Cardiovascular Risk Reduction in Insulin Resistant States

Thozhukat Sathyapalan M.B.B.S. MRCP
Department of Medicine
The University of Hull

A Thesis Submitted for the Degree of Doctor of Medicine to the University of Hull

June 2009

To

This thesis is dedicated to my other half, Esther, for her love and support during both my MD and all of our time together.

Cardiovascular Risk Reduction in Insulin Resistant States

Table of Contents

Table of Contents

	Page
Title Page	1
Dedication	2
Table of Contents	3
List of Tables	8
List of Figures	10
Acknowledgements	11
Publications	13
Abstract	14
Format of Thesis and Aims	17
Chapter 1 Introduction	18
1.1 Conditions associated with insulin resistance	19
1.1.1 The relationship between hyperinsulinemia, insulin resistance and cardiovascular risk factors	23
1.2 Polycystic ovary syndrome	24
1.2.1 Definition of PCOS	25
1.2.2 Clinical profile of PCOS	28
1.2.3 The association between insulin resistance and PCOS	28
1.2.4 The relationship between hyperandrogenemia and hyperinsulinism in PCOS	31
1.2.5 The relationship between metabolic syndrome and PCOS	32

1.2.6 The relationship between obesity and PCOS 34
1.2.7 The role of insulin sensitisers in PCOS 34
1.2.8 Metformin 35
1.2.9 Mechanism of action of metformin 35
1.2.10 The effect of metformin in weight reduction 36
1.2.11 The effect of metformin on androgen levels 37
1.2.12 The effect of metformin in obese PCOS 37
1.2.13 Adverse effect of metformin 37

1.3 The effect of obesity and weight loss in PCOS 38
1.3.1 The effect of weight loss on improvement of insulin resistance in PCOS 39
1.3.2 Does weight reduction lead to other health benefits in PCOS? 39
1.3.3 Are interventions aimed at lifestyle modification effective? 39

1.4 What is the current state of antiobesity drug therapy? 40
1.4.1 Sibutramine 40
1.4.2 Orlistat 41
1.4.3 Phentermine 41
1.4.4 Rimonabant 42
1.4.4.1 Cannabinoid receptors 42
1.4.4.2 Does rimonabant produce weight loss than other currently available weight loss medications? 44
1.4.4.3 What about reduction of cardiovascular and metabolic risk factors with rimonabant? 45

1.5 Does rimonabant modulate incretin hormones to produce weight loss? 47
1.5.1 The incretin effect 47
1.5.2 Incretin homones - GIP and GLP-1 48
1.5.3 GIP 50
1.5.3 GLP-1 52

1.6 Is there any potential role for statin in the treatment of PCOS? 54
1.6.1 What is the pleotrophic effects of statins? 55

1.7 What is the clinical relevance of biological variation? 57

1.7.1 Is there any biological variation of lipids? 60

1.7.2 How can we minimise the source of pre-analytical variation? 66

1.7.3 Is measured LDL more reliable than calculated LDL in patients with type 2 diabetes? 67

Chapter 2 Methods 73

2.1 Laboratory Methods and Reagents 74

2.1.1 Intervention Studies 74

2.1.2 Biological Variability Studies 75

2.2 Statistical Analysis 75

2.2.1 Intervention Studies 76

2.2.2 Biological Variability Studies 76

2.3 Ethics 76

Chapter 3 A Comparison between Rimonabant and Metformin in Reducing Biochemical Hyperandrogenemia and Insulin Resistance in Patients with Polycystic Ovary Syndrome: A randomised open labelled parallel study 77

3.1 Introduction 78

3.2 Research design and Methods 78

3.3 Results 80

3.4 Discussion 82

Chapter 4 Metformin Maintains the Weight Loss and Metabolic Benefits following Rimonabant Treatment in Obese Women with Polycystic Ovary Syndrome 88

4.1 Introduction 89

4.2 Research design and Methods 89

4.3 Results 90

4.4 Discussion 92

Chapter 5 Effect of Rimonabant and Metformin on GIP and GLP-1 in Obese Women with Polycystic Ovary Syndrome. 101

5.1 Introduction 102

5.2 Research design and Methods 102

5.3 Results 104

5.4 Discussion 104

Chapter 6 The Effect of Atorvastatin in Patients with Polycystic Ovary Syndrome: A Randomized Double Blind Placebo Controlled Study. 107

6.1 Introduction 108

6.2 Research design and Methods 109

6.3 Results 110

6.7 Discussion 111

Chapter 7 Atorvastatin Pre-treatment Augments the Effect of Metformin in Patients with Polycystic Ovary Syndrome. 116

7.1 Introduction 117

7.2 Research design and Methods 117

7.3 Results 118

7.4 Discussion 119

Chapter 8 The Biological Variability of Simvastatin and Atorvastatin in patients with Patients with Type 2 Diabetes: Implications for treatment to target. 124

8.1 Introduction 125

8..2 Research design and Methods 126

8.3 Results 127

8.4 Discussion 128

Chapter 9 Biological Variability of Measured LDL Compared to Calculated LDL in patients with Type 2 Diabetes
133

9.1 Introduction 138

9.2 Research design and Methods 139

9.3 Results 140

9.4 Discussion 141

Chapter 10 Summary Discussion 149

References 155

List of Tables

1. Pathological conditions associated with insulin resistance in humans 20
2. Syndromes associated with extreme insulin resistance 22
3. Characteristics of GIP and GLP-1 49
4. NCEP Analytical Goals for Lipid and Lipoprotein Measurements 63
5. Influence of analystical and biological variation of the clinical utility of lipid measurement. 64
6. Important Considerations for the Measurement of Blood Lipids 65
7. Comparison of anthropometric and hormonal parameters before and after treatment with rimonabant and metformin 86
8. Changes in anthropometric parameters in metformin group and rimonabant/metformin group 97
9. Changes in biochemical hyperandrogenemia in metformin group and rimonabant/metformin group 98
10. Changes in metabolic parameters in metformin group and rimonabant/metformin group 99
11. Comparison of anthropometric and hormonal parameters before and after treatment with atorvastatin and placebo in patients with PCOS 115
12. Comparison of anthropometric and hormonal parameters at baseline, 12 weeks of atorvastatin or placebo followed by 12 weeks of metformin in patients with PCOS 122

13. Baseline characteristics of patients in atorvastatin and simvastatin in patients with T2DM study 133

14. The biological variation of lipid parameters in 26 patients on Simvastatin 40mg and Atorvastatin 10mg in patients with T2DM 134

15. Mean values of total cholesterol and LDL-Cholesterol to achieve different targets on 95% of sampling occasions in patients with T2DM 135

16. Single lipid measurement results which give 95% confidence of the true mean value being below target or of each subsequent measurement being below target in patients with T2DM 136

17. Mean values of direct LDL-Cholesterol to consistently achieve different targets in patients with T2DM 146

List of Figures

1. Correlation between reduction in testosterone levels with weight loss with rimonabant 87

2. Flow chart of patients through rimonabant/metformin study 100

3. Glucose-dependent insulinotropic polypeptide (GIP) levels after 3 months of Rimonabant followed by 3 months of Metformin and after 3, 6 months of Metformin 106

4. Variation of LDL cholesterol on atorvastatin and simvastatin in patients with T2DM 137

5. Biological variation of direct LDL on simvastatin and atorvastatin in patients with T2DM 147

Acknowledgements

My deepest feelings of gratitude are directed to Prof. Stephen L Atkin, my guide and mentor for unwavering support and skilful guidance throughout the study. His clear scientific vision, logical thinking and positive attitude to life in general, as well as infectious enthusiasm are all attributes well worth learning. He has provided invaluable guidance in both the preparation of manuscripts and in the writing of this thesis.

I am equally indebted to Prof. Eric S Kilpatrick, despite his multiple responsibilities; he has always managed to find time to help me in the resolution of the scientific, as well as the practical problems of my studies. I am grateful for his invaluable input in preparing and editing the manuscripts. I would also like to extend my thanks to Dr Anne-Marie Coady for performing ultrasonographic examination of ovaries for my study patients with polycystic ovarian syndrome.

During my period of research, I had the opportunity to work with my research colleagues, Dr A Wakil, Dr A M Manuchehri and Dr. L.W. Cho who are now more like my close friends than colleagues. I am really grateful for their support and friendship during and after this research period. I would like to thank Dr M Aye who has patiently helped to improve my computer skills.

I wish to sincerely thank my other colleagues in the research team in the Centre for Diabetes Hull Action Research - Mr. Duane D Mellor, Mrs Lisa Redshaw, Mrs Anneli James, Miss Carrie Willerby, Mrs Maryjane Marshall, Ms Julia Harrison and Ms Nalumon Parnwell who helped facilitate my research, and organise the patients who participated, as well as their friendship. Without their co-operation this work would not have been possible.

I am also deeply appreciative of the all help I received from all the Consultants and Specialist Registrars in the Department of Diabetes, Endocrinology and Metabolism. I am thankful for all the support I received from diabetes specialist nurses, podiatrists, retinal screening team, secretaries, receptionists and porters at the Michael White Diabetes Centre. I would also like to acknowledge the help provided by Mrs Gill Bynes for her secretarial support during my research period. I would like to acknowledge the help of Mrs Anne Anderson who performed many of the assays mentioned in this thesis. I am also deeply indebted to all the patients who participated in the studies, for their selflessness and enthusiasm to further the cause of medical science, I am truly grateful.

I am extremely grateful to my family who has endured many hardships to allow me the chance to pursue my career in medicine and finally, I am also extremely grateful to my other half, Esther, for the patience and understanding shown during the seemingly endless years spent performing the studies and writing of the manuscripts as well as this thesis.

Publications:

Some of the studies described in this thesis have already been published in peer review journals. These include:-

Sathyapalan, T., Kilpatrick, E.S., Coady, A.M. & Atkin, S.L. (2009) The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double-blind placebo-controlled study. *J Clin Endocrinol Metab*, **94**, 103-108.
Hoeger KM 2009 Polycystic Ovary Syndrome, Inflammation, and Statins: Do We Have the right Target? *J Clin Endocrinol Metab,* **94**:35-37 *(Editorial)*

Sathyapalan, T., Cho, L.W., Kilpatrick, E.S., Coady, A.-M. & Atkin, S.L. (2009) Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS). Clinical Endocrinology, 70, 124-128.

Sathyapalan, T., Cho, L.W., Kilpatrick, E.S., Coady, A.-M. & Atkin, S.L. (2009) Effect of rimonabant and metformin on GIP and GLP-1 in obese women with polycystic ovary syndrome. Clinical Endocrinology, 70, 124-128.

Sathyapalan, T., Cho, L.W., Kilpatrick, E.S., Coady, A.M. & Atkin, S.L. (2008) A comparison between rimonabant and metformin in reducing biochemical hyperandrogenaemia and insulin resistance in patients with polycystic ovary syndrome (PCOS): a randomized open-label parallel study. *Clin Endocrinol (Oxf)*, **69**, 931-935.

Sathyapalan, T., Atkin, S.L. & Kilpatrick, E.S. (2008) Variability of lipids in patients with Type 2 diabetes taking statin treatment: implications for target setting. *Diabet Med*, **25**, 909-915.

Abstract

Introduction: Insulin resistance is the hall mark of a number of pathological conditions and is thought to play a major role in the cardiovascular risk associated with them. This thesis critically evaluates two insulin resistant conditions - polycystic ovary syndrome (PCOS) and type 2 diabetes (T2DM) - where there are many unresolved issues. During the course of these studies, the effect of weight loss and medications in modifying cardiovascular risk in these conditions was evaluated.

Methods: The first studies focused on a randomised open labelled parallel study of metformin and rimonabant in obese patients with PCOS. Subsquently, an extension to this study was undertaken where patients who were on rimonabant were changed over to metformin, whereas those on metformin were continued on metformin for another 3 months. As part of this study the effect of rimonabant and metformin on incretin hormones in patients with PCOS was studied.

The next studies focused on a randomised double blind placebo controlled study on the pleotrophic effect of atorvastatin in patients with PCOS. Subsequent metformin therapy after atorvastatin treatment was undertaken. This study led to the investigation of the effect of simvastatin and atorvastatin on biological variation of lipids in patients with T2DM that has got implications in treating to lipid targets. A corollary to this study was whether the biological variation of LDL calculated using Friedewald formula differed from that of direct LDL.

Results:

In the first series of studies, after 12 weeks of rimonabant there was a significant reduction in anthropometric and metabolic parameters as well as biochemical hyperandrogenemia in patients with PCOS. There was no change in any of these parameters in the metformin treated group. In three months extension arm to this study, metformin maintained the weight loss as well as enhanced the metabolic and biochemical parameters achieved by treatment with rimonabant, compared to 6 months of metformin treatment alone. There was a significant and reversible increase in glucose-dependent insulinotropic polypeptide (GIP) levels after 3 months of rimonabant treatment. There were no changes in GIP or glucagon-like peptide-1 (GLP-1) levels with metformin.

In the second series of studies it has shown that atorvastatin was effective in reducing inflammation, biochemical hyperandrogenemia and metabolic parameters in patients with polycystic ovary syndrome after a 12 week period compared to placebo. The subsequent effect of three months metformin treatment was augmented by atorvastatin pre-treatment compared to placebo pre-treatment. In the subsequent study it was shown that the coefficient of variation (CV) of TC, LDL, HDL and TG on simvastatin was significant but comparable to atorvastatin in patients with T2DM. However, subsequent directly measured LDL cholesterol was shown to be an order of magnitude more stable when taking equivalent doses of atorvastatin rather than simvastatin.

Conclusion: Both weight loss using rimonabant and atorvastatin were effective in reducing biochemical hyperandrogenemia and metabolic profile in patients with PCOS. The effect of rimonabant might be partly mediated through modulating GIP levels.

There was a significant biological variation in lipid profile in patients with T2DM who are on simvastatin and atorvastatin that may lead to lipid targets in patients with diabetes which are significantly lower than the current evidence suggests and thereby more difficult to achieve. LDL targets can be consistently met at higher mean LDL concentrations (and with less lipid monitoring) using atorvastatin rather than simvastatin when direct LDL is used.

Format of the thesis

This thesis evaluated cardiovascular risk reduction in two related insulin resistant states - polycystic ovary syndrome and T2DM. The first chapter is the "Introduction" where a literature review is done setting the background for the studies done in this thesis. The second chapter described the methodology involved in the studies. Chapters from 3 – 9 describes the 7 studies which were done for this thesis. Chapter 10 begins by summarising my conclusions before discussing my findings in the context of current management.

Aim of this work

1) To see if the reduction of biochemical hyperandrogenaemia and insulin resistance by rimonabant through weight loss was superior to insulin sensitisation with metformin in obese women with PCOS and if subsequent metformin treatment would maintain any of the initial improvement. We aimed to establish whether rimonabant might have an effect on the incretin system that may augment its weight reduction effect in patients with PCOS.
2) To study the effects of atorvastatin in patients with PCOS and the effect of subsequent metformin treatment.
3) To establish the biological variability of directly measured LDL and calculated LDL in patients with T2DM who are on statin treatment and to determine how this could influence the ability for patients to maintain cholesterol values below target thresholds.

Chapter 1

Introduction

1.1 Conditions associated with Insulin resistance

Although the insight into clinical implications of insulin resistance has dramatically increased in the past 50 years, much uncertainty remains about the association between insulin resistance and human disease (1). The exact prevalence of insulin resistance is unknown since relative hyperinsulinaemia and other features of the insulin resistance syndrome can be identified in a proportion of apparently healthy individuals. This is due to the difficulties of defining insulin resistance in clinical terms and of quantifying insulin action in humans.

Physiological states of insulin resistance

Insulin sensitivity spans a broad range (threefold to fourfold) even among apparently healthy people with normal glucose tolerance. Many inherited and acquired factors can affect insulin sensitivity. Some of these, sex for example, are immutable. However, associated factors such as regional adiposity, skeletal muscle mass and level of physical conditioning are potentially modifiable. Hormonal changes associated with puberty and pregnancy (second and third trimesters) often lead to substantial increases in insulin requirements.

Conditions associated with insulin resistance

Insulin resistance occurs in many aetiologically diverse human disorders (Table 1 and 2). In the extreme insulin resistance syndromes (Table 2) resistance is an important determinant of the clinical phenotype.

Table 1

Pathological conditions associated with insulin resistance in humans

Acquired conditions

Antagonism of insulin action:

Acute counter regulatory hormone excess (trauma, severe sepsis, acute myocardial infarction, diabetic ketoacidosis--common)

Medications (corticosteroids, ß blockers--common)

Thyrotoxicosis (common)

Polycystic ovary syndrome (relatively common)

Acromegaly (rare)

Phaeochromocytoma (rare)

Cushing's syndrome (rare)

Insulinoma (rare)

Glucagonoma syndrome (very rare)

Cardiological syndromes:

Congestive cardiac failure (common)

Atheromatous disease (common)

Microvascular angina (uncommon)

Other major organ failure (relatively common):

Hepatic cirrhosis

Chronic renal failure

Inherited (all uncommon or rare)

Myotonic dystrophy

Prader-Willi syndrome

Alstrom's syndrome

Laurence-Moon-Biedl syndrome

Werner's syndrome

Table 2-Syndromes associated with extreme insulin resistance

Insulin receptor mutations:

Leprechaunism

Rabson-Mendenhall syndrome

Type A insulin resistance (mutations are relatively uncommon)

Post-binding defects in insulin action:

Lipodystrophic diabetes syndromes (includes inherited and acquired forms)

Type C insulin resistance (post-receptor defect; overlaps with type A)

Insulin receptor antibodies:

Type B insulin resistance (usually associated with evidence of other autoimmune disease)

All of these syndromes are uncommon or rare. Glucose tolerance may be only minimally impaired if compensatory hyperinsulinaemia is sufficient to overcome the defect.

1.1.1 Relationship between hyperinsulinemia, insulin resistance and cardiovascular risk factors

Cross-sectional studies have reported an association of insulin resistance (as determined by the hyperinsulinemic euglycemic clamp) with atherosclerosis as measured by carotid ultrasound or coronary angiography(6, 7). Hyperinsulinemia has been identified as a risk factor for coronary heart disease in several (8-11), but not in all, studies. However the negative studies were done on elderly subjects (13) and high risk subjects (12).

Hypertension

Insulin resistance has been strongly associated with hypertension in lean subjects(15). Fasting insulin levels predicted the development of hypertension in lean but not in obese subjects(16).

Dyslipidemia

Hyperinsulinemia and insulin resistance have been consistently related to dyslipidemia (2, 17, 18) with a preponderance of small dense low density-lipoprotein has been associated with coronary heart disease(19). Studies have confirmed that elevated insulin levels are associated, cross-sectionally with increased triglyceride levels, decreased HDL levels and hypertension(17, 20). In the San Antonio Heart study increased fasting insulin levels significantly predicted the development of T2DM, low HDL levels, high triglyceride levels and hypertension over an 8 year follow up. Subjects who developed multiple metabolic disorders had higher insulin concenterations than those who developed only a single disorder.

Impairment of fibrinolysis

High levels of plasma plasminogen activator inhibitor (PAI-1) have been associated consistently with increased insulin concentrations and insulin resistance(21, 22). It has been suggested that an increased PAI-1 could form a link between insulin resistance and coronary heart disease(23).

1.2 Polycystic ovary syndrome

"Giovane rustica, maritata,modicamente pingue, et infeconda, con due ovaie più grandi del normale, come uova di colomba, bernoccolute, lucenti et biancastre..." (Young peasant woman, married, moderately lump and infertile, with ovaries larger than normal, like doves' eggs, lumpy, shiny and whitish...) This description from 1721 by the Italian scientist Antonio Vallisneri is probably the first text of polycystic ovary syndrome PCOS) (24). In 1844 Chereau described sclerotic changes in the ovary (25). The association between hyperandrogenism and diabetes was first described by Achard and Thiers in 1921, in the paper "Le virilism pilaire et son association á l'insuffisance glycolytique" (26). and was called "the diabetes of bearded women (diabete des femmes a barbe)" (27).

In 1935, the two American gynaecologists, Stein and Leventhal published a classic paper on a series of seven patients(28). They described bilaterally enlarged polycystic ovaries, *"two to four times the normal size, sometimes distinctly globular", "tunica thickened, though, and fibrotic", "follicle cysts near the cortex and almost entirely confined to the cortex". "The colour of the ovary was oyster gray with bluish areas where the cysts were superficial and appeared on the surface as sago-like bodies".* Other characteristics included oligo-amenorrhea, hirsutism and infertility.

According to Stein and Leventhal the diagnosis was based on the clinical appearance: hirsuitism, amenorrhea, infertility and histological specimen of polycystic ovaries with prominent theca, fibrotic thickening of the tunica albuginea and multiple cystic follicles(28). In the 1960s it became evident that the "Stein-Leventhal syndrome" represented a variety of clinical manifestations. In the early 1970s, the scientific community focused on the changed function in the hypothalamic- pituitary-ovarian axis, increased serum levels of LH, and elevated LH/FSH ratio(29).

In the late 1970s the concept of PCOS developed. Burghen et al. were first to point out a link between PCOS and insulin resistance. They demonstrated that hyperandrogenism correlate with hyperinsulinemia in obese PCOS women(30). Later, ultrasound became central in visualizing polycystic ovaries (PCO) and diagnosing PCOS. Swanson et al. were the first to describe the typical ultrasonographic appearance of polycystic ovaries in 1981(31), and Adams et al. refined the criteria for the ultrasonographic diagnosis of PCO(32).

The first treatment for PCOS was bilateral wedge resection of the ovaries, suggested by Stein and Leventhal. They reported regain of regular menstruations in seven patients, and pregnancy in two women, after wedge resection. Half a century later Gjønnæss, a Norwegian gynaecologist, introduced laparoscopic ovarian drilling, as a more conservative method with fewer problems with adhesions(33).

1.2.1 Definition of PCOS

Polycystic ovary syndrome (PCOS), a disorder characterised by hyperandrogenism and chronic anovulation(34), is one of the most common endocrinopathies in pre-menopausal women and the most common cause of anovulatory infertility.

University Library Hull

Until 2003 there was no international consensus on the definition of PCOS. In the United States, the National Institute of Health (NIH) Conference on PCOS 1990 recommended that diagnostic criteria should include evidence of hyperandrogenism (clinical or biochemical) and ovulatory dysfunction in the absence of non-classic congenital adrenal hyperplasia (CAH). Polycystic ovarian morphology was not considered essential. A clear definition of ovulatory dysfunction, hirsutism or hyperandrogenism was, however, not given (35). PCOS according to this definition was recognized in three principal phenotypes:

1) Women with hirsutism, hyperandrogenemia and oligo-ovulation,

2) Women with hirsutism and oligo-ovulation and

3) Women with hyperandrogenemia and oligo-ovulation.

In Europe, the definition of PCOS was restricted to a condition with polycystic ovaries, identified by ultrasonography and one or more of the following; oligo/amenorrhea, hyperandrogenism, obesity, elevated serum testosterone and / or elevated LH concentrations (35). Ovulatory dysfunction was not mandatory. The need for a universal agreement on the definition of PCOS was obvious.

In Rotterdam in 2003, the European Society of Human Reproduction and Embryology (ESHRE) and the American Society of Reproductive Medicine (ASRM) achieved a new consensus(36). The new definition reflects the awareness that PCOS represents a multitude of clinical expressions and emphasizes the importance of realising it. According to the Rotterdam 2003 consensus, two of the following three criteria must be fulfilled for the diagnosis:

1. Polycystic ovaries; 12 or more follicles in each ovary, each follicle measuring 2-9 mm in diameter and/or ovarian volume >10ml. One polycystic ovary is sufficient for the diagnosis.

2. Oligo-/anovulation; clinically diagnosed as oligo-/ amenorrhea, i.e. menstrual cycles longer than 35 days or less than 10 menstruations per year.

3. Hyperandrogenism; clinical or biochemical.

The clinical definition of hyperandrogenism includes: hirsutism, acne and androgen alopecia, but the evaluation of hirsutism is difficult, because of racial differences (37, 38). Cosmetic treatment abolishes the expression and although there exists standardised scoring, the Ferrimann-Gallwey score is seldom used (39).

The Rotterdam consensus on the definition of PCOS has not defined clinical hyperandrogenism. The definition of biochemical hyperandrogenism is not without problems;

1. Modern immunoassay methods in routine clinical practice have recently been shown to be inaccurate for measuring testosterone in women(40).

2. Normative ranges are not established, and adjustment for age and BMI should be recommended.

3. Other androgens than testosterone should also be considered, especially DHEAS and androstenedione.

The diagnosis of PCOS should not be based on one single criterion, and it can be argued that PCOS is a diagnosis of exclusions. Congenital adrenal hyperplasia, non-classic congenital adrenal hyperplasia, Cushing syndrome, acromegaly and androgen secreting tumours should be ruled out.

It is important to realise that the Rotterdam criteria has expanded the definition of PCOS compared with the NIH criteria, and created two new phenotypes i.e. 1) women with PCO, hirsutism / hyperandrogenemia and regular ovulations and 2) women with PCO, oligo-ovulations and normal androgens(41).

1.2.2 Clinical profile of PCOS

In clinical practice, women with PCOS present with infertility (mean incidence, 74%), menstrual irregularity (dysfunctional bleeding, 29%; amenorrhoea, 51%), hyperandrogenism (69%), and virilization (21%) (42). The endocrine profile of women with PCOS is characterised by high plasma concentrations of ovarian and adrenal androgens, gonadotropin abnormalities, a relative increase in oestrogen levels derived from conversion of androgens, reduced levels of sex hormone binding globulin (SHBG), and often high levels of insulin (43).

1.2.3 The association between insulin resistance and PCOS

Approximately 60-70% of PCOS patients are obese(44), with a central body fat distribution pattern described as visceral obesity that is well known to be highly associated with insulin resistance (IR). However, PCOS patients have evidence of insulin resistance independent of obesity (45-47). Insulin sensitivity is decreased by 35-40% in women with PCOS, independent of obesity, a decrease similar in magnitude to that seen in T2DM mellitus(48); still, any degree of obesity further impairs insulin action. About 50-70% of all women with PCOS have some degree of insulin resistance (49). It is now evident that PCOS has major metabolic consequences related to insulin resistance. Insulin resistance in PCOS may be considered a risk factor for gestational

diabetes (GD) (50); the prevalence of GD in PCOS patients has been reported to be 40-46%. A link between insulin resistance and hypertensive disorders in pregnancy has been widely reported; pre-eclampsia is reported to be more frequent in PCOS patients than in normal women (51) and in one case control study, the incidence of this disorder was found to be as high as 28.5%(52). Evidence supporting the possibility of insulin resistance playing a role in the development of endometrial cancer has been provided(53, 54); increased risk for endometrial cancer was reported in women with increased serum levels of insulin(55) and lower serum levels of SHBG(56), both prominent features of women with PCOS and of insulin resistance. In addition, an increased prevalence of endometrial cancer among women with PCOS, including young women with the disorder has been reported(57, 58). Insulin resistance is associated with an increased risk for several disorders, including T2DM mellitus (T2DM) or, hypertension, dyslipidemia (low high-density lipoprotein cholesterol and high triglycerides), elevated plasminogen activator inhibitor type 1 (PAI-1), elevated endothelin-1, endothelial dysfunction, and heart disease.

Evidence that PCOS is associated with a high risk for the development of T2DM and heart disease is mounting. Regarding diabetes risk, prospective clinical trials have demonstrated a 31-35% prevalence of impaired glucose tolerance (IGT) and 7.5-10% prevalence of T2DM in women with PCOS(44, 59). Furthermore, studies (59, 60) demonstrated that both obese and lean PCOS patients are at increased risk of IGT or overt diabetes during their third or fourth decade; up to 20%of PCOS patients have IGT or T2DM by the third decade and up to 30-50% of obese women with PCOS will develop IGT or T2DM by the age of 30 years(44, 62). Regarding cardiovascular risk,

PCOS is associated with increased prevalence of several cardiovascular risk factors, including hypertension and dyslipidemia. In addition, women with PCOS display surrogate markers for early atherosclerosis, such as increased PAI-1(59, 69, 70), endothelin-1(71), and CRP concentrations(72). Several studies (73-75) suggest that PCOS is associated with endothelial dysfunction that is linked to insulin resistance and is a risk factor for cardiovascular disease. PCOS women were shown to have higher mean carotid intima media thickness (IMT) compared with age-matched normal women, a striking illustration of the early atherogenic process in PCOS(76). PCOS women have a greater prevalence and extend of coronary artery calcification(77); the extent of which closely correlates with the atherosclerotic plaque burden(78) and predicts an increased risk of cardiovascular events(79). In addition, several studies reported an increased prevalence of cardiovascular heart disease in PCOS (67, 73, 76, 80, 81). Women with PCOS may represent the larges unique female population at high risk for premature atherosclerotic heart disease. The above considerations indicate that PCOS is not only an infertility or cosmetic problem, but perhaps a primary general heath problem at whose root lays insulin resistance.

Similarly, androgens do cause mild insulin resistance in women (82) and lowering circulating androgen levels pharmacologically or by blocking androgen action with receptor antagonists do slightly improve insulin resistance in hyperandrogenemic women (83). However, the magnitude of change is not in the range of the insulin resistance associated with PCOS (83) and therefore, androgens may amplify but do not account for insulin resistance in adult women with PCOS. Most of the evidence on the directionality of the relationship between insulin resistance and hyperandrogenism

would suggest that the direction of causation is from insulin to androgen and not the reverse. For example, weight loss and administration of insulin sensitisers that specifically reduce insulin concentrations, results in a reduction in circulation androgen concentrations(84). However, administration of a gonadotropin-releasing hormone analogue that reduces androgen secretion from the ovary by suppressing gonadotropins, does not result in a reduction in insulin(85).

The paradox of insulin promoting androgen production in ovarian and adrenal tissues in the face of insulin resistance in peripheral tissues has been partly explained by tissue differences in insulin sensitivity in PCOS such that the steroidogenic tissues are insulin-sensitivity, whereas the major tissues involved in carbohydrate metabolism, namely fat and muscle, are insulin resistant(86).

1.2.4 The relationship between hyperinsulinemia and hyperandrogenism in PCOS:

IGF-I is produced by human ovarian tissue, and IGF-I receptors are present in the ovary(87, 88). Insulin in high concentrations can mimic IGF-I actions by occupancy of the IGF-I receptor, and this has been a proposed mechanism for insulin-mediated hyperandrogenism(91, 92). However, it has recently been shown that insulin has specific actions on steroidogenesis acting through its own receptor(93). Moreover, these actions appear to be preserved in insulin-resistant states (93, 94), presumably because of differences in receptor sensitivity to this insulin action or because of differential regulation of the receptor in this tissue.

Studies in which insulin levels have been lowered for prolonged periods have been much more informative. This has been accomplished for 7 days to 3 months with agents that either decrease insulin secretion, diazoxide(95) or somatostatin (96), or that

improve insulin sensitivity, metformin(70) or troglitazone(97). Circulating androgen levels have decreased significantly in women with PCOS in these studies. Sex hormone binding globulin (SHBG) levels have increased(95, 97), compatible with a major role for insulin in regulating hepatic production of this protein(98, 99). However, estrogen levels also decreased significantly, suggesting that insulin has diffuse effects on steroidogenesis(97).

In summary, studies in which insulin levels have been lowered by a variety of modalities indicate that hyperinsulinemia augments androgen production in PCOS. Moreover, this action appears to be directly mediated by insulin acting through its cognate receptor rather than by spill over occupancy of the IGF-I receptor. Intrinsic abnormalities in steroidogenesis appear to be necessary for this insulin action to be manifested since lowering insulin levels does not affect circulating androgen levels in normal women. Further, in many PCOS women, lowering insulin levels ameliorates but does not abolish hyperandrogenism.

On the other hand, modest hyperandrogenism characteristic of PCOS may contribute to the associated IR. Additional factors are necessary to explain the IR, since suppressing androgen levels does not completely restore normal insulin sensitivity(83, 100). Further, androgen administration does not produce IR of the same magnitude as that seen in PCOS(45, 101, 102). Finally, there are clearly defects in insulin action that persist in cultured PCOS skin fibroblasts removed from the hormonal milieu for generations(103).

1.2.5 The relationship between metabolic syndrome and PCOS

PCOS is associated with an increase of cardiovascular risk factors, including dyslipidemia, which in these patients typically consists of elevated total cholesterol and

LDL(66, 68, 104, 105). In the long term, PCOS is associated with increased thickness of the carotid intima and media(76).

The consequences of PCOS extend beyond the reproductive axis; women with the disorder are at substantial risk for the development of metabolic and cardiovascular abnormalities similar to those that make up MS(106). This finding is not surprising, since both PCOS and metabolic syndrome (MS) share IR as a central pathogenetic feature(107). The PCOS might thus be viewed as a sex-specific form of MS, and the term "syndrome XX" has been suggested as an apt term to underscore this association(108).

MS is a consistent feature of the majority of obese women with PCOS, although it can also be detected in many normal-weight affected women.(82, 109) Studies using ATPIII criteria to assess the prevalence of the MS in PCOS women has found prevalence rate ranging from 43% to 46%(106, 110). It has also been described that there is higher free testosterone and lower sex-hormone-binding globulin (SHBG) levels in those women with the MS with respect to those without it, as well as a higher prevalence of acanthosis nigricans and a greater tendency to have a family history of PCOS(110). These results were in accordance with a cross-sectional population-based study which reported a different concentration of some sex hormones between premenopausal women with and without the ATPIII-defined MS (111). Therefore, collectively, 82% of PCOS women had at least one feature of MS, a finding consistent with a very large presence of single or grouped metabolic abnormalities in this disorder. Compared to those without any

criteria, the other two groups were progressively more obese and had a higher prevalence of the abdominal pattern of fat distribution.

In addition, women presenting with MS were characterized by higher systolic and diastolic blood pressure, higher pulse rate, greater frequency of liver enzyme abnormalities, worsened IR, higher glycosylated haemoglobin and a more severe hyperandrogenemia with respect to those without the MS. Taken together, these findings demonstrate that the prevalence of the MS in women with PCOS is higher than that of the general population, regardless of ethnicity and geographical area. They also indicate a strong association between the MS and the hyperandrogenic state(112).

1.2.6 The relationship between obesity and PCOS

The cause of obesity in PCOS remains unknown, but obesity is present in at least 30 percent of cases; in some series, the percentage is as high as 75(113). Increased adiposity, particularly visceral adiposity that is reflected by an elevated waist circumference (>88 cm [35 in.]) or waist-to-hip ratio, has been associated with hyperandrogenemia, IR, glucose intolerance, and dyslipidemia. Attenuation of IR, whether accomplished by weight loss or with medication, ameliorates (but not necessarily normalizes) many of the metabolic aberrations in women with PCOS(107) i.e obesity causes the expression of PCOS phenotype.

1.2.7 The role of insulin sensitisers in PCOS.

A reduction in insulin levels pharmacologically ameliorates sequelae of both hyperinsulinemia and hyperandrogenemia. The place of insulin-reduction therapies in treating PCOS is evolving. These therapies can effectively manage the established

metabolic derangements in PCOS, but whether they can prevent them is not yet established.

1.2.8 Metformin

Galega officinalis or French lilac is a perennial herb that blooms in July and August in most of Europe. It can be grown as far north as Trondheim. The extract of *Galega officinalis* contains isoamyline guanidine, a hypoglycemic compound, which was used to treat diabetes mellitus in medieval Europe (114). The biguanide metformin was discovered in the 1950s to have hypoglycaemic effect. Two guanine molecules that are dimethylated make up metformin. In 1957 metformin was introduced as an agent for treatment of diabetes mellitus type-2. The use of metformin was restricted during the next decades because of reports of deaths associated to lactic acidosis during metformin treatment. The drug had a renaissance in the 1990s, when metformin was proven to be particularly useful in the treatment of diabetes mellitus type 2.

1.2.9 Mechanism of action of Metformin

In non-diabetic patients, metformin does not influence blood glucose levels.

In patients with diabetes mellitus type-2, metformin lowers fasting blood glucose and improves glucose tolerance. The anti hyperglycaemic activity of metformin is achieved by stimulation of peripheral glucose uptake, reduced hepatic gluco-neogenesis and to a minor degree delayed intestinal absorption. Metformin has a major effect on hepatic gluco-neogenesis. In non-diabetic subjects metformin has been demonstrated to lower cholesterol and LDL/HDL cholesterol ratio (116). A recent study suggests that the mechanisms of action of metformin might be through its activation of AMP- activated protein kinase (117).

Although metformin appears to influence ovarian steroidogenesis directly(118, 119) this effect does not appear to be primarily responsible for the attenuation of ovarian androgen production in women with PCOS. Rather, metformin inhibits the output of hepatic glucose, necessitating a lower insulin concentration and thereby probably reducing the androgen production of theca cells. Subject characteristics and control measures for effects of weight change, dose of metformin, and outcome vary widely among published studies of metformin in PCOS.

Metformin also improved fasting insulin levels, blood pressure, and levels of low-density lipoprotein cholesterol(120). These effects were judged to be independent of any changes in weight that were associated with metformin, but controversy persists as to whether the beneficial effects of metformin are entirely independent of the weight loss(121) that is typically seen early in the course of therapy. Finally, the rates of spontaneous miscarriage and gestational diabetes are reportedly lower among women with PCOS who conceive while taking metformin(122-125). But, the long-term effects of metformin in pregnancy are unknown.

However, in one recent randomised trial involving 626 infertile women with PCOS with metformin, clomiphene or combination, clomiphene was superior to metformin in achieving live birth although metformin significantly reduced body weight, IR and hyperandrogenemia compared to clomiphene(126) suggesting that reduction of insulin resistance alone doesn't improve fertility.

1.2.10 The effect of Metformin on weight reduction

Out of seven RCTs, which had weight as endpoint, six demonstrated reduction of body weight with metformin treatment compared with placebo (127). The studies lasted from

one to six months and the average reduction in BMI was 4%. However, in a systematic review and meta-analysis of RCTs, Lord et al. concluded that there was no evidence that metformin caused weight reduction in PCOS women (128). Weight reduction seems to be dose-related in obese PCOS women (129).

1.2.11 Effects of Metformin on androgen levels

In RCTs the average reduction of androgen hormone levels (free testosterone, free androgen index and total testosterone) were around 20%, with wide variations, in the metformin groups (130-135). The data on SHBG are inconclusive (127).

1.2.12 The effect of metformin in obese PCOS

Metformin therapy to ameliorate the hormonal and metabolic consequences of PCOS is common in practice(136, 137). Studies have shown that metformin can reduce body mass index of around 4% and androgen measures of around 20% compared to placebo(138), but may be less effective in overweight patients especially with BMI more than 36 kg/m^2(129, 139, 140)

1.2.13 Adverse effects of Metformin

Gastrointestinal side effects occur in about 20 % of patients. Adverse effects are usually transient and resolve within one month of treatment, but 5 % of the patients cannot tolerate the drug. Gastrointestinal side effects, such as bloatedness, nausea, vomiting, diarrhoea, constipation and metallic taste are most frequent. Increasing the dose slowly and taking with food, could diminish side effects. A severe and feared adverse effect is the development of lactic acidosis. This occurs in less than 1 in 10 000 patient years, and it has never been observed when contraindications to metformin administration are adhered to. The contraindications to metformin are impaired renal and hepatic function,

alcohol abuse, and serious cardiovascular and pulmonary diseases. These are all conditions that predisposes to lactic acidosis per se.

1.3 The effect of weight loss PCOS

Obesity, particularly an abdominal deposition of fat, is common in PCOS patients (141). Together with the presence of insulin resistance, obesity contributes to the 43% prevalence of the metabolic syndrome in PCOS patients(142). Diet-induced weight loss ameliorates the clinical signs and symptoms of PCOS, including hyperandrogenism and insulin resistance(143), menstrual dysfunction(144), and oligoovulation(145). Even modest weight loss of less than 10% of initial body weight has been shown to increase the frequency of ovulation, improve conception, and reduce testosterone, free androgen index, hyperlipidemia, hyperglycaemia and insulin resistance in women with PCOS.(146, 147)

However, the magnitude of the weight loss usually attained after caloric restriction combined with increased physical activity is usually moderate, in the range of 5–10% of the initial body weight, and is frequently not maintained for long periods of time (148, 149). Therefore, PCOS patients usually require treatment with insulin sensitizers or oral contraceptives to control their symptoms even after successful nonpharmacological treatment. It has been shown that hyperandrogenism, menstrual function, and insulin resistance may completely improve after bariatric surgery(150).

1.3.1 The effect of weight loss on improvement of insulin resistance in PCOS:

Since a high percentage of PCOS patients are obese, the role of weight loss in the management of this syndrome may be significant. The literature is encouraging on this point, although most studies have not included a control group(151, 152); statistical samples have also been small(153-155) and heterogeneous(151) but all studies agree that weight loss has a positive effect on hyperinsulinemia in women with PCOS. The effect did not seem to require great weight loss, but became evident with losses of 2–5%(146). In addition to a reduction in insulin resistance, weight loss also involves a parallel improvement in endocrine status of PCOS patients. Significant improvements in hirsutism and ovulation, with restoration of regular cycles and an increased incidence of spontaneous pregnancies in 30% of patients, have also been reported (151-155).

1.3.2 Does weight reduction lead to other health benefits?

Weight loss of 5% to 10% generally lessens many health risks, including cardiovascular risks, although such improvements are most notably demonstrable in studies specifically conducted in high-risk populations, and the benefits are presumed to be greater when healthier weight is maintained for long periods(156). When weight loss is achieved primarily via pharmacological interventions, these benefits have not occurred quite so consistently. Weight loss of 5–10% reduces the long-term risk of diseases associated with obesity(157). For every 1kg an obese person loses, serum concentration of LDL cholesterol falls by 0.02mmol/L, triglyceride falls by 0.015mmol/L and HDL cholesterol rises by 0.009mmol/L(158), and in those who are hypertensive, blood pressure falls by about 1–2mmHg(159).

1.3.3 Are interventions aimed at lifestyle modifications effective?

Reduced caloric intake and increased physical activity are generally accepted as the foundations of any approach directed at weight reduction, but these lifestyle interventions do not appear to provide long-lasting success for obese individuals wishing to lose weight. About half of the weight lost with the help of lifestyle interventions is regained at 1 year; after 3 to 5 years, only about 1 in 5 individuals maintains clinically meaningful weight loss, and more than half of obese patients return to their baseline weights(160). One systematic review concluded that dietary and lifestyle therapy leads to less than 5 kg of weight loss after 2 to 4 years(156).

1.4 What is the current state of antiobesity drug therapy?

1.4.1 Sibutramine

It is a mixed norepinephrine serotonin uptake inhibitor. A recent meta-analysis estimated that 1 year treatment with sibutramine yields an average placebo-subtracted weight loss of 4.5kg(161). Some evidence suggests that sibutramine helps patients maintain initial weight reductions. In the Sibutramine Trial of Obesity Reduction and Maintenance (STORM)(162), Sibutramine is associated with small increases in BP and heart rate in obese patients with and without hypertension (161). It is contraindicated for patients with uncontrolled or poorly controlled hypertension, CHD, congestive heart failure, arrhythmias, and stroke and those taking monoamine oxidase inhibitors. The product label advises caution in using sibutramine for patients receiving the selective serotonin reuptake inhibitor class of antidepressants.

1.4.2 Orlistat

Orlistat (Xenical), a lipase inhibitor that reduces fat absorption in the gut. A recent meta-analysis estimated that orlistat treatment led to an average placebo-subtracted weight loss of 2.7 kg at 1 year (163). Overall, the magnitude of weight loss achievable with orlistat appears to be less than that with sibutramine after 1 to 2 years. However, orlistat is the only antiobesity drug with a published 4-year RCT. In a Swedish study of 3305 obese, nondiabetic patients (21% had impaired glucose tolerance), orlistat treatment was associated with a 3.6-kg weight loss compared with 1.4 kg for placebo at 4 years (intention-to-treat [ITT] analysis)(164). The cumulative incidence of diabetes mellitus was 6.2% with orlistat therapy and 9.0% with placebo; a difference in diabetes incidence was detectable only in the subgroup of patients with impaired glucose tolerance at baseline.

No major safety concerns have been identified with orlistat therapy. Approximately 15% to 30% of those taking orlistat experience oily stool, faecal urgency, or oily spotting, and 7% report faecal incontinence, particularly at the initiation of treatment(163).

1.4.3 Phentermine

Phentermine remains the most prescribed antiobesity drug in the United States, where prescriptions for the drug, which was approved in 1959, outnumber combined prescriptions for sibutramine and orlistat(165). Whereas phentermine is approved for short-term use (generally taken as 12 to 16 weeks), physicians commonly prescribe it for longer periods(165). Its long-term efficacy (for at least 1 year) has never been tested in an RCT.

1.4.4 Rimonabant

The story of rimonabant begins with the understanding that endocannabinoids, cannabis-like substances in the central nervous system, play a significant role in stimulating the drive for food ingestion. The endocannabinoid system interacts with several neuropeptides that modulate hunger and satiety signals, with the net result being stimulation of appetite (166). In 1990, Matsuda et al(167) reported that a specific cannabinoid receptor, CB1, was found extensively in the brain. CB1 receptors appear to regulate the activity of mesolimbic dopamine neurons, thereby possibly modulating hedonistic or reward behaviours mediated by dopamine(168), and to interact with neuropeptides such as the melanocortins and gut peptides such as ghrelin in regulating food intake(169, 170). This knowledge sparked the development of numerous CB1 antagonists, of which rimonabant has by far had the most success in human applications. In animal studies, considerable evidence indicates that rimonabant suppresses eating(171) and reduces the preference for sweet foods(172).

