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Effect of Androgel on Atherogenesis, Inflammation, Cardiovascular Risk Factors And Adiposity in Type 2 Diabetic Males with Hypogonadotrophic Hypogonadism.: a Prospective, Randomized and Controlled-Study

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Complete Research Protocol (HRP-503)

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PROTOCOL TITLE:

Include the full protocol title.

Response:

PRINCIPAL INVESTIGATOR:

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Department
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VERSION:

Include the version date or number.

Response: 11/16/2017

GRANT APPLICABILITY:

Indicate whether this protocol is funded by a grant (e.g. NIH, foundation grant). For a grant with multiple aims, indicate which aims are covered by this research proposal.

NOTE: This question does not apply to studies funded by a sponsor contract.

Include a copy of the grant proposal with your submission.

Response:

Fully funded by Abbvie Incorporated

RESEARCH REPOSITORY:

Indicate where the research files will be kept, including when the study has been closed. The repository should include, at minimum, copies of IRB correspondence (approval, determination letters) as well as signed consent documents. This documentation should be maintained for 3 years after the study has been closed.

Response: Diabetes Endocrinology Center of WNY

Location: 1000 Youngs Road, Suite 105

Williamsville NY 14221

Department: Diabetes Endocrinology & Metabolism

1.0 Objectives

1.1 Describe the purpose, specific aims, or objectives of this research.

Response: We have shown that the prevalence of hypogonadotrophic hypogonadism (HH) in type 2 diabetic (DM) patient population may be around 35%. We have also shown that in an age and glycemia matched group of type 1 diabetic patients, there is no significant hypogonadotrophic hypogonadism. HH in type 2 diabetics is associated with a normal release of LH and FSH following GnRH administration. Thus, the defect in testosterone secretion is specific to type 2 diabetes and is likely to be at the hypothalamic level.

Since insulin may facilitate GnRH secretion from hypothalamic neurons and interference with insulin signaling may result in a reduction in GnRH secretion, it is possible that insulin resistance in type 2 diabetes may play a key role in the pathogenesis of HH. Furthermore, as insulin resistance may be mediated by pro-inflammatory factors which are also known to reduce GnRH and gonadotrophin secretion, it is possible that there may be an increase in pro-inflammatory mediators in association with low testosterone concentrations in type 2 DM. We have shown that there is an inverse relationship between CRP and testosterone concentrations and that patients with HH have CRP concentrations which are twice as high (6.5mg/L) as those in patients with normal cFT and TT concentrations (3.2 mg/L). Since CRP concentrations of 3mg/L or greater are predictive of cardiovascular disease, patients with HH and type 2 DM would be in the highest risk category. Low testosterone concentrations constitute a significant atherogenic risk as shown in an epidemiological study from Britain. However, the combination of a low T and type 2 diabetes mellitus was not examined in this study.

Our work has recently also demonstrated that in patients with type 2 D.M. and HH, hematocrit and bone mineral density are significantly lower while upper abdominal adiposity is significantly greater than normal subjects and those with type 2 DM without HH.

In view of these observations, it is necessary to investigate the cardiovascular risk in patients with HH and type 2 DM prospectively and to examine the effect of Testosterone replacement on atherogenesis, endothelial function, inflammatory and oxidative stress mediators, hematocrit and body composition in this cohort of patients

1.2 Describe the purpose, specific aims, or objectives.

Response: To compare the carotid IMT of type 2 diabetic males with and without HH at baseline.

Aim 1.2: To compare the carotid IMT of type 2 diabetic males with HH before and after treatment with Androgel 1% or placebo daily for 24 months. Measurements will be done at baseline and at 6, 12, 18 and 24 months.

Aim 2.1: To compare the brachial FMD% of type 2 diabetic males with and without HH at baseline.

Aim 3.1: To compare the serum concentrations and expression in adipose tissue of Tumor

Necrosis Factor alpha (TNF-□), Interleukin-6 (IL-6), Intracellular adhesion molecule1(ICAM-1), plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinase-

9 (MMP9), C-reactive protein (CRP), serum Amyloid A (SAA) and Monocyte Chemoattractant protein-1 (MCP-1) in type 2 diabetic males with and without HH at baseline.

Aim 3.2: To compare the levels of inflammatory mediators in aim 3.1 before and after therapy with Androgel 1% or placebo daily for 24 months in type 2 diabetic males with HH. Serum will be obtained at baseline , 6, 12, 18 and 24 months. Adipose tissue and 24 hour urine will be obtained at baseline, 12 and 24 months.

Aim 4.1: To compare the abdominal adipose tissue mass of type 2 diabetic males with and without HH as measured by MRI. Abdominal MRI will be done to measure hepatic, visceral and subcutaneous fat at baseline.

Aim 4.2: To compare the hepatic, visceral and subcutaneous abdominal fat mass of type 2 diabetic males with HH as measured by MRI, before and after treatment with Androgel 1% or placebo daily for 24 months. Measurements will be done at baseline, 12 and 24 months.

Aim 5.1: to compare the hematocrit of type 2 diabetic males with and without HH at baseline.

Aim 5.2: To compare hematocrit levels before and after therapy with Androgel 1% or placebo daily for 24 months in type 2 diabetic males with HH. Measurements will be done at baseline and at 6, 12, 18 and 24 months.

Aim 6.1: to compare the aromatase enzyme expression in adipose tissue of type 2 diabetic males with and without HH at baseline.

Aim 6.2: To compare aromatase enzyme expression in adipose tissue before and after therapy with Androgel 1% or placebo daily for 24 months in type 2 diabetic males with HH. Measurements will be done at baseline, 12 and 24 months.

Aim 7.1: to compare the APP expression in adipose tissue of type 2 diabetic males with and without HH at baseline.

Aim 7.2: To compare APP expression in adipose tissue before and after therapy with Androgel 1% or placebo daily for 24 months in type 2 diabetic males with HH. Measurements will be done at baseline, 12 and 24 months

1.3 State the hypotheses to be tested, if applicable.

NOTE: A hypothesis is a specific, testable prediction about what you expect to happen in your study that corresponds with your above listed objectives.

Response: **Hypothesis 1:** HH in type 2 diabetic males is associated with increased atherogenesis as assessed by carotid intima media thickness (IMT) compared to age and BMI matched type 2 diabetic males without hypogonadism. Testosterone replacement in type 2 diabetic males with HH reduces atherogenesis.

Hypothesis 2: HH in type 2 diabetic males is associated with impaired endothelial function as assessed by brachial artery Flow mediated dilatation (FMD%) compared to age and BMI matched type 2 diabetic males without hypogonadism. Testosterone replacement in type 2 diabetic males with HH improves endothelial function.

Hypothesis 3: HH in type 2 diabetic males is associated with increased inflammatory mediators as compared to age and BMI matched type 2 diabetic males without hypogonadism. Testosterone replacement in type 2 diabetic males with HH decreases inflammatory mediators in serum and adipose tissue.

Hypothesis 4: HH in the diabetic patients is associated with increased abdominal adipose tissue mass as compared to age and BMI matched type 2 diabetic males. Testosterone replacement in type 2 diabetic males with HH decreases abdominal adipose tissue mass as compared to age matched type 2 diabetic patients with untreated hypogonadism.

Hypothesis 5: HH in type 2 diabetic males is associated with lower hematocrit compared to age matched type 2 diabetic males without hypogonadism.

Hypothesis 6: HH in type 2 diabetic men is associated with an increase in aromatase enzyme expression in adipose tissue. Testosterone therapy for 24 months will decrease the expression of aromatase enzyme.

Hypothesis 7: HH is type 2 diabetic men is associated with an increase in amyloid precursor protein (APP) expression in adipose tissue. Testosterone therapy for 24 months will decrease the expression of APP.

Aim 7.1: to compare the APP expression in adipose tissue of type 2 diabetic males with and without HH at baseline.

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2.0 Scientific Endpoints

2.1 Describe the scientific endpoint(s), the main result or occurrence under study.

NOTE: Scientific endpoints are outcomes defined before the study begins to determine whether the objectives of the study have been met and to draw conclusions from the data. Include primary and secondary endpoints. Some example endpoints are: reduction of symptoms, improvement in quality of life, or survival. Your response should **not** be a date.

Response: The focus of the proposed research is to evaluate the effect of hypogonadotrophic hypogonadism on atherogenesis, endothelial function, inflammatory mediators and oxidative stress in type 2 diabetic patients and the effect of testosterone replacement on these parameters. The similarities between the study groups, baseline values for subject's demographics, carotid IMT, FMD%, body composition, hematocrit and inflammatory markers will be compared using appropriate parametric tests. Transformations of the data in order to meet statistical assumptions may be considered. The PRIMARY ENDPOINT of the study is to detect a difference in atherogenesis as measured by carotid IMT between type 2 diabetic subjects with and without HH after 24 months of testosterone replacement with Androgel. There are no prior studies looking at these two populations in humans in vivo, however studies assessing the effect of thiazolidinediones (insulin sensitizers with anti-inflammatory properties) on carotid IMT in type 2 diabetics have shown a reduction in IMT by 0.033mm at 12 weeks and 0.054mm at 24 weeks (178). In a study of 154 diabetic patients, serum free testosterone (F-tes) concentrations were found to be inversely correlated with mean IMT. Patients with low concentrations of FT (<10 pg/ml) had greater mean IMT (1.01 ± 0.29 mm) than those with high concentrations of FT(0.91 ± 0.26 mm) (179). On the basis of these previous observations, we have conservatively estimated a difference in carotid IMT of 0.020mm at 24 months between the type 2 DM with HH and the type 2 DM with HH who is on androgel replacement. A sample size of 30 patients per group (assuming a drop-out rate of 5%) will provide adequate power ($\beta = 0.8$) to detect a significant difference of 0.020mm in carotid IMT ($\alpha = 0.05$), provided the standard deviation of the residuals is not equal to or greater than the mean difference. The results will be computed as mean ± SD. Comparisons for endpoints will be made using repeated measures ANOVA, with Tukey's test used for pair wise comparisons. Thus there will be 30 HH type 2 diabetic subjects each in placebo and treatment group of diabetic subjects with HH (total 60 subjects with HH). 20 eugonadal type 2 diabetic subjects will be recruited to serve as controls. SECONDARY END POINTS: The secondary endpoints for the study will be comparison of the relative change from baseline in FMD%, inflammatory mediators, abdominal fat, waist and hip ratio, after either testosterone or placebo. Comparison of these parameters at baseline will also

be done. Comparison for each endpoint will be made using ANOVA, with Tukey's test used for pairwise comparisons.

3.0 Background

3.1 Provide the scientific or scholarly background, rationale, and significance of the research based on the existing literature and how it will contribute to existing knowledge. Describe any gaps in current knowledge. Include relevant preliminary findings or prior research by the investigator.

Response: Hypogonadism is associated with increased adipose tissue mass, decreased muscle and bone mass, decreased insulin sensitivity and increased inflammatory stress. Testosterone replacement in nondiabetic males is associated with an improvement in some of these parameters and also sexual function and mood (1-4). It has recently been recognized that hypogonadotrophic hypogonadism occurs frequently in type 2 diabetes mellitus(5). We have shown that HH in type 2 DM is associated with increased inflammation compared to eugonadal type 2 DM. It is therefore important to study the effect of HH on cardiovascular risk prospectively and the effect of Testosterone (T) replacement on these parameters in this cohort of patients. If HH is found to be associated with increased cardiovascular risk and T replacement is shown to reduce this risk, this will provide the rationale for treatment of this common defect in type 2 DM.

Hypogonadism and Type 2 Diabetes

Total testosterone concentrations are determined to a large extent by circulating sex hormone binding globulin (SHBG) concentrations. In the blood of normal men, around 44% of total testosterone (T) is bound to SHBG, 2% is unbound (free T) and 54% circulates bound to albumin and other proteins.(6; 7) Circulating SHBG concentrations are decreased in obesity and increase with aging. A complete assessment of hypogonadism should therefore include measurement of free and/or bioavailable testosterone. Equilibrium Dialysis (ED) is considered to be the gold standard for measuring free testosterone. Free testosterone can also be calculated from SHBG and T using the method of Vermeulen *et al*.(8) This calculated free testosterone (cFT) is reliable (9) and has been shown to correlate very well (r=0.92) with free testosterone measured by ED.(5; 10; 11) Bioavailable testosterone can also be calculated from the equation of Vermeulen *et al*(8). For proposed research project, **hypogonadism will be defined as calculated free testosterone below 6.5ng/dL.**

We have recently observed that a significant proportion of type 2 diabetic patients have low free testosterone (measured by ED or calculated using T and SHBG) and the nature of hypogonadism in type 2 diabetics is hypogonadotrophic(5; 12) *(see preliminary data*). This condition is a potential candidate for being the commonest complication of type 2 diabetes (through association rather than causality at this time) and requires further assessment in terms of the etiology of the defect and the possible consequences, complications and treatment. Another recent study (ADA, 2005) has confirmed the high prevalence of hypogonadotrophic hypogonadism in an older group of type 2 diabetic patients, aged over 45 years. The patients with HH had greater prevalence of hypertension, hyperlipidemia, obesity and inadequate sexual function and libido than patients with normal gonadal function. These findings have also been confirmed in type 2 diabetic subjects attending a tertiary clinic for the assessment of erectile dysfunction(13). Low Testosterone in type 2 diabetics in this study was also associated with a reduction in blood flow in the cavernous arteries when assessed by Doppler ultrasound.

Hypogonadism and insulin sensitivity

Insulin sensitivity is closely correlated with intramuscular, intramyocellular and intraabdominal adipose tissue. Hypogonadism is associated with increased subcutaneous

(both trunk and appendicular), intraabdominal and intermuscular adipose tissue(14; 15). Epidemiological studies have suggested an inverse relationship between testosterone concentrations and insulin sensitivity, probably mediated by total or abdominal adiposity (16). Metabolic syndrome, insulin resistance and visceral obesity have all been associated with low SHBG and low total testosterone levels in men.(17; 18) It is believed that the low total testosterone in obesity is due to low SHBG concentrations. However, free testosterone levels have also been found to correlate inversely with body mass index (BMI)(19). Vermeulen *et al*(20) compared LH pulsatility over 12 hours in obese and lean men and found that the mean integrated LH levels over 12 hours were significantly lower in obese men. Free testosterone (FT) levels correlated positively with the sum of LH pulse amplitudes in each individual.(20) Other studies showed a rise in LH or FSH levels after weight loss(21; 22). It appears that in obesity induced hypogonadism, FT levels fall into hypogonadal range only when the obesity is severe (BMI>40kg/m^2)(23). Elevated estradiol and leptin levels observed in obesity probably mediate part of the hypogonadotrophic effect of obesity , but they do not account for the full effect of obesity on hypothalamus/pituitary (22; 24; 25).

As mentioned above, type 2 diabetes is associated with hypogonadotrophic hypogonadism (HH). FT correlates inversely with BMI in type 2 diabetic patients (5). We have recently also shown that BMI is inversely related to FT in Type 1 DM patients although these patients do not have HH. The existence of a hypothalamic defect resulting in HH in type 2 diabetes is of interest in view of its association with insulin resistance. It is known that neuron specific insulin receptor knock out (NIRKO) mice exhibit HH (26). Plasma LH levels are decreased by 60-90% in NIRKO mice as compared to controls. When these mice were injected with lupron, a GnRH receptor agonist, they displayed a normal to twofold increase in LH levels as compared to control mice. Thus, the pituitary reserve in these animals was normal. These mice also had increased adipose tissue and insulin resistance. A recent study demonstrated an association between insulin sensitivity (as measured by hyperinsulinemic euglycemic clamp) and hCG stimulated testicular response among humans with varying degrees of glucose tolerance(27). Thus data from literature suggests that obesity/insulin resistance and type 2 diabetes are associated with hypogonadism, this relationship may be bidirectional, and may occur at multiple levels of hypothalamicpituitary-gonadal axis (28-30). It is interesting that type 1 diabetes mellitus is not associated with hypogonadism (31; 32) *(preliminary data)*.

Testosterone replacement leads to a dose-dependent decrease in adipose tissue and increase in muscle mass and strength(14; 33; 34). This effect appears to be more pronounced if the population studied has higher amounts of adipose tissue. The mechanism by which testosterone replacement produces these effects is not well understood. Inhibition of lipoprotein lipase activity in intraabdominal adipose tissue and the differentiation of pluripotent mesenchymal precursor cells preferentially into myogenic lineage instead of adipocytic lineage may play a role(35; 36). Marin *et al* demonstrated a decrease in visceral adipose tissue and improvement in insulin sensitivity with oral and transdermal testosterone treatment in middle aged obese non-diabetic men (37; 38). The subjects were not hypogonadal but insulin sensitivity improved more in subjects whose testosterone concentrations were in the low normal range at the start of the study. The improvement in insulin sensitivity and plasma testosterone concentrations were inversely related. In one of these studies, oral testosterone undecanoate 80mg twice a day (which increased serum testosterone concentrations to 500-600 ng/dL for a few hours after the dosing) or placebo given for 8 months reduced visceral body fat (measured by CT scan) and total body fat by ~6%. Subcutaneous adipose tissue did not change. Glucose disposal rate (HE clamp) increased by 20%. Change in glucose disposal rate was related to baseline testosterone levels (r= -0.65, P<0.05). In another publication using a similar population, transdermal

testosterone for 3 months (which doubled T concentrations from 418 ng/dL at baseline to 820 ng/dL) increased glucose uptake in HE clamp by 17% and decreased waist/hip circumference. Relatively hypogonadal men had more demonstrable effects. However, other studies have failed to show an improvement in insulin sensitivity(39-41). It is possible that the lack of a low testosterone prior to treatment was responsible for the lack of change in insulin sensitivity in these studies. The effect of testosterone on adipose tissue, muscle mass and insulin sensitivity seems to depend on the population studied, dose of testosterone replacement and duration of therapy. It is possible that the improvement in insulin sensitivity after testosterone replacement is due to an increase in lean body mass and a decrease in adipose tissue mass, or more specifically by a decrease in visceral or intramyocellular adipose tissue, and that this effect is more pronounced in the hypogonadal patients. No studies have been done to evaluate the effect of testosterone on insulin sensitivity in type 2 diabetic patients. We intend to study the effects of testosterone replacement in type 2 diabetic subjects, a population that is likely to benefit from a reduction in adipose tissue and an increase in muscle mass. We have recently demonstrated that HH in type 2 DM is associated with an increase in total, appendicular and truncal fat (preliminary data).

