1. Introduction

1.1 Introduction overview

Tuberculosis (TB) continues to be a major killer in sub-Saharan Africa. Effective therapy is available, but the mortality and morbidity of TB during treatment, especially amongst those with the human immunodeficiency virus (HIV), remains high. There has been speculation for three decades that adrenal insufficiency, possibly compounded by rifampicin-induced steroid catabolism, plays a role in the mortality and morbidity of TB in the first few days and weeks after antituberculous treatment is commenced. However, studies to demonstrate this have been inconclusive.

1.2 Tuberculosis in southern Africa

TB continues to be a major cause of morbidity and mortality in the developing world, where 95% of all global TB cases occur (1). The steady rise in the prevalence of HIV has fueled the TB epidemic in sub-saharan Africa (2, 3, 4). The World Health Organisation has declared TB to be a global emergency. South Africa is estimated to have the 7th highest absolute number of cases in the world, with an incidence estimated at 556/100 000, the third highest in the world (5). 224, 420 cases of TB were notified in 2002 through the South African government's formal notification system (6), 16% up from the previous year. It is estimated that approximately 55% of TB cases in South Africa are HIV-infected (7). HIV is now the biggest risk factor for TB in sub-Saharan Africa, with an overall case load that has increased by 300-400% in the last decade (2). Despite the implementation of improved Directly Observed Therapy Short Course (DOTS) programs in southern Africa, the incidence of TB is still rising (8, 9, 10).

1.3 TB and mortality in the developing world

TB has a high mortality rate in the immediate period after diagnosis, despite treatment with potent antituberculous drugs (11, 12, 13, 14, 15, 16). The reasons for this high mortality are unclear. Postulated causes include (17, 18):

1) Late presentation, with an advanced **systemic inflammatory response** directly due to TB

2) **Herxheimer reaction** due to rapid death of a large TB bacillary load following initiation of treatment

3) **Bacterial superinfection** of damaged lungs, with overwhelming sepsis

4) **Pulmonary embolus**, secondary to hypercoagulability, possibly directly due to TB, or due to general debility

5) Serious predisposing and **concurrent medical conditions**, such as HIV.

6) **Acute hypoadrenalism,** secondary to tuberculous infection of the adrenals, with possible exacerbation by rifampicin-containing treatment regimens (*explored further in this study*)

Tuberculosis remains a common cause of hypoadrenalism (Addison's disease) in the developing world (19, 20). This was previously the case throughout the world (21), but the control of tuberculosis in many countries has meant that auto-immune destruction

is more common in these countries (22). Five percent of people with active TB are estimated to have adrenal involvement (23).

1.4 Adrenal failure

1.4.1 The hypothalamic-pituitary-adrenal (HPA) axis (24, 25, 26, 27) (**Figure 1)**

Normal adrenal function is characterized by the diurnal secretion of cortisol, the principal corticosteroid secreted by the adrenal glands. The secretion of cortisol is controlled by a complex pathway involving a variety of feedback loops, and is critical to the body's immune system, metabolic processes and stress responses.

Cortisol is derived from cholesterol, which is delivered to the adrenal by low-density lipoprotein cholesterol (LDL). The number of LDL receptors is increased by corticotropin (adrenocorticotropic hormone, ACTH). Cholesterol is then modified by the cytochrome P-450 system to produce cortisol. Corticotropin activates the 20,22-desmolase enzyme, which catalyzes the rate-limiting step in adrenal steroidogenesis and increases the nicotinamide adenine dinucleotide phosphate (NADPH) levels necessary for the hydroxylation steps in steroidogenesis

Initial regulation of hypothalamic function occurs at the level of the brain, especially the limbic system. The parvicellular division of the hypothalamic paraventricular nucleus secretes corticotropin-releasing hormone (CRH) and arginine vasopressin which stimulate the anterior pituitary to secrete corticotropin, which then enters the systemic circulation. This is accomplished through a considerable amplification response.

A similar amplification response occurs at the level of the adrenal gland, with large amount of cortisol secreted in response to corticotropin. Corticotropin is also able to stimulate the short-term release of aldosterone. The adrenal response modifies the hypothalamic-pituitary response in turn, and is facilitated by a negative feedback loop to CRH, corticotropin and vasopressin, mediated by glucocorticoid receptors.

Cortisol is synthesized in the adrenal cortex zona fasciculata, along with adrenal androgens such as dehydroepiandrosterone (DHEA) (in the adrenal zona reticularis) and mineralocorticoids (zona glomerulosa). Cortisol is subject to marked diurnal variation in healthy people, with peak levels between 7 and 9am, and trough levels at midnight. Afternoon levels are generally 50-70% of morning levels. The diurnal variation is lost during severe illness. 90% of cortisol is bound to circulating cortisol-binding globulin, which protects it from hepatic clearance. This bound fraction is not available for receptor binding. Cortisol-binding globulin is decreased in cirrhosis, nephrotic syndrome, hypothyroidism, and critical illness, and increased in response to the administration of oestrogens. Decreased levels of cortisol-binding globulin leads to decreased free plasma cortisol.

Cortisol is essential during stress responses, especially during critical illness and sepsis.

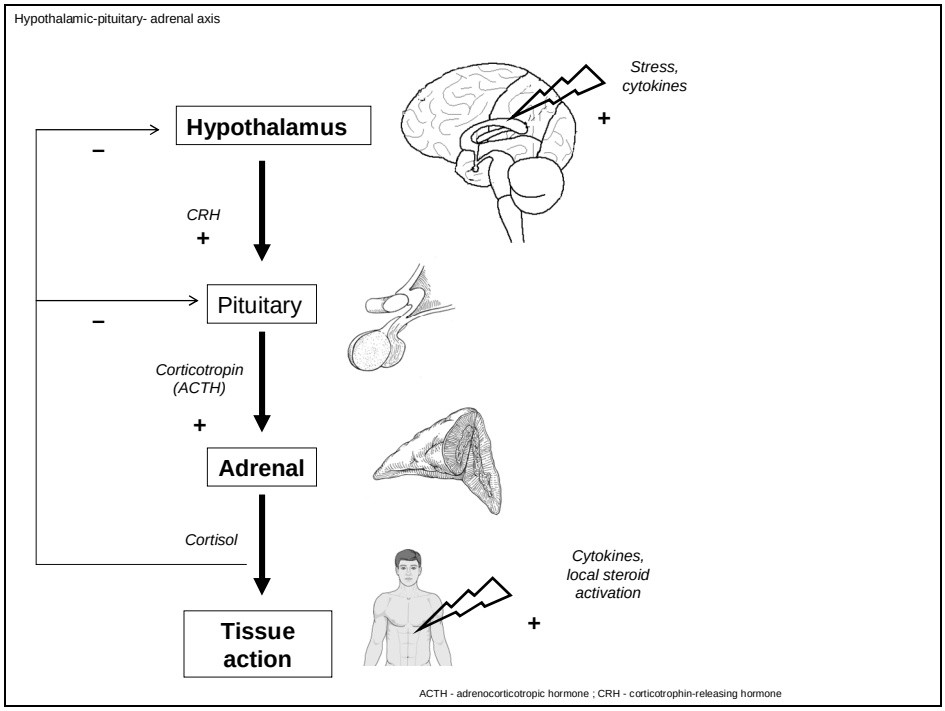

Figure 1: The HPA axis, adapted from Cooper et al (25)

1.4.2 Causes of hypoadrenalism

Thomas Addison described Addison's Disease (primary hypoadrenalism) in his 1855 paper, *"On the Constitutional and Local Effects of Disease of the Supra-renal Capsules"*. The disease at the time was secondary to tuberculosis, although this was not recognized till later, as tubercles in the glands were softened by the high local concentration of steroids, disguising the disease from pathologist scrutiny. His paper lead to intense study of the organs, and the subsequent discovery of steroids for which its finders were awarded two Nobel Prizes (28).