1.4.4.1 Cannabinoid receptors

The endocannabinoid system consists of endogenous cannabinoids (endocannabinoids), cannabinoid receptors and the synthetic and degrading enzymes responsible for synthesis and degradation of endocannabinoids. Endocannabinoids are so named because they were first identified as activating the same receptors as cannabinoids, the primary psychoactive components of cannabis. The first endocannabinoid identified was arachidonoyl ethanolamide (anandamide; from the Sanskrit for 'internal bliss'). Anandamide is only one of a large family of related bioactive acyl ethanolamides. The second endocannabinoid identified was 2-arachidonoyl glycerol (2-AG).

The synthesis, cellular transport and degradation of endocannabinoids are tightly regulated processes (174). A feature that distinguishes endocannabinoids from many other neuromodulators is that they are not synthesised in advance and stored in vesicles. Rather, their precursors exist in cell membranes and are cleaved by specific enzymes. This form of synthesis is often referred to as 'on demand'..

The identification of cannabinoid receptors grew out of a desire to understand the psychoactive effects of -tetrahydro D9 cannabinol (THC), the principal psychoactive component of cannabis. Although several experiments hinted at the existence of specific protein receptors for D9-THC, Allyn Howlett et al.(174) provided definitive proof for a cannabinoid receptor. Their work established that cannabinoids activated a G protein-coupled receptor (GPCR) that inhibited adenylyl cyclase. Furthermore, they developed a binding assay for this receptor and showed that quite high levels of this receptor were present in certain brain regions (174).

The development of high affinity cannabinoid receptor agonists permitted the mapping of cannabinoid receptor distribution in the brain (181, 182). These initial auto-radiographic studies also established that cannabinoid binding sites are highest in the brain regions implicated in the actions of cannabis (181, 182).

The cloning of a cannabinoid receptor by Matsuda et al. (167), provided the final evidence for the existence of a cannabinoid receptor and permitted the identification of cannabinoid receptor-expressing neurones. This cloning was swiftly followed by the cloning of a second cannabinoid receptor, designated CB2, from a promyelocytic cell line (183). (Of course, the first cannabinoid receptor was then designated as CB1).

1.4.4.2 Does rimonabant produce greater weight loss than other currently available antiobesity drugs?

Treatment with rimonabant 20 mg and diet is associated with 3.9- to 5.4-kg (8.6- to 11.9-lb) greater weight loss than could be achieved with placebo and diet after 1 year; this finding is similar to the efficacy noted with sibutramine treatment in 1-year RCTs. The 7.4-kg weight loss observed with continuous 2-year treatment with rimonabant 20 mg/d in the RIO-North America study is an impressive finding because there was further weight loss and not weight regain during the second year. Although the STORM trial (162) showed significant weight reduction with sibutramine treatment for 2 years, only those patients who had achieved at least 5% weight loss in the first 6 months continued further in the study; thus, the net weight loss reported at 2-year follow-up was for the initial responders to sibutramine, and the 2-year sibutramine results could not be compared with RIO-North America study findings. In 4 separate 2-year RCTs (184-187) patients treated with orlistat regained some degree of the weight during the second year of continuous treatment. At this time, RIO-North America is the only 2-year RCT with rimonabant; studies of longer duration (5 years) will provide more valuable information about efficacy, safety, and cost-benefit analysis of rimonabant therapy.

1.4.4.3 What about reduction in cardiovascular and metabolic risk factors with rimonabant?

In the 4 RIO trials, rimonabant treatment led to a negligible reduction in systolic BP that ranged from 0.2 to 2.3 mm Hg relative to placebo and no reduction in diastolic BP. A consistent reduction in TGs has been observed with rimonabant in all RIO trials, with

placebo-subtracted decreases ranging from 12% to 16%. Furthermore, HDL-C showed consistent improvement with rimonabant treatment in all RIO trials, with placebo-subtracted increases ranging from 7% to 9%. However, weight loss achieved with rimonabant has not led to reductions in total cholesterol or LDL-C. It is notable that sibutramine-promoted weight loss also led to improvements in TGs and HDL-C but not in total cholesterol or LDL-C in most studies. In contrast, long-term treatment with orlistat is often associated with greater improvements in total cholesterol and LDL-C relative to placebo and less so to improvements in TG and HDL-C(163, 188).

In the RIO-Diabetes trial that enrolled overweight or obese patients with T2DM who were given metformin or a sulfonylurea, treatment with rimonabant for 1 year reduced HbA1C by 0.7% relative to placebo. Changes in glycemic indices were less remarkable with rimonabant treatment in the other 3 RIO studies. A recent meta-analysis has estimated that sibutramine treatment was associated with an average 0.7% absolute reduction in HbA1C among overweight adults with T2DM in studies of 12 to 26 weeks' duration (189).

In summary, the most notable changes in lipids with rimonabant treatment in RIO trials were a 12% to 16% reduction in TGs and a 7% to 9% increase in HDL-C. Rimonabant has demonstrated somewhat meaningful improvements in TG and HDL-C, but other highly effective interventions are available for this purpose(190). Nicotinic acid and fibric acids reduce TGs by 20% to 50% and raise HDL-C by 10% to 35%. Thus, the value of rimonabant for obese patients with dyslipidemia and/or T2DM as monotherapy remains open to question.

The strengths of rimonabant are as follows:

(1) In 4 well-designed studies with >6600 overweight and obese patients, rimonabant has demonstrated consistent efficacy with regard to weight reduction.

(2) Rimonabant offers a novel mechanism of action, which may make it well suited as an alternative for people who do not respond well to other agents and for combination treatment with other antiobesity agents.

(3) Weight loss achieved with rimonabant also appears to improve some features of metabolic syndrome.

The limitations of rimonabant are the following:

(1) Rimonabant increases the risk of psychiatric adverse events – ie depressed mood disorders and anxiety (191). In view of this the European Medicines Age ncy (EMEA), the European Union (EU) body which is responsible for monitoring the safety of medicines, has recommended the suspension of the marketing authorisation of rimonabant. The EMEA has concluded that the benefits of rimonabant no longer outweigh its risks and the market authorisation was suspended across the EU from July 2008.

(2) Weight-reduction efficacy is not superior to the modest effects observed with currently approved antiobesity drugs.

(3) Although some features of metabolic syndrome have been shown to improve modestly, no reduction in LDL-C occurs, although this appears to be the case with all centrally acting antiobesity drugs.

(4) Whereas rimonabant has been shown to be superior to placebo in helping smokers quit in short duration trials, its overall efficacy is not particularly impressive, and it has been judged to be not approvable for this indication at this time.

1.5 Does rimonabant modulate incretin hormones to produce weight loss?

1.5.1 The Incretin Effect

The role of the gastrointestinal tract in influencing insulin secretion and glucose homeostasis has been recognized since the beginning of the 20th century (192). Zunz and La Barre first proposed the term "incretin" in reference to an insulin-stimulatory hypoglycemic factor found in the extract of duodenum. Incretin hormones have since been defined as hormones produced by the gastrointestinal tract in response to nutrient entry, which then stimulate insulin secretion. The enteroinsular axis refers to the regulation of pancreatic islet hormone secretion by such incretin hormone signals from the gastrointestinal tract (193).

The concept of the incretin effect developed from the observation by Elrick and colleagues and McIntyre and colleagues that insulin responses to oral glucose exceeded those measured after intravenous administration of equivalent amounts of glucose (194, 195). They concluded that gut-derived factors, or incretins, influenced postprandial insulin release. Nutrient entry into the stomach and proximal gastrointestinal tract causes release of incretin hormones, which then stimulate insulin secretion (196). This insulinotropism, or ability to stimulate insulin secretion, can be quantified by comparing insulin or C-peptide responses to oral vs. intravenous glucose loads. In this way, it has been shown that the incretin effect is responsible for about 50% to 70% of the insulin response to oral glucose in healthy individuals (197, 198). Although many postprandial hormones have incretin-like activity, the 2 predominant incretin hormones are glucose-dependent insulinotropic polypeptide, also known as gastric inhibitory polypeptide (GIP), and glucagon-like peptide-1 (GLP-1).

1.5.2 The Incretin Hormones: GIP and GLP-1

GIP and GLP-1 both belong to the glucagon peptide superfamily and thus share amino acid sequence homology. GIP and GLP-1 are secreted by specialized cells in the gastrointestinal tract and have receptors located on islet cells as well as other tissues. As incretins, both are secreted from the intestine in response to ingestion of nutrients, which results in enhanced insulin secretion. The insulinotropic effect of GIP and GLP-1 is dependent on elevations in ambient glucose. Both are rapidly inactivated by the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV). The characteristics of GIP and GLP-1 are summarized in the Table 3.

Table 3. Characteristics of GIP and GLP-1

	GIP	GLP-1
Peptide	42 amino acid	30/31 amino acid
Secreted by	K cells, primarily in duodenum and proximal jejunum	L cells, primarily in ileum and colon
Stimulated by	Oral ingestion of nutrients	Oral ingestion of nutrients
Metabolized by	DPP-IV	DPP-IV
Effects on insulin secretion	Stimulates	Stimulates
Effects on gastric emptying	Accelerates?	Slows
Effects on beta-cell proliferation	Stimulates*	Stimulates*
Effects on glucagon secretion	None significant	Suppresses
Effects on food intake	None significant	Reduces
Effects on insulin sensitivity	?	Improves?
Secretion in type 2 diabetes	Preserved	Impaired
Insulinotropic response to exogenous administration in type 2 diabetes	Impaired	Preserved

*In cell-line studies

1.5.3 GIP

Discovered in 1971, GIP is a single 42-amino acid peptide synthesized in and secreted by specialized enteroendocrine K-cells. These cells are concentrated primarily in the duodenum and proximal jejunum, although they also can be found throughout the intestine (199). The main stimulant for GIP secretion is ingestion of carbohydrate- and lipid-rich meals (200). Following ingestion, circulating plasma GIP levels increase 10- to 20-fold. The half-life of intact GIP is estimated to be approximately 7.3 minutes in healthy subjects and 5.2 minutes in diabetic subjects (201).

GIP secretion reaches peak concentrations 15-30 minutes following ingestion of oral glucose or lipids, even before absorption of nutrients into the gut (202, 203). This suggests a potential role for other influences in GIP secretion. GIP secretion is also closely correlated with GLP-1 secretion, suggesting a paracrine relation between the 2 hormones (204, 205).

Following secretion into the circulation, intact GIP (1-42 amide) is cleaved at the NH2-terminus by DPP-IV, resulting in the formation of the inactive truncated GIP (3-42 amide), which lacks incretin activity and may even act as an antagonist of GIP at its receptor (199).

The GIP receptor also has been cloned and is related to the receptors for other members of the glucagon peptide super family. The GIP receptor is expressed in the pancreatic islets, as well as the gut, adipose tissue, heart, pituitary, adrenal cortex, and the brain (206). GIP was initially thought to act predominantly on the stomach as an inhibitor of gastrointestinal motor activity and acid secretion, hence the name gastric inhibitory

polypeptide. This was based on observations in the canine stomach (207). However, further studies in humans have been unable to demonstrate any significant role of physiologic GIP on gastric acid secretion (208).

The incretin effect of GIP was first appreciated in the 1970s. The physiologic effects of GIP have been elucidated using GIP receptor antagonists, GIP peptide antagonists, and GIP receptor knockout mice, in addition to GIP infusion protocols. Blocking GIP binding to its receptor results in attenuated glucose-dependent insulin secretion following oral glucose load in rats and mice (211). Similarly, administration of GIP antagonists or GIP antisera markedly reduces the postprandial insulin release in rats (212). GIP receptor knockout mice demonstrate normal fasting glucose levels but mild glucose intolerance following oral glucose loads (213, 214). Interestingly, they also exhibit resistance to diet-induced obesity following months of high-fat feeding. Additionally, in the leptin-deficient ob/ob mouse, the GIP receptor knockout genotype appears to decrease the extent of obesity that develops (214).

GIP infusion has consistently demonstrated stimulation of insulin secretion in isolated rat islets, isolated perfused rat pancreas, dogs, and humans (207, 215-217). During stepwise euglycemic, mild hyperglycemic (54 mg/dL above basal), and moderate hyperglycemic (143 mg/dL above basal) clamps, Elahi and colleagues (217) have demonstrated that GIP infusion results in insulin secretion only in the presence of elevated glucose concentrations. Furthermore, they demonstrated that GIP is not glucagonotropic in normal humans during either euglycemic or hyperglycemic conditions. Thus, the effect of endogenously released GIP appears to be an important

mechanism of postprandial insulin secretion and does not appear to play a role in the fasting state.

GIP has many non-incretin effects. Unlike other insulin secretagogues, GIP stimulates beta-cell proliferation and cell survival in INS-1 islet cell-line studies (218, 219). Furthermore, animal studies have suggested a role for GIP in lipid metabolism by stimulating lipoprotein lipase activity, inducing fatty acid incorporation into adipose tissue and stimulating fatty acid synthesis (220-222). However, in humans, there is no clear evidence for an effect of GIP on lipid metabolism. GIP also appears to stimulate glucagon secretion from the isolated perfused rat pancreas, although human studies have not demonstrated any significant influence on glucagon secretion (217). Furthermore, unlike GLP-1, GIP appears to act by accelerating emptying of the stomach rather than by inhibiting gastrointestinal motility (199).

1.5.4 GLP-1

GLP-1, a product of the glucagon gene, was first identified in the early 1980s. GLP-1 is a 30/31 amino acid peptide synthesized and secreted by enteroendocrine L-cells located predominantly in the ileum and colon, although also by L-cells in the duodenum and jejunum. Other incretin products of the glucagon gene include glicentin, which is biologically inactive, and oxyntomodulin, which has some insulinotropic properties (223). Like GIP, the GLP-1 receptor is widely expressed in pancreatic islets, the brain, heart, kidney, and the gastrointestinal tract. There are 2 major forms of biologically active GLP-1 secreted following meal ingestion: GLP-1(7-37) and GLP-1 (7-36) amide, which differ by a single amino acid. The majority of the circulating active GLP-1 appears to be GLP-1 (7-36) amide, although both are equipotent and have similar

biological activities (224). GLP-1 secretion from the distal gut is triggered by neural and endocrine signals initiated by nutrient entry into the lumen of the proximal GI tract. Circulating levels of GLP-1 increase rapidly within minutes of food ingestion and are highly correlated with the release of insulin (225, 226). Like GIP, GLP-1 enhances insulin secretion only in the presence of elevated glucose concentrations. DPP-IV rapidly cleaves GLP-1 to its truncated inactive metabolite. Infused GLP-1 has a shorter half-life than GIP, approximating 2 minutes in both nondiabetic and diabetic human subjects (227).

GLP-1 exerts many biological effects, and most of the GLP-1 actions studied in animal studies also have been demonstrated in human studies. GLP-1 is responsible for a significant part of the insulin response to oral glucose, and both animal and human studies with GLP-1 receptor antagonists suggest that GLP-1 may be essential for normal glucose tolerance. GLP-1 not only enhances insulin secretion but also suppresses the secretion of glucagon in a glucose-dependent fashion (231). There is increasing evidence that, like GIP, GLP-1 increases beta-cell proliferation and promotes beta-cell survival. GLP-1 has also been shown to slow gastric emptying in animal and human studies, resulting in slowed nutrient entry to the intestine and decreased postprandial glucose concentrations.

There is also a significant interest in the role of GLP-1 in the regulation of food intake and weight loss. In rodents, acute intracerebroventricular injection of GLP-1 or GLP-1 receptor agonists results in reduction of food intake. Furthermore, central administration of the GLP-1 receptor antagonist exendin 9-39 results in increased food intake in rats (235).

1.6 Is there any potential role of statins in the treatment of PCOS?

Mevastatin inhibits ovarian theca-interstitial cell proliferation and steroidogenesis (236). The ovaries of women with PCOS are typically enlarged with prominent hyperplasia of ovarian theca-interstitial cells and excessive production of androgens by these cells(237-239).

Statins have been shown to inhibit growth of vascular smooth muscle(240-242), cardiomyocytes(243), and mesangial cells(244). It is likely that statin induced inhibition of growth is related to blockage of HMG-Co (A). Products of HMG-Co (A) include mevalonate and several downstream isoprenoids including geranyl pyrophosphate and farnesyl pyrophosphate. These isoprenoids play an important role in the post-translational modifications (e.g., granulation and farnesylation) of a variety of small GTPase proteins, such as Ras and Rho(244, 245).

Both Ras and Rho are involved in the regulation of various cellular processes such as proliferation, apoptosis, and differentiation. An inhibition of HMG-Co (A) and a consequent decrease of the genanylation or farnesylation of Ras and Rho may inactivate important signal transduction pathways regulating mitotic activity. Studies have indicated that statin-induced inhibition of proliferation of mesanglial cells was associated with a repression of activation of Rho GTPase/p21 signaling; this effect was independent of its cholesterol-lowering actions(244). Comparable mechanisms may be involved in mevastatin-induced inhibition of theca-interstitial proliferation. The effects of mevastatin on steroidogenesis are most likely related to the inhibition of cholesterol

synthesis and a consequent decrease in the availability of the precursors of progesterone and testosterone.

Studies in men produced contradictory findings. Some investigators reported that the use of statins was associated with a decrease of testosterone levels(246, 247); however, other researchers found no significant change in testosterone level after even prolonged use of several statins(248-250). In postmenopausal women, use of statins led to a borderline decrease of progesterone(251). The same study also evaluated the effects of statin in a small population of premenopausal women and detected no significant changes in progesterone or estrogen levels; testosterone levels were not documented. In another small study of seven postmenopausal women, a low dose of simvastatin had no effect on plasma levels of progesterone or testosterone(252)

1.6.1 What are the pleotrophic effects of statins?

Pleiotropic effects of a drug are actions other than those for which the agent was specifically developed. These effects may be related or unrelated to the primary mechanism of action of the drug, and they are usually unanticipated. Pleiotropic effects may be undesirable (such as side effects or toxicity), neutral, or, as is especially the case with HMG-CoA reductase inhibitors (statins), beneficial. Pleiotropic effects of statins include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques. These and several other emergent properties could act in concert with the potent low-density lipoprotein cholesterol-lowering effects of statins to exert early as well as lasting cardiovascular protective effects. Understanding the pleiotropic

effects of statins is important to optimize their use in treatment and prevention of cardiovascular disease.

What potential mechanisms might explain the early effects of statin therapy? Statins inhibit HMG-CoA reductase, which is responsible for the reduction in circulating LDL cholesterol beginning one to two weeks after therapy initiation. Statins also inhibit HMG-CoA reductase within endothelial cells, vascular smooth muscle cells, and inflammatory cells (the monocyte/macrophage system), which affects important signaling pathways. In cell culture, animal, and clinical studies, these effects appear within hours after statin administration and may be dose dependent (253, 254).

As HMG-CoA reductase inhibitors (statins) became more widely used in greater numbers of patients, their effects beyond lipid lowering began to emerge. Such pleiotropic effects include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant effects, anti-inflammatory properties, and stabilization of atherosclerotic plaques. Additional effects of growing interest include the ability to recruit endothelial progenitor cells (EPCs), a putative immunosuppressive activity, and inhibition of cardiac hypertrophy. Research indicates that some of the pleiotropic effects of statins may be unrelated to the cholesterol-lowering properties of the drugs. Others may even be fully dissociated from inhibition of HMG-CoA reductase, and many take place at very low drug concentrations.

The pleotrophic effect of statin includes improvement of endothelial dysfunction, normalized vasomotion, increased bioavailability of nitric oxide, antioxidant effects, anti-inflammatory effects, reduction of serum CRP, reduction of adhesion molecules,

plaque stabilization, stimulation of endothelial progenitor cell recruitment, immunomodulation and inhibition of myocardial hypertrophy.

Although the possibility that statins might have pleiotropic effects was met at first with a healthy skepticism, the vast amount of knowledge accrued over the past few years has moved these effects into the spotlight. Many of the statin pleiotropic effects operate independently of LDL-cholesterol reduction, correlate poorly or not at all with LDL-cholesterol changes, take place rapidly, and are rapidly reversible on discontinuation of the drug. Direct effects in the absence of LDL or total cholesterol modification have been shown both in vitro and in vivo.

1.7 What is the clinical relevance of biological variation?

Most bio-analytes measured in clinical chemistry laboratories change with time. The knowledge of the temporal changes following acute illnesses, for example, in serum troponin concentration after myocardial infarction is necessary for the selection of the most appropriate time to draw samples to aid in the diagnostic process and the correct interpretation of the results.

Changes that occur in health are also important. Many analytes can vary over an individual's lifetime, simply because of natural biological factors involved in the aging process with particularly important periods being neonatal period, childhood, puberty and menopause. In addition, certain analytes have predictable biological rhythms which may be daily (circadian), monthly or seasonal. The knowledge of these rhythms is clearly crucial as the samples must be collected at the appropriate times and the results interpreted with this information.

Most analytes, however, do not have cyclical rhythms that are clearly defined but have variation that can be described as random fluctuation around a homeostatic setting point. For example if a series of samples from one individual for a particular laboratory test is taken the results are often not exactly the same number. This biological variation may have contributors, e.g., pre-analytical influences (variation in sample collection, such as patient posture, exercise and the application of a tourniquet), analytical variation (such as assay imprecision and calibration errors) and inherent biological variation around the homeostatic setting point which is also called the within-subject (or intraindividual) biological variation.

Even amongst healthy individuals if the same test were performed repeatedly on various individuals the mean of each person's results would not exactly match as individuals homeostatic setting points usually vary. This difference between individuals is called between-subject (or inter-individual) variation.

Data on biological variation of physiological response variables are useful for many purposes in clinical chemistry such as to set quality specifications derive reference change values and assess the utility of conventional reference values. An important example of quality specification is the American Diabetes Association documented quality specification for glycated haemoglobin analysis which ensures uniformity of measurement across many countries.

Clinical practice often leads to a situation where serial measurements are made in an individual to monitor health or chronic disease states such as diabetes. The significance

of change in serial measures of an analyte is fundamental to correctly interpreting these serial results. The reference change value or critical difference between two consecutive samples in an individual subject (i.e, the smallest percentage change unlikely to be due to biological variability) for each analyte is needed for this analysis and is dependent on the biological variation data (both in health and in disease) of the analyte being tested (270).

Data on biological variation is also important in testing the utility of conventional reference ranges and n the objective assessment of a tests suitability to be used for the purposes of screening. To be suitable for use as a screening test the relative intra- to inter individual variation of the analyte in the population being tested should be similar (i.e. the within subject variation for the test should be similar to the variation of the population as a whole) and this is mathematically represented by the index of individuality (IoI).(271) The IoI is derived from the ratio of intra- and inter individual variation. When the IoI for a particular test is ≤ 0.6, conventional population based reference intervals are of limited value in the detection of unusual results for a particular individual. When the IoI is ≥ 1.4, the variation of an individual will fit populations' reference limits more closely so being suitable as a screening test. Serum creatinine, for example, has an IoI of only 0.27 which contrasts with a newer marker of renal function, cystatin C, which has got an IoI of 1.64(272). This means the latter test is likely to be the more useful to screen subjects for reduced glomerular filtration rate (GFR) using a population-based (rather than a subjects own) reference interval.

1.7.1 Is there any biological variation of lipids?

In order to assess an individual patient, one should sample the lipids from at least 2 different collections and average the results. Another situation involves the evaluation of change in the patient's lipid values following intervention, whether the intervention is diet, lifestyle changes, or drug therapy. A difference between the baseline and new values should fall greater than 2.77 times the standard deviation of the biological variation, expressed in units of the test measurement, to be significant at a 95% confidence interval. For example, if the baseline value for LDL-C was 167 mg/dL and one assumed a biological variation of 6%, and then the biological variation would be 10.0 mg/dL. This biological variation multiplied times 2.77 yields 27.7 mg/dL. A new value of 150 mg/dL provides a difference of 17 mg/dL, which is less than 27.7 mg/dL, and thus, the difference between the values is not significant. The new value would have to decline to a value of 139 mg/dL or less in order to represent a significant decrease. In this example, one has assumed that the biological variation was 6% for LDL-C. Ideally, one would use a value derived from the patient, because biological variation varies somewhat from person to person.

One can compare the difference between two sequential values with the biological variation. Biological variation is a measure of the random disturbances of an analyte's value, measured at different times. When the difference > Z square root 2 SD (BV) then the difference is due to an underlying disease process or physiologic change. A Z value of 1.96 yields a 95% confidence limit. When using multiple sequential values or time periods exceeding that for the empirically derived biological variance, a random walk model allows one to estimate the spread of the variance. For a difference, (delta) to be

significant, delta > Z square root 2n SD(BV), where n is the ratio of time reflecting the longer time period divided by the shorter time period. Not all variances grow to this degree over time, because restoring forces diminish the extent of random disturbances. The relationship between a biological variance measured over a longer time period to the one measured over a shorter period can be expressed in terms of this restoring force as SD2 BV,n = SD2 BV,1 sigma(n) j=1 e(-2)(j-1)phi, where n is the ratio of time periods. One can calculate phi using this formula and a spreadsheet. From phi one can calculate the biological variance for any time period, within experimental limits, and compare the difference in sequential values with it. Test intervals can be calculated based on these biological variances.

Recently reported estimates of within individual biological variation (CVbiological) for key lipids and lipoproteins are listed in table 4 together with the NCEP recommended goals for precision for these analytes. From these figures the contribution of analytical imprecision to the total result variability has been calculated from the formula given by Fraser and Harris(270) and is shown to range from < 3% for triglyceride, through < 12% for total cholesterol and LDL-c, to < 15% for HDL. This is in line with the recommendation of Harris that it is desirable that CVanalytical should be less than 50% of CVbiological on the empirical basis that total result variability due to the analytical component is less than 11.8% in such circumstances(270). The clinical utility of an investigation is then largely determined by the individual biological variation of the parameter.

On the basis of these figures for biological variation and acceptable imprecision, the Smallest Significant Difference (SSD%) between consecutive measurements has been calculated, together with the number of serial results which must be averaged to estimate the Homeostatic Set Point (HSP) of an individual to a given degree of accuracy(270).(table 4). These statistics are an important consideration in use of lipid and lipoprotein measurements to assess risk, response to therapy or attainment of a therapeutic goal: The SSD between consecutive measurements ($p<0.05$) is approximately 15% for total cholesterol, 20% for HDL-c and LDL-c and 50% for triglycerides.

A single measurement will be within 15% of the HSP for total cholesterol, within 20% for HDL-c and LDL-c. To achieve a more accurate estimate within 10%, however, requires the mean of two, three and four measurements respectively for these analytes. For triglyceride, which has a much greater biological variation, the mean of two results will estimate the HSP to within 30% and it requires five results to be within 20%.

Table 4. NCEP Analytical Goals for Lipid and Lipoprotein Measurements

		Consistent with	
	Total error (%)	Bias (%)	CVa (%)
Cholesterol	9	3	3
Triglyceride	15	5	5
HDL-c	13	5	4*
LDL-c	12	4	4
Total error = % Bias + 1.96 (CVanalytical)			

* When HDL-c >/= 1.04 mmol/L, 40 mg/dL

Key: LDL-c = low density lipoprotein-cholesterol; HDL-c = high density lipoprotein-cholesterol

Table 5. Influence of Analytical and Biological Variation on the Clinical Utility of Lipid Measurements

	Total-c	HDL-c	LDL-c	Triglyceride
CVanalytical (%)	3.0	4.0	4.0	5.0
CVbiological (%)	6.0	7.1	8.3	21.0
Contribution (%) of analytical imprecision to total variability of serial results	11.8	14.8	11.0	2.8
SSD (%) between consecutive test results	15.7	21.5	19.0	50.4
Number of results to estimate				
HSP within ±10%	2	3	4	18
HSP within ±15%	1	2	2	8
HSP within ±20%		1	1	5
HSP within ±30%				2

Key: SSD = smallest significant difference ($p<0.05$), unidirectional; HSP = homeostatic set point ($p<0.05$); Total-c = total cholesterol; HDL-c = high density lipoprotein-cholesterol; LDL-c = low density lipoprotein-cholesterol

Table 6

Important Considerations for the Measurement of Blood Lipids

- The accuracy of lipid measurements must be traceable to the CDC reference methodology accuracy base
- Inclusion of high density lipoprotein-cholesterol (HDL-c) improves specificity and sensitivity in risk calculations
- Direct use of computer programs allows greater accuracy than point score tables and charts derived from the same statistical functions
- To accurately assess total-cholesterol, high density lipoprotein-cholesterol (HDL-c) and LDL-c the mean of three measurements is required
- Several measurements are required for triglyceride
- Non-fasting total and HDL-c measurements are adequate for initial risk assessment
- A follow-up fasting profile including triglyceride is necessary if an elevated total or low HDL-c is identified
- When dyslipidaemia is identified consideration should be given to genetic and secondary causes with family screening and referral to specialists when appropriate

1.7.2 How can we minimise the sources of pre-analytical variation?

Careful attention to patient preparation and blood collection technique can do much to facilitate accurate assessment of patients by controlling factors which induce variability in lipid and lipoprotein concentrations. These include:

- Prandial status:

  A 12-hour fast essential for triglyceride measurement and calculated LDL, but not essential for total- or HDL-c measurements

- Lifestyle:

  The patient should be metabolically stable and advised to maintain their normal diet, alcohol intake, smoking and exercise habits prior to testing

- Pregnancy or illness:

  Investigation should be deferred for two weeks after a minor illness, 2-3 months after pregnancy, major illness or myocardial infarction. A sample taken within 24 hours of the onset of myocardial infarction, however may match the pre-infarction state

- Phlebotomy:

  Increases in lipid concentrations of up to 15%, resulting from diffusion of water from blood vessels into tissues, can occur within 15 minutes of changing from a supine to a standing position or if a tourniquet is applied for more than five minutes. Blood samples should therefore be drawn after the patient has been

seated for at least five, preferably 15 minutes. If necessary, a tourniquet should not be applied for more than one minute

- Multiple Measurements:

  As discussed earlier, medical decisions should be based on the required number of measurements performed within two months and at least one week apart. To avoid inter-laboratory differences in bias, these measurements should be performed by the same laboratory.

1.7.3 Is measured LDL more reliable than calculated LDL in patients with type 2 diabetes?

Hypercholesterolemia is one of the most common risk factors for cardiovascular disease. The guidelines stress the importance of utilizing both diet and drug therapy, if necessary, to achieve LDL-C target concentrations. Therefore, accurate and precise estimations of patients' LDL-C concentrations are necessary to appropriately identify individuals with hypercholesterolemia and to monitor response to diet and drug treatment.

For clinical purposes, LDL-C is generally determined from the Friedewald equation (277), which assumes that the amount of cholesterol in very-low density lipoproteins (VLDL) can be estimated by dividing the blood triglyceride concentration by a factor of five. The Friedewald equation correlates well with LDL-C concentrations determined by ultracentrifugation if blood triglyceride concentrations are <4.52 mmol/L. However, the

reliability of the LDL-C calculation depends upon the accuracy and precision of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride measurements. Poor analytic precision in any or all of these measurements will contribute to the variability observed in LDL-C concentrations. The biologic variability inherent in each of the three lipid measurements will also contribute to the total variability of the LDL-C concentration when the Friedewald equation is used. In particular, the biologic CV of blood triglyceride concentrations may be ≥20% and may interfere with the reliability of the LDL-C calculation. Biologic variation is also a major component of the variability in HDL-C measurements, and is frequently in the range of 7-8%. Although analytic variability can often be reduced with methodological advances in the laboratory, sources of error introduced by excessive biologic variability are not easily overcome.

The total variability for LDL-C measurements calculated by the Friedewald equation has been reported to be as high as 9.6% (278, 279), which implies that individual LDL-C readings could vary by up to 40% (± 2 SD) from one measurement to the next by chance alone. The usual approach to reduce this variability is to calculate the mean of several serial specimens (273, 280). For example, to decrease the LDL-C CV to 5%, a level adequate to detect most LDL-C responses to diet and drug therapy, at least two, and as many as five, serial blood specimens may have to be analyzed and averaged (281).

To address these limitations of the Friedewald equation, a direct LDL-C assay has been developed. By using solid-phase immunocapture, HDL-C and VLDL-C are removed by centrifugation. The LDL-C remaining in the filtrate is quantified by an enzymatic cholesterol assay. The accuracy of this method compared with both standard ultracentrifugal techniques and the Friedewald calculation has been favourable, with an analytic imprecision of <5% (282). Because this assay measures LDL-C independently of other lipid fractions, it may potentially reduce the variability introduced into the Friedewald equation from the cumulative analytic and biologic variability of triglyceride, HDL-C, and total cholesterol measurements. For this reason, a direct assay of LDL-C may be a more useful test even when triglyceride concentrations are not markedly increased. For example, decreasing the variability of LDL-C measurements may reduce the number of serial specimens necessary to accurately reflect LDL-C concentrations in a patient.

Because the calculated LDL-C is derived from total cholesterol, triglycerides, and HDL-C measurements, one may expect that the considerable variability of these three measurements would contribute directly to the observed variability in the calculated LDL-C value. By determining LDL-C directly, the dependence upon three separate and relatively variable lipid measurements, total cholesterol, HDL-C, and triglycerides, is eliminated. The analytic precision of the direct LDL-C assay was excellent, meeting the precision criteria set for total cholesterol of ≤3% and performing better than reported for other direct LDL-C assays involving chemical precipitation methods. The analytic variability for total cholesterol, HDL-C, triglycerides, and the calculated LDL-C measurements were also <3%, comparing favourably with most previously published

reports(279). On the other hand, the high biologic variability present in LDL-C measurements increased the total variability of both the calculated and direct LDL-C estimations. The total variability (CV) for either measurement measurements was greater than the total variability present for the direct LDL-C assay. However, despite the larger total variability present in HDL-C and triglyceride measurements, only the variability derived from total cholesterol determination was significantly associated with the variability of the calculated LDL-C. This suggests that an accurate and reliable total cholesterol measurement is of primary importance to ensure the accuracy and precision of LDL-C estimations with the Friedewald formula. Recommendations to improve both accuracy and precision of the total cholesterol concentration to CVs of <3% have been formulated, and will help to ensure the reliability of the calculated LDL-C determination.

An examination of the Friedewald equation suggests why neither triglyceride nor HDL-C variability is an important determinant of LDL-C variability. By dividing blood triglyceride concentrations by 5 to estimate \TLDLC, the equation limits the impact of triglyceride variability on the LDL-C measurement. Further, both HDL-C and VLDL-C concentrations are usually less than half of LDL-C concentrations, also diminishing their impact on calculated LDL-C variability. Therefore, increasing triglyceride concentrations are likely to add progressively more variability to the calculated LDL-C measurement.

When should the direct LDL-C measurement be ordered? In patients with triglycerides >4.52 mmol/L, VLDL-C cannot be estimated accurately, and the calculated LDL-C becomes less accurate and precise(283). The calculated LDL-C may also be less reliable in patients with T2DM(284) and liver disease (285), possibly because of the propensity towards increased blood triglycerides in these illnesses. On the other hand, the direct LDL-C assay accurately measures LDL-C with triglyceride concentrations to 9.03 mmol/L or higher. Patients requiring lipid determinations while not fasting may have increased triglyceride concentrations and may also benefit from a direct LDL-C measurement.

Although the direct LDL-C assay is less expensive than the calculated LDL-C value, it does not provide the additional information of triglyceride and HDL-C measurements. When this additional information is important, and the triglyceride concentrations are <4.52 mmol/L, a routine lipid panel and calculated LDL-C are probably sufficient. However, if the triglycerides may potentially increase above this concentration, or if only the LDL-C concentration is required, then the direct LDL-C assay should be the test of choice.

According to current recommendations for lipid monitoring in the hypercholesterolemic patient, triglyceride and HDL-C determinations, in addition to LDL-C, should be obtained yearly, whereas total cholesterol can be used to assess therapeutic effectiveness at interim visits. The direct LDL-C assay is less expensive than the standard lipid panel, yet provides a more accurate assessment of LDL-C than does the total cholesterol alone.

Therefore, the direct LDL assay may have a role in routine monitoring of hypercholesterolemia therapy when triglyceride and HDL-C values are not required.

The direct LDL-C assay does not reduce the variation in LDL-C compared with the conventional LDL-C calculation. Therefore, serial specimens are still necessary to accurately assess LDL-C values and gauge response to therapy. However, because the direct assay is accurate even when triglycerides are increased, and because it allows a less expensive assessment of LDL-C (as an isolated test) than does the standard lipid panel, it appears to have a potentially useful role in lipid disorder management.

Chapter 2

Methods

2.1 Laboratory Methods and Reagents

2.1.1 Intervention Studies

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Fasting venous blood was collected into serum gel and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 g for 15 min at 4oC, and the aliquots stored at –20oC. Serum testosterone was measured on an Architect analyzer (Abbott Laboratories, Maidenhead, UK), and SHBG was measured by immunometric assay with fluorescence detection on the DPC Immulite 2000 analyzer using the manufacturer's recommended protocol. The free androgen index was obtained as the total testosterone x100/SHBG. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter, High Wycombe, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2μU/ml, the coefficient of variation was 6%, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyzer (Beckman-Coulter, High Wycombe, UK), using the manufacturer's recommended protocol. The coefficient of variation for the assay was 1.2% at a mean glucose value of 94.6 mg/dl (5.3mmol/liter) during the study period. The insulin resistance was calculated using the homeostasis model assessment (HOMA) method (HOMA-IR= (insulin x glucose)/22.5).

2.1.2Biological Variability Studies

The biological variation of TC, HDL-C, LDL-C and triglycerides was assessed by measuring 12 hour fasting blood samples at four-day intervals on 10 consecutive occasions. Fasting venous blood was collected into serum gel tubes (Becton Dickinson, Oxford, U.K.) at the same time each day (0800–0900) after the patient had been seated for at least 5 minutes and tourniquet was not applied for more than a minute. Samples were separated by centrifugation at 2000g for 15 min at 4°C, and two aliquots of the serum were stored at -20°C within 1 h of collection. The serum samples were split before assay. Before analysis, all of the serum samples were thawed and thoroughly mixed. The duplicate samples (i.e., two per visit) were randomised and then analysed for cholesterol, triglycerides and HDL cholesterol in a continuous batch on a Synchron LX 20 analyser (Beckman-Coulter, High Wycombe, U.K.) using a single batch of reagents. LDL cholesterol was calculated using the Friedewald formula. The analytical sensitivity of the insulin assay was 2μU/ml and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyser (Beckman_Coulter, High Wycombe, UK), using the manufacturer's recommended protocol. The coefficient of variation for this assay was 1.2% at a mean glucose value of 5.3 mmol/L.

2.2 Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 15.0 (SPSS, Chicago, IL) and Microsoft Excel. Results were considered significant if the two-tailed p value was less than 0.05. Details of sample size calculations for the biological

variability studies are given below. The sample size calculation for the intervention studies are described in the relevant chapters.

2.2.1 Interventional studies

Comparisons between the two treatment groups, with respect to percentage changes from baseline were carried out using the paired t test for biochemical data and clinical observations. The Wilcoxon signed rank test was applied to biochemical data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov test.

2.2.2 Biological variability studies

Biological variability data was analyzed by calculating the analytical and within-subject variability according to the methods of Fraser and Harris(270). By this technique, analytical variance (SDA2) was calculated from the difference between duplicate results for each specimen (SDA2 = $\Sigma d2/2N$, where d is the difference between duplicates, and N is the number of paired results). The variance of the first set of duplicate results for each subject on the 10 assessment days was used to calculate the average biological intraindividual variance (SDI2) by subtraction of the mean SDA2 from the observed dispersion (equal to SDI2 + SDA2). The standard deviation of intraindividual variations (SDI) was estimated as square roots of the respective variance component estimates. An individual's coefficient of variation (CV) for each lipid parameter was calculated as the SDI/mean value x 100% and then expressed as a mean value for each treatment.

2.3 Ethics

All subjects gave their informed written consent prior to entering the studies that had been approved by the Hull and East Riding Local Research Ethics Committee and South Humber Local Research Ethics Committee.

Chapter 3

A Comparison between Rimonabant and Metformin in Reducing Biochemical Hyperandrogenaemia and Insulin Resistance in Patients with Polycystic Ovary Syndrome: A Randomised Open Labelled Parallel Study.

3.1 Introduction: Polycystic ovary syndrome (PCOS) is a common disorder of women of reproductive age group affecting more than 10% of Caucasian women and is characterised by insulin resistance, chronic anovulation and androgen excess.(107) Obesity is present in varying degrees in women with PCOS and is associated with hyperandrogenaemia and insulin resistance(286). Even modest weight loss of less than 10% of initial body weight has been shown to increase the frequency of ovulation, improve conception, and reduce testosterone, free androgen index, hyperlipidaemia, hyperglycaemia and insulin resistance in women with PCOS.(146, 147)
Metformin is commonly used in patients with PCOS and is reported to improve body weight, insulin resistance, sex hormone binding globulin (SHBG) and hyperandrogenaemia.(133, 287) Although metformin's actions appear to be mediated by activation of AMP-activated protein kinase, its precise molecular mechanism of action remains unclear (288). However, it has been shown to be of limited use in very obese women with polycystic ovary syndrome in some studies.(140) Rimonabant, a cannabinoid receptor 1 blocker, (289, 290) has shown an improvement in metabolic syndrome, waist circumference, lipid parameters and particularly insulin resistance in obese subjects.(291-293) The aim of this study was to see if the reduction of biochemical hyperandrogenaemia and insulin resistance by rimonabant through weight loss was superior to insulin sensitisation with metformin in obese women with PCOS.

3.2 Research Design and Methods

This was a randomised open labelled parallel study with metformin and rimonabant in 20 patients with PCOS with a body mass index (BMI) $\geq$30kg/m^2. The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely

clinical and biochemical evidence of hyperandrogenaemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound.(36) Subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 year prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. All patients gave informed consent. Randomisation was performed using a random number generator. The study was approved by the Hull and East Riding Local Research Ethics committee

The twenty patients were randomised to either metformin 500mg t.d.s or to rimonabant 20mg daily. Clinical and biochemical assessments were performed at randomisation and at the end of the 3-month period. The primary end point of the study was a change in free androgen index and insulin resistance. The secondary end points were change in weight and waist circumference.

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Fasting venous blood was collected into serum gel and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 g for 15 min at 4°C, and the aliquots stored at –20°C. Serum testosterone was measured on an Architect analyzer (Abbott Laboratories, Maidenhead, UK), and SHBG was measured by immunometric assay with fluorescence detection on the DPC Immulite

2000 analyzer using the manufacturer's recommended protocol. The free androgen index was obtained as the total testosterone x100/SHBG. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter, High Wycombe, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2μU/ml, the coefficient of variation was 6%, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyzer (Beckman-Coulter, High Wycombe, UK), using the manufacturer's recommended protocol. The coefficient of variation for the assay was 1.2% at a mean glucose value of 94.6 mg/dl (5.3mmol/liter) during the study period. The insulin resistance was calculated using the homeostasis model assessment (HOMA) method (HOMA-IR= (insulin x glucose)/22.5). Data are reported as mean ± SEM.

3.3 Statistical analysis and sample size calculation

The power of the study to demonstrate a significant reduction in total testosterone was based on a previous study showing a significant reduction in total testosterone concentration after treatment with metformin(294). Using two-sided 5% significance level, a sample of 10 patients per group was found to be needed (assuming a 20% dropout rate) to detect changes in total testosterone with 90% power. Statistical analysis is detailed in section 2.2.1

3.4 Results

All 20 subjects recruited completed the 3-month study period. The compliance was 98% in both groups. Two subjects given metformin reported having mild nausea and heartburn that resolved within 4 weeks; no subject required any dose reduction. All patients continued to have an irregular cycle and there was no difference in progesterone measurements before and after the study suggesting that phase of the menstrual cycle was not a confounder for the androgen or insulin results.

The mean age group of patients was 28.6 ± 1.2 years (metformin 29.8 ± 1.8 vs. rimonabant 27.4 ± 1.5 years). The mean body mass index of the patients did not differ between treatment groups (36.86 ± 1.0 and 35.7 ± 1.4 kg/m^2) for rimonabant and metformin groups, respectively. Subject characteristics are shown in Table 7.

Anthropometric parameters

Weight reduced significantly after 12 weeks of rimonabant treatment (104.6 ± 4.6 vs. 98.4 ± 4.7 kg, $p<0.01$) with a corresponding reduction in waist circumference (116.0 ± 3.3 vs. 109.2 ± 3.7 cm, $p<0.01$), hip circumference (128.5 ± 4.0 vs. 124.1 ± 4.2 cm, $p<0.03$) and waist hip ratio (0.90 ± 0.02 vs. 0.88 ± 0.01, $p<0.01$). In the metformin group these parameters were unchanged after treatment.

Biochemical hyperandrogenaemia

After 12 weeks of rimonabant there was a significant reduction from baseline in free androgen index (26.6 ± 6.1 vs. 16.6 ± 4.1 $p<0.01$) and testosterone (4.6 ± 0.4 vs. 3.1 ± 0.3 nmol/L (132.7 ± 11.5 vs. 89.4 ± 8.65ng/dL) $p<0.01$) (Figure 1) but there was no significant reduction in the metformin treated group (FAI $p = 0.38$; testosterone p =

0.57). The percentage reduction in testosterone was significantly higher in patients treated with rimonabant for 12 weeks compared to the metformin group (-33.2 ± 5.0 vs. -7.5 ± 1.0 % $p < 0.05$). There were no significant changes in SHBG in either group.

Metabolic parameters

Both treatments reduced glucose levels that reflected in a significant reduction of insulin resistance from baseline for rimonabant, but not with metformin, albeit that, the absolute change in HOMA-IR did not differ between the 2 groups. There was no significant improvement in any of the lipid parameters or for hsCRP within or between study groups.

There was a strong positive correlation between weight loss and reduction of testosterone levels (r=0.821 p value 0.004) (Figure 1). However there was no significant correlation of weight loss with improvement in HOMA-IR (r=0.420 p value 0.23) or between reductions in testosterone levels with decrease in HOMA-IR (r=0.285 p value 0.42).

3.5 Discussion:

This randomised open labelled parallel study showed that weight reduction through rimonabant 20mg per day improved both hyperandrogenaemia and insulin resistance and was a more effective insulin sensitisor than metformin in this obese PCOS group over the 3 month period.

