactive protein and cholesterol profile of volunteers or patients.

**Key words:** HMG-CoA reductase inhibitors, CRP, LDL, HDL, Cardio-chek PA electronic hand model, the timed-end method, DxC800, Microtiter plate reader (ELISA test).
1.1 Background

Various cholesterol-lowering agents had been discovered during the 1950s and 1960s. However, the majority had unwanted side effects. In 1971 Dr Akira Endo and Dr Masao Kuroda began the search for a safer drug to treat hypercholesterolemia. Endo and Kuroda discovered statins in 1973 by searching for microbial metabolites that would competitively inhibit 3-hydroxy 3-methylglutaryl CoA (HMG-CoA) reductase, the rate-limiting step in the synthesis of cholesterol. Having examined over 6000 microbial strains, the antibiotic Citrinin was isolated from *Pythium ultimum* and later mevastatin from *Penicillium Citrinum*. This led to the development of mevastatin analogues namely atorvastatin, lovastatin, simvastatin and pravastatin as well as others. The discovery and development of statins is summarized in a review article published in 1992 (Endo, 1992). Since statins were first approved in 1987, their ability to reduce the risks of vascular death, non-fatal myocardial infarction, stroke, and the need for arterial revascularisation procedures has been shown, in high-quality randomised trials (Cholesterol Treatment Trialists' (CTT) Collaborators, 2005).

1.1.1 Biochemistry of statin

HMG-CoA reductase inhibitors, which are commonly known as statins are a widely used groups of cholesterol-lowering agents that act by inhibiting the enzyme (HMG CoA) reductase, which catalyses the rate-limiting step in cholesterol biosynthesis (Grundy, 1988; Pedersen et al., 2005). Statins are effective inhibitors of the endogenous mevalonate pathway, which direct the biosynthesis of cholesterol (Goldstein and Brown, 1990). Therefore, hepatocytes become depleted of cholesterol and respond by increasing low density lipoprotein (LDL) clearance from
the blood (via up regulation of hepatic LDL receptors) and decreasing entry of LDL into the circulation. These actions, in turn, give rise to lower plasma LDL levels (Knopp, 1999; Steiner, 2003).

1.1.2 Pharmacological correlation between statin and cholesterol profile

In the last six years, statins has been shown to reduce cardiovascular-related disease and death in multiple clinical trials. Cholesterol reduction of 15-60 percent and relative risk reduction for coronary events of 30-35 percent have validated the use of statins in risk reduction for those at increased risk of coronary events and mortality from coronary heart disease (Sacks and Ridker, 1999). Two recent trials demonstrated that intensive lipid-lowering therapy with statins improved clinical outcomes (Cannon et al., 2004) and reduced the progression of atherosclerosis (Nissen et al., 2004). Many authorities attributed the greater benefits of intensive statin therapy, as compared with moderate statin therapy, to greater reductions in the levels of atherogenic lipoproteins, particularly LDL cholesterol (Sacks, 2004). Due to their ability to inhibit the synthesis of cholesterol, statins are widely used in medical practice and are the principal therapy for hypercholesterolemia. Statins have been shown to induce a regression in vascular atherosclerosis and a reduction in cardiovascular-related morbidity and mortality in patients with and without coronary artery disease (Peto et al., 1985; LaRosa et al., 2005; Pedersen et al., 2005; Cannon et al., 2004). HMG-CoA reductase inhibitors has been shown in animal models to act as antioxidants by stabilizing vulnerable plaque, decreasing LDL oxidation, and reducing platelet aggregation and thromboxane production (Rosenson and Tangney, 1998).
1.1.3 Pharmacological correlation between statin and high-sensitivity C-reactive protein

Large observational studies have established a strong relationship between hs-CRP levels and the morbidity and mortality associated with coronary disease (Ridker, 2003). Specific trials with statins have demonstrated a CRP-lowering capacity (Downs et al., 2001). Other findings suggest that statins also exert anti-inflammatory properties and may play a role in regulating the immune system. The immunomodulatory properties of statins could be exerted through interference in the expression and function of a variety of immune relevant molecules. Atorvastatin, which has been shown to lower C-reactive protein (CRP), has also been shown to significantly decrease overall risk for non-hemorrhagic stroke when other forms of cholesterol-lowering agents have not (Plutzky and Ridker, 2001; Trochu et al., 2003).

CRP was 30 to 40 percent lower after intensive statin therapy than after moderate treatment in the mentioned trials (Topol, 2004). This finding raises the question: Do reductions in CRP represent an independent factor influencing the benefits of more intensive statin therapy? Nevertheless, the feasibility, starting speed and dose-response characteristics of this effect have not yet been defined (Ridker and Rifai, 2001). However, the precise mechanism underlying the association between CRP levels and adverse outcomes is not known.

1.2 Aim and Objectives

The aim of this study was to investigate the short term effect of low dose (10mg) atorvastatin on high sensitivity CRP concentration and the relationship between changes in LDL-C and high sensitivity CRP concentrations in a small group of patients with moderate hyperlipidaemia.

In order to achieve this aim certain objectives had to be identified:

- Assessing high sensitivity C-reactive protein in healthy volunteers
- Assessing high sensitivity C-reactive protein in patients not using atorvastatin
Assessing high sensitivity C-reactive protein in patients on atorvastatin
Assessing the correlation between high sensitivity CRP with the cholesterol profile of the different groups
Assessing different methods to determine high sensitivity CRP

1.3 Significance of the study

High-sensitivity C-reactive protein (hs-CRP) is a non-specific acute-phase protein produced by the liver in response to tissue injury, infection, inflammation and has recently emerged as a valuable marker of cardiovascular risk for various cardiovascular-related disorders (Ridker, 2003). Evidence suggests patients with high hs-CRP/normal LDL-C are at greater risk than those with normal hs-CRP/high LDL-C (Ridker, 2008; Albert, 2001). Furthermore elevated hs-CRP levels predict poor prognosis and outcome in patients with cardiovascular disease. The major beneficial effect of statins-reducing the risk for coronary events has primarily been ascribed to reductions in LDL-C but may in part be related to a direct anti-inflammatory action (decreased hs-CRP) concentration (Bonnet et al., 2008; Keles et al., 2008; Riesen et al., 2002).

The study could help to identify individuals with high risk for cardiovascular disease and artery coronary disease using determination of CRP and cholesterol profile. It has been reported that the statins and in particular atorvastatin have anti-inflammatory properties in addition to its cholesterol-lowering effect. Furthermore, low density lipoprotein cholesterol (LDL-C) is purported to have pro-inflammatory effects (Mora et al., 2006; Albert, 2001). Lowering LDL-cholesterol by statins has been proven to be associated with reduction of pro-inflammatory regulators e.g. activation of the transcription factor NF-κB (Syn et al., 2009; Rudofsky et al., 2012).

The measurement of CRP and cholesterol profile would possibly have immediate clinical applicability. Basic research should actively search for therapeutic markers that may provide important targets in preventing cardiovascular diseases, such markers might include CRP. The
identification of patients at risk for developing serious cardiovascular disease, but by measuring
the CRP and cholesterol profile, this may offer best hope reducing what is rapidly becoming a
global epidemic. High sensitivity CRP together with other known cardiac risk factors may be
used as a predictor of cardiovascular disease and can be used in primary prevention as a
cardiovascular risk marker.
2.1 Introduction

The name cholesterol originates from the Greek chole- (bile) and stereos (solid), and the chemical suffix -ol for an alcohol, as François Poulletier de la Salle first identified cholesterol in solid form in gallstones, in 1769. However, it was only in 1815 that chemist Eugène Chevreul named the compound "cholesterine" (Olson, 1998).

Figure 2.1 Structure of cholesterol

Other names: 10R,13R)-10,13-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (Olson, 1998).
2.1.1 The liver and intestine: sources of cholesterol

Cholesterol is a waxy and lipidic steroid found in the cell membranes and transported in the blood plasma of all animals (Emma, 2009). It is an essential component of mammalian cell membranes where it is required to establish proper membrane permeability and fluidity. Cholesterol is the principal sterol synthesized by animals, but small quantities are synthesized in other eukaryotes, such as plants and fungi. It is almost completely absent among prokaryotes, which include bacteria (Pearson et al., 2003). It is present in the membranes of every cell, including cells in the brain, nerves, muscle, skin, liver, intestines, and heart. Without cholesterol, our bodies would not function properly. It acts as the precursor of hormones like estrogen and testosterone, vitamin D and bile. The average adult body contains approximately 140 mg of sterol, mainly in the form of cholesterol (Cook, 1958). Approximately 500 to 1400 mg per day of cholesterol enters the body's cholesterol pool from synthesis (500 to 1000 mg) and intestinal absorption (up to 400 mg per day). However, the cholesterol pool usually changes little because cholesterol input is approximately balanced by cholesterol output via excretion in bile/feces, skin excretion, steroid hormone synthesis, tissue storage, etc (Cook, 1958; Illingworth, 1995).

2.1.2 Physiology of cholesterol

Since cholesterol is essential for life, it is primarily synthesized de novo within the body. However, high levels in blood circulation depending on how it is transported within lipoproteins, are strongly associated with progression of atherosclerosis. For a person of about (68 kg), typical total body cholesterol synthesis is about 1 g per day and total body content is about 35 mg. Typical daily additional dietary intake, in the United States and societies with similar dietary patterns, is 200–300 mg. Cholesterol is recycled. It is excreted by the liver via the bile into the digestive tract. Typically about 50% of the excreted cholesterol is reabsorbed by the small bowel.
back into the blood stream. Intestinal tract absorption is highly selective for cholesterol, excreting plant stanols and sterols, back into the intestinal lumen for elimination (Olson, 1998).

2.2 Hypercholesterolemia

Hypercholesterolemia plays a key role in the development and progression of atherosclerosis and is a proven risk factor for coronary heart disease (CHD) (Steinberg and Witztum, 1990; Castelli et al., 1992; Wilson et al., 1980; Stamler et al., 1986). Pharmacologic interventions to lower cholesterol levels both in primary and secondary prevention trials show a clear reduction in the incidence of CHD as well as stroke (The Lipid Research Clinics Coronary Primary Prevention Trial results, 1984; Shepherd et al., 1995; Collins et al., 2004; Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease, 1994; Downs et al., 2001; Manninen et al., 1988; Frick et al., 1987).

According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), higher concentrations of LDL and lower concentrations of functional high density lipoprotein (HDL) are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This can lead an increase risk of myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood LDL, especially higher LDL particle concentrations and smaller LDL particle size, contribute to this process more than the cholesterol content of the LDL particles (Brunzell et al., 2008), LDL particles are often termed "bad cholesterol". On the other hand, high concentrations of functional HDL, which can remove cholesterol from cells, offer protection and are sometimes referred to as "good cholesterol". These balances are mostly genetically determined but can be changed by body build, medications, food choices and other factors (Durrington, 2003).
The 1987 report of National Cholesterol Education Program, Adult Treatment Panels suggest the total blood cholesterol level should be: < 5mmol/l represents normal blood cholesterol, a value between 5.2-6.2mmol/l represents borderline-high cholesterol, a value more than 6.2mmol/l is regarded as high cholesterol (Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 1988). However in risk patients, total cholesterol should be less than 4.5mmol/l.

2.2.1 Hypercholesterolemia is divided into two categories:

2.2.1.1 Homozygous familial hypercholesterolemia (HoFH)

Homozygous familial hypercholesterolemia (HoFH) is an autosomal dominantly inherited disorder characterized by markedly elevated levels of plasma LDL cholesterol, tendon xanthomas, and severe premature coronary artery disease (Goldstein and Brown, 1992). If untreated, the majority of patients with this disorder die from accelerated atherosclerosis before the age of 30 years (Allen et al., 1980). A High dose of statin therapy has recently been shown to be partially effective in lowering LDL cholesterol levels in this patient group (Raal et al., 1997 and Marais et al., 1997). However, the mechanisms by which high dose statins reduce LDL cholesterol levels in HoFH require further clarification.

2.2.1.2 Heterozygous familial hypercholesterolemia (HeFH)

Heterozygous familial hypercholesterolemia (HeFH) is an autosomal dominant disease characterized by markedly elevated plasma concentrations of low-density lipoprotein cholesterol, typically well above the 95th percentile for age and sex (Brown and Goldstein, 1986).
2.2.2 Physiology of hypercholesterolemia

2.2.2.1 Methods of measuring cholesterol absorption and synthesis

A variety of techniques exist to measure cholesterol absorption and synthesis. The following section briefly describes the most common methods currently implemented, and also touches upon the advantages and limitations of each.

2.2.2.1.1 Cholesterol absorption assessment methods:

2.2.2.1.1.1 Sterol balance approach

The accepted standard approach to measuring cholesterol absorption is the sterol balance method (Grundy and Ahrens, 1969, Wilson and Lindsey, 1965). This technique measures the mass absorption of cholesterol as the difference between dietary intake of exogenous cholesterol and fecal excretion of exogenous cholesterol.

2.2.2.1.1.2 Plasma isotope ratio technique

The plasma isotope ratio technique differs from the balance method in that, instead of quantifying cholesterol absorption, it estimates the fraction of cholesterol absorbed over a
defined period of time (Bosner et al., 1999, Gremaud et al., 2001; Jones et al., 2000). Cholesterol absorption is calculated from the ratio of oral cholesterol tracer enrichment, taken to represent the amount of exogenous cholesterol absorbed, to intravenous tracer enrichment, taken to represent the total cholesterol pool in the blood.

2.2.2.1.2 Cholesterol synthesis assessment methods:

2.2.2.1.2.1 Deuterium incorporation approach

The deuterium incorporation approach determines cholesterol synthesis as the rate of incorporation of deuterium from body water into red cell membrane free cholesterol over a 24 hrs period (Wang et al., 2004b; Woollett et al., 2003). Employing this technique requires that subjects consume a small amount of deuterated water following the study intervention. Shortly after ingestion, the deuterated water equilibrates with intra- and extra-cellular body water pools. In this way, any newly formed cholesterol synthesized within 24 h will have a certain number of H atoms replaced by deuterium. The fractional cholesterol synthesis rate (FSR) (pools/d) over a 24 h period is then calculated as the amount of enriched free cholesterol relative to the total cholesterol pool. Although this method allows for the direct measurement of the cholesterol pool synthesized over a 24 hrs period, the laboratory techniques employed to quantify isotope enrichments are quite time-intensive and laborious.
2.2.2.1.2.2 Cholesterol precursor quantification

Circulating cholesterol precursors quantification, i.e. lathosterol, lanosterol, squalene, and desmosterol levels, may reflect whole body cholesterol synthesis (Kempen et al., 1988; Pfohl et al., 1999). The rationale for using precursor levels as an indicator of cholesterol synthesis lies in the assumption that these compounds leak into plasma lipoproteins at a rate proportional to that of their formation in the cholesterol synthetic pathway (Ordovas and Tai, 2002). Levels of cholesterol precursors are determined by gas–liquid chromatography from the non-saponifiable material of serum or plasma lipids. This cholesterol synthesis assessment method benefits from being efficient and inexpensive. On the other hand, the method is limited in that it does not allow for the direct quantification of the amount of cholesterol synthesized. Instead, this method simply indicates whether or not increases or decreases in cholesterol precursors occur as a result of specific interventions.

2.2.2.1.3 Physiological factors affecting cholesterol absorption and synthesis:

In light of their advantages and disadvantages, the methods used in the assessment of cholesterol kinetic parameters should be considered in reviewing the studies examining these vectors. Certain physiological factors, such as genetics, and body weight, have been shown to affect cholesterol homeostasis by directly modulating either cholesterol absorption or synthesis. The following section aims to discuss the primary effect of each of these factors on either one of these vectors, and attempts to delineate whether a corresponding effect between the two vectors exists:
2.2.2.1.3.1 Genetic factors

Cholesterol metabolism is regulated by a host of different proteins and transporters, many of which have been shown to possess genetic polymorphisms. The involvement of hereditary factors in cholesterol absorption and synthesis is supported by human studies showing inter-individual variability in response to cholesterol lowering by statins (Miettinen and Gylling, 2003a; O'Neill et al., 2001), heritability of indicators of cholesterol synthesis and absorption (Boomsma et al., 2003; Gylling and Miettinen, 2002a). Apolipoprotein E (apoE) has been shown to play a major role in lipid transport and metabolism. Three common isoforms of the apoE gene exist, these being E2, E3, and E4. Carriers of the E2 isoform may have lower rates of cholesterol absorption and higher rates of cholesterol synthesis compared to carriers of the E3 or E4 isoform (Gylling et al., 1995; Kesaniemi et al., 1987; Miettinen et al., 1992). In contrast, carriers of the E4 isoform exhibit increased cholesterol absorption rates, accompanied by a reciprocal decrease in bile acid synthesis (Kesaniemi et al., 1987). As a result, carriers of the E4 isoform may be better candidates for cholesterol lowering drugs that inhibit absorption.