Primary hypoadrenalism (Addison's disease) is due to destruction of the adrenals. 90% of the gland is required to be destroyed before clinical symptoms are manifested. Secondary hypoadrenalism is due to destruction of the pituitary gland, or suppression after prolonged use of corticosteroids. As little as 15mg of prednisone daily for 3 weeks can suppress the HPA axis for 8-12 months. In the absence of corticotropin, blood flow decreases to the gland, with atrophy occurring within weeks (24, 29, 30, 31).

Causes of primary hypadrenalism (24, 25, 27, 32):

1) Tuberculosis. This is the commonest cause in the developing world, usually accompanied by destruction of both the cortex and the medulla. It is commonly associated with calcification of the adrenal gland secondary to inactive disease, although may also be associated with or precipitated by active TB.

2) Immune-mediated hypoadrenalism, as an isolated condition or as part of the auto-immune polyendocrine syndrome (APS). This is now the

commonest cause in the developed world, accounting for 50-70% of cases. Patients with this condition are prone to a variety of auto-immune conditions affecting different organs.

3) Human immunodeficiency virus (HIV), usually due to infiltration of the gland by opportunistic infections (cytomegalovirus, *Mycobacterium avium intracellulare*, cryptococcus) but occasionally secondary to the virus itself.

4) Genetic conditions, including congenital adrenal hyperplasia (21-hydroxylase, 11β-hydroxylase, 3β-HSD type 2, and 17α-hydroxylase deficiencies), familial glucocorticoid deficiency, secondary to an ACTH receptor abnormality, adrenoleukodystrophy, an uncommon but increasingly recognized X-linked neuro-endocrine degenerative disease, and an array of other rare conditions.

5) Traumatic or iatrogenic (surgical) destruction.

6) Anti-phospholipid syndrome, causing multiple thromboses of the adrenals.

7) Adrenal infiltration due to amyloidosis, haemochromatosis, metastatic neoplastic infiltration, especially secondary to carcinoma of the breast and lung, systemic fungal infections, such as cryptococcus, blastomycosis and histoplasmosis, and sarcoid.

8) Haemorrhage into the adrenal (Waterhouse-Friderichsen syndrome in meningococcaemia and other infections).

9) Drug induced inhibition of cortisol production, such as ketoconazole (not seen with other azoles) which inhibits steroidogenesis by inhibiting adrenal cytochrome P450 steroidogenic enzymes.

10) Drug induced induction of cortisol metabolism, such as rifampicin, phenytoin and barbiturates, which accelerate steroid metabolism.

1.4.3 Laboratory evaluation of adrenal function

Finding clear consensus on the exact laboratory values at which to diagnose primary adrenal insufficiency is difficult. A cortisol level above 410-938 nmol/l (depending on the author) excludes the condition, while a level below 82 nmol/l is diagnostic of the condition (25, 26, 27, 31, 33, 34, 35, 36, 37, 38). Stimulation testing is required for values between these levels, with no response to cosyntropin being seen if adrenal function is already maximally stimulated. Mild long-standing primary adrenal insufficiency may be missed using this approach (35).

The cosyntropin -stimulation test is the test most used by clinicians to diagnose primary hypoadrenalism with suspected inadequate stress responses in patients with normal or elevated cortisol levels, using supraphysiologic levels of an ACTH analogue. 250 ug of cosyntropin (ACTH 1-24) is given intravenously or intramuscularly before 10am, with cortisol concentrations measured at 30 and/or 60 minutes thereafter, although there is a wealth of evidence that the 30 minute blood levels are sufficient. In resting conditions in normal subjects, the cortisol level will usually increase 2-5 fold within 15-30 minutes of cosynotropin administration in adults with normal adrenal function. A level above 525 nmol/l is considered to exclude primary hypoadrenalism. In patients with

primary hypoadrenalism, minimal response is seen, as the gland is producing a maximum amount of cortisol in response to endogenous cosyntropin. Some authorities use the absolute increase of twofold above the basal level, or a minimum increase of 137 - 200 nmol/l above basal levels (34, 36, 37, 39, 40), to diagnose hypoadrenalism, but this is not regarded as conventional. A single paper recommends an increment of less than 300 nmol/l as defining an inadequate increases (41). In critically ill patients, a random sample is advised to exclude acute adrenal deficiency (25, 31), prior to introduction of empiric steroid replacement therapy, and a result >938nmol/l is regarded as indicating normal reserve. An increment of less than 250 nmol/l on stimulation testing is associated with a poorer outcome in these patients.

There has been recent concern that the 250ug dose of cosyntropin may be pharmacologically rather than physiologically active, and that a lower dose (1-10μg) may be a more sensitive method for testing for primary hypoadrenalism. Several studies have demonstrated equivalent plasma cortisol responses to 1 ug and 250 ug, in normal subjects (42, 43, 44, 45, 46) and indicated that the lower dose is thus able to detect mild adrenal insufficiency. There has been some suggestion that the 250ug dose is less sensitive than lower doses in the detection of hypoadrenalism (46, 47), but studies are conflicting , with studies showing that the high dose test very rarely gives false positive results in patients with hypoadrenalism (48, 49, 50). Concern has been raised regarding actual injection volume of cosyntropin seen with lower doses, presumably because of adhesion of cosynotropin to the administering catheter or needle (51). A South African study (52) strongly motivated for the use of the 1μg dose, and these authors used a healthy control group to develop a reference figure.

However, standard endocrinology textbooks continue to recommend the higher dose of cosynotropin (53, 54).

Basal ACTH and cortisol levels are conventionally measured in suspected primary hypoadrenalism. The loss of negative feedback on the pituitary results in marked elevated ACTH values above 22 pmol/l (100pg/l), usually above 45 pmol/l, even if cortisol levels are normal, in primary hypoadrenalism. Normal ACTH levels rule out primary but not secondary hypoadrenalism.

Patients with secondary hypoadrenalism may have normal results with the ACTH test, if insufficient adrenal atrophy has developed. However, in long standing disease, severe atrophy with minimal cortisol response develops. There is considerable interest in diagnosing secondary hypoadrenalism using the 'low dose' 1μg cosynotropin test, along with the conventional insulin tolerance, metyrapone and CRH tests.

1.5 TB, rifampicin and the HPA axis

1.5.1 Overview

Studies looking at adrenal function in patients with active TB have shown conflicting results, and there are remarkably few large studies, considering the size of the TB epidemic in the developing world. Early studies showed depressed adrenal function in patients with TB (55, 56), but subsequent studies discussed below have shown widely divergent results.

1.5.2 TB and adrenal function

Many of these studies used different laboratory values for both basal and dynamic testing, reflecting the historically conflicting advice from many authoritative studies and texts.

A study, also done in South Africa, looked at 50 hospitalised adults with pulmonary TB, and showed that all patients had a stimulated cortisol above the 550 nmol/l cut-off used. Mean basal cortisol levels were 625 nmol/l (range of 394 to 1185 nmol/l), and the average increase after cosyntropin stimulation was 256 nmol/l (range 0-650 nmol/l). ACTH was undetectable in 32 patients at baseline, normal in 17 and raised in one. (57)

A study by the same group evaluated ill hospitalised patients with pulmonary TB, using the newer but still controversial low-dose 1μg cosyntropin test, and demonstrated only a single case of hypoadrenalism at presentation amongst 40 patients. (52)

Similar results were reported from Tanzania, where 50 patients with pulmonary TB were assessed after treatment of at least three months, with 30 and 60 minute cortisol levels taken after cosyntropin stimulation. Impaired cortisol responses were defined as a one hour result of below 600nmol/l and/or a less than the relatively high 300nmol/l increment, which is unconventional. Two patients had low cortisol levels at baseline, and one of these patients had a subnormal cosyntropin response. 30% had normal baseline cortisol but subnormal responses to cosyntropin. Responses to cosyntropin showed no correlation with body-mass index, symptoms or length of treatment of TB. However, significant correlation was found between baseline and one-hour cortisol levels, and both systolic and diastolic blood pressure. (38)

A study from Papau New Guinea assessing patients with TB showed very little effect on adrenal function. The study looked at three groups of patients: pulmonary or pleural TB, miliary TB, and extrapulmonary TB, with 30 patients in each group. Prior to starting TB therapy, all 90 patients had normal or raised cortisol levels. Cosyntropin

stimulation tests were defined as normal (a greater than 200nmol/l increase was used) in 74 of the 90 patients. Nine of the remaining 16 patients had raised cortisol levels to begin with. Electrolytes in this study showed depressed serum sodium levels in 37 patients, and no cases of hyperkalaemia. There was no correlation between sodium levels and response to cosyntropin. HIV status was not recorded, but was unlikely to be significant, as HIV prevalence in Papau New Guinea is currently low (58).