Obesity, diabetes and inflammation

Both obesity and diabetes have been shown to be associated with oxidative stress (4245) and inflammation (46-50). The understanding of mechanisms leading to oxidative stress and inflammation is important. One possible reason why obesity and type 2 diabetes are associated with oxidative stress and inflammation is the state of insulin resistance. This is due to the fact that 1) resistance is associated with the presence of pro-inflammatory factors including cytokines (51); 2) insulin has been shown to exert an anti-inflammatory and anti-oxidant effects at the cellular and molecular level both, *in vitro*, and *in vivo* (43; 52-54). A low dose infusion of insulin (2.5 IU/h) has been shown to reduce ROS generation by mononuclear cells (MNC), suppress NADPH oxidase expression and intranuclear NFκB binding, induce IκB expression and suppress plasma ICAM-1 and MCP-1 concentrations (43). It also suppresses intranuclear Egr-1, plasma tissue factor (TF), PAI1 and MCP-1 concentrations (52). An interruption of insulin signal transduction would prevent the anti-inflammatory effect of insulin from being exerted. The anti-inflammatory and pro-fibrinolytic effects of insulin have also been confirmed in the setting of acute ST segment elevation myocardial infarction(55).

We have previously demonstrated that macronutrient intake (glucose, protein and lipids) induce ROS generation and cause oxidative stress in humans. Glucose or a mixed meal intake cause an increase in NF□B and a fall in total cellular I□B□, the two cardinal indices of inflammation at the cellular level (56-58). Mixed meal also induces an increase in C-reactive protein (CRP) (56). Glucose intake has further been shown to increase the ratio of phosphorylated I□B□ to native non-phosphorylated I□B□ and to induce an increase in I□B□ kinase (IKK□) and IKK□. At a cellular level, the changes that occur in the MNC following a glucose challenge are very similar to those which occur after endotoxin challenge.

It is, thus, possible that obesity is a pro-oxidative and pro-inflammatory state resulting from chronically increased macronutrient intake. An increasing amount of epidemiologic data show that persistent, low-grade inflammation is an independent predictor of ischemic heart disease (59; 60), stroke (61; 62), diabetes (63; 64), and all-cause mortality (65; 66). In addition to these epidemiologic findings, experimental evidence shows that markers of chronic inflammation, such as pro-inflammatory cytokines IL-6 and TNFα and the acute-phase reactant CRP, play a direct role in the etiology of atherosclerosis and

insulin resistance (67; 68). Studies are beginning to show that diet-induced weight loss decreases concentrations of CRP, IL-6, and TNFα (46; 69-74). It is also relevant that caloric restriction in the obese and a fast in normal subjects result in a reduction in oxidative stress and inflammatory mediators (43; 46; 75) (**Tables 1 & 2**). In this regard, it is interesting to note that TNF-α and IL-1β have been shown to reduce hypothalamic GnRH and LH secretion in animals and *in vitro*.(76; 77) It is therefore possible that inflammatory cytokines may partly mediate HH of obesity and type 2 diabetes.

	Baseline	24 hr	48 hr
ROS by PMN (% change from the baseline)	100	66 ± 20	46 ± 23§
ROS by MNC (% change from the baseline)	100	62 ± 17	48 ± 17§
$p47^{phox}$ Subunit (% change from the baseline)	100	41 ± 13	32 ± 16§
o-tyrosine	3.26± 0.05	3.23 ± 0.06	3.03 ± 0.05*
m-tyrosine	3.79 ± 0.62	3.80 ± 0.62	3.59 ± 0.66*

Table 1: ROS generation, $p47^{phox}$ Subunit protein expression, *o*- and *m*-tyrosine following 2 day fast; §: $P<0.001$; *: $P<0.05$(75).

	Lean	Obese (0 Week)	4 Weeks
ROS by PMN (mV)	220 ± 60	236 ± 96	103± 36 §
ROS by MNC (mV)	165 ± 80	188 ± 75	75 ± 32 §
TBARS (□M; lipid peroxide)	1.29 ± 0.12 *	1.68 ± 0.17	1.47 ± 0.13 *
13-HODE(□mol/L)	2.04 ± 0.72 §	6.67 ± 3.85	4.15 ± 5.62 *
9-HODE(□mol/L)	1.97 ± 0.84 §	7.10 ± 3.88	4.50 ± 5.70 *
Carbonylated proteins (□mol/mg protein)	0.60 ± 0.10 §	1.39 ± 0.27	1.17 ± 0.12 *
o-tyrosine/phenyalanine ratio (mmol/mol)	0.28 ± 0.02 §	0.42 ± 0.03	0.36 ± 0.02 *
m-tyrosine/phenyalanine ratio (mmol/mol)	0.27 ± 0.03 §	0.45 ± 0.04	0.40 ± 0.03 *

Table 2: Oxidative stress markers in lean vs. obese subjects and the effect of diet restriction for 4 weeks (1000 kcal/day);§:$P<0.001$; *: $P<0.05$ when compared with obese at baseline (43):

Measuring Inflammation and Oxidative Stress

Inflammation at the cellular level can be described as an increase in the pro-inflammatory transcription factor, NFκB, in the nucleus and with a concomitant decrease in its inhibitors IκB□ and/or IκB□. NF□B is a heterodimer, and usually consists of two proteins, a p65 (RelA) subunit and a p50 subunit. In the basal state NF□B is bound to its inhibitor protein I□B, which restricts NF□B to the cytoplasm. Stimulation of cells by cytokines like TNF□ or IL-1 or endotoxin results in phosphorylation of I□B, unbinding of NF□B from I□B and activation of NF□B with its subsequent translocation into the nucleus (78; 79). This in turn induces the transcription of proinflammatory cytokines (such as TNFα, IL-1 and IL-6), adhesion molecules (ICAM, VCAM), chemokines (such as MCP-1 and MIF), metalloproteinases (MMP-1, and MMP-9) and many other genes that regulate transcription, apoptosis and cell proliferation.

Oxidative stress can be defined as an increase in ROS generation. The term ROS is a collective term that includes not only oxygen-centered radicals such as superoxide ($O^{\cdot}_2$□) and hydroxyl (OH), but also some non-radical derivative of oxygen such as hydrogen peroxide (H_2O_2), singlet oxygen and hypochlorous acid (HOCL). A critical balance exists between generation and detoxification of ROS in respiring cells of normal individuals. The various risk factors for atherosclerosis, including hyperlipidemia, hypertension, and diabetes, have in common the generation of oxidative stress. ROS are able to break off cell membrane proteins, fuse membrane lipid and proteins, hardening the

cell membrane and exposing genetic material in the nucleus, leaving the DNA open for mutation or destruction. They may damage the endothelium of blood vessels, thereby leading to atherosclerosis. ROS-mediated lipid peroxidation is a cardinal step in the initiation of atherosclerosis (80; 81). NF□B is one of the transcription factors that may be controlled by the redox status of the cell (82). NADPH oxidase complex, composed of cytoplasmic ($p47^{phox}$ $p67^{phox}$, small GTP-binding p21rac) and plasma membrane componenets (heterodimer of gp91 & $p22^{phox}$) is a key enzyme involved in the production of ROS.(83) Oxidative stress can also be measured by the presence of isoprostanes in urine.

Lipid Peroxidation and Protein Oxidation: Lipid peroxidation is an autocatalytic free radicalmediated chemical mechanism in which polyunsaturated fatty acids (PUFA) undergo oxidation to form lipid hydroperoxide (LHP)(84; 85). Levels of 15-isoprostane F2 in urine are useful for the non-invasive assessment of oxidant stress *in vivo* (86). There is evidence that oxidative modification of proteins may be physiologically important, serving as a "marking" step for initiating protein degradation. Oxidation of proteins by microsomal oxidases and metal-catalyzed oxidation treatment is known to produce carbonyl modifications of amino acids (87). Phe reacts with the hydroxyl radical (OH) to form the hydroxylated products ortho-, meta-, and para-tyrosine (*o*-, *m*-, and *p*-tyr). The aromatic hydroxylation of Phe can be used to detect OH formation and is considered as a marker of protein oxidation (88).

Hypogonadism and Inflammation

It has been suggested that hypogonadism is associated with an increase in inflammatory mediators and treatment of hypogonadism leads to a reduction in inflammation(89). *In vitro* studies have demonstrated that testosterone induces an inhibition of IL-6 production by human monocytes(90). Testosterone treatment of human aortic endothelial cells results in an inhibition of TNF-α induced VCAM-1 mRNA expression and NF-κB activation.(91) Intramuscular testosterone replacement in hypogonadal males results in a decrease in pro-inflammatory cytokines(IL-1 and TNF-α) and an increase in anti-inflammatory cytokine(IL-10) levels in serum(92). However, some studies were unable to demonstrate an anti-inflammatory effect of testosterone(39; 93). It is possible that the effects of testosterone replacement on inflammation depend on the population studied. A comprehensive study on inflammation and testosterone replacement needs to be done to clarify the issue.

We have demonstrated clearly for the first time that low plasma TT and cFT concentrations are associated with elevated CRP concentrations in patients with type 2 diabetes (Diabetes Care 2006 and Preliminary data). In addition, there is an inverse relationship between plasma TT and plasma CRP concentrations. This relationship is independent of the known relationship between BMI and CRP, which has previously been observed by others and by us during studies on obesity (47-50; 94-97). It is noteworthy that this relationship between CRP and testosterone concentrations was observed in spite of the intake of statins, ACE-inhibitors, ARBs and aspirin that have antiinflammatory effects.

While the establishment of this important relationship does not provide us with a causal link between HH and inflammation, it provides us with several potential novel hypotheses and avenues of investigation. Firstly, it is possible that a low T contributes to the overall inflammatory state of a patient with type 2 diabetes. This can be tested by administering T to hypogonadal patients to determine whether CRP concentrations and other indices of inflammation fall as proposed in this investigation. Secondly, it is possible that the pro-inflammatory state, of which elevated CRP concentration is an indicator, may lead to the suppression of GnRH secretion by the hypothalamic neurons and thus lead to HH. The pro-inflammatory cytokine, TNF□, known to be elevated in type 2 diabetes and obesity(47; 63; 94; 98; 99), suppresses the secretion of hypothalamic hormones including gonadotropins.(76; 100-102).

In a previous study on young, non-obese healthy men in whom endogenous testosterone production was suppressed by giving GnRH analogue and graded doses of testosterone were given (39), the circulating levels of CRP did not change with testosterone administration, nor were they related to T concentrations. This can be probably explained by the low level of inflammatory mediators including CRP in younger healthy subjects with lower fat mass. Furthermore, the study probably

did not allow a sufficient period of hypogonadism for a change in CRP concentration to emerge. It is relevant that specific insulin receptor deletion in neurons has recently been shown to result in hypogonadotrophic hypogonadism in mice (26). It would, therefore appear that insulin has a facilitatory role in gonadotrophin secretion, possibly through GnRH secretion in the hypothalamus. Recently, insulin has been shown to exert other specific effects on the hypothalamus. For example, it mediates satiety(103; 104) and also regulates gluconeogenesis and hepatic glucose production through the hypothalamus and its vagal outflow(105). It is possible that insulin also has a facilitatory, trophic effect on the release of GnRH from hypothalamic neurons and that inflammatory mediators like TNF and IL-6, known to interfere with insulin signaling(106-108), thus suppress GnRH secretion in conditions associated with insulin resistance like obesity and the metabolic syndrome. These conditions are characterized by insulin resistance at least in part due to pro-inflammatory mediators like TNF and IL-6 which interfere with insulin signal transduction(109; 110). Furthermore, glucose also induces oxidative stress (111; 112)and inflammation, especially if endogenous insulin secretion is impaired or inadequate and may thus contribute to insulin resistance.

In this context, it is also important that insulin has recently been shown to exert a powerful and rapid, anti-inflammatory effect with a suppression of pro-inflammatory transcription factors (NFkB, Egr-1), chemokines (MCP-1), adhesion molecules (ICAM-1), matrix metalloproteinases and CRP(52; 54; 108; 113-115). Resistance to the action of insulin would therefore, facilitate a proinflammatory state, which in turn would further interfere with insulin signal transduction. Since the patients with hypogonadism had a higher BMI, tended to have lower HDL apart from having diabetes and a higher CRP, they probably had the features of the metabolic syndrome. Hypertension cannot be commented on since blood pressure was controlled with anti-hypertensive drugs. This makes it likely that hypogonadism may be a feature of the metabolic syndrome. Indeed, nondiabetic patients with metabolic syndrome, have been shown to have low testosterone concentrations.(95; 116-118). Whether these low testosterone concentrations in patients with metabolic syndrome are associated with low LH and FSH concentrations as in type 2 diabetes has not yet been investigated. Since hypogonadism promotes obesity(19; 22; 24; 25), it is possible that the increase in obesity secondary to a low T, further promotes inflammation and an increase in CRP. It is possible that T administration may both reduce obesity and decrease inflammation and oxidative stress. Recent work has shown that a low testosterone/cortisol ratio may be a risk factor for an increase in atherosclerotic cardiovascular events(119). Our data provides a potential mechanism for this increased risk through inflammation since atherosclerosis is a chronic inflammation of the arterial wall(120-124). Clearly, we need to investigate whether testosterone administration will not only reduce inflammation (CRP) but also retard the rate of atherogenesis. Elevated CRP concentrations are known to be associated with an increased risk of cardiovascular events(125; 126). There is also the evidence that CRP may induce the release of pro-inflammatory factors like plasminogen Activator Inhibitor-1 and intracellular adhesion molecule-1(ICAM-1) from endothelial cells in vitro, through the activation of FcII receptor on the endothelium(127-130). Infusion of CRP has recently been shown to induce an increase in pro-inflammatory mediators in humans, in vivo(131). The markedly elevated plasma CRP concentrations (mean: 6.13 mg/l) in the hypogonadal type 2 DM thus would place them at a much higher risk for cardiovascular disease than the eugonadal group (mean CRP 3.2 mg/L). One previous study in non-diabetic subjects has shown that cardiovascular risk factors like higher BMI, systolic hypertension, high blood glucose and insulin concentrations occur more frequently with low testosterone concentrations(132).

It is of interest that testosterone has been shown to be protective of endothelial function in experimental animals(133). Thus, bilateral orchidectomy in rats is associated with a reduction in NO generation by the aorta while administration of testosterone to orchidectomized animals leads to partial prevention of the loss of NO generation(134). Since NO exerts vasodilatory, platelet antiaggregatory, anti-inflammatory and anti-atherogenic actions, it is possible that testosterone is also anti-atherogenic in the male. Orchidectomy in rats result in insulin resistance which can be reversed by testosterone administration at physiological doses(135; 136). The loss of testosterone in human male is associated with a loss in muscle mass and an increase in plasma free fatty acid

concentration(15; 137; 138) (16; 17; 117). Free fatty acids are known to interfere with insulin signaling and to induce inflammation and oxidative stress in association with insulin resistance(139-141). Oral and transdermal testosterone administration to obese, hypogonadal patients leads to a fall in blood pressure(38). Conversely, androgen deprivation in patients with prostatic cancer leads to increased arterial stiffness(142),(143). A Japanese study has shown that low testosterone concentration predicts abdominal adiposity(144).

Inflammation is known to be a causative factor in inducing atherosclerosis(145; 146). Adherence of circulating monocytes and lymphocytes to the arterial endothelial lining is one of the earliest detectable events in human and experimental atherosclerosis. The initial entrapment of the monocyte to the endothelium is dependent upon the increased expression of the adhesion molecules, P-selectin, VCAM-1 and ICAM-1, on the endothelium (147). This expression is mediated by the activation of NF□B which modulates the transcription of pro-inflammatory cytokines, TNF□, IL1□, IL-6; adhesion molecules ICAM-1, VCAM-1; chemokines like MCP-1; and enzymes generating ROS such as NADPH oxidase (78). NF□B is now considered cardinal in the pathogenesis of both acute inflammation and chronic inflammation like that seen in atherosclerosis (82).

