

**Project Number:** 11-CH-0208

**Drug/Device:**

Mifepristone IND 113698

9,11,12,12-[<sup>2</sup>H]<sub>4</sub>-cortisol IND 113698

**Subject:** Effects of the glucocorticoid antagonist, mifepristone, on glucose intolerance in obese and overweight individuals

**Identifying words:** Glucose intolerance, cortisol, Mifepristone, glucocorticoid antagonist

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**Proposed Start Date:** June 1, 2011

**Estimated Duration of Accrual:** Five years

**Subjects of Study:** Men and women (35 - 70 yrs) with glucose intolerance

**Number:** 80

**Project uses ionizing radiation?** No

**Off-Site project?** No

**Multi-Institutional Project?** No

**Precis**

The hormone cortisol is a key regulator of metabolism that influences the use of glucose (sugar) and fat as fuels. Persistently increased cortisol levels, as in Cushing's syndrome, lead to obesity, type 2 diabetes mellitus and lipid abnormalities including elevated triglyceride levels and low high-density lipoprotein (HDL) levels. These same disorders are also present in patients without Cushing's syndrome, suggesting that cortisol may be involved in their pathogenesis. Mifepristone is a cortisol-like drug that blocks cortisol action in the body. It can reverse lipid abnormalities, diabetes and obesity in Cushing's syndrome patients but its effects on these conditions have not been tested in patients without the syndrome.

The long-term aim of this clinical trial is to evaluate the ability of mifepristone to reverse or improve glucose intolerance, dyslipidemia, hypertension and weight gain. An initial 7-day

prospective, randomized, placebo-controlled, crossover study is proposed here to look at the effect of short-term administration of oral mifepristone or placebo on glucose intolerance. Given that there are no human data available on the effect of mifepristone on insulin sensitivity, this will be a pilot study of 15 subjects. Data from this study will then be used to design a larger trial to evaluate long-term effects on blood pressure and weight, as well as glucose and triglyceride control.

Overweight or obese subjects with abnormal glucose tolerance will undergo each of the two treatments in a randomized order, including mifepristone by mouth and a look-alike inert tablet by mouth. Each treatment study will include two or three days of baseline tests that will be repeated after seven days of treatment. Treatments will be separated by at least six and no more than eight weeks. The tests will include blood drawing, urine collection, administration of glucose and insulin by vein, and a cortisol-like material to evaluate the metabolism of cortisol and a related hormone, corticosterone.

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## BACKGROUND

Cortisol is an important regulator of metabolism that modulates the use of glucose and fat as fuels. Elevated cortisol levels in Cushing's syndrome lead to obesity, hypertension, dyslipidemia with elevated triglyceride (TG) levels and low high-density lipoprotein (HDL) levels, and type 2 diabetes mellitus (1). These disorders also are common in the general population, in the absence of Cushing's syndrome. In people without Cushing's syndrome, the constellation of abnormalities is often referred to as the "Metabolic Syndrome" based on the extent of the abnormalities. Some individuals with Metabolic Syndrome seem to have mild functional hypercortisolism, which may contribute to the pathogenesis of the syndrome (2 - 5). Also, some individuals with abdominal obesity have increased urinary free cortisol (UFC) levels, loss of diurnal cortisol rhythm, and abnormal HPA axis suppression after dexamethasone (5 - 6), similar to the biochemical abnormalities seen in Cushing's syndrome. Excess glucocorticoids in Cushing's syndrome are known to favor visceral fat deposition (7). However, many individuals with obesity do not have abnormal cortisol tests (8). Taken together, these data have led to the hypothesis that cortisol may be involved in development of abdominal obesity, glucose intolerance and other components of the Metabolic Syndrome, either because of subtle increases in circulating levels, or because of increased tissue exposure due to local metabolism.

### *Associations between tissue levels of cortisol, obesity and glucose tolerance*

Circulating cortisol levels are mostly normal in people with central obesity, insulin resistance Metabolic Syndrome (2 - 3), leading to speculation that increased local tissue exposure to cortisol or its active metabolites may be critical in development of these disorders. One possibility is that there is increased local regeneration of cortisol from corticosterone. The 11 $\beta$ -hydroxysteroid dehydrogenase (11BHS) enzymes interconvert hormonally inactive glucocorticoids such as cortisone and dehydrocorticosterone and their active forms, cortisol and corticosterone. The renal 11BHS2 enzyme catalyzes the conversion of cortisol to (inactive) cortisone, thus reducing mineralocorticoid effects of cortisol. The 11BHS1 enzyme is more widely expressed (in liver, adipose, bone, and the central nervous system) and functions primarily as a reductase (converting cortisone to cortisol) (9 - 10). This 11BHS1 reductase activity requires subcellular NADPH cofactor generation by hexose-6-phosphate dehydrogenase, and in its absence, this bi-directional enzyme can convert cortisol to cortisone. Intracellular cortisol levels may thus be modulated by conversion of cortisone to cortisol by 11BHS1, and this may contribute to the development of glucose intolerance and the Metabolic Syndrome (11).

Because urinary levels of cortisol, cortisone, and their metabolites reflect integrated (total) body metabolism (including the kidney), they cannot be used to assess the contribution of specific tissue components. As a result, 11BHS effects have been assessed using enzymatic assays of activity, measurement of mRNA expression in specific tissues, or measurement of conversion of cortisol or cortisone labeled with stable isotopes.

11BHS1 expression and activity has been found to be elevated in both visceral and subcutaneous fat depots in obese individuals (12 - 13). Purnell and colleagues studied eight obese men before and after weight loss and looked at daily cortisol production and clearance rates, intraabdominal fat (IAF) and subcutaneous fat (SQF) by computerized tomography, insulin

sensitivity ( $S_i$ ) by frequently sampled intravenous glucose tolerance tests, and subcutaneous adipocyte 11BHS1 gene expression by quantitative RT-PCR. They found that increased cortisol production rates and free cortisol levels correlated with increased IAF and decreased insulin sensitivity but not with subcutaneous fat. Increased 11BHS1 expression correlated with both IAF and SQF and with decreased  $S_i$ . With greater loss in body fat than lean mass with weight loss, 11BHS1 expression was found to be decreased from baseline (13). These data support a role for increased local (tissue) cortisol exposure leading to visceral fat deposition and insulin resistance.