A study in Hong Kong prospectively evaluated 39 patients with pulmonary TB, and found that none of them had subnormal basal cortisol levels (570.4 ± 243.8 nmol/l). 41% had a suboptimal rise (defined as >200 nmol/l rise on cosyntropin testing). Interestingly, 13 patients died within two months of starting treatment, and this group had significantly higher basal cortisol levels when compared to the survivors (743.7 ± 288.5). Nine of the 13 underwent autopsy, and only one patient had granulomas in the adrenals. This patient had the highest basal cortisol level in the group who died within two months (16).

A large study from South Africa published in 1986 (prior to HIV becoming a major clinical problem in this country) showed 55% of black patients with pulmonary TB having suboptimal responses to cosyntropin stimulation testing, using a relatively high cortisol increase of less than 300 nmols/l as indicating poor response (41).

In a hormonal and radiological study of both acute and chronic pulmonary tuberculosis, only two patients out of 61 (‘acute’ referred to 20 patients recently diagnosed, ‘chronic’ to 41 patients who had TB for at least three years) had subnormal ACTH responses (defined in this study as a cortisol over 550 nmol/l), both in the chronic group. Basal cortisol in the acute group was 554 ± 317 nmol/l (mean ± SD). 20 control

patients were used, and there was no difference in the increase in cortisol in the three groups in response to stimulation testing. In the acute TB group, computerised tomography displayed increased length and thickness of the adrenals (59).

In an Indian study half of all the 105 patients with TB (the study looked at patients in 3 groups, with pulmonary TB, disseminated /miliary TB or multidrug resistant TB) had compromised responses to ACTH testing. This study defined a normal response as a peak cortisol value >410 nmol/l with an increment of >137 nmol/l. If a peak value >550 nmol/l was obtained, the response was considered normal regardless of the increment (60).

22 patients with pulmonary TB in Turkey were investigated. One was assessed as having Addison's disease, based on a stimulation test result of <550 nmol/l. Decreased DHEA and urinary cortisol were measured after a month of rifampicin treatment, suggesting significant impact on steroid catabolism, according to the authors, and is discussed further in section 1.5.3 (61).

Disseminated extrapulmonary TB is very common in HIV-infected patients. Adrenal involvement seems plausible in these patients, as it has been the site of infection of a host of other opportunistic infections. In a large study from Kenya, looking at 184 patients, over 50% of the patients had subnormal cortisol responses to ACTH stimulation, defined as a cortisol over 195 nmol/l and an increment on cosyntropin testing of less than 195 nmol/l, but there was no difference between the HIV-positive and HIV-negative patients. Median cortisol was over 600 nmol/l, and most patients with 'subnormal' responses (66%) had baseline cortisol above normal. Ten patients had subnormal basal

cortisols (below 195 nmol/l in this study), but seven had normal increments on stimulation testing (62).

There is limited autopsy data on TB and adrenal disease. An autopsy study of 871 patients with active tuberculosis in a low HIV-prevalence area (Hong Kong), showed that 261 (30%) patients had extrapulmonary TB, and 52 (6%) had TB of the adrenals. Seven of these patients had presented with signs and symptoms of Addison's disease (23).

In summary, most studies seem to suggest that while adrenal involvement by TB is possible, it is uncommon to see abnormal results during stimulation testing. The Hong Kong study (16) suggests, worryingly, that a hypoadrenal crisis can occur despite normal assessment with stimulation testing.

1.5.3 Effects of rifampicin on steroid metabolism

Rifampicin induces hepatic oxygenase enzyme and cytochrome p450 activity in the endoplasmic reticulum (63). The liver cytochrome p450 system is responsible largely for phase I oxidative metabolism.

Rifampicin-containing antituberculous medication has been reported to increase cortisol production (64), decrease prednisolone half-life (65, 66), and increase urinary excretion of corticosteroid metabolites (67).

Healthy volunteers given rifampicin and isoniazid for 14 days showed a decrease of 34% of circulating steroid 25-hydroxy vitamin D (25-OHD), and a 23% decrease in 1-alpha 25-dihydroxy vitamin D (1.25 (OH)2D). These volunteers showed a decrease in 25-OHD and 1.25 (OH)2D, with an increase in parathyroid hormone; a similar hormonal pattern was seen in the same study in nine TB patients treated for one month with antituberculous drugs (68).

A study demonstrated a 48% decrease in prednisolone levels with rifampicin administration, and a decrease in elimination half-life from 3.72 to 2.11 hours (69). Areas under the curve of prednisolone after rifampicin administration, over time, showed a 66% decrease when both drugs were given together (66).

Rifampicin increased the metabolism of dexamethasone fivefold, and prednisolone by 50-60% (70). A similar effect on dexamethasone by rifampicin was confirmed in a further study (71).

In the Turkish studies, significant impact by rifampicin on steroid metabolism was found. Tuberculosis treatment had no impact on basal cortisol or ACTH levels, but marked decreases in DHEA and urinary free cortisol occurred after rifampicin administration. Dexamethasone suppression tests showed normal suppression prior to commencing tuberculous treatment in 9/22 patients, but no patients showed suppression after 20 to 30 days of treatment (61).

1.5.4 Clinical cases of hypoadrenalism secondary to rifampicin

Clinical consequences of rifampicin-induced cortisol metabolism have been limited to well-documented but isolated case reports.

A child with nephrotic syndrome, controlled on prednisolone, was given rifampicin and isoniazid after accidental administration of a BCG vaccine, with relapse of the nephrotic syndrome. Increased prednisolone subsequently controlled the nephrotic syndrome, and the dosage was reduced once the antimycobacterial agents were withdrawn (72).

A patient on cortisone and fludrocortisone for hypoadrenalism developed clinical evidence of steroid excess after ethambutol was substituted for rifampicin. (64).

In the Papau New Guinea group, 77 patients out of the original 90 had cosyntropin stimulation tests three to four weeks after starting a rifampicin-based regimen. Five patients had raised cortisol levels prior to starting treatment. Only a single patient had a subnormal response (120 nmol/l increment) to cosyntropin stimulation (58).

In the Hong Kong study described above, three cases were identified who died within six days of initiating rifampicin containing treatment and reviewed. All had developed severe hypotension and had cortisol levels measured as they were suspected to have Addison's disease. All had basal cortisol levels above 1000 nmol/L. Two had disseminated granulomas, including in the adrenals at autopsy, while the other had normal adrenals (16).

1.6 Role of rifampicin in treating TB

Rifampicin, along with isoniazid (INH), is a key antibiotic as part of the four-drug first line regimen advocated for the treatment of TB in South Africa, and most other places in the world (8, 73). The introduction of a multidrug approach, including the use of these two drugs, has allowed for the duration of treatment to be reduced from 18 months to 6 months, with almost 100% cure rates (74, 75, 76). Studies have demonstrated that rifampicin-containing regimens are more effective than older non-rifamycin based regimens, especially in HIV-prevalent populations (77). Rifampicin, however, has been shown to be less effective in TB bactericidal activity in the first few days of therapy, with activity very similar to that seen with ciprofloxacin (78).