Since hypogonadism may be a pro-inflammatory state, it is worth mentioning that hypogonadism is associated with increased atherosclerosis(148). In this regard, it is interesting that testosterone has been shown to have anti-anginal properties in humans(149-151) Animal studies have shown that high physiological doses of androgens (testosterone or DHEA) decrease aortic atherosclerosis in cholesterol-fed castrated rabbits.(152) It is possible that treatment of hypogonadism can lead to decreased atherosclerosis in humans, but long-term studies looking at this effect have not been done. This study therefore presents a unique opportunity to study the effect of testosterone replacement on inflammatory and oxidative stress in type 2 diabetes, a known pro-inflammatory, pro-oxidative and pro-atherosclerotic state.

Hypogonadism and Hematocrit

We have recently demonstrated a significantly lower hematocrit in hypogonadal males and a direct relationship between the hematocrit and testosterone in type 2 diabetics (JCEM 2006 and preliminary data). This suggests that a low testosterone may contribute to the pathogenesis of the mild anemia in these patients. Testosterone is known to stimulate erythropoiesis in the bone marrow and to increase the hematocrit.(153)

We also showed a significant inverse relationship between CRP concentrations and the hematocrit which suggests that inflammatory processes in type 2 diabetes probably regulate the hematocrit in a negative fashion. CRP has for long been considered a marker/indicator of systemic inflammation and therefore, it is also a prognosticator of cardiovascular events(125; 126; 154). However, there is now evidence that CRP may be a mediator of inflammation(127-129). It induces ICAM-1 and MCP-1 in endothelial cells, in vitro, and probably exerts this effect through the FcII receptor(130). More recently it has been shown to exert a pro-inflammatory effect following an injection into normal human subjects, in vivo(131). Thus the relationship of CRP with hematocrit may either be through the direct action of CRP or through other inflammatory mediators associated with CRP or both. Inflammatory mechanisms may affect the hematocrit in two ways. Firstly, they may suppress erythropoietin secretion(155) and secondly, there may be increased apoptotic death of red cell precursors resulting in no increase in erythropoiesis in spite of elevated erythropoietin levels(155158). These mechanisms are relevant to the pathogenesis of anemia of chronic inflammatory disease. Thus, the mild anemia of type 2 diabetes may in part be attributable to processes similar to those involved in chronic inflammatory disease in addition to the contribution by low testosterone concentrations. The overwhelming predominance of normocytic normochromic picture in the anemic patients is also consistent with marrow suppression secondary to inflammatory mechanisms. Elevated erythropoietin levels associated with anemia, suggest an inadequate response to erythropoietin, a feature of anemia of chronic disease.The relationship of inflammatory mechanisms with hematocrit becomes even more intricate since these mechanisms may be involved in the pathogenesis of hypogonadotrophic hypogonadism itself in patients with type 2 diabetes as

discussed above. Thus, both a low testosterone and inflammatory mechanisms play an important role in the pathogenesis of the low grade anemia observed in patients with type 2 diabetes. **Type 2 diabetes, HH and Estrogen**

The patients with HH tend to be obese, and, there is an inverse relationship between BMI and total and free testosterone concentrations(5). Since adipose tissue expresses the enzyme aromatase which converts testosterone to estradiol, it has been suggested that the state of HH in these patients may be due to an excessive aromatase dependent conversion of testosterone into estradiol(159162). Elevated concentrations of estradiol may in turn suppress hypothalamic GnRH and gonadotrophin secretion from the pituitary gland (163; 164). This would then explain the pathogenesis of HH. We, therefore, hypothesize that the plasma concentration of estradiol and aromatase expression in adipose tissue in patients with type 2 diabetes and HH is elevated when compared with those who have normal testosterone concentrations. We will also evaluate the aromatase expression after Androgel therapy.

Type 2 diabetes, Alzheimer's disease and Hypogonadism

With a progressively aging population, the total load of Alzheimer's disease (AD) and the characteristic dementia, the burden to society is likely to be phenomenally large. AD is a clinical diagnosis characterized by progressive dementia. A definitive diagnosis can only be made by histopathological examination of brain tissue. MRI findings suggestive of AD (amyloid plaques; reduced brain volumes in hippocampus, amygdala and entorhinal cortex) are detected when the disease is well established. There is a great need for biochemical tests that can detect AD early in its course. We have recently discovered that mononuclear cells (MNC) express amyloid precursor protein (APP). APP (a transmembrane protein) is sequentially cleaved by β- and γ-secretases in the neuronal cells to form amyloid-β (Aβ) peptide, the principal component of senile plaques.

Type 2 diabetes and male hypogonadism are both known to independently increase the risk of AD markedly (165; 166). Since the pathogenesis of AD is dependent upon oxidative and inflammatory stress, patients with HH and DM would be expected to have a markedly enhanced risk of AD. Consistent with this, our preliminary data show that type 2 diabetic men with low free T have increased expression of APP in their peripheral blood MNC. Treatment of these patients with testosterone for 8 weeks suppresses APP. We now want to study the expression of APP and correlate it with the expression of inflammatory mediators in the adipose tissue

3.2 Include complete citations or references.

Response:

1. Snyder PJ, Peachey H, Berlin JA, Hannoush P, Haddad G, Dlewati A, Santanna J, Loh L, Lenrow DA, Holmes JH, Kapoor SC, Atkinson LE, Strom BL: Effects of testosterone replacement in hypogonadal men. *J Clin Endocrinol Metab* 85:2670-2677, 2000
2. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Berman N, Hull L, Swerdloff RS: Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab* 89:2085-2098, 2004
3. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL: Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *J Clin Endocrinol Metab* 84:2647-2653, 1999
4. Marin P: Testosterone and regional fat distribution. *Obes Res* 3 Suppl 4:609S-612S, 1995
5. Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A, Dandona P: Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. *J Clin Endocrinol Metab* 89:5462-5468, 2004
6. Dunn JF, Nisula BC, Rodbard D: Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab* 53:58-68, 1981
7. Pardridge WM: Serum bioavailability of sex steroid hormones. *Clin Endocrinol Metab* 15:259278, 1986

8. Vermeulen A, Verdonck L, Kaufman JM: A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 84:3666-3672, 1999
9. Matsumoto AM, Bremner WJ: Serum testosterone assays-accuracy matters. *J Clin Endocrinol Metab* 89:520-524, 2004
10. Morley JE, Patrick P, Perry HM, 3rd: Evaluation of assays available to measure free testosterone. *Metabolism* 51:554-559, 2002
11. Vermeulen A, Kaufman JM: Diagnosis of hypogonadism in the aging male. *Aging Male* 5:170176, 2002
12. Tripathy D, Dhindsa S, Garg R, Khaishagi A, Syed T, Dandona P: Hypogondotrophic Hypogonadism in Erectile Dysfunction Associated with Type 2 Diabetes Mellitus: A Common Defect? *Metabolic Syndrome and Related Disorders* 1:75-81, 2003
13. Corona G, Mannucci E, Petrone L, Ricca V, Balercia G, Mansani R, Chiarini V, Giommi R, Forti G, Maggi M: Association of hypogonadism and type II diabetes in men attending an outpatient erectile dysfunction clinic. *Int J Impot Res* 18:190-197, 2006
14. Woodhouse LJ, Gupta N, Bhasin M, Singh AB, Ross R, Phillips J, Bhasin S: Dose-dependent effects of testosterone on regional adipose tissue distribution in healthy young men. *J Clin Endocrinol Metab* 89:718-726, 2004
15. Seidell JC, Bjorntorp P, Sjostrom L, Kvist H, Sannerstedt R: Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism* 39:897-901, 1990
16. Tsai EC, Matsumoto AM, Fujimoto WY, Boyko EJ: Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat. *Diabetes Care* 27:861-868, 2004
17. Haffner SM, Karhapaa P, Mykkanen L, Laakso M: Insulin resistance, body fat distribution, and sex hormones in men. *Diabetes* 43:212-219, 1994
18. Laaksonen DE, Niskanen L, Punnonen K, Nyyssonen K, Tuomainen TP, Salonen R, Rauramaa R, Salonen JT: Sex hormones, inflammation and the metabolic syndrome: a population-based study. *Eur J Endocrinol* 149:601-608, 2003
19. Zumoff B, Strain GW, Miller LK, Rosner W, Senie R, Seres DS, Rosenfeld RS: Plasma free and non-sex-hormone-binding-globulin-bound testosterone are decreased in obese men in proportion to their degree of obesity. *J Clin Endocrinol Metab* 71:929-931, 1990
20. Vermeulen A, Kaufman JM, Deslypere JP, Thomas G: Attenuated luteinizing hormone (LH) pulse amplitude but normal LH pulse frequency, and its relation to plasma androgens in hypogonadism of obese men. *J Clin Endocrinol Metab* 76:1140-1146, 1993
21. Lima N, Cavaliere H, Knobel M, Halpern A, Medeiros-Neto G: Decreased androgen levels in massively obese men may be associated with impaired function of the gonadostat. *Int J Obes Relat Metab Disord* 24:1433-1437, 2000
22. Strain GW, Zumoff B, Miller LK, Rosner W, Levit C, Kalin M, Hershcopf RJ, Rosenfeld RS: Effect of massive weight loss on hypothalamic-pituitary-gonadal function in obese men. *J Clin Endocrinol Metab* 66:1019-1023, 1988
23. Giagulli VA, Kaufman JM, Vermeulen A: Pathogenesis of the decreased androgen levels in obese men. *J Clin Endocrinol Metab* 79:997-1000, 1994
24. Stanik S, Dornfeld LP, Maxwell MH, Viosca SP, Korenman SG: The effect of weight loss on reproductive hormones in obese men. *J Clin Endocrinol Metab* 53:828-832, 1981
25. Isidori AM, Caprio M, Strollo F, Moretti C, Frajese G, Isidori A, Fabbri A: Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. *J Clin Endocrinol Metab* 84:3673-3680, 1999
26. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, MullerWieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 289:2122-2125, 2000
27. Pitteloud N, Hardin M, Dwyer AA, Valassi E, Yialamas M, Elahi D, Hayes FJ: Increasing insulin resistance is associated with a decrease in Leydig cell testosterone secretion in men. *J Clin Endocrinol Metab* 90:2636-2641, 2005

28. Burcelin R, Thorens B, Glauser M, Gaillard RC, Pralong FP: Gonadotropin-releasing hormone secretion from hypothalamic neurons: stimulation by insulin and potentiation by leptin. *Endocrinology* 144:4484-4491, 2003
29. Adashi EY, Hsueh AJ, Yen SS: Insulin enhancement of luteinizing hormone and folliclestimulating hormone release by cultured pituitary cells. *Endocrinology* 108:1441-1449, 1981
30. Lin T, Haskell J, Vinson N, Terracio L: Characterization of insulin and insulin-like growth factor I receptors of purified Leydig cells and their role in steroidogenesis in primary culture: a comparative study. *Endocrinology* 119:1641-1647, 1986
31. van Dam EW, Dekker JM, Lentjes EG, Romijn FP, Smulders YM, Post WJ, Romijn JA, Krans HM: Steroids in adult men with type 1 diabetes: a tendency to hypogonadism. *Diabetes Care* 26:1812-1818, 2003
32. Tomar R, Dhindsa S, Chaudhuri A, Mohanty P, Garg R, Dandona P: Contrasting testosterone concentrations in type 1 and type 2 diabetes. *Diabetes Care* 29:1120-1122, 2006
33. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R: The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N Engl J Med* 335:1-7, 1996
34. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-Hikim I, Shen R, Storer TW: Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 281:E1172-1181, 2001
35. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S: Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology* 144:5081-5088, 2003
36. Marin P, Oden B, Bjorntorp P: Assimilation and mobilization of triglycerides in subcutaneous abdominal and femoral adipose tissue in vivo in men: effects of androgens. *J Clin Endocrinol Metab* 80:239-243, 1995
37. Marin P, Krotkiewski M, Bjorntorp P: Androgen treatment of middle-aged, obese men: effects on metabolism, muscle and adipose tissues. *Eur J Med* 1:329-336, 1992
38. Marin P, Holmang S, Jonsson L, Sjostrom L, Kvist H, Holm G, Lindstedt G, Bjorntorp P: The effects of testosterone treatment on body composition and metabolism in middle-aged obese men. *Int J Obes Relat Metab Disord* 16:991-997, 1992
39. Singh AB, Hsia S, Alaupovic P, Sinha-Hikim I, Woodhouse L, Buchanan TA, Shen R, Bross R, Berman N, Bhasin S: The effects of varying doses of T on insulin sensitivity, plasma lipids, apolipoproteins, and C-reactive protein in healthy young men. *J Clin Endocrinol Metab* 87:136143, 2002
40. Liu PY, Wishart SM, Celermajer DS, Jimenez M, Pierro ID, Conway AJ, Handelsman DJ: Do reproductive hormones modify insulin sensitivity and metabolism in older men? A randomized, placebo-controlled clinical trial of recombinant human chorionic gonadotropin. *Eur J Endocrinol* 148:55-66, 2003
41. Tripathy D, Shah P, Lakshmy R, Reddy KS: Effect of testosterone replacement on whole body glucose utilisation and other cardiovascular risk factors in males with idiopathic hypogonadotrophic hypogonadism. *Horm Metab Res* 30:642-645, 1998
42. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T: Oxidative damage to DNA in diabetes mellitus. *Lancet* 347:444-445, 1996
43. Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, Prabhala A, Afzal A, Garg R: The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab* 86:355-362, 2001
44. Higdon JV, Frei B: Obesity and oxidative stress: a direct link to CVD? *Arterioscler Thromb Vasc Biol* 23:365-367, 2003
45. Vincent HK, Powers SK, Stewart DJ, Shanely RA, Demirel H, Naito H: Obesity is associated with increased myocardial oxidative stress. *Int J Obes Relat Metab Disord* 23:67-74, 1999

46. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T: Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 83:2907-2910, 1998
47. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factoralpha: direct role in obesity-linked insulin resistance. *Science* 259:87-91, 1993
48. Katsuki A, Sumida Y, Murashima S, Murata K, Takarada Y, Ito K, Fujii M, Tsuchihashi K, Goto H, Nakatani K, Yano Y: Serum levels of tumor necrosis factor-alpha are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 83:859-862, 1998
49. Madrid LV, Mayo MW, Reuther JY, Baldwin AS, Jr.: Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-kappa B through utilization of the Ikappa B kinase and activation of the mitogen-activated protein kinase p38. *J Biol Chem* 276:18934-18940, 2001
50. Davi G, Guagnano MT, Ciabattoni G, Basili S, Falco A, Marinopiccoli M, Nutini M, Sensi S, Patrono C: Platelet activation in obese women: role of inflammation and oxidant stress. *Jama* 288:2008-2014, 2002
51. Dandona P, Aljada A, Bandyopadhyay A: Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 25:4-7, 2004
52. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P: Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (Egr)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. *J Clin Endocrinol Metab* 87:1419-1422, 2002
53. Aljada A, Ghanim H, Saadeh R, Dandona P: Insulin Inhibits NFkappaB and MCP-1 Expression in Human Aortic Endothelial Cells. *J Clin Endocrinol Metab* 86:450-453., 2001
54. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P: Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. *J Clin Endocrinol Metab* 85:2572-2575, 2000
55. Chaudhuri A, Janicke D, Wilson MF, Tripathy D, Garg R, Bandyopadhyay A, Calieri J, Hoffmeyer D, Syed T, Ghanim H, Aljada A, Dandona P: Anti-inflammatory and profibrinolytic effect of insulin in acute ST-segment-elevation myocardial infarction. *Circulation* 109:849-854, 2004
56. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, Dandona P: Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. *Am J Clin Nutr* 79:682-690, 2004
57. Dandona P, Aljada A, Mohanty P: The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm. *Diabetologia* 45:924-930, 2002
58. Aljada A, Ghanim H, Mohanty P, Assian E, Dandona P: Glucose intake stimulates intranuclear NFkB and p47phox in mononuclear cells. *ENDO '2000, the 82nd Annual Meeting of the Endocrine Society*, 2000
59. Ridker PM: High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 103:1813-1818, 2001
60. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH: Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101:17671772, 2000
61. Elkind MS, Cheng J, Boden-Albala B, Rundek T, Thomas J, Chen H, Rabbani LE, Sacco RL: Tumor necrosis factor receptor levels are associated with carotid atherosclerosis. *Stroke* 33:3137, 2002
62. Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PW: Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke* 32:2575-2579, 2001
63. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama* 286:327-334, 2001

64. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 353:1649-1652, 1999
65. Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH, Jr., Heimovitz H, Cohen HJ, Wallace R: Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med* 106:506-512, 1999
66. Strandberg TE, Tilvis RS: C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. *Arterioscler Thromb Vasc Biol* 20:1057-1060, 2000
67. Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM: C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood* 82:513-520, 1993
68. Robbie L, Libby P: Inflammation and atherothrombosis. *Ann N Y Acad Sci* 947:167-179; discussion 179-180, 2001
69. Ziccardi P, Nappo F, Giugliano G, Esposito K, Marfella R, Cioffi M, D'Andrea F, Molinari AM, Giugliano D: Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 105:804-809, 2002
70. Zahorska-Markiewicz B, Janowska J, Olszanecka-Glinianowicz M, Zurakowski A: Serum concentrations of TNF-alpha and soluble TNF-alpha receptors in obesity. *Int J Obes Relat Metab Disord* 24:1392-1395, 2000
71. Heilbronn LK, Noakes M, Clifton PM: Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. *Arterioscler Thromb Vasc Biol* 21:968-970, 2001
72. Bastard JP, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B: Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab* 85:3338-3342, 2000
73. Abad LW, Schmitz HR, Parker R, Roubenoff R: Cytokine responses differ by compartment and wasting status in patients with HIV infection and healthy controls. *Cytokine* 18:286-293, 2002
74. Nicklas BJ, Ambrosius W, Messier SP, Miller GD, Penninx BW, Loeser RF, Palla S, Bleecker E, Pahor M: Diet-induced weight loss, exercise, and chronic inflammation in older, obese adults: a randomized controlled clinical trial. *Am J Clin Nutr* 79:544-551, 2004
75. Dandona P, Mohanty P, Hamouda W, Ghanim H, Aljada A, Garg R, Kumar V: Inhibitory effect of a two day fast on reactive oxygen species (ROS) generation by leucocytes and plasma orthotyrosine and meta-tyrosine concentrations. *J Clin Endocrinol Metab* 86:2899-2902, 2001
76. Watanobe H, Hayakawa Y: Hypothalamic interleukin-1 beta and tumor necrosis factor-alpha, but not interleukin-6, mediate the endotoxin-induced suppression of the reproductive axis in rats. *Endocrinology* 144:4868-4875, 2003
77. Russell SH, Small CJ, Stanley SA, Franks S, Ghatei MA, Bloom SR: The in vitro role of tumour necrosis factor-alpha and interleukin-6 in the hypothalamic-pituitary gonadal axis. *J Neuroendocrinol* 13:296-301, 2001
78. Baeuerle PA, Baltimore D: NF-kappa B: ten years after. *Cell* 87:13-20, 1996
79. Baldwin AS, Jr.: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 14:649-683, 1996
80. Halliwell B: Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 344:721-724., 1994
81. McCord JM: The evolution of free radicals and oxidative stress. *Am J Med* 108:652-659., 2000
82. Barnes PJ, Karin M: Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336:1066-1071, 1997
83. Leusen JH, Verhoeven AJ, Roos D: Interactions between the components of the human NADPH oxidase: intrigues in the phox family. *J Lab Clin Med* 128:461-476, 1996
84. Yagi K: *Lipid peroxides in biology and medicine.* New York and London:, Academic Press, 1982

85. Armstrong D, Abdella N, Salman A, Miller N, Rahman EA, Bojancyzk M: Relationship of lipid peroxides to diabetic complications. Comparison with conventional laboratory tests. *J Diabetes Complications* 6:116-122, 1992
86. Roberts LJ, Morrow JD: Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med* 28:505-513, 2000
87. Stadtman ER, Oliver CN: Metal-catalyzed oxidation of proteins. Physiological consequences. *J Biol Chem* 266:2005-2008., 1991
88. Blount BC, Duncan MW: Trace quantification of the oxidative damage products, meta- and ortho-tyrosine, in biological samples by gas chromatography-electron capture negative ionization mass spectrometry. *Anal Biochem* 244:270-276., 1997
89. Yesilova Z, Ozata M, Kocar IH, Turan M, Pekel A, Sengul A, Ozdemir IC: The effects of gonadotropin treatment on the immunological features of male patients with idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 85:66-70, 2000
90. Kanda N, Tsuchida T, Tamaki K: Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clin Exp Immunol* 106:410-415, 1996
91. Hatakeyama H, Nishizawa M, Nakagawa A, Nakano S, Kigoshi T, Uchida K: Testosterone inhibits tumor necrosis factor-alpha-induced vascular cell adhesion molecule-1 expression in human aortic endothelial cells. *FEBS Lett* 530:129-132, 2002
92. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH: The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 89:3313-3318, 2004
93. Ng MK, Liu PY, Williams AJ, Nakhla S, Ly LP, Handelsman DJ, Celermajer DS: Prospective study of effect of androgens on serum inflammatory markers in men. *Arterioscler Thromb Vasc Biol* 22:1136-1141, 2002
94. Dandona P, Thusu K, Hafeez R, Abdel-Rahman E, Chaudhuri A: Effect of hydrocortisone on oxygen free radical generation by mononuclear cells. *Metabolism* 47:788-791, 1998
95. Tong PC, Ho CS, Yeung VT, Ng MC, So WY, Ozaki R, Ko GT, Ma RC, Poon E, Chan NN, Lam CW, Chan JC: Association Of Testosterone, Insulin-Like Growth Factor-1 And C-Reactive Protein With Metabolic Syndrome In Chinese Middle-Aged Men With A Family History Of Type 2 Diabetes. *J Clin Endocrinol Metab*, 2005
96. Lipid, lipoproteins, C-reactive protein, and hemostatic factors at baseline in the diabetes prevention program. *Diabetes Care* 28:2472-2479, 2005
97. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19:972-978, 1999
98. Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P: Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* 110:1564-1571, 2004
99. Kern WV, Engel A, Kern P: Soluble tumor necrosis factor receptors in febrile neutropenic cancer patients. *Infection* 23:64-65, 1995
100. Daniel JA, Elsasser TH, Martinez A, Steele B, Whitlock BK, Sartin JL: Interleukin-1beta and tumor necrosis factor-alpha mediation of endotoxin action on growth hormone. *Am J Physiol Endocrinol Metab* 289:E650-657, 2005
101. Yoo MJ, Nishihara M, Takahashi M: Tumor necrosis factor-alpha mediates endotoxin induced suppression of gonadotropin-releasing hormone pulse generator activity in the rat. *Endocr J* 44:141-148, 1997
102. Pang XP, Yoshimura M, Hershman JM: Suppression of rat thyrotroph and thyroid cell function by tumor necrosis factor-alpha. *Thyroid* 3:325-330, 1993
103. Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG: Central nervous system control of food intake. *Nature* 404:661-671, 2000
104. Porte D, Jr., Baskin DG, Schwartz MW: Leptin and insulin action in the central nervous system. *Nutr Rev* 60:S20-29; discussion S68-84, 85-27, 2002

105. Pocai A, Lam TK, Gutierrez-Juarez R, Obici S, Schwartz GJ, Bryan J, Aguilar-Bryan L, Rossetti L: Hypothalamic K(ATP) channels control hepatic glucose production. *Nature* 434:1026-1031, 2005
106. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95:2409-2415, 1995
107. Rui L, Yuan M, Frantz D, Shoelson S, White MF: SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* 277:42394-42398, 2002
108. Aljada A, Ghanim H, Saadeh R, Dandona P: Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* 86:450-453, 2001
109. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271:665-668, 1996
110. Aljada A, Ghanim H, Assian E, Dandona P: Tumor necrosis factor-alpha inhibits insulininduced increase in endothelial nitric oxide synthase and reduces insulin receptor content and phosphorylation in human aortic endothelial cells. *Metabolism* 51:487-491, 2002
111. Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P: Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab* 85:2970-2973, 2000
112. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D: Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 106:2067-2072, 2002
113. Aljada A, Dandona P: Effect of insulin on human aortic endothelial nitric oxide synthase. *Metabolism* 49:147-150, 2000
114. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, Ahmad S: Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 86:3257-3265, 2001
115. Dandona P, Aljada A, Mohanty P, Ghanim H, Bandyopadhyay A, Chaudhuri A: Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care* 26:3310-3314, 2003
116. Blouin K, Despres JP, Couillard C, Tremblay A, Prud'homme D, Bouchard C, Tchernof A: Contribution of age and declining androgen levels to features of the metabolic syndrome in men. *Metabolism* 54:1034-1040, 2005
117. Pitteloud N, Mootha VK, Dwyer AA, Hardin M, Lee H, Eriksson KF, Tripathy D, Yialamas M, Groop L, Elahi D, Hayes FJ: Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. *Diabetes Care* 28:1636-1642, 2005
118. Muller M, Grobbee DE, den Tonkelaar I, Lamberts SW, van der Schouw YT: Endogenous sex hormones and metabolic syndrome in aging men. *J Clin Endocrinol Metab* 90:2618-2623, 2005
119. Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P: Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. *Circulation* 112:332-340, 2005
120. van der Wal AC, Becker AE, van der Loos CM, Das PK: Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 89:36-44, 1994
121. Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V, Fallon JT: Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. *Circulation* 90:775-778, 1994
122. Libby P: Molecular bases of the acute coronary syndromes. *Circulation* 91:2844-2850, 1995
123. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, Fallon JT, Regnstrom J, Fuster V: Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* 92:1565-1569, 1995

124. Buffon A, Biasucci LM, Liuzzo G, D'Onofrio G, Crea F, Maseri A: Widespread coronary inflammation in unstable angina. *N Engl J Med* 347:5-12, 2002
125. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336:973-979, 1997
126. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH: Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 97:425-428, 1998
127. Woollard KJ, Phillips DC, Griffiths HR: Direct modulatory effect of C-reactive protein on primary human monocyte adhesion to human endothelial cells. *Clin Exp Immunol* 130:256-262, 2002
128. Nan B, Yang H, Yan S, Lin PH, Lumsden AB, Yao Q, Chen C: C-reactive protein decreases expression of thrombomodulin and endothelial protein C receptor in human endothelial cells. *Surgery* 138:212-222, 2005
129. Yang H, Nan B, Yan S, Li M, Yao Q, Chen C: C-reactive protein decreases expression of VEGF receptors and neuropilins and inhibits VEGF165-induced cell proliferation in human endothelial cells. *Biochem Biophys Res Commun* 333:1003-1010, 2005
130. Singh U, Devaraj S, Jialal I: C-reactive protein decreases tissue plasminogen activator activity in human aortic endothelial cells: evidence that C-reactive protein is a procoagulant. *Arterioscler Thromb Vasc Biol* 25:2216-2221, 2005
131. Bisoendial RJ, Kastelein JJ, Levels JH, Zwaginga JJ, van den Bogaard B, Reitsma PH, Meijers JC, Hartman D, Levi M, Stroes ES: Activation of inflammation and coagulation after infusion of C-reactive protein in humans. *Circ Res* 96:714-716, 2005
132. Simon D, Preziosi P, Barrett-Connor E, Roger M, Saint-Paul M, Nahoul K, Papoz L: Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study. *Diabetologia* 35:173-177, 1992
133. Yue P, Chatterjee K, Beale C, Poole-Wilson PA, Collins P: Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91:1154-1160, 1995
134. Ajayi AA, Ogungbade GO, Okorodudu AO: Sex hormone regulation of systemic endothelial and renal microvascular reactivity in type-2 diabetes: studies in gonadectomized and shamoperated Zucker diabetic rats. *Eur J Clin Invest* 34:349-357, 2004
135. Singh R, Pervin S, Shryne J, Gorski R, Chaudhuri G: Castration increases and androgens decrease nitric oxide synthase activity in the brain: physiologic implications. *Proc Natl Acad Sci U S A* 97:3672-3677, 2000
136. Holmang A, Bjorntorp P: The effects of testosterone on insulin sensitivity in male rats. *Acta Physiol Scand* 146:505-510, 1992
137. Katznelson L, Rosenthal DI, Rosol MS, Anderson EJ, Hayden DL, Schoenfeld DA, Klibanski A: Using quantitative CT to assess adipose distribution in adult men with acquired hypogonadism. *AJR Am J Roentgenol* 170:423-427, 1998
138. Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, Veldhuis JD, Urban RJ: Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab* 83:1886-1892, 1998
139. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253-259, 1999
140. Itani SI, Ruderman NB, Schmieder F, Boden G: Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes* 51:2005-2011, 2002
141. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, Dandona P: Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes* 52:2882-2887, 2003
142. Dockery F, Bulpitt CJ, Agarwal S, Rajkumar C: Testosterone suppression in men with prostate cancer is associated with increased arterial stiffness. *Aging Male* 5:216-222, 2002

143. Dockery F, Bulpitt CJ, Agarwal S, Donaldson M, Rajkumar C: Testosterone suppression in men with prostate cancer leads to an increase in arterial stiffness and hyperinsulinaemia. *Clin Sci (Lond)* 104:195-201, 2003
144. Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY: Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. *Int J Obes Relat Metab Disord* 24:485-491, 2000
145. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. *Circulation* 105:1135-1143, 2002
146. Ross R: Atherosclerosis--an inflammatory disease. *N Engl J Med* 340:115-126, 1999
147. Lusis AJ: Atherosclerosis. *Nature* 407:233-241., 2000
148. Hak AE, Witteman JC, de Jong FH, Geerlings MI, Hofman A, Pols HA: Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab* 87:3632-3639, 2002
149. Rosano GM, Leonardo F, Pagnotta P, Pelliccia F, Panina G, Cerquetani E, della Monica PL, Bonfigli B, Volpe M, Chierchia SL: Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 99:1666-1670, 1999
150. Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P: Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 100:1690-1696, 1999
151. English KM, Steeds RP, Jones TH, Diver MJ, Channer KS: Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: A randomized, doubleblind, placebo-controlled study. *Circulation* 102:1906-1911, 2000
152. Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C: Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res* 84:813-819, 1999
153. Shahidi NT: Androgens and erythropoiesis. *N Engl J Med* 289:72-80, 1973
154. Yeh ET: CRP as a mediator of disease. *Circulation* 109:II11-14, 2004
155. Means RT, Jr., Krantz SB: Progress in understanding the pathogenesis of the anemia of chronic disease. *Blood* 80:1639-1647, 1992
156. Papadaki HA, Kritikos HD, Gemetzi C, Koutala H, Marsh JC, Boumpas DT, Eliopoulos GD: Bone marrow progenitor cell reserve and function and stromal cell function are defective in rheumatoid arthritis: evidence for a tumor necrosis factor alpha-mediated effect. *Blood* 99:16101619, 2002
157. Papadaki HA, Kritikos HD, Valatas V, Boumpas DT, Eliopoulos GD: Anemia of chronic disease in rheumatoid arthritis is associated with increased apoptosis of bone marrow erythroid cells: improvement following anti-tumor necrosis factor-alpha antibody therapy. *Blood* 100:474482, 2002
158. Schilling RF: Anemia of chronic disease: a misnomer. *Ann Intern Med* 115:572-573, 1991
159. Hofstra J, Loves S, van Wageningen B, Ruinemans-Koerts J, Jansen I, de Boer H: High prevalence of hypogonadotropic hypogonadism in men referred for obesity treatment. *Neth J Med* 66:103-109, 2008
160. Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, Andrew R, YkiJarvinen H, Olsson T, Walker BR: Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. *Clin Endocrinol (Oxf)* 66:440-446, 2007
161. Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I: Estradiol in elderly men. *Aging Male* 5:98-102, 2002
162. Cohen PG: The hypogonadal-obesity cycle: role of aromatase in modulating the testosteroneestradiol shunt--a major factor in the genesis of morbid obesity. *Med Hypotheses* 52:49-51, 1999
163. Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF, Jr., Hayes FJ: Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropinreleasing hormone-deficient men. *J Clin Endocrinol Metab* 93:784-791, 2008