Many investigators have suggested that increased cortisol release from the omentum into the portal vein contributes to hepatic insulin resistance associated with central obesity. However *in vivo* studies of obese non-diabetic individuals using stable isotopes suggest that splanchnic cortisol is derived entirely from the liver and that there is no cortisol release from the visceral tissue into the portal vein (–14 - 15). On the contrary, visceral tissue releases cortisone into the portal venous system, thus potentially providing increased substrate for hepatic conversion to cortisol (9). This cortisone release from the visceral tissue occurs in the presence of low levels of 11BHS2 mRNA in visceral fat. Therefore the exact mechanism by which cortisone is generated is not clear. It is possible that in some situations 11BHS1 has a predominately dehydrogenase (inactivating) activity leading to cortisone production (16 - 17).

It should be mentioned that some studies do not show a depot difference in 11BHS1 mRNA expression and have suggested that under steady state conditions, increased local production of cortisol is unlikely to contribute to omental adipose tissue function (18). These discrepancies in study results are likely related to differences in assays for measuring 11BHS activity, the use of different cells or explants and the effects of renal 11BHS2. Also, *in vitro* studies may not faithfully reproduce the *in vivo* environment, and therefore may result in a false preponderance of reductase or dehydrogenase activity.

Although glucocorticoids have been shown to induce 11BHS1 expression *in vitro*, a single study looking at omental levels of 11BHS1 in Cushing's syndrome patients found its expression to be similar to those of lean individuals (19). However, as omental fat mass is generally increased in patients with Cushing's syndrome, the net hormonal conversion cannot be ascertained. Moreover, it is possible that high cortisol levels overwhelm the 11BHS1 enzyme, as seen with 11BHS2 enzyme in the kidneys (20), so that excess cortisol is delivered to the liver.

Genetically engineered mouse models provide additional evidence for a potential pathologic role for 11BHS. Mice that over-express 11BHS1 in the liver develop fatty liver, hypertension, and mild insulin resistance without altered fat depot mass (21). On the other hand, transgenic mice overexpressing 11BHS1 selectively in adipose tissue exhibit a full metabolic syndrome with visceral obesity, hyperlipidemia, insulin resistance/diabetes and hypertension (22).

Other types of local cortisol metabolism may contribute to excess tissue glucocorticoid exposure. Reduction of glucocorticoids to A-ring reduced dihydro- and tetrahydro-derivatives by means of hepatic 5  $\alpha$ - and 5  $\beta$ -reductases was previously regarded as the final inactivation pathway. However, studies in rats have shown that corticosterone, 5  $\alpha$ - tetrahydrocorticosterone (5 $\alpha$ THB), and 5  $\alpha$  –dihydrocorticosterone (5 $\alpha$ DHB) are similarly effective in displacing tritiated dexamethasone from binding sites in hepatocytes while the 5 $\beta$  metabolites have less effective binding. In addition, 5 $\alpha$ THB, but not the 5 $\beta$ -reduced metabolites, activated the glucocorticoid receptor *in vitro* and suppressed ACTH *in vivo*, and these effects were inhibited by mifepristone (23). Increased 5 $\alpha$  reduced metabolites have also been seen in patients with diabetes and women with polycystic ovary syndrome (PCOS). These findings suggest that this pathway may be involved

in the development of glucose intolerance and Metabolic Syndrome (24 - 25).

The link of cortisol to insulin resistance and the Metabolic Syndrome is further substantiated by studies looking at the effects of mifepristone. Liu and colleagues investigated the role of tissue glucocorticoids in type 2 diabetes mellitus and obesity by analyzing the expression of glucocorticoid receptor and 11BHS1 in the hepatocytes of db/db mice (a model of type 2 diabetes mellitus). Increased expression of glucocorticoid receptor and 11BHS1 was associated with elevated levels of corticosterone, insulin, and blood glucose. They also found that intraperitoneal administration of mifepristone to the db/db mice for 3 weeks improved glucose tolerance with simultaneous decrease in the expression of 11BHS1 and glucocorticoid receptor in the liver (26). These findings were corroborated in another study on ob/ob diabetic mice (27). Also, administration of mifepristone to obese Zucker rats and db/db mice prevents high fat diet-induced weight gain and type-2 diabetes (28 - 29).

In humans, mifepristone successfully ameliorated hypercortisolemia-related effects in patients with Cushing's syndrome (30). More recently mifepristone was approved by the FDA for treatment of chronic hyperglycemia of uncontrolled Cushing's syndrome (31). In healthy men, administration of 400 mg of mifepristone at 2200h led to a decrease in triglyceride levels, despite compensatory increases in cortisol (32). Bertagna et al. looked at the antiglucocorticoid effect of mifepristone in four healthy men and found that ACTH and cortisol values increased 6 - 10 hours after administration of 400 mg of mifepristone at 0200 h, but not at 1400 h. Although this was a small study, it demonstrates the fact that the antiglucocorticoid effect of mifepristone on the hypothalamic-pituitary unit may occur only at certain times of the day (33), and supports the use of a split dosing regimen rather than a single daily dose, to minimize effects on ACTH.

Based on the putative role of excessive exposure to cortisol, we hypothesized that reduction in cortisol action with mifepristone might reverse glucose intolerance and some of the other components of the metabolic syndrome, just as it reverses the features of Cushing's syndrome (30).

The long-term aim of this study is to evaluate the ability of mifepristone to reverse or improve the components of Metabolic Syndrome, specifically impaired fasting glucose, dyslipidemia, hypertension and visceral obesity. This pilot study will specifically investigate the effects of short-term administration of mifepristone on insulin sensitivity. A second phase of the study will then look at the effects of long-term treatment on lipids, blood pressure, weight as well as glucose intolerance.