1.7 Rifampicin and the cytochrome systems

Cytochrome P450 (CYP) is a group of enzymes involved in the metabolism of many compounds. Increasing numbers of these enzymes have been isolated over the last two decades, in both plants and animals. Their origin arises from the ancient archaebacteria, with some enzymes being extremely stable even at extremes of temperature, implying their role at the beginnings of evolution (79).

CYP enzymes utilize two atoms of oxygen with one atom being bound to hydrogen to yield water, and the other used to bind to the target substrate. All cytochromes have peroxidase potential. The reaction is summarized in the equation:

Reagent+NADPH+hydrogen+O2 ->ReagentOH+NADP+water (H2O)

Mammalian cytochrome enzymes are broadly divided into:

1) Biological regulators, involved in a broad number of functions including multiple steps in steroid hormone synthesis

2) Enzymes involved in compound metabolism, including toxins and drugs.

Numbering of cytochrome enzymes is according to the sequence of each enzyme's discovery, or according to the site of action (CYP21A1 catalyses hydroxylation of cholesterol at position 21). The "superfamily" of P450 cytochrome enzymes is subdivided into families (eg: CYP1) and then subfamilies (differentiated by letters – A,B,C etc).

The second group, enzymes involved in compound metabolism, operate in different organs, including the liver, gastrointestinal tract, lung, skin, kidney and blood components. The cytochrome system can:

1) Be induced by one drug, which can influence the metabolism of another drug. Hypericum (St John's Wort) can induce CYP3A4, decreasing cyclosporin levels in patients with transplants, with a resultant risk of rejection, or;

2) Have drugs compete for binding sites, with resultant increases in one or the other or both compounds. A clinical example is when certain antiretroviral protease inhibitors and terfenadine are combined. Protease inhibitors are more successful in competing for the CYP3A4 site, with a resultant increase in terfenadine levels, resulting in potential malignant arrythmias.

CYP3A4, and closely related CYP3A5, are the most important enzymes in humans, due to their abundance. These enzymes account for the majority of human liver CYP enzyme, although the amount seems to differ fairly markedly between individuals, depending on genetics, diet, and other poorly-understood factors. 30% of liver and 70% of intestinal CYP are CYP3A4,. 30% of individuals have significant liver CYP3A5. The broad but inter-individual variation in the distribution of the enzyme, and its interaction with such a variety of substrates leads to difficulty in predicting individual outcomes. Drug regulatory agencies have increasingly asked for details about potential drug impacts on the cytochrome system. Animal models have demonstrated varying levels of usefulness, as distribution of different enzymes varies between species.

Rifampicin falls into the first group above, by inducing CYP3A. Glucocorticoids are also inducers of CYP3A4 (79, 80, 81, 82, 83).

1.8 Impact and use of fluoroquinolones in treating TB

Fluoroquinolones, specifically ciprofloxacin and ofloxacin, have been advocated (84, 85) for the treatment of TB in patients who cannot tolerate the side effects of other antituberculous drugs, or as part of regimens in patients resistant to conventional therapy. Ciprofloxacin has been demonstrated to have a similar bactericidal activity against TB, both *in-vitro* and in clinical studies when compared to rifampicin and isoniazid in the first days after commencing therapy (84).

1.9 Summary of literature review

Tuberculosis is common in Southern Africa, and adrenal involvement at autopsy appears to be common. The prevalence of adrenal hypofunction in people with acute TB is unclear, with biochemical and clinical studies showing widely divergent results. Diagnosing adrenal insufficiency is not straightforward, with no clear agreement on what is regarded as the indication for and the adequate responses to stimulation testing. There are conflicting reports about the biochemical impact of rifampicin on steroid metabolism, and isolated but convincing case reports concerning the clinical consequences of this interaction.

1.10 Background to the study

Acute hypoadrenalism as a consequence of tuberculosis is well described in Africa. While there are a few possible mechanisms, it most commonly occurs in the setting of past adrenal tuberculosis with subsequent calcified adrenal glands. New onset tuberculosis may precipitate hypoadrenalism in patients with compromised adrenal function, as with any infection Furthermore, there is evidence that rifampicin can induce the catabolism of corticosteroids.

In patients with compromised steroid production this accelerated catabolism may lead to acute steroid deficiency when they are initiated on a rifampicin-based anti-tuberculosis treatment regimen for active tuberculosis. Should accelerated catabolism of corticosteroids be found to be common among patients initiated on treatment with a rifampicin-based anti-tuberculosis regimen, alternative strategies, such as supplementing these patients with corticosteroids or choosing an initial treatment regimen that does not contain rifampicin may be options, particularly in patients in whom there is concern about the adequacy of adrenal function.

1.11 Aim of the study

The aim of the study was to determine whether rifampicin induced accelerated catabolism of corticosteroids. This was done by measuring serum levels of ACTH and both basal and stimulated cortisol in patients with newly diagnosed active pulmonary tuberculosis, before and for five days after initiation of anti-tuberculosis therapy, comparing those treated with a rifampicin-based regimen versus those in whom rifampicin was replaced with a fluoroquinolone.

2 Materials and Methods

2.1 Summary:

A prospective, randomised trial was done comparing adrenal function responses over five days in hospitalised participants with pulmonary TB, treated either with a rifampicin or ciprofloxacin-containing regimen. Ciprofloxacin was used in place of rifampicin, as it does not induce the cytochrome p450 system. Ciprofloxacin has equivalent in-vitro mycobacterial killing ability to rifampicin in the acute phase of use, and it was therefore felt that this drug could be used without compromising patient TB treatment for the purposes of this trial, hence creating a control group.

2.1.1 Study population

Hospitalised patients with pulmonary TB were recruited for the study. Patients were recruited if they were admitted to the Johannesburg Hospital's medical admission ward, had acid-fast bacilli in their sputa in the presence of an abnormal chest radiograph, and required several days of in-patient care before discharge. Additional entry criteria included informed consent and age over 18 years. Exclusion criteria included current exposure to corticosteroids, prior antituberculous therapy, drugs known to interfere with steroid metabolism, or pregnancy.

2.1.2 Informed consent

Written informed consent was obtained from each participant prior to any study procedure, by the principal investigator. Prior approval was obtained from the University of the Witwatersrand's Committee for Research on Human Subjects (reference number 000809), now called the Human Research Ethics Committee. One participant withdrew consent, as he wanted to leave the hospital early, and was replaced at the end of the study.

2.1.3 Randomisation

Participants were assigned numbers sequentially as they were recruited. Each number had been assigned a specific drug regimen prior to the commencement of the trial, by removing the numbers from a hat by an uninvolved third party. The trial was initially double-blinded with the randomised drug being given to the patient in a sealed envelope. However, inadvertent unblinding, especially due to concerns about urine discolouration and pill dosing being voiced by participants to the investigator conducting the study, meant that this strategy had to be abandoned.

2.1.4 Baseline data collection

Hospitalised patients with acid-fast bacilli in their sputa were identified by doctors responsible for the clinical care of the patient. Clinical data were obtained from the participant after signed informed consent was obtained, by a review of the clinical file and a clinical examination. Laboratory data at baseline were collected from the admission notes and laboratory database.

On the first day, an intravenous, heparinised catheter was placed in a major forearm or antecubital vein at least one hour prior to specimen collection. Baseline serum corticotropin, aldosterone, cortisol, DHEA, urine osmolality and urine sodium, were obtained immediately prior to injection of 250ug Synacthen (cosyntropin) through the catheter accompanied by a flush of intravenous heparinised saline. Synacthen® depot is made by Novartis Pharmaceuticals, and is a long-acting analogue of corticotropin. Cortisol sampling at 30 and 60 minutes was then done, to assess the response to cosyntropin stimulation. There is no difference in the cortisol response if Synacthen is given IM or IV (86, 87).