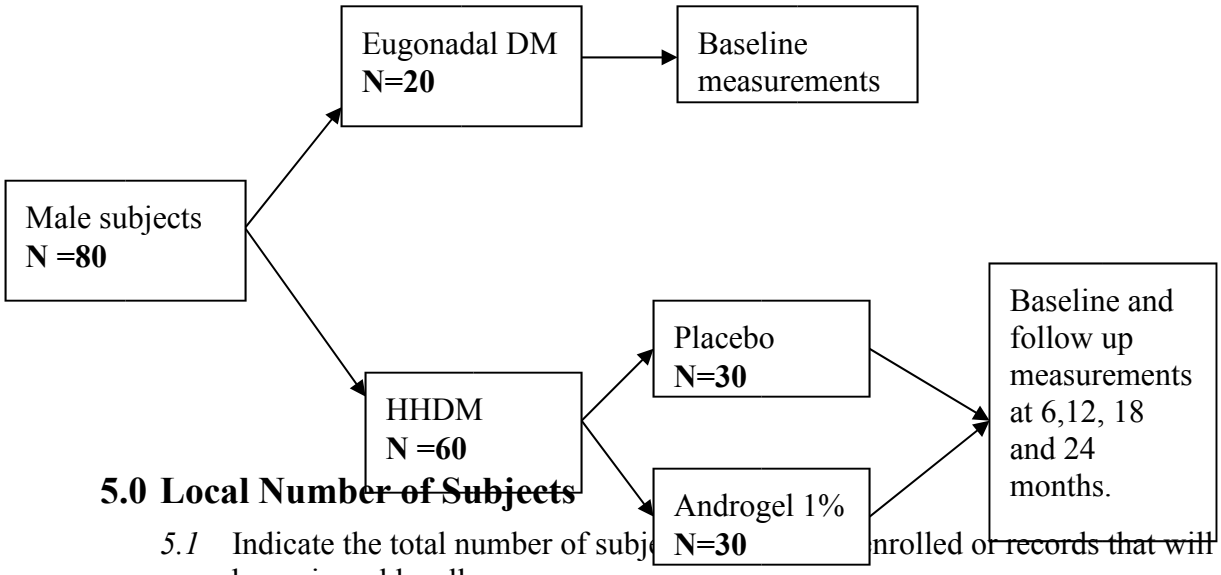
164. Pitteloud N, Dwyer AA, Decruz S, Lee H, Boepple PA, Crowley WF, Jr., Hayes FJ: The relative role of gonadal sex steroids and gonadotropin-releasing hormone pulse frequency in the regulation of follicle-stimulating hormone secretion in men. *J Clin Endocrinol Metab* 93:26862692, 2008
165. Arvanitakis Z, Wilson RS, Bienias JL, Evans DA, Bennett DA: Diabetes mellitus and risk of Alzheimer disease and decline in cognitive function. *Arch Neurol* 61:661-666, 2004
166. Rosario ER, Pike CJ: Androgen regulation of beta-amyloid protein and the risk of Alzheimer's disease. *Brain Res Rev* 57:444-453, 2008
167. Janssen I, Fortier A, Hudson R, Ross R: Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care* 25:431-438, 2002
168. Kanters SD, Algra A, van Leeuwen MS, Banga JD: Reproducibility of in vivo carotid intimamedia thickness measurements: a review. *Stroke* 28:665-671, 1997
169. Van Trijp MJ, Uiterwaal CS, Bos WJ, Oren A, Grobbee DE, Bots ML: Noninvasive arterial measurements of vascular damage in healthy young adults: relation to coronary heart disease risk. *Ann Epidemiol* 16:71-77, 2006
170. Cheng KS, Mikhailidis DP, Hamilton G, Seifalian AM: A review of the carotid and femoral intima-media thickness as an indicator of the presence of peripheral vascular disease and cardiovascular risk factors. *Cardiovasc Res* 54:528-538, 2002
171. Lo J, Dolan SE, Kanter JR, Hemphill LC, Connelly JM, Lees RS, Grinspoon SK: Effects of Obesity, Body Composition, and Adiponectin on Carotid Intima-Media Thickness in Healthy Women. *J Clin Endocrinol Metab*, 2006
172. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R: Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 39:257-265, 2002
173. Perregaux D, Chaudhuri A, Rao S, Airen A, Wilson M, Sung BH, Dandona P: Brachial vascular reactivity in blacks. *Hypertension* 36:866-871, 2000
174. Garg R, Kumbkarni Y, Aljada A, Mohanty P, Ghanim H, Hamouda W, Dandona P: Troglitazone reduces reactive oxygen species generation by leukocytes and lipid peroxidation and improves flow-mediated vasodilatation in obese subjects. *Hypertension* 36:430-435, 2000
175. Perregaux D, Chaudhuri A, Mohanty P, Bukhari L, Wilson MF, Sung BH, Dandona P: Effect of gender differences and estrogen replacement therapy on vascular reactivity. *Metabolism* 48:227-232, 1999
176. Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J, Kilgore M: Estrogen regulation of adipose tissue lipoprotein lipase--possible mechanism of body fat distribution. *Am J Obstet Gynecol* 178:101-107, 1998
177. Yki-Jarvinen H, Nikkila EA, Kubo K, Foley JE: Assay of glucose transport in human fat cells obtained by needle biopsy. *Diabetologia* 29:287-290, 1986
178. Langenfeld MR, Forst T, Hohberg C, Kann P, Lubben G, Konrad T, Fullert SD, Sachara C, Pfutzner A: Pioglitazone decreases carotid intima-media thickness independently of glycemic control in patients with type 2 diabetes mellitus: results from a controlled randomized study. *Circulation* 111:2525-2531, 2005
179. Fukui M, Kitagawa Y, Nakamura N, Kadono M, Mogami S, Hirata C, Ichio N, Wada K, Hasegawa G, Yoshikawa T: Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. *Diabetes Care* 26:1869-1873, 2003

4.0 Study Design

4.1 Describe and explain the study design (e.g. case-control, cross-sectional, ethnographic, experimental, interventional, longitudinal, observational).

Response:. The proposed study will prospectively investigate, in a randomized controlled manner, the changes in carotid intima-media thickness (IMT), brachial flow mediated dilatation (FMD%), inflammatory mediators, and adiposity over a period of 24 months in hypogonadotrophic hypogonadal type 2 diabetics (HHDM, n=60) randomized equally to either placebo or Androgel 1% (50mg daily). Fasting blood samples will be collected and carotid IMT, brachial FMD%, MRI measurements will be taken at baseline and at selected time points up to 24 months. 20 subjects with type 2 diabetes who are eugonodal will be recruited as controls for comparison of carotid IMT, brachial FMD%, inflammatory mediators and adiposity at baseline with the HHDM group. The study will be approved by the State University of New York at Buffalo School of Medicine Institutional Review Board.

Study Design



5.0 Local Number of Subjects

5.1 Indicate the total number of subjects to be enrolled or records that will be reviewed locally.

Response: 80

5.2 If applicable, indicate how many subjects you expect to screen to reach your target sample (i.e. your screen failure rate).

Response: 100-110. All screened and qualified patients will be enrolled and randomized up to 80 enrolled patients.

5.3 Justify the feasibility of recruiting the proposed number of eligible subjects within the anticipated recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?

Response: The Diabetes and Endocrinology Center of WNY is the largest Diabetes center in the WNY area, seeing between 100 to 120 type 2 diabetes patients every week. 5%-10% of the patients will be males with Hypogonadism who might qualify for this study. Therefore, majority of recruited patients are our Kaleida Health and UBMD clinic patients. We will also recruit patients through advertisement, Buffalo Research Registry and researchmatch.org. These sources will suffice to recruit the needed number to subjects.

6.0 Inclusion and Exclusion Criteria

6.1 Describe the criteria that define who will be **included** in your final study sample.

NOTE: This may be done in bullet point fashion.

Response:

1- Type 2 diabetes
2- males
3- Age 30-60 years inclusive. The lower age limit was decided on the fact that in our study on hypogonadotrophic hypogonadism in type 2 diabetic patients, the youngest subject was 31 years old. The upper age limit has been restricted to 60 to avoid including subjects with significant age-related declines in testosterone concentrations. 4- PSA < 4 ng/ml 5- IPSS ≤ 19.
6- Subjects on thiazolidinediones, statins, ACE inhibitors, angiotensin receptor blockers or antioxidants will be allowed as long as they are on stable doses (for last 3 months) of these compounds and the dosage in not changed during the study. Subjects on insulin, metformin or sulfonylureas can participate in the study, provided that minimal changes are made to the doses during the study.

6.2 Describe the criteria that define who will be **excluded** from your final study sample.

NOTE: This may be done in bullet point fashion.

Response:

1)Coronary event or procedure (myocardial infarction, unstable angina, coronary artery bypass, surgery or coronary angioplasty) in the previous 6 months
2)Hemoglobin A1c >10%
3)h/o prostate carcinoma
4)Hepatic disease (transaminase > 3 times normal) or cirrhosis
5)Renal impairment (defined as GFR<30)
6)HIV or Hepatitis C positive status
7)Participation in any other concurrent clinical trial
8)Any other life-threatening, non-cardiac disease
9)Use of over the counter health supplements which contain androgens
10)Use of an investigational agent or therapeutic regimen within 30 days of study.
11) Hematocrit > 50%.
12) PSA > 4 ng/ml

6.3 Indicate specifically whether you will include any of the following special populations in your study using the checkboxes below.

NOTE: Members of special populations may not be targeted for enrollment in your study unless you indicate this in your inclusion criteria.

Response: None of the below populations will be enrolled

☐ Adults unable to consent

☐ Individuals who are not yet adults (infants, children, teenagers)

☐ Pregnant women

☐ Prisoners

6.4 Indicate whether you will include non-English speaking individuals in your study. **Provide justification if you will exclude non-English speaking individuals.**

In order to meet one of the primary ethical principles of equitable selection of subjects, non-English speaking individuals may **not** be routinely excluded from research as a matter of convenience.

In cases where the research is of therapeutic intent or is designed to investigate areas that would necessarily require certain populations who may not speak English, the researcher is required to make efforts to recruit and include non-English speaking individuals. However, there are studies in which it would be reasonable to limit subjects to those who speak English. Some examples include pilot studies, small unfunded studies with validated instruments not available in other languages, studies with numerous questionnaires, and some non-therapeutic studies which offer no direct benefit.

Response: We have no non-English speaking patients in this population. We have patients that English is a second language, but they are able to read, write and understand it. This population is less than 10% of the total population.

7.0 Vulnerable Populations

If the research involves special populations that are considered vulnerable, **describe the safeguards included to protect their rights and welfare.**

NOTE: You should refer to the appropriate checklists, referenced below, to ensure you have provided adequate detail regarding safeguards and protections. You do not, however, need to provide these checklists to the IRB.

7.1 For research that involves **pregnant women,** safeguards include: NOTE CHECKLIST: Pregnant Women (HRP-412)

Response: We will not be using subjects from vulnerable populations

☒ **N/A**: This research does not involve pregnant women.

7.2 For research that involves **neonates of uncertain viability or non-viable neonates,** safeguards include:
NOTE CHECKLISTS: Non-Viable Neonates (HRP-413), or Neonates of Uncertain Viability (HRP-414)

Response:

☒ **N/A:** This research does not involve non-viable neonates or neonates of uncertain viability.

7.3 For research that involves **prisoners**, safeguards include:
NOTE CHECKLIST: Prisoners (HRP-415)

Response:

☒ **N/A:** This research does not involve prisoners.

7.4 For research that involves **persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children")**, safeguards include:
NOTE CHECKLIST: Children (HRP-416)

Response:

☒ **N/A:** This research does not involve persons who have not attained the legal age for consent to treatments or procedures ("children").

7.5 For research that involves **cognitively impaired adults**, safeguards include:
NOTE CHECKLIST: Cognitively Impaired Adults (HRP-417)

Response:

☒ **N/A:** This research does not involve cognitively impaired adults.

7.6 Consider if other specifically targeted populations such as students, employees of a specific firm, or educationally or economically disadvantaged persons are vulnerable. **Provide information regarding their safeguards and protections, including safeguards to eliminate coercion or undue influence.**

Response: No specific populations or vulnerable groups will be targeted. All subjects enrolled in this study will be of legal adult consenting age with the ability to speak, read and interrupt the English language. Patients will have the ability to speak with the research team regarding any questions or concern they have before signing the consent. Patients are made aware that this study is voluntary and they are able to stop participating at any time they feel uncomfortable. Patients are not be pressured into participating and their clinic standard of care will remain the same if they participate or choose not to participate.

8.0 Eligibility Screening

8.1 Describe **screening procedures** for determining subjects' eligibility.
Screening refers to determining if prospective participants meet inclusion and exclusion criteria.

Include all relevant screening documents with your submission (e.g. screening protocol, script, questionnaire).

Response: :

Prospective participants will be asked to read and understand the consent and any questions they may have regarding the protocol will be answered. If the subject wants to participate in the study, they will be asked to sign the informed consent form. Each participant will complete a physical exam including Digital Rectal Examination, Medical History and baseline labs to measure CBC, SMA, HbA1c, lipid profile, PSA, testosterone, SHBG, Estradiol, LH and FSH. A total of 80 male subjects will be recruited (60 type 2 diabetics with Hypogonadotrophic hypogonadism, 20 eugonadal type 2 diabetics). There will be two groups in the study: -

Hypogonadal group: Sixty type 2 diabetic male subjects with hypogonadotrophic hypogonadism (as defined below) will be recruited into the study. **Hypogonadotrophic hypogonadism (HH) will be defined as low calculated free testosterone (below 6.5ng/dL)** with normal or low LH and FSH concentrations.

Eugonadal group*:* Twenty type 2 diabetic male patients with normal calculated free testosterone concentrations will also be recruited in the study to serve as an age-matched comparison group. They will not be treated with testosterone.

☐ **N/A:** There is no screening as part of this protocol.

9.0 Recruitment Methods

☐ **N/A:** This is a records review only, and subjects will not be recruited. NOTE: If you select this option, please make sure that all records review procedures and inclusion/exclusion screening are adequately described in other sections.

9.1 Describe when, where, and how potential subjects will be recruited.

NOTE: Recruitment refers to how you are identifying potential participants and introducing them to the study. Include specific methods you will use (e.g. searching charts for specific ICD code numbers, Research Participant Groups, posted advertisements, etc.).

Response: Participants will be identified by prescreening clinical charts, patient doctor interaction at the time of their visits, flyers advertisements and researchmatch.org. Diabetes Endocrinology Center of WNY Locations include:

1. 1020 Youngs Road, Williamsville NY 14221

2. 705 Maple Road, Williamsville NY 14221

3. 1000 Youngs Road, Suite 105, Williamsville NY 14221

4. 462 Grider Street, Buffalo NY 14215

The study clinical team will evaluate their clinic patients for possible participation in this study according to the inclusion and exclusion criteria at the Diabetes and Endocrinology Center of WNY. Patients that may qualify for the study are referred to the research team for further eligibility evolution. Patients meeting the inclusion and exclusion criteria based on preliminary phone evaluation will be invited to participate in the study.

9.2 Describe how you will protect the privacy interests of prospective subjects during the recruitment process.

NOTE: Privacy refers to an individual's right to control access to him or herself.

Response: Patient charts will be screened according to the study inclusion and exclusion criteria by our trained clinical staff and physicians. If the patient qualifies and is of consenting age, the physicians will speak to them about their interests in participating in research. If the patient agrees, their information will be given to the research coordinator to be contacted for further evaluation. All personal information will be kept confidential and locked in the coordinator office.

9.3 Identify any materials that will be used to recruit subjects.

NOTE: Examples include scripts for telephone calls, in person announcements / presentations, email invitations.

For advertisements, include the final copy of printed advertisements with your submission. When advertisements are taped for broadcast, attach the final

audio/video tape. NOTE: You may submit the wording of the advertisement prior to taping to ensure there will be no IRB-required revisions, provided the IRB also reviews and approves the final version.

Response: In addition to screening clinical charts, participations will be identified through; flyer advertisement and researchmatch.org

10.0 Procedures Involved

10.1 Provide a description of **all research procedures or activities** being performed and when they are performed once a subject is screened and determined to be eligible. Provide as much detail as possible.

NOTE: This should serve as a blueprint for your study and include enough detail so that another investigator could pick up your protocol and replicate the research. For studies that have multiple or complex visits or procedures, consider the addition of a schedule of events table in in your response.

Response:

Screening Day (week -1)

Each subject will have completed the following procedures prior to participating in the study.

1) Informed consent;
2) Physical Exam including Digital Rectal examination.
3) Medical History;
4) Baseline lab draws to measure CBC, SMA, HbA1c, lipid profile, PSA, testosterone, SHBG, estradiol, LH and FSH.

Study design for the hypogonadal group

After the screening visit, subjects will be randomized to receive Androgel 1% (5grams) daily (starting at week 0) or placebo for 24 months.

Randomization Method: Patients who qualify for the study will be assigned a number by a computerized randomization program (Random Allocation Software) and will be therefore randomized to either of the two study groups. Randomization will be done in blocks of 4 and there will thus be 15 blocks. The subjects will be blinded to the treatment.

The subjects in the hypogonadal group will have following visits during the study:-

1. Baseline visit 1: Vitals and blood pressure, MRI will be done estimate total body and regional adiposity and lean body mass. Waist and Hip measurements will be taken to determine the waist to hip ratio. Subjects will be given a container for 24-hour urine collection for measurement of isoprostanes.
2. Baseline visit 2: This visit will occur within 30 days of visit 1. The participants will have blood drawn for research lab **(1.5 tablespoons (23ml))** and Vitals and blood pressure. Carotid IMT will be measured and brachial artery flow mediated dilatation will be done as per methodology described in the protocol. A fat biopsy will be obtained. Subjects will also receive instructions on standardized diet and exercise. Subjects will turn in their 24-hour urine collection container.
3. Titration: Visit 3, 4, (week 4, week 10): A blood sample will be taken to measure calculated free testosterone concentration (**1.5 tablespoons (23ml))** and Vitals and blood pressure. During the study, we will attempt to keep the free testosterone concentrations in the range of 14-17 ng/dL for subjects randomized to drug group. This range has been chosen because it falls in middle of the normal range for lean healthy young men. Dose response studies of testosterone replacement have shown that in the mid-normal range of testosterone, subjects have demonstrable beneficial effects on lean body mass (34). If the free testosterone concentration is less than 14ng/dL in subjects randomized to drug group, then the dose of Androgel 1% will be increased to 7.5 grams at week 6 and if still lower than 14 ng/dl at week 10, the dose will be increased to Androgel 1% (10grams) at week 12. If the total testosterone concentration is more than 17ng/dL in subjects randomized to drug group, then the dose of Androgel 1% will be decreased by 2.5 mg starting from week 6. On

visit 4, blood samples will be taken for PSA and subjects will be questioned for BPH symptoms to estimate the IPSS.

4. Visit 5, 6, 7 (month 6, 12, 18): The participants will have to come fasting and blood will be drawn for research labs (**1.5 tablespoons (23ml))** and Vitals and blood pressure. CBC, PSA, testosterone, SHBG and liver function test will be done at each visit and subjects will be questioned for BPH symptoms to estimate the IPSS. Carotid IMT will be measured and brachial artery flow mediated dilatation will be done as per methodology described in the protocol. On visit 6 (month 12), fat biopsy and MRI will be done. Subjects will also receive instructions on standardized diet and exercise at visit 5 and 6. At every visit, compliance with study drug will be assessed and a 180 day supply of study drug will be dispensed and we will adjust the dose 2 week after each visit as mentioned above. Subjects will give 24-hour urine at month 12. At each of these visits, dose of Androgel will be adjusted (as above) if needed.
5. End of study Visit 8 (month 24): The participants will come fasting and blood will be collected for research labs(**1.5 tablespoons (23ml))** and Vitals and blood pressure. The subjects will be questioned for BPH symptoms to estimate the IPSS. Carotid IMT will be measured and brachial artery flow mediated dilatation will be done as per methodology described in the protocol. A MRI will be done to estimate total body and regional adiposity and waist and hip measurements will be taken. Blood for CBC, SMA, HbA1c, PSA, testosterone, estradiol, SHBG, LH and FSH and lipid profile will also be drawn. Subjects will turn in their 24-hour urine collection container. A fat biopsy will be obtained.