2.2.2.1.3.2 Body weight [Body mass index (BMI)]

Body mass index affect both the physiological and therapeutic factor. Dyslipidemia is common in persons who are overweight and obese (Dattilo and Kris-Etherton, 1992). These abnormal fasting lipid profiles may be due to altered cholesterol metabolism commonly found in overweight populations (Di Buono et al., 1999; Miettinen and Gylling, 2000). Compared to normal weight individuals, obese subjects demonstrate elevated cholesterol synthesis (Miettinen and Gylling, 2000). Liver biopsies taken from normal and overweight individuals reveal that the expression of HMG CoA reductase, the enzyme catalyzing the rate-limiting step in hepatic cholesterol synthesis, is augmented in those who are overweight (Stahlberg et al., 1997). In
addition, the activity of other enzymes participating in the cholesterol synthesis pathway is elevated in obese individuals. Moreover, it was observed that the livers of obese participants are enlarged (Stahlberg et al., 1997). Since synthesis of cholesterol per g of hepatic tissue is similar in lean and obese individuals, the larger livers of obese compared to lean individuals results in an overall augmentation in synthesis (Angel and Bray, 1979; Angelin et al., 1982). This increase in cholesterol synthesis in obese subjects has been associated with a decrease in cholesterol absorption (Miettinen and Gylling, 2000).

2.2.2.1.4 Therapeutic factors affecting cholesterol absorption and synthesis:

2.2.2.1.4.1 Weight loss

The statement that weight loss decreases cholesterol synthesis is supported by the work of which showed that a weight loss of 3 to 8 kg by diet and obese men resulted in a decline in whole body cholesterol synthesis (Di Buono et al., 1999). The mutual effect of weight loss on cholesterol absorption, however, has yet to be clarified. Although the action of insulin and glucose on cholesterol metabolism may confound the results (Pihlajamaki et al., 2004), weight loss of approximately 15 kg in type 2 diabetics decrease total cholesterol by 21%. The decrease in cholesterol was accompanied by suppression in cholesterol synthesis and elevated cholesterol absorption (Simonen et al., 2002). Additionally, the question still remains as to how a decrease in hepatic synthesis signals an up regulation in intestinal cholesterol absorption. Future investigation should aim to establish a clear mechanistic cause and effect relationship between synthesis at the liver, and absorption at the intestine.
2.2.2.1.4.2 Statin therapy

The reduction of HMG CoA reductase at the hepatocytes leads to increased expression of LDL receptors, which bind circulating LDL in turn effecting plasma LDL levels (Lennernas and Fager, 1997; Ray and Cannon, 2004). As a result of this inhibition of hepatic cholesterol synthesis, statin therapy has been shown to reduce cholesterol concentrations by 20–30% (Miettinen et al., 2003). Since statin therapy aims to lower cholesterol synthesis, the effect of these pharmaceutical agents on overall cholesterol metabolism has been investigated. A study was conducted where 31 patients with gallstone participated in a randomized, parallel-arm, placebo-controlled trial examining the effect of statin therapy on cholesterol kinetic parameters. Seventeen of these patients received 20 mg/d of atorvastatin and the other 14 served as controls. Cholesterol precursor-to-cholesterol ratios were used as indicators of hepatic cholesterol synthesis. After 3 weeks of treatment, the plasma lathosterol-to-cholesterol ratio decreased, suggesting a decrease in cholesterol synthesis. Despite these reductions, no changes in cholesterol absorption occurred. More recently, the absorption markers, campesterol, sitosterol and cholestanol, were used to determine the effects of statin therapy in subjects with high initial cholesterol absorption versus those with low absorption (Gylling and Miettinen, 2002b). After one year of statin treatment, no differences in circulating total cholesterol levels were observed between the two groups. Conversely, a greater suppression in synthesis in those with low baseline cholesterol absorption levels was noted following treatment. Furthermore, cholesterol absorption was shown to increase more in those who had greater declines in synthesis. Thus, although the study did not observe an association (Smith et al, 2000).

2.2.2.1.4.3 Ezetimibe therapy

Ezetimibe, also known as SCH 58235, is the first of a series of new pharmaceutical agents developed to reduce cholesterol absorption (Jeu and Cheng, 2003). The inhibition of cholesterol absorption by ezetimibe is thought to occur through its interference with the function of the
NPC1L1 transporter (Altmann et al., 2004; Davies et al., 2005). Ten mg/d of ezetimibe may lower total cholesterol up to 15% and LDL cholesterol up to 20% (Jeu and Cheng, 2003). Recently the effect of ezetimibe on cholesterol absorption and synthesis in humans were examined (Sudhop et al., 2002). The study was a randomized, double-blind, placebo-controlled cross-over trial in individuals with mild to moderate hypercholesterolemia. In comparing ezetimibe with placebo, researchers found a 54% decrease in cholesterol absorption. Not surprisingly, the decline in absorption was accompanied by a corresponding rise in cholesterol synthesis. Thus, as with plant sterols, inhibiting cholesterol absorption through ezetimibe results in compensatory increases in cholesterol synthesis.

2.2.3 Treating two sources of cholesterol — cholesterol production and absorption

An innovative progression in cholesterol-lipid lowering therapy has occurred in the past few years. Identifying that treating both cholesterol production in the liver and absorption in the intestine, either by administering ezetimibe/simvastatin as a single tablet or co-administering ezetimibe together with any dose of statin, results in superior LDL lowering efficacy and more patients achieving or getting below LDL treatment goals compared with inhibition of a single cholesterol source with statins alone. Statins (e.g., fluvastatin, lovastatin, pravastatin, simvastatin, atorvastatin, and rosuvastatin) deliver only single inhibition of cholesterol production in the liver by blocking HMG-CoA reductase, the rate-limiting step in cholesterol synthesis, but do not impact intestinal cholesterol absorption (Shepherd, 2004). Accordingly, hepatocytes become depleted of cholesterol and respond by increasing LDL clearance from the blood and decreasing entry of LDL into the circulation (Knopp, 1999; Steiner, 2003). These actions, in turn, give rise to lower plasma LDL.
2.2.4 Maintaining very low cholesterol levels

Monitoring cohort studies have consistently shown that people with low total cholesterol levels (e.g., <4·0 mmol/l) are at a higher risk of subsequent death from cancers, respiratory causes, haemorrhagic stroke, and non-medical causes of death than are people with higher baseline cholesterol levels (Jacobs et al., 1992; Neaton et al., 1992). Collective results from large randomised controlled trials of statin treatment have now provided confirmation that reducing cholesterol and maintaining low cholesterol levels for at least 5 years is not only safe but beneficial (Cholesterol Treatment Trialists' (CTT) Collaborators, 2005). Some of these associations can be explained by reverse causality (e.g., cancer-reducing cholesterol levels while increasing risk of subsequent death), but concerns remain that low total cholesterol levels, as well as lowering cholesterol to very low levels, could be harmful (Muldoon and Manuck, 1994; Muldoon et al, 2001). However, populations eating diets which are low in saturated fats often have average total cholesterol level of about 4mmol/l, very low rates of coronary heart disease, and no clear excess of deaths from other causes (Chen et al., 1990; Chen et al., 1991). Also, neonates have LDL cholesterol of about 1mmol/l (Sankatsing et al., 2005).

All demonstrated sustained benefits from cholesterol-lowering therapy on cardiovascular mortality or morbidity, and reassuring long-term safety effects.

Trials of more intensive statin therapy published since 2004 achieved substantial and sustained LDL cholesterol reductions, typically to below 2·0 mmol/l in those allocated intensive treatment (LaRosa et al., 2005; Pedersen et al., 2005; Cannon et al., 2004; de Lemos et al., 2005; Wiviott, 2006; Cannon et al., 2006).
3.1 Introduction

3.1.1 History of CRP

C-reactive protein (CRP) is an acute-phase protein that was discovered in 1930 by William S. Tillet and Thomas Francis at the Rockefeller Institute for Medical Research (Tillet and Francis, 1930). After studying the immune response of patients with pneumococcal pneumonia, Tillet and Francis found that sera of these patients precipitated with a soluble extract of the *Pneumococcus pneumoniae*. This soluble extract was called fraction C, later identified as a polysaccharide of the cell wall. After resolution of the pneumonia, no precipitation reaction occurred when the serum of recovered patients was mixed with fragment C, while in sera of patients with a fatal outcome, the precipitation reaction remained positive. In 1933, Rachel Welsh found strongly positive precipitation tests with gram-negative microorganisms, her youngest patient being 6 months of age. Two arguments pointed in the direction of a nonspecific physiochemical reaction to bacterial infection instead of a specific antigen–antibody reaction: first, the disappearance of a positive test after recovery of the infection and, second, a positive test result in a 6-month-old baby (Gotschlich, 1982). In 1941, Avery and Theodore Abernethy found that the reactive substance responsible for the precipitation with fraction C was a protein: CRP. In addition, they discovered that Ca$^{2+}$ is an essential participant in the reaction (Gotschlich, 1982).

CRP was the first protein that was found in the acute phase of an infection. The concentrations of these proteins increased from 50% to as much as 1000-fold. The acute-phase response, i.e. the changes in concentrations of the acute-phase proteins, is a non-specific innate defense mechanism of the host. There are many other conditions besides bacterial infections that lead to
an acute-phase response including inflammation, necrosis, malignancies, burns, surgery, trauma, childbirth, strenuous exercise, stress, and psychiatric disease (Gabay and Kushner, 1999; Kushner, 1982).

CRP levels are also elevated in the presence of metabolic risk factors such as obesity (Visser, Bouter, McQuillan et al., 1999). Adipose tissue is a major producer of proinflammatory cytokines and hormones and is thought to induce low-grade systemic inflammation (Greenberg and Obin, 2006). However, while several studies demonstrate that levels of CRP are elevated in persons with higher BMI (Visser, Bouter, McQuillan et al., 1999), and that weight loss reduces CRP, few studies have examined whether obesity might have a differential impact on CRP levels in different racial and ethnic groups (Esposito, Pontillo, Di Palo et al., 2003; Selvin, Paynter, and Erlinger, 2007).

3.2 The source of the C-reactive protein

C-reactive protein is referred as an acute phase reactant expressed principally by the liver. In healthy, lean individuals CRP circulates at low concentrations in plasma (<3 mg/l). These levels rise dramatically in response to injury, infection and inflammation (Steel and Whitehead, 1994), in response to bacterial lipopolysaccarides (LPS), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), oncostatin M and leukemia inhibitory factor (LIF) (Weinhold and Ruther, 1997). Slightly increased CRP concentrations, detected with high sensitivity kits, but still within what has traditionally been regarded as the normal range (1–10 mg/l) may reflect chronic low-grade inflammation.
3.3 Structure and binding sites of CRP

In the last decade, the protein experienced a revival in attention due to the inflammatory pathogenesis of atherosclerosis. In particular, the role of CRP in the vulnerability and instability of atherosclerotic plaques, leading to rupture, thrombosis and thus to occlusive arterial disease, has been studied.

CRP is a protein of the highly conserved pentraxin family with a striking sequence homology between species going back as far as the horseshoe crab. Another striking feature is the lack of polymorphism within a species. It is built up of five identical subunits (protomers) aggregated in a symmetric pentameric form by noncovalent binding between the subunits. CRP is an allosteric protein. Other ligands of the calcium-dependent binding property of CRP are nuclear constituents: histones, chromatin, and small nuclear ribonucleoproteins (snRNPs) (Du clos et al., 1988; Du clos, 1989). Therefore, CRP may play an important role in the clearance and processing of nuclear antigens, thus preventing autoimmune responses to nuclear material. A schematic illustration of CRP is depicted in Fig 3.3.

![Schematic illustration of pentameric CRP with possible binding sites](Du clos et al., 1988 and Du clos, 1989).

Figure 3.3 Schematic illustration of pentameric CRP with possible binding sites (Du clos et al., 1988 and Du clos, 1989).
3.4 Determination of C-reactive protein

C-reactive protein detection has been used for many years in the diagnosis of tissue injury or inflammation as high concentrations can be found in patients suffering from infection, inflammation, trauma, malignancies, stress, arthritis, surgery and acute myocardial infarction. Until recently, the prediction of cardiovascular disease relied on factors such as hypercholesterolemia and hypertension, and so a non-conventional method such as CRP detection was considered. CRP is produced due to underlying vascular inflammation and it was found that baseline levels of CRP can predict future risk of stroke, myocardial infarction and peripheral arterial disease in both healthy and high-risk individuals such as smokers, the elderly and angina patients (Kuller et al., 1996).

Several pharmacological agents proven to reduce vascular risk influence of CRP levels, of these, the statin drugs are the most important drug to reduce the levels of CRP. Studies with pravastatin, lovastatin, cerivastatin, simvastatin, and atorvastatin have all shown that, on average, median CRP levels decline 15% to 25% as early as 6 weeks after initiation of therapy. As shown in the large-scale Cholesterol and Recurrent Events (CARE) (Ridker et al., 1999) and Pravastatin Inflammation/CRP Evaluation (PRINCE) (Albert et al, 2001) trials and subsequently confirmed in other settings, there is little evidence that the magnitude of LDL reduction predicts the magnitude of CRP reduction. On the other hand, aggressive LDL reduction remains a critical therapeutic goal, and thus serial LDL evaluation should remain the primary method to monitor statin compliance. However, whereas all subjects taking statins achieve a beneficial reduction in LDL levels, there seems to be responders and non-responders for statins in terms of CRP reduction (Albert et al, 2001).
For individuals with LDL levels above >5mmol/l, an elevated CRP level should aggressively encourage physicians and patients to institute pharmacological therapy in those instances where none is currently being used or where compliance is poor. For individuals with LDL levels between 5-5.2mmol/l, the additional finding of an elevated CRP indicates an elevated risk. For individuals with LDL levels below <5mmol/l, the finding of an elevated CRP implies substantially higher risk than predicted on the basis of LDL alone, such individuals will have risk estimates as high as some individuals with overt hyperlipidemia. Patients with this profile should be advised to adhere carefully with their lifestyle interventions, despite "low" LDL cholesterol levels. Individuals with the low LDL/high CRP phenotype are at elevated risk of having the metabolic syndrome and should have fasting glucose levels measured. (Ridker, 2001).

3.5 Biosynthesis and kinetics of CRP

The major site of CRP synthesis is the hepatocyte (Taylor et al., 1981). Under physiological circumstances, human CRP is a protein with a median serum concentration of 0.8 mg/l. The human CRP gene is located on the long arm of the chromosome. Plasma CRP is mainly regulated at the transcriptional level induced by interleukin-6 (Volanakis, 2001; Toniatti et al., 1981). When released in the circulation, the protein is equally distributed in the vascular compartment without substantial tissue sequestration at sites of inflammation (Hutchinson et al., 1994). This could be explained by the ‘detoxification’ hypothesis: binding and thereby neutralizing/detoxifying harmful substances that escape from the site of inflammation to the circulation. The dramatic rise in CRP levels may exceed 300 mg/l within 48 h after the acute event. High levels may persist during the presence of the stimulus. There is a strong positive correlation between the duration and the intensity of the stimulus (e.g. tissue injury) and the number of hepatocytes synthesizing CRP. The latter phenomenon is due to the activation of the hepatocytes in the direction of the blood flow: cytokines first arrive at the hepatocytes in the vicinity of the portal triangle and further activation of the hepatocytes takes place in the direction
of the central vein. This results in a higher peak level and also a protracted increase in serum CRP whenever the degree of the stimulus is stronger and longer. Most of the CRP is taken up and degraded at the same site of production of the hepatocyte. A small part (bound to its ligands) is taken up and processed by neutrophils and macrophages. C-reactive protein has a biological half-time of 19 h (Vigushin et al., 1993).

Functionally, CRP has several direct effects that may influence progression of vascular disease. Native CRP binds to oxidized LDL and to partly degraded LDL, and then activates complement of HDL (Pepys et al., 2003). CRP also induces expression of several cell adhesion molecules, mediates uptake of LDL by macrophages, induces monocyte recruitment into the arterial wall, enhances production of MCP-1, and up regulates the angiotensin type 1 receptor (Bhakdi et al., 1999; Pasceri et al., 2000; Pasceri et al., 2001; Torzewski et al., 2000; Zwaka et al., 2001; Wang et al., 2003). In addition, evidence suggests that CRP expression is up regulated in atheromatous plaque compared with normal arterial tissue (Yasojima et al., 2001).

### 3.6 Lowering CRP levels: a new therapeutic goal

The inflammatory pathogenesis of atherosclerotic disease would seem to indicate that CRP measurements and the goal of lowering CRP levels may be tools in the treatment strategy of the physician. The following are the different possibilities with regard to primary and secondary prevention:

#### 3.6.1 Statins and CRP

HMG-CoA reductase inhibitors (statins) are extensively used as lipid-lowering drugs in patients with atherosclerosis. The clinical and pathological effects of statins cannot be attributed to the lowering of circulating LDL alone. Among their other effects (antithrombotic, anti-oxidant), statins may also have anti-inflammatory properties (Lyn and Uzick, 2000). Several studies have demonstrated the CRP-lowering capacity of statins, independent of changes in lipid profiles
(Ridker et al., 2001). Moreover, the effect of statins is more profound in patients with CRP levels in the highest quartiles or quintiles in combination with low lipid levels (Albert et al., 2001). To distinguish high-risk from low-risk patients, CRP measurements may be a good target in monitoring the effectiveness of statins (Jialal et al., 2001). This may also have implications for the use of statins in primary prevention in patients without hyperlipidemia.