Rimonabant led to an average 22% reduction in insulin resistance and patients had a 6% mean weight loss in just 12 weeks. These data are in accord with several studies showing a reduction in the weight and waist circumference in patients with metabolic

syndrome treated with rimonabant(291-293), and are also in agreement with the metabolic improvements that weight loss in PCOS would predict.(146, 147) However, this is the first study that has directly demonstrated these improvements with rimonabant in patients with PCOS.

As expected, the subjects treated with rimonabant had a significantly greater degree of weight loss than those treated with metformin. There was a 6.2 kg reduction in weight (6%) after 12 weeks in patients treated with rimonabant which compares with previous studies of rimonabant in obese patients with metabolic syndrome which showed a 4.64 kg weight loss after one year compared to placebo(295) The weight reduction found here with rimonabant translated into a reduction of 6.8 cm or 5.9% in waist circumference. Abdominal obesity is associated with an increase in cardiovascular risk(296, 297), which is mainly reflected in waist circumference. The reduction in insulin resistance by rimonabant may be due to the weight loss alone. However, additional less well defined mechanisms have been suggested to contribute to its metabolic effects. (298, 299)

In the rimonabant group study there was a 37.6% decrease in the free androgen index levels compared to baseline, primarily due to a reduction in the serum testosterone level that fell by 33.2% that was significantly greater than that seen with metformin that showed fall of 7.5%. The reduction in serum testosterone levels in rimonabant group strongly correlated with weight loss but not with decrease in insulin resistance measured by HOMA-IR. This data is in accord with reports of weight reduction reducing biochemical hyperandrogenism in PCOS(146, 300, 301). This is also in accord with the data suggesting that more obese patients are less responsive to metformin [7,19] There were

no changes in the SHBG levels with either metformin or the rimonabant treatment groups.

In this study metformin had a more modest effect on weight, with the 1.6% reduction being comparable with some previous data. (287) However, other studies have shown that while metformin can reduce body mass index of around 4% and androgen measures of around 20% compared to placebo(138), it may be less effective in more overweight patients.(129, 139, 140) This latter point may help account for the lack of response here.

The lack of efficacy through insulin sensitisation with metformin is likely to be due to the obesity of this patient group as noted before. Neither treatment affected cholesterol or hsCRP over the 3 month period in accord with other studies (294), although rimonabant treatment has been reported to reduced triglycerides and increase HDL-C concentration over a year period.(295)

This short duration of the study meant that no assessment of clinical hyperandrogenaemia could be undertaken, nor of menstrual change and ovulation. The irregular menses and lack of change in the progesterone measurements before and after the study suggests that the 6kg weight loss with rimonabant had not affected menstrual periods over the 12 week period. It must be emphasised that rimonabant is not recommended while contemplating pregnancy.

No patient on the study reported side effects to rimonabant and mild side effects in a few patients were reported for metformin. In other studies involving rimonabant approximately 15% of treated patients (twice as many as with placebo) withdrew from trials because of unwanted effects, mainly because of psychiatric disorders especially

depression.(292, 293, 302, 303) This is of relevance in view of the finding that the number of new cases of depressive disorders was 21% in women with PCOS and the adjusted odd ratio for depressive disorders in women with PCOS, independent of family history, obesity, infertility and other factors, was 4.23 compared to patients without PCOS(304). In this study we specifically excluded patients with any history of depression or other psychiatric disorders.

In conclusion, this study has demonstrated that rimonabant may have a therapeutic role in patients with PCOS by improving anthropometric parameters, insulin resistance and biochemical hyperandrogenaemia over a short term 12 weeks period, and was superior to insulin sensitisation by metformin in this obese patient group.

Table 7: Comparison of anthropometric and hormonal parameters before and after treatment

Parameter	Metformin group (n=10)			Rimonabant group (n=10)			% change		
	Baseline	12 weeks	*p*-value	Baseline	12 weeks	P value	Metformin	Rimonabant	*p*-value
Weight (kg)	103.8 ± 3.9	102.2 ± 4.1	0.08	104.6 ± 4.6	98.4 ± 4.7	<0.01 ***a***	-1.6 ± 1.0	-6.0 ± 1.1	0.02 ***b***
BMI (kg/m^2)	35.7 ± 1.4	35.09 ± 1.5	0.08	36.86 ± 1.0	34.52 ± 1.0	<0.01 ***a***	-1.6 ± 1.0	-6.0 ± 1.1	0.02 ***b***
Waist (cm)	110.4 ± 3.4	110.0 ± 2.4	0.78	116.0 ± 3.3	109.2 ± 3.7	<0.01 ***a***	-1.6 ± 1.0	-5.9 ± 1.0	0.11
Hip (cm)	121.3 ± 3.2	120.3 ± 3.4	0.06	128.5 ± 4.0	124.1 ± 4.2	0.03 ***a***	-0.4 ± 1.0	-3.4 ± 1.0	0.10
Waist-Hip ratio	0.91 ± 0.02	0.92 ± 0.02	0.57	0.90 ± 0.02	0.88 ± 0.01	<0.01 ***a***	-0.9 ± 1.0	-2.6 ± 1.0	0.07
Glucose(mmol/L)	*4.9 ± 0.1*	*4.7 ± 0.1*	*0.03* ***a***	*5.0 ± 0.1.*	*4.8 ± 0.2*	*0.04* ***a***	*0.1 ± 1.4*	*-3.8 ± 1.6*	*0.06*
Insulin (µIU/mL)	*15.2 ± 3.*	*15.5 ± 3.4*	*0.81*	*19.6 ± 2.2*	*16.0 ± 1.8*	*0.08*	*1.0 ± 5.2*	*-13.9 ± 8.8*	*0.21*
HOMA-IR	*3.4 ± 0.7*	*3.4 ± 0.8*	*0.77*	*4.4 ± 0.5*	*3.4 ± 0.4*	*0.05* ***a***	*1.5 ± 4.0*	*-22.2 ± 9.0*	*0.10*
TC (mmol/L)	*6.0 ± 0.3*	*5.6 ± 0.2*	*0.12*	*5.1 ± 0.3*	*4.7 ± 0.3*	*0.19*	*-8.0 ± 4.0*	*-7.6 ± 8.0*	*0.41*
LDL-C (mmol/L)	*3.9 ± 0.3*	*3.5 ± 0.1*	*0.06*	*3.3 ± 0.2.*	*3.1 ± 0.3*	*0.21*	*-8.4 ± 3.0*	*-2.8 ± 1.0*	*0.13*
HDL-C (mmol/L)	*1.2 ± 0.2*	*1.2 ± 0.1*	*0.70*	*1.2 ± 0.1*	*1.1 ± 0.1*	*0.38*	*-9.5 ± 11.0*	*-8.2 ± 8.0*	*0.10*
TG (mmol/L)	*2.0 ± 0.3*	*1.9 ± 0.4*	*0.34*	*1.4 ± 0.2*	*1.3 ± 0.1*	*0.44*	*-8.4 ± 9.0*	*-9.1 ± 22.0*	*0.45*
Hs-CRP (mg/L)	*4.2 ± 1.7.*	*4.0 ± 1.3*	*0.87*	*5.6 ± 1.2*	*5.2 ± 1.2*	*0.70*	*-3.4 ± 33.0*	*-6.9 ± 4.0*	*0.71*
FAI	20.5 ± 4.0	18.2 ± 3.0	0.38	26.6 ± 6.1	16.6 ± 4.1	<0.01 ***a***	-11.5 ±1.2	-37.6 ± 6.0	0.07
Testosterone (nmol/L)	3.8 ± 0.6	3.5 ± 0.5	0.57	4.6 ± 0.4	3.1 ± 0.3	<0.01 ***a***	-7.5 ± 1.0	-33.2 ± 5.0	0.05 ***b***
SHBG (nmol/L)	18.4 ± 2.1	19.2 ± 2.0	0.65	21.5 ± 2.8	23.4 ± 2.9	0.08	4.5 ± 1.0	8.8 ± 5	0.97

Data are presented as mean ± SEM. All serum results are obtained from fasting variables. Change, percent difference compared with baseline.

a: Significant difference from baseline.

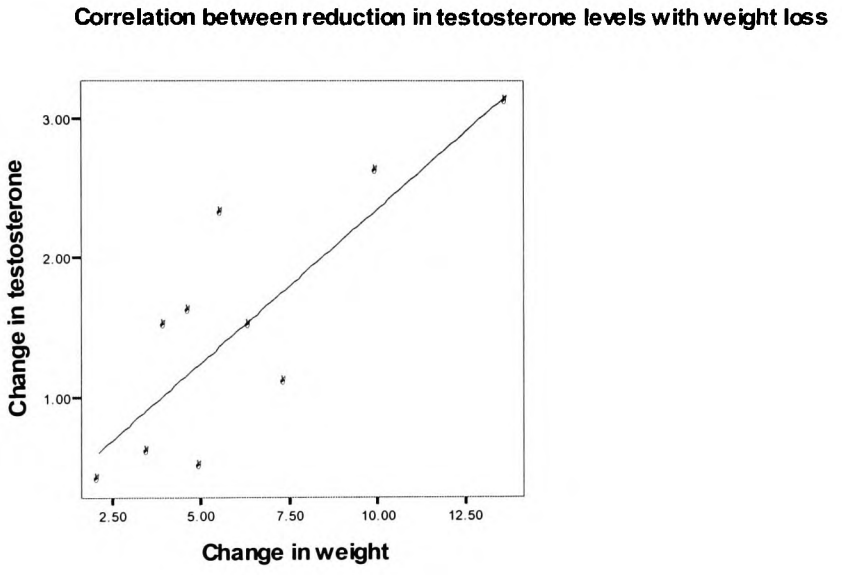
b: Significant differences for the comparison between treatments.

To convert values for glucose to milligrams per deciliter, divide by 0.056.

To convert values for insulin to picomoles per liter, multiply by 6.

Figure 1 Correlation between reduction in testosterone levels with weight loss

There was a strong positive correlation between weight loss and reduction of testosterone levels (R 0.821 p value 0.004) (Figure 1). However there was no significant correlation of weight loss with improvement in HOMA-IR (R 0.420 p value 0.23) or between reductions in testosterone levels with decrease in HOMA-IR (R 0.285 p value 0.42).



Pearson Correlation R= 0.821 p value 0.004 (2 tailed significance)
Change in weight expressed in kilograms
Change in testosterone expressed in nmol/L
To convert values for testosterone to nanograms per deciliter, divide by 0.03467

Chapter 4

Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in patients with polycystic ovary syndrome.

4.1 Introduction: Subsequent to the previous study showing rimonabant is superior to metformin in improving insulin resistance and hyperandrogenemia in obese patients with polycystic ovary syndrome. As discussed in section 1.4.4.3, significant weight regain, over 2kg occurs, in the 12 weeks following cessation of rimonabant (303). This study was undertaken to see if subsequent metformin treatment after rimonabant would maintain any of the initial improvement for weight, insulin resistance and hyperandrogenemia in patients with PCOS.

4.2: Research Design and Methods: This was an extension arm with the addition of metformin to the randomised open labelled parallel study of metformin and rimonabant in 20 patients with PCOS with a body mass index (BMI) $\geq$30kg/m^2. All the patients who were on rimonabant were changed over to metformin 500mg three times daily for 3 months (rimonabant/metformin group), whereas all the patients who were on metformin were continued on metformin for another 3 months (metformin only group) (Figure 2).

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound.(36) Subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 year prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting

tumours were excluded by appropriate tests. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee.

Clinical and biochemical assessments were performed at the end of the 3-month period of the extension arm. The primary end point of the study was a change weight and the secondary end points were a change in free androgen index and insulin resistance.

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Blood samples were processed and analysed as per our previous study (305). Data are reported as mean ± SEM.

4.3 Statistical analysis

Statistical analysis is described in section 2.2.1.

4.4 Results: All the 20 subjects completed the 3-month extension period. The compliance was 98% in both groups. All patients tolerated metformin without any side effects. All patients continued to have an irregular cycle.

Anthropometric parameters (Table 1)

There was no significant change in weight (98.4 ± 4.7 vs. 98.6 ± 4.8 kg $p<0.96$) or the waist circumference and the waist-hip ratio for the rimonabant/metformin group after 3 months of metformin (Figure 2), Conversely, there was a significant weight loss between 3 and 6 months after metformin treatment (102.2 ± 4.1 vs. 100 ± 4.2 kg $p<0.01$), together with a reduction in waist circumference (110.0 ± 2.4 vs. 109.4 ± 2.3 cm $p=0.05$) and waist-hip ratio (0.92 ± 0.02 vs. 0.91 ± 0.02 $p=0.05$. However, there was

a significant reduction in weight, waist circumference and waist-hip ratio in both groups at 6 months compared to baseline. The percentage reduction in weight, waist circumference and waist hip ratio was significantly higher in rimonabant/metformin group compared to metformin only group at 6 months compared to baseline [weight (-6.0 ± 0.1 vs. -2.8 ± 0.1% p=0.04); waist circumference (-5.0 ± 0.1 vs. -0.6± 0.1% p=0.02); waist-hip ratio (-1.7 ± 0.4 vs. -0.3 ± 0.1% p=0.02%)].

Metabolic parameters (Table 2)

There was a significant reduction for glucose, insulin and HOMA-IR in the rimonabant/metformin group between baseline, 3 and 6 months. However, there was no change in any of these parameters after 6 months of metformin alone. The percentage change of glucose was significantly more in rimonabant/metformin group compared to the metformin group (-7.0 ± 0.12 vs. -0.8 ± 0.1 % p <0.01).

There was a significant reduction in total cholesterol and LDL after 6 months in both the groups (Table 2). There was also a reduction in triglycerides and improvements in HDL in the rimonabant/metformin group that was not seen after 6 months of metformin alone.

Biochemical hyperandrogenemia (Table 3)

There was a significant reduction in the testosterone and FAI between 3 and 6 months in both rimonabant/metformin group and metformin group. There was no change in sex hormone binding globulin (SHBG) either group. The percentage change from baseline to 6 months were greater in the rimonabant/metformin group compared to metformin

only group [testosterone (-45.0 ± 5.0 vs. -16 ± 2.0% p=0.02); FAI (-53.0 ±5.0 vs. -17.0 ± 12.2% p=0.02)].

There was no significant correlation between weight loss with testosterone (r=0.438 p=0.21), HOMA-IR (r=0.19 p=0.83) and total cholesterol (r=0.349 p=0.32) in the rimonabant pre-treatment group or in the metformin alone group after 6 months (testosterone r=0.252 p=0.49; HOMA-IR r=0.12 p=0.75; total cholesterol r=0.124 p=0.93).

4.5 Discussion

This study demonstrates that in obese patients with PCOS the weight reduction and decrease in waist circumference following rimonabant therapy can be maintained if patients are subsequently changed onto metformin, while the initial improvements in testosterone and insulin resistance can be augmented. The reduction in weight and waist circumference was also superior in the rimonabant/metformin group compared to 6 months of metformin therapy alone.

In the Rio-North America trial, which studied treatment with rimonabant in obese patients, those who were re-randomised to placebo following 12 months of treatment regained around 2kg of their previous weight loss in 12 weeks which was accompanied by a deterioration in their metabolic parameters (303). This highlighted that the cardio metabolic risk factors were only improved with sustained weight loss and that long-term treatment with rimonabant appeared to be necessary(303). In this study the improvement in anthropometric and metabolic parameters achieved with 3 months of

rimonabant therapy was maintained for a further 3 months by treating patients with metformin therapy after the discontinuation of rimonabant.

There was an improvement in weight and waist circumference after 6 months of metformin therapy which was not there after the initial 3 months of therapy. The results from randomised studies are conflicting on an effect of metformin on weight loss, especially obese patients with PCOS (129, 133, 139, 140) where metformin may be less effective in patients with a BMI greater than 37. Variations in study groups in pre-treatment BMI, metformin dose, duration of metformin therapy, concomitant life style changes and patient adherence to treatment may account for many of these differences (129, 137).

There was a significant reduction in glucose, insulin and HOMA-IR with metformin after rimonabant, over and above the initial changes through rimonabant treatment. Rimonabant has been shown to reduce fasting insulin(292) and glucose(302) over a period of 1-2 years, but this is the first study to show that the subsequent substitution with metformin may maintain and improve the insulin resistance. This suggests that the insulin sensitisation action of metformin was complementary to the weight loss caused by rimonabant. In comparison, there was no change in any of these parameters after 6 months of sole metformin treatment, despite the small though significant weight loss, a finding that has been found before in obese group patients with PCOS (139).

There was a significant correlation between reduction of weight and testosterone at 3 months of rimonabant therapy that was lost following subsequent metformin treatment. This suggests that the initial weight loss was responsible for the reduction in

testosterone with rimonabant, but that there may be a weight independent action of metformin for further testosterone reduction (306, 307). Rimonabant has also been found to have weight independent effects which might be mediated through less well defined direct pharmacological effects, but these were not obvious during the first phase of treatment here (308-313).

There was a significant reduction of total cholesterol and LDL cholesterol after 6 months of metformin that may have been due more to the reduction in weight than a direct effect of metformin as not changes in FAI or insulin resistance were seen. The reduction in LDL is consistent with another meta analysis which showed significant reduction of LDL with metformin, but no changes in total cholesterol(137). Rimonabant has been reported to reduce triglycerides and increase HDL in other studies after 1-2 year (292, 302, 303, 312). There was certainly a significant reduction of triglycerides and improvement in HDL on metformin in rimonabant pre-treated PCOS patients in this study. The apparent lack of improvement in the first 3 months on rimonabant is more difficult to explain but might be due to shorter duration of therapy. However, this shows that metformin therapy after rimonabant pre-treatment might sustain, or even enhance, the beneficial effects of rimonabant in these parameters. Surprisingly there was a significant reduction of total cholesterol and LDL cholesterol after metformin therapy in rimonabant pre-treated patients which has not been found by others (292, 302, 303, 312).

Rimonabant is not recommended during pregnancy whereas infertility is an issue in a significant proportion of patients with PCOS (314, 315). Since we know weight loss

has a significant effect on ovulation and the chance of pregnancy (316), rimonabant pre-treatment followed by metformin therapy could be an option in this group of patients. Moreover, as there is growing evidence that endocannabinoids are involved in implantation, pregnancy and miscarriage in animal models and human tissues (317-320), it is possible to tentatively speculate that rimonabant priming may actually prove beneficial in pathological situations like miscarriage(320) and infertility.

We made no assessment of clinical hyperandrogenemia, nor of menstrual change and ovulation. As such, the blood tests were done without respect to the bleeding pattern so there may have been temporary hormonal fluctuations interfering with the results. Nevertheless, the irregular menses and lack of change in the progesterone measurements before and after the study suggests that rimonabant and metformin had not affected menstrual periods over the 24 week period in this obese group.

In summary, this study has demonstrated the potential for using a single 'course' of rimonabant preceding the longer term prescription of metformin. While it could be argued that with a longer period of follow-up the separation between rimonabant/metformin versus metformin alone may ultimately diminish, there seems little doubt that the improvements seen by replacing rimonabant with metformin are likely lead to many months of clinically significant anthropometric reduction and risk factor benefit. Such short courses of rimonabant may also go some way to addressing the concerns surrounding longer term use of cannabinoid receptor blockers.*(292, 293, 302, 303)*. Indeed, at worst, it may prove that further courses of rimonabant- either instead of or in addition to metformin- are all that is required.

In summary, metformin for 3 months in obese patients with PCOS maintained the improvement of weight loss and enhanced the metabolic and biochemical parameters achieved by treatment with rimonabant compared to 6 months of metformin treatment.

Table 8
Changes in anthropometric parameters in metformin group and rimonabant/metformin group

Parameter	Metformin only group (n=10)						Rimonabant / Metformin group (n=10)					
	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1/V2 (p value)	V2-V3 (p value)	V1- V3 (p value)	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1&V2 (p value)	V2&V3 (p value)	V1- V3 (p value)
Weight (kg)	103.8 ± 3.9	102.2 ± 4.1	100.9 ± 4.2	0.08	<0.01*	0.02*	104.6 ± 4.6	98.4 ± 4.7	98.6 ± 4.8	<0.01*	0.96	<0.01*
BMI (kg/m^2)	35.7 ± 1.4	35.09 ± 1.5	34.1 ± 1.2	0.08	<0.01*	0.02*	36.86 ± 1.0	34.52 ± 1.0	34.59 ± 1.5	<0.01 *	0.09	<0.01*
Waist (cm)	110.4 ± 3.4	110.0 ± 2.4	109.4 ± 2.3	0.78	0.05*	0.50	116.0 ± 3.3	109.2 ± 3.7	110.2 ± 3.6	<0.01*	0.11	<0.01*
Hip (cm)	121.3 ± 3.2	120.3 ± 3.4	120.3 ± 3.4	0.06	No-change	0.30	128.5 ± 4.0	124.1 ± 4.2	124.3 ± 4.2	0.03 *	0.34	0.04*
W/H ratio	0.91 ± 0.02	0.92 ± 0.02	0.91 ± 0.02	0.57	0.05*	0.87	0.90 ± 0.02	0.88 ± 0.01	0.89 ± 0.01	<0.01 *	0.17	0.19

Data are presented as mean ± SEM. All serum results are obtained from fasting variables.
All variables were normally distributed
To convert values for glucose to milligrams per deciliter, divide by 0.056.
To convert values for insulin to picomoles per liter, multiply by 6.
To convert values for cholesterol to milligrams per deciliter, divide by 0.0259.
To convert values for triglycerides to milligrams per deciliter, divide by 0.0113.
To convert values for testosterone to nanograms per deciliter, divide by 0.03467.
To convert values for SHBG to micrograms per deciliter, divide by 34.7.
TC - Total cholesterol; LDL-C - LDL-cholesterol; HDL-C - HDL cholesterol; TG-Triglycerides; FAI – Free Androgen Index

Table 9
Changes in biochemical hyperandrogenemia in metformin group and rimonabant/metformin group

Parameter	Metformin only group (n=10)						Rimonabant / Metformin group (n=10)					
	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1/V2 (p value)	V2-V3 (p value)	V1- V3 (p value)	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1&V2 (p value)	V2&V3 (p value)	V1- V3 (p value)
Testosterone (nmol/L)	3.8 ± 0.6	3.5 ± 0.5	2.5 ± 0.3	0.57	0.04*	0.02*	4.6 ± 0.4	3.1 ± 0.3	2.4 ± 0.8	<0.01*	0.03*	<0.01*
SHBG (nmol/L)	18.4 ± 2.1	19.2 ± 2.1	23.9 ± 4.7	0.65	0.41	0.30	21.5 ± 2.8	23.4 ± 2.9	23.9 ± 3.7	0.08	0.70	0.1
FAI	20.5 ± 4.0	18.2 ± 3.0	10.8 ± 2.0	0.38	0.04*	0.02*	26.6 ± 6.1	16.6 ± 4.1	11.4 ± 2.3	<0.01*	0.02*	<0.01*

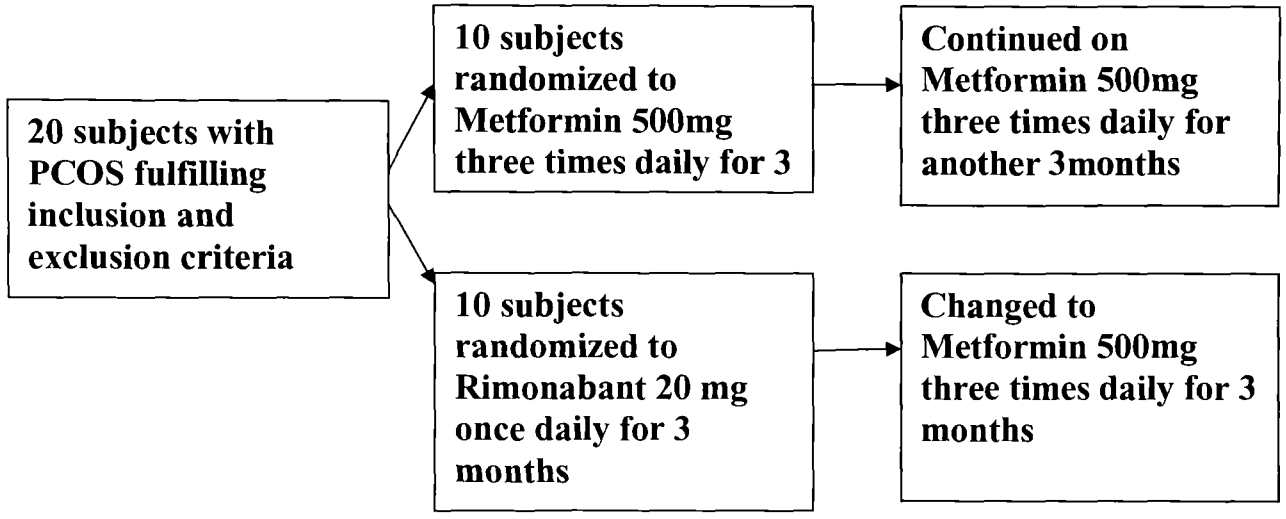
Data are presented as mean ± SEM. All serum results are obtained from fasting variables.
All variables were normally distributed
To convert values for testosterone to nanograms per deciliter, divide by 0.03467.
To convert values for SHBG to micrograms per deciliter, divide by 34.7.
FAI – Free Androgen Index

Table 10
Changes in metabolic parameters in metformin group and rimonabant/metformin group

Parameter	Metformin group (n=10)						Rimonabant / Metformin group (n=10)					
	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1/V2 (p value)	V2-V3 (p value)	V1- V3 (p value)	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1&V2 (p value)	V2&V3 (p value)	V1- V3 (p value)
Glucose (mmol/L)	4.9 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	0.20	0.30	0.50	5.0 ± 0.1	4.8 ± 0.2	4.6 ± 0.4	0.04*	0.01*	<0.01*
Insulin (μIU/mL)	15.2 ± 2.9	15.5 ± 3.4	17.3 ± 3.9	0.81	0.80	0.70	19.6±2.2	16.0 ± 1.8	13.4 ± 1.8	0.08	0.02*	0.01
HOMA-IR	3.4 ± 0.7	3.4 ± 0.8	3.7 ± 0.8	0.77	0.80	0.81	4.4 ± 0.5	3.4 ± 0.4	2.7 ± 0.3	0.05*	0.02*	<0.01*
TC (mmol/L)	6.0 ± 0.3	5.6 ± 0.2	4.9 ± 0.5	0.12	0.20	0.05*	5.1 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	0.20	0.61	0.05*
LDL-C (mmol/L)	3.9 ± 0.3	3.5 ± 0.1	2.8 ± 0.4	0.06	0.07	0.02*	3.3 ± 0.2	3.1 ± 0.3	2.9 ± 0.3	0.21	0.52	0.05*
HDL-C (mmol/L)	1.2 ± 0.2	1.2 ± 0.1	1.4 ± 0.2	0.7	0.42	0.52	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	0.38	0.04*	0.02*
TG (mmol/L)	2.0 ± 0.3	1.9 ± 0.4	1.4 ± 0.2	0.34	0.50	0.22	1.4 ± 0.2	1.3 ± 0.1	1.0 ± 0.1	0.44	<0.01*	0.05*
Hs-CRP (mg/L)	4.2 ± 1.7	4.0 ± 1.3	3.8 ± 0.8	0.87	0.94	0.81	5.6 ± 0.9	5.20 ± 1.2	7.1 ± 1.8	0.70	0.4	0.4

Data are presented as mean ± SEM. All serum results are obtained from fasting variables.
All variables were normally distributed
To convert values for glucose to milligrams per deciliter, divide by 0.056.
To convert values for insulin to picomoles per liter, multiply by 6.
To convert values for cholesterol to milligrams per deciliter, divide by 0.0259.
To convert values for triglycerides to milligrams per deciliter, divide by 0.0113.

Figure 2.
Flow chart of patients through rimonabant/metformin study



Chapter 5

Effect of Rimonabant and Metformin on GIP and GLP-1 in Obese Women with Polycystic Ovary Syndrome.

5.1 Introduction

In chapter 3 rimonabant has been shown to reduce weight, free androgen index (FAI) and insulin resistance in obese patients with polycystic ovary syndrome (PCOS) compared to metformin during a 12 week period. When subsequently treated for another 3 months as described in chapter 4, metformin maintained the weight loss and enhanced the metabolic and biochemical parameters achieved by treatment with rimonabant, compared to 6 months of metformin treatment alone.

Metformin's actions appear to be mediated by activation of AMP-activated protein kinase(288) where as rimonabant is a cannabinoid receptor 1 blocker. (289, 290) Exenitide, an incretinmimetic that shares similar glucoregulatory properties of the hormone glucagon-like peptide-1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP) has been shown to improve FAI and insulin sensitivity measures predominantly through weight loss in patients with PCOS(321). We aimed to establish whether rimonabant might have an effect on the incretin system that may augment its weight reduction effect in patients with PCOS.

5.2 Research Design and Methods

A randomized open labelled parallel study of metformin and rimonabant for 12 weeks in 20 patients with PCOS with a body mass index (BMI) $\geq$30kg/m^2 was undertaken (described in chapter 3). This was followed by an extension arm with the addition of metformin for another 12 weeks (chapter 4). All the patients who were on rimonabant were changed over to metformin 500mg three times daily for 3 months, whereas all the patients who were on metformin were continued on metformin for another 3 months.

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound.(36) Subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 year prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee.

Clinical and biochemical assessments were performed at baseline, 12 weeks and 24 weeks. Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Blood samples were processed and analysed. GIP and GLP-1 were measured using ELISA methods (Linco Research, Missouri, USA) with an intra-assay CV of 7.3% at 4.2 pmol/L and 7% at 28 pmol/L respectively. Data are reported as mean ± SEM.

Statistical analyses were carried out using the paired t test. The biochemical data was normally distributed when tested using the Kolmogorov-Smirnov test. For all analysis, a two-tailed $P \leq 0.05$ was considered to indicate statistical significance. Statistical analysis was performed using SPSS for Windows NT, version 14.0 (SPSS Inc., Chicago, IL).

5.3 Results

All the 20 subjects completed the study. The compliance was 98% in both groups. All patients tolerated metformin and rimonabant without any side effects. The mean age group of patients was 28.6 ± 1.2 years (metformin 29.8 ± 1.8 *vs.* rimonabant 27.4 ± 1.5 years).

There was a significant increase in GIP levels after rimonabant treatment for 3 months (7.78 ± 0.38 vs. 21.62 ± 1.96 pmol/L p value – 0.04) that decreased when changed over to metformin (21.62 ± 1.96 vs. 8.94 ± 0.4 pmol/L p value – 0.08). There were no significant changes in GIP levels either at 3 months (6.88 ± 0.28 vs. 6.08 ± 0.2 pmol/L p value – 0.23) or at 6 months (6.08 ± 0.2 vs. 6.22 ± 0.34 pmol/L p value – 0.89) with metformin (Figure 3).

There were no significant changes in GLP-1 levels after rimonabant treatment for 3 months (18.6 ± 0.9 vs. 21.4 ± 1.2 pmol/L p value – 0.42) and 6 months (21.4 ± 1.2 vs. 21.6 ± 0.9 pmol/L p value – 0.92) or after metformin treatment at 3 months (22.2 ± 1.5 vs. 21.0 ± 1.4 pmol/L p value – 0.72) and 6 months (21.0 ± 1.4 vs. 19.6 ± 1.8 pmol/L p value – 0.54). There was no significant correlation between the increase in GIP and weight loss with rimonabant (r=0.12 p=0.89)

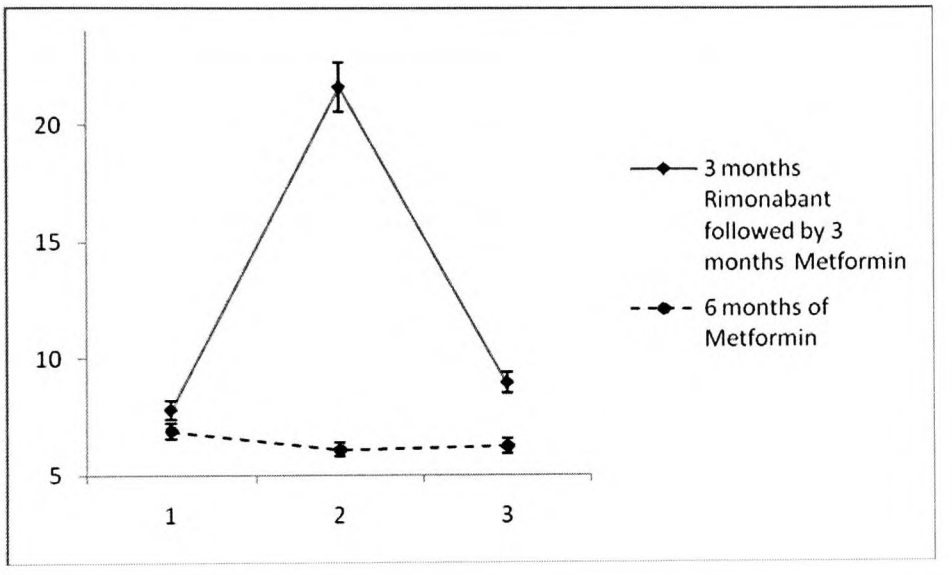
5.4 Discussion

This study showed a significant (and reversible) increase in GIP levels after 3 months of rimonabant treatment. There were no changes in either GLP-1 or GIP levels with metformin.

The increase in GIP levels could be due to rimonabant stimulating incretin hormones rather than secondary to weight loss since there was no correlation between weight loss and increase in GIP levels. However, this latter finding may simply be a consequence of the number of study participants.

Curiously, this study demonstrates that rimonabant affects GIP levels but not GLP – 1 in this group of obese patients with PCOS. GLP-1 is produced by L cells located mainly in the ileum and colon, and to a lesser extent by L cells in the duodenum and jejunum, whereas GIP is produced by K cells in the proximal gut(322). CB1 receptors are also present in the duodenum and jejunum and activation of CB1 receptors depresses gastro-intestinal motility by inhibiting contractile transmitter release. Moreover, CB1 receptor activation/agonists inhibit gastric emptying and intestinal transit, delays gastric emptying in humans and rodents and also inhibit gastric acid secretion(323), functions that precisely mirror those of GIP. In conclusion therefore, it is likely that the increase in GIP by rimonabant in patients with PCOS may contribute to the metabolic changes found with the drug, but that the rise may simply be a compensatory response to maintain gastro-intestinal homeostasis.

Figure 3
Glucose-dependent insulinotropic polypeptide (GIP) levels after 3 months of Rimonabant followed by 3 months of Metformin and after 3, 6 months of Metformin



X axis – visit 1, visit 2, visit 3
Y-axis – Glucose-dependent insulinotropic polypeptide in pmol/L

Chapter 6

The Effect of Atorvastatin in Patients with Polycystic Ovary Syndrome: A Randomized Double Blind Placebo Controlled Study.

6.1 Introduction: Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age with a reported prevalence of 5-7% (107, 324, 325). PCOS is associated with a broad range of adverse sequelae, including dyslipidemia, hypertension, insulin resistance, hyperandrogenaemia, gestational and T2DM, which ultimately increase the risk of cardiovascular morbidity (76, 77, 80, 326-331). HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors (statins) have been shown to reduce cardiovascular morbidity and mortality in several studies (332-334). They also have other, non-lipid lowering effects, demonstrated by their benefits amongst hypertensive patients with normal lipids (335) as well as their anti-inflammatory effect in patients with rheumatoid arthritis(336). Other pleiotropic effects of statins include improvement in endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques(337).

In patients with PCOS, simvastatin, with concomitant oral contraceptive (OCP) therapy, has recently been shown to reduce testosterone, LH and markers of systemic inflammation incrementally more than OCP treatment alone. Both regimes (with and without statin treatment) had an adverse effect on glucose metabolism (338). However, the OCP accounted for the the majority of the biochemical benefit, which was consistent with other OCP studies showing a reduction in testosterone and improvement in SHBG in PCOS patients (339-342).

Hypothetically the reduction of inflammation through statin therapy should have a beneficial effect in PCOS where the inflammatory marker hsCRP has been shown to be elevated. However, the effects of statins alone in patients with PCOS who are treatment

naïve is unknown, and therefore we have performed a double blind randomized placebo control study with atorvastatin to investigate this..

6.2 Methods

A randomized double blind placebo controlled study was undertaken using atorvastatin 20mg daily. The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus being present for each patient, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound(36). Subjects had no concurrent illness, were not on any medication for the preceding 6 months, were not planning to conceive and were using barrier contraception. Patients did not use any oral or implantable contraceptives or any other treatments likely to affect ovarian function, insulin sensitivity or lipids for at least 3 months before entering the study. Subjects were advised not to change their lifestyle, including physical activity or dietary habits, during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. Compliance with treatment was calculated by counting the returned medications. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee. This study was registered ISRCTN registry - ISRCTN24474824.

Clinical and fasting biochemical assessments were performed at baseline and at the end of the 3-month period. The primary end point of the study was a change in high sensitivity (hs) CRP and the secondary end points were a change in HOMA-IR and total

testosterone. Blood sample collection and analysis is described in section 2.1.1. Data are reported as mean ± SEM.

6.3 Statistical analysis

The sample size was based on the study on the effect of atorvastatin on hsCRP in patients with impaired fasting glucose (343). Powered specifically for CRP the minimum difference worth detecting/observed difference was 32.7%, estimated within group SD was 11.1; therefore, for 90% power and a significance level of 5%, a sample size of 16 per group was calculated. Adjusting for a possible 20% drop out rate meant a total of 40 patients needed to be recruited.

Statistical tests are described in section 2.2.1

6.4 Results:

Thirty seven patients completed the study. Two patients from the placebo group and one patient from atorvastatin group dropped out of the study due to non-compliance. Following their exclusion, compliance was 99% in both groups. None of the subjects developed significant side-effects in the course of the study. None of them developed symptoms of muscle toxicity, and liver function tests and creatine kinase remained normal throughout the study.

The mean age group of patients was 27.7 ± 1.4 years (atorvastatin 26.6 ± 1.2 vs. placebo 28.8 ± 1.8). The BMI were comparable in both atorvastatin and placebo group (33.20 ± 1.4 vs. 33.92 ± 1.4 kg/m^2).

There was a significant absolute reduction in total cholesterol, LDL cholesterol, triglycerides, FAI, SHBG and total testosterone in patients randomized to atorvastatin, while there were no changes in any of these parameters in the placebo group. (Table

10). The percentage change in total cholesterol, triglycerides, LDL, FAI, SHBG and total testosterone were greater in the atorvastatin group compared to placebo group. There was a significant reduction in serum insulin levels and HOMA-IR in patients taking atorvastatin whilst there was significant increase in both these parameters in patients randomised to placebo.

There was no linear correlation between reduction in total cholesterol with improvement of FAI (r^2=0.015; p=0.95), testosterone (r^2=0.1; p=0.69) or SHBG (r^2=0.29; p=0.22). However there was a significant correlation between reduction in triglycerides and reduction of HOMA-IR in atorvastatin group (r^2 0.68; p<0.01)

6.5 Discussion

In patients with PCOS 12 weeks treatment with atorvastatin 20mg resulted in a significant reduction in inflammatory markers, insulin resistance and hyperandrogenemia, in addition to the expected improvement in lipids. The reduction of the hyperandrogenemia was also independent of the improvement of the lipid profile with atorvastatin.

The 26% reduction in total cholesterol, 36% reduction in LDL and 21% reduction in triglycerides with atorvastatin is comparable with other trials (344, 345), although in this study there were no detectable changes in HDL. PCOS is associated with an increase of cardiovascular risk factors, including dyslipidemia that typically is reflected in an elevated total cholesterol and LDL(66, 68, 104, 105). This is in accord with an report using simvastatin 20 mg daily and an OCP containing 20μg ethinyl estradiol and 150μg desogestrel over 12 weeks that reduced total cholesterol and LDL by 7.5% and 20%, respectively(346). In contrast the OCP alone induced a modest increase of TC by 5%

without any marked effect on LDL cholesterol. In that study triglycerides remained virtually unchanged after statin and OCP treatment, but increased significantly by 20% after OCP alone in accord with other studies(347, 348).

Of note is the finding that the improvement in biochemical hyperandrogenemia with atorvastatin in this study is comparable to anti-androgen agents (349, 350). The reduction in testosterone is comparable to the reduction in testosterone by the combined OCP (Ethinyl oestradiol/Levonorgestrel combination) that may reduce testosterone by 27%. However OCPs are more effective in improving SHBG by up to 100% when given for 3 months(339, 340, 347). Simvastatin 20mg daily concomitant with an OCPgive a 38% decrease in total testosterone (346) compared to a 25% reduction with atorvastatin 20mg daily alone. These suggest that there might be a dose dependent effect or it might be due to difference in potency and class. Ethinyl oestradiol/cyproterone acetate pill has shown to reduce testosterone by around 42% after 6 month period(351). However, anti-androgens reduce hirsuitism not only by reducing hyperandrogenemia, but also by other mechanisms including androgen receptor blockage, effect on LH secretion and 5α reductase activity(352). This study was too short to investigate the improvement of clinical hyperandrogenemia.

Weight loss of <10% of initial body weight has been shown to reduce testosterone in women with PCOS (353). There was no weight change with atorvastatin in this study; however, the reduction in testosterone was more than that seen with orlistat (17% reduction) and increased SHBG by 4% after 3 months of treatment. However there was a 33.2% reduction in testosterone with the endocannabinoid blocker, rimonabant, after 3 months that correlated with weight loss.

The reduction of testosterone is more comparable to improvement with insulin sensitizers like metformin that gave a 14% decrease in total testosterone in 3 months (294) and thiazolindinediones that gave a 6 - 15% reduction in serum testosterone (354-357). No improvement in hirsuitism with metformin may be seen(137).

There was a 25% reduction in hs-CRP with atorvastatin in this group of patients with PCOS. PCOS is associated with increased levels of indices of low-grade chronic inflammation such as hs-CRP (72, 358) that appears to be a predictor of cardiovascular events in women. (359, 360). Atorvastatin has been shown to significantly reduce hsCRP with a trend to reducing insulin resistance in patients with impaired fasting glucose (361).

A reduction in insulin resistance may be central to the improvements seen for hyperandrogenaemia and hsCRP. There was a 21% reduction in serum insulin levels and a 20% improvement in HOMA-IR with atorvastatin. This improvement in HOMA-IR was correlated positively with the degree of reduction in triglyceride levels and is in accord with other studies which have demonstrated a similar link in patients with the metabolic syndrome and T2DMtreated with atorvastatin (362, 363). Hypothetically, the reduction in triglyceride availability leads to an increased use of glucose as the main intracellular substrate(363) thereby improving insulin sensitivity. Whatever the reason, this mechanism may explain other findings such as reduced development of diabetes amongst patients treated with pravastatin in the WOSCOPS trial (364, 365).

Improvement in biochemical hyperandrogenemia appears to be independent of the reduction in total cholesterol, LDL or triglycerides. There was no correlation between reduction of lipid levels and improvement in biochemical hyperandrogenemia that supports the concept of a pleotrophic effect of statins. Statins inhibit ovarian theca-interstitial cell proliferation and steroidogenesis in vitro (236). The ovaries of women with PCOS are typically enlarged with prominent hyperplasia of ovarian theca-interstitial cells and excessive production of androgens by these cells(237-239). The effects of statins on steroidogenesis are most likely related to the inhibition of cholesterol synthesis by the mevalonate pathway and a consequent decrease in the availability of the precursors of progesterone and testosterone(236).

Although a short study, patients continued to have irregular periods suggesting atorvastatin has not overtly affected menstrual function over the 3 months period. We made no assessment of ovulatory function in this study. It must be emphasised that atorvastatin is not recommended while contemplating pregnancy.

In conclusion, atorvastatin 20mg daily improved biochemical hyperandrogenemia, insulin resistance and markers of inflammation in patients with polycystic ovary syndrome when given over a 12 week period. Statin treatment may therefore prove to be a useful adjunct for women with PCOS.