Study design for Eugonadal Diabetic group

1. Baseline visit 1(day 0): 24 hour urine will be given and MRI will be done to estimate total body and regional adiposity and lean body mass.

1.2. Baseline visit 2 (day 1): The participants will have blood drawn for research labs(**1.5 tablespoons (23ml))**, Waist and Hip measurements will be taken to determine the waist to hip ratio. Carotid IMT will be measured and brachial artery flow mediated dilatation will be done as per methodology described in the protocol. Fat Biopsy will be obtained. Subjects will turn in their 24-hour urine collection container. This visit will be the end of study visit for this group

LABORATORY PROCEDURES

2. Plasma concentrations of inflammatory mediators: Concentrations of TNF-□, IL-6, ICAM1, MMP-9, PAI-1, MCP-1 are measured in plasma using sensitive and high sensitive ELISA kits (R&D Systems, MN). CRP concentrations will be measured using an ELISA kit from Alpha Diagnostics International Inc. (San Antonio, TX). SAA will be measured in serum using an ELISA kit from Biosource (CA).
3. Plasma glucose, insulin and free fatty acids measurements: Insulin levels are determined using an ELISA kit from Diagnostic Systems Laboratories Inc. (Webster, TX). Glucose levels are measured in plasma by YSI 2300 STAT Plus glucose analyzer (Yellow Springs, Ohio). Free fatty acid levels are measured by a colorimetric assay (Wako, Richmond, VA).
4. Serum testosterone, SHBG, estradiol, LH and FSH, CBC, CMP, PSA and HbA1c measurements: All these tests will be done by Quest Laboratories. Total testosterone and estradiol will be done by liquid chromatography followed mass spectrometry. LH, FSH, SHBG, CBC, CMP, PSA and hbA1c will be done well established clinical assays. Free testosterone will be calculated from SHBG and T using the method of Vermeulen et al (8).
5. MRI: Multislice MRI of abdomen will be obtained (14). Abdominal subcutaneous adipose tissue, visceral adipose tissue (adipose tissue in the intraabdominal cavity delineated at the innermost aspect of the abdominal and oblique muscle walls surrounding the cavity and the anterior aspect of the vertebral body) and hepatic fat will be analyzed in a blinded fashion using customized software (167).MRI measurements will be expressed as volume in cm^3.
6. Carotid Intima-media thickness (IMT): Carotid IMT is used to assess subclinical and clinical atherosclerosis. The carotid arteries are most suitable for IMT measurements given their superficial location, size and limited movement (168). IMT is measured in both the right and

left common carotid arteries and a 7.5 MHz linear array transducer is used for the purpose(169). All vascular images will be acquired using a Hewlett Packard (HP) Image Point Hx Multispecialty Ultrasound System. Following imaging by B-mode Duplex scanning, IMT measurement is defined by two parallel echogenic lines, corresponding to the interfaces between the lumen-intima and the media-adventitia. While imaging is done of both the near and far walls, these interfaces are best defined in the far wall of the carotid arteries(170). The IMT thus is the combined thickness of both the intima and the media.

Subjects are positioned with a wedge of approximately 35 degrees to reduce respiratory variation and motion of the jugulars. Initially, the transducer is swept in cross-section to note the position and orientation of the carotid artery bifurcation. Following this, the transducer is applied in the longitudinal plane and images acquired at two angles, 90- and 45-degrees. The 90-degree imaging plane is a frontal plane of the head of the common carotid artery. In each plane, the transducer is manipulated till the best image of the far wall of the distal 1 cm of the common carotid is obtained (171). Images will be digitized using a frame-grabber card and a PC and specialized software (IntiMate, Pixen Technologies, India) will be used for edge detection and subsequent IMT measurement. This technique is being applied to the assessment of atherosclerosis in several of our studies in obesity and diabetes.

7. Brachial flow-mediated dilatation (FMD): FMD is the capacity of blood vessels to respond to an increase in flow, or more precisely shear stress, by dilating. Endothelium-derived nitric oxide (NO) is a principal mediator of FMD. A 7.5 – 10 MHz broadband multi-frequency linear array transducer will be used to acquire images. The subject is positioned supine with the arm in a comfortable position for imaging the brachial artery. To create a flow stimulus, a blood pressure cuff is placed on the forearm. After acquiring a baseline rest image, arterial occlusion is created by cuff inflation to suprasystolic pressure. To occlude arterial inflow for a standardized length of time, the cuff is inflated to at least 50 mm Hg above systolic pressure. The resultant ischemia triggers dilatation of downstream resistance vessels via autoregulatory mechanisms. Following 5 minutes of occlusion, the cuff is deflated. This results in reactive hyperemia (a brief high-flow state) in the brachial artery to accommodate the dilated resistance vessels. The shear stress that results causes dilatation of the brachial artery. The recording of the arterial image is done continuously from 30 seconds before to 2 minutes following cuff deflation (172). This procedure has been utilized in several studies carried out in our unit, including the first studies describing abnormalities in African Americans(173), brachial reactivity improvements related to reversal of oxidative stress in the obese and type 2 diabetics following troglitazone (174), rosiglitazone (submitted for publication) and pioglitazone therapy; and gender differences in brachial reactivity and the effect of hormone replacement therapy (HRT) in post-menopausal women (175).
8. Fat aspiration procedure: Adipocytes will be harvested from the subjects by methods previously described in literature (176; 177). Subcutaneous fat tissue aspiration will be performed on abdomen at a 10 cm distance from umbilicus. The subjects will not have taken aspirin or NSAIDS in the last 72 hours. If that is the case, then the procedure will not be done. The skin will first be prepared with povidone-iodine (Betadine) and alcohol. A sterile drape will be placed around the appropriate area. 3 cc of 1% lidocaine will then be administered subcutaneously. After adequate anesthesia has been achieved, approximately 20-50cc of 0.5% lidocaine will be injected in the adipose tissue. Dose of lidocaine will not exceed 4.5mg/kg body weight. Aspiration of fat tissue will then be performed with a 3-holed cannula (Tulip Instrumentation, length: 15cm, diameter: 2.1mm) fixed to a 10mL syringe. More than one attempt at aspiration can be done at the same site during the procedure to get adequate sample. After getting adequate fat tissue (500mg-3g), the puncture site will be pressed for at least ten minutes before the patient rises up from supine position (to minimize bruising). The study subjects will then be discharged home. The adipose tissue will be centrifuged to remove blood and fluid contaminants. The upper adipose tissue will be collected into a separate sterile tube, washed twice with cold sterile Phosphate Buffered Saline (PBS) and centrifuged to remove the PBS. The adipose tissue sample will be weighed

and approximately 500 mg transferred to a separate tube for analysis. Total RNA, nuclear extracts and total cell lysates will be prepared from the adipose tissue.

9. During the study visits subjects would be screened for side effects .Blood test results would be reviewed by physician and appropriate adjustments would be made as per protocol.

10.2 Describe what data will be collected.

NOTE: For studies with multiple data collection points or long-term follow up, consider the addition of a schedule or table in your response.

Response:. **Study 1: *Type 2 diabetes and hypogonadism(5)***

Table 1

	Hypogonadal	Eugonadal
n	34	69
Age(years)	57.2±2.4	53.5±1.5
BMI(kg/m^2)	35.7±1.7	31.7±1.0
T (nmol/L)	8.07±0.65	14.58±0.62$^{\#}$
FT(nmol/L)	0.146±0.011	0.306±0.015$^{\#}$
cFT(nmol/L)	0.172±0.007	0.326±0.013$^{\#}$
LH (MIU/mL)	3.15±0.26	3.91±0.24*
FSH (MIU/mL)	4.25±0.45	5.53±0.40*
PRL (MIU/mL)	6.69±0.58	6.69±0.46
SHBG (nmol/L)	28.87±2.79	27.31±1.96
HbA1c%	8.5±0.3	8.42±0.3
Duration of diabetes(years)	9.03±1.31	7.12±0.97

$^{\#}$ $P<0.001$ versus Hypogonadal group *$P<0.05$ versus Hypogonadal group.

To convert testosterone from SI units (nmol/L) into conventional units (ng/dL) multiply by 28.8. We systematically investigated 103 consecutive male patients with type 2 diabetes mellitus who had been referred to our center (Diabetes Endocrinology Center of Western New York) by measuring total testosterone, free testosterone, SHBG, LH, FSH and prolactin (PRL) in order to determine the prevalence of hypogonadism (as defined by a low free testosterone) in type 2 diabetes and to differentiate whether the nature of hypogonadism was hypogonadotrophic or hypergonadotrophic (5) *(please see attached manuscript)*. All patients had either free testosterone (FT) measured by Equilibrium Dialysis (ED) or calculated FT (free testosterone calculated using SHBG and T). Hypogonadism was defined as low FT or cFT. Out of a total of 103 patients who had either FT or cFT measured, 34 patients (33%) were hypogonadal. *Table 1* depicts the clinical and biochemical features of patients with normal or low free (FT or cFT) testosterone. As shown above, LH concentrations and FSH concentrations were significantly lower in the hypogonadal group. LH levels in our study correlated significantly and positively with FT concentrations ($r=0.287$, $P<0.05$). FT correlated inversely with weight ($r = -0.413$, $P<0.01$) and BMI ($r = -0.382$, $P<0.01$).

Study 2: Comparative study of hypogonadism in type 1 and type 2 diabetes(32)

We examined the testosterone, sex hormone binding globulin (SHBG) and gonadotrophin concentrations in patients with type 1 diabetes and compared with those in age matched type 2 diabetes. Type 1 diabetics had normal total and free testosterone (measured by equilibrium dialysis or calculated using total testosterone and SHBG) concentrations. Both were significantly higher than those in type 2 diabetes (*Table 2*). None of the type 1 diabetics had subnormal total testosterone concentrations while 48% of type 2 diabetic patients had low total testosterone concentrations. 6% of type 1 and 26% of type 2 diabetic patients ($P<0.01$ vs. type 1) had hypogonadism (low free

testosterone concentrations). SHBG concentrations were at the upper end of the normal range and were significantly higher in type 1 diabetics (49.32±2.83 nmol/L) when compared with those in type 2 diabetes (20.44±1.68 nmol/L, *P*<0.001 vs. type 1). Serum LH and prolactin concentrations were in the normal range in type 1 diabetes but were significantly higher than those in type 2 diabetics with hypogonadism. Thus, type 1 diabetics have a normal function of hypothalamicohypophysio-gonadal axis but with elevated SHBG concentrations. In contrast, type 2 diabetics have hypogonadotrophic hypogonadism. The normal but significantly lower prolactin concentrations in type 2 diabetics may point to a further hypothalamico-hypophyseal defect in the type 2 diabetic patients. We conclude that the pathogenesis of frequent hypogonadotrophic hypogonadism in type 2 diabetes is not related to diabetes or hyperglycemia per se but may be related to a combination of insulin resistance and hyperglycemia. ***Table 2***

	Type 1 Diabetes	Type 2 Diabetes	*P* (vs type 1 diabetes)	Type 2 diabetes with HH	*P* (vs type 1 Diabetes)
subjects(n)	50	50		12	
Hypogonadal subjects (%)	3 (6%)	13 (26%)		12 (100%)	
Age (years)	42.78±1.4	43.74±0.8	0.261	42.85±1.4	0.982
BMI (kg/m^2)	26.09±0.75	34.91±1.26	**<0.001**	37.53±3.7	**<0.001**
Total T (nmol/L)	22.97±0.99	11.20±0.60	**<0.001**	6.76±0.65	**<0.001**
Free Testosterone(nmol/L)	0.382±0.025	0.262±0.022	**0.001**	0.144±0.021	**<0.001**
cFT (nmol/L)	0.398±0.019	0.278±0.0.017	**<0.001**	0.171±0.010	**<0.001**
Bioavailable T (nmol/L)	9.28±0.44	6.46±0.43	**<0.001**	4.08±0.19	**<0.001**
LH (IU/L)	4.12±0.28	3.94±0.33	0.39	2.79±0.37	**0.04**
FSH(IU/L)	4.46±0.51	5.57±0.61	0.121	3.84±0.52	0.79
Prolactin(□g/L)	11.21±2.1	6.20±0.54	**<0.001**	5.78±1.13	**0.038**
SHBG (nmol/L)	49.32±2.83	20.44±1.68	**<0.001**	20.43±3.54	**<0.001**
HbA1c%	7.57±0.20	8.40±0.25	**0.024**	8.95±0.52	**0.015**

Study 3: Type 2 diabetes, hypogonadism and inflammation:

We examined the relationship of hsCRP with total testosterone (TT) in 50 subjects with type 2 diabetes mellitus. Hypogonadism was defined as TT < 300 ng/dl. Hs CRP was significantly higher in the hypogonadal (4.6 ±0.8 mg/l) compared to the eugonadal group (2.4 ±0.5 mg/l). There was also an inverse relationship between TT and hsCRP which was significant after adjusting for BMI on multivariate analysis (Figure 2 and 3). These preliminary findings suggest that the hypogonadal type 2 diabetics may have greater inflammatory stress than the eugonadal type 2 diabetic (Hypothesis 2).

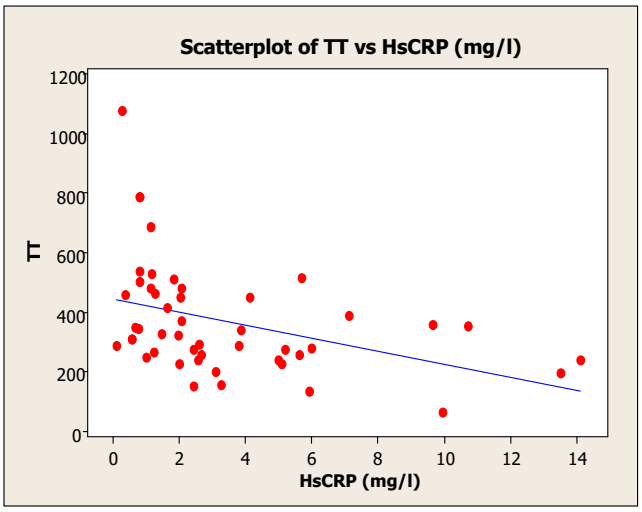


Figure 2: Correlations: HsCRP (mg/l), TT.

Pearson correlation of HsCRP (mg/l) and TT = -0.402. P-Value = 0.005

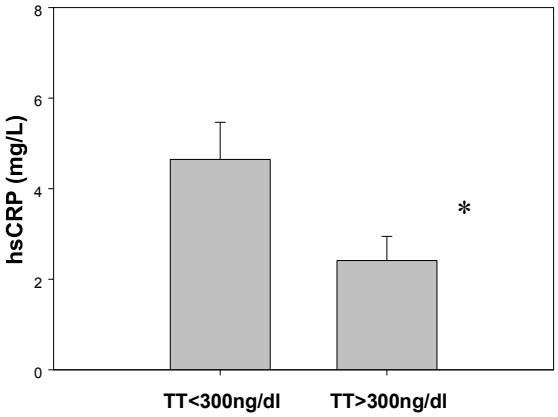


Figure 3: HsCRP (mg/L) in type 2 diabetes

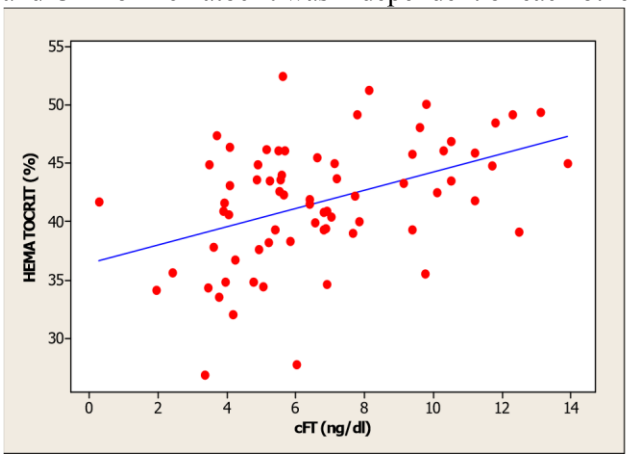
(hypogonadal TT < 300ng/dl vs eugonadal TT > 300ng/dl. * p=0.003 by students t-test

<u>Study 4: Relationship of low testosterone and high CRP to hematocrit in type 2 Diabetes Mellitus.</u>

Background: We explored the biological relevance of HH in another direction by evaluating its effect on hemoglobin/hematocrit since testosterone increases hemoglobin concentrations and the hematocrit.

Methods: In 70 consecutive male patients with type 2 diabetes mellitus (DM), fasting blood samples were collected to measure Hemoglobin, Hematocrit, serum Total Testosterone (TT), Sex Hormone Binding Globulin (SHBG), LH, FSH, Prolactin, glucose, glycosylated hemoglobin and hs C-Reactive protein (CRP). Calculated free Testosterone (cFT) was calculated by the method of Vermuelen et al [described in the grant application]. Hypogonadotophic Hypogonadism (HH) was defined as cFT < 6.5 ng/dl, with normal LH and FSH. Anemia was defined as hemoglobin < 13g/dl or hematocrit (Hct) < 39% as per WHO definition.