3.6.2 Aspirin and CRP

Aspirin is primarily a platelet aggregation inhibitor, but it also has anti-inflammatory actions. In 1997, Ridker and colleagues demonstrated that the reduction in risk of myocardial infarction in patients using aspirin was directly correlated with the decrease in CRP level. That is, CRP is a tool for measuring the success of therapy.

3.6.3 Antibiotics and CRP

A recent study showed a positive association between viable C. pneumoniae (positive RNA-PCR) within plaques and high CRP levels. Measuring plasma CRP, however, could not discriminate between patients with viable Chlamydia and patients with only positive serological tests (Johnston et al., 2001).

3.6.4 Antioxidants and CRP

Oxidative stress is considered to be one of the most common causes of the inflammatory process. Due to oxidation of triglyceride molecules within LDL particles, the LDL is modified and, as a process of clearance, taken up by macrophages in atherosclerotic lesions. Thus, theoretically, reducing oxidative stress could bring the inflammatory process to a lower level, resulting in
plaque stabilization. α-Tocopherol (α-TOH) is the most abundant lipid-soluble antioxidant in LDL and it protects LDL from lipid peroxidation by scavenging α-tocopheroxyl radicals. Natural polyphenols and synthetic probucol inhibit LDL oxidation in vitro and in animal studies and may therefore have inhibitory effects on atherosclerosis (Johnston et al., 2001).

3.7 Potential clinical application of C-reactive protein measurement

The suitability of plasma CRP assay for clinical application compares favourably with determination of other inflammatory markers (Ridker, 1999). The predictive abilities of both plasma CRP and fibrinogen levels add to that of plasma lipid measures (e.g., total cholesterol–HDL ratio); however, it remains unclear whether risk assessment can be improved by the addition of assays for lipoprotein(a), homocysteine, or such fibrinolytic markers as tissue plasminogen activator and plasminogen activator inhibitor–1. The performance assessment of markers for cardiovascular risk prediction in the Women's Health Study showed that an elevated plasma CRP value was the single best marker and, when combined with the plasma total cholesterol–HDL ratio, provided an even more accurate prediction of prospective risks (Ridker et al., 2000).

CRP is easily and inexpensively measured, and standardized high-sensitivity assays are commercially available that provide similar results in stored, fresh or frozen plasma (Rifai et al., 1999; Khuseyinova et al., 2003). However, taking into account the moderate repeatability of CRP measurement over time, its predictive value could be even further improved by making several serial measurements (Koenig et al., 2003). In the range needed for vascular risk detection, the variability and classification accuracy of CRP is similar to that of total cholesterol (Ockene et al., 2001). For all these reasons, a recent statement from the Centers for Disease Control and Prevention and the American Heart Association (CDC/AHA) recommended that CRP had the analyte and assay characteristics most conducive to use in clinical practice compared with other inflammatory markers identified (Pearson et al., 2003).
3.8 C- reactive protein levels after statin therapy

Statin therapy lowers the risk of cardiovascular events by reducing plasma cholesterol levels, and practice guidelines for patients with known cardiovascular disease emphasize the importance of reaching target goals for low-density lipoprotein cholesterol (Expert Panel on Detection et al., 2001). However, it shown that statin therapy results in a greater clinical benefit when levels of the inflammatory biomarker C-reactive protein are elevated (Ridker et al., 1998, and Ridker et al., 1959) and that statins lower CRP levels in a manner largely independent of LDL cholesterol levels (Ridker et al., 1959). These findings, along with basic laboratory evidence, have led to the hypothesis that, in addition to being potent lipid-lowering agents, statins may also have anti-inflammatory properties that are important for prognosis and treatment (Albert et al., 2001). If so, then the level of CRP achieved as a result of statin therapy may have clinical relevance analogous to that of the LDL cholesterol levels achieved through the use of statin therapy (Ridker et al., 2001).
4.1 Introduction

HMG CoA reductase inhibitors have been largely used in patients with atherosclerotic disease and hyperlipidemia (Mahley et al., 1999; Vaughan et al., 2000). The lipid-related effects include reduced serum levels of total cholesterol, LDL cholesterol, triglyceride, and an increased level of HDL cholesterol (Hunninghake et al., 1990; Watts, 2001). Furthermore, statins have also been found to exert both anti-inflammatory and anti-angiogenic effects, both of which are relevant to vascular disease (Inoue et al., 2000; Jialal et al, 2001; Kwak et al., 2001; Pruefer et al., 1999). It is these non-lipid-related effects that are thought to act rapidly and produce a significant cardiovascular benefit long before their lipid-lowering effects could possibly exert their effects (Kuvin and Kara, 2003; Plenge et al., 2002; Wassman et al., 2003).

4.2 Classification

Atorvastatin, cerivastatin, pitavastatin, rosuvastatin and fluvastatin are synthetic. Lovastatin and pravastatin are fermentation-derived. Simvastatin is synthetic derivate of a fermentation products. Mevastatin is a natural compound found in red yeast rice.
4.2.2 The endogenous mevalonate pathway leading to cholesterol biosynthesis

Figure 4.2.2 the endogenous mevalonate pathway leading to cholesterol biosynthesis (Vaughan et al., 2000).

4.3 Mechanism of action

Statins block the conversion of HMG-CoA to mevalonate by inhibiting the enzyme HMG-CoA reductase. This inhibition ultimately leads to decreased production of cholesterol and isoprenoid intermediates, such as farnesyl-PP and geranylgeranyl-PP. The inhibition of cholesterol
biosynthesis by statins leads to lowered production of sterol products and impaired formation or disruption of lipid rafts. By inhibiting the production of isoprenoids, which serve as lipid attachments for intracellular signaling molecules such as the GTP-binding proteins Ras and Rho, statins interfere with a number of cellular processes, including cell proliferation, differentiation and migration, and factor secretion. Inhibition of HMG CoA reductase reduces intracellular cholesterol levels; this activates a protease, which in turn cleaves sterol regulatory element-binding proteins (SREBP's) from the endoplasmic reticulum. The SREBP's translocate to the nucleus where they upregulate expression of the LDL receptor gene. Enhanced LDL receptor expression increases receptor-mediated endocytosis of LDL and thus lowers serum LDL. Inhibition of HMG CoA reductase also reduces intracellular levels of isoprenoids, which are intermediates in cholesterol biosynthesis. Anti-inflammatory action of statins is thought to occur via a number of mechanisms, they exert an inhibitory effect on macrophage growth and secretion of proinflammatory cytokines (Vaughan et al., 2000). They inhibit proliferation of mononuclear cells, and modulate the differentiation and function of T lymphocytes in peripheral blood (Neuhaus et al., 2002), and the central nervous system (Youssef et al., 2002). Their use may also lead to a reduced number of inflammatory cells in atherosclerotic plaques (Scuteri et al., 2001).

4.4 Statin and Cholesterol level

Beside widely used in lipid lowering therapy, statins (when administered at high doses) are frequently sufficient to achieve guideline-recommended LDL targets for many patients with hypercholesterolemia in everyday clinical practice (Goettsch et al., 2004). Over half of patients (52%) did achieve the LDL target on the initial dose of statin, and 86% of these patients had reached the target after 6 months (Foley et al., 2003).
4.5 Statin and C-reactive protein level

Reduced serum level of the acute phase protein C-reactive protein has also been linked to statin use (Jialal et al., 2001). This is important, as elevated levels of CRP have been found to be an independent high risk factor in the development of cardiovascular disease (Kuvin and Kara, 2003; Li and Chen, 2003). Recently, it has been shown that this may be due to a direct pro-inflammatory effect that CRP has on human vascular endothelial cells (Li and Chen, 2003). It has been reported that after 5 years' treatment with statin, CRP levels decrease by 22% in comparison to placebo (Ridker et al., 1998). In that study, the mean CRP level in the atorvastatin group was significantly lower than that of placebo sub-group where LDL was low. In similar long-term studies, significant reductions in plasma CRP levels have been reported after statin administration (Ridker and Rifai, 2001; Ridker et al., 1999).

Theoretically, by decreasing the levels of atherogenic lipoproteins, statins could decrease systemic inflammation, thereby reducing CRP levels, which resulting in lowering cholesterol. An alternative hypothesis proposes that statins have direct anti-inflammatory effects, independent of their lipid-lowering capabilities. In this model, CRP plays a more direct role in the pathogenesis of atherosclerosis, and a statin-mediated reduction in inflammation contributes directly to reduce disease activity (Ridker et al., 2000).

4.6 Statin and cardiovascular disease

Multiple human trials utilizing HMG-CoA reductase inhibitors, have repeatedly confirmed that changing lipoprotein transport patterns from unhealthy to healthier patterns significantly lowers cardiovascular disease event rates, even for people with cholesterol values currently considered low for adults. As a result, people with a history of cardiovascular disease may derive benefit from statins irrespective of their cholesterol levels (MRC/BHF Heart Protection Study of
cholesterol lowering with simvastatin in 20,536 high-risk individuals, 2002), and in men without cardiovascular disease there is benefit from lowering abnormally high cholesterol levels ("primary prevention") (Shepherd et al., 1995). Primary prevention in women is practiced only by extension of the findings in studies on men (Grundy, 2007), since in women none of the large statin trials has shown a reduction in overall mortality or in cardiovascular diseases (Kendrick, 2007).

4.7 Adverse effects of statin use

The lack of effect of statins on adverse hepatic outcomes (with the possible exception of atorvastatin 80 mg) raises several clinical questions about the increased transaminases. Are all statins the same in this respect? If a patient develops raised enzymes with one statin should another be tried or should statin treatment be continued? Is it safe to start a statin in individuals with raised enzymes?

Is there any risk of clinical hepatitis? Is it safe to start statin treatment in people with raised γ-glutamyl transferase due to excessive alcohol intake? Statins produce information that recommends baseline measurement of liver function and contraindicates the drugs in active liver disease, so in patients with baseline liver abnormalities, active disease must first be excluded. At standard doses, effects on liver enzymes are rare (<1%), but at higher doses different statins vary in the degree to which they raise liver enzymes (Illingworth et al., 2001). This may just parallel their LDL cholesterol-lowering efficacy, or could be some specific effect of particular statins. A logical approach is to increase the statin dose slowly in those at risk of transaminase rises. Routine monitoring of liver function after starting statin treatment is no longer recommended for simvastatin, pravastatin, or lovastatin up to 40 mg daily (since the extensive controlled trial data are reassuring), but remains recommended in product information for the other statins and higher doses, despite the lack of evidence of adverse outcomes. If alanine or aspartate transaminases are more than three times the upper limit of normal in an asymptomatic patient with no other liver abnormalities, the enzymes should be checked within a week and statin treatment stopped.
temporarily if alanine transaminase is still at this level. Increases to between two to three times the upper limit of normal in an asymptomatic patient necessitate monitoring, but will often resolve while on treatment. Adverse reactions have usually been mild and transient. In controlled clinical studies of 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to atorvastatin. The most frequent adverse events thought to be related to atorvastatin were constipation, flatulence, dyspepsia, and abdominal pain. Uncomplicated myalgia not associated with elevated creatine kinase has been reported in atorvastatin-treated patients. In clinical trials <1% of patients had confirmed transaminase elevations greater than three times the upper limit of normal. Most elevations occurred within 16 weeks of starting treatment. The ocular lens is completely avascular and depends largely on endogenous cholesterol synthesis. Therefore, reductase inhibitors could have an adverse effect on the human lens after long-term treatment. Rhabdomyolysis with acute renal failure secondary to myoglobinuria has been reported in this class. Atorvastatin therapy should be temporarily withheld or discontinued in any patient with an acute, serious condition suggestive of a myopathy or having a risk factor predisposing to the development of renal failure secondary to rhabdomyolysis (e.g. severe acute infection, hypotension, major surgery, trauma, severe metabolic, endocrine and electrolyte disorders (www.med-decisions.com/cvtool/phys/phys.html).
5.1 Introduction

Atorvastatin is a synthetic inhibitor of HMG-CoA reductase which catalyzes the conversion of HMG-CoA to mevalonate, the rate-limiting step in de novo cholesterol synthesis (Gibson et al., 1996). Atorvastatin is administered as the calcium salt of the active hydroxy acid usually at a dosage of 10–80 mg daily. Nevertheless, atorvastatin acid is converted to its lactone (Kearney et al., 1993). Atorvastatin is metabolized in the liver via the cytochrome P450 3A4 enzyme system in ortho- and parahydroxylated derivatives, which are active metabolites, and a beta-oxidation product, which is inactive. The active metabolites inhibit HMG-CoA reductase to the same degree as atorvastatin. Atorvastatin and its metabolites are mostly excreted in the bile and urinary excretion is low (Kantola et al., 1998; Black et al., 1999). As a result, renal insufficiency is unlikely to influence the pharmacokinetics of atorvastatin. Atorvastatin is one of several drugs in the “statin” class of compounds that is used for the treatment of hypercholesterolemia.
5.2 Chemical Structure of atorvastatin

![Chemical Structure of atorvastatin](http://www.rxlist.com/cgi/generic/atorvastatin.htm)

**Figure 5.2 Chemical Structure of atorvastatin**

5.3 Pharmacotherapy

Atorvastatin decrease total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations by up to 60% (Jones et al., 1998; Dart et al., 1997; Davidson et al., 1997).

The low density lipoprotein cholesterol target (2.6mmol/l) in coronary artery disease prevention specified by the National Cholesterol Educational Program (NCEP) can be reached by atorvastatin in the majority of patients (Athyros et al., 2002). However, the benefits (significant reduction in mortality and events) associated with achieving this goal may not be exclusively
dependent on a fall in LDL levels. One possibility is that statins influence the inflammatory process (Sacks and Ridker., 1999). In addition to cholesterol and LDL-cholesterol, this drug also decreases triglyceride concentrations. Total cholesterol was reduced by 45% at that dose level. Results suggest that there are age-related differences in systemic exposure of atorvastatin (Jones et al., 1998; Dart et al., 1997; Davidson et al., 1997).
6.1 Volunteers and patients

6.1.1 Healthy volunteers

Volunteers and patients were recruited from the Medunsa Community and the patients from the Pharmacology Clinic. Sixty one healthy volunteers (male and female age 21-58) were included into the study.

6.1.1 The inclusion criteria were:

- healthy volunteers age 18-70
- male or female
- no abnormalities in clinical medical examination
- normal full blood count, urea and electrolytes, liver function test,
- non-smoking
- not on any chronic medication
- no chronic or acute diseases

On the first visit a complete history and consent form (see appendix 1) were completed and a medical examination was carried out to include or to exclude volunteers from the study.

6.1.2 Patients

Forty two naïve patients age 58± 8 with documented moderate hyperlipidaemia (total cholesterol level ranging from 5.1-7.8 mmol/L; LDL-C level ranging from 3.1-5.5mmol/L; triglyceride level ranging from 0.6-3.5mmol/L; HDL-C level ranging from 0.9-2.2mmol/L) were enrolled in the
study. Unfortunately six patients were lost to follow up. Total cholesterol (TC), triglycerides (TG), LDL-cholesterol, HDL-cholesterol and hs-CRP were measured at baseline and after 3 months of atorvastatin (20mg daily) treatment. Cardiovascular risk was graded as low risk if CRP was < 1mg/L, moderate if 1-3mg/L and high risk if 3-10mg/L.

These patients were grouped as follows:

33 healthy Volunteers (controls) and 42 patients

- No. of Healthy volunteers (control) = 33
- No. Patients with both high cholesterol and CRP =6
- No. Patients with normal cholesterol and high CRP =10
- Patients with high cholesterol and normal CRP=26

No medication.

- No. of patients on 20mg Atorvastatin=3
- No. of patients on 20 mg Atorvastatin=12

No follow up.

- No follow up.
- No follow up.

Figure 6.1.2: Flow diagram of the study
6.1.2.1 Measurements

Blood samples 5ml (EDTA tubes) of peripheral venous blood was be taken, after a 12-h overnight fast. Samples were centrifuged and plasma stored at -73°C for later analysis of CRP. Tubes were numbered maintain confidentiality. Cholesterol profile was done by using 30µl of blood from a finger prick. These profile included LDL, HDL, and total triglyceride level determination. Blood glucose was also done. The above was determined by using a Cardiocheck P-A hand held digital analyzer.

6.1.2.2 Statistical analysis

Comparisons between the control (treatment-naïve patients) and the experimental group (patients on 10 mg atorvastatin for 3 months) were done by using Wilcoxon Signed Rank Test (p value < 0.05 was considered as significant). Pearson’s coefficient was used to determine correlations between LDL-C, HDL-C, TC-C and hs-CRP.