Table 11: Comparison of anthropometric and hormonal parameters before and after treatment with atorvastatin and placebo

Parameter	Atorvastatin group (n=19)			Placebo group (n=18)			% change		
	Baseline	12 weeks	*p*-value	Baseline	12 weeks	P value	Atorvastatin	Placebo	*p*-value
Weight (kg)	91.29 ± 3.4	91.20 ± 3.4	0.42	93.12 ± 4.8	93.05 ± 4.7	0.67	0.1 ± 0.1	0.03 ± 0.02	0.82
BMI (kg/m^2)	33.20 ± 1.4	33.16 ± 1.4	0.42	33.94 ± 1.5	33.92 ± 1.4	0.67	0.1 ± 0.1	0.03 ± 0.02	0.82
Waist (cm)	98.1 ± 3.2	98.9 ± 2.2	0.59	99.3 ± 2.4	98.7 ± 2.1	0.54	0.07 ± 0.02	0.02 ± 0.01	0.78
Glucose(mmol/L)	4.8 ± 0.1	4.9 ± 0.1	0.52	4.7 ± 0.5	4.8 ± 0.1	0.32	1.0 ± 2.0	2.0 ± 1.0	0.90
Insulin (μIU/mL)	15.6 ± 1.8	12.4 ± 1.7	<0.01 ***a***	14.4 ± 2.0	17.6 ± 2.4	0.04 ***a***	-21.4 ± 4.0	34.2 ± 19.7	<0.01 ***b***
HOMA-IR	3.3 ± 0.4	2.7 ± 0.4	<0.01 ***a***	3.0 ± 0.4	3.8 ± 0.5	0.04 ***a***	-19.8 ± 5.2	37.2 ± 20.4	<0.01 ***b***
TC (mmol/L)	4.6 ± 0.2	3.4 ± 0.2	<0.01 ***a***	4.5 ± 0.2	4.6 ± 0.2	0.13	- 26.4 ± 3.0	3.1 ± 1.1	<0.01 ***b***
LDL-C (mmol/L)	2.9 ± 0.2	1.8 ± 0.2	<0.01 ***a***	2.7 ± 0.2	2.7 ± 0.1	0.77	- 36.6 ± 5.0	3.1 ± 0.9	<0.01 ***b***
HDL-C (mmol/L)	1.07 ± 0.1	1.08 ± 0.1	0.17	1.1 ± 0.08	1.1 ± 0.09	0.47	- 4.0 ± 3.9	3.0 ± 3.2	0.17
TG (mmol/L)	1.34 ± 0.08	1.08 ± 0.13	<0.01 ***a***	1.39 ± 0.24	1.69 ± 0.27	0.19	- 20.9 ± 3.8	36.8 ± 21.0	<0.01 ***b***
Hs-CRP (mg/L)	4.9 ± 1.4	3.4 ± 1.1	0.04 ***a***	5.8 ± 1.4	5.7 ± 1.6	0.90	- 25.8 ± 7.0	- 8.8 ± 8.2	0.05
FAI	13.4 ± 0.6	8.7 ± 0.4	<0.01 ***a***	13.9 ± 0.6	13.3 ± 0.5	0.07	-32.7 ± 2.6	- 3.1 ± 2.0	<0.01 ***b***
Testosterone (nmol/L)	4.1 ± 0.2	2.9 ± 0.1	<0.01 ***a***	4.4 ± 0.2	4.3 ± 0.2	0.73	-24.6 ± 2.6	-0.1 ± 1.5	<0.01 ***b***
SHBG (nmol/L)	31.1 ± 1.0	35.3 ± 1.2	<0.01 ***a***	31.9 ± 0.9	32.9 ± 0.9	0.14	13.7 ± 2.4	3.6 ± 2.0	<0.01 ***b***

Data are presented as mean ± SEM. All serum results are obtained from fasting variables.
All variables were normally distributed.
a: Significant difference from baseline.
b: Significant differences for the comparison between treatments.
Change, percent difference compared with baseline.
To convert values for glucose to milligrams per deciliter, divide by 0.056.
To convert values for insulin to picomoles per liter, multiply by 6.

Chapter 7

Atorvastatin Pre-treatment Augments the Effect of Metformin in Patients with Polycystic Ovary Syndrome.

7.1 Introduction:

The pleotrophic effects of HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors (statins) are increasingly recognized (335-337) including the improvement of biochemical hyperandrogenemia in patients with polycystic ovary syndrome (PCOS) (346). In Chapter 3 it was shown that atorvastatin improves biochemical hyperandrogenemia, insulin resistance and inflammatory markers in patients with PCOS (366) .

Metformin has been shown to have a beneficial effect in women with PCOS by reducing serum insulin concentrations, lowering androgen levels and improving reproductive outcome (133, 137, 287). In chapter 4 metformin has been shown to maintain the metabolic benefits following treatment with other medications including endocannabinoid blockers in PCOS (367). This study was undertaken to determine if metformin would maintain the improvement in insulin resistance and hyperandrogenemia in patients with PCOS following atorvastatin, and whether there would be an improvement in these parameters in the placebo pre-treatment group.

7.2 Research Design and Methods

This was an extension arm of a randomized double blind placebo controlled study with atorvastatin 20mg daily in patients with PCOS. Immediately after stopping the trial medication 37 patients (19 patients from the atorvastatin group and 18 patients from the placebo group) who completed the study were given metformin 500mg three times daily for 3 months.

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenemia (Ferriman-Gallwey score >8; free androgen index >8 respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound (36). Subjects had no concurrent illness, were not on any medication for the preceding 9 months except study medications and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 year prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. All patients gave informed consent. The study was approved by the South Humber Research Ethics committee.

Clinical and biochemical assessments were performed at the end of the 3-month period of the extension arm. The primary end points of the study were the change in HOMA-IR and total testosterone.

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Blood samples were processed and analysed as per our previous study (366). Data are reported as mean ± SEM. Statististical analysis are done as per Section 2.2.

7.3 Results:

All the 37 patients completed the study. The mean age group of patients was 27.7 ± 1.4 years and the mean BMI was 33.42 ± 1.6 kg/m^2. There was no change in cycle

length in the atorvastatin pre-treatment group (50 ± 6 vs. 48 ± 10 days) or placebo pre-treated group (52 ± 10 vs. 50 ± 10days).

There were significant improvements in insulin and the HOMA-IR index, total testosterone, FAI, SHBG and hsCRP with metformin in the atorvastatin pre-treated group (Table 1). There were no significant changes in any of these parameters with metformin in the placebo pre-treatment group. The percentage change in HOMA-IR, FAI and hsCRP, were greater in atorvastatin pre-treated group compared to patients who were randomized to placebo pre-treatment.

There was a significant increase in LDL and a non significant increase in total cholesterol following the cessation of atorvastatin and starting metformin. However, there was a further improvement in triglyceride levels with metformin in atorvastatin pre-treated patients. There were no changes in any of the lipid parameters with metformin in placebo pre-treated patients. There was no significant improvement of weight with metformin in either group.

7.4 Discussion

In this study atorvastatin pre-treatment both augmented and facilitated the effect of metformin in the improvement of the metabolic parameters, biochemical hyperandrogenemia and inflammatory markers in patients with PCOS. The improvements of these parameters were independent of the deterioration in LDL and total cholesterol following cessation of atorvastatin. There was a significant reduction in total testosterone with metformin after atorvastatin, over and above the initial changes through atorvastatin treatment. Three months of metformin following 3 months of atorvastatin reduced total testosterone by 31%, FAI by 41% and increased SHBG by

18%, whilst there were no significant improvements in any of these parameters with 12 weeks of metformin following placebo pre-treatment. Statins have been reported to inhibit ovarian theca-interstitial cell proliferation and steroidogenesis in vitro most likely due to reduced availability of testosterone precursors. (236, 238, 239) In addition, statins have also been shown to rapidly activate AMP-activated protein kinase (AMPK), a protein kinase that modulates metabolic homeostasis and energy balance in individual cells and multiple organs (288), both in-vivo and in-vitro (368). Interestingly, this is the same mechanism reported for metformin action and this may explain the augmented effects of metformin following atorvastatin treatment.

This study is also the first to demonstrate the enhanced improvement of insulin resistance and hsCRP with metformin after atorvastatin pre-treatment. There was a reduction of insulin by 16% and HOMA index by 21% that was not seen in patients who had been placebo pre-treated. In total, 12 weeks of atorvastatin followed by 12 weeks of metformin reduced the insulin levels by 33% and HOMA-IR by 35%. There was also a 33% reduction of hsCRP with metformin treatment following atorvastatin. There was a 25% reduction in hs-CRP with atorvastatin in this group of patients with PCOS. This may be important for patients with PCOS who have increased levels hs-CRP (72, 358) that is a predictor of cardiovascular events in women (359, 360).

As expected there was a rise in total cholesterol by 18% and LDL cholesterol by 37%, after stopping the atorvastatin and having 3 months of metformin. However, triglycerides improved further (4%) on metformin after stopping atorvastatin. In total, there was a 24% reduction in triglycerides after 12 weeks of metformin following 12 weeks of atorvastatin that paralleled the improvement in insulin resistance.

There were no significant changes in insulin resistance, biochemical hyperandrogenemia and hsCRP in patients who went on to 12 weeks of metformin treatment following placebo. This is consistent with other studies which shows that metformin may not be effective in all patients with PCOS especially those who are overweight (129, 139, 140).

We made no assessment of clinical hyperandrogenemia or ovulation. As such, the blood tests were done without respect to the bleeding pattern so there may have been temporary hormonal fluctuations interfering with the results. All the patients continued to have irregular periods suggesting that neither atorvastatin pre-treatment nor metformin has overtly affected menstrual function over the 6 months period. Due to study design we have also not looked whether the combination of atorvastatin and metformin would be synergistic.

Statin therapy is contraindicated in any stage of gestation and is recommended to discontinue in anticipation of pregnancy (369). On the other hand, infertility is often an issue in PCOS, contributed by insulin resistance (314, 315). Atorvastatin pre-treatment followed by metformin would be an option in this group of patients in view of augmentation of beneficial effects of metformin following atorvastatin.

In conclusion, the effect of three months metformin treatment was augmented by atorvastatin pre-treatment compared to placebo pre-treatment.

Table 12 Comparison of anthropometric and hormonal parameters at baseline, 12 weeks of atorvastatin or placebo followed by 12 weeks of metformin.

arameter	Atrovastatin pre-treatment group (n=19)							Placebo pretreatment group (n=18)						
	Baseline (V1)	12 weeks (V2) (Atorvastatin 20mg daily)	24 weeks (V3) (Metformin 1.5gm daily)	V1/V2 (pvalue)	V2-V3 (p value)	%change V2-V3	V1-V3 (p value)	Baseline (V1)	12 weeks (V2) (Placebo)	24 weeks (V3) (Metformin 1.5gm daily)	V1/V2 (p value)	V2/V3 (p value)	%change V2-V3	V1- V3 (pvalue)
Veight (kg)	91.29 ± 3.4	91.20 ± 3.4	90.3 ± 3.1	0.42	0.06	-1.0 ± 0.1	0.08	93.12 ± 4.8	93.05 ± 4.7	92.6 ± 3.2	0.67	0.2	-1.0 ± 0.1	0.12
MI (kg/m^2)	33.20 ± 1.4	33.16 ± 1.4	32.83 ± 1.2	0.42	0.06	-0.8 ± 0.1	0.08	33.94 ± 1.5	33.92 ± 1.4	33.52 ± 1.5	0.67	0.2	-0.8 ± 0.1	0.12
Vaist (cm)	98.1 ± 3.2	98.9 ± 2.2	97.70 ± 0.8	0.59	0.62	-1.0 ± 0.3	0.44	99.3 ± 2.4	98.7 ± 2.1	98.2 ± 1.2	0.54	0.32	-1.0 ± 0.2	0.20
'estosterone ımol/L)	4.1 ± 0.2	2.9 ± 0.1	2.7 ± 0.1	<0.01	0.05	-1.0 ± 0.1	<0.01	4.4 ± 0.2	4.3 ± 0.2	4.2 ± 0.8	0.73	0.30	-0.08 ± 0.2	0.18
HBG (nmol/L)	31.1 ± 1.0	35.3 ± 1.2	36.6 ± 1.7	<0.01	0.05	2.0 ± 0.2	<0.01	31.9 ± 0.9	32.9 ± 0.9	32.2 ± 0.8	0.14	0.72	-0.9 ± 0.1	0.8
AI	13.4 ± 0.6	8.7 ± 0.4	7.6 ± 0.9	<0.01	0.04	-13 ± 0.9* p*=0.02	<0.01	13.9 ± 0.6	13.3 ± 0.5	13.1 ± 0.9	0.07	0.72	-3.0 ± 0.1	0.14
;lucose (mmol/L)	4.8 ± 0.1	4.9 ± 0.1	4.6 ± 0.1	0.52	0.64	-5.0 ± 0.8	0.47	4.7 ± 0.5	4.8 ± 0.1	4.6 ± 0.4	0.32	0.42	-2.0 ± 0.9	0.33
ısulin (μIU/mL)	15.6 ± 1.8	12.4 ± 1.7	10.2 ± 1.8	<0.01	0.04	-18.0 ± 1.0	<0.01	14.4 ± 2.0	17.6 ± 2.4	16.4 ± 1.8	0.04	0.88	-6.0 ± 2.0	0.32
[OMA-IR	3.3 ± 0.4	2.7 ± 0.4	2.0 ± 0.8	<0.01	0.04	-24.0 ± 1.0* p*=0.03	<0.01	3.0 ± 0.4	3.8 ± 0.5	3.4 ± 0.3	0.04	0.72	-9.0 ± 1.2	0.36
'C (mmol/L)	4.6 ± 0.2	3.4 ± 0.2	3.9 ± 0.5	<0.01	0.82	4.0 ± 0.9	0.08	4.5 ± 0.2	4.6 ± 0.2	4.5 ± 0.3	0.13	0.34	-0.8 ± 0.1	0.82
DL-C (mmol/L)	2.9 ± 0.2	1.8 ± 0.2	2.8 ± 0.4	<0.01	0.04	9.0 ± 2.0	0.92	2.7 ± 0.2	2.7 ± 0.1	2.9 ± 0.3	0.77	0.62	2.2 ± 0.1	0.7
[DL-C (mmol/L)	1.07 ± 0.1	1.08 ± 0.1	1.07 ± 0.2	0.17	0.20	3.0 ± 0.8	0.43	1.1 ± 0.08	1.1 ± 0.09	1.1 ± 0.1	0.47	0.52	-1.0 ± 0.4	0.70
'G (mmol/L)	1.34 ± 0.08	1.08 ± 0.1	1.01 ± 0.1	<0.01	0.05	-3.0 ± 0.4	<0.01	1.39 ± 0.24	1.69 ± 0.27	1.34 ± 0.1	0.19	0.44	-2.7 ± 0.3	0.82
[s-CRP ng/L)	4.9 ± 1.4	3.4 ± 1.1	2.7 ± 0.8	0.04	0.04	-15 ± 0.1* p*=0.02	0.02	5.8 ± 1.4	5.7 ± 1.6	5.2 ± 1.8	0.90	0.66	-5.0 ± 0.8	0.58

Table 12 - Legend

Atorvastatin pre-treatment group – Atorvastatin for 12 weeks followed by Metformin for 12 weeks.
Placebo pre-treatment group – Placebo for 12 weeks followed by Metformin for 12 weeks.
V1 – Baseline; V2 – 12 weeks from baseline on either atorvastatin or placebo; V3 - 24 weeks from baseline (12 weeks from visit 2 on Metformin 1.5g daily)
p* - p value for percentage difference between both group using unpaired t test
*pvalue < 0.01
Data are presented as mean ± SEM. All serum results are obtained from fasting variables.
All variables were normally distributed
To convert values for testosterone to nanograms per deciliter, divide by 0.03467.
To convert values for SHBG to micrograms per deciliter, divide by 34.7.
To convert values for glucose to milligrams per deciliter, divide by 0.056.
To convert values for insulin to picomoles per liter, multiply by 6.
To convert values for cholesterol to milligrams per deciliter, divide by 0.0259.
To convert values for triglycerides to milligrams per deciliter, divide by 0.0113.
TC - Total cholesterol; LDL-C - LDL-cholesterol; HDL-C - HDL cholesterol; TG-Triglycerides; FAI – Free Androgen Index

Chapter 8

Variability of lipids in patients with type 2 diabetes taking statin treatment: implications for target setting.

8.1 Introduction

While there is very strong evidence that low density lipoprotein (LDL) cholesterol lowering using statin treatment can reduce the incidence of coronary and other major vascular events(332-334, 370), in the United Kingdom there is current controversy and confusion about what lipid targets should be aimed for once a statin drug is initiated. Current guidance from the National Service Framework for Coronary Heart Disease and the National Institute for Health and Clinical Excellence (NICE) recommends targets for serum total cholesterol (TC) of <5mmol/L and LDL of <3mmol/L, and this has formed the basis of the Quality and Outcomes Framework (QOF) of the new General Medical Services (nGMS)(371, 372). However, more recent guidance from the Joint British Societies, published in December 2005(373), recommends patients should aim for a lower target of TC <4mmol/L and an LDL<2mmol/L. This disparity has led the UK's National Director for Heart Disease & Stroke to issue a statement in November 2006 declaring that the previous targets of 5 and 3mmol/L should be kept, at least until revised NICE guidance is published(374).

Whether a patient consistently achieves any target depends on both the lipid lowering ability of the statin and the variability of lipid parameters while on the drug. Thus, a statin drug may be effective at lowering the mean cholesterol in a patient, but if the measurement is extremely variable, then they may not always be below their target when tested. It has been shown that lipid measurements in healthy volunteers and in patients with T2DM who are not on lipid lowering treatment can indeed vary on a day-to-day basis(375-377). However, no study to date has looked at lipid variability in patients already on statin treatment. Indeed, arguments can be made for statin treatment leading to either possible increases or reductions in cholesterol variation within an individual. Added to this is the fact that short half-life statins, such as simvastatin, may have an effect on lipid variability which is quite different to that of a long half-life

statin, such as atorvastatin. This study has therefore aimed to establish how the biological variability of lipids in patients with T2DM who are on statin treatment could influence the ability for patients to maintain cholesterol values below target. In order to assess any difference between long and short half-life statins, we have conducted the investigation as a cross-over study with equivalent doses of simvastatin and atorvastatin.

8.2 Research Design and Methods

Thirty caucasian patients with T2DM for at least 3 years and HbA1c between 6 and 9% were recruited into the study with informed consent. Nineteen patients were taking 10mg atorvastatin before bed and 11 patients on simvastatin 40mg before bed. All the patients were on stable doses of medications for at least 3 months. None of the patients were on additional lipid lowering therapy or over the counter medications. The insulin doses of patients who took insulin were not changed by >10% throughout the study. The patients were advised to maintain their normal diet, alcohol intake, smoking and exercise habits during the study period. Patients with untreated hypothyroidism or nephrotic syndrome were excluded. The biological variation of TC, HDL, LDL and triglycerides was assessed by measuring 12 hour fasting blood samples at four-day intervals on 10 consecutive occasions. Thereafter the patients on simvastatin were changed to the equivalent dose of atorvastatin and vice versa(378). After 3 months, the biological variation of lipid parameters were again assessed by measuring fasting blood samples at four day intervals on 10 consecutive occasions in these patients. Fasting venous blood was collected into serum gel tubes (Becton Dickinson, Oxford, U.K.) at the same time each day (0800–0900) after the patient had been seated for at least 5 minutes and tourniquet was not applied for more than a minute. Samples were separated by centrifugation at 2000*g* for 15 min at 4°C, and two aliquots of the serum were stored at

-20°C within 1 h of collection. All samples were analysed within 12 months of collection, and studies have found no stability issues when stored for this long at this temperature(379). The serum samples were split before assay. Before analysis, all of the serum samples were thawed and thoroughly mixed. The duplicate samples (i.e., two per visit) were randomised and then analysed for cholesterol, triglycerides and HDL cholesterol in a continuous batch on a Synchron LX 20 analyser (Beckman-Coulter, High Wycombe, U.K.) using a single batch of reagents according to our previous studies(380). Lipid assays used calibrators assigned from CDC standards and LDL cholesterol was calculated using the Friedewald formula(277). All subjects gave their informed written consent before entering the study, which had been approved by the Hull and East Riding Local Research Ethics Committee.

Statistical analysis

The CV was used to calculate which mean lipid values would be required to maintain a total cholesterol of <5mmol/L (4.9mmol/L or lower) or <4mmol/L and an LDL of <3mmol/L or <2mmol/L on 95% or more of testing occasions using a one-sided analysis (mean ±1.645xCV)(270). A similar method was used to calculate the value that a single lipid measurement would have to be in order to be 95% confident that (a) the mean for a patient was below target or (b) subsequent measurements would consistently be below target.

8.3: Results

The baseline demographics of patients are given in Table 12. The baseline lipid profile where comparable in both groups. One patient from each group dropped out after completing one arm because of difficulty to adhere to study protocols. One patient who was on atorvastatin initially withdrew from the study due to development of myalgia without any rise in creatine kinase (CK) when changed over to simvastatin. Another

patient on atorvastatin initially withdrew from the study due to development of lethargy while on simvastatin which got better after changing back to atorvastatin. None of the patients developed elevated liver transminases or CK during the study. There was no significant change in glycaemic control during the course of the study in any patients (median ± IQR) (7.72 ± 0.98 vs. 7.69 ± 0.88%, p=0.60).

Table 13 shows the mean lipid values and the biological variability in lipids expressed as standard deviation (SD) and coefficient of variation (CV) in each treatment group. It shows no statistical difference in lipid variability (SD) when the same patients take either simvastatin or atorvastatin. Whether patients started on simvastatin or atorvastatin made no difference to the results.

Table 14 shows the mean values of total cholesterol required to maintain values of <5 or <4mmol/L and to maintain an LDL cholesterol of<3mmol/L or <2mmol/L on up to 95% of occasions. Table 15 shows the concentrations of total cholesterol and LDL required from a single lipid measurement on statin treatment so that two different criteria are met. The first criterion is to be 95% confident that the mean lipid value for a patient is truly below target e.g. that their mean total cholesterol is 4.9mmol/L or lower when the target is 5mmol/L. The second is to be 95% confident that 95% of subsequent lipid measurements are below the specified target. In this regard, Table 14 gives values which show 50% confidence that 95% of subsequent measurements are below target.

8.5 Discussion

This study is the first to show that there is clinically significant biological variability in the lipid profiles of patients with T2DMwho are on statin treatment, and that this does not significantly differ between the short and long half-life statins of simvastatin and atorvastatin. Taken together, the coefficient of variation of total cholesterol suggests that values can vary by approximately ±15% (2 standard deviations from the mean value) in

the same individual on treatment, while that of LDL cholesterol can vary by ±24% before any laboratory analytical variability is also taken into consideration.

Studies which examine the normal biological variation in lipids and lipoproteins have generally been conducted on healthy subjects (375-377), but the biological component should be studied for each disease or treatment state as this may influence the extent of intra-individual variation. A meta-analysis of previously published studies has found that the mean biological variability (CV) found in healthy individuals is ≤6.1%, ≤9.5%, ≤7.4%, and <22.6% for TC, LDL, HDL and TG respectively(279), whereas these figures in patients with T2DM who are not on statins were 5.1%, 8.3%, 4.4% and 17% respectively. In this study the CV of TC on atorvastatin and simvastatin was comparable at 6.9% and 8.2% respectively, while that for LDL is somewhat higher at 10.3 and 13.1% respectively.

The variability in lipids on statin treatment found here have several clinical implications, the first of which relates to the ongoing debate regarding setting lipid targets in patients with or without T2DM. The National Service Framework (NSF) for Coronary Heart Disease (CHD) in 2000 laid down standards of care for the prevention and treatment of CHD (381). It set a target for TC lowering of <5mmol/L and LDL <3mmol/L, or by 20–25% (LDL by 30%), whichever results in the lowest absolute level (381). In contrast, in December 2005 the Joint British Societies' Guidelines (JBS2) guidelines set similar audit targets, but recommended an optimal total cholesterol target level of <4.0mmol/L and an LDL<2.0mmol/L(373). Since the publication of JBS2, a statement from Professor Roger Boyle, National Director for Heart Disease and Stroke(374), points out that current UK national policy for lipid management and current targets for cholesterol levels remain those recommended in the NSF for CHD, and not those recommended by the JBS2. This has been followed by a response from the Association of British Clinical Diabetologists (ABCD) which criticised this statement arguing that in the CARDS study(382) the 30% of patients who had TC and LDL-C levels were below

5 and 3 mmol/L respectively still benefited from statins(383). Diabetes UK has also criticised this decision as current NICE guidance does not account of new evidence in patients with T2DM(384).

The evidence for the new JBS2 targets themselves arose from the findings of the Heart Protection Study, the ASCOT-LLA, PROVE-IT, TNT, REVERSAL and GREACE studies(385-390). In these studies the mean LDL cholesterol in the active or intensively treated groups was 2.3mmol/L, 2.3mmol/L, 1.6mmol/L, 2.0mmol/L, 2.1mmol/L and 2.5mmol/L respectively. However, the target for LDL cholesterol did not become a mean of 2mmol/L, but *less than* 2mmol/L. From our study, as shown in Table 3, we can determine that in order to consistently maintain an LDL of 1.9mmol/L or lower, the mean LDL for someone taking atorvastatin or simvastatin has to be around 1.5-1.6mmol/L or lower. This means the average LDL needs to be lower than that found in the clinical studies from which the target itself is based. This, in turn, is likely to have a knock-on effect regarding the potency, dosage and cost of statins (as well as possibly other agents) required. In addition, if the aim is to achieve a total cholesterol <5mmol/L (as suggested by the Quality and Outcomes Framework (QOF) for UK general practitioners) or an LDL<3mmol/L on a consistent basis then the mean values for a patient needs to be 4.3-4.4 and 2.4-5mmol/L respectively. This means that existing NICE lipid targets may not be as far removed from current evidence than the values of 5 and 3mmol/L suggest, especially given the fact that adding laboratory analytical variation to the biological variation determined here will only reduce these mean values further.

This study has thus highlighted the difference between aiming to consistently have a patient below a cholesterol target at each visit (as demanded by financial incentives schemes such as the QOF) compared to aiming for just their mean value be below the

same target. By definition, the latter means that a patient whose mean is the same as the target value will have half their individual measurements above it, whereas with the former any result above target is unacceptable. One effect of this is difference is shown in Table 4 where a single total cholesterol reading on treatment must be under 3.7-3.9mmol/L before it can be assumed that subsequent measurements will remain below 5mmol/L, whereas this value can be around 0.5mmol/L higher if just a mean below 5mmol/L is desired. Repeated measurements help in this regard but, for example, the mean of 3 measurements still has to be below 4.0-4.1mmol/L to be sure 95% of ensuing results are under 5mmol/L.

There are potential limitations to this study. LDL was calculated using the Friedewald formula rather than using direct measurement or analysis after ultracentrifugation. This means the variability in cholesterol, triglycerides and HDL could each be contributing to the overall variation in LDL found here. There is also a possibility that the Friedewald formula may have limitations when applied to a population with diabetes (391). Nevertheless, this is the means by which most laboratories currently report LDL and none of the patients in the study had triglycerides above the 4.5mmol/L which would prohibit use of the calculation. There is also some evidence that calculating LDL in this way may not in fact lead to any spurious increase in variability, at least compared to direct measurement (392).

Given these difficulties in using estimated LDL in T2DMpatients it is perhaps an opportunity to reassess the utility of non-HDL and apolipoprotein B (apoB) measurement which seem to be equally useful in detecting high-risk phenotypes in hypertriglyceridaemic type 2 diabetic patients, with apoB possibly being superior in normotriglyceridaemic subjects(393). The fact that ApoB can be accurately measured in non-fasting individuals would only strengthen its appeal.

It is reassuring that none of the patients in the study developed serious adverse effects on statins. The 2 patients withdrawals due to non-severe adverse effects is probably in keeping with the 5-10% of patients who develop problems on statin treatment outside trial situations (394).

Our data is also reassuring for patients where a switch from 10mg of atorvastatin to the less costly generic simvastatin 40mg is being considered. Not only were the mean values of TC and LDL achieved by individuals on simvastatin not inferior to that of atorvastatin (which is in accordance with other studies (378)), but there were no significantly differences in lipid variability. This means that the relatively short half-life of simvastatin (2-3 hours) compared to atorvastatin (up to 24 hours)(395) does not seem to influence its ability to keep a patient below their lipid target, at least when taken at night time (396).

In summary, this study has found that in patients with T2DM taking either simvastatin or atorvastatin the mean TC and LDL concentrations needed to keep below target levels are much lower than the target value itself. When evidence for lipid targets are derived from the mean values obtained in patients participating in the different treatment groups of clinical studies, the mean value often becomes the upper target limit for patients.(332-334, 378, 397, 398) In doing so, we have shown that this may lead to lipid targets in patients with diabetes which are significantly lower than the current evidence suggests and thereby more difficult to achieve.

Table13. Baseline characteristics of patients in atorvastatin and simvastatin in patients with T2DM study

	Simvastatin crossed over to Atorvastatin group	Atorvastatin crossed over to Simvastatin group
Total Number of patients completed the study	10	16
Duration of Diabetes Median (25-75 Inter Quartile Range) months	84 (108)	108 (108)
HbA1c % (Mean ± SD)	7.75 ± 0.98	7.69 ± 0.91
Sex (Male:Female)	7:3	10:6
Age (years) Median (Range)	58 (48-76)	64 (46-73)
Body Mass Index (Mean ± SD) Kg/m^2	34.67 ± 6.64	34.45 ± 7.23
Waist circumference (Mean ± SD) cm	119.80 ± 16.01	114.78 ± 17.29
Baseline TC (Mean ± SD) mmol/L	4.08 ± 0.71	3.98 ± 0.49
Baseline LDL-C (Mean ± SD) mmol/L	2.2 ± 0.4	2.18 ± 0.44
Baseline HDL-C (Mean ± SD) mmol/L	1.08 ± 0.23	1.08 ± 0.24
Baseline TG (Median ± IQL) mmol/L	1.9 ± 0.6	1.7 ± 0.7

Table 14
The biological variation of lipid parameters in 26 patients on Simvastatin 40mg and Atorvastatin 10mg.

Lipid Parameters	Simvastatin			Atorvastatin			
	Mean (mmol/L)	SD (mmol/L)	CV (%)	Mean (mmol/L)	SD (mmol/L)	CV (%)	p-value *
Total Cholesterol	3.8	0.3	8.2	4.0	0.3	6.9	0.21
LDL- Cholesterol	2.1	0.3	13.1	2.2	0.2	10.3	0.19
HDL-Cholesterol	1.0	0.1	7.7	1.0	0.1	6.2	0.17
Triglycerides	2.7	0.3	12.1	1.7	0.3	19.7	0.95

SD - Standard deviation of lipid parameter
CV - coefficient of variation
* SD simvastatin vs SD atorvastatin

Table 15

Mean values of total cholesterol and LDL-Cholesterol to achieve different targets on 95% of sampling occasions

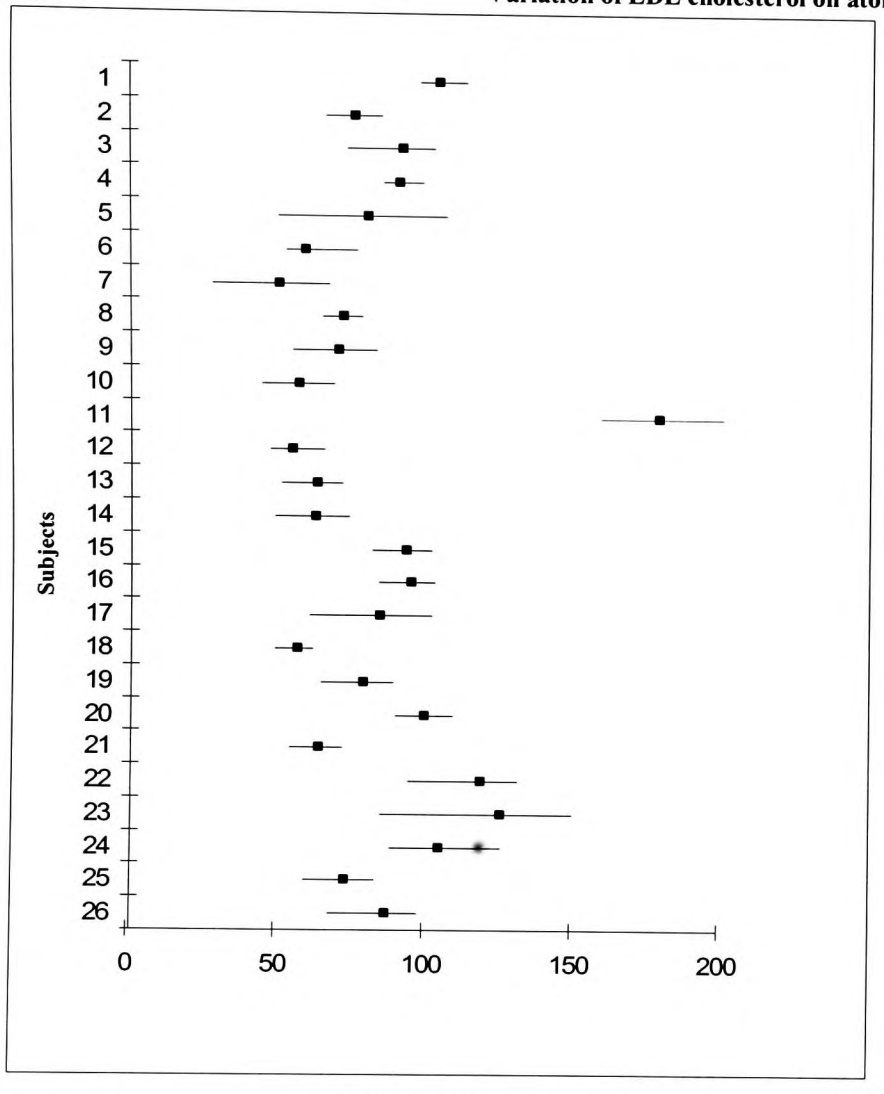
	On Simvastatin 40mg (mmol/L)	On Atorvastatin 10mg (mmol/L)
Mean TC to be achieved to produce a consistent TC < 5mmol/L	4.32	4.40
Mean TC to be achieved to produce a consistent TC < 4mmol/L	3.43	3.50
Mean LDL-C to be achieved to produce a consistent LDL-C < 3 mmol/L	2.39	2.48
Mean LDL-C to be achieved to produce a consistent TC < 2mmol/L	1.56	1.62

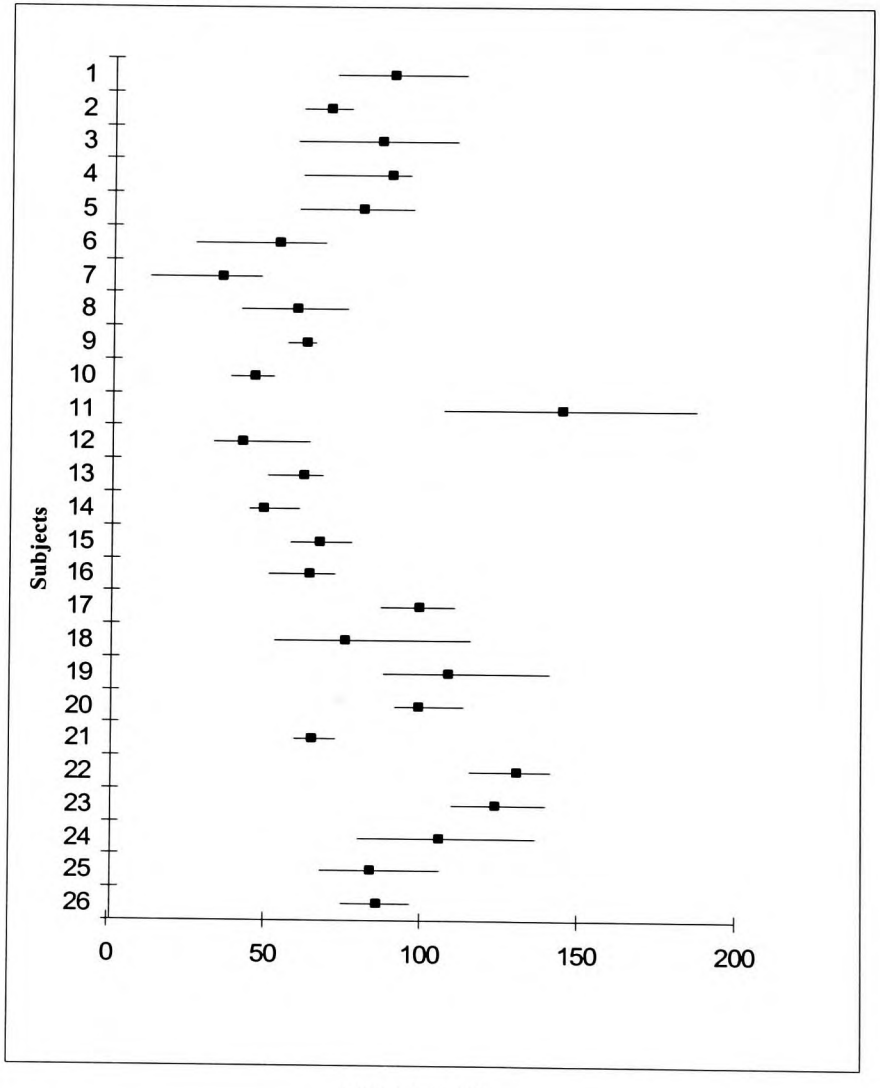
Table 16

Single lipid measurement results which give 95% confidence of the true mean value being below target or of each subsequent measurement being below target

	Simvastatin		Atorvastatin	
	True mean below target	Subsequent measurements below target	True mean below target	Subsequent measurements below target
TC <5mmol/L	<4.11	<3.62	<4.23	<3.80
TC <4mmol/L	<3.27	<2.88	<3.37	<3.02
LDL-C <3mmol/L	<2.16	<1.78	<2.31	<1.98
LDL-C <2mmol/L	<1.41	<1.16	<1.51	<1.23

Figure 4: **Variation of LDL cholesterol on atorvastatin and simvastatin**





Chapter 9

LDL cholesterol variability in patients with type 2 diabetes taking atorvastatin compared to simvastatin: justification for direct measurement?

9.1 Introduction

Hypercholesterolemia is one of the most common risk factors for cardiovascular disease and elevated low density lipoprotein cholesterol (LDL) is the primary target of cholesterol-lowering therapy (399). There is very strong evidence that low density lipoprotein (LDL) cholesterol lowering using statin treatment can reduce the incidence of coronary and other major vascular events (332-334) including patients with type 2 diabetes (382, 400). As a consequence of these studies, lipid targets for patients with diabetes have been established which, in the US, are mainly centered on LDL.

Whether a patient consistently achieves any target depends on both the lipid lowering ability of the statin and the variability of lipid parameters while on the drug. Thus, a statin drug may be effective at lowering the mean cholesterol in a patient, but if the measurement is extremely variable, then they may not always be below their target when tested. It is also not known whether a patient with more variable lipids is at any different a risk of a cardiovascular event than someone with the same mean but much more stable lipid values.

Theoretically, in a situation analogous to antihypertensive medication (401), it could be argued that the stability of lipids while taking a relatively short half-life statin such as simvastatin (2-3 hours) might be different to that of a longer half-life statin like atorvastatin (up to 24 hours)(402). However, in the last chapter we found no statistically significant difference in LDL variability between simvastatin and atorvastatin when calculating LDL using the Friedewald formula (403)

Nevertheless, this equation has many potential sources of error which could lead to a spuriously increased estimation of variability in calculated LDL. This is because the

formula derives LDL from the total cholesterol, the HDL cholesterol and estimates the very-low density lipoproteins (VLDL) cholesterol from the serum triglyceride concentration. Biological variability of all 3 components will therefore be included in the apparent LDL variation. The calculated LDL has also been shown to be less reliable in patients with type 2 diabetes (284).

Homogeneous (direct) assays for LDL have been available for more than a decade, but although they offer potential advantages, uptake of these methods has been slow, partly because it has been difficult to demonstrate any clear clinical benefit over the derived value, despite the latter's inherent limitations (404). One area of promise for the direct assay was the potential to reduce within-individual variability and so lessen the need for averaging serial specimens, but variability was found to be no less than with calculated LDL among untreated individuals (392). No data on the biological variability of directly measured LDL in patients who are taking statin treatment exists, so this study has sought to establish this in patients with type 2 diabetes. In order to assess any difference between long and short half-life statins, we have conducted the investigation as a cross-over study with equivalent doses of simvastatin and atorvastatin.

9.2 Research Design and Methods

Thirty Caucasian patients with type 2 diabetes for at least 3 years and HbA1c between 6 and 9% were recruited into the study with informed consent. Nineteen patients were taking 10mg atorvastatin before bed and 11 patients on simvastatin 40mg before bed. All the patients were on stable doses of medications for at least 3 months. None of the patients were on additional lipid lowering therapy or over the counter medications. The insulin doses of patients who took insulin were not changed by >10% throughout the

study. The patients were advised to maintain their normal diet, alcohol intake, smoking and exercise habits during the study period. Patients with untreated hypothyroidism or nephrotic syndrome were excluded. The biological variation of LDL was assessed by measuring 12 hour fasting blood samples at four-day intervals on 10 consecutive occasions. Thereafter the patients on simvastatin were changed to the equivalent dose of atorvastatin and vice versa. After 3 months, the biological variation of lipid parameters were again assessed by measuring fasting blood samples at four day intervals on 10 consecutive occasions in these patients. Fasting venous blood was collected into serum gel tubes (Becton Dickinson, Oxford, U.K.) at the same time each day (0800–0900) after the patient had been seated for at least 5 minutes and tourniquet was not applied for more than a minute. Samples were separated by centrifugation at 2000*g* for 15 min at 4°C, and two aliquots of the serum were stored at -20°C within 1 h of collection. The serum samples were split before assay. Before analysis, all of the serum samples were thawed and thoroughly mixed. According to our previous studies(405, 406), duplicate samples (i.e., two per visit) were randomised and then analysed using a single batch of reagents for direct LDL using a Synchron DxC analyser (Beckman-Coulter, High Wycombe, U.K.) using LDL reagents and calibrators. It is a homogeneous assay which uses a detergent to solubilize the non-LDL lipoprotein particles. After removal, a second detergent then solubilizes the remaining LDL which is then measured. The sensitivity for the direct LDL assay is <8 mg/dL (<0.21 mmol/L). The imprecision of the assay was as determined using duplicate sample as described below. All subjects gave their informed written consent before entering the study, which had been approved by the

Hull and East Riding Local Research Ethics Committee. Statistical analysis was done as per Section 2.

9.3 Results

The baseline demographics of patients are given in Table 12. One patient from each group dropped out after completing one arm because of difficulty to adhere to study protocols. One patient who was on atorvastatin initially withdrew from the study due to development of myalgia without any rise in creatine kinase (CK) when changed over to simvastatin. Another patient on atorvastatin initially withdrew from the study due to development of lethargy while on simvastatin which got better after changing back to atorvastatin. None of the patients developed elevated liver transminases or CK during the study.

The (mean ± SEM) LDL concentration on atorvastatin 10mg was no different than when taking simvastatin 10mg (1.67±0.60mmol/L vs. 1.69±0.60 respectively, $p=0.19$ using an unpaired t-test). In contrast, the variability in LDL, expressed as SD, was much lower on atorvastatin (average SD ± SEM) (0.17±0.02mmol/L) than on simvastatin (0.01±0.003mmol/L, $p<0.0001$) (figure 1). This equated to a coefficient of variance (CV) of 0.85% for atorvastatin and 10.7% for simvastatin.

Table 2 shows the mean values of LDL required to maintain values of <70, <77 and <100 mg/dL on 95% of occasions.

9.4 Discussion

This study has shown that the biological variability of LDL cholesterol, when measured using a direct assay method, is substantially lower when patients with type 2 diabetes take atorvastatin 10mg compared to simvastatin 40mg daily. This is despite the mean LDL values on both treatments being the same.

These findings contrast with the biological variability we found when LDL was calculated using the Friedewald equation on the same samples (277). On simvastatin, the CV for calculated LDL was 13.1% compared to 10.7% found here using the direct LDL method. For atorvastatin the difference was much larger, with a CV for calculated LDL being 10.3%, but <1% for direct LDL. Indeed, in every patient the variability was less when taking atorvastatin than with simvastatin. The reason for the reduction in variability with the direct assay is probably because the measurement is, as discussed above, not influenced by the cumulative variabilities of total cholesterol, HDL cholesterol and serum triglycerides.

This difference seen between the 2 statins has clinical implications for achieving LDL targets. The American Diabetes Association position statement on dyslipidemia management in adults with diabetes recommended lowering LDL cholesterol to <100 mg/dL (2.6 mmol/L) as the primary goal of therapy for patients with type 2 diabetes (407). There is an argument that this should be lower still (332), with the recent LDL target from the UK National Institute for Health and Clinical Excellence (NICE) reflecting this by being <2mmol/L (<77g/dL)(408). Based on this study, since direct LDL within the same individual varies by approximately ± 20% when taking simvastatin compared to only ± 2% for atorvastatin then a much lower mean LDL needs to be aimed for with simvastatin than atorvastatin to ensure the patient consistently achieves their target goal over time (table 2). Looked at in another way, it means that while a single measurement on atorvastatin (in a perfectly performing LDL assay) could predict the true mean LDL for that patient within ±2%, the same individual would require their LDL to be measured more than 100 times before the same could be said of

a patient taking simvastatin. In turn, this means that a patient taking atorvastatin may require less in the way of statin dose titration and monitoring, especially if a direct LDL assay is being used, that will also reflect in an economic benefit. Also, the extremely low biological variability of direct LDL rather than total cholesterol (CV<1% vs. 6.9%), may indicate this is the preferred way of assessing lipid response to statin treatment.

It has recently been suggested that lipid monitoring amongst patients taking statin treatment may be of limited value because of the biological and analytical variability of lipids present while taking pravastatin in the LIPID (Long-Term Intervention with Pravastatin in Ischaemic Disease) study (409). This current study may go someway towards explaining the LIPID findings, firstly because a short half-life drug was studied and secondly because a direct LDL assay was not used.