## **STUDY HYPOTHESIS**

The primary study hypothesis is that glucose abnormalities in overweight and obese individuals will improve with short-term administration of the glucocorticoid antagonist mifepristone.

A secondary study hypothesis is that activation of the HPA axis will be minimal when mifepristone is administered in four divided daily doses of 50 mg.

## **STUDY DESIGN AND METHODS**

Prospective, randomized, placebo-controlled, cross-over study

Inclusion Criteria:

1. Men and women 35 – 70 years of age
2. Overweight or obese subjects with BMI ranging from 25 - 37 kg/m<sup>2</sup>.
3. Subjects will have pre-diabetes defined as fasting glucose  $\geq 100$  mg/dL or a 2-hour glucose value  $\geq 140$  mg/dl during an oral glucose tolerance test (OGTT)

OR

Mild diabetes defined as patients with a Hba1c  $\leq 7\%$  on no medications (diet-controlled) or on a stable dose of metformin and no other hypoglycemic agents for  $\geq 3$  months before study entry.

4. Willing and able to comply with study requirements.

Exclusion Criteria:

1. Pregnancy and lactation
2. Change in dose of metformin or use of hypoglycemic agents other than metformin for treatment of diabetes within 3 months of study entry. Diagnosis of diabetes will be based on the 2011 American Diabetes Association guidelines: Hba1c  $\geq 6.5\%$ , fasting plasma glucose  $\geq 126$  mg/dl, 2-hour glucose  $\geq 200$  mg/dl during an OGTT, or a random blood glucose  $\geq 200$  mg/dl along with classic symptoms of hyperglycemia (34)
3. Uncontrolled hypertension (blood pressure  $\geq 180/110$  mmHg)
4. Current unstable medical conditions including clinically significant impaired cardiac function (Stage III and IV Cardiac failure), cardiac ischemia, severe respiratory insufficiency requiring oxygen therapy as assessed on history and/or physical exam
5. Liver function tests (ALT, AST) more than 3-times the upper normal limit
6. Severe renal impairment (creatinine clearance  $< 30$  ml/min)
7. Evidence of human immunodeficiency virus (HIV) based on history and physical examination and/or known positive HIV antibodies
8. Evidence of hepatitis C based on history and physical examination and/or known positive hepatitis C (HCV) antibody
9. History of hemorrhagic disorders or on anticoagulants
10. History of endometrial cancer, endometrial hyperplasia, unexplained vaginal bleeding, or endometrial thickness greater than 6 mm
11. Change in dose of lipid-lowering medications (including HMG Co-A inhibitors, fibrates, niacin, ezetemibe, and over-the-counter fish oil supplements) within one month of study entry and during the study period
12. Current administration of medications known to be strong CYP3A4 inhibitors including ketoconazole, itraconazole, and erythromycin
13. Use of herbal supplements or grapefruit juice within 14 days of study drug initiation
14. Use of medications or dietary supplements that inhibit or induce CYP3A4 activity within 14 days of study drug initiation
15. Use of oral, injectable, or inhaled glucocorticoids or megestrol in the past six months
16. Use of estrogen-containing hormone therapy
17. Potential pseudocushing's states: depression or intake of  $> 2$  alcoholic drinks a day for women and  $> 3$  alcoholic drinks per day for men. Subjects will be screened for depression

using the well-validated physician health questionnaire-9 (PHQ-9) with a score cut-off of  $\geq 10$  for moderate depression (35).

18. Subjects who are actively dieting or are in a weight loss program
19. Midnight salivary cortisol  $> 100$  ng/dl on two separate occasions
20. Untreated thyroid dysfunction (TSH and Free T4 not within normal range). If abnormal on screening labs, they will be repeated to confirm that not due to lab error or non-thyroidal illness.
21. Moderate to severe anemia (hemoglobin  $< 10$  g/dl)
22. Blood donation of more than 500 ml within one month prior to study enrollment
23. Subjects with a prolonged QTc interval on electrocardiogram
24. Unable to give informed consent

## **Study protocol**

### *Screening*

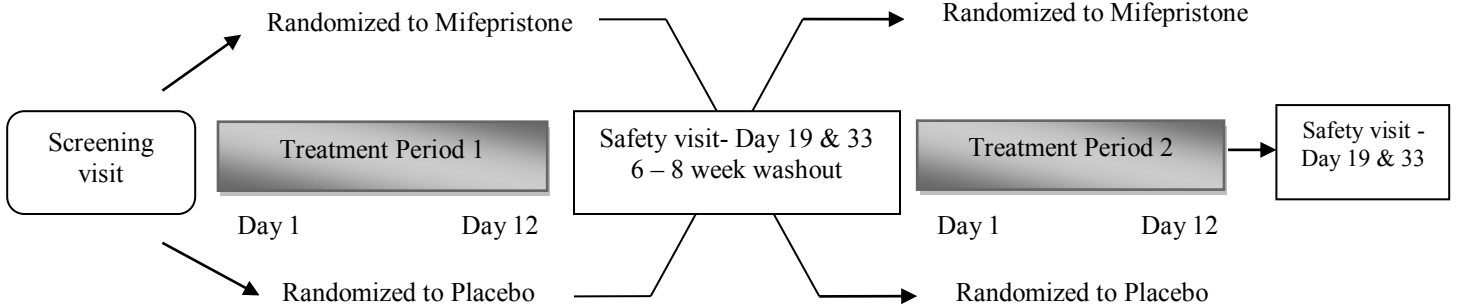
Subjects will undergo evaluation at the Clinical Center for eligibility. This will include measurement of height, weight, waist circumference, blood pressure, and a detailed history and physical examination. Subjects will be screened for depression using the PHQ-9 questionnaire. If a subject is found to have severe depression or suicidal ideation, they will be seen by a psychiatrist at NIH on an emergent basis and further management will be guided by their recommendations. An electrocardiogram will be obtained to identify any subjects with QTc interval prolongation. Screening laboratory tests will include a serum pregnancy test for females, fasting plasma glucose, HgbA1c, OGTT (2 hour, after 75 g dextrose), comprehensive metabolic panel, CBC with differential, coagulation profile, midnight salivary cortisol, TSH, Free T4, FSH and LH levels. HIV 1&2 antibody and hepatitis C antibody levels will also be checked if on history taking the subject is found to have risk factors for HIV or Hepatitis C. A transvaginal ultrasound will be obtained to evaluate endometrial thickness in postmenopausal women (defined as amenorrhea for  $\geq 12$  months and an FSH level in the postmenopausal range) as well as those in the perimenopausal phase (amenorrhea for  $\geq 6$  months and FSH level in the postmenopausal range). An MRI Pelvis will be performed only if the transvaginal ultrasound is unable to provide an accurate measurement of the endometrial stripe. Subjects in whom a definite assessment of endometrial thickness cannot be obtained with either ultrasound or MRI (as may be the case in patients with multiple fibroids) will be followed closely for any signs of abnormal vaginal bleeding and will undergo a repeat transvaginal ultrasound upon completion of the study.