6.2 Apparatus and methods

6.2.1 Determination of C-reactive protein

6.2.1.1 Immunoradiometric assay (IRMA)

It is a test that measures the concentration of antigens in a specimen through serological analysis that involves mixing radioactive antibodies with the antigen in question. This method was designed specifically to measure serum/plasma CRP in the previously established reference interval for healthy individuals (Shine et al., 1981).
6.2.1.2 Immunonephelometric (Dade Behring N Latex High Sensitivity CRP\textsuperscript{TM} mono assay

It is a method for quantifying serum protein concentration and compares it to a previous immunoblotting method. The detection limit for CRP was 0.17 mg/L, and the measuring range is 0.175–1100 mg/l, according to the manufacturer. The interassay is at 11.5 and 47.9 mg/l and the percentage are 6.1% and 3.5%, respectively (Hosmer, 1981).

6.2.1.3 Immunoturbidimetric (Tina-quant CRP detection method; Roche Diagnostics)

This method is used for the quantitative in vitro determination of Lipoprotein in human serum or plasma. It is performed on a Hitachi 717 automated analyzer. The detection limit is 0.1 mg/l and the extended measuring range (with reruns) is 0.1–240 mg/l. The intermediate assay is 2.6% at 4.65 mg/l CRP (Martin, 2000).

Three methods to measure were implemented in this study, i.e. DxC800 system a chemistry/biochemistry analyzer marketed by Beckman Coulter Inc for clinical diagnosis used in this study to measure cholesterol profile and C-reactive protein in serum, Cardio-check test manufactured by Polymer Technology Systems, Inc. (PTS, Inc.), was used to measure cholesterol profile only, and Elisa test used to determine the C-reactive protein.

6.3 Blood analyses of cholesterol profile using a Cardio-check test

Volunteers had to be fasting over-night. Five ml of peripheral venous blood samples were collected with EDTA capillaries for analysis with Cardio-check PA electronic hand technique.
Test strips with imbedded reagents and a pre-programmed electronic memo chip were used. Forty µl of blood was placed on the test. Samples were centrifuged and plasma stored at -73°C for later analysis of C-reactive protein (CRP). Tubes were numbered to maintain confidentiality. Cholesterol profile was done by using 30µl of blood from a finger prick. This profile includes LDL, HDL, TC and triglyceride. Blood glucose was also done.

6.4 Blood analysis of the Cholesterol profile using the DxC800 system

DxC800 system was intended for quantitative determination of cholesterol profile concentration and CRP in human serum or plasma.

6.5 Methodology of total cholesterol (TC) determination

Cholesterol reagent was used to measure cholesterol concentration (Allain, 1974; Roeschlau et al, 1974; Trinder, 1969). In the reaction, cholesterol esterase (CE) hydrolyzes cholesterol esters to free cholesterol and fatty acids. Free cholesterol was oxidized to cholestene-3-one and hydrogen peroxide by cholesterol oxidase (CO). Peroxidase catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine (4-AAP) and phenol to produce a colored quinoneimine product.

The synchron system automatically proportions the appropriate sample and reagent volumes into the cuvette.

The ratio used was one part sample to 100 parts reagent. The system monitors changes in absorbance at 520 nanometers. This change in absorbance was directly proportional to the concentration of cholesterol in the sample and was used by the System to calculate and express cholesterol concentration.
6.5.1. Chemical reaction scheme

Cholesterol ester $\xrightarrow{\text{CE}}$ Cholesterol + Fatty Acid

Cholesterol + O$_2$ $\rightarrow$ CO $\rightarrow$ Cholestene-3-one + H$_2$O$_2$

2H$_2$O$_2$ + 4-AAP + Phenol $\rightarrow$ Peroxidase $\rightarrow$ Quinoneimine + H$_2$O

6.5.2 Reagents

Each kit contains the following items: Two cholesterol reagent cartridges (2 x 300 tests)

6.5.2.1 Reagent storage and stability

Cholesterol reagents were stored unopened at +2°C to +8°C. Once opened, the reagents were stable for 30 days at +2°C to +8°C unless the expiration date was exceeded.

6.5.3 Performance characteristics

6.5.3.1 Analytic range

The SYNCHRON® System method for the determination of this analyte provided the following analytical ranges:
### Table 6.5.3.1 Analytic range of the SYNCHRON® system

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Conventional units</th>
<th>S.I units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum or Plasma</td>
<td>5-750 mg/dl</td>
<td>0.13-19.43 mmol/l</td>
</tr>
<tr>
<td>Serum or Plasma (ORDAC)</td>
<td>600-1000 mg/dl</td>
<td>15.54-25.90 mmol/l</td>
</tr>
</tbody>
</table>

Samples with concentrations exceeding the high end of the analytical range were rerun with overrange detection and correction (ORDAC) enabled or diluted with saline and reanalyzed.

#### 6.5.3.2 Sensitivity

Sensitivity was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for cholesterol determination was 5 mg/dl (0.13 mmol/l).

#### 6.5.3.3 Equivalency

Equivalency to the Abell-Kendall procedure was assessed by Deming regression of Abell-Kendall values to those obtained with the SYNCHRON LX Systems using patient samples. The Total Cholesterol test on SYNCHRON Systems had been certified by the National Cholesterol Education Program (NCEP) [National Committee for Clinical Laboratory Standards, 1995].
6.5.3.4 Precision

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

The mean of the test precision data was less than or equal to the changeover value, compare the test standard deviation (SD) to the SD guideline given above to determine the acceptability of the precision testing. The mean of the test precision data was greater than the changeover value; compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100 (National Committee for Clinical Laboratory Standards, 1992).

6.6 Methodology of low density lipoprotein (LDL) determination

LDL reagent was used to measure the cholesterol concentration by a timed-endpoint method. The SYNCHRON® System was automatically proportioned the appropriate LDL cholesterol sample and reagent volumes into a cuvette. The ratio used was one part sample to 93 parts reagent. The System monitors changes in absorbance at 560 nanometers.

This change in absorbance was directly proportional to the concentration of LDL cholesterol in the sample and was used by the System to calculate and express the LDL cholesterol concentration.
6.6.1 Chemical reaction scheme

HDL-C, VLDL-C, CM-C → Non-Color Product

LDL Cholesterol → \( \triangle^4 \) Cholesterol + \( \text{H}_2\text{O}_2 \)

\( \text{H}_2\text{O}_2 + \text{DSBmT} + 4\text{-AA} \) → Color Product

6.6.2 Specimen

6.6.2.1 Specimen storage and stability

LDL cholesterol was stable for up to 5 days at +2°C to +8°C. Storage at -15°C to -20°C was not recommended. Frozen samples were thawed only once (National Committee for Clinical Laboratory Standards, 1990).

6.6.2.2 Reagents

Each kit contained the following items: Two LDL Reagent Cartridges (2 x 100 tests), two LDL Calibrator Bottles (2 x 1.0 ml), and Preparation Insert (Calibrator), Calibrator, Diskette, and Value-assignment Sheet.
6.6.2.2.1 Volumes per test

Sample Volume was 3 μl and Total Reagent Volume was 280 μl.

6.6.2.2 Reagent storage and stability

LDL reagent was stored unopened at +2°C to +8°C to obtain the shelf-life. Once opened, the reagent was stable for 30 days at +2°C to +8°C.

6.6.2.3 Performance characteristics of LDL determination

6.6.2.3.1 Analytic range

The SYNCHRON® System was a method for the determination of LDL; samples with concentrations exceeding the high end of the analytical range were diluted with saline and reanalyzed.

6.6.2.3.2 Sensitivity

Sensitivity was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for LDL determination was <8 mg/dl (<0.21 mmol/l).
6.6.2.3.3 Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods (National Committee for Clinical Laboratory Standards, 1995).

6.6.2.3.4 Precision

The mean of the test precision data was less than or equal to the changeover value, compared the test standard deviation to the standard deviation guideline given above to determine the acceptability of the precision testing. The mean of the test precision data was greater than the changeover value, compared the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

6.6.4 Methodology of high density lipoprotein (HDL) determination

HDL in human serum or plasma was precipitated by dextran sulfate (50 000 Mw) and magnesium in the separating reagent (Assman et al, 1983). The HDL portions were then removed by centrifugation. The cholesterol in the HDL fraction which remains in the supernatant was assayed with an enzymatic cholesterol reagent. HDL reagent was used to measure the cholesterol concentration by a timed-endpoint method (Allain et al, 1974; Roeschlau et al, 1974). In the reaction, the Cholesterol esterase (CE) hydrolyzes cholesterol estered to free cholesterol and fatty acids. The free cholesterol was oxidized to cholestene-3-one and hydrogen peroxide by cholesterol oxidase (CO). Peroxidase (HPO) catalyzes the reaction of hydrogen peroxide with 4-aminoantipyrine (4-AAP) and phenol to produce a colored quinoneimine product.
The SYNCHRON® System automatically proportioned the appropriate HDL cholesterol sample and reagent volumes into a cuvette. The ratio used was one part sample to 60 parts reagent. The system monitored the change in absorbance at 520 nanometers. This change in absorbance was directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL cholesterol concentration.

6.6.4.1 Chemical reaction scheme

\[
\text{Cholesterol ester} \xrightarrow{\text{CE}} \text{Cholesterol + Fatty Acid}
\]

\[
\text{Cholesterol + O}_2 \xrightarrow{\text{CO}} \text{Cholesten-3-one + H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 4\text{-AAP + Phenol} \xrightarrow{\text{HPO}} \text{Quinoneimine + H}_2
\]

6.6.4.2 Specimen

6.6.4.2.1 Sample preparation

a. 0.50 ml of each control and sample was pipetted and were assayed into appropriately labeled 10 x 75 mm glass test tubes or Microtube™ Tubecups.

b. 0.10 ml HDL Cholesterol separation reagent was pipette into each tube, was briefly vortexed for three seconds, incubated at room temperature for 10 minutes, and centrifuged for 10 minutes at an RCF of 1500g at +25°C.
6.6.4.3 Reagents

Each kit contains the following items: Two HDL Reagent Cartridges (2 x 300 tests), two HDL Separation Reagent Bottles, and One Sample Preparation Insert.

6.6.4.3.1 Volumes per test

Sample Volume was 5 μl, and total Reagent Volume was 300 μl.

6.6.4.3.2 Reactive ingredient

Dextran Sulfate (50,000 Mw) 6%, and magnesium Chloride Hexahydrate 0.24 mol/L were used as HDL cholesterol separation reagents.

6.6.4.4 Performance characteristics

6.6.4.4.1 Analytic range

Samples with concentrations that exceeded the analytical range and were clear (not cloudy) after pretreatment with the precipitating reagent were having the supernatant diluted one part sample with one part physiological saline. These diluted supernatants were reanalyzed.
6.6.4.4.2  Sensitivity

Sensitivity was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for HDL determination was 5 mg/dl (0.13mmol/l).

6.6.4.4.3  Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

The calibrator for HDL was value-assigned by CDC-certified Lipid Laboratories using the Abell-Kendall procedure (National Committee for Clinical Laboratory Standards, 1995).

6.6.4.4.4  Precision

The mean of the test precision data was less than or equal to the changeover value, compared the test SD to the SD guideline given above to determine the acceptability of the precision testing.

The mean of the test precision data was greater than the changeover value, compared the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.
6.6.5 Methodology of triglyceride (TG) determination

Triglyceride reagent was used to measure the triglycerides concentration by a timed endpoint method (Bucolo and David, 1973; Pinter et al 1965). Triglyceride in the sample was hydrolyzed to glycerol and frees fatty acids by the action of lipase. A sequence of three coupled enzymatic steps using glycerol kinase (GK), glycerophosphate oxidase (GPO), and horseradish peroxidase (HPO) caused the oxidative coupling of 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS) with 4-aminoantipyrine to form a red quinoneimine dye.

The triglyceride-blanked assay parameters were an alternate parameter set designed to be used with the triglycerides reagent. The triglyceride-blanked assay reduced the effects of free glycerol in serum which may be seen with the triglycerides assay parameters. The triglyceride-blanked assay employed the use of a reaction trigger cycle for glycerol blanking. The blanking step in the triglyceride-blanked assay reduced the sample throughput when compared to the non-blank triglyceride assay. In some cases, free glycerol was having clinically significant effect on the final result (Cole, 1990; Jessen, 1990). The SYNCHRON® System automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used was one part sample to 100 parts reagent. The System monitored the change in absorbance at 520 nanometers just prior to the addition of lipase and for a fixed time interval after lipase addition. This change in absorbance was directly proportional to the concentration of triglycerides in the sample and was used by the System to calculate and express the triglycerides concentration.

6.6.5.1 Chemical reaction scheme

(a) Triglycerides $\xrightarrow{\text{Lipase}}$ Glycerol + Fatty Acids

(b) Glycerol + ATP $\xrightarrow{\text{GK}}_{\text{Mg}^{++}}$ Glycerol-3-phosphate + ADP

(c) Glycerol-3-phosphate + O$_2$ $\xrightarrow{\text{GPO}}$ Dihydroxyacetone + H$_2$O$_2$

(d) 2H$_2$O$_2$ + 4-aminoantipyrine + DHBS $\xrightarrow{\text{HPO}}$ Quinoneimine Dye + HCl + 2H$_2$O
6.6.5.2  Reagents

Each kit contains the following items: Two TG-B Reagent Cartridges (2 x 300 tests), and One Instruction Inserted.

6.6.5.2.1  Volumes per test

Sample Volume was 3 μl, and total Reagent Volume was 300 μl.

6.6.5.3  Performance characteristic

6.6.5.3.1  Analytic range

Samples with concentrations exceeding the high end of the analytical range were diluted with saline and reanalyzed.

6.6.5.3.2  Sensitivity

Sensitivity was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for TG-B determination was 10 mg/dl (0.1 mmol/l).
6.6.5.3.3 Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods (National Committee for Clinical Laboratory Standards, 1995).

6.6.5.3.4 Precision

The mean of the test precision data was less than or equal to the changeover value, compared the test SD to the SD guideline given above to determine the acceptability of the precision testing. The mean of the test precision data was greater than the changeover value, compared the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

6.6.5.1.1.5 Performance characteristics of TG

6.6.5.1.1.5.1 Analytical range

Samples with concentrations outside of the analytical range were reported as "<0.10 mg/dl" ("<1.0 mg/l") or ">25.00 mg/dl" (">250.0 mg/l"). Samples with concentrations outside the ORDAC range were reported as "<20.00 mg/dl" or ">50.00 mg/dl".

Samples reported out as greater than the analytical range were confirmed by enabling ORDAC, or diluting with saline, and reanalyzing.
6.6.5.1.5.2 Analytical sensitivity

Sensitivity was defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for CRP determination was 0.10 mg/dL (1.0 mg/l).

6.6.5.1.5.3 Functional sensitivity

Functional sensitivity was defined as the lowest concentration that can be measured with an interassay CV of ≤20%. Functional sensitivity was estimated to be ≤0.08 mg/dl (≤0.8 mg/l).

6.6.5.1.5.4 Equivalence

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods (National Committee for Clinical Laboratory Standards 1995)

6.6.5.1.5.5 Precision

The mean of the test precision data was less than or equal to the changeover value, compared the test standard deviation (SD) to the SD guideline given above to determine the acceptability of the precision testing, when the mean of the test precision data were greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100 (National Committee for Clinical Laboratory Standards, 1992).
6.7 Blood analysis of C-reactive protein using DxC800 system

C-reactive protein reagents when used in conjunction with DxC800 system, the system was intended for quantitative determination of C-reactive protein concentration in human serum or plasma.

6.7.1 Methodology of C-reactive protein (CRP) determination

CRP reagent was used to measure C-reactive protein concentration by a turbidimetric method. In the reaction, an anti-CRP antibody-coated particle binds to C-reactive protein in the patient sample resulting in the formation of insoluble aggregates causing turbidity.

The DxC800 system automatically proportioned the appropriate sample and reagent volumes into a cuvette.

The ratio used was one part sample to 50 parts reagent. The System monitors change in absorbance at 600 nanometers. This change in absorbance was proportional to the concentration of CRP in the sample and was used by the System to calculate and express CRP concentration based upon a single-point adjusted, predetermined calibration curve.

6.7.1.1 Chemical scheme reaction

C-reactive protein (sample) + Particle bound anti-CRP (antibody) → C-reactive protein (sample)-antibody Complex
6.7.1.2 Specimen

6.7.1.2.1 Type of specimen

Serum samples were recommended. Plasma samples (EDTA), Lithium Heparin, and Sodium Heparin) were used. Serum or plasma samples were collected in the manner routinely used for any clinical laboratory test (Burtis et al., 1999). Freshly drawn serum or plasma from a fasting individual was preferred.

6.7.1.2.2 Specimen storage and stability

a. Tubes of blood were kept closed at all times and in a vertical position. It was recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection (National Committee for Clinical Laboratory Standards, 1990).

b. Serum samples were assayed within 72 hours; samples were stored frozen at -15°C to -20°C were same as for total cholesterol, high density lipoprotein and triglyceride (National Committee for Clinical Laboratory Standards, 1990).