There is consistent evidence that intermittent or non-adherence to statin treatment- no doubt associated with abrupt variations in lipids- is associated with poorer outcomes for patients (410, 411). However, it remains a matter of speculation as to whether the greater variability of LDL with simvastatin found here may have an influence on plaque stability and, ultimately, vascular events. Certainly, cardiovascular risk does rise exponentially, rather than linearly, as LDL rises (412-414). Thus, although a patient on a treatment causing more variable LDL will be spending the same time above and below their mean value as another with comparatively stable LDL, their average risk may be higher because their periods of LDL far above their mean will be placing them at especially high risk. This will more than cancel out any reduction in risk caused by them also having equal periods far below their mean. It has also been shown that cardiovascular events rate was favourable with atorvastatin compared to simvastatin in

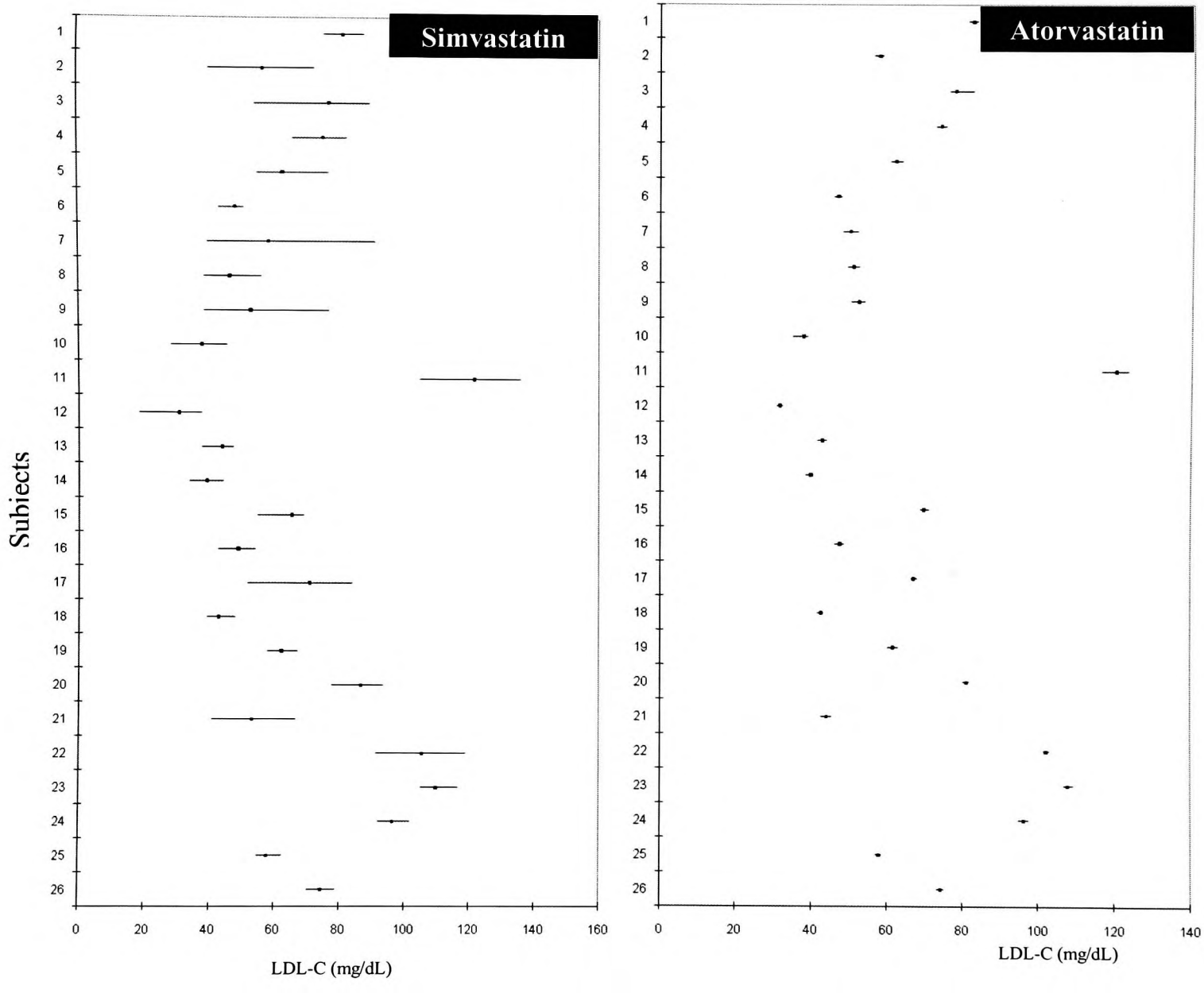
both randomized controlled studies(415) and observational studies (416-418). However, with the five 'treat to lower targets' studies the 2 least impressive reductions in CV risk per mmol/L reduction in LDL were SEARCH(419) and A to Z(420) which used simvastatin. The studies which used atorvastatin, PROVE-IT(421), TNT(422) and IDEAL(333) produced greater reductions in cardiovascular risk per mmol/L reduction in LDL. This could be probably attributed to lesser variability of LDL with atorvastatin compared to simvastatin. Until now there has been no proven advantage of direct vs. calculated LDL (404) but that we have shown direct LDL is potentially most useful in patients on statin treatment.

In summary, this study has found marked differences in the biological variability of directly measured LDL when taking simvastatin compared to atorvastatin, which is in clear contrast the lack of difference found when calculating LDL. While it is unknown whether the increased variability of LDL on simvastatin can influence cardiovascular risk, it certainly means that maintaining an LDL concentration below target will be more difficult to achieve than when using longer half-life treatments such as atorvastatin. Our findings have also shown that direct LDL measurement seems to have a potential clinical advantage over calculated LDL when used in patients taking statin treatment.

Table 17. Mean values of direct LDL-Cholesterol to consistently achieve different targets

	On Simvastatin 40mg mg/dL(mmol/L)	**On Atorvastatin 10mg mg/dL (mmol/L)**
Target of LDL cholesterol to be achieved to produce an LDL consistently < 100 mg/dL (2.6 mmol/L)	85 (2.21)	99 (2.56)
Target of LDL cholesterol to be achieved to produce an LDL consistently < 77 mg/dL (2.0 mmol/L)	65 (1.69)	76 (1.98)
Target of LDL cholesterol to be achieved to produce an LDL consistently < 70 mg/dl (1.8 mmol/L)	60 (1.56)	69 (1.79)

Figure 5 Legend
Means (range) of direct LDL-Cholesterol on Simvastatin 40mg and Atorvastatin 10mg daily.


Simvastatin
Atorvastatin
Subjects
LDL-C (mg/dL)
LDL-C (mg/dL)

Chapter 10

Summary Discussion

This thesis has examined three distinct but related aspects of endocrinology where currently many unresolved issues exist. First is the effect of weight loss with the endocannabinoid receptor blocker, rimonabant, when compared to metformin which is the standard therapy of choice in patients with PCOS. Second is the potential pleotrophic effect of medication, atorvastatin in therapeutic reduction of insulin resistance and hyperandrogenemia in patients with PCOS. Third is the biological variability of various lipid parameters including direct LDL with simvastatin and atorvastatin in patients with T2DM which will have implications for achieving specific lipid targets and may influence cardiovascular risk.

Obesity is present in varying degrees in women with PCOS and is associated with hyperandrogenaemia and insulin resistance (286). Even modest weight loss of less than 10% of initial body weight has been shown to increase the metabolic parameters and biochemical hyperandrogenemia in women with PCOS (146, 147). Metformin is commonly used in patients with PCOS and is reported to improve body weight, insulin resistance, sex hormone binding globulin (SHBG) and hyperandrogenaemia (133, 287). However, it has been shown to be of limited use in very obese women with polycystic ovary syndrome in some studies (140). However rimonabant, has shown an improvement in metabolic syndrome, waist circumference, lipid parameters and particularly insulin resistance in obese subjects (291-293). It was shown that weight reduction through rimonabant 20mg per day improved both hyperandrogenaemia and insulin resistance and was a more effective insulin sensitisor than metformin in this obese PCOS group over the 3 month period. Subsequent treatment with metformin for 3 months in obese patients with PCOS maintained the improvement of weight loss and

enhanced the metabolic and biochemical parameters achieved by treatment with rimonabant compared to 6 months of metformin treatment. There was a significant and reversible increase in GIP levels after 3 months of rimonabant treatment but no changes in either GLP-1 or GIP levels with metformin. This suggests that the metabolic effects of rimonabant in patients with PCOS might be partly due to its influence on GIP metabolism in addition to its known endocannabinoid blocking effect.

Rimonabant has been recently withdrawn from market in view of its psychiatric side effects since rimonabant increases the risk of psychiatric adverse events – ie depressed mood disorders and anxiety (191). In view of this the European Medicines Age ncy (EMEA), the European Union (EU) body which is responsible for monitoring the safety of medicines, has concluded that the benefits of rimonabant no longer outweigh its risks and the market authorisation was suspended across the EU from July 2008. However, there are new endocannabinoid receptor blockers in development. It is unknown whether the newer agents will have the same beneficial effects in patients with PCOS with comparitevely less side effects. It is also not studied whether the weight loss effect of endocannabinoid blockers in PCOS is mediated via modulating oroxogenic hormones like ghrelin and peptide YY (PYY). It is also not clear regarding the cellular effect of rimonabant in altering adipokines, cytokines and interleukins.

Statins have been shown to reduce cardiovascular morbidity and mortality in several studies (332-334). They also have other, non-lipid lowering effects, demonstrated by their benefits amongst hypertensive patients with normal lipids (335) as well as their anti-inflammatory effect in patients with rheumatoid arthritis(336). Other pleiotropic effects of statins include improvement in endothelial dysfunction, increased nitric oxide

bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques(337). We looked into the potential pleotrophic effect of atorvastatin in patients with polycystic ovary syndrome. It was shown that atorvastatin is effective in reducing inflammation, biochemical hyperandrogenemia and metabolic parameters in patients with polycystic ovary syndrome after a 12 week period compared to placebo. The effect of three months metformin treatment was augmented by atorvastatin pre-treatment compared to placebo pre-treatment.

A reduction in insulin resistance with atorvastatin may explain other findings such as reduced development of diabetes amongst patients treated with pravastatin in the WOSCOPS trial (364, 365). There is a potential avenue to explore the precise mechanism involved in improvement of insulin resistance with statins in patients with insulin resistant states using techniques such as insulin clamp studies.

Statin therapy is contraindicated in any stage of gestation and is recommended to discontinue in anticipation of pregnancy (369). On the other hand, infertility is often an issue in PCOS, contributed by insulin resistance (314, 315). Atorvastatin pre-treatment followed by metformin could be an option in this group of patients in view of augmentation of beneficial effects of metformin following atorvastatin.

This study has also raised a number of unanswered questions which could be explored by further studies. The effect of combination of atorvastatin and metformin has not been looked at. Whether any other statin like short half life statin, simvastatin or more potent statin like rosuvastatin could be better than atorvastatin is not known. Similar to study with rimonabant, this study is or relatively shorter duration and longer duration studies are needed to look into the effect of statins on ovulation and clinical hirsutism.

It would also be interesting to look into the effect of statins on adipocytokines and interleukins in patients with PCOS. Of course, longer time studies are needed to see whether this beneficial effect of statins translates into improved cardiovascular outcome.

T2DM is another area with relative high insulin resistant state where statins have been shown to reduce cardiovascular morbidity and mortality. There is very strong evidence that low density lipoprotein (LDL) cholesterol lowering using statin treatment can reduce the incidence of coronary and other major vascular events (332-334, 370). Hence, accurate and precise estimations of LDL concentrations are necessary to appropriately identify individuals with hypercholesterolemia and to monitor response to treatment. There were no statistically significant difference in LDL variability between simvastatin and atorvastatin when calculating LDL using the Friedewald formula. However, in patients with T2DM taking either simvastatin or atorvastatin the mean TC and LDL concentrations needed to consistently remain below a target are much lower than the target value itself which means that guideline target limits extrapolated from the mean values of patients participating in clinical studies may overestimate the lipid reductions required. Interestingly, directly measured LDL cholesterol is an order of magnitude more stable when taking equivalent doses of atorvastatin rather than simvastatin. This means LDL targets can be consistently met at higher mean LDL concentrations (and with less lipid monitoring) using atorvastatin rather than simvastatin. Whether this relative instability of LDL on simvastatin may influence its ability to reduce cardiovascular events should be explored further.

Given these difficulties in using estimated LDL in T2DM patients, the variability of non-HDL and apolipoprotein B (apoB) measurement which could be accurately measured in non-fasting individuals should be explored further which seem to be equally useful in detecting high-risk phenotypes in hypertriglyceridaemic type 2 diabetic patients, with apoB possibly being superior in normotriglyceridaemic subjects (393).

In conclusion both weight loss using rimonabant and atorvastatin is effective in reducing biochemical hyperandrogenemia and metabolic profile in patients with PCOS. The effect of rimonabant might be partly mediated through modulating Glucose-dependent insulinotropic polypeptide (GIP) levels. There is a significant biological variation in lipid profile in patients with T2DM who are on simvastatin and atorvastatin which may lead to lipid targets in patients with diabetes which are significantly lower than the current evidence suggests and thereby more difficult to achieve. However LDL targets can be consistently met at higher mean LDL concentrations (and with less lipid monitoring) using atorvastatin rather than simvastatin.

References

1. **Bell PM** 1996 Clinical significance of insulin resistance. Diabet Med 13:504-509
2. **Zavaroni I, Bonora E, Pagliara M, Dall'Aglio E, Luchetti L, Buonanno G, Bonati PA, Bergonzani M, Gnudi L, Passeri M, et al.** 1989 Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 320:702-706
3. **Reaven GM** 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607
4. **Yki-Jarvinen H** 1995 Role of insulin resistance in the pathogenesis of NIDDM. Diabetologia 38:1378-1388
5. **Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV** 1986 Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. N Engl J Med 315:215-219
6. **Laakso M, Sarlund H, Salonen R, Suhonen M, Pyorala K, Salonen JT, Karhapaa P** 1991 Asymptomatic atherosclerosis and insulin resistance. Arterioscler Thromb 11:1068-1076
7. **Bressler P, Bailey SR, Matsuda M, DeFronzo RA** 1996 Insulin resistance and coronary artery disease. Diabetologia 39:1345-1350
8. **Welborn TA, Wearne K** 1979 Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diabetes Care 2:154-160
9. **Pyorala K, Savolainen E, Kaukola S, Haapakoski J** 1985 Plasma insulin as coronary heart disease risk factor: relationship to other risk factors and predictive value during 9 1/2-year follow-up of the Helsinki Policemen Study population. Acta Med Scand Suppl 701:38-52
10. **Eschwege E, Richard JL, Thibult N, Ducimetiere P, Warnet JM, Claude JR, Rosselin GE** 1985 Coronary heart disease mortality in relation with diabetes, blood glucose and plasma insulin levels. The Paris Prospective Study, ten years later. Horm Metab Res Suppl 15:41-46
11. **Despres JP, Lamarche B, Mauriege P, Cantin B, Dagenais GR, Moorjani S, Lupien PJ** 1996 Hyperinsulinemia as an independent risk factor for ischemic heart disease. N Engl J Med 334:952-957
12. **Orchard TJ, Eichner J, Kuller LH, Becker DJ, McCallum LM, Grandits GA** 1994 Insulin as a predictor of coronary heart disease:

interaction with apolipoprotein E phenotype. A report from the Multiple Risk Factor Intervention Trial. Ann Epidemiol 4:40-45

13. **Welin L, Eriksson H, Larsson B, Ohlson LO, Svardsudd K, Tibblin G** 1992 Hyperinsulinaemia is not a major coronary risk factor in elderly men. The study of men born in 1913. Diabetologia 35:766-770
14. **Ferrara A, Barrett-Connor EL, Edelstein SL** 1994 Hyperinsulinemia does not increase the risk of fatal cardiovascular disease in elderly men or women without diabetes: the Rancho Bernardo Study, 1984-1991. Am J Epidemiol 140:857-869
15. **Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S** 1987 Insulin resistance in essential hypertension. N Engl J Med 317:350-357
16. **Haffner SM, Ferrannini E, Hazuda HP, Stern MP** 1992 Clustering of cardiovascular risk factors in confirmed prehypertensive individuals. Hypertension 20:38-45
17. **Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP** 1992 Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes 41:715-722
18. **Laws A, King AC, Haskell WL, Reaven GM** 1991 Relation of fasting plasma insulin concentration to high density lipoprotein cholesterol and triglyceride concentrations in men. Arterioscler Thromb 11:1636-1642
19. **Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM** 1988 Low-density lipoprotein subclass patterns and risk of myocardial infarction. Jama 260:1917-1921
20. **Zavaroni I, Bonora E, Pagliara M, Dall'Aglio E, Luchetti L, Buonanno G, Bonati PA, Bergonzani M, Gnudi L, Passeri M, et al.** 1989 Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. N Engl J Med 320:702-706
21. **Vague P, Juhan-Vague I, Aillaud MF, Badier C, Viard R, Alessi MC, Collen D** 1986 Correlation between blood fibrinolytic activity, plasminogen activator inhibitor level, plasma insulin level, and relative body weight in normal and obese subjects. Metabolism 35:250-253
22. **Mykkanen L, Ronnemaa T, Marniemi J, Haffner SM, Bergman R, Laakso M** 1994 Insulin sensitivity is not an independent

determinant of plasma plasminogen activator inhibitor-1 activity. Arterioscler Thromb 14:1264-1271

23. **Juhan-Vague I, Alessi MC, Vague P** 1991 Increased plasma plasminogen activator inhibitor 1 levels. A possible link between insulin resistance and atherothrombosis. Diabetologia 34:457-462
24. **Vallisneri A** 1721 Storia della generazione dell'uomo e dell 'animale.
25. **Chereau A** 1844 Maladies des ovaries. Fortin, Masson et Libraires-Editeurs
26. **Archard MC TM** 1921 Le virilism pilaire et son associacion a' l' 'insuffiisance glycolytique (Diabetes de femmes a barbe.). Bull Acad Natl Med 86:51-64
27. **Achard C Thiers J** 1921 Le Virilisme pilaire et son association a l'insuffisance glycolytique (diabete des femmes abarb). Bull Acad Natl Med 86:51-64
28. **Stein I.F LML** 1935 Amenorrhea associated with bilateral polycystic ovaries. . Am J Obstet Gynecol 29:181-191
29. **Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F** 1976 Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 57:1320-1329
30. **Burghen GA, Givens JR, Kitabchi AE** 1980 Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 50:113-116
31. **Swanson M, Sauerbrei EE, Cooperberg PL** 1981 Medical implications of ultrasonically detected polycystic ovaries. J Clin Ultrasound 9:219-222
32. **Adams J, Polson DW, Franks S** 1986 Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. Br Med J (Clin Res Ed) 293:355-359
33. **Gjonnaess H** 1984 Polycystic ovarian syndrome treated by ovarian electrocautery through the laparoscope. Fertil Steril 41:20-25
34. **Carmina E, Lobo RA** 1999 Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. J Clin Endocrinol Metab 84:1897-1899
35. **Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, Jacobs HS** 1995 Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Hum Reprod 10:2107-2111
36. 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41-47

37. **Lunde O** 1984 A study of body hair density and distribution in normal women. American journal of physical anthropology 64:179-184
38. **Lunde O, Grottum P** 1984 Body hair growth in women: normal or hirsute. American journal of physical anthropology 64:307-313
39. **Ferriman D, Gallwey JD** 1961 Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 21:1440-1447
40. **Taieb J, Mathian B, Millot F, Patricot MC, Mathieu E, Queyrel N, Lacroix I, Somma-Delpero C, Boudou P** 2003 Testosterone measured by 10 immunoassays and by isotope-dilution gas chromatography-mass spectrometry in sera from 116 men, women, and children. Clin Chem 49:1381-1395
41. **Azziz R** 2005 Diagnostic criteria for polycystic ovary syndrome: a reappraisal. Fertil Steril 83:1343-1346
42. **Goldzieher JW, Axelrod LR** 1963 Clinical and Biochemical Features of Polycystic Ovarian Disease. Fertil Steril 14:631-653
43. **De Leo V, la Marca A, Petraglia F** 2003 Insulin-Lowering Agents in the Management of Polycystic Ovary Syndrome. Endocr Rev 24:633-667
44. **Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J** 1999 Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 22:141-146
45. **Dunaif A, Segal KR, Futterweit W, Dobrjansky A** 1989 Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. Diabetes 38:1165-1174
46. **Chang RJ, Nakamura RM, Judd HL, Kaplan SA** 1983 Insulin resistance in nonobese patients with polycystic ovarian disease. J Clin Endocrinol Metab 57:356-359
47. **Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS** 1992 Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 75:577-583
48. **O'Driscoll JB, Mamtora H, Higginson J, Pollock A, Kane J, Anderson DC** 1994 A prospective study of the prevalence of clear-cut endocrine disorders and polycystic ovaries in 350 patients presenting with hirsutism or androgenic alopecia. Clin Endocrinol (Oxf) 41:231-236
49. **Legro RS, Castracane VD, Kauffman RP** 2004 Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstet Gynecol Surv 59:141-154

50. **Lanzone A, Caruso A, Di Simone N, De Carolis S, Fulghesu AM, Mancuso S** 1995 Polycystic ovary disease. A risk factor for gestational diabetes? J Reprod Med 40:312-316
51. **de Vries MJ, Dekker GA, Schoemaker J** 1998 Higher risk of preeclampsia in the polycystic ovary syndrome. A case control study. Eur J Obstet Gynecol Reprod Biol 76:91-95
52. **Diamant YZ, Rimon E, Evron S** 1982 High incidence of preeclamptic toxemia in patients with polycystic ovarian disease. Eur J Obstet Gynecol Reprod Biol 14:199-204
53. **Nagamani M, Stuart CA, Dunhardt PA, Doherty MG** 1991 Specific binding sites for insulin and insulin-like growth factor I in human endometrial cancer. Am J Obstet Gynecol 165:1865-1871
54. **Nagamani M, Stuart CA, Doherty MG** 1992 Increased steroid production by the ovarian stromal tissue of postmenopausal women with endometrial cancer. J Clin Endocrinol Metab 74:172-176
55. **Troisi R, Potischman N, Hoover RN, Siiteri P, Brinton LA** 1997 Insulin and endometrial cancer. Am J Epidemiol 146:476-482
56. **Potischman N, Hoover RN, Brinton LA, Siiteri P, Dorgan JF, Swanson CA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD, Persky V, Lurain JR** 1996 Case-control study of endogenous steroid hormones and endometrial cancer. J Natl Cancer Inst 88:1127-1135
57. **Smyczek-Gargya B, Geppert M** 1992 Endometrial cancer associated with polycystic ovaries in young women. Pathol Res Pract 188:946-948; discussion 948-950
58. **Ho SP, Tan KT, Pang MW, Ho TH** 1997 Endometrial hyperplasia and the risk of endometrial carcinoma. Singapore Med J 38:11-15
59. **Legro RS, Kunselman AR, Dodson WC, Dunaif A** 1999 Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 84:165-169
60. **Elting MW, Korsen TJ, Bezemer PD, Schoemaker J** 2001 Prevalence of diabetes mellitus, hypertension and cardiac complaints in a follow-up study of a Dutch PCOS population. Hum Reprod 16:556-560
61. **Dunaif A** 1993 Insulin resistance in polycystic ovarian syndrome. Ann N Y Acad Sci 687:60-64
62. **Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ** 2001 Relative risk of conversion from normoglycaemia to impaired

glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod 16:1995-1998

63. **Bjorntorp P** 1996 The android woman--a risky condition. J Intern Med 239:105-110
64. **Holte J, Gennarelli G, Wide L, Lithell H, Berne C** 1998 High prevalence of polycystic ovaries and associated clinical, endocrine, and metabolic features in women with previous gestational diabetes mellitus. J Clin Endocrinol Metab 83:1143-1150
65. **Rebuffe-Scrive M, Cullberg G, Lundberg PA, Lindstedt G, Bjorntorp P** 1989 Anthropometric variables and metabolism in polycystic ovarian disease. Horm Metab Res 21:391-397
66. **Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, Kuller L** 1995 Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol 15:821-826
67. **Talbott E, Clerici A, Berga SL, Kuller L, Guzick D, Detre K, Daniels T, Engberg RA** 1998 Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. J Clin Epidemiol 51:415-422
68. **Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB** 1985 Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J Clin Endocrinol Metab 61:946-951
69. **Wild RA** 1995 Obesity, lipids, cardiovascular risk, and androgen excess. Am J Med 98:27S-32S
70. **Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ** 1994 Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. Metabolism 43:647-654
71. **Diamanti-Kandarakis E, Spina G, Kouli C, Migdalis I** 2001 Increased endothelin-1 levels in women with polycystic ovary syndrome and the beneficial effect of metformin therapy. J Clin Endocrinol Metab 86:4666-4673
72. **Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N** 2001 Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 86:2453-2455

73. **Paradisi G, Steinberg HO, Hempfling A, Cronin J, Hook G, Shepard MK, Baron AD** 2001 Polycystic ovary syndrome is associated with endothelial dysfunction. Circulation 103:1410-1415
74. **Lakhani K, Constantinovici N, Purcell WM, Fernando R, Hardiman P** 2000 Internal carotid-artery response to 5% carbon dioxide in women with polycystic ovaries. Lancet 356:1166-1167
75. **Kelly CJ, Speirs A, Gould GW, Petrie JR, Lyall H, Connell JM** 2002 Altered vascular function in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 87:742-746
76. **Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH** 2000 Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thromb Vasc Biol 20:2414-2421
77. **Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy PF, 2nd, Fitzpatrick LA** 2003 Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:2562-2568
78. **Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA, Schwartz RS** 1998 Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol 31:126-133
79. **Beadenkopf WG, Daoud AS, Love BM** 1964 Calcification in the Coronary Arteries and Its Relationship to Arteriosclerosis and Myocardial Infarction. Am J Roentgenol Radium Ther Nucl Med 92:865-871
80. **Dahlgren E, Janson PO, Johansson S, Lapidus L, Oden A** 1992 Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. Acta Obstet Gynecol Scand 71:599-604
81. **Wild S, Pierpoint T, McKeigue P, Jacobs H** 2000 Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf) 52:595-600
82. **Dunaif A** 1997 Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 18:774-800

83. **Moghetti P, Tosi F, Castello R, Magnani CM, Negri C, Brun E, Furlani L, Caputo M, Muggeo M** 1996 The insulin resistance in women with hyperandrogenism is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. J Clin Endocrinol Metab 81:952-960
84. **Nestler JE, Singh R, Matt DW, Clore JN, Blackard WG** 1990 Suppression of serum insulin level by diazoxide does not alter serum testosterone or sex hormone-binding globulin levels in healthy, nonobese women. Am J Obstet Gynecol 163:1243-1246
85. **Geffner ME, Kaplan SA, Bersch N, Golde DW, Landaw EM, Chang RJ** 1986 Persistence of insulin resistance in polycystic ovarian disease after inhibition of ovarian steroid secretion. Fertil Steril 45:327-333
86. **Ciaraldi TP, Morales AJ, Hickman MG, Odom-Ford R, Yen SS, Olefsky JM** 1998 Lack of insulin resistance in fibroblasts from subjects with polycystic ovary syndrome. Metabolism 47:940-946
87. **el-Roeiy A, Chen X, Roberts VJ, LeRoith D, Roberts CT, Jr., Yen SS** 1993 Expression of insulin-like growth factor-I (IGF-I) and IGF-II and the IGF-I, IGF-II, and insulin receptor genes and localization of the gene products in the human ovary. J Clin Endocrinol Metab 77:1411-1418
88. **el-Roeiy A, Chen X, Roberts VJ, Shimasakai S, Ling N, LeRoith D, Roberts CT, Jr., Yen SS** 1994 Expression of the genes encoding the insulin-like growth factors (IGF-I and II), the IGF and insulin receptors, and IGF-binding proteins-1-6 and the localization of their gene products in normal and polycystic ovary syndrome ovaries. J Clin Endocrinol Metab 78:1488-1496
89. **Froesch ER, Zapf J** 1985 Insulin-like growth factors and insulin: comparative aspects. Diabetologia 28:485-493
90. **LeRoith D, Werner H, Beitner-Johnson D, Roberts CT, Jr.** 1995 Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocr Rev 16:143-163
91. **Barbieri RL, Ryan KJ** 1983 Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. Am J Obstet Gynecol 147:90-101
92. **Poretsky L, Kalin MF** 1987 The gonadotropic function of insulin. Endocr Rev 8:132-141
93. **Willis D, Franks S** 1995 Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin

receptor and not the type-I insulin-like growth factor receptor. J Clin Endocrinol Metab 80:3788-3790

94. **Willis D, Mason H, Gilling-Smith C, Franks S** 1996 Modulation by insulin of follicle-stimulating hormone and luteinizing hormone actions in human granulosa cells of normal and polycystic ovaries. J Clin Endocrinol Metab 81:302-309
95. **Nestler JE, Barlascini CO, Matt DW, Steingold KA, Plymate SR, Clore JN, Blackard WG** 1989 Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 68:1027-1032
96. **Prelevic GM, Wurzburger MI, Balint-Peric L, Nesic JS** 1990 Inhibitory effect of sandostatin on secretion of luteinising hormone and ovarian steroids in polycystic ovary syndrome. Lancet 336:900-903
97. **Dunaif A, Scott D, Finegood D, Quintana B, Whitcomb R** 1996 The insulin-sensitizing agent troglitazone improves metabolic and reproductive abnormalities in the polycystic ovary syndrome. J Clin Endocrinol Metab 81:3299-3306
98. **Plymate SR, Matej LA, Jones RE, Friedl KE** 1988 Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. J Clin Endocrinol Metab 67:460-464
99. **Nestler JE** 1993 Sex hormone-binding globulin: a marker for hyperinsulinemia and/or insulin resistance? J Clin Endocrinol Metab 76:273-274
100. **Dunaif A, Green G, Futterweit W, Dobrjansky A** 1990 Suppression of hyperandrogenism does not improve peripheral or hepatic insulin resistance in the polycystic ovary syndrome. J Clin Endocrinol Metab 70:699-704
101. **Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T** 1992 Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. Diabetes 41:1257-1266
102. **Polderman KH, Gooren LJ, Asscheman H, Bakker A, Heine RJ** 1994 Induction of insulin resistance by androgens and estrogens. J Clin Endocrinol Metab 79:265-271
103. **Dunaif A, Xia J, Book CB, Schenker E, Tang Z** 1995 Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest 96:801-810

104. **Mahabeer S, Naidoo C, Norman RJ, Jialal I, Reddi K, Joubert SM** 1990 Metabolic profiles and lipoprotein lipid concentrations in non-obese and obese patients with polycystic ovarian disease. Horm Metab Res 22:537-540
105. **Guzick DS, Talbott EO, Sutton-Tyrrell K, Herzog HC, Kuller LH, Wolfson SK, Jr.** 1996 Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. Am J Obstet Gynecol 174:1224-1229; discussion 1229-1232
106. **Glueck CJ, Papanna R, Wang P, Goldenberg N, Sieve-Smith L** 2003 Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. Metabolism 52:908-915
107. **Ehrmann DA** 2005 Polycystic ovary syndrome. N Engl J Med 352:1223-1236
108. **Sam S, Dunaif A** 2003 Polycystic ovary syndrome: syndrome XX? Trends Endocrinol Metab 14:365-370
109. **Poretsky L, Cataldo NA, Rosenwaks Z, Giudice LC** 1999 The insulin-related ovarian regulatory system in health and disease. Endocr Rev 20:535-582
110. **Apridonidze T, Essah PA, Iuorno MJ, Nestler JE** 2005 Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 90:1929-1935
111. **Korhonen S, Hippelainen M, Vanhala M, Heinonen S, Niskanen L** 2003 The androgenic sex hormone profile is an essential feature of metabolic syndrome in premenopausal women: a controlled community-based study. Fertil Steril 79:1327-1334
112. **Pasquali R, Gambineri A** 2006 Insulin-sensitizing agents in polycystic ovary syndrome. Eur J Endocrinol 154:763-775
113. **Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN** 2001 Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86:1626-1632
114. **Bailey CJ, Day C** 1989 Traditional plant medicines as treatments for diabetes. Diabetes Care 12:553-564
115. **Carlsen SM, Grill V, Folling I** 1998 Evidence for dissociation of insulin- and weight-reducing effects of metformin in non-diabetic male patients with coronary heart disease. Diabetes Res Clin Pract 39:47-54

116. **Carlsen SM, Rossvoll O, Bjerve KS, Folling I** 1996 Metformin improves blood lipid pattern in nondiabetic patients with coronary heart disease. J Intern Med 239:227-233
117. **Musi N, Hirshman MF, Nygren J, Svanfeldt M, Bavenholm P, Rooyackers O, Zhou G, Williamson JM, Ljunqvist O, Efendic S, Moller DE, Thorell A, Goodyear LJ** 2002 Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 51:2074-2081
118. **Mansfield R, Galea R, Brincat M, Hole D, Mason H** 2003 Metformin has direct effects on human ovarian steroidogenesis. Fertil Steril 79:956-962
119. **Attia GR, Rainey WE, Carr BR** 2001 Metformin directly inhibits androgen production in human thecal cells. Fertil Steril 76:517-524
120. **Lord JM, Flight IH, Norman RJ** 2003 Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. Cochrane Database Syst Rev:CD003053
121. **Crave JC, Lejeune H, Brebant C, Baret C, Pugeat M** 1995 Differential effects of insulin and insulin-like growth factor I on the production of plasma steroid-binding globulins by human hepatoblastoma-derived (Hep G2) cells. J Clin Endocrinol Metab 80:1283-1289
122. **Glueck CJ, Phillips H, Cameron D, Sieve-Smith L, Wang P** 2001 Continuing metformin throughout pregnancy in women with polycystic ovary syndrome appears to safely reduce first-trimester spontaneous abortion: a pilot study. Fertil Steril 75:46-52
123. **Glueck CJ, Goldenberg N, Pranikoff J, Loftspring M, Sieve L, Wang P** 2004 Height, weight, and motor-social development during the first 18 months of life in 126 infants born to 109 mothers with polycystic ovary syndrome who conceived on and continued metformin through pregnancy. Hum Reprod 19:1323-1330
124. **Glueck CJ, Wang P, Kobayashi S, Phillips H, Sieve-Smith L** 2002 Metformin therapy throughout pregnancy reduces the development of gestational diabetes in women with polycystic ovary syndrome. Fertil Steril 77:520-525
125. **Glueck CJ, Wang P, Goldenberg N, Sieve-Smith L** 2002 Pregnancy outcomes among women with polycystic ovary syndrome treated with metformin. Hum Reprod 17:2858-2864
126. **Legro RS, Barnhart HX, Schlaff WD, Carr BR, Diamond MP, Carson SA, Steinkampf MP, Coutifaris C, McGovern PG,**

Cataldo NA, Gosman GG, Nestler JE, Giudice LC, Leppert PC, Myers ER 2007 Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. N Engl J Med 356:551-566

127. **Harborne L, Fleming R, Lyall H, Sattar N, Norman J** 2003 Metformin or antiandrogen in the treatment of hirsutism in polycystic ovary syndrome. J Clin Endocrinol Metab 88:4116-4123
128. **Nimgade A, Costello MC** 2003 Return to work for a company president with traumatic brain injury. The Journal of head trauma rehabilitation 18:464-467
129. **Harborne LR, Sattar N, Norman JE, Fleming R** 2005 Metformin and Weight Loss in Obese Women with Polycystic Ovary Syndrome: Comparison of Doses. J Clin Endocrinol Metab 90:4593-4598
130. **Musi N, Goodyear LJ** 2002 Targeting the AMP-activated protein kinase for the treatment of type 2 diabetes. Current drug targets 2:119-127
131. **Pasquali R, Gambineri A, Biscotti D, Vicennati V, Gagliardi L, Colitta D, Fiorini S, Cognigni GE, Filicori M, Morselli-Labate AM** 2000 Effect of long-term treatment with metformin added to hypocaloric diet on body composition, fat distribution, and androgen and insulin levels in abdominally obese women with and without the polycystic ovary syndrome. J Clin Endocrinol Metab 85:2767-2774
132. **Gambineri A, Pelusi C, Genghini S, Morselli-Labate AM, Cacciari M, Pagotto U, Pasquali R** 2004 Effect of flutamide and metformin administered alone or in combination in dieting obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 60:241-249
133. **Fleming R, Hopkinson ZE, Wallace AM, Greer IA, Sattar N** 2002 Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. J Clin Endocrinol Metab 87:569-574
134. **Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R** 1998 Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engl J Med 338:1876-1880
135. **Ng EH, Wat NM, Ho PC** 2001 Effects of metformin on ovulation rate, hormonal and metabolic profiles in women with clomiphene-resistant polycystic ovaries: a randomized, double-blinded placebo-controlled trial. Hum Reprod 16:1625-1631
136. **De Sloover Koch Y, Ernst ME** 2001 Use of metformin in polycystic ovary syndrome. Ann Pharmacother 35:1644-1647

137. **Lord JM, Flight IH, Norman RJ** 2003 Metformin in polycystic ovary syndrome: systematic review and meta-analysis. Bmj 327:951-953
138. **Harborne L, Fleming R, Lyall H, Norman J, Sattar N** 2003 Descriptive review of the evidence for the use of metformin in polycystic ovary syndrome. Lancet 361:1894-1901
139. **Maciel GAR, Soares Junior JM, Alves da Motta EL, Abi Haidar M, de Lima GR, Baracat EC** 2004 Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. Fertility and Sterility 81:355-360
140. **Tang T, Glanville J, Hayden CJ, White D, Barth JH, Balen AH** 2006 Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multicentre study. Hum Reprod 21:80-89
141. **Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R** 2002 Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord 26:883-896
142. **Apridonidze T, Essah PA, Iuorno MJ, Nestler JE** 2005 Prevalence and Characteristics of the Metabolic Syndrome in Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 90:1929-1935
143. **Crave JC, Fimbel S, Lejeune H, Cugnardey N, Dechaud H, Pugeat M** 1995 Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. J Clin Endocrinol Metab 80:2057-2062
144. **Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G** 2003 Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. Hum Reprod 18:1928-1932
145. **Clark AM, Ledger W, Galletly C, Tomlinson L, Blaney F, Wang X, Norman RJ** 1995 Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 10:2705-2712
146. **Huber-Buchholz MM, Carey DG, Norman RJ** 1999 Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. J Clin Endocrinol Metab 84:1470-1474
147. **Moran LJ, Noakes M, Clifton PM, Tomlinson L, Galletly C, Norman RJ** 2003 Dietary composition in restoring reproductive and

metabolic physiology in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:812-819

148. **Yanovski SZ, Yanovski JA** 2002 Obesity. N Engl J Med 346:591-602
149. **Norman RJ, Davies MJ, Lord J, Moran LJ** 2002 The role of lifestyle modification in polycystic ovary syndrome. Trends Endocrinol Metab 13:251-257
150. **Escobar-Morreale HF, Botella-Carretero JI, Alvarez-Blasco F, Sancho J, San Millan JL** 2005 The Polycystic Ovary Syndrome Associated with Morbid Obesity May Resolve after Weight Loss Induced by Bariatric Surgery. J Clin Endocrinol Metab 90:6364-6369
151. **Pasquali R, Antenucci D, Casimirri F, Venturoli S, Paradisi R, Fabbri R, Balestra V, Melchionda N, Barbara L** 1989 Clinical and hormonal characteristics of obese amenorrheic hyperandrogenic women before and after weight loss. J Clin Endocrinol Metab 68:173-179
152. **Kiddy DS, Hamilton-Fairley D, Bush A, Short F, Anyaoku V, Reed MJ, Franks S** 1992 Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 36:105-111
153. **Harlass FE, Plymate SR, Fariss BL, Belts RP** 1984 Weight loss is associated with correction of gonadotropin and sex steroid abnormalities in the obese anovulatory female. Fertil Steril 42:649-652
154. **Holte J, Bergh T, Berne C, Wide L, Lithell H** 1995 Restored insulin sensitivity but persistently increased early insulin secretion after weight loss in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 80:2586-2593
155. **Guzick DS, Wing R, Smith D, Berga SL, Winters SJ** 1994 Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women. Fertil Steril 61:598-604
156. **Douketis JD, Macie C, Thabane L, Williamson DF** 2005 Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. International journal of obesity (2005) 29:1153-1167
157. **Williamson DF, Pamuk E, Thun M, Flanders D, Byers T, Heath C** 1995 Prospective Study of Intentional Weight Loss and Mortality in Never-Smoking Overweight US White Women Aged 40-64 Years. Am J Epidemiol 141:1128-1141

158. **Dattilo AM, Kris-Etherton PM** 1992 Effects of weight reduction on blood lipids and lipoproteins: a meta- analysis. Am J Clin Nutr 56:320-328
159. **Goldstein DJ** 1992 Beneficial health effects of modest weight loss. Int J Obes Relat Metab Disord 16:397-415
160. **Curioni CC, Lourenco PM** 2005 Long-term weight loss after diet and exercise: a systematic review. International journal of obesity (2005) 29:1168-1174
161. **Arterburn DE, Crane PK, Veenstra DL** 2004 The efficacy and safety of sibutramine for weight loss: a systematic review. Arch Intern Med 164:994-1003
162. **James WP, Astrup A, Finer N, Hilsted J, Kopelman P, Rossner S, Saris WH, Van Gaal LF** 2000 Effect of sibutramine on weight maintenance after weight loss: a randomised trial. STORM Study Group. Sibutramine Trial of Obesity Reduction and Maintenance. Lancet 356:2119-2125
163. **Padwal R, Li SK, Lau DC** 2003 Long-term pharmacotherapy for overweight and obesity: a systematic review and meta-analysis of randomized controlled trials. Int J Obes Relat Metab Disord 27:1437-1446
164. **Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L** 2004 XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. Diabetes Care 27:155-161
165. **Stafford RS, Radley DC** 2003 National trends in antiobesity medication use. Arch Intern Med 163:1046-1050
166. **Di Marzo V, Matias I** 2005 Endocannabinoid control of food intake and energy balance. Nature neuroscience 8:585-589
167. **Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI** 1990 Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561-564
168. **Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P** 2002 SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behavioural pharmacology 13:451-463
169. **Verty AN, McFarlane JR, McGregor IS, Mallet PE** 2004 Evidence for an interaction between CB1 cannabinoid and oxytocin receptors in food and water intake. Neuropharmacology 47:593-603

170. **Tucci SA, Rogers EK, Korbonits M, Kirkham TC** 2004 The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. Br J Pharmacol 143:520-523
171. **Vickers SP, Webster LJ, Wyatt A, Dourish CT, Kennett GA** 2003 Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. Psychopharmacology 167:103-111
172. **Simiand J, Keane M, Keane PE, Soubrie P** 1998 SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. Behavioural pharmacology 9:179-181
173. **Freund TF, Katona I, Piomelli D** 2003 Role of endogenous cannabinoids in synaptic signaling. Physiological reviews 83:1017-1066
174. **Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG** 2002 International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161-202
175. **Mackie K** 2006 Cannabinoid receptors as therapeutic targets. Annual review of pharmacology and toxicology 46:101-122
176. **Tan B, Bradshaw HB, Rimmerman N, Srinivasan H, Yu YW, Krey JF, Monn MF, Chen JS, Hu SS, Pickens SR, Walker JM** 2006 Targeted lipidomics: discovery of new fatty acyl amides. The AAPS journal 8:E461-465
177. **Alexander SP, Kendall DA** 2007 The complications of promiscuity: endocannabinoid action and metabolism. Br J Pharmacol 152:602-623
178. **Basavarajappa BS** 2007 Critical enzymes involved in endocannabinoid metabolism. Protein and peptide letters 14:237-246
179. **Simon GM, Cravatt BF** 2006 Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. J Biol Chem 281:26465-26472
180. **Okamoto Y, Wang J, Morishita J, Ueda N** 2007 Biosynthetic pathways of the endocannabinoid anandamide. Chemistry & biodiversity 4:1842-1857
181. **Herkenham M** 1991 Characterization and localization of cannabinoid receptors in brain: an in vitro technique using slide-mounted tissue sections. NIDA research monograph 112:129-145

182. **Jansen EM, Haycock DA, Ward SJ, Seybold VS** 1992 Distribution of cannabinoid receptors in rat brain determined with aminoalkylindoles. Brain research 575:93-102
183. **Munro S, Thomas KL, Abu-Shaar M** 1993 Molecular characterization of a peripheral receptor for cannabinoids. Nature 365:61-65
184. **Sjostrom L, Rissanen A, Andersen T, Boldrin M, Golay A, Koppeschaar HP, Krempf M** 1998 Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. European Multicentre Orlistat Study Group. Lancet 352:167-172
185. **Davidson MH, Hauptman J, DiGirolamo M, Foreyt JP, Halsted CH, Heber D, Heimburger DC, Lucas CP, Robbins DC, Chung J, Heymsfield SB** 1999 Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: a randomized controlled trial. Jama 281:235-242
186. **Hauptman J, Lucas C, Boldrin MN, Collins H, Segal KR** 2000 Orlistat in the long-term treatment of obesity in primary care settings. Archives of family medicine 9:160-167
187. **Rossner S, Sjostrom L, Noack R, Meinders AE, Noseda G** 2000 Weight loss, weight maintenance, and improved cardiovascular risk factors after 2 years treatment with orlistat for obesity. European Orlistat Obesity Study Group. Obes Res 8:49-61
188. **Hutton B, Fergusson D** 2004 Changes in body weight and serum lipid profile in obese patients treated with orlistat in addition to a hypocaloric diet: a systematic review of randomized clinical trials. Am J Clin Nutr 80:1461-1468
189. **Norris SL, Zhang X, Avenell A, Gregg E, Schmid CH, Kim C, Lau J** 2004 Efficacy of pharmacotherapy for weight loss in adults with type 2 diabetes mellitus: a meta-analysis. Arch Intern Med 164:1395-1404
190. **Expert Panel on Detection EaToHBCiA** 2001 Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285:2486-2497
191. **Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A** 2007 Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 370:1706-1713

192. **Moore B** 1906 On the treatment of Diabetus mellitus by acid extract of Duodenal Mucous Membrane. Biochem J 1:28-38
193. **Vahl T, D'Alessio D** 2003 Enteroinsular signaling: perspectives on the role of the gastrointestinal hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide in normal and abnormal glucose metabolism. Curr Opin Clin Nutr Metab Care 6:461-468
194. **Elrick H, Stimmler L, Hlad CJ, Jr., Arai Y** 1964 Plasma Insulin Response to Oral and Intravenous Glucose Administration. J Clin Endocrinol Metab 24:1076-1082
195. **McIntyre N, Holdsworth CD, Turner DS** 1965 Intestinal factors in the control of insulin secretion. J Clin Endocrinol Metab 25:1317-1324
196. **Creutzfeldt W, Nauck M** 1992 Gut hormones and diabetes mellitus. Diabetes Metab Rev 8:149-177
197. **Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W** 1986 Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 63:492-498
198. **Nauck M, Stockmann F, Ebert R, Creutzfeldt W** 1986 Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia 29:46-52
199. **Meier JJ, Nauck MA, Schmidt WE, Gallwitz B** 2002 Gastric inhibitory polypeptide: the neglected incretin revisited. Regulatory peptides 107:1-13
200. **Ross SA, Dupre J** 1978 Effects of ingestion of triglyceride or galactose on secretion of gastric inhibitory polypeptide and on responses to intravenous glucose in normal and diabetic subjects. Diabetes 27:327-333
201. **Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ** 2000 Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. J Clin Endocrinol Metab 85:3575-3581
202. **Cataland S, Crockett SE, Brown JC, Mazzaferri EL** 1974 Gastric inhibitory polypeptide (GIP) stimulation by oral glucose in man. J Clin Endocrinol Metab 39:223-228
203. **Falko JM, Crockett SE, Cataland S, Mazzaferri EL** 1975 Gastric inhibitory polypeptide (GIP) stimulated by fat ingestion in man. J Clin Endocrinol Metab 41:260-265