### *Study for eligible subjects*

This crossover study has two treatment arms (placebo or Mifepristone 50 mg tablets every six hours) with each subject serving as her own control. After eligibility is confirmed, subjects will undergo baseline testing and will be randomized to one of the treatment arms. Testing will be performed before and at the end of seven days of treatment with the study agent. Study drug will be continued until completion of post-drug testing. Thus, subjects will take the study drug for a total of nine days during each treatment arm. There will be a washout period of at least six and no more than eight weeks after which subjects will be randomized to the other arm of the study with an identical testing schedule. Subjects will record any symptoms or adverse events on a calendar



throughout the study. Women will be asked to keep a record of any abnormal menstrual bleeding. Subjects will be asked not to start on any new diet or exercise regimen during the study and maintain a stable weight for at least 2 weeks prior to study entry.



**Figure 1: Study Design overview**

*Procedures during each treatment*

Subjects will be admitted to the 5SWN metabolic unit or 5NW in the Clinical Center on a Sunday for testing the following day. To accommodate menstrual cycle timing, and for subjects for whom weekend testing is preferable, admission may occur on Friday or Saturday; additionally the Day 2 and 3 testing may be swapped if needed to accommodate nursing scheduling. To reduce potential variability associated with estrogen in premenopausal women, testing will be performed in the early follicular phase (days 1-7 of menstrual cycle). In case of perimenopausal women with irregular menstrual cycles, an attempt will be made to begin testing in the early follicular phase with the rest of the testing performed at pre-determined study intervals. Height, weight, and waist, hip and neck circumference (mean of three measurements) will be measured. Waist circumference will be measured at the intersection of midaxillary line with the uppermost lateral border of the right iliac crest at the end of a normal expiration (36). The hip circumference will be measured in a horizontal plane at the level of the maximal circumference of hips and buttocks. Neck circumference will be measured at mid-neck height between the mid-cervical spine and mid anterior neck to within 1 mm (37). Weight will be measured at the same time of the day on the same scale. All measurements will be taken after an overnight fast with the subject standing upright, both arms hanging loosely by the side of body, and eyes directed straight ahead. Subjects will be maintained on an isocaloric regular diet during the inpatient part of the study. Those subjects who are on metformin at the time of study entry will be maintained on the same dose of metformin throughout the study.

Day 1 (Monday)

- Fasting blood pressure will be measured consecutively three times five minutes apart. Blood pressure will be measured in a standardized manner in the morning using equipment that meets certification criteria. Measurements will begin after at least 5 minutes of rest in a supine position. Subjects will be asked to refrain from smoking or ingesting caffeine during the 30 minutes preceding the blood pressure measurement. Blood pressure will be measured in the same arm on

each occasion using an appropriate cuff size with the bladder in the cuff encircling at least 80 percent of the arm for accurate measurement.

- Morning fasting baseline laboratory measurements: serum levels of lipids (total cholesterol, HDL and low density lipoprotein (LDL) cholesterol, triglycerides, VLDL), glucose, total and free testosterone, androstenedione, DHEAS, comprehensive metabolic panel, ACTH and cortisol.
- ACTH and cortisol levels will be measured hourly for 24 hours from 0900h to 0900h the next day; urine will be obtained during this time for measurement of 5 alpha and beta-reduced cortisol metabolites, cortisol, cortisone and their tetra-hydro metabolites in collaboration with Dr. B. Walker.
- Glucose levels will be measured before and two hours after breakfast, lunch, and dinner.

#### Day 2 (Tuesday):

Subjects will undergo an insulin-modified frequently sampled intravenous glucose tolerance test (FSIVGTT) when the urine collection is finished. Two indwelling intravenous catheters will be placed – one for sampling and other for infusing glucose and insulin. A bolus of 50% dextrose (0.3 gm/kg) will be administered over ~ 1 minute. Blood samples will be obtained for measurement of glucose, insulin, and free fatty acids (FFA) at -10, -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, and 19 minutes. At 20 minutes, an insulin bolus (0.03 units/kg) will be administered and samples for insulin, glucose, and FFA will be collected at 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 minute time points. The data will be analyzed by Bergman method to calculate the insulin sensitivity index (Si) (38 - 39). Subjects will be allowed to go on pass if they can return by 8 AM.

A fasting lipid panel will also be obtained along with baseline labs.

#### Day 3 (Wednesday):

A 75 gram 3 hour OGTT with measurement of insulin, c-peptide and glucose levels at -5, 0, +30, +60, +90 +120, +150 and +180 min will be performed on day 3 before and after the study drug.

Serial blood draws for Day 1 (serial ACTH/cortisol measurements) and Day 3 (OGTT) will be obtained within 5 minutes of the appointed time. After the conclusion of Day 1 through Day 3 testing, subjects will be sent on pass with the study drug with instructions to begin at 6 PM, and to continue at 6-hour intervals.