2.1.5 Treatment initiation

Antituberculous treatment was started immediately after the 60 minute cortisol sample had been taken.

Patients randomised to the rifampicin group were given 300mg rifampicin orally daily if they weighed less than 50kg, and 450mg if over 50kg, in line with local TB guidelines (6). Patients randomised to the ciprofloxacin group were given 500mg twice orally daily, irrespective of weight. The rest of the regimen was given according to local TB guidelines, and included isoniazid, ethambutol and pyrazinamide, with dose adjusted for weight.

On day 2, day 3 and day 4, serum samples for ACTH, aldosterone, cortisol and DHEA were obtained. In addition, electrolytes were measured on day 3.

On day 5, a repeat of day 1 testing was performed with measurement of baseline serum electrolytes, corticotropin, aldosterone, cortisol and DHEA, followed by the cosyntropin stimulation test.

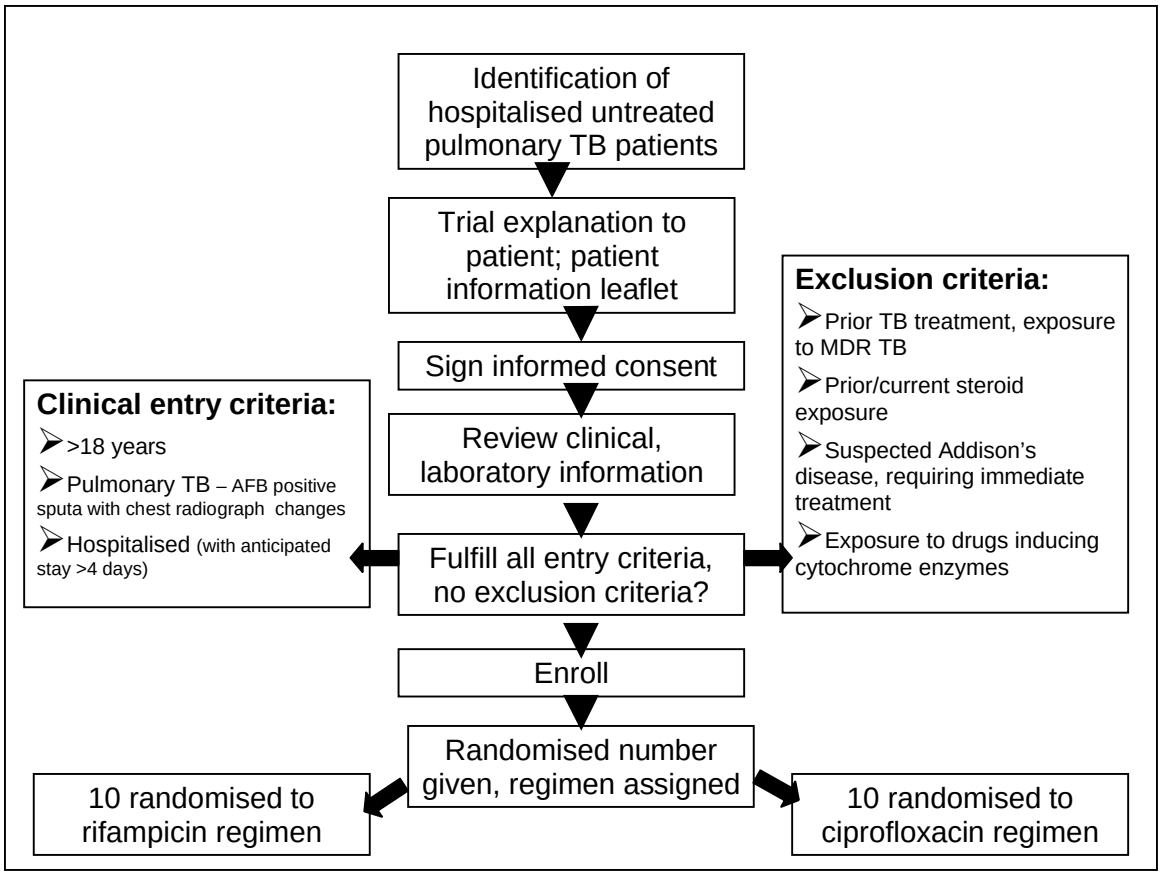

Figure 2.1: Recruitment procedure

	Pretreatment	Day 1	Day 2	Day 3	Day 4	Day 5	Post
Enrolment procedure	X						
Regimen assigned	X						
Demographic information							
Clinical examination	X						
Laboratory review	X						
Intravenous catheter		X					
Cortisol, corticotropin, aldosterone, DHEA		X	X	X	X	X	
Electrolytes, glucose	X			X		X	
Urine sodium, osmolality	X						
Cosyntropin stimulation test		X				X	
Assessment of clinical status at discharge							X

Table 2: Study procedure post consent

Note: The time between 'pretreatment' and day 1 was never greater than 24 hours, and was<12 hours in most cases.

2.1.6 Biochemical and hormone assays

Serum electrolytes (sodium, potassium, chloride, urea, creatinine and total carbon dioxide) were measured with a COBAS Integra 400 autoanalyser, using reagents made by Roche Diagnostic, Mannheim. Plasma glucose levels were determined by a standard glucose oxidase method. Urine sodium was measured using automatic micro-osmometer (Roebling, Berlin). Serum cortisol concentrations were measured by radioimmunoassay using kits supplied by ICN Biochemicals Inc, Costa Mesa, California. Serum levels of ACTH, DHEA and aldosterone were measured by immunoassay using direct chemiluminometric technology and reagents supplied by Diagnostic Products Corporation, Los Angeles, California. Inter-assay co-efficients of variation were between 5 and 10% for all assays.

The following formula was used to calculate serum osmolality : 2 x (sodium plus potassium) + urea +glucose.

The syndrome of inappropriate antidiuretic hormone (SIADH) was defined by the combination of hyponatraemia (sodium < 130 mmol/l) with low serum osmolality (<280 mmol/kg), inappropriately high urine osmolality compared with serum osmolality, and urinary sodium >20 mmol, in the absence of clinical volume depletion.

Normal values for all of the above are included in the results tables.

2. 2 Interpretation of results

The diagnosis of adrenal insufficiency was made by means of a cosyntropin stimulation test. A cortisol increase of >250 nmol/l was used to indicate sufficient adrenal reserve.

Corticotropin was collected, immediately placed on ice, and spun down within one hour. Storage occurred in the endocrinology research laboratory at -20°C.

2.3 Statistical analysis

Results were analysed using the one way analysis of variance (ANOVA) for repeated measures, followed by the two-tailed t-test for parametric data and the Wilcoxon Rank Sum test or Signed Rank Test where distribution of the data was non-parametric. The effect of the cosyntropin stimulation was analysed by comparison of the incremental rises in cortisol, which were calculated by subtracting the basal level from the peak level attained at the 30 minute and 60 minute (Δ change). Results are represented as mean of SEM or as 95% confidence intervals (CI), and a value of $p<0.05$ was regarded as significant.

3 Results

3.1 Baseline results of participants

3.1.1 Clinical characteristics (Table 3.1)

The demographics of the two groups were similar. There were 5 men and 5 women in each group. All participants were black Africans. Age and duration of symptoms was similar. Clinical data were also very similar. Patients tended to be pyrexial with low blood pressures. The ciprofloxacin group had borderline statistically significant lower systolic and diastolic blood pressures and temperature than the rifampicin group (p=0.05)). All patients had abnormal chest radiographs.