204. **Damholt AB, Buchan AM, Kofod H** 1998 Glucagon-like-peptide-1 secretion from canine L-cells is increased by glucose-dependent-insulinotropic peptide but unaffected by glucose. Endocrinology 139:2085-2091
205. **Damholt AB, Kofod H, Buchan AM** 1999 Immunocytochemical evidence for a paracrine interaction between GIP and GLP-1-producing cells in canine small intestine. Cell Tissue Res 298:287-293
206. **Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI** 1993 Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. Endocrinology 133:2861-2870
207. **Meier JJ, Gallwitz B, Nauck MA** 2003 Glucagon-like peptide 1 and gastric inhibitory polypeptide: potential applications in type 2 diabetes mellitus. BioDrugs 17:93-102
208. **Nauck MA, Bartels E, Orskov C, Ebert R, Creutzfeldt W** 1992 Lack of effect of synthetic human gastric inhibitory polypeptide and glucagon-like peptide 1 [7-36 amide] infused at near-physiological concentrations on pentagastrin-stimulated gastric acid secretion in normal human subjects. Digestion 52:214-221
209. **Dupre J, Ross SA, Watson D, Brown JC** 1973 Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab 37:826-828
210. **Andersen DK, Elahi D, Brown JC, Tobin JD, Andres R** 1978 Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. J Clin Invest 62:152-161
211. **Lewis JT, Dayanandan B, Habener JF, Kieffer TJ** 2000 Glucose-dependent insulinotropic polypeptide confers early phase insulin release to oral glucose in rats: demonstration by a receptor antagonist. Endocrinology 141:3710-3716
212. **Tseng CC, Zhang XY, Wolfe MM** 1999 Effect of GIP and GLP-1 antagonists on insulin release in the rat. Am J Physiol 276:E1049-1054
213. **Miyawaki K, Yamada Y, Yano H, Niwa H, Ban N, Ihara Y, Kubota A, Fujimoto S, Kajikawa M, Kuroe A, Tsuda K, Hashimoto H, Yamashita T, Jomori T, Tashiro F, Miyazaki J, Seino Y** 1999 Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. Proc Natl Acad Sci U S A 96:14843-14847

214. **Miyawaki K, Yamada Y, Ban N, Ihara Y, Tsukiyama K, Zhou H, Fujimoto S, Oku A, Tsuda K, Toyokuni S, Hiai H, Mizunoya W, Fushiki T, Holst JJ, Makino M, Tashita A, Kobara Y, Tsubamoto Y, Jinnouchi T, Jomori T, Seino Y** 2002 Inhibition of gastric inhibitory polypeptide signaling prevents obesity. Nat Med 8:738-742
215. **Siegel EG, Creutzfeldt W** 1985 Stimulation of insulin release in isolated rat islets by GIP in physiological concentrations and its relation to islet cyclic AMP content. Diabetologia 28:857-861
216. **Pederson RA, Schubert HE, Brown JC** 1975 Gastric inhibitory polypeptide. Its physiologic release and insulinotropic action in the dog. Diabetes 24:1050-1056
217. **Elahi D, Andersen DK, Brown JC, Debas HT, Hershcopf RJ, Raizes GS, Tobin JD, Andres R** 1979 Pancreatic alpha- and beta-cell responses to GIP infusion in normal man. Am J Physiol 237:E185-191
218. **Ehses JA, Casilla VR, Doty T, Pospisilik JA, Winter KD, Demuth HU, Pederson RA, McIntosh CH** 2003 Glucose-dependent insulinotropic polypeptide promotes beta-(INS-1) cell survival via cyclic adenosine monophosphate-mediated caspase-3 inhibition and regulation of p38 mitogen-activated protein kinase. Endocrinology 144:4433-4445
219. **Trumper A, Trumper K, Trusheim H, Arnold R, Goke B, Horsch D** 2001 Glucose-dependent insulinotropic polypeptide is a growth factor for beta (INS-1) cells by pleiotropic signaling. Mol Endocrinol 15:1559-1570
220. **Eckel RH, Fujimoto WY, Brunzell JD** 1979 Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. Diabetes 28:1141-1142
221. **Beck B, Max JP** 1983 Gastric inhibitory polypeptide enhancement of the insulin effect on fatty acid incorporation into adipose tissue in the rat. Regulatory peptides 7:3-8
222. **Oben J, Morgan L, Fletcher J, Marks V** 1991 Effect of the entero-pancreatic hormones, gastric inhibitory polypeptide and glucagon-like polypeptide-1(7-36) amide, on fatty acid synthesis in explants of rat adipose tissue. J Endocrinol 130:267-272
223. **Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC** 1983 Exon duplication and divergence in the human preproglucagon gene. Nature 304:368-371

224. **Orskov C, Wettergren A, Holst JJ** 1993 Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. Diabetes 42:658-661
225. **Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B** 1995 Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion 56:117-126
226. **Orskov C, Wettergren A, Holst JJ** 1996 Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. Scand J Gastroenterol 31:665-670
227. **Vilsboll T, Agerso H, Krarup T, Holst JJ** 2003 Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. J Clin Endocrinol Metab 88:220-224
228. **Vilsboll T, Holst JJ** 2004 Incretins, insulin secretion and Type 2 diabetes mellitus. Diabetologia 47:357-366
229. **Kolligs F, Fehmann HC, Goke R, Goke B** 1995 Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. Diabetes 44:16-19
230. **Edwards CM, Todd JF, Mahmoudi M, Wang Z, Wang RM, Ghatei MA, Bloom SR** 1999 Glucagon-like peptide 1 has a physiological role in the control of postprandial glucose in humans: studies with the antagonist exendin 9-39. Diabetes 48:86-93
231. **Matsuyama T, Komatsu R, Namba M, Watanabe N, Itoh H, Tarui S** 1988 Glucagon-like peptide-1 (7-36 amide): a potent glucagonostatic and insulinotropic hormone. Diabetes Res Clin Pract 5:281-284
232. **Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ** 1993 Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig Dis Sci 38:665-673
233. **Naslund E, Bogefors J, Skogar S, Gryback P, Jacobsson H, Holst JJ, Hellstrom PM** 1999 GLP-1 slows solid gastric emptying and inhibits insulin, glucagon, and PYY release in humans. Am J Physiol 277:R910-916
234. **Wishart JM, Horowitz M, Morris HA, Jones KL, Nauck MA** 1998 Relation between gastric emptying of glucose and plasma concentrations of glucagon-like peptide-1. Peptides 19:1049-1053

235. **Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR** 1996 A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379:69-72
236. **Izquierdo D, Foyouzi N, Kwintkiewicz J, Duleba AJ** 2004 Mevastatin inhibits ovarian theca-interstitial cell proliferation and steroidogenesis. Fertil Steril 82 Suppl 3:1193-1197
237. **Hughesdon PE** 1982 Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis". Obstet Gynecol Surv 37:59-77
238. **Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss JF, 3rd, McAllister JM** 2000 Differential activity of the cytochrome P450 17alpha-hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. J Clin Endocrinol Metab 85:2304-2311
239. **Nelson VL, Legro RS, Strauss JF, 3rd, McAllister JM** 1999 Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 13:946-957
240. **Porter KE, Naik J, Turner NA, Dickinson T, Thompson MM, London NJ** 2002 Simvastatin inhibits human saphenous vein neointima formation via inhibition of smooth muscle cell proliferation and migration. J Vasc Surg 36:150-157
241. **Axel DI, Riessen R, Runge H, Viebahn R, Karsch KR** 2000 Effects of cerivastatin on human arterial smooth muscle cell proliferation and migration in transfilter cocultures. J Cardiovasc Pharmacol 35:619-629
242. **Buemi M, Allegra A, Senatore M, Marino D, Medici MA, Aloisi C, Di Pasquale G, Corica F** 1999 Pro-apoptotic effect of fluvastatin on human smooth muscle cells. Eur J Pharmacol 370:201-203
243. **El-Ani D, Zimlichman R** 2001 Simvastatin induces apoptosis of cultured rat cardiomyocytes. J Basic Clin Physiol Pharmacol 12:325-338
244. **Danesh FR, Sadeghi MM, Amro N, Philips C, Zeng L, Lin S, Sahai A, Kanwar YS** 2002 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors prevent high glucose-induced proliferation of mesangial cells via modulation of Rho GTPase/ p21 signaling pathway: Implications for diabetic nephropathy. Proc Natl Acad Sci U S A 99:8301-8305

245. **Kato K, Cox AD, Hisaka MM, Graham SM, Buss JE, Der CJ** 1992 Isoprenoid addition to Ras protein is the critical modification for its membrane association and transforming activity. Proc Natl Acad Sci U S A 89:6403-6407
246. **Dobs AS, Schrott H, Davidson MH, Bays H, Stein EA, Kush D, Wu M, Mitchel Y, Illingworth RD** 2000 Effects of high-dose simvastatin on adrenal and gonadal steroidogenesis in men with hypercholesterolemia. Metabolism 49:1234-1238
247. **Hyyppa MT, Kronholm E, Virtanen A, Leino A, Jula A** 2003 Does simvastatin affect mood and steroid hormone levels in hypercholesterolemic men? A randomized double-blind trial. Psychoneuroendocrinology 28:181-194
248. **Jay RH, Sturley RH, Stirling C, McGarrigle HH, Katz M, Reckless JP, Betteridge DJ** 1991 Effects of pravastatin and cholestyramine on gonadal and adrenal steroid production in familial hypercholesterolaemia. Br J Clin Pharmacol 32:417-422
249. **Bernini GP, Argenio GF, Gasperi M, Vivaldi MS, Franchi F, Salvetti A** 1994 Effects of long-term simvastatin treatment on testicular and adrenal steroidogenesis in hypercholesterolemic patients. J Endocrinol Invest 17:227-233
250. **Travia D, Tosi F, Negri C, Faccini G, Moghetti P, Muggeo M** 1995 Sustained therapy with 3-hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitors does not impair steroidogenesis by adrenals and gonads. J Clin Endocrinol Metab 80:836-840
251. **Bairey Merz CN, Olson MB, Johnson BD, Bittner V, Hodgson TK, Berga SL, Braunstein GD, Pepine CJ, Reis SE, Sopko G, Kelsey SF** 2002 Cholesterol-lowering medication, cholesterol level, and reproductive hormones in women: the Women's Ischemia Syndrome Evaluation (WISE). Am J Med 113:723-727
252. **Ide H, Fujiya S, Aanuma Y, Agishi Y** 1990 Effects of simvastatin, an HMG-CoA reductase inhibitor, on plasma lipids and steroid hormones. Clin Ther 12:410-420
253. **Ray KK, Cannon CP** 2005 Early time to benefit with intensive statin treatment: could it be the pleiotropic effects? Am J Cardiol 96:54F-60F
254. **Ray KK, Cannon CP** 2004 Pathological changes in acute coronary syndromes: the role of statin therapy in the modulation of inflammation, endothelial function and coagulation. J Thromb Thrombolysis 18:89-101

255. **Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA** 2002 Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 105:1567-1572
256. **Schachinger V, Britten MB, Zeiher AM** 2000 Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. Circulation 101:1899-1906
257. **O'Driscoll G, Green D, Taylor RR** 1997 Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. Circulation 95:1126-1131
258. **Feron O, Dessy C, Desager JP, Balligand JL** 2001 Hydroxy-methylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. Circulation 103:113-118
259. **Romano M, Mezzetti A, Marulli C, Ciabattoni G, Febo F, Di Ienno S, Roccaforte S, Vigneri S, Nubile G, Milani M, Davi G** 2000 Fluvastatin reduces soluble P-selectin and ICAM-1 levels in hypercholesterolemic patients: role of nitric oxide. J Investig Med 48:183-189
260. **Fuhrman B, Koren L, Volkova N, Keidar S, Hayek T, Aviram M** 2002 Atorvastatin therapy in hypercholesterolemic patients suppresses cellular uptake of oxidized-LDL by differentiating monocytes. Atherosclerosis 164:179-185
261. **Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, Flaker GC, Braunwald E** 1998 Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation 98:839-844
262. **Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L** 2000 Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. N Engl J Med 343:1139-1147
263. **Albert MA, Danielson E, Rifai N, Ridker PM** 2001 Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. Jama 286:64-70
264. **Plenge JK, Hernandez TL, Weil KM, Poirier P, Grunwald GK, Marcovina SM, Eckel RH** 2002 Simvastatin lowers C-reactive

protein within 14 days: an effect independent of low-density lipoprotein cholesterol reduction. Circulation 106:1447-1452

265. **Weitz-Schmidt G, Welzenbach K, Brinkmann V, Kamata T, Kallen J, Bruns C, Cottens S, Takada Y, Hommel U** 2001 Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. Nat Med 7:687-692
266. **Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J** 2001 Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. Circulation 103:926-933
267. **Kobashigawa JA, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA, et al.** 1995 Effect of pravastatin on outcomes after cardiac transplantation. N Engl J Med 333:621-627
268. **Laurat E, Poirier B, Tupin E, Caligiuri G, Hansson GK, Bariety J, Nicoletti A** 2001 In vivo downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice. Circulation 104:197-202
269. **Takemoto M, Node K, Nakagami H, Liao Y, Grimm M, Takemoto Y, Kitakaze M, Liao JK** 2001 Statins as antioxidant therapy for preventing cardiac myocyte hypertrophy. J Clin Invest 108:1429-1437
270. **Fraser CG, Harris EK** 1989 Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 27:409-437
271. **Harris EK** 1974 Effects of intra- and interindividual variation on the appropriate use of normal ranges. Clin Chem 20:1535-1542
272. **Keevil BG, Kilpatrick ES, Nichols SP, Maylor PW** 1998 Biological variation of cystatin C: implications for the assessment of glomerular filtration rate. Clin Chem 44:1535-1539
273. **Cooper GR, Myers GL, Smith SJ, Schlant RC** 1992 Blood lipid measurements. Variations and practical utility. Jama 267:1652-1660
274. **Fraser CG, Hyltoft Peterson P, Larsen ML** 1990 Setting analytical goals for random analytical error in specific clinical monitoring situations. Clin Chem 36:1625-1628
275. **Kroll MH** 2002 Evaluating sequential values using time-adjusted biological variation. Clin Chem Lab Med 40:499-504
276. **Ricos C, Alvarez V, Cava F, Garcia-Lario JV, Hernandez A, Jimenez CV, Minchinela J, Perich C, Simon M** 1999 Current

databases on biological variation: pros, cons and progress. Scandinavian journal of clinical and laboratory investigation 59:491-500

277. **Friedewald WT, Levy RI, Fredrickson DS** 1972 Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clin Chem 18:499-502
278. **Mogadam M, Ahmed SW, Mensch AH, Godwin ID** 1990 Within-person fluctuations of serum cholesterol and lipoproteins. Arch Intern Med 150:1645-1648
279. **Smith SJ, Cooper GR, Myers GL, Sampson EJ** 1993 Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. Clin Chem 39:1012-1022
280. **Thompson SG, Pocock SJ** 1990 The variability of serum cholesterol measurements: implications for screening and monitoring. Journal of clinical epidemiology 43:783-789
281. **Schectman G, Sasse E** 1993 Variability of lipid measurements: relevance for the clinician. Clin Chem 39:1495-1503
282. **McNamara JR, Cole TG, Contois JH, Ferguson CA, Ordovas JM, Schaefer EJ** 1995 Immunoseparation method for measuring low-density lipoprotein cholesterol directly from serum evaluated. Clin Chem 41:232-240
283. **McNamara JR, Cohn JS, Wilson PW, Schaefer EJ** 1990 Calculated values for low-density lipoprotein cholesterol in the assessment of lipid abnormalities and coronary disease risk. Clin Chem 36:36-42
284. **Rubies-Prat J, Reverter JL, Senti M, Pedro-Botet J, Salinas I, Lucas A, Nogues X, Sanmarti A** 1993 Calculated low-density lipoprotein cholesterol should not be used for management of lipoprotein abnormalities in patients with diabetes mellitus. Diabetes Care 16:1081-1086
285. **Matas C, Cabre M, La Ville A, Prats E, Joven J, Turner PR, Masana L, Camps J** 1994 Limitations of the Friedewald formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease. Clin Chem 40:404-406
286. **Barber TM, McCarthy MI, Wass JAH, Franks S** 2006 Obesity and polycystic ovary syndrome. Clinical Endocrinology 65:137-145
287. **Moghetti P, Castello R, Negri C, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M** 2000 Metformin effects on clinical features,

endocrine and metabolic profiles, and insulin sensitivity in polycystic ovary syndrome: a randomized, double-blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. J Clin Endocrinol Metab 85:139-146

288. **Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE** 2001 Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 108:1167-1174
289. **Di Marzo V, Bifulco M, De Petrocellis L** 2004 The endocannabinoid system and its therapeutic exploitation. Nat Rev Drug Discov 3:771-784
290. **Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U** 2003 The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest 112:423-431
291. **Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J, for the RIONASG** 2006 Effect of Rimonabant, a Cannabinoid-1 Receptor Blocker, on Weight and Cardiometabolic Risk Factors in Overweight or Obese Patients: RIO-North America: A Randomized Controlled Trial. JAMA 295:761-775
292. **Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S** 2005 Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet 365:1389-1397
293. **Despres J-P, Golay A, Sjostrom L, the Rimonabant in Obesity-Lipids Study G** 2005 Effects of Rimonabant on Metabolic Risk Factors in Overweight Patients with Dyslipidemia. N Engl J Med 353:2121-2134
294. **Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL** 2005 Orlistat is as beneficial as metformin in the treatment of polycystic ovarian syndrome. J Clin Endocrinol Metab 90:729-733
295. **Curioni C, Andre C** 2006 Rimonabant for overweight or obesity. Cochrane Database Syst Rev:CD006162
296. **Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Bjorntorp P, Tibblin G** 1984 Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow up of

participants in the study of men born in 1913. Br Med J (Clin Res Ed) 288:1401-1404

297. **Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjostrom L** 1984 Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. Br Med J (Clin Res Ed) 289:1257-1261
298. **Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrie P** 2003 The Cannabinoid CB1 Receptor Antagonist SR141716 Increases Acrp30 mRNA Expression in Adipose Tissue of Obese fa/fa Rats and in Cultured Adipocyte Cells. Mol Pharmacol 63:908-914
299. **Salmenniemi U, Ruotsalainen E, Pihlajamaki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E, Laakso M** 2004 Multiple Abnormalities in Glucose and Energy Metabolism and Coordinated Changes in Levels of Adiponectin, Cytokines, and Adhesion Molecules in Subjects With Metabolic Syndrome. Circulation 110:3842-3848
300. **Hamilton-Fairley D, Kiddy D, Anyaoku V, Koistinen R, Seppala M, Franks S** 1993 Response of sex hormone binding globulin and insulin-like growth factor binding protein-1 to an oral glucose tolerance test in obese women with polycystic ovary syndrome before and after calorie restriction. Clin Endocrinol (Oxf) 39:363-367
301. **Kiddy DS, Hamilton-Fairley D, Seppala M, Koistinen R, James VH, Reed MJ, Franks S** 1989 Diet-induced changes in sex hormone binding globulin and free testosterone in women with normal or polycystic ovaries: correlation with serum insulin and insulin-like growth factor-I. Clin Endocrinol (Oxf) 31:757-763
302. **Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF** 2006 Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet 368:1660-1672
303. **Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J** 2006 Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. Jama 295:761-775
304. **Kerchner AM, Lester W, Stuart SP, Dokras A** 2007 Longitudinal study detects high risk of depression and other mental health

disorders in women with polycystic ovary syndrome (PCOS). Fertility and Sterility 88:S83

305. **Sathyapalan T, Cho LW, Kilpatrick ES, Coady AM, Atkin SL** 2008 A comparison between rimonabant and metformin in reducing biochemical hyperandrogenaemia and insulin resistance in patients with polycystic ovary syndrome (PCOS): a randomized open-label parallel study. Clin Endocrinol (Oxf) 69:931-935
306. **Mansfield R, Galea R, Brincat M, Hole D, Mason H** 2003 Metformin has direct effects on human ovarian steroidogenesis. Fertility and Sterility 79:956-962
307. **Arslanian SA, Lewy V, Danadian K, Saad R** 2002 Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. J Clin Endocrinol Metab 87:1555-1559
308. **Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrie P** 2003 The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol 63:908-914
309. **Liu YL, Connoley IP, Wilson CA, Stock MJ** 2005 Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. Int J Obes (Lond) 29:183-187
310. **Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L, Wang L, Kunos G** 2005 Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest 115:1298-1305
311. **Salmenniemi U, Ruotsalainen E, Pihlajamaki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E, Laakso M** 2004 Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation 110:3842-3848
312. **Despres JP, Golay A, Sjostrom L** 2005 Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med 353:2121-2134

313. **Berg AH, Combs TP, Scherer PE** 2002 ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13:84-89
314. **Kousta E, White DM, Cela E, McCarthy MI, Franks S** 1999 The prevalence of polycystic ovaries in women with infertility. Hum Reprod 14:2720-2723
315. **Allen SE, Potter HD, Azziz R** 1997 Prevalence of hyperandrogenemia among nonhirsute oligo-ovulatory women. Fertil Steril 67:569-572
316. **Clark AM, Ledger W, Galletly C, Tomlinson L, Blaney F, Wang X, Norman RJ** 1995 Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. Hum Reprod 10:2705-2712
317. **Liu WM, Duan EK, Cao YJ** 2002 Effects of anandamide on embryo implantation in the mouse. Life sciences 71:1623-1632
318. **Dennedy MC, Friel AM, Houlihan DD, Broderick VM, Smith T, Morrison JJ** 2004 Cannabinoids and the human uterus during pregnancy. Am J Obstet Gynecol 190:2-9; discussion 3A
319. **Maccarrone M, Valensise H, Bari M, Lazzarin N, Romanini C, Finazzi-Agro A** 2000 Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. Lancet 355:1326-1329
320. **Bifulco M, Grimaldi C, Gazzerro P, Pisanti S, Santoro A** 2007 Rimonabant: just an antiobesity drug? Current evidence on its pleiotropic effects. Mol Pharmacol 71:1445-1456
321. **Elkind-Hirsch K, Marrioneaux O, Bhushan M, Vernor D, Bhushan R** 2008 Comparison of Single and Combined Treatment with Exenatide and Metformin on Menstrual Cyclicity in Overweight Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 93:2670-2678
322. **Drucker DJ** 2003 Enhancing incretin action for the treatment of type 2 diabetes. Diabetes Care 26:2929-2940
323. **Pertwee RG** 2001 Cannabinoids and the gastrointestinal tract. Gut 48:859-867
324. **Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO** 2004 The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 89:2745-2749
325. **Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF** 2000 A prospective study of the prevalence

of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab 85:2434-2438

326. **Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS** 2006 Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab 91:1357-1363
327. **Dahlgren E, Johansson S, Lindstedt G, Knutsson F, Oden A, Janson PO, Mattson LA, Crona N, Lundberg PA** 1992 Women with polycystic ovary syndrome wedge resected in 1956 to 1965: a long-term follow-up focusing on natural history and circulating hormones. Fertil Steril 57:505-513
328. **Son HY, Nishikawa A, Ikeda T, Imazawa T, Kimura S, Hirose M** 2001 Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet. Jpn J Cancer Res 92:103-108
329. **Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS** 1998 Mortality of women with polycystic ovary syndrome at long-term follow-up. Journal of clinical epidemiology 51:581-586
330. **Wild S, Pierpoint T, Jacobs H, McKeigue P** 2000 Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. Hum Fertil (Camb) 3:101-105
331. **Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, Batakis N, Phenekos C, Dionyssiou-Asteriou A** 2005 Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 90:2740-2746
332. **Shepherd J, Barter P, Carmena R, Deedwania P, Fruchart J-C, Haffner S, Hsia J, Breazna A, LaRosa J, Grundy S, Waters D, for the Treating to New Targets I** 2006 Effect of Lowering LDL Cholesterol Substantially Below Currently Recommended Levels in Patients With Coronary Heart Disease and Diabetes: The Treating to New Targets (TNT) study. Diabetes Care 29:1220-1226
333. **Pedersen TR, Faergeman O, Kastelein JJP, Olsson AG, Tikkanen MJ, Holme I, Larsen ML, Bendiksen FS, Lindahl C, Szarek M, Tsai J, for the Incremental Decrease in End Points Through Aggressive Lipid Lowering Study G** 2005 High-Dose Atorvastatin vs Usual-Dose Simvastatin for Secondary Prevention After Myocardial Infarction: The IDEAL Study: A Randomized Controlled Trial. JAMA 294:2437-2445

334. 2003 MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. The Lancet 361:2005-2016
335. **Sever PS, Poulter NR, Dahlof B, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes G, Mehlsen J, Nieminen MS, O'Brien ET, Ostergren J** 2008 The Anglo-Scandinavian Cardiac Outcomes Trial lipid lowering arm: extended observations 2 years after trial closure. Eur Heart J 29:499-508
336. **McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakova O, Ford I, Capell HA, Sattar N** 2004 Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. The Lancet 363:2015-2021
337. **Davignon J** 2004 Beneficial Cardiovascular Pleiotropic Effects of Statins. Circulation 109:III-39-43
338. **Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ** 2007 Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial. J Clin Endocrinol Metab 92:456-461
339. **Burkman RT, Jr.** 1995 The role of oral contraceptives in the treatment of hyperandrogenic disorders. Am J Med 98:130S-136S
340. **Wild RA, Demers LM, Applebaum-Bowden D, Lenker R** 1991 Hirsutism: metabolic effects of two commonly used oral contraceptives and spironolactone. Contraception 44:113-124
341. **Hardiman P, Pillay OC, Atiomo W** 2003 Polycystic ovary syndrome and endometrial carcinoma. Lancet 361:1810-1812
342. **Balen A** 2001 Polycystic ovary syndrome and cancer. Human reproduction update 7:522-525
343. **Costa A, Casamitjana R, Casals E, Alvarez L, Morales J, Masramon X, Hernandez G, Gomis R, Conget I** 2003 Effects of atorvastatin on glucose homeostasis, postprandial triglyceride response and C-reactive protein in subjects with impaired fasting glucose. Diabet Med 20:743-745
344. 2001 Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 285:2486-2497
345. **Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM** 2006 Effect of very high-intensity statin therapy on

regression of coronary atherosclerosis: the ASTEROID trial. Jama 295:1556-1565

346. **Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ** 2007 Effects of Simvastatin and Oral Contraceptive Agent on Polycystic Ovary Syndrome: Prospective, Randomized, Crossover Trial. J Clin Endocrinol Metab 92:456-461
347. **Crook D, Godsland IF, Worthington M, Felton CV, Proudler AJ, Stevenson JC** 1993 A comparative metabolic study of two low-estrogen-dose oral contraceptives containing desogestrel or gestodene progestins. Am J Obstet Gynecol 169:1183-1189
348. **van Rooijen M, von Schoultz B, Silveira A, Hamsten A, Bremme K** 2002 Different effects of oral contraceptives containing levonorgestrel or desogestrel on plasma lipoproteins and coagulation factor VII. Am J Obstet Gynecol 186:44-48
349. **Venturoli S, Marescalchi O, Colombo FM, Macrelli S, Ravaioli B, Bagnoli A, Paradisi R, Flamigni C** 1999 A prospective randomized trial comparing low dose flutamide, finasteride, ketoconazole, and cyproterone acetate-estrogen regimens in the treatment of hirsutism. J Clin Endocrinol Metab 84:1304-1310
350. **Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M, Castello R** 2000 Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. J Clin Endocrinol Metab 85:89-94
351. **Falsetti L, Gambera A, Tisi G** 2001 Efficacy of the combination ethinyl oestradiol and cyproterone acetate on endocrine, clinical and ultrasonographic profile in polycystic ovarian syndrome. Hum Reprod 16:36-42
352. **Fruzzetti F, Bersi C, Parrini D, Ricci C, Genazzani AR** 1999 Treatment of hirsutism: comparisons between different antiandrogens with central and peripheral effects. Fertil Steril 71:445-451
353. **Moran LJ, Noakes M, Clifton PM, Tomlinson L, Norman RJ** 2003 Dietary Composition in Restoring Reproductive and Metabolic Physiology in Overweight Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 88:812-819
354. **Brettenthaler N, De Geyter C, Huber PR, Keller U** 2004 Effect of the Insulin Sensitizer Pioglitazone on Insulin Resistance, Hyperandrogenism, and Ovulatory Dysfunction in Women with Polycystic Ovary Syndrome. J Clin Endocrinol Metab 89:3835-3840

355. **Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN** 2001 Troglitazone Improves Ovulation and Hirsutism in the Polycystic Ovary Syndrome: A Multicenter, Double Blind, Placebo-Controlled Trial. J Clin Endocrinol Metab 86:1626-1632
356. **Ortega-Gonzalez C, Luna S, Hernandez L, Crespo G, Aguayo P, Arteaga-Troncoso G, Parra A** 2005 Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome. J Clin Endocrinol Metab 90:1360-1365
357. **Brettenthaler N, De Geyter C, Huber PR, Keller U** 2004 Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 89:3835-3840
358. **Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, Katsilambros N, Kreatsas G, Panidis D** 2006 Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. Hum Reprod 21:1426-1431
359. **Ridker PM, Rifai N, Rose L, Buring JE, Cook NR** 2002 Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 347:1557-1565
360. **Ridker PM, Buring JE, Cook NR, Rifai N** 2003 C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 107:391-397
361. **Costa A, Casamitjana R, Casals E, Alvarez L, Morales J, Masramon X, Hernandez G, Gomis R, Conget I** 2003 Effects of atorvastatin on glucose homeostasis, postprandial triglyceride response and C-reactive protein in subjects with impaired fasting glucose. Diabetic Medicine 20:743-745
362. **Huptas S, Geiss H-C, Otto C, Parhofer KG** 2006 Effect of Atorvastatin (10 mg/day) on Glucose Metabolism in Patients With the Metabolic Syndrome. The American Journal of Cardiology 98:66-69
363. **Paolisso G, Barbagallo M, Petrella G, Ragno E, Barbieri M, Giordano M, Varricchio M** 2000 Effects of simvastatin and atorvastatin administration on insulin resistance and respiratory

quotient in aged dyslipidemic non-insulin dependent diabetic patients. Atherosclerosis 150:121-127

364. **Takagi T, Matsuda M, Abe M, Kobayashi H, Fukuhara A, Komuro R, Kihara S, Caslake MJ, McMahon A, Shepherd J, Funahashi T, Shimomura I** 2008 Effect of pravastatin on the development of diabetes and adiponectin production. Atherosclerosis 196:114-121
365. **Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A** 2001 Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. Circulation 103:357-362
366. **Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL** 2009 The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double-blind placebo-controlled study. J Clin Endocrinol Metab 94:103-108
367. **Sathyapalan T, Cho LW, Kilpatrick ES, Coady AM, Atkin SL** 2009 Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS). Clin Endocrinol (Oxf) 70:124-128
368. **Sun W, Lee T-S, Zhu M, Gu C, Wang Y, Zhu Y, Shyy JYJ** 2006 Statins Activate AMP-Activated Protein Kinase In Vitro and In Vivo. Circulation 114:2655-2662
369. **Kitzmiller JL, Block JM, Brown FM, Catalano PM, Conway DL, Coustan DR, Gunderson EP, Herman WH, Hoffman LD, Inturrisi M, Jovanovic LB, Kjos SI, Knopp RH, Montoro MN, Ogata ES, Paramsothy P, Reader DM, Rosenn BM, Thomas AM, Kirkman MS** 2008 Managing Preexisting Diabetes for Pregnancy: Summary of evidence and consensus recommendations for care. Diabetes Care 31:1060-1079
370. **Costa J, Borges M, David C, Vaz Carneiro A** 2006 Efficacy of lipid lowering drug treatment for diabetic and non-diabetic patients: meta-analysis of randomised controlled trials. BMJ 332:1115-1124
371. **DOH** 2003 Investing in General Practice: The New General Medical Services Contract. In: Department of Health, London
372. **DOH** 2000 National Service Framework for coronary Heart disease. In: Stationary Office L ed.

373. **Prepared by: British Cardiac Society BHSDUKHUKPCCSTSA** 2005 JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. Heart 91:v1-52
374. **Boyle R** 2006 National policy on statin prescribing. In: DOH ed.
375. **Bookstein L, Gidding SS, Donovan M, Smith FA** 1990 Day-to-day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. Arch Intern Med 150:1653-1657
376. **Friedlander Y, Kark JD, Stein Y** 1985 Variability of plasma lipids and lipoproteins: the Jerusalem Lipid Research Clinic Study. Clin Chem 31:1121-1126
377. **Ford RP** 1989 Essential data derived from biological variation for establishment and use of lipid analyses. Ann Clin Biochem 26 (Pt 3):281-285
378. **Law MR, Wald NJ, Rudnicka AR** 2003 Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. BMJ 326:1423-
379. **Rehak NN, Chiang BT** 1988 Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 34:2111-2114
380. **Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL** 2002 Biological Variation of Homeostasis Model Assessment-Derived Insulin Resistance in Type 2 Diabetes. Diabetes Care 25:2022-2025
381. **DOH** 2000 National service framework for coronary heart disease: modern standards and service models. . In: DOH ed.
382. **Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, W Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH** Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. The Lancet 364:685-696
383. **ABCD** 2006 Association of British Clinical Diabetologists (ABCD) response to Department of Health Statement – 7th November 2006 - 'National Policy on Statin Prescribing'. In: ABCD ed.
384. **Valerkou S** 2007 Evidence submitted by Diabetes UK (NICE 78) In: DUK ed.: WWW.parliment.UK

385. 2002 MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 360:7-22
386. **Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes GT, Mehlsen J, Nieminen M, O'Brien E, Ostergren J** 2003 Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial--Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. Lancet 361:1149-1158
387. **Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM** 2004 Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 350:1495-1504
388. **Waters DD, Guyton JR, Herrington DM, McGowan MP, Wenger NK, Shear C** 2004 Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? Am J Cardiol 93:154-158
389. **Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN** 2004 Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. Jama 291:1071-1080
390. **Athyros VG, Papageorgiou AA, Mercouris BR, Athyrou VV, Symeonidis AN, Basayannis EO, Demitriadis DS, Kontopoulos AG** 2002 Treatment with atorvastatin to the National Cholesterol Educational Program goal versus 'usual' care in secondary coronary heart disease prevention. The GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. Curr Med Res Opin 18:220-228
391. **Wagner AM, Sanchez-Quesada JL, Perez A, Rigla M, Cortes M, Blanco-Vaca F, Ordonez-Llanos J** 2000 Inaccuracy of Calculated LDL-Cholesterol in Type 2 Diabetes: Consequences for Patient Risk Classification and Therapeutic Decisions. Clin Chem 46:1830-1832
392. **Schectman G, Patsches M, Sasse EA** 1996 Variability in cholesterol measurements: comparison of calculated and direct LDL cholesterol determinations. Clin Chem 42:732-737

393. **Wagner AM, Perez A, Zapico E, Ordonez-Llanos J** 2003 Non-HDL Cholesterol and Apolipoprotein B in the Dyslipidemic Classification of Type 2 Diabetic Patients. Diabetes Care 26:2048-2051
394. **Simons LA, Levis G, Simons J** 1996 Apparent discontinuation rates in patients prescribed lipid-lowering drugs. The Medical journal of Australia 164:208-211
395. **Naoumova RP, Dunn S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB, Taylor GW, Thompson GR** 1997 Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. J Lipid Res 38:1496-1500
396. **Wallace A, Chinn D, Rubin G** 2003 Taking simvastatin in the morning compared with in the evening: randomised controlled trial. BMJ 327:788-
397. **Waters DD, Guyton JR, Herrington DM, McGowan MP, Wenger NK, Shear C** 2004 Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? The American Journal of Cardiology 93:154-158
398. **Law MR, Wald NJ, Thompson SG** 1994 By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? BMJ 308:367-372
399. 2002 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. Circulation 106:3143-3155
400. **Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G** 1997 Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). Diabetes Care 20:614-620
401. **Wang J-G, Li Y, Franklin SS, Safar M** 2007 Prevention of Stroke and Myocardial Infarction by Amlodipine and Angiotensin Receptor Blockers: A Quantitative Overview. Hypertension 50:181-188
402. **Furberg CD** 1999 Natural Statins and Stroke Risk. Circulation 99:185-188
403. **Sathyapalan T, Atkin SL, Kilpatrick ES** 2008 Variability of lipids in patients with Type 2 diabetes taking statin treatment: implications for target setting. Diabetic Medicine 25:909-915

404. **Nauck M, Warnick GR, Rifai N** 2002 Methods for measurement of LDL-cholesterol: a critical assessment of direct measurement by homogeneous assays versus calculation. Clin Chem 48:236-254
405. **Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL** 2003 The Biological Variation of Testosterone and Sex Hormone-Binding Globulin (SHBG) in Polycystic Ovarian Syndrome: Implications for SHBG as a Surrogate Marker of Insulin Resistance. J Clin Endocrinol Metab 88:1528-1533
406. **Jayagopal V, Kilpatrick ES, Jennings PE, Holding S, Hepburn DA, Atkin SL** 2004 The Biological Variation of Sex Hormone-Binding Globulin in Type 2 Diabetes: Implications for sex hormone-binding globulin as a surrogate marker of insulin resistance. Diabetes Care 27:278-280
407. 2004 Dyslipidemia Management in Adults With Diabetes. Diabetes Care 27:68S-71
408. **NICE** May, 2008 Lipid modification: Cardiovascular risk assessment and the modification of blood lipids for the primary and secondary prevention of cardiovascular disease . In. London: National Institute for Health and Clinical Excellence.
409. **Glasziou PP, Irwig L, Heritier S, Simes RJ, Tonkin A, for the LSI** 2008 Monitoring Cholesterol Levels: Measurement Error or True Change? Ann Intern Med 148:656-661
410. **Ho PM, Rumsfeld JS, Masoudi FA, McClure DL, Plomondon ME, Steiner JF, Magid DJ** 2006 Effect of Medication Nonadherence on Hospitalization and Mortality Among Patients With Diabetes Mellitus. Arch Intern Med 166:1836-1841
411. **Jian-Jun L, Yi-Shi L, Jue C, Jin-Qi Y** 2006 Rebound phenomenon of inflammatory response may be a major mechanism responsible for increased cardiovascular events after abrupt cessation of statin therapy. Medical hypotheses 66:1199-1204
412. **Castelli WP, Anderson K, Wilson PW, Levy D** 1992 Lipids and risk of coronary heart disease. The Framingham Study. Ann Epidemiol 2:23-28
413. **Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D** 1986 Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361,662 men. Lancet 2:933-936
414. **Assmann G, Schulte H, von Eckardstein A** 1996 Hypertriglyceridemia and elevated lipoprotein(a) are risk factors for major coronary events in middle-aged men. Am J Cardiol 77:1179-1184

415. **Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, Larsen ML, Bendiksen FS, Lindahl C, Szarek M, Tsai J** 2005 High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. Jama 294:2437-2445
416. **Jacobson TA, Wertz DA, Hoy T, Kuznik A, Grochulski D, Cziraky M** 2008 Comparison of cardiovascular event rates in patients without cardiovascular disease in whom atorvastatin or simvastatin was newly initiated. Mayo Clin Proc 83:1316-1325
417. **Foody JM, Joyce AT, Rudolph AE, Liu LZ, Benner JS** 2008 Cardiovascular outcomes among patients newly initiating atorvastatin or simvastatin therapy: A large database analysis of managed care plans in the United States. Clin Ther 30:195-205
418. **Dieleman JP, van Wyk JT, van Wijk MA, van Herpen G, Straus SM, Dunselman H, Sturkenboom MC** 2005 Differences between statins on clinical endpoints: a population-based cohort study. Curr Med Res Opin 21:1461-1468
419. 2007 Study of the effectiveness of additional reductions in cholesterol and homocysteine (SEARCH): Characteristics of a randomized trial among 12 064 myocardial infarction survivors. American Heart Journal 154:815-823, 823.e811-823.e816
420. **de Lemos JA, Blazing MA, Wiviott SD, Lewis EF, Fox KAA, White HD, Rouleau J-L, Pedersen TR, Gardner LH, Mukherjee R, Ramsey KE, Palmisano J, Bilheimer DW, Pfeffer MA, Califf RM, Braunwald E, for the AtZI** 2004 Early Intensive vs a Delayed Conservative Simvastatin Strategy in Patients With Acute Coronary Syndromes: Phase Z of the A to Z Trial. JAMA 292:1307-1316
421. **Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM, the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction I** 2004 Intensive versus Moderate Lipid Lowering with Statins after Acute Coronary Syndromes. N Engl J Med 350:1495-1504
422. **LaRosa JC, Grundy SM, Waters DD, Shear C, Barter P, Fruchart J-C, Gotto AM, Greten H, Kastelein JJP, Shepherd J, Wenger NK, the Treating to New Targets I** 2005 Intensive Lipid Lowering with Atorvastatin in Patients with Stable Coronary Disease. N Engl J Med 352:1425-1435

Clinical Endocrinology (2009) 70, 124–128 doi: 10.1111/j.1365-2265.2008.03345.x

ORIGINAL ARTICLE

Metformin maintains the weight loss and metabolic benefits following rimonabant treatment in obese women with polycystic ovary syndrome (PCOS)

Thozhukat Sathyapalan*, Li Wei Cho*, Eric S. Kilpatrick†, Anne-Marie Coady‡ and Stephen L. Atkin*

*Department of Diabetes and Endocrinology, University of Hull, Hull, UK, †Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK, and ‡Department of Obstetric Ultrasound, Hull and East Yorkshire Women's and Children's Hospital, Hull, UK

Summary

Objective Rimonabant has been shown to reduce weight, free androgen index (FAI) and insulin resistance in obese patients with polycystic ovary syndrome (PCOS) compared to metformin. Studies have shown that significant weight regain occurs following the cessation of rimonabant therapy. This study was undertaken to determine if subsequent metformin treatment after rimonabant would maintain the improvement in weight, insulin resistance and hyperandrogenaemia in PCOS.

Design An extension study for 3 months with the addition of metformin to the randomised open labelled parallel study of metformin and rimonabant in 20 patients with PCOS with a body mass index ≥ 30 kg/m^2. Patients who were on 3 months of rimonabant were changed over to metformin for 3 months, whereas those on 3 months of metformin were continued on metformin for another 3 months.

Measurements The primary end-point was a change in weight; secondary end-points were a change in FAI and insulin resistance.

Results The mean weight loss of 6·2 kg associated with 3 months of rimonabant treatment was maintained by 3 months of metformin treatment (mean change +0·2 kg, $P = 0·96$). Therefore, the percentage reduction in weight remained significantly higher in the rimonabant/metformin group compared to metformin only subjects at 6 months compared to baseline (−6·0 ± 0·1% *vs.* −2·8 ± 0·1%, $P = 0·04$). The percentage change in testosterone and FAI from baseline to 6 months was also greater in the rimonabant/metformin group. [Testosterone (−45·0 ± 5·0% *vs.* −16 ± 2·0%, $P = 0·02$); FAI (−53·0 ± 5·0% *vs.* −17·0 ± 12·2%, $P = 0·02$)]. HOMA-IR continued to fall significantly in the rimonabant/metformin group between 0, 3 and 6 months (4·4 ± 0·5 *vs.* 3·4 ± 0·4 *vs.* 2·7 ± 0·3, respectively, $P < 0·01$) but not at all in the metformin only group (3·4 ± 0·7 *vs.* 3·4 ± 0·8 *vs.* 3·7 ± 0·8, respectively, $P = 0·80$). Total cholesterol and LDL reduced significantly in both groups, but improvements in triglycerides and HDL were limited to the rimonabant/metformin group.

Conclusions In these obese patients with PCOS, metformin maintained the weight loss and enhanced the metabolic and biochemical parameters achieved by treatment with rimonabant, compared to 6 months of metformin treatment alone.

(Received 28 May 2008; returned for revision 27 June 2008; finally revised 29 June 2008; accepted 30 June 2008)

Correspondence: Thozhukat Sathyapalan, Michael White Diabetes Centre, 220–236 Analby Road, Hull Royal Infirmary, Hull, HU3 2JZ, UK. Tel.: +44 1482675387; Fax: +44 1482675385; E-mail: tsathyapal@rediffmail.com

Introduction

Rimonabant and metformin have been shown to improve insulin resistance and hyperandrogenaemia in patients with polycystic ovary syndrome (PCOS) though metformin may not be effective in obese patients with PCOS.[1–3] Recently, we have shown that rimonabant will reduce weight and was superior to insulin sensitization by metformin in reducing free androgen index (FAI) and insulin resistance in obese PCOS patients treated over a 12-weeks period.[4] However, previous studies have shown that significant weight regain, over 2 kg occurs, in the 12 weeks following cessation of rimonabant.[5] This study was undertaken to see if subsequent metformin treatment after rimonabant would maintain any of the initial improvement for weight, insulin resistance and hyperandrogenaemia in patients with PCOS.

Methods

Research design and methods

This was an extension arm with the addition of metformin to the randomised open labelled parallel study of metformin and rimonabant in 20 patients with PCOS with a body mass index (BMI) ≥ 30 kg/m^2. All the patients who were on rimonabant were changed over to metformin 500 mg three times daily for 3 months (rimonabant/metformin group), whereas all the patients who were on metformin were continued on metformin for another 3 months (metformin only group).