6.7.2.2 Reagents

Each kit contained the following items: Two C-RP Reagent Cartridges (2 x 200 tests), and One lot-specific Parameter Card.
6.7.2.2.1 Sample volume

The optimum volume, when using a 0.5 mL sample cup was 0.3 mL of sample was the same as for cholesterol profile.

6.7.2.2 Volume per test

Sample Volume was 3 μl, ORDAC Sample Volume was 2μl, and total Reagent Volume 300 μl was same as of total cholesterol.

6.7.2.3 Reagent preparation

Preparations were same as of cholesterol profile.

a) Cartridge was inverted several times prior to loading onto the SYNCHRON LX System.
b) Bubbles were checked or foamed in compartments; and bubbles were break.

6.8 Blood analysis of CRP using Enzyme-linked immunosorbent assay

(ELISA test).

C-reactive protein (CRP) used the quantitative sandwich enzyme immunoassay technique. Briefly, the 96-well microtiter plate provided has been pre-coated with polyclonal antibodies directed against CRP. Samples, standards, and controls were pipetted into the wells and incubated for 30 minutes at 37°C, and any CRP present was bound by the immobilized CRP-
specific antibodies. After washing to remove any unbound substances, to quantitate the amount of CRP present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated antibody specific for CRP was added to each well to pack in the CRP immobilized during the second incubation for 30 minutes at 37°C. After a wash to remove any unbound, HRP-conjugated antibodies and 3, 3',5, 5’ tetramethyl-benzidine (TMB) substrate solution was added to each well. The enzyme (HRP) and substrate were allowed to react over a short incubation period for 15 minutes at 37°C. After incubation period, only those wells that contain CRP and enzyme-conjugated antibody will develops a change in color. An acidic stopping solution was added to each well, stopping the color development. Resultant color intensity was measured by (micro titer plate reader). A dose-response curve of absorbance unit versus CRP concentration was generated. The CRP present in the subject samples was determined directly from this calibration curve.

6.9 Statistical analysis

Data were analyzed using Statistical Analysis System software SPSS 17.0. Data were presented as the mean (standard deviation) for normally distributed variables, as percentages for categorical variables. The information was either presented in frequency tables, pie graphs and bar charts for all variables in order to determine the distribution of variables. ANOVA was used to compare the groups, and the Spearman Rank correlation test was used to determine correlations. P value of less than 0.05 was considered as significant.

Project number: MREC/M/37/2008: PG, approved by MREC with clearance certificate.
7.1 Baseline

7.1.1 Description of study population

Data were collected from 33 volunteers and 42 patients. The following graphs (Table 7.1; Figure 7.1-7.2) summarize the overall distribution of age, BMI and CRP level of the volunteers as well as patients.

**Table 7.1.1 Subject characteristics of volunteers groups**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>19</td>
<td>21-48</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>21-58</td>
</tr>
</tbody>
</table>

**Table 7.1.2 Subject characteristics of patients groups**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>15</td>
<td>21-63</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>27-61</td>
</tr>
<tr>
<td>Follow up ( male)</td>
<td>5</td>
<td>44-63</td>
</tr>
<tr>
<td>Follow up ( Female)</td>
<td>10</td>
<td>35-60</td>
</tr>
</tbody>
</table>
7.1.2 Cardiovascular risk stratification and cholesterol targets.

<table>
<thead>
<tr>
<th>Fasting</th>
<th>Category 1 risk</th>
<th>Category 2 risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>&lt; 4.5mmol/l</td>
<td>&lt; 5.0mmol/l</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>&lt; 2.5mmol/l</td>
<td>&lt; 3.0mmol/l</td>
</tr>
<tr>
<td>HDL cholesterol&lt;sup&gt;*&lt;/sup&gt; male</td>
<td>&gt; 1.0mmol/l</td>
<td>&gt; 1.0mmol/l</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>&gt; 1.2mmol/l</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;1.7mmol</td>
<td>&lt;1.7mmol</td>
</tr>
</tbody>
</table>

7.1.2.1 Category 1: High risk

In the high risk category there are included: established atherosclerosis that is coronary heart disease, cerebrovascular atherosclerotic disease, peripheral vascular disease, diabetes type 2, and diabetes type 1 with microalbuminuria or proteinuria, genetic dyslipidaemias e.g. familial hypercholesterolaemia.
7.1.2.2 Category 2:

The category 2 is the primary prevention of healthy volunteer dyslipidaemia (from NHLS).

Figure 7.1.2.1 Body mass index (BMI) of healthy volunteers.

Most of healthy volunteers had normal body mass index of 18.5-25 kg$^2$m (57.6%), following overweight volunteers with body mass index ranging from 26-30 kg$^2$m, underweight and obesity were very few (<18.5kg$^2$m (6.1%) and >30 kg$^2$m with (9.1%).
Figure 7.1.2.2 Body mass index (BMI) of patients

In this figure, most patients had normal body mass index of 20-25 kg\(^2\)m (54.8%), overweight patients ranging from 26-30 kg\(^2\)m (29%), and obesity patients with the BMI > 30kg\(^2\)m (6.5%).

Of the thirty three participants having healthy cholesterol level, 85% had normal BMI, while 9% were obese, and 6% were underweight (figure 7.1.2.1). Of the forty two patients 84% had normal BMI, while 6.5% were obese (figure 7.1.2.2).
7.1.3 Correlation between cholesterol profile [Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL) and C-reactive protein (CRP)] of healthy volunteers (n=33).

Figure 7.1.3.1 Correlation between TC and CRP of healthy volunteers (n=33), this figure shows that there was no significant correlation between TC and CRP of the healthy volunteers.
Figure 7.1.3.2 Correlation between TG and CRP of healthy volunteers (n=33), this figure also shows that there was no significant correlation between TG and CRP of the healthy volunteers.

Figure 7.1.3.3 Correlation between HDL and CRP of healthy volunteers (n=33), this figure also shows that the was no significant correlation between HDL and CRP of the healthy volunteers.
Figure 7.1.3.4 Correlation between LDL and CRP of healthy volunteers (n=33), this figure also shows that there was no correlation between LDL and CRP of the healthy volunteers.
7.1.4 Correlation between patients with normal cholesterol profile and high C-reactive protein (not on Atorvastatin) n=10

Figure 7.1.4.1 Correlation between TC and CRP (patients not on atorvastatin) n=10, this figure shows that there was no significant correlation between TC and CRP of the patients with normal cholesterol profile and high CRP.
Figure 7.1.4.2 Correlation between TG and CRP (patients not on atorvastatin) n=10, this figure also shows that there was no significant correlation between TG and CRP of the patients with normal cholesterol profile and high CRP.

Figure 7.1.4.3 Correlation between HDL and CRP (patients not on atorvastatin) n=10, this figure also shows that there was no significant correlation between HDL and CRP of the patients with normal cholesterol profile and high CRP.
Figure 7.1.4.4 Correlation between LDL and CRP (Patients not on atorvastatin) n=10, this figure shows that there was no significant correlation between LDL and CRP of the patients with normal cholesterol profile and high CRP.

7.1.5 Correlation between cholesterol profiles and CRP of patients on atorvastatin (n=32)

Figure 7.1.5.1-7.1.5.4 shows that there was no significant correlation between the cholesterol profile and CRP of patients on atorvastatin after three months of treatment (figure 7.1.5.1; \( r^2 = 0.04 \) with \( p \) value of 0.06, figure 7.1.5.2; \( r^2 = 0.0005 \) with \( p \) value of 0.864, figure 7.1.5.3; \( r^2 = 0.104 \) with \( p \) value of 0.76, and figure 7.1.5.4; \( r^2 = 0.017; p=0.844. \)
Figure 7.1.5.1 Correlation between TC and CRP in patients on atorvastatin after three months of treatment (n=32)

Figure 7.1.5.2 Correlations between TG and CRP in patients on atorvastatin after three months of treatment (n=32)
Figure 7.1.5.3 Correlations between HDL and CRP in patients on atorvastatin after three months of treatment (n=32)

Figure 7.1.5.4 Correlations between LDL and CRP in patients on atorvastatin after three months of treatment (n=32)
7.1.6 Correlation between two systems (DxC800 and Cardio-check)

Figure 7.1.6.1-7.1.6.4 shows that there was no significant correlation between the two techniques (figure 7.1.7.1; \( r^2 = 0.052; p=0.06 \), figure 7.1.7.2; \( r^2 = 0.227; p=0.05 \), figure 7.1.7.3; \( r^2 = 0.318; p=0.05 \), and figure 7.1.7.4; \( r^2 = 0.005; p=0.896 \)).

Figure 7.1.6.1 Correlation between the TG using DxC800 and Cardio-chek techniques.
Figure 7.1.6.2 Correlation between the TC using DxC800 and Cardio-check techniques.

Figure 7.1.6.3 Correlation between the HDL using DxC800 and Cardio-check techniques.
7.1.6 (a) Descriptive statistics of volunteers with normal Cholesterol using Cardio-chek technique (n=36)

Using cardio-chek technique measuring the cholesterol level of healthy volunteers the minimum age of volunteers was twenty and maximum was fifty two years with the minimum TC = 0.56 mmol/l; maximum= 1.33mmol/l, TG minimum= 2.62mmol/l; maximum=4.97mmol/l, HDL minimum=0.56mmol/l; maximum=1.89mmol/l, LDL minimum=1.82mmol/l; maximum 2.77mmol/l.

7.1.6 (b) Descriptive Statistics of volunteers with high Cholesterol using Cardio-check technique (n=25)

Using cardio-check techniques measuring the cholesterol level of healthy volunteers the minimum age of volunteers was nineteen and maximum was sixty two years with the minimum TC =0.56mmol/l; maximum= 2.42mmol/l, TG minimum= 5.00mmol/l;
maximum=9.00mmol/l, HDL minimum=0.54mmol/l; maximum=2.56mmol/l, LDL minimum=3.86mmol/l; maximum 6.99mmol/l.

* Between Cardio and NHLS, > 2 STD; n= number participants who fell outside 2 standard deviations.

### 7.1.7 Correlation between DxC800 and Microtiter plate reader techniques measuring C-reactive protein (n=78)

Figure 7.1.7.1-7.1.7.5 shows that there was no significant correlation between the two techniques (figure 7.1.7.1; $r^2=0.2000; p=0.5$, figure 7.1.7.2; $r^2=0.023; p=0.05$, figure 7.1.7.3; $r^2=0.443; p=0.05$, figure 7.1.7.4; $r^2=0.2881; p=0.05$, and figure 7.1.7.5; $r^2=0.0948; p=0.05$).

![Graph showing correlation between DxC800 and microtiter plate reader techniques measuring C-reactive protein](image)

Figure 7.1.7.1 Correlation between DxC800 and microtiter plate reader techniques measuring CRP of healthy volunteers (n=33).
Figure 7.1.7.2 Correlation between DxC800 and microtiter plate reader techniques measuring CRP of patients with normal cholesterol and high C-reactive protein (n=10)

Figure 7.1.7.3 Correlation between DxC800 and microtiter plate reader techniques measuring CRP of patients with high cholesterol and normal C-reactive protein (n=26)
Figure 7.1.7.4 Correlation between DxC800 and microtiter plate reader techniques measuring CRP with both high cholesterol and high C-reactive protein (n=6)

Figure 7.1.7.5 Correlation between DxC800 and microtiter plate reader techniques measuring C-reactive protein of patients after three months of 20mg atorvastatin (n=6)
7.2 Summary of the results

CRP decreased significantly from $8.4 \pm 8.19$ to $6.66 \pm 7.49$ mg/L (p < 0.021). Total cholesterol decreased from $5.93 \pm 0.96$ to $4.49 \pm 0.59$ mmol/L (p < 0000.3). LDL-cholesterol decreased from $3.79 \pm 0.99$ to $2.7 \pm 0.57$ mmol/L (p < 0000.7). HDL-cholesterol and total triglycerides did not change. There was no significant correlation between total cholesterol and hs-CRP, LDL-cholesterol and hs-CRP or HDL-cholesterol and hs-CRP before and after 3 months of treatment. However the decrease in hs-CRP was fully established by 3 months of treatment.

Table 7.2 The relationship between atorvastatin, C-reactive protein and lipid profile in patients with moderate hyperlipidaemia

<table>
<thead>
<tr>
<th>Variables</th>
<th>M0 (Means ±SD Patients N=15)</th>
<th>M3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>5.93 ± 0.96</td>
<td>4.49 ± 0.59</td>
<td>p&lt;0000.3</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.25 ± 0.74</td>
<td>1.14 ± 0.59</td>
<td>p=0.516</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.79 ± 0.99</td>
<td>2.7 ± 0.57</td>
<td>p &lt; 0000.7</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.36 ± 0.39</td>
<td>1.27 ± 0.47</td>
<td>p=0.147</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>8.4 ± 8.19</td>
<td>6.66 ± 7.49</td>
<td>p &lt; 0.021</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (SD); TC, total cholesterol, TG, triglycerides, low density lipoprotein cholesterol LDL-C, high density lipoprotein cholesterol HDL-C and high-sensitivity C-reactive protein hs-CRP

Base line -M0; after 3months of therapy- M1 (p < 0.05 Wilcoxon Signed Rank Test, 95% confidence interval).
Discussion and conclusion

Statins and in particular atorvastatin have anti-inflammatory properties in addition to its cholesterol-lowering effect while it is suggested that low density lipoprotein cholesterol (LDL-C) have pro-inflammatory effects. Lowering LDL-cholesterol by statins has been proven to be associated with reduction of pro-inflammatory regulators. The evidence has indicated that rapid reduction of inflammatory marker, such as C-reactive protein could be achieved by the treatment with statins.

High-sensitivity C-reactive protein (hs-CRP) is a non-specific acute-phase protein produced by the liver in response to tissue injury, infection, inflammation and has recently emerged as a valuable marker of cardiovascular risk for various cardiovascular-related disorders. Evidence suggests patients with high hs-CRP/normal LDL-C are at greater risk than those with normal hs-CRP/high LDL-C.

The elevated hs-CRP levels predict poor prognosis and outcome in patients with cardiovascular disease. The major beneficial effect of statins- reducing the risk for coronary events has primarily been ascribed to reductions in LDL-C but may in part be related to a direct anti-inflammatory action (decreased hs-CRP) concentration. However there is still limited information available in evaluating the short term course of CRP reduction on statin treatment.

Twenty one naïve patients age 58± 8 with documented moderate hyperlipidaemia were enrolled in the study. Total cholesterol (TC), triglycerides (TG), LDL-cholesterol, HDL-cholesterol and hs-CRP were measured at baseline and after 3 months of atorvastatin (20mg daily) treatment. Cardiovascular risk was graded as low risk if CRP was < 1mg/L, moderate if 1-3mg/L and high risk if 3-10mg/L.

The major finding of this study was a significant decrease in hs-CRP level from 8.4 ± 8.19 to 6.66 ± 7.49 mg/L± (p< 0.021). Total cholesterol decreased from 5.93 ± 0.96 to 4.49 ± 0.59
mmol/L (p<0.0003). LDL-cholesterol decreased from 3.79 ± 0.99 to 2.7 ± 0.57 mmol/L (p<0.0007) (table 7.2).

However in the 3 patients CRP level have remained high (15-27 mg/l) despite being on the 20 mg atorvastatin for 3 months. That could be explain by the possibility of other factors or diseases (eg: acute infection, inflammation) influencing the level of CRP independent of the statin use. Also the intra-individual variation of CRP concentration could potentially interfere with interpretation of the data.

In this study the output showed that there was no significant correlation between total cholesterol, triglyceride, HDL level and CRP levels of the healthy volunteers (figure 7.1.3.1-7.1.3.4).

There was also no significant correlation between the total cholesterol level and hs-CRP level, LDL-cholesterol and hs-CRP, HDL- cholesterol and hs-CRP in patients before (figure 7.1.4.1-7.1.4.4) and after 3 months of treatment figure 7.1.5.1-7.1.5.4) with 20 mg of atorvastatin although the decrease in hs-CRP was fully established after 3 months of treatment and is strongly suggestive that lipids can produce inflammatory changes. In this study the changes in CRP were independent of lipid changes, so the reason for the anti-inflammatory effect of statins needs to be still elucidated.

It seems that BMI could be a predictor for changes in CRP level but not a predictor for cholesterol profile level changes. Volunteers with BMI above 30 kg/m² had CRP above 7.5mg/l but further investigation is warranted to see if there is any correlation between BMI, cholesterol level and CRP level. (See appendix III).

In this study, HDL level of patients with high cholesterol level was slightly reduced from 1.36 ± 0.39 to 1.27 ± 0.47 mmol/l (table 7.2) instead of increasing after three months of 20mg atorvastatin. This may be caused by the patient s’ life style, drinking of alcohol and smoking and using oral contraceptive drugs.

Clinical trials have established that lowering LDL cholesterol level with statin therapy reduces the risk of cardiovascular events (Scandinavian Simvastatin Survival Study Group., 1994; Long-
Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group., 1998; MRC/BHF Heart Protection Study Investigators., 2002; Downs et al., 1998; Sacks et al., 1996; Shepherd et al., 1995).