Results: cFT concentrations were shown to be significantly related to hematocrit (r = 0.46; p< 0.0001) [Figure 1] and HH patients with type 2 DM had significantly lower hematocrit (40.6 ± 1.1% vs. 43.3 ± 0.72%; p =0.01) than those with normal cFT (figure 2). Hematocrit was also related inversely to CRP (r = -0.41; p < 0.0004) [fig 3]. Anemia was found in 37. 8% of men with CRP > 3mg/L, compared to 15.8 % of men with CRP < 3mg/L (fig 4), consistent with the suppressive effect of chronic inflammation on hematocrit. On multivariate analysis, the effect of testosterone and CRP on hematocrit was independent of each other.



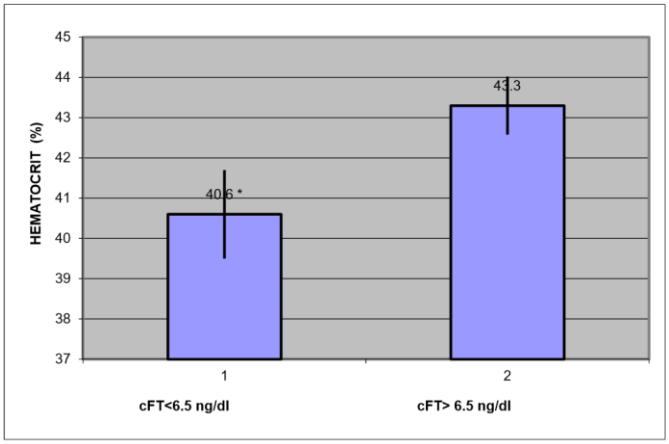


Figure 1: Correlation of Hct with cFT without HH

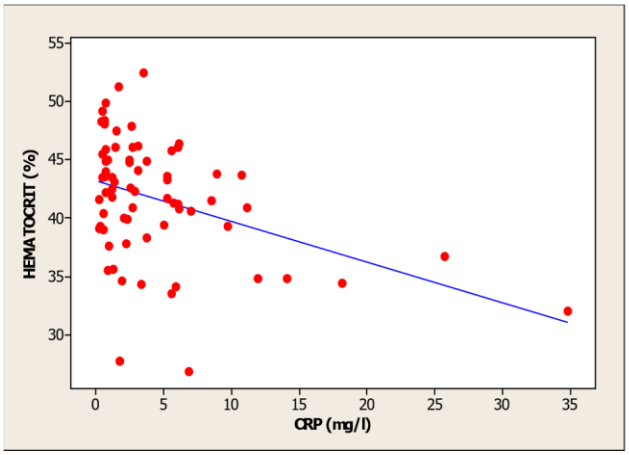


Figure 2: Hct in subjects with and

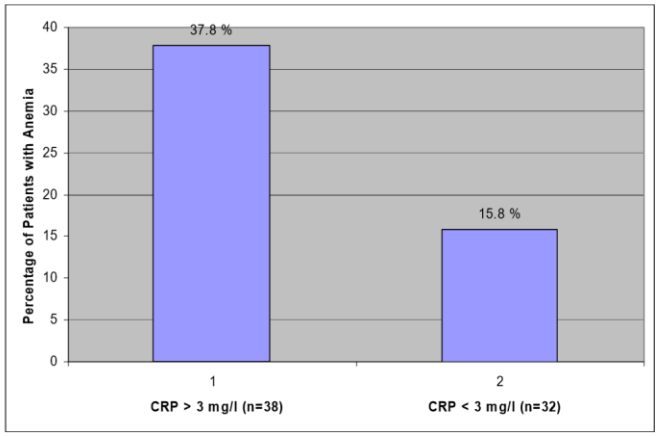
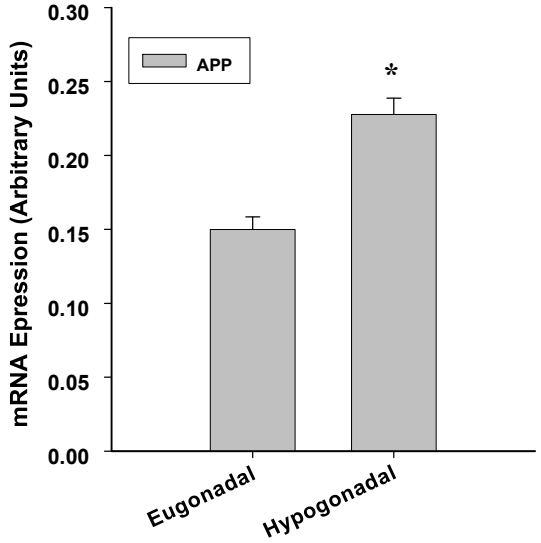


Figure 3: Correlation of Hct with CRP CRP < or > 3mg/L

Figure 4: Anemia in subjects with

Study 5

We therefore hypothesized that the expression of amyloid precursor protein (APP) is increased in patients with type 2 diabetes and hypogonadism. We used the peripheral blood mononuclear cell (MNC) for the expression of APP since we have shown in our previous work that it functions as an excellent representative cell for the assessment of oxidative stress and inflammation. Our preliminary data show that the expression of APP in MNC is significantly increased in diabetic hypogonadal men when compared with either eugonadal diabetic subjects **(Figure 1)**. This increase is consistent with the potential dual increase in the risk of AD as suggested by the concomitant presence of diabetes and hypogonadism. Although the increased expression of APP in MNC does not necessarily represent an increase in the expression of this protein in the brain, it is an indication that the marked pro-inflammatory state of hypogonadism with diabetes is conducive to an increase in APP expression. The significance of the increase in the expression of APP in hypogonadal diabetic men is amplified further by our data showing that T treatment (5g Androgel/day) suppresses the expression of APP **(Figure 2)**. The suppression was observed within 4 weeks of the initiation of T therapy and persisted for the 8 weeks of treatment with T. Clearly, thus, the lack of T in the male results in the propensity to accumulate APP while the replenishment of this hormone reverses this process.



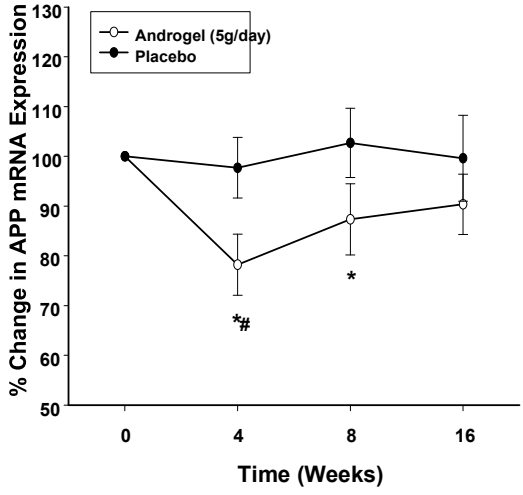


Figure 1: Basal APP mRNA expression in

Figure 2: APP mRNA expression in MNC in hypogonadal

MNC of euogonadal and hypogonadal DM DM patients following 8 weeks of testosterone therapy patients (n=23 vs. 33) * P<0.05 by t-test. (androgel 5g/day) vs. placebo (n=13 vs. 13) * P<0.05 by One Way Repeated Measures ANOVA for comparison versus the # baseline, P<0.05 by Two Way Repeated Measures ANOVA for comparison versus control group.

.

10.3 List any instruments or measurement tools used to collect data (e.g. questionnaire, interview guide, validated instrument, data collection form).

Include copies of these documents with your submission.

Response: Source documents will be used to collect patient information. Patients ,, BP, weight are monitored every visit to ensure safety. The subjects will be questioned for BPH symptoms to estimate the IPSS. .

10.4 Describe any source records that will be used to collect data about subjects (e.g. school records, electronic medical records).

Response: electronic medical records and research files.

10.5 Indicate whether or not **individual** subject results, such as results of investigational diagnostic tests, genetic tests, or incidental findings will be shared with subjects or others (e.g., the subject's primary care physician) and if so, describe how these will be shared.

Response: Individual participant lab results will be disclosed to the participant upon their request. If the participant requests documentation be shared with another physician, physician office or hospital the participant must come to the research center to collect said documentation or the documentation can be mailed to their given home address.

10.6 Indicate whether or not **study** results will be shared with subjects or others, and if so, describe how these will be shared.

Response: Not Applicable. Study results will not be shared with the subjects. However, unidentifiable study results could be published in the form of a manuscript or abstract and will be reported to Abbvie and to clinicaltrilas.gov.

11.0 Study Timelines

11.1 Describe the anticipated duration needed to enroll all study subjects.

Response: 36 months

11.2 Describe the duration of an individual subject's participation in the study. Include length of study visits, and overall study follow-up time.

Response: After the screening visit, subjects who meet the inclusion and exclusion criteria will be expected to participate from 1-24 months depending on which part of the study the participant is randomized into..

11.3 Describe the estimated duration for the investigators to complete this study (i.e. all data is collected and all analyses have been completed).

Response: 60 months

12.0 Setting

12.1 Describe all facilities/sites where you will be conducting research procedures. Include a description of the security and privacy of the facilities (e.g. locked facility, limited access, privacy barriers). Facility, department, and type of room are relevant. Do not abbreviate facility names.

NOTE: Examples of acceptable response may be: "A classroom setting in the Department of Psychology equipped with a computer with relevant survey administration software," "The angiogram suite at Buffalo General Medical Center, a fully accredited tertiary care institution within New York State with badge access," or, "Community Center meeting hall."

Response: Research will be conducted at the Diabetes Endocrinology Research Center of WNY, located at 1000 Youngs Road, Suite 105, Williamsville NY 14221 and at the CTRC located in 875 Ellicott St. Buffalo NY14203. The Diabetes Research Center has facilities and exam rooms available for insulin pump download, CGM device download, meal and infusion studies and presence of study coordinator and registered nurse for data collection and blood work at all times. One of the investigators will be available at all times to address patients' related issues. CTRC location is a fully equipped laboratory with equipment include ultra-low freezers for sample storage, centrifuges, microscopes for sample preparation, infusion pumps, ELISA, PCR and immunoblotting instrumentation.

12.2 For research conducted outside of UB and its affiliates, describe:

- Site-specific regulations or customs affecting the research
- Local scientific and ethical review structure

NOTE: This question is referring to UB affiliated research taking place outside UB, i.e. research conducted in the community, school-based research, international research, etc. It is not referring to multi-site research. UB affiliated institutions include Kaleida Health, ECMC, and Roswell Park Cancer Institute.

Response:

☒ **N/A:** This study is not conducted outside of UB or its affiliates.

13.0 Community-Based Participatory Research

13.1 Describe involvement of the community in the design and conduct of the research.

NOTE: Community-Based Participatory Research (CBPR) is a collaborative approach to research that equitably involves all partners in the research process and recognizes the unique strengths that each brings. CBPR begins with a research topic of importance to the community, has the aim of combining knowledge with action and achieving social change to improve health outcomes and eliminate health disparities.

Response:

☒ **N/A:** This study does not utilize CBPR.

13.2 Describe the composition and involvement of a community advisory board.

Response:

☒ **N/A:** This study does not have a community advisory board.

14.0 Resources and Qualifications

14.1 Describe the qualifications (e.g., education, training, experience, expertise, or certifications) of the Principal Investigator **and** staff to perform the research. When applicable describe their knowledge of the local study sites, culture, and society. Provide enough information to convince the IRB that you have qualified staff for the proposed research.

NOTE: If you specify a person by name, a change to that person will require prior approval by the IRB. If you specify a person by role (e.g., coordinator, research assistant, co-investigator, or pharmacist), a change to that person will not usually require prior approval by the IRB, provided that the person meets the qualifications described to fulfill their roles.

Response: All study personnel are educated, trained, and licensed as required for their delegated role in this study. All study personnel have also received the required university training and will be trained by the PI before the study starts.

Describe other resources available to conduct the research.

14.2 Describe the time and effort that the Principal Investigator and research staff will devote to conducting and completing the research.

NOTE: Examples include the percentage of Full Time Equivalents (FTE), hours per week. The question will elicit whether there are appropriate resources to conduct the research.

Response: The principal investigator supervises the research project and weekly research meetings are conducted to discuss the recruitment rate, resolve and discuss issues related to the conduct, safety, analysis of the study and related publications. PI is expected to spend 5% of his academic time on this research. The co-investigators and study coordinator provide coverage to the research related activity for 365 days a year.

14.3 Describe the availability of medical or psychological resources that subjects might need as a result of anticipated consequences of the human research, if applicable.

NOTE: One example includes: on-call availability of a counselor or psychologist for a study that screens subjects for depression.

Response: Available medical literature will be provided as deemed appropriate or requested by patient through UB libraries, Pubmed, Google scholar as all the investigators have access to medical literature through listed resources above

The patient will also have access to physician (Investigators and Co-Investigators) who will be available to address any adverse effects or other questions during the course of the study who will be available to address any adverse effects or other questions during the course of the study

14.4 Describe your process to ensure that all persons assisting with the research are adequately informed about the protocol, the research procedures, and their duties and functions.

Response: Education through training, meetings, conferences and discussions.

15.0 Other Approvals

15.1 Describe any approvals that will be obtained prior to commencing the research (e.g., school, external site, funding agency, laboratory, radiation safety, or biosafety).

Response:

☒ **N/A:** This study does not require any other approvals.

16.0 Provisions to Protect the Privacy Interests of Subjects

16.1 Describe how you will protect subjects' privacy interests during the course of this research.

NOTE: Privacy refers to an individual's right to control access to him or herself. Privacy applies to the person. Confidentiality refers to how data collected about individuals for the research will be protected by the researcher from release. Confidentiality applies to the data.

Examples of appropriate responses include: "participant only meets with a study coordinator in a classroom setting where no one can overhear", or "the participant is reminded that they are free to refuse to answer any questions that they do not feel comfortable answering."

Response: Our clinical providers involved in the study will identify potential patients for recruitment from the Diabetes-Endocrinology Center of WNY Clinics according to the inclusions and exclusion criteria and through advertisements. Patient who qualify will be asked in private during their one on one consultation time with the physician if they wish to participate in the research study. If the patient agrees, the research coordinator will contact them for a telephone screening privately. The patients who call for potential participation in the study due to advertisement flyers will be screened over the phone with the research coordinator, using our telephone screening form.

When the patient is being seen at our clinics for the first time they sign the "Consent to use and disclosure of protected health information" form which clearly states that their protected health information (PHI) can be used for review in preparation for possible research.

If the patient passes the telephone screening, they will be asked to make an appointment to review and sign the consent. Patient will do this in a private, screen off area of the research department and will be allowed to discuss the consent in detail with the research coordinator and or study doctor. Patient will be no notified that it is completely voluntary to participate in the research study and can withdraw at any time.

We will not be accessing any medical information of the patients for whom the services are not provided by our clinic providers.

16.2 Indicate how the research team is permitted to access any sources of information about the subjects.

NOTE: Examples of appropriate responses include: school permission for review of records, consent of the subject, HIPAA waiver. This question **does apply** to records reviews.

Response: Consent of the subject and partial HIPAA waiver.

17.0 Data Management and Analysis

17.1 Describe the data analysis plan, including any statistical procedures. This section applies to both quantitative and qualitative analysis.

Response: The focus of the proposed research is to evaluate the effect of hypogonadotrophic hypogonadism on atherogenesis, endothelial function, inflammatory mediators and oxidative stress in type 2 diabetic patients and the effect of testosterone replacement on these parameters. The similarities between the study groups, baseline values for subject's demographics, carotid IMT, FMD%, body composition, hematocrit and inflammatory markers will be compared using appropriate parametric tests. Transformations of the data in order to meet statistical assumptions may be considered. The PRIMARY ENDPOINT of the study is to detect a difference in atherogenesis as measured by carotid IMT between type 2 diabetic subjects with and without HH after 24 months of testosterone replacement with Androgel. There are no prior studies looking at these two populations in humans in vivo, however studies assessing the effect of thiazolidinediones (insulin sensitizers with anti-inflammatory properties) on carotid IMT in type 2 diabetics have shown a reduction in IMT by 0.033mm at 12 weeks and 0.054mm at 24 weeks (178). In a study of 154 diabetic patients, serum free testosterone (F-tes) concentrations were found to be inversely correlated with mean IMT. Patients with low concentrations of FT (<10 pg/ml) had greater mean IMT (1.01 ± 0.29 mm) than those with high concentrations of FT(0.91 ± 0.26 mm) (179). On the basis of these previous observations, we have conservatively estimated a difference in carotid IMT of 0.020mm at 24 months between the type 2 DM with HH and the type 2 DM with HH who is on androgel replacement. A sample size of 30 patients per group (assuming a drop-out rate of 5%) will provide adequate power (β = 0.8) to detect a significant difference of 0.020mm in carotid IMT (α = 0.05), provided the standard deviation of the residuals is not equal to or greater than the mean difference. The results will be computed as mean ± SD. Comparisons for endpoints will be made using repeated measures ANOVA, with Tukey's test used for pair wise comparisons. Thus there will be 30 HH type 2 diabetic subjects each in placebo and treatment group of diabetic subjects with HH (total 60 subjects with HH). 20 eugonadal type 2 diabetic subjects will be recruited to serve as controls. SECONDARY END POINTS: The secondary endpoints for the study will be comparison of the relative change from baseline in FMD%, inflammatory mediators, abdominal fat, waist and hip ratio, after either testosterone or placebo. Comparison of these parameters at baseline will also be done. Comparison for each endpoint will be made using ANOVA, with Tukey's test used for pairwise comparisons

17.2 If applicable, provide a power analysis.

NOTE: This may not apply to certain types of studies, including chart/records reviews, survey studies, or observational studies. This question is asked to elicit whether the investigator has an adequate sample size to achieve the study objectives and justify a conclusion.