Table 3.1: Baseline results: Demographic and Clinical

Characteristics	**Rifampicin group (10 patients) – (mean, range, confidence intervals)**	**Ciprofloxacin group (10 patients) – (mean, range, confidence intervals)**	**Significance (p-value)**
Female:Male	Ratio 5:5	Ratio 5:5	
Age (years)	28.5 (18-43; CI ± 4.9)	35 (20-53; CI ± 5.7)	0.12
Duration of symptoms (months)	2.8 (0.5-12; CI ± 2.3)	2.6 (0.25-12;CI ± 2.1)	0.87
Respiratory rate/min	21.4 (18-30; CI ± 2.8)	20.4 (16-28; CI ± 2.6)	0.59
Temperature (Centigrade)	38.3 (36.6-40; CI ± 0.8)	37.6 (36.5-38.5; CI ±0.5)	**0.03**
Weight (kilograms)	51.5 (41-65.5; CI ± 5.4)	51 (31-70; CI ± 8.1)	0.93
Pulse rate/min	101 (88-120; CI ± 5.8)	99 (84-112; CI ± 5.9)	0.63
Systolic blood pressure (mmHg)	114 (90-140; CI ± 13.6)	93 (75-110; CI ± 8.2)	**0.05**
Diastolic blood pressure (mmHg)	76 (60-100; CI ± 8.2)	63 (50-80; CI ± 5.1)	**0.05**

.

3.1.2 Baseline laboratory data (Table 3.2)

There were no significant differences in serum electrolytes, bicarbonate, urea, creatinine or serum osmolality between the two groups. The only significant difference was in the initial C-reactive protein (CRP) levels, where patients in the rifampicin group had a higher mean CRP. Arterial blood gases were done but oxygen supplementation percentages were not recorded in the clinical notes, making interpretation difficult.

One patient refused HIV testing in the ciprofloxacin group, but had clinical features suggestive of HIV disease. CD4 counts were variable, with most patients (7/9 in the rifampicin group, 6/9 in the ciprofloxacin group) having CD4 counts below 200 cells/μl. White cell counts tended to be slightly raised. Mild SIADH was present in 4/9 patients in the rifampicin group, and 1/9 in the ciprofloxacin group.

All patients improved clinically during the five days they spent in hospital, as assessed by their attending doctor based on symptom and clinical signs, and all were discharged home within one week of admission.

Table 3.2: Baseline results: Laboratory; n is the number of patients whose data were available.. This relates to CD4 counts, liver enzymes, protein, bilirubin, ESR, CRP, glucose, and urine sodium and osmolality.

Characteristics (normal value, units)	**Rifampicin group – result with mean, range, confidence intervals, n reported if data incomplete**	**Ciprofloxacin group – result with mean, range, confidence intervals, n reported if data incomplete**	**Significance (P value)**
Immunology			
HIV status	9/10 positive (1 negative)	9/10 positive (1 refused testing)	
CD4 (500-2010 cells/µl)	211 (3-820; CI ± 156) n=9	171 (5-538; CI ± 130) n=9	0.74
Gases			
pH (7.36-7.4	7.461 (7.40-7.52)	7.454 (7.28-7.55)	0.73
O2 (11-13.3kPa)	8.6 (6.7-14.1; CI ± 1.4)	9.6 (4.8-17.9; CI ± 2.3)	0.87
PCO2 (4.6-6.0 kPa)	3.7 (3-4.7; CI ± 0.3)	3.9 (3-5.6; CI ± 0.5)	0.43
Saturation	94 (88-98; CI ± 2)	92 (78-97; CI ± 5)	0.4

(90-100%)			
Haematology			
Haemoglobin (12.1-16.3 g/dl)	9.5 (7.9-12.3; CI ± 0.8)	9.5 (7.3-12.3; CI ± 1.1)	0.97
White cell count (4-10 cellsx10^6/l)	10.6 (2.5-21.9; CI ± 4.5)	8.4 (4.9-19.3; CI ± 2.5)	0.46
Platelets (178-400 cellsx10^9/l)	434 (116-665; CI ± 110)	334 (245-499; CI ± 53)	0.13
Red cell distribution width (11.6-14.0%)	18.1 (13.3-24.3; CI ± 1.9)	15.8 (12.8-18.3; CI ± 1.1)	0.07
Mean cell volume (79.1 -98.9 fl)	78.9 (70.6-96.7; CI ± 6.2)	81.5 (72.9-92.1; CI ± 4.3)	0.52
Serum enzymes and biochemistry			
Aspartate transaminase (5-40 units/l)	47 (19-132; CI ± 25) n=8	58 (28-114; CI ± 23) n=8	0.44
Alanine transaminase (5-40 units/l)	27 (7-53; CI ± 13) n=8	29 (16-54; CI ± 9) n=8	0.52
Alkaline phosphate (40-120 units/l)	113 (62-352; CI ± 68) n=8	122 (62-244; CI ± 44) n=8	0.70

Gamma-glutamyltranspeptidase (0-35 units/l)	60 (17-262; CI ± 57) n=8	72 (17-205; CI ± 43) n=8	0.70
Total bilirubin (0-21 µmol/l)	7 (4-11; CI ± 2) n=8	10 (4-21; CI ± 4) n=8	0.38
Unconjugated bilirubin (0-6 µmol/l)	3 (1-8; CI ± 2) n=8	5 (2-14; CI ± 3) n=8	0.32
Total protein (60-85 g/l)	78 (64-91; CI ± 6) n=8	72 (57-92; CI ± 7) n=8	0.28
Albumin (35-52 g/l)	26 (20-35; CI ± 4) n=8	25 (15-29; CI ± 3) n=8	0.57
Erythrocyte sedimentation rate (1-20 mm/hr)	104 (88-126; CI ± 11) n=8	86 (8-130; CI ± 23) n=10	0.34
C-reactive protein (<10mg/l)	155 (93-222; CI ± 28) n=8	99 (44-181; CI ± 28) n=9	**0.025**
Sodium (135-145 mmol/l)	129 (121-135; CI ± 2)	133 (125-141; CI ± 3)	0.08
Potassium (3.5-5 mmol/l)	3.7 (3.7-3.7; CI ± 0)	3.9 (3.2-4.8; CI ± 0.3)	0.11

Chloride (98-106 mmol/l)	97 (92-106; CI ± 3)	99 (89-110; CI ± 4)	0.46
Bicarbonate (18-23 mmol/l)	20 (14-25; CI ± 2)	22 (20-26; CI ± 1)	0.10
Urea (3-8 mmol/l)	4.5 (2.3-12.4; CI ± 2.1)	3.2 (1.7-8.3; CI ± 1.2)	0.34
Creatinine (60-120µmol/l)	84 (66-129; CI ± 12)	75 (59-115; CI ± 11)	0.29
Glucose (3.1-5 mmol/l)	5.3 (3.8-75; CI ± 0.8) n=10	5.1 (2.6-8; CI ± 1.0) n=9	0.78
Serum osmolality (285-295 mmol/kg)	276.5 (265.7-287.6; CI±4.0) n=9	283.3 (268.7-290; CI±5.8) n=9	0.06
Urine results			
Urine osmolality (mmol/kg)	456 (120-695; CI ± 129) n=9	505 (205-877; CI ± 138) n=10	0.68
Sodium	69 (6-205; CI ± 48) n=9	140 (10-699); CI ± 128) n=10	0.34
SIADH present	4/9	1/9	

3.1.3 Non-hormonal laboratory values on treatment (Table 3.3)

There was no significant difference in electrolytes, bicarbonate, urea or creatinine between the two groups and no significant difference between the values on day 1, day 3 and day 5 within the group (all $p>0.05$).