Statins have been also reported to increase HDL levels by 5% to 15% (Scandinavian Simvastatin Survival Study Group., 1994; Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group., 1998; Downs et al, 1998; Sacks et al, 1996).

Atorvastatin has been proven to reduce CRP levels in this study (table 7.2). The present analysis shows that a more intensive statin regimen consistently achieved lower CRP levels at three months of treatment.

It has also recently been observed that achieving a lower CRP levels with statin therapy is associated with a lower risk of recurrent events in patients with acute coronary syndromes (Ridker et al., 2005). It was confirmed that statin therapy lowers CRP levels (Albert et al., 2001), and a recent analysis has suggested that achieving a lower CRP levels with statin therapy in patients with acute coronary syndromes (ACS) is associated with a lower rate of recurrent cardiovascular events and less progression of atherosclerotic plaques (Ridker et al., 2005; Nissen et al., 2005). Clinical trials have shown that statins reduce patient levels of CRP by 15% to 28% as early as six weeks after treatment begins independent of the magnitude of reduction in LDL levels (Jialal et al., 2001; Albert et al., 2001; Ridker et al., 2001).

A number of large, prospective epidemiologic studies have indicated that CRP level is a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death among individuals without known cardiovascular disease (Ridker, 2001 and 2003).

Several recent studies have evaluated the ability of statins to reduce CRP level in individuals with acute coronary syndrome. In addition, there was a resultant statistically significant 16% reduction ($P=.005$) in cardiovascular events at two years (Cannon et al., 2004). Patients in the highest CRP level quintile (>4.18 mg/L) in this study had more than a threefold increased risk of diabetes mellitus after five years (95% CI, 1.33-7.10) [Freeman et al., 2002]. Finally, elevated
CRP level was an independent predictor of diabetes mellitus and the metabolic syndrome in women in the Mexico City Diabetes Study (Han et al., 2005). Compared with women in the lowest CRP level tertile, women in the highest tertile had a relative risk of 4.0 of the metabolic syndrome developing (95% CI, 2.0-7.9) and a relative risk of 5.5 of type 2 diabetes mellitus developing (95% CI, 2.2-13.5) [Han et al., 2005].

In the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial, which compared aggressive lipid lowering with atorvastatin (80 mg) versus moderate lipid lowering with pravastatin (40 mg), atorvastatin reduced CRP and LDL levels 38% and 35% more, respectively, than pravastatin (Cannon et al., 2004).

Additional, greater reductions in CRP level with statin therapy have been associated with less progression of atherosclerosis in patients with stable coronary artery disease (Nissen et al., 2005). 13.3% of CRP level decreases in an 8-week (20mg cerivastatin) [Ridker et al., 1999]. In the Cholesterol and Recurrent Events study (CARE) a similar decrease was found in a 5-year follow-up (Ridker et al., 1998). In the Physicians' Health Study, CRP level added to the predictive value of lipid parameters for determining future risk of myocardial infarction (Ridker et al., 1998).

In the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study, atorvastatin (80 mg) significantly reduced CRP level by 83%, versus 74% ($P<.001$) with placebo, at 16 weeks (Kinlay et al., 2003).

More recently, reductions in levels of the inflammatory biomarker CRP in patients treated with statins have been associated with clinical benefits, including the slowing of disease progression and reduction in morbidity and mortality (Nissen et al., 2005).

In this study atorvastatin has an anti-inflammatory effect as confirm with the lowering of hs-CRP (table 7.2). Since no correlation could be found between LDL-C and hs-CRP it could be concluded that statins lower plasma levels of hs-CRP in a manner largely independent of LDL cholesterol lowering. High sensitivity CRP together with other known cardiac risk factors may
be used as a predictor of cardiovascular disease and can be used in primary prevention, although caution should be taken to regard hs-CRP as highly specific cardiovascular risk marker.

This study also has compared two established analytical methods: DxC800 system and cardiocheck test for determination of the cholesterol profile. This study also compared two techniques (micro titer plate reader, DxC800) on the determination of the CRP level.

There was no significant correlation in cholesterol profile comparing DxC800 and Cardio-check (figure 7.1.8.1-7.1.8.4). There was no significant correlation between DxC800 and Microtiter Plate reader techniques measuring CRP level of healthy volunteers and CRP level of patients.

In conclusion, statin therapy is recommended as a drug of the management strategy for the primary prevention of cardiovascular diseases (CVD) from the point of view of the researcher. Strong evidence from the study showed that CRP is associated with CVD events. Sufficient evidence that atorvastatin reduce CRP level was proficient in this study.

Results suggest that hs-CRP could be used to target high-risk patients who may benefit from early statin use. However it should be investigated on a larger group of patient whether hs-CRP reduction, independent of LDL cholesterol reduction, results in a clinical benefit.

Arteriosclerosis is a highly complex disease involving not only inflammatory changes. CRP levels can be only used as an additional risk assessment to the already existing one. It is still not clear by which mechanism of action the statins reduce CRP concentration producing anti-inflammatory effects. Since recent data indicate that endothelial dysfunction can be improved by the statin therapy within days, it could be possible that chronic vascular inflammation could be also improved even after such a short period of 3 months’ time.


Appendices

Appendix I a: Consent form (English version)

UNIVERSITY OF LIMPOPO (Medunsa Campus) CONSENT FORM

Statement concerning participation in a Clinical Trial/Research Project*.

Name of Project / Study / Trial*

Correlation between C-reactive protein, cholesterol profile and statin use

I have read the information on */heard the aims and objectives of* the proposed study and was provided the opportunity to ask questions and given adequate time to rethink the issue. The aim and objectives of the study are sufficiently clear to me. I have not been pressurized to participate in any way.

I understand that participation in this Clinical Trial / Study / Project* is completely voluntary and that I may withdraw from it at any time and without supplying reasons. This will have no influence on the regular treatment that holds for my condition neither will it influence the care that I receive from my regular doctor.

I know that this Trial / Study / Project* has been approved by the Research, Ethics and Publications Committee of Faculty of Medicine, University of Limpopo (Medunsa Campus) / Dr George Mukhari Hospital. I am fully aware that the results of this results of this Trial / Study / Project* will be used for scientific purposes and may be published.
I agree to this, provided my privacy is guaranteed.

I hereby give consent to participate in this Trial / Study / Project*.

............................................................  ........................................................

Name of patient/volunteer  Signature of patient or guardian.

........................................  ........................................  ........................................

Place.  Date.  Witness

________________________________________________________________________

Statement by the Researcher

I provided verbal and/or written* information regarding this Trial / Study / Project*

I agree to answer any future questions concerning the Trial / Study / Project* as best as I am able.

I will adhere to the approved protocol.

Name of Researcher: Rudzani Madzivhe  Signature: __________________________

Date:  26/02/08  Place:  Department of Pharmacology and Therapeutics
Appendix I b: Consent form (Tshwane version)

UNIVERSITY OF LIMPOPO (Medunsa Campus) LEKWALE LA TETLELELO

Tsebiso ya go tsenela Projeke/Teko ya Bophelo*

Lebitso la Projeke/Teko/Dipatlisiso*

Correlation between C-reactive protein, cholesterol profile and statin use

Ke badile lekwalo le le tihalosang ka */botlalo dipatlisiso le maikaelelo ka* botlalo le tshono ya go botsisa mabbapi le dipatlisiso tseo le go ipotsolotsa sentle ka go tsea karolo gape mo dipatlisiso tseo.

Ke utlwisisa Dipatlisisong/Projeke/Tekong tsa, *mme le gore ga ke pateletsege go tsaya karolo. Go tsaya karolo game go tswa mo go nna, ka nako e ngwe le e ngwe nka ikgogela morago ka ntle le go fa mabaka. Se se ka se nkgoreletse mo kalafing yaka ya ka methla e ke e fiwang ke ngaka yaka ya ka methla.

Ke itsi le gore dipatlisiso tse di dumeletswe ke “Research Ethics and Publications. Committee of the Faculty of Medicine, University of Limpopo (Medunsa Campus/Dr George Mukhari Hospital).

Ke itse sentle gore dipholo “results” tsa dipatlisiso tse di tlo diriswa mo kitsong tsa mahlale “science” le gore di tla phatlalatswa ke dumelana le gore leina laka se itsesiwe, ke sephiri.

Kefa tumello ya go tsaya karolo mo dipatlisisong Dipatlisisong/Projeke/Tekong tsa*

____________________________________  __________________________________________

Leina la molwetsi/moithaupi Goitlama ga molwetsi kgotsa mothokomedi

____________________________________  ____________________________  ____________________________
Lefelo  Letsatsi  Moemedi

Tsebiso ka Mobatlisisi

Ke fane ka polelo/le ka mongwalo* ketsiso ya Dipatlisisong/Projeke/Tekong*.

Ke dumela go araba dipotso tse tlang mabapi le Dipatlisisong/Projeke/Tekong tsa* ka mokgwa o nka kgonang.

Ke tla tshwarella mo tekong e dumelletseng.

Rudzani  Madzivhe________________________

Leina la Mobatlisisi  Goitlama ga Mobatlisisi

Pretoria (Medunsa Campus)  26/02/08

Lefelo  Letsatsi
Appendix II: Study Approval form

UNIVERSITY OF LIMPOPO
Medunsa Campus

MEDUNSA RESEARCH & ETHICS COMMITTEE
CLEARANCE CERTIFICATE

MEETING: 03/2008
PROJECT NUMBER: MREC/M/37/2008:PG.

PROJECT:
Title: Correlation between C - reactive protein, Cholesterol profile and statin use
Researcher: Ms R. Madzivhe
Supervisor: Prof W. du Plooy
Hospital Superintendent: Dr P. Shembe (Dr George Mukhari Hospital)
Department: Pharmacology
School: Medicine
Degree: MSc (Med) (Pharmacology)

DATE CONSIDERED: April 09, 2008

DECISION OF THE COMMITTEE:
MREC approved the project.

DATE: April 09, 2008

PROF GA Ogünbanjo
DIRECTOR: RESEARCH & CHAIRPERSON MREC

Note:
1) Should any departure be contemplated from the research procedure as approved, the researcher(s) must re-submit the protocol to the committee.
2) The budget for the research will be considered separately from the protocol. PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES.
Appendix III: Patients questionnaire

CONFIDENTIAL

HISTORY FORM

NAME: .............................................. TEL. NO. ..........................

Dr E Osuch
Chief Investigator
Dept of Pharmacology & Therapeutics
University of Limpopo
MEDUNSA CAMPUS
Box 225, Medunsa 0204
Voluntary Health Assessment

GENERAL INFORMATION

Patient: Name ................................................. Surname .............................................

Sex: Male: ........ Female: ........ Date of birth: ........................................... Age: ............

Residential address: ........................................................................................................

Classification: Rural  Urban  Suburban  Other:

Marital Status: Single  Married  Divorced  Widowed

Number of children: .............................................

Occupation: ..........................................................................................................................

Formal education: .............................................

HISTORY

Previous illnesses: ..............................................................................................................

........................................................................................................................................

........................................................................................................................................

........................................................................................................................................
Hospitalization: ............................................................................................................
..............................................................................................................................
..............................................................................................................................

Chronic medication: Are you taking any form of medication regularly? ....................
..............................................................................................................................
If yes, give details: ........................................................................................................
..............................................................................................................................

Psychiatric history: ....................................................................................................
..............................................................................................................................

Allergies: ......................................................................................................................
..............................................................................................................................

Do you smoke? .............................. How many per day? .....................................

If not, did you smoke previously? ......... How many per day? .................................

Do you use alcohol on a regular basis? ........ How much per week? ......................

Have you, or are you suffering from any of the following?
High blood pressure/Hoë bloeddruk

Asthma/Asma

Hay fever/Hooikoors

Epilepsy or any other convulsions/Epilepsie of enige ander vorm van stuiptrekkings

Kidney disease/Nierstene

Liver disease/Lewersiekte

Peptic ulcer/Maagseer

Diabetes/Suikersiekte

Anaemia/Bloedarmoede

Jaundice/Geelsug

Thyroid disease/Skildkliersiekte

Adrenal disease/Byniersiekte

Psychiatric problems/Psigiatriese probleme

Headache or Migraine/Hoofpyn of Migraine
GENERAL EXAMINATION

Weight: ........................ BMI: .......................... Height: ..........................

Temperature: ............. Pulse: ......................... BP: ..............................

PHYSICAL EXAMINATION

Skin:  ..............................................................................................................

Jaundice:  ......................................... Clubbing:  ..............................................

Anaemia:  ........................................ Cyanosis:  ..............................................

Oedema:  ............................................ Lymphadenopathy:  ..............................

Head and Neck:  ...........................................................................................

JVP = N / ↑ = ............ cm  Thyroid:  ..............................................................

Ear, Nose and Throat:  ......................................................................................

Cardiovascular System:  ..................................................................................

Blood pressure: Right arm ..................... mmHg  Left arm ......................... mmHg

Pulses:  Tempo = ...................... /min  Regular/ Irregular  ..............................

Heart sounds:  ................................................................................................
Murmurs: ................................................. Apex beat: ...........................................

Foot pulses: .................................................................................................................

**Respiratory System:**

Respiratory rate ........................../min

Auscultation: ..............................................................................................................

**Abdomen:**

Scars: .............................................. Vessels: .......................................................

Liver: ................................................. Spleen: .......................................................

Ascites: ............................................. Abdominal masses: .................................

**Musculoskeletal System:** .................................................................

..........................................................................................................................

**Genito-urinary:** ...........................................................

..........................................................................................................................

**Nervous System:** ..............................................................

..........................................................................................................................

**SPECIAL INVESTIGATION:** ...........................................................

Urinalysis: .............................................................................................................
Blood tests:

1. FBC  
2. U & E  
3. LFT  
4. Lipid profile  
5. Glucose  
6. HBA1C

DIFFERENTIAL DIAGNOSIS:  .................................................................

...........................................................................................................

FINAL DIAGNOSIS:  .................................................................

...........................................................................................................

...........................................................................................................
Appendix IV: Raw Healthy Volunteers and Patients data collection

Healthy volunteers (Controls)