#### Day 4 – 9:

Study participants will take the study drug as instructed for seven days and will return on day 10 of the study, bringing the remaining study medication with them. Study drug will be continued until all study procedures are complete on day 12 (study drug given for a total of 9 days).

Day 9 (Tuesday): Subjects will be readmitted to 5SWN or 5NW

#### Day 10 – 12 (Wednesday-Friday):

After an overnight fast, all study procedures indicated for day 1 -3 will be repeated on day 10 - 12. Blood (5 ml) will be obtained on day 10 for measurement of post-treatment mifepristone level and to hold for other potential measurements related to cortisol metabolism. Subjects will continue on the study drug until the completion of all study procedures on day 12.

Day 19 and 33 (Friday): Safety monitoring

After discontinuation of study drug, subjects will return approximately one week (Day 19) and then 3 weeks (Day 33) after the last day of study drug intake for measurement of blood chemistries and CBC. The research team will make every effort to ensure that these safety follow-up visits are within 2 days of day 19 and day 33. However, for subject convenience, we will allow for the follow-up visits to be within 5 days of the expected date of follow-up (day 19 ± 5 days and day 33 ± 5 days). In these cases, a member of the study team will contact the study subject on the expected date of follow-up to ensure that the subject is doing well. If any potential treatment-related abnormalities/adverse events are noted at the safety visits, subjects will receive appropriate treatment for these abnormalities and will be followed closely as clinically indicated until the abnormalities/adverse events have fully resolved.

Second study treatment visit: These study procedures will be repeated with each study drug after a wash-out period of 6-8 weeks.

For subject convenience, we will allow for the pre-study drug testing (Days 1 through 3) in each treatment arm to be done on days other than as specified above as long as it is done no more than 10 days prior to study drug initiation.

Table 1: Schema for first admission testing. The second admission will be identical.

<b>Sunday</b>	<b>Monday</b>	<b>Tuesday</b>	<b>Wednesday</b>	<b>Thursday</b>	<b>Friday</b>	<b>Saturday</b>
Admit to 5SWN or 5NW	<b><u>DAY 1</u></b> Fasting labs 24 hr urine  0900 Serial 24 h ACTH/Cortisol  Pre & post prandial glucose levels	<b><u>DAY 2</u></b> 0900 Complete serial ACTH/Cortisol  0930 Fasting lipids FSIVGTT  Can go out on pass	<b><u>DAY 3</u></b> 0800 3 hr OGTT  1800 START MIFEPRISTONE OR PLACEBO	<b><u>DAY 4</u></b> On pass	<b><u>DAY 5</u></b> On pass	<b><u>DAY 6</u></b> On pass
<b><u>DAY 7</u></b> On pass	<b><u>DAY 8</u></b> On pass	<b><u>DAY 9</u></b> Return from pass to 5SWN	<b><u>DAY 10</u></b> Fasting labs 24 hr urine  0900 Serial 24h ACTH/Cortisol  Pre & post prandial	<b><u>DAY 11</u></b> 0900 Complete serial ACTH/Cortisol  0930 Fasting lipids FSIVGTT	<b><u>Day 12</u></b> 3h OGTT STOP MIFEPRISTONE OR PLACEBO  Discharge	

			glucose levels			
					<b>DAY 19 and 33</b> Follow-up	

Measurement of 11BHSD activity

Subjects will also undergo measurement of 11BHSD activity at least after one and no more than two months after the last day of study drug intake in the second treatment arm. This will be performed using 20% 9,11,12,12-[<sup>2</sup>H]<sub>4</sub>-cortisol and 80% hydrocortisone, as reported by Andrew et al (kindly provided by Dr. Brian Walker) (40). At time zero (~0830 h), a primed intravenous infusion of cortisol (20% 9,11,12,12-[<sup>2</sup>H]<sub>4</sub>-cortisol and cortisol) is given (3.6 mg priming bolus, then continuous 1.74 mg/h infusion) for three hours and 20 minutes. Blood is obtained at times -5, 0, 60, 120, 180, 185, 190, 195 and 200 minutes. This test will only be performed once during the study. This part of the study is currently on clinical hold.

**Study Outcomes**

**Primary Outcome**

1. Change in insulin sensitivity index based on the effect of insulin on glucose metabolism in the FSIVGTT.

**Secondary Outcomes**

1. Change in fasting plasma glucose and insulin levels.
2. Change in area under the curve (AUC) insulin, AUCglucose/AUCinsulin, and insulin sensitivity index based on OGTT.
3. Change in pre- and post-prandial glucose levels.
4. Change in basal triglyceride level.
5. Change in free fatty clearance based on the effect of insulin on FFA in the FSIVGTT.
6. Change in HDL, LDL, and total cholesterol levels.
4. Change in weight, BMI, neck, waist and hip circumference. It is unlikely that these parameters will change with the short duration of administration, however.
5. Change in blood pressure. It is unlikely that these parameters will change with the short duration of administration, however.
6. Whole-body rate of regenerating cortisol (measured with 9,11,12,12-[(2)H(4)]cortisol tracer) will be analyzed in collaboration with Dr. Brian Walker. On metabolism by dehydrogenation, d4F loses 11alpha- deuterium, forming trideuterated cortisone (d3E) and is regenerated by reduction to tri-deuterated cortisol (d3F). 11BHSD1 reductase activity can be measured specifically as conversion of d3E to d3F (40). The amount of regenerated cortisol at baseline will be assessed for correlation with basal measures of insulin sensitivity and triglyceride levels, and the change in these parameters in response to mifepristone.
7. Adverse events will be tallied and compared between treatments.

8. Change in total and free testosterone, androstenedione, DHEAS, ACTH and cortisol will be assessed between treatments as a measure of mifepristone effect.
9. Change in 5 alpha and beta-reduced cortisol metabolites, cortisol, cortisone and their tetra-hydro metabolites will be assessed between treatments.
10. Correlation between post-treatment mifepristone level and the change in triglyceride levels and insulin sensitivity in response to mifepristone.