Table 3.3: Electrolytes and renal function at baseline, day 1, 3 and 5: Comparison between two groups. Results are mean ± SEM (Standard error of the mean), ($p>0.05$ for all values measured between day 1, 3 and 5)

	Rifampicin			**Ciprofloxacin**		
Day	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5
Sodium (mmol/l)	129±1	133±1	133±1	133±2	133±1	135±1
Potassium (mmol/l)	3.7±0.1	3.7±0.1	3.9±0.1	3.9±0.1	3.7±0.1	3.8±0.1
Chloride (mmol/l)	97±2	102±1	102±1	99±2	102±2	105±2
Bicarbonate (mmol/l)	20±1	20±1	20±1	22±1	21±1	20±1
Urea (mmol/l)	4.5±1	4.2±0.7	3.1±0.4	3.2±0.6	3.3±0.3	2.7±0.3
Creatinine (umol/l)	84±6	80±6	73±7	75±6	74±3	68±2
Calculated osmolality	277±2	283±2	282±2	283±3	282±2	287±3

3.1.4 Hormonal laboratory values on treatment (Table 3.4)

Mean basal cortisol was substantially elevated, reflecting the stress of the underlying illness. The lowest recorded baseline cortisol was 577 nmol/l among the 20 patients. Cortisol levels were higher in the rifampicin group prior to treatment (not significant).

The rifampicin group overall showed a significant decrease in cortisol over the full five day treatment period ($p=0.001$). The ciprofloxacin group showed a non-significant decrease, and there was a significant degree of variability in this group.

There was no significant difference between the rifampicin and the ciprofloxacin groups in terms of baseline corticotropin, aldosterone and DHEA values. Corticotropin levels fluctuated widely on treatment in both groups, despite there being no significant difference between them, and were higher on all other days compared to day 1. DHEA levels were low at all time points, and showed a slight statistically significant decrease downward in the rifampicin group ($p=0.007$). Aldosterone levels varied widely on a daily basis, all within normal ranges.

Table 3.4: Serum concentrations of pituitary and adrenal hormones in the two groups of patients during treatment. Results are mean ± SEM (Standard error of the mean)

	Rifampicin					**Ciprofloxacin**				
Day	1	2	3	4	5	1	2	3	4	5
Cortisol (mmol/l)	1258±180	1232±130	1144±86	1098±127	918±97	989±124	1090±104	864±113	953±129	793±102
Corticotropin (ng/l)	26±5	46±20	30±7	68±26	54±6	26±25	62±25	37±9	66±22	32±8
DHEA (µmol/l)	2.7±1	2.3±0.8	1.8±0.6	1±0.2	1±0.1	1.4±0.3	1.5±0.3	1.4±0.4	1.5±0.3	1.2±0.2
Aldosterone (pmol/l)	372±108	297±86	393±101	328±117	135±34	144±56	256±112	212±75	265±183	138±52

Cortisol normal values: 190-660 umol/l; Corticotropin:<46 ng/dl; DHEA: Female age range: 18-40 years: 0.9-8.7 µmol/l; Male age range: 24-53 years: 4-13.3 µmol/l; Aldosterone: <440 pmol/l

3.1.5 Responses to cosyntropin stimulation testing (Table 3.5, 3.6 and 3.7, Figure 3)

If an increment of 250nmol/l at 30 or 60 minutes from baseline is used as the definition of inadequate response to cosyntropin stimulation testing, eight patients out of the 20 (three in the rifampicin group (patient 4 (increment Δ105 nmol/l), patient 5 (Δ149 nmol/l) and patient 6 (dropped 23 nmol/l)) and five patients in the ciprofloxacin group (patient 1 (increment Δ 210nmol/l), patient 2 (dropped 94nmol/l), patient 3 (Δ232 nmol/l), patient 4 (Δ 221 nmol/l) and patient 10 (Δ229 nmol/l) had impaired adrenal reserve on day 1. Two patients (one in each group; patient 6 in the rifampicin group, patient 2 in the ciprofloxacin group) had baseline cortisol levels that *dropped* on stimulation testing (but only one significantly; from 936 nmol/l at baseline to 842nmol/l at 30 minutes and 709nmol/l at 60 minutes - ciprofloxacin patient 2). Of the eight patients, only three had readings below 1000nmol/l.

In both groups, responses to ACTH stimulation were observed, after 4 days of treatment. At day 5, two patients (one in each group; patient 6 in the rifampicin group, patient 3 in the ciprofloxacin group) had impaired responses to ACTH stimulation, if the 200 nmol/l increment is used. All 20 patients demonstrated cortisol increases at 60 minutes, the lowest increment being an increase from 1557 to 1600 nmol/l (rifampicin patient 6).

There was no statistical difference in the overall increments between the two groups. Increments were statistically higher in both groups on day 5 versus day 1, due to a fall in the basal cortisol on day 5.

Table 3.5: Cortisol concentrations during stimulation testing, Results are mean ± SEM, all nmol/l

Ciprofloxacin						Rifampicin					
Day 1			Day 5			Day 1			Day 5		
0 min	30 min	60 min	0 min	30 min	60 min	0 min	30 min	60 min	0 min	30 min	60 min
988 ± 124	1169 ± 123	1269 ± 138	793 ± 102	1077 ± 107	1175 ± 98	1258 ± 180	1416 ± 168	1606 ± 166	918 ± 97	1277 ± 93	1532 ± 94

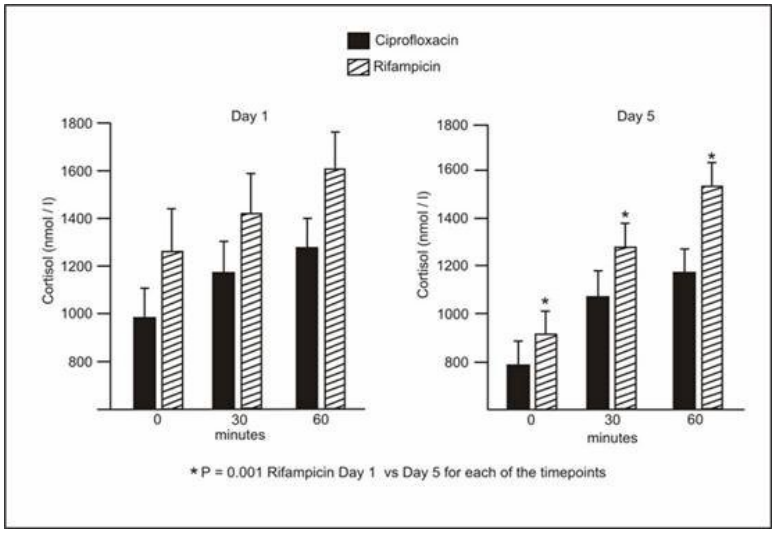

Figure 3: Cortisol responses during stimulation testing

Table 3.6: Rifampicin group: cortisol levels, nmol/l

Rifampicin	Day1 (ACTH stimulation)			Day2	Day3	Day4	Day5 (ACTH stimulation)		
Patient number	0min	30min	60min	0min	0min	0min	0min	30min	60min
1	1007	1466	1551	1035	1278	851	781	1065	1479
2	894	1308	1427	894	1010	1018	737	1217	1322
3	817	861	1132	1035	858	513	809	930	1038
4	784	795	889	1016	1065	988	745	858	1104
5	1441	1590	1540	1642	1380	1656	938	1628	1990
6	2156	2133	2098	2089	1164	1372	1557	1609	1600
7	2385	2454	2793	1645	1455	1802	1405	1526	1692
8	858	1159	1719	831	690	977	748	1386	1722
9	1074	1330	1457	1035	988	729	646	1027	1733
10	1165	1060	1457	1101	1551	1076	811	1532	1642

Table 3.7: Ciprofloxacin group: cortisol levels, nmol/l

Ciprofloxacin	Day1 (ACTH stimulation)			Day2	Day3	Day4	Day5 (ACTH stimulation)		
Patient number	0min	30min	60min				0min	30min	60min
1	1170	1344	1380	1670	1435	1570	916	1452	1372
2	936	842	709	831	657	535	566	781	911
3	770	814	1002	1179	806	1038	654	676	778
4	596	817	792	773	502	671	513	742	789
5	922	1167	1278	1024	439	596	828	1239	1308
6	1344	1604	1697	1054	861	955	1046	1184	1377
7	577	856	905	566	555	787	505	770	1018
8	966	1197	1551	1402	1435	1667	660	983	1104
9	729	1065	1270	1016	1060	1159	1559	1678	1750
10	1877	1987	2106	1386	886	555	682	1261	1350

DISCUSSION

The primary objective of this clinical investigation was to assess adrenocortical function during the initial phase of antituberculous therapy in hospitalised patients with active pulmonary TB in an attempt to demonstrate if there was any compromise of adrenal function by rifampicin. A control group was created using ciprofloxacin in place of rifampicin. Ciprofloxacin does not induce cytochrome systems and hence is assumed not to impact on adrenal hormone metabolism by the liver.