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Age</th>
<th>weight</th>
<th>date tested</th>
<th>TC mmol/l</th>
<th>TG mmol/l</th>
<th>HDL mmol/l</th>
<th>LDL mmol/l</th>
<th>CRP mg/l</th>
<th>BMI Kgm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5978</td>
<td>21</td>
<td>66</td>
<td>08/11/06</td>
<td>3.2</td>
<td>0.5</td>
<td>0.8</td>
<td>1.9</td>
<td>&lt;0.2</td>
<td>24.2</td>
</tr>
<tr>
<td>ADJG5981</td>
<td>28</td>
<td>56</td>
<td>08/11/06</td>
<td>4.2</td>
<td>0.4</td>
<td>1.6</td>
<td>2</td>
<td>&lt;0.2</td>
<td>17.5</td>
</tr>
<tr>
<td>ADJG5971</td>
<td>25</td>
<td>60</td>
<td>08/11/05</td>
<td>4.6</td>
<td>1.1</td>
<td>1.1</td>
<td>2.8</td>
<td>0.2</td>
<td>21.3</td>
</tr>
<tr>
<td>ADJG5960</td>
<td>25</td>
<td>44</td>
<td>08/11/04</td>
<td>4.8</td>
<td>0.8</td>
<td>1.3</td>
<td>3.1</td>
<td>5</td>
<td>19</td>
</tr>
<tr>
<td>ADJG5912</td>
<td>38</td>
<td>80</td>
<td>08/11/18</td>
<td>3.6</td>
<td>0.7</td>
<td>1.2</td>
<td>1.9</td>
<td>4</td>
<td>31.6</td>
</tr>
<tr>
<td>ADJG5944</td>
<td>58</td>
<td>75</td>
<td>08/12/04</td>
<td>4.4</td>
<td>2</td>
<td>0.8</td>
<td>2.3</td>
<td>5</td>
<td>26.9</td>
</tr>
<tr>
<td>ADJG5975</td>
<td>24</td>
<td>63</td>
<td>08/11/06</td>
<td>3.8</td>
<td>0.6</td>
<td>1.2</td>
<td>2.1</td>
<td>5</td>
<td>23.1</td>
</tr>
<tr>
<td>ADJG5954</td>
<td>22</td>
<td>48</td>
<td>08/11/04</td>
<td>3.5</td>
<td>1</td>
<td>1.1</td>
<td>1.5</td>
<td>4</td>
<td>28.3</td>
</tr>
<tr>
<td>ADJG5957</td>
<td>27</td>
<td>60</td>
<td>08/11/04</td>
<td>4.5</td>
<td>0.3</td>
<td>1.6</td>
<td>2.5</td>
<td>5</td>
<td>21.8</td>
</tr>
<tr>
<td>ADJG5992</td>
<td>21</td>
<td>58</td>
<td>08/11/07</td>
<td>4</td>
<td>0.3</td>
<td>1.3</td>
<td>2.3</td>
<td>5</td>
<td>18.5</td>
</tr>
<tr>
<td>ADJG5923</td>
<td>26</td>
<td>57</td>
<td>08/11/19</td>
<td>3.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.8</td>
<td>&lt;0.2</td>
<td>19.7</td>
</tr>
<tr>
<td>ADJG5979</td>
<td>26</td>
<td>63</td>
<td>08/11/06</td>
<td>4</td>
<td>1.2</td>
<td>1</td>
<td>2.2</td>
<td>&lt;0.2</td>
<td>21.8</td>
</tr>
<tr>
<td>ADJG5980</td>
<td>32</td>
<td>64</td>
<td>08/11/06</td>
<td>3.4</td>
<td>0.5</td>
<td>0.6</td>
<td>2.3</td>
<td>4</td>
<td>26.3</td>
</tr>
<tr>
<td>ADJG5956</td>
<td>28</td>
<td>69</td>
<td>08/11/04</td>
<td>4.1</td>
<td>1</td>
<td>0.9</td>
<td>2.5</td>
<td>2</td>
<td>21.1</td>
</tr>
<tr>
<td>ADJG5962</td>
<td>26</td>
<td>59</td>
<td>08/11/04</td>
<td>4.1</td>
<td>1</td>
<td>1.2</td>
<td>2.4</td>
<td>5</td>
<td>21.4</td>
</tr>
<tr>
<td>ADJG5907</td>
<td>33</td>
<td>100</td>
<td>08/11/18</td>
<td>3.4</td>
<td>1.6</td>
<td>0.6</td>
<td>1.9</td>
<td>2</td>
<td>38.6</td>
</tr>
<tr>
<td>ADJG5985</td>
<td>24</td>
<td>52</td>
<td>08/11/06</td>
<td>3.5</td>
<td>0.4</td>
<td>1.1</td>
<td>2</td>
<td>&lt;0.2</td>
<td>18.4</td>
</tr>
<tr>
<td>ADJG5984</td>
<td>22</td>
<td>64</td>
<td>08/11/06</td>
<td>3.6</td>
<td>0.7</td>
<td>1.2</td>
<td>1.7</td>
<td></td>
<td>20.6</td>
</tr>
<tr>
<td>Code</td>
<td>Value</td>
<td>Date</td>
<td>Component 1</td>
<td>Component 2</td>
<td>Component 3</td>
<td>Component 4</td>
<td>Component 5</td>
<td>Component 6</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>----------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>ADJG5936</td>
<td>37</td>
<td>08/12/01</td>
<td>4.3</td>
<td>1.2</td>
<td>0.9</td>
<td>2.9</td>
<td>4</td>
<td>39.8</td>
<td></td>
</tr>
<tr>
<td>ADJG5930</td>
<td>22</td>
<td>08/11/05</td>
<td>4.1</td>
<td>0.3</td>
<td>1.3</td>
<td>2.3</td>
<td>5</td>
<td>22.8</td>
<td></td>
</tr>
<tr>
<td>ADJG5994</td>
<td>23</td>
<td>08/11/10</td>
<td>4.9</td>
<td>1.5</td>
<td>0.9</td>
<td>2.2</td>
<td>5</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>ADJG5997</td>
<td>48</td>
<td>08/11/11</td>
<td>4.1</td>
<td>1.1</td>
<td>0.6</td>
<td>2.4</td>
<td>6</td>
<td>26.2</td>
<td></td>
</tr>
<tr>
<td>ADJG5964</td>
<td>24</td>
<td>08/11/04</td>
<td>4.7</td>
<td>1.4</td>
<td>1.1</td>
<td>2.4</td>
<td>5</td>
<td>23.6</td>
<td></td>
</tr>
<tr>
<td>ADJG5938</td>
<td>43</td>
<td>08/12/01</td>
<td>3.8</td>
<td>0.6</td>
<td>0.7</td>
<td>2.5</td>
<td>2</td>
<td>27.4</td>
<td></td>
</tr>
<tr>
<td>ADJG5961</td>
<td>32</td>
<td>08/11/04</td>
<td>4.4</td>
<td>0.6</td>
<td>1.4</td>
<td>2.5</td>
<td>1</td>
<td>23.4</td>
<td></td>
</tr>
<tr>
<td>ADJG5977</td>
<td>20</td>
<td>08/11/06</td>
<td>3.2</td>
<td>0.8</td>
<td>0.9</td>
<td>1.9</td>
<td>&lt;0.2</td>
<td>18.7</td>
<td></td>
</tr>
<tr>
<td>ADJG5972</td>
<td>20</td>
<td>08/11/05</td>
<td>4.1</td>
<td>0.4</td>
<td>1.3</td>
<td>2.4</td>
<td>5</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td>ADJG5983</td>
<td>21</td>
<td>08/11/06</td>
<td>4.1</td>
<td>0.7</td>
<td>1</td>
<td>2.6</td>
<td>1</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>ADJG5945</td>
<td>41</td>
<td>08/12/04</td>
<td>4.1</td>
<td>0.5</td>
<td>0.8</td>
<td>2.5</td>
<td>7</td>
<td>30.5</td>
<td></td>
</tr>
<tr>
<td>ADJG5924</td>
<td>45</td>
<td>08/11/24</td>
<td>3.4</td>
<td>0.9</td>
<td>1.2</td>
<td>1.9</td>
<td>4</td>
<td>37.8</td>
<td></td>
</tr>
<tr>
<td>ADJG5913</td>
<td>51</td>
<td>08/11/18</td>
<td>4.5</td>
<td>0.6</td>
<td>1.1</td>
<td>2.8</td>
<td>5</td>
<td>28.7</td>
<td></td>
</tr>
<tr>
<td>ADJG5909</td>
<td>24</td>
<td>08/11/18</td>
<td>2.5</td>
<td>0.4</td>
<td>0.6</td>
<td>1.5</td>
<td>5</td>
<td>23.7</td>
<td></td>
</tr>
<tr>
<td>ADJG5927</td>
<td>37</td>
<td>08/11/24</td>
<td>3.3</td>
<td>0.9</td>
<td>0.6</td>
<td>2.1</td>
<td>2</td>
<td>22.2</td>
<td></td>
</tr>
</tbody>
</table>
Patients with normal cholesterol and high C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Age</th>
<th>weight kg</th>
<th>date tested</th>
<th>TC mmol/l</th>
<th>TG mmol/l</th>
<th>HDL CS mmol/l</th>
<th>LDL CS mmol/l</th>
<th>CRP mg/l</th>
<th>BMI kg² m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5918</td>
<td>46</td>
<td>92</td>
<td>08/11/19</td>
<td>4.5</td>
<td>1.6</td>
<td>1.1</td>
<td>1.5</td>
<td>8</td>
<td>35.9</td>
</tr>
<tr>
<td>ADJG5911</td>
<td>52</td>
<td>94</td>
<td>08/11/18</td>
<td>2.4</td>
<td>0.6</td>
<td>0.8</td>
<td>1.2</td>
<td>13</td>
<td>38.4</td>
</tr>
<tr>
<td>ADJG5922</td>
<td>41</td>
<td>88</td>
<td>08/11/19</td>
<td>4.5</td>
<td>0.9</td>
<td>0.9</td>
<td>3.3</td>
<td>20</td>
<td>34.4</td>
</tr>
<tr>
<td>ADJG5931</td>
<td>51</td>
<td>71</td>
<td>08/11/25</td>
<td>4.1</td>
<td>0.8</td>
<td>1.8</td>
<td>1.7</td>
<td>8</td>
<td>28.3</td>
</tr>
<tr>
<td>ADJG5947</td>
<td>48</td>
<td>95</td>
<td>08/12/05</td>
<td>4.2</td>
<td>0.6</td>
<td>1</td>
<td>2.8</td>
<td>16</td>
<td>33.7</td>
</tr>
<tr>
<td>ADJG5905</td>
<td>22</td>
<td>120</td>
<td>08/11/18</td>
<td>3.8</td>
<td>0.7</td>
<td>1.1</td>
<td>2.2</td>
<td>8</td>
<td>37.5</td>
</tr>
<tr>
<td>ADJG5928</td>
<td>51</td>
<td>90</td>
<td>08/11/24</td>
<td>3.7</td>
<td>0.8</td>
<td>1</td>
<td>2.3</td>
<td>15</td>
<td>35.6</td>
</tr>
<tr>
<td>ADJG5919</td>
<td>42</td>
<td>100</td>
<td>08/11/19</td>
<td>4.1</td>
<td>0.8</td>
<td>0.7</td>
<td>2.9</td>
<td>8</td>
<td>34.2</td>
</tr>
<tr>
<td>ADJG5941</td>
<td>37</td>
<td>87</td>
<td>08/12/03</td>
<td>4.4</td>
<td>0.5</td>
<td>1.2</td>
<td>2.6</td>
<td>9</td>
<td>30.5</td>
</tr>
<tr>
<td>ADJG5948</td>
<td>47</td>
<td>82</td>
<td>08/12/05</td>
<td>3.4</td>
<td>1.2</td>
<td>1.3</td>
<td>1.6</td>
<td>8</td>
<td>28.4</td>
</tr>
</tbody>
</table>

Patients with both high cholesterol and high C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Age</th>
<th>weight kg</th>
<th>date tested</th>
<th>TC mmol/l</th>
<th>TG mmol/l</th>
<th>HDL CS mmol/l</th>
<th>LDL CS mmol/l</th>
<th>CRP mg/l</th>
<th>BMI Kg²m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5914</td>
<td>35</td>
<td>75</td>
<td>08/11/18</td>
<td>5</td>
<td>1.1</td>
<td>1</td>
<td>3.4</td>
<td>28</td>
<td>30.8</td>
</tr>
<tr>
<td>ADJG5970</td>
<td>45</td>
<td>99</td>
<td>08/11/05</td>
<td>5</td>
<td>0.6</td>
<td>0.9</td>
<td>3.7</td>
<td>27</td>
<td>40.2</td>
</tr>
<tr>
<td>ADJG5915</td>
<td>53</td>
<td>74</td>
<td>08/11/18</td>
<td>5.6</td>
<td>1.6</td>
<td>1</td>
<td>3.7</td>
<td>8</td>
<td>27.6</td>
</tr>
<tr>
<td>ADJG5989</td>
<td>50</td>
<td>140</td>
<td>08/11/07</td>
<td>5.7</td>
<td>1.6</td>
<td>0.9</td>
<td>4</td>
<td>22</td>
<td>50.2</td>
</tr>
<tr>
<td>ADJG5955</td>
<td>46</td>
<td>118</td>
<td>08/11/04</td>
<td>5</td>
<td>1</td>
<td>1.3</td>
<td>3.3</td>
<td>20</td>
<td>44.4</td>
</tr>
<tr>
<td>ADJG5908</td>
<td>35</td>
<td>95</td>
<td>08/11/18</td>
<td>5.5</td>
<td>3</td>
<td>0.9</td>
<td>3.1</td>
<td>12</td>
<td>32.5</td>
</tr>
</tbody>
</table>

114
Patients with high cholesterol and normal C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Age</th>
<th>Weight (kg)</th>
<th>date tested</th>
<th>TC (mmol/l)</th>
<th>TG (mmol/l)</th>
<th>HDL CS (mmol/l)</th>
<th>LDL CS (mmol/l)</th>
<th>CRP (mg/l)</th>
<th>BMI Kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5920</td>
<td>51</td>
<td>78</td>
<td>08/11/19</td>
<td>5.5</td>
<td>1.4</td>
<td>1.1</td>
<td>3.8</td>
<td>6</td>
<td>29.7</td>
</tr>
<tr>
<td>ADJG5925</td>
<td>60</td>
<td>59</td>
<td>09/06/04</td>
<td>6.6</td>
<td>2.1</td>
<td>1.5</td>
<td>4.7</td>
<td>5</td>
<td>23.9</td>
</tr>
<tr>
<td>ADJG5929</td>
<td>54</td>
<td>60</td>
<td>08/11/24</td>
<td>7.7</td>
<td>1.8</td>
<td>1.4</td>
<td>5.5</td>
<td>5</td>
<td>21.8</td>
</tr>
<tr>
<td>ADJG5916</td>
<td>55</td>
<td>58</td>
<td>08/11/19</td>
<td>5.3</td>
<td>0.7</td>
<td>1.6</td>
<td>3.3</td>
<td>&lt;0.2</td>
<td>20.3</td>
</tr>
<tr>
<td>ADJG5995</td>
<td>61</td>
<td>67</td>
<td>09/03/10</td>
<td>5.9</td>
<td>1.6</td>
<td>1.3</td>
<td>4</td>
<td>4</td>
<td>26.5</td>
</tr>
<tr>
<td>ADJG5926</td>
<td>58</td>
<td>78</td>
<td>08/11/24</td>
<td>5.6</td>
<td>0.6</td>
<td>1.4</td>
<td>3.7</td>
<td>6</td>
<td>30.5</td>
</tr>
<tr>
<td>ADJG5993</td>
<td>19</td>
<td>57</td>
<td>08/11/07</td>
<td>5.4</td>
<td>0.8</td>
<td>1</td>
<td>3.8</td>
<td>1</td>
<td>22.8</td>
</tr>
<tr>
<td>AEPI5815</td>
<td>37</td>
<td>58</td>
<td>09/06/04</td>
<td>5.5</td>
<td>0.8</td>
<td>1.7</td>
<td>3.3</td>
<td>5</td>
<td>23.5</td>
</tr>
<tr>
<td>ADJG5991</td>
<td>24</td>
<td>72</td>
<td>08/11/07</td>
<td>5</td>
<td>1.1</td>
<td>1.2</td>
<td>3</td>
<td>2</td>
<td>22.5</td>
</tr>
<tr>
<td>ADJG5982</td>
<td>21</td>
<td>102</td>
<td>08/11/06</td>
<td>5.3</td>
<td>1.4</td>
<td>0.7</td>
<td>3.7</td>
<td>2</td>
<td>28.9</td>
</tr>
<tr>
<td>ADJG5966</td>
<td>47</td>
<td>72</td>
<td>08/11/05</td>
<td>7</td>
<td>3.5</td>
<td>0.9</td>
<td>4.5</td>
<td>1</td>
<td>23.2</td>
</tr>
<tr>
<td>ADJG5906</td>
<td>33</td>
<td>74</td>
<td>08/11/18</td>
<td>5.6</td>
<td>1.1</td>
<td>1</td>
<td>3.6</td>
<td>5</td>
<td>29.3</td>
</tr>
<tr>
<td>ADJG5904</td>
<td>53</td>
<td>73</td>
<td>08/11/18</td>
<td>5.4</td>
<td>0.6</td>
<td>1.4</td>
<td>3.3</td>
<td>3</td>
<td>25.3</td>
</tr>
<tr>
<td>ADJG5943</td>
<td>45</td>
<td>69</td>
<td>08/12/03</td>
<td>5.4</td>
<td>0.5</td>
<td>1.9</td>
<td>3.1</td>
<td>5</td>
<td>21.9</td>
</tr>
<tr>
<td>ADJG5990</td>
<td>21</td>
<td>69</td>
<td>08/11/07</td>
<td>5.4</td>
<td>0.8</td>
<td>1.1</td>
<td>3.7</td>
<td>1</td>
<td>21.5</td>
</tr>
<tr>
<td>AEPI5918</td>
<td>48</td>
<td>68</td>
<td>09/03/24</td>
<td>5.3</td>
<td>2.1</td>
<td>1</td>
<td>3.3</td>
<td>3</td>
<td>24.7</td>
</tr>
<tr>
<td>ADJG5967</td>
<td>61</td>
<td>65</td>
<td>08/11/05</td>
<td>5.4</td>
<td>0.9</td>
<td>1.9</td>
<td>3.1</td>
<td>5</td>
<td>24.2</td>
</tr>
<tr>
<td>ADJG5910</td>
<td>39</td>
<td>88</td>
<td>08/11/18</td>
<td>6.9</td>
<td>2.1</td>
<td>1.2</td>
<td>4.8</td>
<td>1</td>
<td>30.9</td>
</tr>
<tr>
<td>ADJG5987</td>
<td>27</td>
<td>94</td>
<td>08/11/07</td>
<td>5.8</td>
<td>1.6</td>
<td>0.9</td>
<td>42</td>
<td>7</td>
<td>32.9</td>
</tr>
<tr>
<td>ADJG5958</td>
<td>22</td>
<td>64</td>
<td>08/11/04</td>
<td>5.1</td>
<td>0.7</td>
<td>1.4</td>
<td>3.3</td>
<td></td>
<td>24.7</td>
</tr>
</tbody>
</table>
Follow up patients on statin for 3 months