## STUDY ANALYSIS

The study will be analyzed in collaboration with Dr. Ninet Sinaii, Ph.D. (Biostatistics and Clinical Epidemiology Service, Clinical Center). Subjects will be randomized in blocks of 6 into the two treatment arms. Means and standard deviations will be used to describe continuous data. Proportions and frequencies will be used to describe categorical data. All continuous data will be examined for normality and homogeneity of variances, and will be either transformed as appropriate or assessed using non-parametric tests. The change in continuous variables (including triglyceride, HDL, LDL and total cholesterol levels, insulin sensitivity index, pre- and post-prandial glucose values, whole body cortisol production rates, blood pressure, weight, and waist circumference) before and after study intervention will be analyzed using paired t-tests or Wilcoxon signed-rank test. Correlation of continuous data (e.g., regenerated cortisol and insulin sensitivity and triglyceride levels, and mifepristone level and insulin sensitivity and triglyceride response) will be carried out by Pearson's correlation coefficient or Spearman's rho, as appropriate. Between and within treatment comparisons will be done using Mixed Models for a crossover design that will also consider sequence effect, if any. Difference in the proportion of subjects who meet criteria for metabolic syndrome, or other categorical data, before and after study intervention will be analyzed using McNemar's test. Two-sided p-values will be used for all analysis and the level of statistical significance will be set a priori at 0.05. The stepdown Bonferroni correction will be used to adjust for multiple comparisons. All statistical analysis will be performed using SAS v. 9.2 (SAS Institute, Cary, NC).

Human data on the effect of mifepristone on insulin sensitivity index are lacking. This will therefore be a pilot study of 17 subjects. Data from this study will then be used to design a long-term study looking at the effect of mifepristone on glucose intolerance, lipids, weight and blood pressure. With an estimated loss-to-follow-up rate of 15%, we would need to recruit a total of 25 patients. Thus, we plan to screen approximately 80 patients to account for the high ineligibility rate and patient drop-outs prior to study initiation.

Based on current evidence, a compensatory rise in ACTH levels has been seen with mifepristone in healthy individuals at doses as low as 100 mg/day (when used as a single dose over 3 months) and 200 mg/day over 8 days (41 - 42). Given the uncertainty in the effect of the dose, we plan to do an interim analysis looking at the effect of the proposed dose (50 mg every 6 hours) on the HPA axis in the first 6 subjects (one randomization block). The data from the first 6 subjects will be unblinded to Dr. Ninet Sinaii, the study statistician, only. If the 24 hour UFC value post mifepristone treatment is more than or equal to twice the upper limit of normal in four or more of the six subjects, then we will decrease the mifepristone dose to 25 mg every 6 hours (100 mg/day). However, if there is no significant increase in the levels (< twice the upper limit of normal), same dose of mifepristone will be continued throughout the study. If the mifepristone

dose is changed based on the results of the interim analysis, then the data from the first six subjects will not be used in the final analysis and 15 additional subjects will be enrolled prior to completion of the study. On the other hand, if no dose adjustment is required, the study analysis will be done as previously planned.

## PHARMACEUTICAL SAFETY AND RANDOMIZATION

Mifepristone (HRA 052015; INN Mifepristone) is a cortisol and progesterone analog with antiprogestin and antiglucocorticoid activity. Healthy subjects receiving long-term mifepristone administration showed antiglucocorticoid effects at a daily dose of 10 mg/kg, which is about 5 – 10 fold greater than that required to demonstrate antiprogestin effects (43). The agent effectively reverses the clinical and glucocorticoid-dependent biochemical features of Cushing's syndrome (30). The proposed doses in this protocol are about 2 – 3 mg/kg/d.

Mifepristone will be given under an active IND (113,698) sponsored by Laboratoire HRA-Pharma. Laboratoire HRA-Pharma, NICHD's cooperative research and development partner, will provide mifepristone compound. The Pharmaceutical Development Service will formulate the compound into 50 mg capsules. Mifepristone will be manufactured, controlled and released according to current GMP guidelines. A look-alike inert placebo will be formulated into capsules by the Pharmaceutical Development Service.

The Pharmaceutical Development Service will randomize subjects in blocks of 6 to receive the two treatments. The investigators will not be informed of the treatment group of any participant while she receives study compound unless there is an adverse event requiring "unblinding" of the treatment. At the interim analysis, the treatment arm assignment for the first 6 subjects will only be revealed to Dr. Ninet Sinaii, the study statistician. The rest of the study investigators will remain blinded.

The 20% 9,11,12,12-[<sup>2</sup>H]<sub>4</sub>-cortisol and 80% hydrocortisone solution will be given under an active IND (113,698) sponsored by HRA-Pharma. The deuterated cortisol will be purchased from Cambridge Isotope Laboratories, Inc (50 Frontage Rd, Andover, MA. 01810-5413; phone 800-322-1174; WEB: [www.isotope.com](http://www.isotope.com)). The Pharmaceutical Development Service will perform sterility and pyrogenicity testing and will formulate the 20% 9,11,12,12-[<sup>2</sup>H]<sub>4</sub>-cortisol and 80% hydrocortisone solution.

## EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

### Benefits

Subjects may benefit by receiving a physical examination, and laboratory testing. In addition this study will contribute to our understanding of the physiology pertaining to the HPA axis and metabolic syndrome.

### Risks/Discomforts

We believe that this study does not involve significant risk for the volunteer subjects. The risks are anticipated to be greater than minimal, with the 7 day course of mifepristone too brief to offer the prospect of direct benefit to subjects. The medical procedures include physical examination, non-invasive tests (eg UFC and saliva collection), venipuncture and study agents with minimal side

effects at doses being used in the study. The risk: benefit ratio is low. The intravenous glucose tolerance test can be associated with greater than minimal risk, but these risks will be mitigated as noted below.