© 2009 The Authors
Journal compilation © 2009 Blackwell Publishing Ltd

The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus, namely clinical and biochemical evidence of hyperandrogenaemia (Ferriman-Gallwey score > 8; FAI > 8, respectively), oligomenorrhoea or amenorrhea and polycystic ovaries on transvaginal ultrasound.[6] Subjects had no concurrent illness, were not on any medication for the preceding 6 months and were not planning to conceive. None of the patients had successful pregnancy or miscarriage at least 5 years prior to the study entry. Subjects were advised not to change their lifestyle including physical activity or dietary habits during the study period. Non-classical 21-hydroxylase deficiency, hyperprolactinaemia, Cushing's disease and androgen-secreting tumours were excluded by appropriate tests. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee.

Clinical and biochemical assessments were performed at the end of the 3-month period of the extension arm. The primary end-point of the study was a change weight and the secondary end-points were a change in FAI and insulin resistance.

Study bloods and measurement were done after an overnight fast. Compliance was monitored by counting returned medication. Blood samples were processed and analysed as per our previous study.[4] Data are reported as mean ± SEM.

Statistical analysis

Comparisons between the metformin only group and the rimonabant/metformin group from baseline were carried out using the paired *t*-test for biochemical data and clinical observations. The Wilcoxon signed rank test was applied to biochemical data that violated the assumptions of normality when tested using the Kolmogorov–Smirnov test. The effect of treatment was evaluated by first computing the percentage change from baseline in all variables studied and then the percentage change seen for each variable in both groups was compared, thus negating the differences in the baseline values of the two groups. Between-group comparison of percent changes was performed using independent samples *t*-test. For all analysis, a two-tailed $P = 0{\cdot}05$ was considered to indicate statistical significance. Statistical analysis was performed using SPSS for Windows NT, version 14·0 (SPSS Inc., Chicago, IL).

Results

All the 20 subjects completed the 3-month extension period. The compliance was 98% in both groups. All patients tolerated metformin without any side-effects. All patients continued to have an irregular cycle.

Anthropometric parameters (Table 1)

The mean age group of patients was 28·6 ± 1·2 years (metformin 29·8 ± 1·8 *vs.* rimonabant 27·4 ± 1·5 years). There was no significant change in weight or the waist circumference and the waist : hip ratio for the rimonabant/metformin group after 3 months of metformin. Conversely, there was a significant weight loss between 3 and 6 months after metformin treatment, together with a reduction in waist circumference and waist : hip ratio. However, there was a significant reduction in weight, waist circumference and waist : hip ratio in both groups at 6 months compared to baseline. The percentage reduction in weight, waist circumference and waist : hip ratio was significantly higher in rimonabant/metformin group compared to metformin only group at 6 months compared to baseline [weight (–6·0 ± 0·1 *vs.* –2·8 ± 0·1% $P = 0{\cdot}04$); waist circumference (–5·0 ± 0·1 *vs.* –0·6 ± 0·1% $P = 0{\cdot}02$); waist : hip ratio (–1·7 ± 0·4 *vs.* –0·3 ± 0·1% $P = 0{\cdot}02$)].

Metabolic parameters (Table 2)

There was a significant reduction for glucose, insulin and HOMA-IR in the rimonabant/metformin group between baseline, 3 and 6 months. However, there was no change in any of these parameters after 6 months of metformin alone. The percentage change of glucose was significantly more in rimonabant/metformin group compared to the metformin group (–7·0 ± 0·12% *vs.* –0·8 ± 0·1%, $P < 0{\cdot}01$).

There was a significant reduction in total cholesterol and LDL after 6 months in both the groups (Table 2). There was also a reduction in triglycerides and improvements in HDL in the rimonabant/metformin group that was not seen after 6 months of metformin alone.

Biochemical hyperandrogenaemia (Table 2)

There was a significant reduction in the testosterone and FAI between 3 and 6 months in both rimonabant/metformin group and metformin group. There was no change in SHBG either group. The percentage change from baseline to 6 months were greater in the rimonabant/metformin group compared to metformin only group [testosterone (–45·0 ± 5·0% *vs.* –16 ± 2·0%, $P = 0{\cdot}02$); FAI (–53·0 ± 5·0% *vs.* –17·0 ± 12·2%, $P = 0{\cdot}02$)].

There was no significant correlation between weight loss with testosterone ($r = 0{\cdot}438$, $P = 0{\cdot}21$), HOMA-IR ($r = 0{\cdot}19$, $P = 0{\cdot}83$) and total cholesterol ($r = 0{\cdot}349$, $P = 0{\cdot}32$) in the rimonabant pretreatment group or in the metformin alone group after 6 months (testosterone $r = 0{\cdot}252$, $P = 0{\cdot}49$; HOMA-IR $r = 0{\cdot}12$, $P = 0{\cdot}75$; total cholesterol $r = 0{\cdot}124$, $P = 0{\cdot}93$).

Discussion

This is the first study to demonstrate that in obese patients with PCOS the weight reduction and decrease in waist circumference following rimonabant therapy can be maintained with subsequent metformin initiation, whilst improving further both testosterone levels and insulin resistance. The reduction in weight and waist circumference was also superior in the rimonabant/metformin group compared to 6 months of metformin therapy alone.

In the Rio-North America trial with rimonabant treatment in obesity, there was 2 kg weight gain within 12 weeks of stopping rimonabant, with an associated deterioration in their metabolic parameters.[5] Thus, cardio metabolic risk factor improvement was only sustained with weight loss maintenance, leading to the suggestion that long-term treatment rimonabant therapy was necessary.[5] In this

© 2009 The Authors

Table 1. Anthropometric parameters

Parameter	Metformin only group (n = 10)						Rimonabant/Metformin group (n = 10)					
	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1/V2 (P value)	V2–V3 (P value)	V1–V3 (P value)	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1&V2 (P value)	V2&V3 (P value)	V1–V3 (P value)
Weight (kg)	103·8 ± 3·9	102·2 ± 4·1	100·9 ± 4·2	0·08	0·02*	< 0·01*	104·6 ± 4·6	98·4 ± 4·7	98·6 ± 4·8	< 0·01*	0·96	< 0·01*
BMI (kg/m^2)	35·7 ± 1·4	35·09 ± 1·5	34·1 ± 1·2	0·08	0·02*	< 0·01*	36·86 ± 1·0	34·52 ± 1·0	34·59 ± 1·5	< 0·01*	0·09	< 0·01*
Waist (cm)	110·4 ± 3·4	110·0 ± 2·4	109·4 ± 2·3	0·78	0·05*	0·05*	116·0 ± 3·3	109·2 ± 3·7	110·2 ± 3·6	< 0·01*	0·11	< 0·01*
Hip (cm)	121·3 ± 3·2	120·3 ± 3·4	120·3 ± 3·4	0·06	No change	0·30	128·5 ± 4·0	124·1 ± 4·2	124·3 ± 4·2	0·03*	0·34	0·04*
Waist : Hip ratio	0·91 ± 0·02	0·92 ± 0·02	0·91 ± 0·02	0·57	0·05*	0·87	0·90 ± 0·02	0·88 ± 0·01	0·89 ± 0·01	< 0·01*	0·17	0·04

Data are presented as mean ± SEM. All serum results are obtained from fasting variables. All variables were normally distributed. To convert values for glucose to milligrams per decilitre, divide by 0·056. To convert values for insulin to picomoles per litre, multiply by 6. To convert values for cholesterol to milligrams per decilitre, divide by 0·0259. To convert values for triglycerides to milligrams per decilitre, divide by 0·0113. To convert values for testosterone to nanograms per decilitre, divide by 0·03467. To convert values for SHBG to micrograms per decilitre, divide by 34·7. TC, Total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; FAI, free androgen index.

Table 2. Metabolic parameters

Parameter	Metformin only group (n = 10)						Rimonabant / Metformin group (n = 10)					
	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1/V2 (P value)	V2–V3 (P value)	V1–V3 (P value)	Baseline (V1)	12 weeks (V2)	24 weeks (V3)	V1&V2 (P value)	V2&V3 (P value)	V1–V3 (P value)
Testosterone (nmol/l)	3·8 ± 0·6	3·5 ± 0·5	2·5 ± 0·3	0·57	0·04*	0·02*	4·6 ± 0·4	3·1 ± 0·3	2·4 ± 0·8	< 0·01*	0·03*	< 0·01*
SHBG (nmol/l)	18·4 ± 2·1	19·2 ± 2·1	23·9 ± 4·7	0·65	0·41	0·30	21·5 ± 2·8	23·4 ± 2·9	23·9 ± 3·7	0·08	0·70	0·1
FAI	20·5 ± 4·0	18·2 ± 3·0	10·8 ± 2·0	0·38	0·04*	0·02*	26·6 ± 6·1	16·6 ± 4·1	11·4 ± 2·3	< 0·01*	0·02*	< 0·01*
Glucose (mmol/l)	4·9 ± 0·1	4·7 ± 0·1	4·9 ± 0·1	0·20	0·30	0·50	5·0 ± 0·1	4·8 ± 0·2	4·6 ± 0·4	0·04*	0·01*	<0·01*
Insulin (μIU/ml)	15·2 ± 2·9	15·5 ± 3·4	17·3 ± 3·9	0·81	0·80	0·70	19·6 ± 2·2	16·0 ± 1·8	13·4 ± 1·8	0·08	0·02*	0·01*
HOMA-IR	3·4 ± 0·7	3·4 ± 0·8	3·7 ± 0·8	0·77	0·80	0·81	4·4 ± 0·5	3·4 ± 0·4	2·7 ± 0·3	0·05*	0·02*	< 0·01*
TC (mmol/l)	6·0 ± 0·3	5·6 ± 0·2	4·9 ± 0·5	0·12	0·20	0·05*	5·1 ± 0·3	4·7 ± 0·3	4·6 ± 0·3	0·20	0·61	0·05*
LDL-C (mmol/l)	3·9 ± 0·3	3·5 ± 0·1	2·8 ± 0·4	0·06	0·07	0·02*	3·3 ± 0·2	3·1 ± 0·3	2·9 ± 0·3	0·21	0·52	0·05*
HDL-C (mmol/l)	1·2 ± 0·2	1·2 ± 0·1	1·4 ± 0·2	0·7	0·42	0·52	1·2 ± 0·1	1·1 ± 0·1	1·2 ± 0·1	0·38	0·04*	No change
TG (mmol/l)	2·0 ± 0·3	1·9 ± 0·4	1·4 ± 0·2	0·34	0·50	0·22	1·4 ± 0·2	1·3 ± 0·1	1·0 ± 0·1	0·44	< 0·01*	0·05*
Hs-CRP (mg/l)	4·2 ± 1·7	4·0 ± 1·3	3·8 ± 0·8	0·87	0·94	0·81	5·6 ± 0·9	5·20 ± 1·2	7·1 ± 1·8	0·70	0·4	0·4

Data are presented as mean ± SEM. All serum results are obtained from fasting variables. All variables were normally distributed. To convert values for testosterone to nanograms per decilitre, divide by 0·03467. To convert values for SHBG to micrograms per decilitre, divide by 34·7. To convert values for glucose to milligrams per decilitre, divide by 0·056. To convert values for insulin to picomoles per litre, multiply by 6. To convert values for cholesterol to milligrams per decilitre, divide by 0·0259. To convert values for triglycerides to milligrams per decilitre, divide by 0·0113. TC, Total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; FAI, free androgen index.

© 2009 The Authors

study the addition of metformin after rimonabant treatment appeared to provide weight maintenance preventing weight rebound.

The improvement in weight and waist circumference was seen at 6 months but not after 3 months of metformin therapy. Study results of metformin on weight loss, especially obese patients with PCOS[1–3,7] are conflicting, particularly a BMI > 37 may make metformin ineffective. Variations in study groups in pretreatment BMI, metformin dose, duration of metformin therapy, concomitant life style changes and patient adherence to treatment may account for many of these differences and makes comparison difficult.[2,8]

The significant reduction in glucose, insulin and HOMA-IR with metformin after rimonabant was unexpected and may have reflected that weight loss may have facilitated its effect, and that the insulin sensitization action of metformin was complementary to the weight loss caused by rimonabant. However, rimonabant has been shown to reduce fasting insulin[9] and glucose[10] over a period of 1–2 years. There was no change in any of these parameters after 6 months of sole metformin treatment, despite the small though significant weight loss, a finding that has been found before in obese group patients with PCOS.[1]

There was a significant correlation between weight reduction and testosterone at 3 months of rimonabant therapy that was lost following subsequent metformin treatment. This suggests that the initial weight loss was responsible for the reduction in testosterone with rimonabant, but that there may be a weight independent action of metformin for further testosterone reduction.[11,12] Rimonabant has also been found to have weight independent effects which might be mediated through less well defined direct pharmacological effects.[13,14]

The significant reduction of total cholesterol and LDL cholesterol after 6 months of metformin was more likely to be due to weight reduction than a direct effect of metformin as FAI and insulin resistance were unchanged. meta analysis which showed significant reduction of LDL with metformin, but no changes in total cholesterol.[8] Rimonabant has been reported to reduce triglycerides and increase HDL in other studies after 1–2 years.[5,9,10,17] There was certainly a significant reduction of triglycerides and improvement in HDL on metformin in rimonabant pretreated PCOS patients in this study. The apparent lack of improvement in the first 3 months on rimonabant is more difficult to explain but might be due to shorter duration of therapy. However, this shows that metformin therapy after rimonabant pretreatment might sustain, or even enhance, the beneficial effects of rimonabant in these parameters. Surprisingly there was a significant reduction of total cholesterol and LDL cholesterol after metformin therapy in rimonabant pretreated patients which has not been found by others.[5,9,10,17]

Rimonabant is contraindicated in pregnancy; however, infertility is often a significant issue in PCOS.[19,20] Weight loss has a significant effect on ovulation and the chance of pregnancy;[21] therefore, rimonabant pretreatment followed by metformin therapy could hypothetically be an option in this group of patients. Moreover, as there is growing evidence that endocannabinoids are involved in implantation, pregnancy and miscarriage in animal models and human tissues,[22–25] though whether they are involved in miscarriage[25] and infertility is unknown.

We made no assessment of clinical hyperandrogenaemia, or of menstrual change and ovulation. As such, the blood tests were done without respect to the bleeding pattern so there may have been temporary hormonal fluctuations interfering with the results. Nevertheless, the irregular menses and lack of change in the progesterone measurements before and after the study suggests that rimonabant and metformin had not affected menstrual periods over the 24-week period in this obese group.

In summary, weight maintenance by metformin was achieved after 3 months of rimonabant therapy and there was an enhancement of metabolic parameters, over and above that seen by metformin also. This may be a novel strategy in this patient group where obesity is a major issue.

References

1 Maciel, G.A.R., Soares Junior, J.M., Alves da Motta, E.L., Abi Haidar, M., de Lima, G.R. & Baracat, E.C. (2004) Nonobese women with polycystic ovary syndrome respond better than obese women to treatment with metformin. *Fertility and Sterility*, **81**, 355–360.

2 Harborne, L.R., Sattar, N., Norman, J.E. & Fleming, R. (2005) metformin and weight loss in obese women with polycystic ovary syndrome: comparison of doses. *Journal of Clinical Endocrinology and Metabolism*, **90**, 4593–4598.

3 Tang, T., Glanville, J., Hayden, C.J., White, D., Barth, J.H. & Balen, A.H. (2006) Combined lifestyle modification and metformin in obese patients with polycystic ovary syndrome. A randomized, placebo-controlled, double-blind multicentre study. *Human Reproduction*, **21**, 80–89.

4 Sathyapalan, T., Cho, L.W., Kilpatrick, E.S., Coady, A.M. & Atkin, S.L. (2008) A comparison between rimonabant and metformin in reducing biochemical hyperandrogenaemia and insulin resistance in patients with polycystic ovary syndrome (PCOS): a randomized open-label parallel study. *Clinical Endocrinology*, **69**, 931–935.

5 Pi-Sunyer, F.X., Aronne, L.J., Heshmati, H.M., Devin, J. & Rosenstock, J. (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *The Journal of the American Medical Association*, **295**, 761–775.

6 The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Human Reproduction*, **19**, 41–47.

7 Fleming, R., Hopkinson, Z.E., Wallace, A.M., Greer, I.A. & Sattar, N. (2002) Ovarian function and metabolic factors in women with oligomenorrhea treated with metformin in a randomized double blind placebo-controlled trial. *Journal of Clinical Endocrinology and Metabolism*, **87**, 569–574.

8 Lord, J.M., Flight, I.H. & Norman, R.J. (2003) Metformin in polycystic ovary syndrome: systematic review and meta-analysis. *British Medical Journal*, **327**, 951–953.

9 Van Gaal, L.F., Rissanen, A.M., Scheen, A.J., Ziegler, O. & Rossner, S. (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet*, **365**, 1389–1397.

10 Scheen, A.J., Finer, N., Hollander, P., Jensen, M.D. & Van Gaal, L.F. (2006) Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet*, **368**, 1660–1672.

© 2009 The Authors

11 Mansfield, R., Galea, R., Brincat, M., Hole, D. & Mason, H. (2003) Metformin has direct effects on human ovarian steroidogenesis. *Fertility and Sterility*, **79**, 956–962.

12 Arslanian, S.A., Lewy, V., Danadian, K. & Saad, R. (2002) Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1555–1559.

13 Bensaid, M., Gary-Bobo, M., Esclangon, A., Maffrand, J.P., Le Fur, G., Oury-Donat, F. & Soubrie, P. (2003) The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Molecular Pharmacology*, **63**, 908–914.

14 Liu, Y.L., Connoley, I.P., Wilson, C.A. & Stock, M.J. (2005) Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep (ob)/Lep (ob) mice. *International Journal of Obesity*, **29**, 183–187.

15 Osei-Hyiaman, D., DePetrillo, M., Pacher, P., Liu, J., Radaeva, S., Batkai, S., Harvey-White, J., Mackie, K., Offertaler, L., Wang, L. & Kunos, G. (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *Journal of Clinical Investigation*, **115**, 1298–1305.

16 Salmenniemi, U., Ruotsalainen, E., Pihlajamaki, J., Vauhkonen, I., Kainulainen, S., Punnonen, K., Vanninen, E. & Laakso, M. (2004) Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation*, **110**, 3842–3848.

17 Despres, J.P., Golay, A. & Sjostrom, L. (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *New England Journal of Medicine*, **353**, 2121–2134.

18 Berg, A.H., Combs, T.P. & Scherer, P.E. (2002) ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends in Endocrinology and Metabolism*, **13**, 84–89.

19 Kousta, E., White, D.M., Cela, E., McCarthy, M.I. & Franks, S. (1999) The prevalence of polycystic ovaries in women with infertility. *Human Reproduction*, **14**, 2720–2723.

20 Allen, S.E., Potter, H.D. & Azziz, R. (1997) Prevalence of hyperandrogenemia among nonhirsute oligo-ovulatory women. *Fertility and Sterility*, **67**, 569–572.

21 Clark, A.M., Ledger, W., Galletly, C., Tomlinson, L., Blaney, F., Wang, X. & Norman, R.J. (1995) Weight loss results in significant improvement in pregnancy and ovulation rates in anovulatory obese women. *Human Reproduction*, **10**, 2705–2712.

22 Liu, W.M., Duan, E.K. & Cao, Y.J. (2002) Effects of anandamide on embryo implantation in the mouse. *Life Science*, **71**, 1623–1632.

23 Dennedy, M.C., Friel, A.M., Houlihan, D.D., Broderick, V.M., Smith, T. & Morrison, J.J. (2004) Cannabinoids and the human uterus during pregnancy. *American Journal of Obstetrics and Gynecology*, **190**, 2–9; discussion 3A.

24 Maccarrone, M., Valensise, H., Bari, M., Lazzarin, N., Romanini, C. & Finazzi-Agro, A. (2000) Relation between decreased anandamide hydrolase concentrations in human lymphocytes and miscarriage. *Lancet*, **355**, 1326–1329.

25 Bifulco, M., Grimaldi, C., Gazzerro, P., Pisanti, S. & Santoro, A. (2007) Rimonabant: just an antiobesity drug? Current evidence on its pleiotropic effects. *Molecular Pharmacology*, **71**, 1445–1456.

© 2009 The Authors

Editorial

Polycystic Ovary Syndrome, Inflammation, and Statins: Do We Have the Right Target?

Kathleen M. Hoeger

Department of Obstetrics and Gynecology, University of Rochester Medical Center, Rochester, New York

(*J Clin Endocrinol Metab* 94: 35–37, 2009)

Polycystic ovary syndrome (PCOS) is a common and complex endocrinopathy in reproductive-aged women presenting multiple clinical challenges. Women with PCOS experience menstrual cycle irregularity, androgen excess in the form of acne and hirsutism, as well as infertility (1), and are at significantly increased risk of diabetes (2). There is also evidence of increased cardiovascular risk demonstrated by abnormal lipid profiles (3) and early evidence of preclinical cardiovascular disease such as increased carotid artery intima media thickness (4) and coronary artery calcification (5). Several studies have demonstrated an increased inflammatory state, noted by increased high-sensitivity C-reactive protein (hs-CRP) levels (6, 7). hs-CRP has been shown to be predictive of an elevated risk for cardiovascular disease in women (8), but there is controversy as to whether this marker is on the causal pathway to cardiovascular disease and whether it remains an appropriate target for therapy (9).

Although there is ongoing debate as to whether CRP is an appropriate target for medical therapy, a recent randomized trial of rosuvastatin or placebo in an older population at increased risk for cardiovascular disease but with normal low-density lipoprotein (LDL) cholesterol (<130 mg/dl) and elevated hs-CRP (>2 mg/liter) (JUPITER trial) was recently halted at 2 yr into a 4-yr trial when interim analysis suggested that treatment with rosuvastatin was associated with an unequivocal reduction in cardiovascular morbidity and mortality (10). Although the details of the study results are still pending, it is suggestive that lipid parameters alone are insufficient as a measure of cardiovascular risk.

PCOS is well recognized to be associated with increased cardiovascular disease risk, although not all studies support an increased rate of cardiovascular events (11, 12). Nonetheless, given the increase in measures of cardiovascular risk, effort toward cardiovascular disease prevention and reduction in diabetes risk are recommended in PCOS. Despite the increased prevalence of dyslipidemia in women with PCOS, there are few studies examining the impact of statin therapy and none in the absence of oral contraceptive cotreatment (13). In the current issue of the *Journal of Endocrinology and Metabolism*, Sathyapalan *et al.* (14) report on a placebo-controlled trial of 40 young women with PCOS treated with 20 mg of atorvastatin or placebo for 12 wk. The authors chose hs-CRP as their primary outcome measure based on the concern regarding increased markers of inflammation that have been reported in women with PCOS in prior studies (6). Inflammation has been associated with increased risk of atherosclerosis and with development of type 2 diabetes (15). Women included in the current trial had a mean age of 28 yr and a body mass index of 33.7 kg/m^2. PCOS was diagnosed by presence of irregular cycles, androgen excess, and ultrasound findings of polycystic ovarian morphology.

At the end of the 12-wk trial, subjects receiving atorvastatin had a 25% reduction in hs-CRP compared with a nonsignificant reduction in the placebo group. Despite the reduction, however, hs-CRP remained significantly elevated in the treatment group at study conclusion (3.4 mg/liter). Additionally, subjects receiving atorvastatin demonstrated a 24.6% reduction in total testosterone and a 32.7% reduction in free androgen index, significantly more than in the placebo group. Notably, total cholesterol, LDL cholesterol, and triglycerides all improved in the atorvastatin group compared with placebo, as would be anticipated with statin use, as did fasting insulin and homeostasis model assessment of insulin resistance measures, both of which rose in the placebo group. Testosterone improvement was significantly correlated with changes in insulin measures but did not correlate with lipid changes.

Current recommendations for statin therapy are based on lipid measures, primarily LDL cholesterol. In this population of young women with PCOS, the mean lipid parameters were within the normal range, and therefore these women would not have been targeted by traditional recommendations to start sta-

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2009 by The Endocrine Society
doi: 10.1210/jc.2008-2376 Received October 31, 2008 Accepted November 3, 2008.

For article see page 103

Abbreviations: CRP, C-reactive protein; hs-CRP, high-sensitivity CRP; LDL, low-density lipoprotein; PCOS, polycystic ovary syndrome

Downloaded from jcem.endojournals.org by Thozhukat Sathyapalan on January 8, 2009

tin therapy. Specific cardiovascular risk factors otherwise in this young population are not detailed, but it is likely they would be relatively low risk on Framingham scoring (<10% risk of myocardial infarction in the next 10 yr). This study did not look at other intermediate cardiovascular endpoints such as measures of carotid artery plaques or coronary artery calcium scores, both of which have been shown to be abnormal in young women with PCOS (4, 5). This was a short trial that was not designed to look for reduction in cardiovascular disease, and implications of this finding with respect to cardiovascular disease prevention for this population are not clear. Although reduction in CRP may ultimately prove to be beneficial to young women with PCOS with respect to cardiovascular protection, the level was not normalized by this trial, and at study conclusion CRP remained elevated. The benefit to cardiovascular protection of a lower but not normal CRP is not clear.

What of the impact of statins on other common features of PCOS? This may be relatively more important given the overall concerns of young women with PCOS, who experience irregular cycles and hirsutism as key features of the disease. In this trial, there was no significant improvement in menstrual cycle length, which remained at over 50 d in the statin treatment group and no difference from placebo. Additionally, whereas the authors did find a significant reduction in serum testosterone and free androgen index, there was no clinical measure of hirsutism reported. Indeed in a trial of 12-wk duration, it is unlikely that a long-term measure such as hirsutism would be impacted. It would be useful for future studies of longer duration, however, to examine this endpoint.

Obesity is a common finding in women with PCOS. In this trial, subjects were significantly obese at the trial onset and were instructed not to change their baseline activity, lifestyle, or diet during the 12-wk trial. Therefore, despite the metabolic and endocrine improvements, there were no improvements in body mass index during the trial. Several studies have demonstrated reduction in CRP with weight reduction (16). Weight reduction in PCOS is also associated with improvements in androgens, menstrual cycle, and fertility (17). Overall recommendations for first-line treatment of PCOS in the presence of obesity include lifestyle change. In other populations, a comparison of lifestyle change and statin therapy proved equally effective in reduction of cholesterol (18), although maintenance of lifestyle changes over the long term is notoriously challenging.

Fertility is impaired in PCOS, and treatments for PCOS such as lifestyle therapy, and possibly insulin sensitizers, are associated with improvement in ovulatory function. Spontaneous pregnancies occur in PCOS and often without prediction because women are often oligo-ovulatory. There is a possible teratogenic risk for statin therapy in early pregnancy, and therefore there is concern raised when these agents are given to young women in the absence of contraception. This study did not measure ovulatory function, but statin use was not associated with improvement in menstrual cycle length, suggesting no impact on ovulation. Nonetheless, statins should be used in reproductive-aged with the appropriate cautions.

What can we therefore conclude with respect to the role of statin therapy in PCOS? Current recommendations for statin use are based on lipid parameters and family history of cardiovascular disease. These recommendations may change as larger and more inclusive trials examine the impact of statins for other indications. Inflammation may indeed prove to be the right target to improve insulin sensitivity and ultimately reduce cardiovascular disease risk. The American Academy of Pediatricians now recommends giving consideration to statin use for children as young as 8 yr in the presence of severe hyperlipidemia (19). This recommendation has met with considerable controversy because there are no long-term studies to suggest that early treatment in childhood prevents cardiovascular events better than attention in adulthood, nor are there long-term studies assessing the risk in children and adolescents. Further evidence in young adult populations at risk, such as those with PCOS, may show benefit in statin therapy over the long term. For now, however, first-line therapy in obese women with PCOS should focus on lifestyle and dietary change with additional antiandrogen or ovulatory therapy as indicated. Although additional therapeutic choices such as statin therapy may prove to have substantial benefit, the evidence is still pending.

Acknowledgments

Address all correspondence and requests for reprints to: Kathleen M. Hoeger, M.D., University of Rochester Medical Center, Department of Obstetrics and Gynecology, Box 668, 601 Elmwood Avenue, Rochester, New York 14642. E-mail: Kathy_hoeger@urmc.rochester.edu.

The author reports no conflicts of interest for this editorial.

References

1. Ehrmann DA 2005 Polycystic ovary syndrome. N Engl J Med 352:1223–1236
2. Legro RS, Kunselman AR, Dodson WC, Dunaif A 1999 Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. J Clin Endocrinol Metab 84:165–169
3. Talbott E, Clerici A, Berga SL, Kuller L, Guzick D, Detre K, Daniels T, Engberg RA 1998 Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. J Clin Epidemiol 51:415–422
4. Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH 2000 Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thiomb Vasc Biol 20:2414–2421
5. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy 2nd PF, Fitzpatrick LA 2003 Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:2562–2568
6. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N 2001 Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 86:2453–2455
7. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, Blumenfeld Z 2004 Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab 89:2160–2165
8. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE 2005 Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. JAMA 294:326–333
9. Scirica BM, Morrow DA 2006 Is C-reactive protein an innocent bystander or proatherogenic culprit? The verdict is still out. Circulation 113:2128–2134; discussion, 2151
10. JUPITER—Crestor 20 mg versus placebo in prevention of cardiovascular (CV) events. www.clinicaltrials.gov/ct2/show/NCT00239681. Accessed October 27, 2008

Downloaded from jcem.endojournals.org by Thozhukat Sathyapalan on January 8, 2009

11. **Wild S, Pierpoint T, McKeigue P, Jacobs H** 2000 Cardiovascular disease in women with polycystic ovary syndrome at long-term follow-up: a retrospective cohort study. Clin Endocrinol (Oxf) 52:595–600
12. **Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS** 1998 Mortality of women with polycystic ovary syndrome at long-term follow-up. J Clin Epidemiol 51:581–586
13. **Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ** 2007 Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial. J Clin Endocrinol Metab 92:456–461
14. **Sathyapalan T, Kilpatrick ES, Coady AM, Atkin SL** 2008 The effect of atorvastatin in patients with polycystic ovary syndrome: a randomized double blind placebo controlled study. J Clin Endocrinol Metab 94:103–108
15. **Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM** 2001 C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286:327–334
16. **Jae SY, Fernhall B, Heffernan KS, Jeong M, Chun EM, Sung J, Lee SH, Lim YJ, Park WH** 2006 Effects of lifestyle modifications on C-reactive protein: contribution of weight loss and improved aerobic capacity. Metabolism 55:825–831
17. **Hoeger KM** 2006 Role of lifestyle modification in the management of polycystic ovary syndrome. Best Pract Res Clin Endocrinol Metab 20: 293–310
18. **Becker DJ, Gordon RY, Morris PB, Yorko J, Gordon YJ, Li M, Iqbal N** 2008 Simvastatin vs therapeutic lifestyle changes and supplements: randomized primary prevention trial. Mayo Clin Proc 83:758–764
19. **Daniels SR, Greer FR** 2008 Lipid screening and cardiovascular health in childhood. Pediatrics 122:198–208

Downloaded from jcem.endojournals.org by Thozhukat Sathyapalan on January 8, 2009

The Effect of Atorvastatin in Patients with Polycystic Ovary Syndrome: A Randomized Double-Blind Placebo-Controlled Study

Thozhukat Sathyapalan, Eric S. Kilpatrick, Anne-Marie Coady, and Stephen L. Atkin

Department of Diabetes and Endocrinology (T.S., S.L.A.), University of Hull, Hull HU6 7RX, United Kingdom; Department of Clinical Biochemistry (E.S.K.), Hull Royal Infirmary, Hull HU3 2JZ, United Kingdom; and Department of Obstetric Ultrasound (A.-M.C.), Hull and East Yorkshire Women's and Children's Hospital, Hull HU3 2PZ, United Kingdom

Context: Polycystic ovary syndrome (PCOS) is associated with increased risk of cardiovascular morbidity, whereas statins are proven to reduce cardiovascular mortality and morbidity through lipid-lowering and perhaps through their pleiotropic effects. Statins can also reduce testosterone *in vitro* by inhibiting ovarian theca-interstitial cell proliferation and steroidogenesis and reducing inflammation *in vivo*.

Objective: Our objective was to assess the effect of atorvastatin on inflammatory markers, insulin resistance, and biochemical hyperandrogenemia in patients with PCOS.

Design and Setting: We conducted a randomized, double-blind, placebo-controlled study at a tertiary care setting in United Kingdom.

Patients: Patients included 40 medication-naive patients with PCOS and biochemical hyperandrogenemia.

Methods: Patients were randomized to either atorvastatin 20 mg daily or placebo.

Main Outcome Measures: The primary endpoint of the study was a change in the inflammatory marker high-sensitivity C-reactive protein. The secondary endpoints were a change in insulin resistance and total testosterone.

Results: After 12 wk atorvastatin, there was a significant reduction (mean ± SEM) in total cholesterol (4.6 ± 0.2 *vs.* 3.4 ± 0.2 mmol/liter, $P < 0.01$), low-density lipoprotein cholesterol (2.9 ± 0.2 *vs.* 1.8 ± 0.2 mmol/liter, $P < 0.01$), triglycerides (1.34 ± 0.08 *vs.* 1.08 ± 0.13 mmol/liter, $P < 0.01$), high-sensitivity C-reactive protein (4.9 ± 1.4 *vs.* 3.4 ± 1.1 mg/liter, $P = 0.04$), free androgen index (13.4 ± 0.6 *vs.* 8.7 ± 0.4, $P < 0.01$), testosterone (4.1 ± 0.2 *vs.* 2.9 ± 0.1 nmol/liter, $P < 0.01$) and insulin resistance as measured by homeostasis model assessment for insulin resistance (HOMA-IR) (3.3 ± 0.4 *vs.* 2.7 ± 0.4). There was a significant increase in SHBG (31.1 ± 1.0 *vs.* 35.3 ± 1.2 nmol/liter, $P < 0.01$). There was a positive correlation between the reduction in HOMA-IR in the atorvastatin group with the reduction in triglycerides and the reduction of free androgen index. There was a significant deterioration of HOMA-IR in the placebo group (3.0 ± 0.4 *vs.* 3.8 ± 0.5).

Conclusions: This study suggests that atorvastatin is effective in reducing inflammation, biochemical hyperandrogenemia, and metabolic parameters in patients with PCOS after a 12-wk period. (*J Clin Endocrinol Metab* **94: 103–108, 2009)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2009 by The Endocrine Society
doi: 10.1210/jc.2008-1750 Received August 11, 2008. Accepted October 10, 2008.
First Published Online October 21, 2008

For editorial see page 35

Abbreviations: BMI, Body mass index; FAI, free androgen index; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; OCP, oral contraceptive pill; PCOS, polycystic ovary syndrome.

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age with a reported prevalence of 5–7% (1–3). PCOS is associated with a broad range of adverse sequelae, including dyslipidemia, hypertension, insulin resistance, hyperandrogenemia, and gestational and type 2 diabetes, which ultimately increase the risk of cardiovascular morbidity (4–12). High-sensitivity C-reactive protein (hsCRP) is an indicator or cardiovascular mortality, and the elevated levels of this inflammatory marker hsCRP have been reported in PCOS patients compared with controls (13–15). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have been shown to reduce cardiovascular morbidity and mortality in several studies (16–19). They also may have other, non-lipid-lowering effects, suggested by their benefits among hypertensive patients with normal lipids (20) as well as their anti-inflammatory effect in patients with rheumatoid arthritis (21). Other pleiotropic effects of statins include an improvement in CRP, endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory responses, and stabilization of atherosclerotic plaques (22).

In patients with PCOS, simvastatin with concomitant oral contraceptive pill (OCP) therapy has recently been shown to reduce testosterone, LH, and markers of systemic inflammation incrementally more than OCP treatment alone (23). In that study, OCP treatment showed an adverse effect on glucose metabolism with an increase in fasting glucose and insulin, without a statin-attributable effect (23). Furthermore, the addition of statins to OCP treatment had a significant beneficial effect on testosterone, and although the OCP alone had no significant effect on CRP, the addition of a statin led to a significant decrease of CRP below the baseline level.

Hypothetically, the reduction of inflammation through statin therapy should have a beneficial effect in PCOS where the inflammatory marker hsCRP has been shown to be elevated. However, the effects of statins alone in patients with PCOS who are treatment naive are unknown, so we have therefore performed a double-blind, randomized, placebo-control study with atorvastatin to investigate this, powered so as to detect a change in hsCRP.

Patients and Methods

A randomized, double-blind, placebo-controlled study was undertaken using atorvastatin 20 mg daily. The diagnosis of PCOS was based on all three diagnostic criteria of the Rotterdam consensus being present for each patient, namely clinical and biochemical evidence of hyperandrogenemia [Ferriman-Gallwey score >8; free androgen index (FAI) >8], oligomenorrhea or amenorrhea, and polycystic ovaries on transvaginal ultrasound (24). Subjects had no concurrent illness, were not on any prescription or over-the-counter medication that was likely to affect insulin sensitivity, lipids, or ovarian function including hormonal contraceptives for the preceding 6 months. None of the patients had statin therapy in the past. Subjects were not planning to conceive and were using barrier contraception. Subjects were advised not to change their lifestyle, including physical activity or dietary habits, during the study period. Nonclassical 21-hydroxylase deficiency, hyperprolactinemia, Cushing's disease, and androgen-secreting tumors were excluded by appropriate tests. Forty-four patients were screened before the study, and two patients were excluded due to use of oral contraceptives within the previous 6 months and two patients due to use of metformin in the previous 6 months. Forty subjects who fulfilled both inclusion and exclusion criteria were randomly assigned to the atorvastatin or the placebo group based on a computer-generated randomization list. Each randomization number corresponded with one of the two possible interventions, and labeling was done by personnel not involved in the trial. Compliance with treatment was calculated by counting the returned medications. All patients gave informed consent. The study was approved by the Hull and East Riding Local Research Ethics committee. This study was registered in the International Standard Randomized Controlled Trial Number (ISRCTN) registry as ISRCTN24474824.

Clinical and fasting biochemical assessments were performed at baseline and at the end of the 3-month period. The primary endpoint of the study was a change in hsCRP, and the secondary endpoints were a change in homeostasis model assessment for insulin resistance (HOMA-IR) and total testosterone.

Fasting venous blood was collected into serum gel and fluoride oxalate tubes. Samples were separated by centrifugation at 2000 × *g* for 15 min at 4 C, and the aliquots stored at −20 C. Serum testosterone was measured on an Architect analyzer (Abbott Laboratories, Maidenhead, UK) and SHBG by an immunometric assay with fluorescence detection on the DPC Immulite 2000 analyzer using the manufacturer's recommended protocol. The FAI was calculated as the total testosterone × 100/SHBG. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using a Synchron LX20 analyzer (Beckman-Coulter, High Wycombe, UK). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation. Serum insulin was assayed using a competitive chemiluminescent immunoassay performed on the manufacturer's DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, UK). The analytical sensitivity of the insulin assay was 2 μU/ml, the coefficient of variation was 6%, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyzer (Beckman-Coulter), using the manufacturer's recommended protocol. The coefficient of variation for the assay was 1.2% at a mean glucose value of 5.3 mmol/liter during the study period. The insulin resistance was calculated using the HOMA method [HOMA-IR = (insulin × glucose)/22.5]. Data are reported as mean ± SEM.

Statistical analysis

The sample size was based on the study on the known effect of atorvastatin on hsCRP in patients with impaired fasting glucose (25), with the assumption that a similar effect would occur in those patients with PCOS. Powered specifically for CRP, the minimum difference worth detecting/observed difference was 32.7%, estimated within-group SD was 11.1; therefore, for 90% power and a significance level of 5%, a sample size of 16 per group was calculated. Adjusting for a possible 20% dropout rate meant a total of 40 patients needed to be recruited.

Comparisons between both the groups from baseline were carried out using the paired *t* test for biochemical data and clinical observations. The Wilcoxon signed rank test was applied to biochemical data that violated the assumptions of normality when tested using the Kolmogorov-Smirnov test. The effect of treatment was evaluated by first calculating the percentage change from baseline for all variables studied and then the percentage change for each variable in each patient group, thus negating the differences in the baseline values of the two groups. Between-group comparison of percent changes was performed using independent-samples *t* test. For all analyses, a two-tailed $P \leq 0.05$ was considered to indicate statistical significance. Statistical analysis was performed using SPSS for Windows NT, version 14.0 (SPSS Inc., Chicago, IL).

Results

Thirty-seven patients completed the study. Two patients from the placebo group [patient 10, age 27, body mass index (BMI) 32.9, and patient 14, age 26, BMI 33.4] and one patient from

atorvastatin group (patient 37, age 26, BMI 32.9) dropped out of the study within 4 wk due to noncompliance. After their exclusion, compliance was 99% in both groups. None of the subjects developed significant side effects in the course of the study. None of them developed symptoms of muscle toxicity, and liver function tests and creatine kinase remained normal throughout the study.

The mean age group of patients was 27.7 ± 1.4 yr (atorvastatin 26.6 ± 1.2 *vs.* placebo 28.8 ± 1.8; $P = 0.44$). The BMI were comparable in both atorvastatin and placebo group (33.20 ± 1.4 *vs.* 33.92 ± 1.4 kg/m^2, $P = 0.62$). There were no significant differences in baseline parameters between the two groups.

There was a significant absolute reduction in total cholesterol, LDL-C, triglycerides, FAI, SHBG, and total testosterone in patients randomized to atorvastatin, whereas there were no changes in any of these parameters in the placebo group (Table 1). The percent change in total cholesterol, triglycerides, LDL, FAI, SHBG, and total testosterone was greater in the atorvastatin group compared with placebo group. There was a significant reduction in serum insulin levels and HOMA-IR in patients taking atorvastatin, whereas there was a significant increase in both these parameters in patients randomized to placebo.

There was no change in cycle length before and after treatment with atorvastatin (52 ± 12 *vs.* 50 ± 6 d) or placebo (55 ± 8 *vs.* 52 ± 10 d).

There was a significant correlation between reduction in HOMA-IR and improvement in FAI ($r^2 = 0.56$; $P = 0.04$) in the atorvastatin group. There was also a significant correlation between reduction of HOMA-IR with reduction in triglycerides ($r^2 = 0.68$; $P < 0.01$). However, there was no linear correlation between reduction in total cholesterol with improvement of FAI ($r^2 = 0.015$; $P = 0.95$), testosterone ($r^2 = 0.1$; $P = 0.69$) or SHBG ($r^2 = 0.29$; $P = 0.22$).

Discussion

In patients with PCOS, 12 wk treatment with atorvastatin 20 mg resulted in a significant reduction in inflammatory markers, insulin resistance, and hyperandrogenemia in addition to the expected improvement in lipids. The reduction in hyperandrogenemia was also independent of the lipid improvement due to atorvastatin treatment.

The 26% reduction in total cholesterol, 36% reduction in LDL, and 21% reduction in triglycerides with atorvastatin is comparable with other trials (26, 27), although in this study there were no detectable changes in HDL. PCOS is associated with an increase of cardiovascular risk factors, including dyslipidemia that typically is reflected in an elevated total cholesterol and LDL (28–31). This is in accord with a report using simvastatin 20 mg daily and an OCP containing 20 μg ethinyl estradiol and 150 μg desogestrel over 12 wk that reduced total cholesterol and LDL by 7.5 and 20%, respectively (23). In contrast, the OCP alone induced a modest increase in TCs of 5% without any marked effect on LDL cholesterol. In that study, triglycerides remained virtually unchanged after statin and OCP treatment but increased

TABLE 1. Comparison of anthropometric and hormonal parameters before and after treatment

Parameter	Atorvastatin group (n = 19)			Placebo group (n = 18)			% change		
	Baseline	12 wk	*P* value	Baseline	12 wk	*P* value	Atorvastatin	Placebo	*P* value
Weight (kg)	91.29 ± 3.4	91.20 ± 3.4	0.42	93.12 ± 4.8	93.05 ± 4.7	0.67	0.1 ± 0.1	0.03 ± 0.02	0.82
BMI (kg/m^2)	33.20 ± 1.4	33.16 ± 1.4	0.42	33.94 ± 1.5	33.92 ± 1.4	0.67	0.1 ± 0.1	0.03 ± 0.02	0.82
Waist (cm)	98.1 ± 3.2	98.9 ± 2.2	0.59	99.3 ± 2.4	98.7 ± 2.1	0.54	0.07 ± 0.02	0.02 ± 0.01	0.78
Glucose (mmol/liter)	4.8 ± 0.1	4.9 ± 0.1	0.52	4.7 ± 0.5	4.8 ± 0.1	0.32	1.0 ± 2.0	2.0 ± 1.0	0.90
Insulin (μIU/ml)	15.6 ± 1.8	12.4 ± 1.7	<0.01[a]	14.4 ± 2.0	17.6 ± 2.4	0.04[a]	−21.4 ± 4.0	34.2 ± 19.7	<0.01[b]
HOMA-IR	3.3 ± 0.4	2.7 ± 0.4	<0.01[a]	3.0 ± 0.4	3.8 ± 0.5	0.04[a]	−19.8 ± 5.2	37.2 ± 20.4	<0.01[b]
TC (mmol/liter)	4.6 ± 0.2	3.4 ± 0.2	<0.01[a]	4.5 ± 0.2	4.6 ± 0.2	0.13	−26.4 ± 3.0	3.1 ± 1.1	<0.01[b]
LDL-C (mmol/liter)	2.9 ± 0.2	1.8 ± 0.2	<0.01[a]	2.7 ± 0.2	2.7 ± 0.1	0.77	−36.6 ± 5.0	3.1 ± 0.9	<0.01[b]
HDL-C (mmol/liter)	1.07 ± 0.1	1.08 ± 0.1	0.17	1.1 ± 0.08	1.1 ± 0.09	0.47	−4.0 ± 3.9	3.0 ± 3.2	0.17
TG (mmol/liter)	1.34 ± 0.08	1.08 ± 0.13	<0.01[a]	1.39 ± 0.24	1.69 ± 0.27	0.19	−20.9 ± 3.8	36.8 ± 21.0	<0.01[b]
hsCRP (mg/liter)	4.9 ± 1.4	3.4 ± 1.1	0.04[a]	5.8 ± 1.4	5.7 ± 1.6	0.90	−25.8 ± 7.0	−8.8 ± 8.2	0.05
FAI	13.4 ± 0.6	8.7 ± 0.4	<0.01[a]	13.9 ± 0.6	13.3 ± 0.5	0.07	−32.7 ± 2.6	−3.1 ± 2.0	<0.01[b]
Testosterone (nmol/liter)	4.1 ± 0.2	2.9 ± 0.1	<0.01[a]	4.4 ± 0.2	4.3 ± 0.2	0.73	−24.6 ± 2.6	−0.1 ± 1.5	<0.01[b]
SHBG (nmol/liter)	31.1 ± 1.0	35.3 ± 1.2	<0.01[a]	31.9 ± 0.9	32.9 ± 0.9	0.14	13.7 ± 2.4	3.6 ± 2.0	<0.01[b]

Data are presented as mean ± SEM. All serum results are obtained from fasting variables. All variables were normally distributed. Percent change is the percent difference compared with baseline. To convert values for glucose to milligrams per deciliter, divide by 0.056. To convert values for insulin to picomoles per liter, multiply by 6. To convert values for cholesterol to milligrams per deciliter, divide by 0.0259. To convert values for triglycerides to milligrams per deciliter, divide by 0.0113. To convert values for testosterone to nanograms per deciliter, divide by 0.03467. To convert values for SHBG to micrograms per deciliter, divide by 34.7. TC, Total cholesterol; TG, triglycerides.