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>Age</th>
<th>weight (kg)</th>
<th>date tested</th>
<th>TC mmol/l</th>
<th>TG mmol/l</th>
<th>HDL CS (mmol/l)</th>
<th>LDL CS (mmol/l)</th>
<th>CRP (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5914</td>
<td>35</td>
<td>75</td>
<td>09/03/09</td>
<td>4.4</td>
<td>1.5</td>
<td>0.7</td>
<td>2.9</td>
<td>27</td>
</tr>
<tr>
<td>ADJG5929</td>
<td>54</td>
<td>60</td>
<td>09/03/16</td>
<td>5</td>
<td>0.6</td>
<td>1.4</td>
<td>3.5</td>
<td>5</td>
</tr>
<tr>
<td>ADJG5995</td>
<td>62</td>
<td>67</td>
<td>09/06/04</td>
<td>4.2</td>
<td>1.1</td>
<td>1.3</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>ADJG5926</td>
<td>58</td>
<td>78</td>
<td>09/03/09</td>
<td>4.1</td>
<td>0.4</td>
<td>1.3</td>
<td>2.3</td>
<td>6</td>
</tr>
<tr>
<td>ADJG5943</td>
<td>44</td>
<td>69</td>
<td>09/03/24</td>
<td>4.1</td>
<td>0.3</td>
<td>1.9</td>
<td>1.9</td>
<td>5</td>
</tr>
<tr>
<td>ADJG5966</td>
<td>47</td>
<td>72</td>
<td>09/03/11</td>
<td>4</td>
<td>2.2</td>
<td>0.7</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>ADJG5989</td>
<td>50</td>
<td>140</td>
<td>09/03/09</td>
<td>4.8</td>
<td>1.3</td>
<td>1.1</td>
<td>3.2</td>
<td>18</td>
</tr>
<tr>
<td>AEPI5918</td>
<td>48</td>
<td>68</td>
<td>09/06/09</td>
<td>3.8</td>
<td>1.8</td>
<td>1.1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>ADJG5904</td>
<td>53</td>
<td>73</td>
<td>09/06/09</td>
<td>4.7</td>
<td>1.2</td>
<td>1.3</td>
<td>2.7</td>
<td>1</td>
</tr>
<tr>
<td>ADJG5955</td>
<td>46</td>
<td>118</td>
<td>09/03/12</td>
<td>3.3</td>
<td>0.7</td>
<td>0.8</td>
<td>2.1</td>
<td>15</td>
</tr>
<tr>
<td>ADJG5951</td>
<td>52</td>
<td>57</td>
<td>09/03/12</td>
<td>4.4</td>
<td>0.9</td>
<td>1.5</td>
<td>2.2</td>
<td>1</td>
</tr>
<tr>
<td>AEPI5817</td>
<td>48</td>
<td>64</td>
<td>09/06/04</td>
<td>5.6</td>
<td>0.5</td>
<td>2.2</td>
<td>3.1</td>
<td>2</td>
</tr>
<tr>
<td>ADJG5996</td>
<td>63</td>
<td>62</td>
<td>09/03/27</td>
<td>4.9</td>
<td>2</td>
<td>0.6</td>
<td>3.8</td>
<td>4</td>
</tr>
<tr>
<td>ADJG5952</td>
<td>53</td>
<td>48</td>
<td>09/03/12</td>
<td>4.9</td>
<td>1</td>
<td>1.3</td>
<td>3.1</td>
<td>5</td>
</tr>
<tr>
<td>ADJG5930</td>
<td>60</td>
<td>74</td>
<td>09/06/04</td>
<td>5.1</td>
<td>1.7</td>
<td>1.9</td>
<td>2.9</td>
<td>5</td>
</tr>
</tbody>
</table>
C - reactive protein of healthy volunteers using DxC800 and Micro titer plate reader techniques.

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>CRP (DxC800) mg/l</th>
<th>CRP (Microtiter plate Reader) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5978</td>
<td>&lt;0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>ADJG5981</td>
<td>&lt;0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>ADJG5971</td>
<td>0.2</td>
<td>0.035</td>
</tr>
<tr>
<td>ADJG5960</td>
<td>5</td>
<td>0.007</td>
</tr>
<tr>
<td>ADJG5912</td>
<td>4</td>
<td>0.106</td>
</tr>
<tr>
<td>ADJG5944</td>
<td>5</td>
<td>0.070</td>
</tr>
<tr>
<td>ADJG5975</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td>ADJG5954</td>
<td>4</td>
<td>0.145</td>
</tr>
<tr>
<td>ADJG5957</td>
<td>5</td>
<td>0.004</td>
</tr>
<tr>
<td>ADJG5992</td>
<td>5</td>
<td>0.007</td>
</tr>
<tr>
<td>ADJG5923</td>
<td>&lt;0.2</td>
<td>0.009</td>
</tr>
<tr>
<td>ADJG5979</td>
<td>&lt;0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>ADJG5980</td>
<td>4</td>
<td>0.082</td>
</tr>
<tr>
<td>ADJG5956</td>
<td>2</td>
<td>0.038</td>
</tr>
<tr>
<td>ADJG5962</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td>ADJG5907</td>
<td>2</td>
<td>0.037</td>
</tr>
<tr>
<td>ADJG5985</td>
<td>&lt;0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>ADJG5984</td>
<td>&lt;0.2</td>
<td>0.004</td>
</tr>
<tr>
<td>ADJG5936</td>
<td>4</td>
<td>0.053</td>
</tr>
</tbody>
</table>
### Patients with normal cholesterol and high C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>CRP (DxC800i) Mg/l</th>
<th>CRP (Microtiter plate Reader) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5918</td>
<td>8</td>
<td>0.197</td>
</tr>
<tr>
<td>ADJG5911</td>
<td>13</td>
<td>0.244</td>
</tr>
<tr>
<td>ADJG5922</td>
<td>20</td>
<td>0.395</td>
</tr>
<tr>
<td>ADJG5931</td>
<td>8</td>
<td>0.245</td>
</tr>
<tr>
<td>ADJG5947</td>
<td>16</td>
<td>0.204</td>
</tr>
<tr>
<td>ADJG5905</td>
<td>8</td>
<td>0.184</td>
</tr>
<tr>
<td>ADJG5928</td>
<td>15</td>
<td>0.280</td>
</tr>
<tr>
<td>ADJG5919</td>
<td>8</td>
<td>0.223</td>
</tr>
<tr>
<td>ADJG5941</td>
<td>9</td>
<td>0.813</td>
</tr>
<tr>
<td>ADJG5948</td>
<td>8</td>
<td>0.248</td>
</tr>
</tbody>
</table>
Patients with both high cholesterol and high C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>CRP (DxC800) mg/l</th>
<th>CRP (Microtiter plate Reader) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5914</td>
<td>28</td>
<td>0.789</td>
</tr>
<tr>
<td>ADJG5970</td>
<td>27</td>
<td>0.656</td>
</tr>
<tr>
<td>ADJG5915</td>
<td>8</td>
<td>0.197</td>
</tr>
<tr>
<td>ADJG5989</td>
<td>22</td>
<td>0.482</td>
</tr>
<tr>
<td>ADJG5955</td>
<td>20</td>
<td>0.599</td>
</tr>
<tr>
<td>ADJG5908</td>
<td>12</td>
<td>0.329</td>
</tr>
</tbody>
</table>

Patients with high cholesterol and normal C-reactive protein

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>CRP (DxC800) mg/l</th>
<th>CRP (Microtiter plate Reader) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5920</td>
<td>6</td>
<td>0.159</td>
</tr>
<tr>
<td>ADJG5925</td>
<td>5</td>
<td>0.022</td>
</tr>
<tr>
<td>ADJG5929</td>
<td>5</td>
<td>0.008</td>
</tr>
<tr>
<td>ADJG5916</td>
<td>&lt;0.2</td>
<td>0.012</td>
</tr>
<tr>
<td>ADJG5995</td>
<td>4</td>
<td>0.113</td>
</tr>
<tr>
<td>ADJG5926</td>
<td>6</td>
<td>0.095</td>
</tr>
<tr>
<td>ADJG5993</td>
<td>1</td>
<td>0.013</td>
</tr>
<tr>
<td>AEPI5815</td>
<td>5</td>
<td>0.019</td>
</tr>
<tr>
<td>Code</td>
<td>Number</td>
<td>Value</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-------</td>
</tr>
<tr>
<td>ADJG5991</td>
<td>2</td>
<td>0.014</td>
</tr>
<tr>
<td>ADJG5982</td>
<td>2</td>
<td>0.043</td>
</tr>
<tr>
<td>ADJG5966</td>
<td>1</td>
<td>0.024</td>
</tr>
<tr>
<td>ADJG5906</td>
<td>5</td>
<td>0.001</td>
</tr>
<tr>
<td>ADJG5904</td>
<td>3</td>
<td>0.081</td>
</tr>
<tr>
<td>ADJG5943</td>
<td>5</td>
<td>0.007</td>
</tr>
<tr>
<td>ADJG5990</td>
<td>1</td>
<td>0.026</td>
</tr>
<tr>
<td>AEPI5918</td>
<td>3</td>
<td>0.047</td>
</tr>
<tr>
<td>ADJG5967</td>
<td>5</td>
<td>0.179</td>
</tr>
<tr>
<td>ADJG5910</td>
<td>1</td>
<td>0.038</td>
</tr>
<tr>
<td>ADJG5987</td>
<td>7</td>
<td>0.172</td>
</tr>
<tr>
<td>ADJG5958</td>
<td>&lt;0.2</td>
<td>0.000</td>
</tr>
<tr>
<td>ADJG5988</td>
<td>2</td>
<td>0.037</td>
</tr>
<tr>
<td>AEPI5817</td>
<td>2</td>
<td>0.055</td>
</tr>
<tr>
<td>ADJG5951</td>
<td>7</td>
<td>0.172</td>
</tr>
<tr>
<td>ADJG5996</td>
<td>2</td>
<td>0.107</td>
</tr>
<tr>
<td>ADJG5930</td>
<td>4</td>
<td>0.134</td>
</tr>
<tr>
<td>ADJG5952</td>
<td>6</td>
<td>0.007</td>
</tr>
</tbody>
</table>
### Follow up patients on statin for 3 months

<table>
<thead>
<tr>
<th>Lab no.</th>
<th>CRP (DxC800) mg/l</th>
<th>CRP (Microtiter plate Reader) µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADJG5914</td>
<td>27</td>
<td>0.730</td>
</tr>
<tr>
<td>ADJG5929</td>
<td>5</td>
<td>0.009</td>
</tr>
<tr>
<td>ADJG5995</td>
<td>3</td>
<td>0.012</td>
</tr>
<tr>
<td>ADJG5926</td>
<td>6</td>
<td>0.100</td>
</tr>
<tr>
<td>ADJG5943</td>
<td>5</td>
<td>0.006</td>
</tr>
<tr>
<td>ADJG5966</td>
<td>0.9</td>
<td>0.006</td>
</tr>
<tr>
<td>ADJG5989</td>
<td>18</td>
<td>0.231</td>
</tr>
<tr>
<td>AEPI5918</td>
<td>2</td>
<td>0.049</td>
</tr>
<tr>
<td>ADJG5904</td>
<td>1</td>
<td>0.034</td>
</tr>
<tr>
<td>ADJG5955</td>
<td>15</td>
<td>0.378</td>
</tr>
<tr>
<td>ADJG5951</td>
<td>1</td>
<td>0.034</td>
</tr>
<tr>
<td>AEPI5817</td>
<td>2</td>
<td>0.057</td>
</tr>
<tr>
<td>ADJG5996</td>
<td>4</td>
<td>0.082</td>
</tr>
<tr>
<td>ADJG5952</td>
<td>5</td>
<td>0.005</td>
</tr>
<tr>
<td>ADJG5930</td>
<td>5</td>
<td>0.076</td>
</tr>
</tbody>
</table>
Descriptive Statistics of healthy volunteers when correlating their cholesterol profile and CRP

<table>
<thead>
<tr>
<th>Cholesterol Profile</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>33</td>
<td>2.5</td>
<td>4.9</td>
<td>3.9</td>
<td>0.5</td>
</tr>
<tr>
<td>CRP</td>
<td>33</td>
<td>0.2</td>
<td>6.0</td>
<td>3.1</td>
<td>2.1</td>
</tr>
<tr>
<td>TG</td>
<td>33</td>
<td>0.3</td>
<td>2.0</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>CRP</td>
<td>33</td>
<td>0.2</td>
<td>6.0</td>
<td>3.1</td>
<td>2.1</td>
</tr>
<tr>
<td>HDL</td>
<td>33</td>
<td>0.6</td>
<td>1.6</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>CRP</td>
<td>33</td>
<td>0.2</td>
<td>6.0</td>
<td>3.1</td>
<td>2.1</td>
</tr>
<tr>
<td>LDL</td>
<td>33</td>
<td>1.5</td>
<td>3.1</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td>CRP</td>
<td>33</td>
<td>0.2</td>
<td>6.0</td>
<td>3.1</td>
<td>2.1</td>
</tr>
</tbody>
</table>
### Descriptive statistics of patients with normal cholesterol profile and high CRP when correlating their cholesterol profile and CRP

<table>
<thead>
<tr>
<th>Cholesterol Profile</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>10</td>
<td>2.4</td>
<td>4.5</td>
<td>3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>CRP</td>
<td>10</td>
<td>7.5</td>
<td>20</td>
<td>10.9</td>
<td>4.3</td>
</tr>
<tr>
<td>TG</td>
<td>10</td>
<td>0.5</td>
<td>1.6</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CRP</td>
<td>10</td>
<td>7.5</td>
<td>20</td>
<td>10.9</td>
<td>4.3</td>
</tr>
<tr>
<td>HDL</td>
<td>10</td>
<td>0.7</td>
<td>1.8</td>
<td>1.1</td>
<td>0.3</td>
</tr>
<tr>
<td>CRP</td>
<td>10</td>
<td>7.5</td>
<td>20</td>
<td>10.9</td>
<td>4.3</td>
</tr>
<tr>
<td>LDL</td>
<td>10</td>
<td>1.2</td>
<td>3.3</td>
<td>2.2</td>
<td>0.6</td>
</tr>
<tr>
<td>CRP</td>
<td>10</td>
<td>7.5</td>
<td>20</td>
<td>10.9</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Descriptive statistics of patients with high cholesterol profile and normal/ high CRP when correlating their cholesterol profile after 20mg atorvastatin for three months

<table>
<thead>
<tr>
<th>Cholesterol profile</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>15</td>
<td>4.4867</td>
<td>0.59024</td>
</tr>
<tr>
<td>CRP</td>
<td>15</td>
<td>6.6600</td>
<td>7.48559</td>
</tr>
<tr>
<td>TG</td>
<td>15</td>
<td>1.1467</td>
<td>0.59745</td>
</tr>
<tr>
<td>CRP</td>
<td>15</td>
<td>6.6600</td>
<td>7.48559</td>
</tr>
<tr>
<td>HDL</td>
<td>15</td>
<td>1.2733</td>
<td>0.47127</td>
</tr>
<tr>
<td>CRP</td>
<td>15</td>
<td>6.6600</td>
<td>7.48559</td>
</tr>
<tr>
<td>LDL</td>
<td>15</td>
<td>2.7067</td>
<td>0.56879</td>
</tr>
<tr>
<td>CRP</td>
<td>15</td>
<td>6.6600</td>
<td>7.48559</td>
</tr>
</tbody>
</table>
Descriptive statistics of patients before and after 20mg atorvastatin for three months

<table>
<thead>
<tr>
<th>Cholesterol profile</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>R-square</th>
<th>R-square</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCBEFORE</td>
<td>15</td>
<td>5.00</td>
<td>7.80</td>
<td>5.9800</td>
<td>.89299</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCAFTER</td>
<td>15</td>
<td>3.30</td>
<td>5.60</td>
<td>4.4867</td>
<td>.59024</td>
<td>0.358</td>
<td>0.01</td>
<td>22</td>
</tr>
<tr>
<td>TGBEFORE</td>
<td>15</td>
<td>.50</td>
<td>2.10</td>
<td>1.1467</td>
<td>.47789</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGAFTER</td>
<td>15</td>
<td>.30</td>
<td>2.20</td>
<td>1.1467</td>
<td>.59745</td>
<td>0.207</td>
<td>0.09</td>
<td>0</td>
</tr>
<tr>
<td>HDLBEFORE</td>
<td>15</td>
<td>.90</td>
<td>2.20</td>
<td>1.3533</td>
<td>.40154</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>HDLAFTER</td>
<td>15</td>
<td>.60</td>
<td>2.20</td>
<td>1.2733</td>
<td>.47127</td>
<td>0.788</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>LDLBEFORE</td>
<td>15</td>
<td>3.00</td>
<td>5.50</td>
<td>3.9067</td>
<td>.79234</td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>LDLAFTER</td>
<td>15</td>
<td>1.90</td>
<td>3.80</td>
<td>2.7067</td>
<td>.56879</td>
<td>0.412</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>CRPBEFORE</td>
<td>15</td>
<td>1.00</td>
<td>28.00</td>
<td>8.0667</td>
<td>8.31064</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRPAFTER</td>
<td>15</td>
<td>.90</td>
<td>27.00</td>
<td>6.6600</td>
<td>7.48559</td>
<td>0.926</td>
<td>0.03</td>
<td>21</td>
</tr>
</tbody>
</table>