1. *Adrenal insufficiency* can be associated with the use of mifepristone given its antiglucocorticoid action. In a previous subchronic study, Laue and colleagues found that 8 of 11 healthy volunteers receiving mifepristone 10mg/kg/day for 7- 14 days exhibited generalized exanthem and one developed signs and symptoms of adrenal insufficiency. In all cases symptoms resolved 5 - 6 days after discontinuation of mifepristone (44). In another study by Lambert et al, chronic daily treatment with 200 mg for months, mifepristone daily was associated with mild nausea and fatigue but no other signs and symptoms of adrenal insufficiency (45). However, given the short duration of administration and smaller doses of mifepristone being used in this study, these effects are unlikely. No adverse effects were reported after single or two-day doses of up to 400 mg in previous studies, or after 200 mg daily for 8 days (32, 33, 46). All subjects will be educated on the signs and symptoms of adrenal insufficiency and will be instructed to contact study personnel if they develop any such symptoms. Detailed history with regards to signs and symptoms of adrenal insufficiency will also be taken during the study visits.
2. *Hypoglycemia* is a risk associated with the frequently sampled intravenous glucose tolerance test after insulin administration. The dose of insulin will be calculated meticulously and the test will be performed in a strictly monitored setting, with frequent measurement of glucose and the ability to administer additional glucose should hypoglycemia occur. Also, we believe that the risk of hypoglycemia with anticipated improvement in glucose intolerance on study drug in subjects on a stable dose of metformin is minimal. Glucose levels will however be monitored at every follow-up visit during the study.
3. *Deuterated isotopes* used to evaluate cortisol and cortisone dynamics are not radioactive and are not expected to have adverse effects.
4. *Endometrial hyperplasia and vaginal bleeding* is a theoretical risk with long-term administration of mifepristone. However, given the short duration of drug administration in this study we do not anticipate this effect. Postmenopausal women will be screened for pre-existing endometrial hyperplasia by ultrasound and any abnormal vaginal bleeding during the study will be investigated. An MRI Pelvis will be performed if the transvaginal ultrasound is unable to provide an accurate measurement of the endometrial stripe. Subjects in whom a definite assessment of endometrial thickness cannot be made with either ultrasound or MRI (as may be the case in patients with multiple fibroids) will be followed closely for any abnormal vaginal bleeding and will undergo a repeat transvaginal ultrasound upon completion of the study.
5. *Phlebotomy* is associated with mild discomfort and the possibility of bruising. There is a small risk of fainting, bleeding, or of infection. These will be minimized by placing the patient in a seated or supine posture and by the use of clean technique.
6. *Time* involved in clinic visit and inpatient stay may be a discomfort. Every effort will be made to streamline the visits, and the amount of time required will be specified in the consent form as clearly as possible. Women will be allowed to go out on pass when no testing is scheduled.

7. *Anemia* is a possible risk of phlebotomy. The total blood withdrawal over a six week period will be 425 ml which is within the NIH guideline of 450 ml for adults or 7ml/kg/6 weeks in all subjects. Every effort will be made to minimize the amount of blood drawn.
8. *Termination of pregnancy* is possible with mifepristone. Only non-pregnant women will be enrolled in the study. A pregnancy test will be obtained for all women at the screening visit and prior to study drug initiation. Women will be counseled regarding the risk of termination of pregnancy associated with study drug intake and will be asked to use non-hormonal contraceptive methods if they are sexually active during the study and for one month after stopping the study medication.
9. *Hypokalemia* has been seen with the use of mifepristone in Cushing's syndrome. However, this is related to the significantly elevated cortisol levels seen in Cushing's syndrome which overwhelm the 11BHS2 enzyme leading to hypokalemia via its action at the mineralocorticoid receptor. Even if a compensatory rise in ACTH and cortisol level is seen with the low dose of mifepristone being used in this study, we do not anticipate the cortisol levels to go high enough to cause hypokalemia. However, a comprehensive metabolic panel that will include a potassium level will be checked both at the post-treatment visit (after 7 days of treatment with drug) as well as at the time of the safety visit (1 week after discontinuation of the study drug).
10. *MRI* uses a strong magnetic field and radiowaves instead of x-rays and therefore does not have any associated risk of radiation exposure. Patients are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves and cochlear implants), implanted delivery pump or shrapnel fragments. All subjects will be screened for these conditions prior to the study. Women of child bearing potential will have a pregnancy test performed before the MRI. Gadolinium-based contrast agents may be used with the MRI study. Subjects will be screened for any history of allergic reactions to these agents prior to administration. MRI evaluations may cause claustrophobia. Should this occur the study will be stopped and re-scheduled with administration of a sedative if needed.

### **Subject Monitoring**

Detailed history will be obtained from the subjects both before and at the end of each treatment with regards to medication compliance and any side effects associated with the medication. The NICHD Data Safety and Monitoring Board (DSMB) will review lab results after the first 10 patients have completed the study, and any unanticipated adverse events. Safety labs including comprehensive metabolic panel and complete blood count will be checked before and one week after each treatment.

All serious adverse events occurring during the study, both patient-reported and those observed by the physician or nursing staffs will be recorded and reported to the regulatory agencies.

### **Withdrawal Criteria**



1. Pregnancy
2. Inability to comply with study visits or requirements
3. Serious adverse reaction to study medication
4. Abnormal Vaginal bleeding
5. Thromboembolic event or new onset condition requiring anticoagulation
6. Subject withdraws informed consent

Patients who develop a serious adverse reaction to the study medication will be evaluated and treated at the Clinical Center.

### **Adverse Events and Protocol Violations and Deviations**

An adverse event is any unfavorable or unintended symptom, sign, or disease temporally associated with the use of the medication, whether or not it is considered to be related to the medication. The occurrence of adverse events will be sought by non-directed questioning of the subject at each study visit and by review of the menstrual and adverse event calendar. Adverse events can also be detected on physical examination and laboratory tests. Each adverse event will be evaluated with regards to its severity, relationship to study medication, duration, action taken, and clinical outcome in the subject.