[a] Significantly difference from baseline.

[b] Significant differences for the comparison between treatments.

significantly by 20% after OCP alone, again in accord with other studies (32, 33).

Of note is the finding that the magnitude of improvement in biochemical hyperandrogenemia with atorvastatin in this study (by up to 33%) is comparable to that of antiandrogen agents (34, 35). The reduction in testosterone is also similar to that found when using the combined OCP (ethinyl estradiol/levonorgestrel combination), where falls of 27% have been described. However, OCPs are more effective in improving SHBG (by up to 100%) when given for 3 months (32, 36, 37). Simvastatin 20 mg daily concomitant with an OCP gave a 38% decrease in total testosterone (23) compared with a 25% reduction found by us with atorvastatin 20 mg daily alone. This study was too short to investigate the improvement of clinical hyperandrogenemia.

Even modest weight loss of less than 10% of initial body weight has been shown to reduce testosterone in women with PCOS (38). However, it seems unlikely that this is the mechanism for the changes found here, because there was no weight change associated with atorvastatin treatment. Indeed, the reduction in testosterone in this study was more than that seen by targeting weight using orlistat (17% reduction) for 3 months (39).

Among other agents known to influence hyperandrogenemia in PCOS, the reduction of testosterone is better with atorvastatin comparable to improvement with insulin sensitizers like metformin that gave a 14% decrease in total testosterone in 3 months (39) and thiazolidinediones that gave a 6–15% reduction in serum testosterone (40–42). No improvement in hirsutism with metformin may be seen (43).

There was a 25% reduction in hsCRP with atorvastatin in this group of patients with PCOS. PCOS has already been associated with increased levels of indices of low-grade chronic inflammation such as hsCRP (13, 44) that appears to be a predictor of cardiovascular events in women (45, 46). Atorvastatin has also been shown to significantly reduce hsCRP with a trend to reducing insulin resistance in patients with impaired fasting glucose (25). In the study using simvastatin and OCP, OCP alone had no significant effect on CRP, whereas the addition of simvastatin led to a significant decrease of CRP below the baseline level (23).

A reduction in insulin resistance may be central to the improvements seen for hyperandrogenemia and hsCRP. There was a 21% reduction in serum insulin levels and a consequent 20% improvement in HOMA-IR with atorvastatin. This improvement in insulin resistance correlated with the improvement in FAI. In addition, the HOMA-IR changes were also positively correlated with the degree of reduction in triglyceride levels and is consistent with other studies that have demonstrated a similar link in patients with the metabolic syndrome and type 2 diabetes treated with atorvastatin (47, 48). Hypothetically, the reduction in triglyceride availability leads to an increased use of glucose as the main intracellular substrate (48), thereby improving insulin sensitivity. Whatever the reason, this mechanism may explain other findings such as reduced development of diabetes among patients treated with pravastatin in the WOSCOPS trial (49, 50).

Improvement in biochemical hyperandrogenemia appears to be independent of the reduction in total cholesterol, LDL, or triglycerides. There was no correlation between reduction of lipid levels and improvement in biochemical hyperandrogenemia that supports the concept of a pleiotropic effect of statins. Statins inhibit ovarian theca-interstitial cell proliferation and steroidogenesis *in vitro* (51). The ovaries of women with PCOS are typically enlarged with prominent hyperplasia of ovarian theca-interstitial cells and excessive production of androgens by these cells (52–54). The effects of statins on steroidogenesis are most likely related to the inhibition of cholesterol synthesis by the mevalonate pathway and a consequent decrease in the availability of the precursors of progesterone and testosterone (51).

We made no assessment of ovulatory function in this study, and because blood tests were done without respect to the bleeding pattern, there may have been temporary hormonal fluctuations that we have not identified. What can be said is that patients continued to have irregular periods, suggesting atorvastatin has not overtly affected menstrual function over the 3-month period. Irrespective of any changes in fertility brought about by atorvastatin, we would emphasize that this drug is not recommended while contemplating pregnancy because of its potentially teratogenic effects.

In conclusion, atorvastatin 20 mg daily improved biochemical hyperandrogenemia, insulin resistance, and markers of inflammation in patients with PCOS when given over a 12-wk period. Statin treatment may therefore prove to be a useful adjunct for women with PCOS.

Acknowledgments

Address all correspondence and requests for reprints to: Thozhukat Sathyapalan, Michael White Diabetes Centre, 220-236 Analby Road, Hull Royal Infirmary, Hull HU3 2JZ, United Kingdom. E-mail: tsathyapal@rediffmail.com.

This study was supported by an unrestricted grant from Pfizer.

Disclosure Summary: Pfizer has supplied atorvastatin 20-mg tablets and placebo for the study. Otherwise, sponsors had no input into study design, its execution, or interpretation of the findings. All of the authors (T.S., E.S.K., A.-M.C., and S.L.A.) have got nothing else to disclose.

References

1. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO 2004 The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 89:2745–2749
2. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF 2000 A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab 85:2434–2438
3. Ehrmann DA 2005 Polycystic ovary syndrome. N Engl J Med 352:1223–1236
4. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS 2006 Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab 91:1357–1363
5. Dahlgren E, Janson PO, Johansson S, Lapidus L, Oden A 1992 Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. Acta Obstet Gynecol Scand 71:599–604
6. Dahlgren E, Johansson S, Lindstedt G, Knutsson F, Oden A, Janson PO, Mattson LA, Crona N, Lundberg PA 1992 Women with polycystic ovary syndrome wedge resected in 1956 to 1965: a long-term follow-up focusing on natural history and circulating hormones. Fertil Steril 57:505–513
7. Son HY, Nishikawa A, Ikeda T, Imazawa T, Kimura S, Hirose M 2001 Lack of effect of soy isoflavone on thyroid hyperplasia in rats receiving an iodine-deficient diet. Jpn J Cancer Res 92:103–108

8. Pierpoint T, McKeigue PM, Isaacs AJ, Wild SH, Jacobs HS 1998 Mortality of women with polycystic ovary syndrome at long-term follow-up. J Clin Epidemiol 51:581–586
9. Wild S, Pierpoint T, Jacobs H, McKeigue P 2000 Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. Hum Fertil (Camb) 3:101–105
10. Talbott EO, Guzick DS, Sutton-Tyrrell K, McHugh-Pemu KP, Zborowski JV, Remsberg KE, Kuller LH 2000 Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. Arterioscler Thromb Vasc Biol 20:2414–2421
11. Vryonidou A, Papatheodorou A, Tavridou A, Terzi T, Loi V, Vatalas IA, Batakis N, Phenekos C, Dionyssiou-Asteriou A 2005 Association of hyperandrogenemic and metabolic phenotype with carotid intima-media thickness in young women with polycystic ovary syndrome. J Clin Endocrinol Metab 90: 2740–2746
12. Christian RC, Dumesic DA, Behrenbeck T, Oberg AL, Sheedy 2nd PF, Fitzpatrick LA 2003 Prevalence and predictors of coronary artery calcification in women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:2562–2568
13. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N 2001 Low grade chronic inflammation in women with polycystic ovarian syndrome. J Clin Endocrinol Metab 86:2453–2455
14. Fenkci V, Fenkci S, Yilmazer M, Serteser M 2003 Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. Fertil Steril 80:123–127
15. Boulman N, Levy Y, Leiba R, Shachar S, Linn R, Zinder O, Blumenfeld Z 2004 Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. J Clin Endocrinol Metab 89:2160–2165
16. Shepherd J, Barter P, Carmena R, Deedwania P, Fruchart J-C, Haffner S, Hsia J, Breazna A, LaRosa J, Grundy S, Waters D 2006 Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the Treating to New Targets (TNT) Study. Diabetes Care 29:1220–1226
17. Pedersen TR, Faergeman O, Kastelein JJ, Olsson AG, Tikkanen MJ, Holme I, Larsen ML, Bendiksen FS, Lindahl C, Szarek M, Tsai J; Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) Study Group 2005 High-dose atorvastatin vs usual-dose simvastatin for secondary prevention after myocardial infarction. The IDEAL Study: a randomized controlled trial. JAMA 294:2437–2445
18. Collins R, Armitage J, Parish S, Sleigh P, Peto R; Heart Protection Study Collaborative Group 2003 MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 361:2005–2016
19. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, Orazem J, Magorien RD, O'Shaughnessy C, Ganz P; Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) Investigators 2005 Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 352:29–38
20. Sever PS, Poulter NR, Dahlof B, Wedel H, Beevers G, Caulfield M, Collins R, Kjeldsen SE, Kristinsson A, McInnes G, Mehlsen J, Nieminen MS, O'Brien ET, Ostergren J 2008 The Anglo-Scandinavian Cardiac Outcomes Trial lipid lowering arm: extended observations 2 years after trial closure. Eur Heart J 29: 499–508
21. McCarey DW, McInnes IB, Madhok R, Hampson R, Scherbakova O, Ford I, Capell HA, Sattar N 2004 Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 363: 2015–2021
22. Davignon J 2004 Beneficial cardiovascular pleiotropic effects of statins. Circulation 109(Suppl 1):III39–III43
23. Banaszewska B, Pawelczyk L, Spaczynski RZ, Dziura J, Duleba AJ 2007 Effects of simvastatin and oral contraceptive agent on polycystic ovary syndrome: prospective, randomized, crossover trial. J Clin Endocrinol Metab 92:456–461
24. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47
25. Costa A, Casamitjana R, Casals E, Alvarez L, Morales J, Masramon X, Hernandez G, Gomis R, Conget I 2003 Effects of atorvastatin on glucose homeostasis, postprandial triglyceride response and C-reactive protein in subjects with impaired fasting glucose. Diabet Med 20:743–745
26. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001 Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 285:2486–2497
27. Nissen SE, Nicholls SJ, Sipahi I, Libby P, Raichlen JS, Ballantyne CM, Davignon J, Erbel R, Fruchart JC, Tardif JC, Schoenhagen P, Crowe T, Cain V, Wolski K, Goormastic M, Tuzcu EM 2006 Effect of very high-intensity statin therapy on regression of coronary atherosclerosis: the ASTEROID trial. JAMA 295:1556–1565
28. Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB 1985 Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. J Clin Endocrinol Metab 61:946–951
29. Mahabeer S, Naidoo C, Norman RJ, Jialal I, Reddi K, Joubert SM 1990 Metabolic profiles and lipoprotein lipid concentrations in non-obese and obese patients with polycystic ovarian disease. Horm Metab Res 22:537–540
30. Guzick DS, Talbott EO, Sutton-Tyrrell K, Herzog HC, Kuller LH, Wolfson Jr SK 1996 Carotid atherosclerosis in women with polycystic ovary syndrome: initial results from a case-control study. Am J Obstet Gynecol 174:1224–1229; discussion 1229–1232
31. Talbott E, Guzick D, Clerici A, Berga S, Detre K, Weimer K, Kuller L 1995 Coronary heart disease risk factors in women with polycystic ovary syndrome. Arterioscler Thromb Vasc Biol 15:821–826
32. Crook D, Godsland IF, Worthington M, Felton CV, Proudler AJ, Stevenson JC 1993 A comparative metabolic study of two low-estrogen-dose oral contraceptives containing desogestrel or gestodene progestins. Am J Obstet Gynecol 169:1183–1189
33. van Rooijen M, von Schoultz B, Silveira A, Hamsten A, Bremme K 2002 Different effects of oral contraceptives containing levonorgestrel or desogestrel on plasma lipoproteins and coagulation factor VII. Am J Obstet Gynecol 186:44–48
34. Venturoli S, Marescalchi O, Colombo FM, Macrelli S, Ravaioli B, Bagnoli A, Paradisi R, Flamigni C 1999 A prospective randomized trial comparing low dose flutamide, finasteride, ketoconazole, and cyproterone acetate-estrogen regimens in the treatment of hirsutism. J Clin Endocrinol Metab 84: 1304–1310
35. Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M, Castello R 2000 Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. J Clin Endocrinol Metab 85:89–94
36. Burkman Jr RT 1995 The role of oral contraceptives in the treatment of hyperandrogenic disorders. Am J Med 98:130S–136S
37. Wild RA, Demers LM, Applebaum-Bowden D, Lenker R 1991 Hirsutism: metabolic effects of two commonly used oral contraceptives and spironolactone. Contraception 44:113–124
38. Moran LJ, Noakes M, Clifton PM, Tomlinson L, Norman RJ 2003 Dietary composition in restoring reproductive and metabolic physiology in overweight women with polycystic ovary syndrome. J Clin Endocrinol Metab 88:812–819
39. Jayagopal V, Kilpatrick ES, Holding S, Jennings PE, Atkin SL 2005 Orlistat is as beneficial as metformin in the treatment of polycystic ovarian syndrome. J Clin Endocrinol Metab 90:729–733
40. Brettenthaler N, De Geyter C, Huber PR, Keller U 2004 Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 89:3835–3840
41. Azziz R, Ehrmann D, Legro RS, Whitcomb RW, Hanley R, Fereshetian AG, O'Keefe M, Ghazzi MN 2001 Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial. J Clin Endocrinol Metab 86:1626–1632
42. Ortega-Gonzalez C, Luna S, Hernandez L, Crespo G, Aguayo P, Arteaga-Troncoso G, Parra A 2005 Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome. J Clin Endocrinol Metab 90:1360–1365
43. Lord JM, Flight IH, Norman RJ 2003 Metformin in polycystic ovary syndrome: systematic review and meta-analysis. BMJ 327:951–953
44. Diamanti-Kandarakis E, Paterakis T, Alexandraki K, Piperi C, Aessopos A, Katsikis I, Katsilambros N, Kreatsas G, Panidis D 2006 Indices of low-grade chronic inflammation in polycystic ovary syndrome and the beneficial effect of metformin. Hum Reprod 21:1426–1431
45. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR 2002 Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med 347:1557–1565
46. Ridker PM, Buring JE, Cook NR, Rifai N 2003 C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 107:391–397
47. Huptas S, Geiss HC, Otto C, Parhofer KG 2006 Effect of atorvastatin (10 mg/day) on glucose metabolism in patients with the metabolic syndrome. Am J Cardiol 98:66–69

48. Paolisso G, Barbagallo M, Petrella G, Ragno E, Barbieri M, Giordano M, Varricchio M 2000 Effects of simvastatin and atorvastatin administration on insulin resistance and respiratory quotient in aged dyslipidemic non-insulin dependent diabetic patients. Atherosclerosis 150:121–127
49. Takagi T, Matsuda M, Abe M, Kobayashi H, Fukuhara A, Komuro R, Kihara S, Caslake MJ, McMahon A, Shepherd J, Funahashi T, Shimomura I 2008 Effect of pravastatin on the development of diabetes and adiponectin production. Atherosclerosis 196:114–121
50. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A 2001 Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. Circulation 103:357–362
51. Izquierdo D, Foyouzi N, Kwintkiewicz J, Duleba AJ 2004 Mevastatin inhibits ovarian theca-interstitial cell proliferation and steroidogenesis. Fertil Steril 82(Suppl 3):1193–1197
52. Hughesdon PE 1982 Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis." Obstet Gynecol Surv 37:59–77
53. Wickenheisser JK, Quinn PG, Nelson VL, Legro RS, Strauss 3rd JF, McAllister JM 2000 Differential activity of the cytochrome P450 17α-hydroxylase and steroidogenic acute regulatory protein gene promoters in normal and polycystic ovary syndrome theca cells. J Clin Endocrinol Metab 85: 2304–2311
54. Nelson VL, Legro RS, Strauss 3rd JF, McAllister JM 1999 Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol 13:946–957

DIABETICMedicine

DOI: 10.1111/j.1464-5491.2008.02519.x

Original Article: Treatment

Variability of lipids in patients with Type 2 diabetes taking statin treatment: implications for target setting

T. Sathyapalan, S. L. Atkin and E. S. Kilpatrick*

Department of Diabetes and Endocrinology, University of Hull and *Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK

Accepted 4 June 2008

Abstract

Aims To determine the biological variability of lipids in patients with Type 2 diabetes (T2DM) who are on statin treatment and then to assess any implications for current lipid targets.

Methods A cross-over study of biological variation of lipids in 26 patients with T2DM taking either simvastatin 40 mg or atorvastatin 10 mg. After 3 months on one statin, fasting lipids were measured on 10 occasions over a 5-week period. Following 3 months on the other statin, 10 further samples were taken over 5 weeks. The main outcome measures were biological variability of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides.

Results The coefficient of variation (CV) of TC, LDL, HDL and triglycerides on simvastatin was 8.17, 13.11, 7.95 and 12.06%, respectively, whereas the CV on atorvastatin was 6.92, 10.30, 5.13 and 19.71%, respectively, with no statistically significant differences between statins. Treating to sustain a target TC < 5.0 mmol/l or LDL < 3.0 mmol/l means needing to maintain a mean TC of 4.3–4.4 mmol/l or LDL of 2.4–2.5 mmol/l. Treating to consistently achieve an LDL < 2.0 mmol/l means aiming for a mean of only 1.5–1.6 mmol/l.

Conclusion In patients with T2DM taking either simvastatin or atorvastatin, the mean TC and LDL concentrations needed to consistently remain below a target are much lower than the target value itself. This means that guideline target limits extrapolated from the mean values of patients participating in clinical studies may overestimate the lipid reductions required.

Diabet. Med. 25, 909–915 (2008)

Keywords Biological variation, Coefficient of variation, statins, Type 2 Diabetes, lipids

Abbreviations apoB, apolipoprotein B; CHD, coronary heart disease; CV, coefficient of variation; HDL, high-density lipoprotein; JBS2, Joint British Societies' guidelines; LDL, low-density lipoprotein; NICE, National Institute for Health and Clinical Excellence; NSF, National Service Framework; QOF, Quality and Outcomes Framework; TC, total cholesterol; T2DM, Type 2 diabetes

Introduction

While there is very strong evidence that low-density lipoprotein (LDL) cholesterol lowering using statin treatment can reduce the incidence of coronary and other major vascular events [1–4], in the UK there is current controversy and confusion about what lipid targets should be aimed for once a statin drug is initiated. Current guidance from the National Service Framework for Coronary Heart Disease and the National Institute for Health and Clinical Excellence (NICE) recommends targets for serum total cholesterol (TC) < 5.0 mmol/l and LDL < 3.0 mmol/l, and this has formed the basis of the Quality and Outcomes Framework (QOF) of the new General Medical Services (nGMS) [5,6]. However, more recent guidance from the Joint British Societies, published in December 2005 [7], recommends patients should aim for a lower target of TC < 4.0 mmol/l and an LDL < 2.0 mmol/l. This disparity has led the UK's National Director for Heart Disease and Stroke to issue a statement in November 2006 declaring that the previous targets of 5.0 and 3.0 mmol/l should be kept, at least until revised NICE guidance is published [8].

Whether a patient consistently achieves any target depends on both the lipid-lowering ability of the statin and the variability

Correspondence to: Dr. T. Sathyapalan, Michael White Centre for Diabetes, Brocklehurst Building, Hull Royal Infirmary, Anlaby Road, Hull HU3 2JZ, UK. E-mail: tsathyapal@rediffmail.com

© 2008 The Authors.
Journal compilation © 2008 Diabetes UK.

of lipid parameters while on the drug. Thus, a statin drug may be effective in lowering the mean cholesterol in a patient, but if the measurement is extremely variable, then they may not always be below their target when tested. Lipid measurements in healthy volunteers and in patients with Type 2 diabetes (T2DM) who are not on lipid-lowering treatment can indeed vary on a day-to-day basis [9–11]. However, no study to date has looked at lipid variability in patients already on statin treatment. Indeed, arguments can be made for statin treatment leading to either possible increases or reductions in cholesterol variation within an individual. Added to this is the fact that short half-life statins, such as simvastatin, may have an effect on lipid variability which is quite different to that of a long half-life statin, such as atorvastatin. This study has therefore aimed to establish how the biological variability of lipids in patients with T2DM who are on statin treatment could influence the ability for patients to maintain cholesterol values below target. In order to assess any difference between long and short half-life statins, we have conducted the investigation as a cross-over study with equivalent doses of simvastatin and atorvastatin.

Patients and methods

Thirty Caucasian patients with Type 2 diabetes for at least 3 years and glycated haemoglobin (HbA_{1c}) of 6.0–9.0% were recruited into the study with informed consent. Nineteen patients were taking 10 mg atorvastatin before bed and 11 patients took simvastatin 40 mg before bed. All the patients were on stable doses of the medications for at least 3 months. None of the patients was on additional lipid-lowering therapy or over-the-counter medications. The insulin doses of patients who took insulin were not changed by > 10% throughout the study. The patients were advised to maintain their normal diet, alcohol intake, smoking and exercise habits during the study period. Patients with untreated hypothyroidism or nephrotic syndrome were excluded. The biological variation of TC, high-density lipoprotein (HDL), LDL and triglycerides was assessed by measuring 12-h fasting blood samples at 4-day intervals on 10 consecutive occasions. Thereafter, the patients on simvastatin were changed to the equivalent dose of atorvastatin and vice versa [12]. After 3 months, the biological variation of lipid parameters were again assessed by measuring fasting blood samples at 4-day intervals on 10 consecutive occasions in these patients.

Fasting venous blood was collected into serum gel tubes (Becton Dickinson, Oxford, UK) at the same time each day (08.00–09.00 h) after the patient had been seated for at least 5 min and a tourniquet was not applied for more than 1 min. Samples were separated by centrifugation at 2000 *g* for 15 min at 4°C and two aliquots of the serum were stored at −20°C within 1 h of collection. All samples were analysed within 12 months of collection and studies have found no stability issues when stored for this period of time at this temperature [13]. The serum samples were split before assay. Before analysis, all of the serum samples were thawed and thoroughly mixed. The duplicate samples (i.e. two per visit) were randomized and then analysed for cholesterol, triglycerides and HDL cholesterol in a continuous batch on a Synchron LX 20 analyser (Beckman-Coulter, High Wycombe, UK) using a single batch of reagents according to our previous studies [14]. Lipid assays used calibrators assigned from Centers for Disease Control (CDC) standards and LDL cholesterol was calculated using the Friedewald formula [15]. All subjects gave their informed written consent before entering the study, which had been approved by the Hull and East Riding Local Research Ethics Committee.

Statistical analysis

Statistical analysis was performed using SPSS for Windows NT, version 15.0 (SPSS, Chicago, IL, USA) and Microsoft Excel. Biological variability data was analysed by calculating the analytical and within-subject variability according to the methods of Fraser and Harris [16]. Using this technique, analytical variance (SD_A^2) was calculated from the difference between duplicate results for each specimen ($SD_A^2 = \Sigma d^2/2N$, where d is the difference between duplicates and N is the number of paired results). The variance of the first set of duplicate results for each subject on the 10 assessment days was used to calculate the average biological intra-individual variance (SD_I^2) by subtraction of the mean SD_A^2 from the observed dispersion (equal to $SD_I^2 + SD_A^2$). The standard deviation of intra-individual variations (SD_I) was estimated as square roots of the respective variance component estimates. An individual's coefficient of variation (CV) for each lipid parameter was calculated as the SD_I/mean value × 100% and then expressed as a mean value for each treatment (simvastatin and atorvastatin).

The CV was used to calculate which mean lipid values would be required to maintain total cholesterol < 5.0 mmol/l (4.9 mmol/l or lower) or < 4.0 mmol/l and LDL < 3.0 mmol/l or < 2.0 mmol/l on 95% or more of testing occasions using a one-sided analysis (mean ± 1.645xCV) [16]. A similar method was used to calculate the value that a single lipid measurement would have to be in order to be 95% confident that (i) the mean for a patient was below target or (ii) subsequent measurements would consistently be below target.

Results

The baseline demographic data of patients are given in Table 1. The baseline lipid profiles were comparable in both groups. One patient from each group dropped out after completing one arm because of difficulty in adhering to study protocols. One patient who was on atorvastatin initially withdrew from the study because of development of myalgia without any rise in creatine kinase when changed over to simvastatin. Another patient on atorvastatin initially withdrew from the study as a result of development of lethargy while on simvastatin, which got better after changing back to atorvastatin. None of the patients developed elevated liver transaminases or creatine kinase during the study. There was no significant change in glycaemic control during the course of the study in any patients (median ± interquartile range) (7.7 ± 0.98 vs. 7.7 ± 0.88%, $P = 0.60$).

Table 2 shows the mean lipid values and the biological variability in lipids expressed as standard deviation (SD) and

© 2008 The Authors.
Journal compilation © 2008 Diabetes UK.

Table 1 Baseline characteristics

	Simvastatin crossed over to atorvastatin group	Atorvastatin crossed over to simvastatin group
Total number of patients completing the study	10	16
Duration of diabetes median (25–75 inter-quartile range) months	84 (108)	108 (108)
HbA_{1c} % (mean ± SD)	7.8 ± 0.98	7.7 ± 0.91
Sex (male : female)	7 : 3	10 : 6
Age, years and median (range)	58 (48–76)	64 (46–73)
Body mass index (mean ± SD) kg/m^2	34.7 ± 6.64	34.5 ± 7.23
Waist circumference (mean ± SD) cm	119.8 ± 16.01	114.8 ± 17.29
Baseline TC (mean ± SD) mmol/l	4.1 ± 0.71	4.0 ± 0.49
Baseline LDL cholesterol (mean ± SD) mmol/l	2.2 ± 0.4	2.2 ± 0.44
Baseline HDL cholesterol (mean ± SD) mmol/l	1.08 ± 0.23	1.08 ± 0.24
Baseline triglycerides (median ± inter-quartile range) mmol/l	1.9 ± 0.6	1.7 ± 0.7

HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation; TC, total cholesterol.

Table 2 The biological variation of lipid parameters in 26 patients on simvastatin 40 mg and atorvastatin 10 mg

	Simvastatin			Atorvastatin			
Lipid Parameters	Mean (mmol/l)	SD (mol/l)	CV (%)	Mean (mmol/l)	SD (mmol/l)	CV (%)	*P*-value*
Total cholesterol	3.8	0.3	8.2	4.0	0.3	6.9	0.21
LDL cholesterol	2.1	0.3	13.1	2.2	0.2	10.3	0.19
HDL cholesterol	1.0	0.1	7.7	1.0	0.1	6.2	0.17
Triglycerides	2.7	0.3	12.1	1.7	0.3	19.7	0.95

CV, coefficient of variation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation of lipid parameter.
*SD simvastatin vs. SD atorvastatin.

CV in each treatment group. It shows no statistical difference in lipid variability (SD) when the same patients take either simvastatin or atorvastatin (Figure 1). Whether patients started on simvastatin or atorvastatin made no difference to the results.

Table 3 shows the mean values of total cholesterol required to maintain values < 5.0 or < 4.0 mmol/l and to maintain LDL cholesterol < 3.0 mmol/l or < 2.0 mmol/l on up to 95% of occasions. Table 4 shows the concentrations of total cholesterol and LDL required from a single lipid measurement on statin treatment so that two different criteria are met. The first criterion is to be 95% confident that the mean lipid value for a patient is truly below target; for example, that their mean total cholesterol is 4.9 mmol/l or lower when the target is 5.0 mmol/l. The second is to be 95% confident that 95% of subsequent lipid measurements are below the specified target. In this regard, Table 3 gives values which show 50% confidence that 95% of subsequent measurements are below target.

Discussion

To our knowledge, this study is the first to show that there is clinically significant biological variability in the lipid profiles of patients with Type 2 diabetes who are on statin treatment and that this does not significantly differ between the short and long half-life statins simvastatin and atorvastatin. Taken together, the coefficient of variation of total cholesterol suggests that values can vary by approximately ± 15% (2 SD from the mean value) in the same individual on treatment, while that of LDL cholesterol can vary by ± 24% before any laboratory analytical variability is also taken into consideration.

Studies which examine the normal biological variation in lipids and lipoproteins have generally been conducted on healthy subjects [9–11], but the biological component should be studied for each disease or treatment state as this may influence the extent of intra-individual variation. A meta-analysis of previously published studies has found that the mean biological variability (CV) found in healthy individuals is ≤ 6.1, ≤ 9.5, ≤ 7.4 and < 22.6% for TC, LDL, HDL and triglycerides, respectively [17], whereas these figures in patients with T2DM who are not on statins were 5.1, 8.3, 4.4 and 17%, respectively. In this study, the CV of TC on atorvastatin and simvastatin was comparable at 6.9 and 8.2%, respectively, while that for LDL is somewhat higher at 10.3 and 13.1%, respectively.

The variability in lipids on statin treatment found here have several clinical implications, the first of which relates to the ongoing debate regarding setting lipid targets in patients with

© 2008 The Authors.

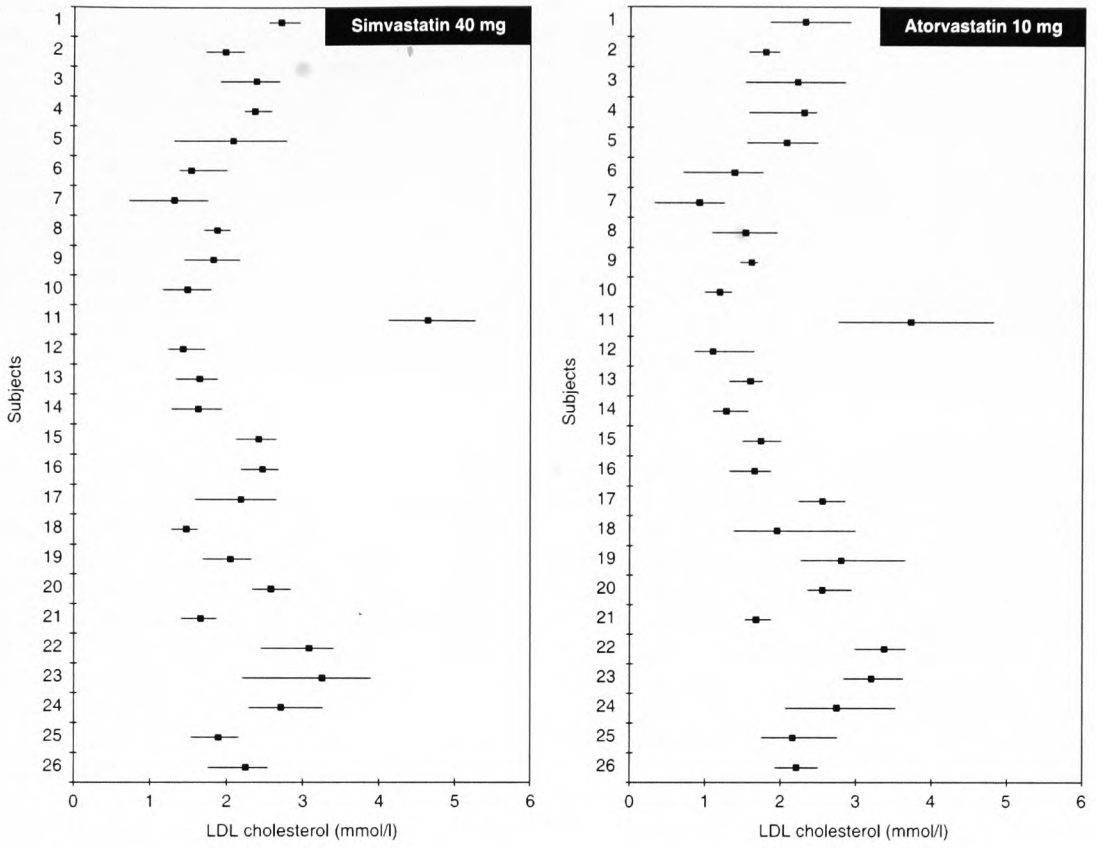


FIGURE 1 Means (range) of low-density lipoprotein (LDL) cholesterol on simvastatin 40 mg and atorvastatin 10 mg daily.

Table 3 Mean values of total cholesterol and LDL cholesterol to achieve different targets on 95% of sampling occasions

	On simvastatin 40 mg (mmol/l)	On atorvastatin 10 mg (mmol/l)
Mean TC to be achieved to produce a consistent TC < 5.0 mmol/l	4.32	4.40
Mean TC to be achieved to produce a consistent TC < 4.0 mmol/l	3.43	3.50
Mean LDL-C to be achieved to produce a consistent LDL-C < 3.0 mmol/l	2.39	2.48
Mean LDL-C to be achieved to produce a consistent LDL-C < 2.0 mmol/l	1.56	1.62

LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

or without T2DM. The National Service Framework (NSF) for Coronary Heart Disease (CHD) in the year 2000 laid down standards of care for the prevention and treatment of CHD [18]. It set a target for TC lowering of < 5.0 mmol/l and LDL < 3.0 mmol/l, or by 20–25% for TC and 30% for LDL, whichever results in the lowest absolute level [18]. In contrast, in December 2005 the Joint British Societies' (JBS2) guidelines set similar audit targets, but recommended an optimal total cholesterol target level < 4.0 mmol/l and an LDL < 2.0 mmol/l [7]. Since the publication of JBS2, a statement from Professor Roger Boyle, National Director for Heart Disease and Stroke [8], points out that current UK national policy for lipid management and current targets for cholesterol levels remain those recommended in the NSF for CHD and not those recommended by the JBS2. This has been followed by a response from the Association of British Clinical Diabetologists (ABCD), which criticized this statement, arguing that in the Collaborative Atorvastatin Diabetes Study (CARDS) [19] the 30% of patients

© 2008 The Authors.

Table 4 Single lipid measurement results which give 95% confidence of the true mean value being below target or of each subsequent measurement being below target

	Simvastatin		Atorvastatin	
	True mean below target	Subsequent measurements below target	True mean below target	Subsequent measurements below target
TC < 5.0 mmol/l	< 4.24	< 3.74	< 4.34	< 3.90
TC < 4.0 mmol/l	< 3.37	< 2.97	< 3.46	< 3.10
LDL-C < 3.0 mmol/l	< 2.28	< 1.87	< 2.41	< 2.06
LDL-C < 2.0 mmol/l	< 1.49	< 1.23	< 1.58	< 1.35

LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

who had TC and LDL cholesterol levels < 5.0 and 3.0 mmol/l, respectively, still benefited from statins [20]. Diabetes UK has also criticized this decision, as current NICE guidance does not take account of new evidence in patients with T2DM [21].

The evidence for the new JBS2 targets themselves arose from the findings of the Heart Protection Study, the ASCOT-LLA, PROVE-IT, TNT, REVERSAL and GREACE studies [22–27]. In these studies, the mean LDL cholesterol in the active or intensively treated groups was 2.3, 2.3, 1.6, 2.0, 2.1 and 2.5 mmol/l, respectively. However, the target for LDL cholesterol did not become a mean of 2.0 mmol/l, but less than 2.0 mmol/l. From our study, as shown in Table 3, we can determine that, in order to consistently maintain an LDL of 1.9 mmol/l or lower, the mean LDL for someone taking atorvastatin or simvastatin has to be around 1.5–1.6 mmol/l or lower. This means the average LDL needs to be lower than that found in the clinical studies from which the target itself is based. This, in turn, is likely to have a knock-on effect regarding the potency, dosage and cost of statins (as well as possibly other agents) required. In addition, if the aim is to achieve a TC < 5.0 mmol/l (as suggested by the QOF for UK general practitioners) or an LDL < 3.0 mmol/l on a consistent basis, then the mean values for a patient needs to be 4.3–4.4 and 2.4–2.5 mmol/l, respectively. This means that existing NICE lipid targets may not be as far removed from current evidence than the values of 5.0 and 3.0 mmol/l suggest, especially given the fact that adding laboratory analytical variation to the biological variation determined here will only reduce these mean values further.

This study has thus highlighted the difference between aiming to consistently have a patient below a cholesterol target at each visit (as demanded by financial incentives schemes such as the QOF) compared with aiming for just their mean value to be below the same target. By definition, the latter means that a patient whose mean is the same as the target value will have half their individual measurements above it, whereas with the former any result above target is unacceptable. One effect of this difference is shown in Table 4, where a single total cholesterol reading on treatment must be under 3.7–3.9 mmol/l before it can be assumed that subsequent measurements will remain below 5.0 mmol/l, whereas this value can be around 0.5 mmol/l higher if only a mean below 5.0 mmol/l is desired. Repeated measurements help in this regard but, for example, the mean of three measurements still has to be below 4.0–4.1 mmol/l to be sure that 95% of ensuing results are < 5.0 mmol/l.

There are potential limitations to this study. LDL was calculated using the Friedewald formula rather than using direct measurement or analysis after ultracentrifugation. This means the variability in cholesterol, triglycerides and HDL could each be contributing to the overall variation in LDL found here. There is also a possibility that the Friedewald formula may have limitations when applied to a population with diabetes [28]. Nevertheless, this is the means by which most laboratories currently report LDL and none of the patients in the study had triglycerides > 4.5 mmol/l, which would prohibit use of the calculation. There is also some evidence that calculating LDL in this way may not in fact lead to any spurious increase in variability, at least compared with direct measurement [29].

Given these difficulties in using estimated LDL in Type 2 diabetic patients, it is perhaps an opportunity to reassess the utility of non-HDL and apolipoprotein B (apoB) measurement which seem to be equally useful in detecting high-risk phenotypes in hypertriglyceridaemic Type 2 diabetic patients, with apoB possibly being superior in normotriglyceridaemic subjects [30]. The fact that apoB can be accurately measured in non-fasting individuals would only strengthen its appeal.

It is reassuring that none of the patients in the study developed serious adverse effects on statins. The two patients who withdrew because of non-severe adverse effects is probably in keeping with the 5–10% of patients who develop problems on statin treatment outside trial situations [31].

Our data are also reassuring for patients where a switch from 10 mg of atorvastatin to the less costly generic simvastatin 40 mg is being considered. Not only were the mean values of TC and LDL achieved by individuals on simvastatin not inferior to that of atorvastatin (which is in accordance with other studies [12]), but there were no significant differences in lipid variability. This means that the relatively short half-life of simvastatin (2–3 h) compared with atorvastatin (up to 24 h) [32] does not

© 2008 The Authors.

seem to influence its ability to keep a patient below their lipid target, at least when taken at night-time [33].

In summary, this study has found that, in patients with T2DM taking either simvastatin or atorvastatin, the mean TC and LDL concentrations needed to keep below target levels are much lower than the target value itself. When evidence for lipid targets are derived from the mean values obtained in patients participating in the different treatment groups of clinical studies, the mean value often becomes the upper target limit for patients [1–3,12,25,34]. In doing so, we have shown that this may lead to lipid targets in patients with diabetes which are significantly lower than the current evidence suggests and thereby more difficult to achieve.

Competing interests

The study was funded by an unrestricted educational grant from Pfizer.

References

1 MRC/BHF. Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 2003; **361**: 2005–2016.

2 Pedersen TR, Faergeman O, Kastelein JJP, Olsson AG, Tikkanen MJ, Holme I *et al.* High-dose atorvastatin vs. usual-dose simvastatin for secondary prevention after myocardial infarction: the IDEAL study: a randomized controlled trial. *J Am Med Assoc* 2005; **294**: 2437–2445.

3 Shepherd J, Barter P, Carmena R, Deedwania P, Fruchart J-C, Haffner S *et al.* Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the treating to new targets (TNT) study. *Diabetes Care* 2006; **29**: 1220–1226.

4 Costa J, Borges M, David C, Vaz Carneiro A. Efficacy of lipid-lowering drug treatment for diabetic and non-diabetic patients: meta-analysis of randomised controlled trials. *Br Med J* 2006; **332**: 1115–1124.

5 Department of Health. *Investing in General Practice: The New General Medical Services Contract.* London: Department of Health, 2003.

6 Department of Health. National service framework for coronary heart disease. London: UK. Available at: http://www.doh.gov.uk/pdfs/chdnsf.pdf Last accessed 12 September 2007.

7 Prepared by: British Cardiac Society, British Hypertension Society, Diabetes UK, Heart UK, Primary Care Cardiovascular Society, Stroke Association. JBS 2: Joint British Societies' guidelines on prevention of cardiovascular disease in clinical practice. *Heart* 2005; **91**: v1–52.

8 Boyle R. *National Policy on Statin Prescribing.* London: Department of Health, 2006.

9 Bookstein L, Gidding SS, Donovan M, Smith FA. Day-to-day variability of serum cholesterol, triglyceride, and high-density lipoprotein cholesterol levels. Impact on the assessment of risk according to the National Cholesterol Education Program guidelines. *Arch Intern Med* 1990; **150**: 1653–1657.

10 Friedlander Y, Kark JD, Stein Y. Variability of plasma lipids and lipoproteins: the Jerusalem Lipid Research Clinic Study. *Clin Chem* 1985; **31**: 1121–1126.

11 Ford RP. Essential data derived from biological variation for establishment and use of lipid analyses. *Ann Clin Biochem* 1989; **26**: 281–285.

12 Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low-density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *Br Med J* 2003; **326**: 1423.

13 Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. *Clin Chem* 1988; **34**: 2111–2114.

14 Jayagopal V, Kilpatrick ES, Jennings PE, Hepburn DA, Atkin SL. Biological variation of homeostasis model assessment-derived insulin resistance in Type 2 diabetes. *Diabetes Care* 2002; **25**: 2022–2025.

15 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.

16 Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989; **27**: 409–437.

17 Smith SJ, Cooper GR, Myers GL, Sampson EJ. Biological variability in concentrations of serum lipids: sources of variation among results from published studies and composite predicted values. *Clin Chem* 1993; **39**: 1012–1022.

18 Department of Health. National service framework for coronary heart disease: modern standards and service models. London: Department of Health, 2000.

19 Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ *et al.* Primary prevention of cardiovascular disease with atorvastatin in Type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* 2004; **364**: 685–696.

20 Association of British Clinical Diabetologists. *Association of British Clinical Diabetologists response to Department of Health Statement—7 November 2006—'National Policy on Statin Prescribing'.* Association of British Clinical Diabetologists, 2006. Available at: http://www.diabetologists.org.uk/Shared_Documents/notice_board/notices.htm Last accessed 12 September 2007.

21 Valerkou S. *Evidence Submitted by Diabetes UK (NICE 78).* Diabetes UK, 2007. Available at http://www.parliament.uk Last accessed 12 September 2007.

22 Medical Research Council/British Heart Foundation (MRC/BHF). Heart Protection Study of cholesterol lowering with simvastatin in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; **360**: 7–22.

23 Sever PS, Dahlof B, Poulter NR, Wedel H, Beevers G, Caulfield M *et al.* Prevention of coronary and stroke events with atorvastatin in hypertensive patients who have average or lower-than-average cholesterol concentrations, in the Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA): a multicentre randomised controlled trial. *Lancet* 2003; **361**: 1149–1158.

24 Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R *et al.* Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med* 2004; **350**: 1495–1504.

25 Waters DD, Guyton JR, Herrington DM, McGowan MP, Wenger NK, Shear C. Treating to New Targets (TNT) Study: does lowering low-density lipoprotein cholesterol levels below currently recommended guidelines yield incremental clinical benefit? *Am J Cardiol* 2004; **93**: 154–158.

26 Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA *et al.* Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *J Am Med Assoc* 2004; **291**: 1071–1080.

27 Athyros VG, Papageorgiou AA, Mercouris BR, Athyrou VV, Symeonidis AN, Basayannis EO *et al.* Treatment with atorvastatin to the National Cholesterol Educational Program goal versus 'usual' care in secondary coronary heart disease prevention. The GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study. *Curr Med Res Opin* 2002; **18**: 220–228.

© 2008 The Authors.
Journal compilation © 2008 Diabetes UK.

28 Wagner AM, Sanchez-Quesada JL, Perez A, Rigla M, Cortes M, Blanco-Vaca F *et al.* Inaccuracy of calculated LDL-cholesterol in Type 2 diabetes: consequences for patient risk classification and therapeutic decisions. *Clin Chem* 2000; **46**: 1830–1832.
29 Schectman G, Patsches M, Sasse EA. Variability in cholesterol measurements: comparison of calculated and direct LDL cholesterol determinations. *Clin Chem* 1996; **42**: 732–737.
30 Wagner AM, Perez A, Zapico E, Ordonez-Llanos J. Non-HDL cholesterol and apolipoprotein B in the dyslipidemic classification of Type 2 diabetic patients. *Diabetes Care* 2003; **26**: 2048–2051.
31 Simons LA, Levis G, Simons J. Apparent discontinuation rates in patients prescribed lipid-lowering drugs. *Med J Aust* 1996; **164**: 208–211.
32 Naoumova RP, Dunn S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB *et al.* Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. *J Lipid Res* 1997; **38**: 1496–1500.
33 Wallace A, Chinn D, Rubin G. Taking simvastatin in the morning compared with in the evening: randomised controlled trial. *Br Med J* 2003; **327**: 788.
34 Law MR, Wald NJ, Thompson SG. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? *Br Med J* 1994; **308**: 367–372.

© 2008 The Authors.
Journal compilation © 2008 Diabetes UK.