Patients were randomised to both arms of the study, and the two groups had similar baseline demographic, clinical and laboratory data. All responded to TB treatment, and were discharged successfully from hospital. No patient had overt or suspected hypoadrenalism.

Mean basal serum cortisol was substantially elevated before and after initiation of antituberculous therapy, reflecting the stress of severe illness meriting hospital admission in all patients. No patient had subnormal cortisol levels at any time point. The lowest recorded baseline cortisol was 577 nmol/l among the 20 patients.

Many texts use levels of 600nmol/l (26, 38) as a cortisol level suggestive of adequate adrenal reserve and it would appear retrospectively that this patient group had adequate adrenal function before commencing therapy. This is further supported by the normal or elevated ACTH levels recorded in both groups. The highest suggested random serum cortisol level of 938 nmol/l (25) applies to critically ill patients, and while the patients used in this study were all sufficiently ill to be hospitalized, no patient was sufficiently ill to be considered for intensive care monitoring, and the mean cortisol before starting treatment was above this level in both groups. However, even using the

938 nmol/l cut-off, 16/20 of the patients in this study had baseline or stimulated serum cortisol levels of over this level. In the remaining four patients, the lowest recorded baseline cortisol response was 784 nmol/l. The lowest recorded cortisol level throughout the measurement period was 439 nmol/l, well within normal limits.

The above suggests that primary hypoadrenalism is relatively uncommon in this patient population, using the criteria above. This is in keeping with the low incidence of primary hypoadrenalism reported in other recent African studies involving similar patient cohorts (16, 38, 52, 57, 58, 59, 60, 61). A very high number of our patients were HIV-positive, and many had advanced disease, as measured by CD4 count. This group of patients with advanced immunosuppression and who were ill enough to be hospitalised, would be thought to be at risk of disseminated TB, and hence adrenal involvement and compromise of stress hormone responses. The lack of any clear adrenal compromise is hence even more significant in this group.

The mean cortisol response to cosyntropin stimulation was well above the normal cut-off limit, both on day 1 before and 5 days after commencing the rifampicin-based regimen. There was no difference between the rifampicin and ciprofloxacin groups. There was no difference between the two groups in terms of cortisol responses during the treatment period, or on ACTH stimulation on the final day. It appears that rifampicin does not significantly induce cortisol catabolism.

However, as discussed, the dose of synacthen (250µg) that was injected was supra-physiological, which may have demonstrated reduced sensitivity compared with low dose (1µg) administration, and this might have masked any subtle impairment of function produced by rifampicin.

Moreover, the significant reduction in mean post-stimulation cortisol responses at each of the time points on day 5 in our patients could indeed be an indication of accelerated cortisol catabolism. However, this occurred in the ciprofloxacin group, which should not have impacted on catabolism. It seems far more likely that improved clinical state, due to more effective anti-tuberculous therapy, accounted for the decreased cortisol levels. Interpretation of free cortisol levels in any ill patient may need to be tempered, as total and free cortisol may fluctuate in response to albumin and other protein changes seen in acute illness.

It is difficult to interpret the other electrolyte and hormonal measures in the study. Electrolyte measures are gross measures of indirect hormonal influence, and may be influenced by other factors, including SIADH. In this study, there was no significant change in either biochemical or hormonal parameters between the two groups.

DHEA (S) levels remained depressed throughout the study period, probably reflecting the illness of the patients, and again there was no difference between the groups. ACTH levels were normal pre-treatment, and then exhibited transient elevations before again returning to normal, for reasons that are unclear. Aldosterone levels were all within normal levels, and exhibited no consistent trend. Again, the fact that there was no difference between the two groups is reassuring in terms of adrenal function, but the length of the study was short. Ideally, it would have been useful to measure DHEA (S) and ACTH levels after a longer period, to see if levels returned to normal, and whether they lagged behind the trend we saw with cortisol levels. Other studies have noted a return to normal of hormonal parameters over months and years after commencement of treatment (16, 41, 58, 88).

DHEA-S concentrations were consistently subnormal, probably as a result of the poor health status of the majority of patients in our study. The discrepancy between high cortisol and low DHEA-S levels in our patients is interesting and might have clinical relevance. Reduced output of DHEA metabolites in patients with severe TB could be detrimental for several reasons. Effective immunity to TB requires a T helper-1 (Th1) pattern of cytokine release and experimental animal studies have demonstrated that even a minor T helper-2 (Th2) component is associated with impaired immunity and a non-protective cytokine profile. Exposure of T-cells to glucocorticoids such as cortisol drives them towards a Th2 response, which may both reactivate and exacerbate TB.

In contrast, DHEA and its derivatives have antiglucocorticoid effects in vivo and, in the experimental situation, have been shown to protect against shifts towards Th2 effects and to be associated with enhanced granuloma formation. In short, the high cortisol/DHEA-S ratio that occurred in our patients may be crucial to both the susceptibility and the pathology of disease in TB, and warrants further investigation. (88, 89, 90).

The occurrence of hyponatraemia and SIADH was the commonest metabolic abnormality encountered in our cohort of patients, having been diagnosed in 5/18 of cases on admission to hospital. This is higher than the 10% SIADH prevalence rate reported in a recent South African study (52) and may be ascribed to 90% of our patients being documented HIV sero-positive (in the other study, 18/40 patients were positive) and possibly having had more advanced disease, despite similar TB diagnostic criteria being employed. The mechanisms involved in SIADH relate to increased total body water with

decreased total body sodium as a consequence of enhanced or an abnormal pattern of ADH release. However, the impact of SIADH on adrenal function is unclear.

Conclusion

During the first few days of therapy, rifampicin did not impair adrenal responses to conventional cosyntropin stimulation tests in this group of patients with active pulmonary TB, most of whom were HIV-positive. Furthermore, most patients with pulmonary TB and HIV appear to have adequate adrenal reserve, as measured by conventional testing.

Finally, the controversy and debate over accepted measurements of adrenal reserve as measured by the cosyntropin-stimulation test must be tempered by evidence (16) that TB patients die of adrenal crisis despite normal stimulation testing. Our study, while reassuring in that rifampicin does not seem to significantly induce cortisol catabolism, does not answer the question of aetiology regarding sudden death in the first few days of TB treatment. Adrenal insufficiency may still be a significant reason for mortality, and may be significantly under-diagnosed till we have more sensitive and specific tools that have been properly validated to diagnose the condition.

Future research into the reasons for the high death rates during the initial period of TB treatment needs to be done, looking at the role of thrombosis and possibly the role of rifampicin in promotion of thrombosis, the immunological changes that occur immediately after treatment with TB treatment, with specific reference to cortisol/DHEA-S ratios, and the role of associated comorbidities. Clinical interventions as adjuncts to TB treatment need to be explored, including the role of routine anticoagulation in hospitalized patients, the routine use of corticosteroids and other analogues, and the use

of different anti-tuberculous therapies. Finally, improved diagnostic tests for reliably diagnosing adrenal insufficiency are needed.

Appendices