Serious adverse event or reaction will be defined as any untoward medical occurrence that at any medication dose or formulation causes death, is life-threatening, requires hospitalization, prolongs existing hospitalization, or results in significant disability. Medical judgment will be exercised in deciding whether expedited reporting is appropriate in case of medical events that may not be immediately life-threatening or require hospitalization but may require intervention to prevent such an outcome. All serious adverse events occurring during the study, both patient-reported and those observed by the physician and nursing staff will be recorded and reported to the FDA within 10 days of our own notification. Non-serious adverse events will be reported annually to the FDA.

An unanticipated problem will be defined as any incident, experience, or outcome that is unexpected, related/possibly related to participation in the study and suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized. These events will be reported to the IRB and Clinical Director within 7 (serious) or 14 (non-serious) days.

Protocol violations and deviations will be reported to the IRB, except for the following: The protocol involves several serial tests, some with blood draws over 24 hours. Every effort is made to ensure that the subject has a reliable IV access prior to initiation of study procedures and the IV line is flushed intermittently to maintain access. However, we anticipate that blood draws may still be missed or delayed due to IV access issues ~10% of the time. We will monitor and keep a record of these but will report it as a protocol deviation to the IRB only if this happens > 10% of the time.

### **HUMAN SUBJECTS PROTECTION**

*Gender, ethnicity and age considerations for subject selection:*

This study will focus on men and women of all ethnicities in the age group of 35 – 70 years. We have no reason to believe that the effect of treatment with mifepristone will be different based on gender or ethnicity.

*Recruitment strategies:* Subjects will be obtained through referrals from individuals studied under the NIDDK phenotyping protocol 07-DK-0077, and may be employees. Subjects may also be recruited through a variety of other mechanisms, including but not limited to bulletin board postings, the Human subjects volunteer program, listing in the NIH record, mailings to physicians, advertisements in local newspapers, posting messages on listserves, social networking sites (like facebook and twitter), local online classified pages (like Craigslist) and via the clinical center web page. We will submit these postings and proposed language to the IRB for approval. If employees are recruited, they will receive the NIH Information Sheet on Staff Research Participation per NIH HRPP SOP 14E, Research Involving NIH Staff as Subjects. Every effort will be made to ensure confidentiality of collected information; we do not anticipate a need to collect sensitive information such as sexual behavior, or HIV status. Consent will be obtained as outlined in SOP 14E.

*Consent Procedures:* The Patient Recruitment and Patient Liaison group will initially screen potential subjects. The research team will call subjects for further screening, provide the consent form, and offer participation. The study will be explained verbally and all questions and concerns addressed by phone conversation and at the first visit. This telephone call will be used to explore eligibility for participation, as well as feasibility, given the time commitment, and whether the subject is able to travel to NIH.

Written informed consent will be obtained from the participant prior to any study procedures or treatments. The Principal Investigator and other designated qualified protocol investigators (listed on the protocol's face page) are clinically trained and have experience in obtaining informed consent, and know the protocol very well. The informed consent process and protections (e.g. lack of coercion) will be carried out as per NIH HRPP SOP 12, Requirements for Informed Consent.

We do not plan or anticipate the enrollment of non-English speaking subjects; however they are not excluded from participation either. Should a non-English speaking subject be eligible for enrollment, IRB approval will be obtained for use of the short form consent process in the absence of a fully translated consent document as outlined in SOP 12.9.1, under the provisions of 45 CFR 46.117(b)(2). IRB approval will be obtained according to IRB guidance prior to obtaining informed consent from the potential study participant/s.

#### **CONFIDENTIALITY AND USE OF RETAINED SPECIMENS**

Study subjects will be informed at the time of consent that the investigators will not provide direct care or counseling for conditions revealed by physical exam or protocol test results, but that the subject will be referred appropriately for further medical evaluation and treatment of any abnormal findings.

Samples will be stored according to NICHD and NIH policy in the NICHD biorepository (serum/plasma/urine). Subjects will be asked if samples obtained during the study can be used in

the future, for studies not described here without obtaining new informed consent. Only studies related to the physiology of the HPA axis will be undertaken. Blood samples may be shared with collaborators, without personal identifiers.

### REMUNERATION OF SUBJECTS

Subjects will be taking an investigational drug and will have the inconvenience of travel to the NIH for study visits. Thus we propose to remunerate subjects for participation according to NIH guidelines. As recommended, inconvenience units will be used for invasive or non-standard procedures that would not be included in routine physical examination. The reimbursement amount based on mandatory NIH compensation for inpatient and outpatient visits will be as follows: 12 inpatient visit days (12 x \$40 = \$480), 2 outpatient visits lasting > 1 hour – screening visit and transvaginal ultrasound (\$30 x 2 = \$60) and 4 outpatient safety visits lasting < 1 hour duration (\$20 x 4 = \$80). Thus according to the mandated NIH compensation, they would receive \$620. Additional compensation of \$60 (\$40 + \$20) will be given to subjects who undergo an MRI Pelvis when the endometrial thickness cannot be assessed precisely on the transvaginal ultrasound (as in the presence of multiple fibroids). Inconvenience units will be assigned as follows: Investigational drug (4 units = \$40), 12 days of multiple blood sampling (1 unit x 12 = \$120), and 4 days of outpatient blood draws (2 units = \$20) and a completion bonus of \$100. Thus, total compensation would be up to \$960 for those who complete the study.

We also propose to provide travel compensation for all subjects, either metro fare or mileage.

### OTHER INTERESTED PARTIES AND COLLABORATORS

The NIH has a Cooperative Research and Development Agreement (CRADA) with Laboratoire HRA-Pharma, in Paris, France. The intention of the CRADA is to develop mifepristone for the treatment of Cushing's syndrome, for use as an adjunctive agent for Octreoscan, and for use as a diagnostic agent.

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