Response: There are no prior studies looking at these two populations in humans in vivo, however studies assessing the effect of thiazolidinediones (insulin sensitizers with antiinflammatory properties) on carotid IMT in type 2 diabetics have shown a reduction in IMT by 0.033mm at 12 weeks and 0.054mm at 24 weeks (178). In a study of 154 diabetic patients, serum free testosterone (F-tes) concentrations were found to be inversely correlated with mean IMT. Patients with low concentrations of FT (<10 pg/ml) had greater mean IMT (1.01 ± 0.29 mm) than those with high concentrations of FT(0.91 ± 0.26 mm) (179). On the basis of these previous observations, we have conservatively estimated a difference in carotid IMT of 0.020mm at 24 months between the type 2 DM with HH and the type 2 DM with HH who is on androgel replacement. A sample size of 30 patients per group (assuming a drop-out rate of 5%) will provide adequate power (β = 0.8) to detect a significant difference of 0.020mm in carotid IMT (α = 0.05), provided the standard deviation of the residuals is not equal to or greater than the mean difference.

17.3 Describe any procedures that will be used for quality control of collected data.

Response: Three investigators and research nurse will double check the accuracy of collected data. All laboratory testing will be standardized using references and standards.

18.0 Confidentiality

A. Confidentiality of Study Data

Describe the local procedures for maintenance of confidentiality of **study data and any records that will be reviewed for data collection**.

18.1 A. Where and how will all data and records be stored? Include information about: password protection, encryption, physical controls, authorization of access, and separation of identifiers and data, as applicable. Include physical (e.g. paper) **and** electronic files.

Response: All data records will be stored on password protected computers and or in locked cabinets within the research department. These files will only be accessible by authorized study personnel.

18.2 A. How long will the data be stored?

Response: Data and specimens storage has no expiration date and will be stored for a minimum of 7 years. The researchers may continue to rely on this for future use in research study

18.3 A. Who will have access to the data?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens

18.4 A. Who is responsible for receipt or transmission of the data?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens and can handle transfer of data and samples

18.5 A. How will the data be transported?

Response: All data are stored at one location and is not transported unless it is being archived. At that point files will be transferred to Iron Mountain for storage and archiving. Samples that are transported will be done so using dry ice in a properly labeled Styrofoam container by the laboratory technician.

B. Confidentiality of Study Specimens

Describe the local procedures for maintenance of confidentiality of **study specimens**.

☐ **N/A:** No specimens will be collected or analyzed in this research. (Skip to Section 19.0)

18.6 B. Where and how will all specimens be stored? Include information about: physical controls, authorization of access, and labeling of specimens, as applicable.

Response: The data and specimens will be stored in the laboratory located at 1000 Youngs Road, Suite 105, Williamsville NY 14221 and at the CTRC located in 875 Ellicott St. Buffalo NY14203. Samples will be stored in a locked -80^{o} C freezer. Data will be stored on computers that are password protected.

18.7 B. How long will the specimens be stored?

Response: Data and specimens storage has no expiration date and will be stored for a minimum of 7 years. The researchers may continue to rely on this for future use in research study

18.8 B. Who will have access to the specimens?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens.

18.9 B. Who is responsible for receipt or transmission of the specimens?

Response: Those physicians, nurses, and laboratory staff that are on all documentation for the study will have access to the data and specimens and can handle transfer of data and samples

18.10 B. How will the specimens be transported?

Response: All data are stored at one location and is not transported unless it is being archived. At that point files will be transferred to Iron Mountain for storage and archiving. Samples that are transported will be done so using dry ice in a properly labeled Styrofoam container by the laboratory technician

19.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

NOTE: Minimal risk studies may be required to monitor subject safety if the research procedures include procedures that present unique risks to subjects that require monitoring. Some examples include: exercising to exertion, or instruments that elicit suicidality or substance abuse behavior. In such cases, N/A is not an acceptable response.

19.1 Describe the plan to periodically evaluate the data collected regarding both harms and benefits to determine whether subjects remain safe.

Response: The principal investigator Paresh Dandona, MD, PhD and coinvestigators Husam Ghanim, PhD and Manav Batra, MD will review the data every 3 months to assess the safety of the participants. Furthermore they will also assess other risks including the physical, psychological, social, legal and economic harm to these patients. The investigators listed above will carefully watch for any invasion of privacy and breach of confidentiality

1- Testosterone levels, PSA, CBC would be checked periodically as mentioned in study protocol.

2- Dose of Androgel would be adjusted as follows:

If the free testosterone concentration is less than 14ng/dL in subjects randomized to drug group, then the dose of Androgel 1% will be increased to 7.5 grams at week 6 and if still lower than 14 ng/dl at week 10, the dose will be increased to Androgel 1% (10grams) at week 12. If the total testosterone concentration is more than 17ng/dL in subjects randomized to drug group, then the dose of Androgel 1% will be decreased by 2.5 mg starting from week 6.Blood samples will be taken for PSA and subjects will be questioned for BPH symptoms to estimate the IPSS.

The dose of androgel 1% will be decreased if the hematocrit is >50%. If PSA concentration rises by more than 1.5 ng/ml in 12 month period or if subjects complain of symptoms suggestive of severe BPH (IPSS > 19), the subjects will be excluded from the study and referred to their physician. The patient will remain blinded to the study drug or dose.

3- Study subjects would routinely be evaluated for any side effects during study visits as per protocol.

19.2 Describe what data are reviewed, including safety data, untoward events, and efficacy data.

Response:

Safety data: All reports from patients or from diagnostic labs will be reviewed for safety. Any skin reaction to the gel or other side effects reported by patients, laboratory safety tests including PSA or Hct will be documented. Any new FDA or NIH guidelines and warnings regarding the drug and testosterone will be reviewed and adopted.

Efficacy data: The effect of testosterone replacement on IMT, FMD%, body composition, hematocrit, endothelial function, inflammatory mediators and oxidative stress

19.3 Describe any safety endpoints.

Response: This study will not be evaluating any safety endpoints. However safety of all participating subjects will be monitored

19.4 Describe how the safety information will be collected (e.g., with case report forms, at study visits, by telephone calls with participants).

Response: The safety information will be collected at the time of the participants visit, and or during telephone calls with the participant

19.5 Describe the frequency of safety data collection.

Response: The data collection will be done at all study visits which will be at intervals of either one or two weeks depending on the number of study visit. The patients, however, will be asked to report any adverse event or safety related information via phone as soon as it occurs and it will be reviewed the same day.

19.6 Describe who will review the safety data.

Response: The principal investigator Paresh Dandona, MD, PhD and coinvestigators, Manav Batra, MD and Husam Ghanim, PhD will review the data at the completion of all visits by each subject and every 3 months to assess the safety and any potential risks to the participants. Furthermore they will also assess other risks including the physical, psychological, social, legal and economic harm

to these patients. The investigators listed above will carefully watch for any invasion of privacy and breach of confidentiality. The principal investigator will be sharing the results of safety analysis of the study with the sponsor (Abbvie) and with the IRB. The study groups will remain blinded. If there are any safety concerns then co-investigator Husam Ghanim, PhD who is not directly involved with the study participants will unblind the study groups on the discretion of principal investigator and the team will assess potential harm to the patients and inform the IRB and sponsor of this potential harm. The corrective actions will then be taken and research subjects will be withdrawn from the study if risks outweigh the benefits. The IRB will be kept well-informed at all times.

19.7 Describe the frequency or periodicity of review of cumulative safety data.

Response: Safety data will be reviewed every 3 months. Study endpoint data will be reviewed once after half of the recruited patients have completed the study and then at the end of the study

19.8 Describe the statistical tests for analyzing the safety data to determine whether harm is occurring.

Response: The statistical analysis will be carried out using student t-test, Chisquare and Wilcoxson's test for paired data

19.9 Describe any conditions that trigger an immediate suspension of the research.

Response:

1- New information about the safety of the used drug (Testosterone) 2- Sponsor suspension of the funds.
3- Significant high incidence of SAE and events leading to withdrawal of subjects determined based on the continuous review by the investigators.

20.0 Withdrawal of Subjects

☐ **N/A:** This study is not enrolling subjects. This section does not apply.

20.1 Describe **anticipated** circumstances under which subjects may be withdrawn from the research without their consent.

Response: Drug intolerability (severe nausea and/or vomiting) developing any of the exclusionary condition listed in the inclusion and exclusion criteria.

The principal investigator of the study can remove a participant from the research study without their approval if for any reason he/she feels is appropriate, including: severe side effect, injury or medical condition which may place the patient at risk of further complications if patient continues to participate, failure to take the medication as instructed, failure to keep your scheduled appointments, cancellation of the study by the sponsor, or other administrative reasons.

Participation in this research study is voluntary. Subjects have the right to refuse to participate or to withdraw from participation at any time for any reason. Refusal to participate or withdrawal from the study will involve no penalty or loss of entitled benefits, nor affect the subjects ongoing medical care

20.2 Describe any procedures for orderly termination.

NOTE: Examples may include return of study drug, exit interview with clinician. Include whether additional follow up is recommended for safety reasons for physical or emotional health.

Response: If a subject withdraws from the research, the data collected to that point will be used toward the research finding. If applicable the subject will have to bring back any unused research drug. If necessary, they will be asked to complete an end of study visit for their safety.

20.3 Describe procedures that will be followed when subjects withdraw from the research, including retention of already collected data, and partial withdrawal from procedures with continued data collection, as applicable.

Response: If a subject withdraws from the research, the data collected to that point will be used toward the research finding. Efficacy and safety data will continue to be collected for the parts of study that patients agree to participate in.

21.0 Risks to Subjects

21.1 List the reasonably foreseeable risks, discomforts, hazards, or inconveniences to the subjects related to their participation in the research. Consider physical, psychological, social, legal, and economic risks. Include a description of the probability, magnitude, duration, and reversibility of the risks.

NOTE: Breach of confidentiality is always a risk for identifiable subject data.

Response:

1- Subjects will arrive after having fasted overnight. A fasting blood sample will be taken. Blood samples will be drawn by venipuncture (60 mL/ visit). This study requires that blood be drawn by venipuncture. Drawing blood may result in pain, a feeling of faintness, irritation of the vein, bruising, or bleeding at the site of puncture. MRI study should not pose any risks. Testosterone replacement can lead to increases in hemoglobin, liver enzymes and PSA. These will be monitored by lab tests before, during and after the study. Serum testosterone concentrations will be checked during the study and dose adjustments will be made. Mood changes (such as aggressiveness) and an increased incidence of sleep apnea may also occur after testosterone replacement. Patients will be asked to report any changes.

2- There have been some post marketing reports of CVD after testosteorone replacement .FDA has reviewed the data and agreed that the CV signal is weak and advised the need for more randomized studies to identify the risk. We will follow the study subjects closely for any CV event. 3-

4- There have been post marketing reports of clots in legs and lungs, in patients using testosterone products. Subjects would be advised to report symptoms of pain, swelling, warmth and redness in the legs or acute shortness of breath and discontinue treatment with AndroGel 1% and inform doctor immediately.

5- With large doses of exogenous androgens, including AndroGel 1%, sperm production may be suppressed through feedback inhibition of pituitary hormones which could possibly lead to adverse effects on semen parameters including sperm count.

6- Androgens, including AndroGel 1%, may promote retention of sodium and water. Alcohol based products, including AndroGel 1%, are flammable; therefore, patients would be advised to avoid fire, flame or smoking until the AndroGel 1% has dried. Androgel can transfer from body to others. This can happen if other people come in contact with the area where Androgel was applied. This can be harmful to the people who get exposed. Subjects will be explained the precautions to take to avoid this from happening.

7- The vascular or blood vessel studies of the neck and arm should not present any risks. Sodium nitroglycerin that will be used as part of the brachial reactivity studies may cause headache, lightheadedness and a lowering of blood pressure. There may be mild discomfort when the ultrasound probe is placed on neck. Moderate discomfort or pain may result from prolonged blood pressure cuff inflation on forearm.
8- MRI may involve some discomfort as subject has to lie still for several minutes at a time and may feel "closed in" during the procedure. MRI study should not pose any risks as MRI does not use any radiation. Ultrasound again does not use any radiation.
9- Fat tissue aspiration will probably lead to a bruise at the site of aspiration. The site will therefore maybe painful for 1-2 weeks. Subjects are advised to call us if they have a lot of pain or swelling at the site after the procedure. Rarely some people have side effects such as low blood pressure or heart rate and allergic reaction to lidocaine including swelling of the throat.

In addition to the risks listed above, the study drugs and procedures may have unknown, unforeseen or unanticipated side effects, including life-threatening reactions. There is always the possibility that may have a reaction that is currently not known and not expected. It is important for subjects to report all symptoms or reactions to the study doctor as well as to their personal doctor. Subjects will be monitored for side effects by study staff and study doctor may decide to withdrawn from the study as per protocol.

21.2 Describe procedures performed to lessen the probability or magnitude of risks, including procedures being performed to monitor subjects for safety.

Response:
Monitoring of testosterone replacement *: The dose of androgel 1% will be decreased if the hematocrit is >50%. If PSA concentration rises by more than 1.5 ng/ml in 12 month period or if subjects complain of symptoms suggestive of severe BPH (IPSS > 19), the subjects will be excluded from the study and referred to their physician. The patient will remain blinded to the study drug or dose. CBC, CMP, lipid profile would be periodically monitored and necessary adjustments would be made as per study protocol.*

21.3 If applicable, indicate **which procedures** may have risks to the subjects that are currently unforeseeable.

Response: Any adverse effects of Androgel that are not currently known may be some of the unforeseeable risks.

21.4 If applicable, indicate which research procedures may have risks to an embryo or fetus should the subject be or become pregnant.

Response: Not applicable

21.5 If applicable, describe risks to others who are not subjects.

Response: Androgel can transfer from body to others. This can happen if other people come in contact with the area where Androgel was applied. This can be harmful to the people who get exposed. Participants will be explained the precautions to take to avoid this from happening

22.0 Potential Benefits to Subjects

22.1 Describe the potential benefits that individual subjects may experience by taking part in the research. Include the probability, magnitude, and duration of the potential benefits. Indicate if there is no direct benefit.

NOTE: Compensation **cannot** be stated as a benefit.

Response: Testosterone replacement in hypogonadism may improve energy, stamina, decrease adiposity, increase muscle mass, decrease insulin resistance and decrease inflammatory markers.

23.0 Compensation for Research-Related Injury

☐ **N/A:** The research procedures for this study do not present risk of research related injury (e.g. survey studies, records review studies). This section does not apply.

23.1 **If the research procedures carry a risk of research related injury,** describe the available compensation to subjects in the event that such injury should occur.

Response: Routinely, Buffalo General Hospital, Erie County Medical Center, and/or the University at Buffalo, State University of New York, its agents, or its employees do not compensate for or provide free medical care for human subjects/participants in the event that any injury results from participation in a human research project. In the unlikely event that they become ill or injured as a direct result of participating in this study, they may receive medical care, that will be covered by study.

23.2 Provide a copy of contract language, if any, relevant to compensation for research related injury.

NOTE: If the contract is not yet approved at the time of this submission, submit the current version here. If the contract is later approved with **different language regarding research related injury**, you must modify your response here and submit an amendment to the IRB for review and approval.

Response: No contract injury language available.

24.0 Economic Burden to Subjects

24.1 Describe any costs that subjects may be responsible for because of participation in the research.

NOTE: Some examples include transportation or parking.

Response: All research expenses will be covered. Participants will not be subjected to any out of pocket cost.

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

25.0 Compensation for Participation

25.1 Describe the amount and timing of any compensation to subjects, including monetary, course credit, or gift card compensation.

Response:

Men with low testosterone: $25.00 for each blood sample drawn (of which there are 7), $25.00 for carotid and brachial ultrasound (of which there are 5), $ 25.00 for each 24-hour urine collection (of which there are 3) and $25.00 for each MRI (of which there are 3) and $50.00 for each fat biopsy (of which there are 3). Total of up to $600.00 for the study. The study drug will be provided to free of cost. Subjects will not be paid for the screening visit

☐ **N/A:** This study is not enrolling subjects, or is limited to records review procedures only. This section does not apply.

☐ **N/A:** There is no compensation for participation. This section does not apply.

26.0 Consent Process

26.1 Indicate whether you will be obtaining consent.

NOTE: This does not refer to consent documentation, but rather whether you will be obtaining permission from subjects to participate in a research study. Consent documentation is addressed in Section 27.0.

☒ **Yes** (If yes, Provide responses to each question in this Section)
☐ **No** (If no, Skip to Section 27.0)

26.2 Describe where the consent process will take place. Include steps to maximize subjects' privacy.

Response: All participants will come to the research department to be consented. Participants will be placed in a private, screened off area and or room where they can review the consent. Participant questions and or concerns will be address with a member of the study team or research doctor if applicable. The research coordinator will discuss in length the participants requests for privacy of their PHI.

26.3 Describe how you will ensure that subjects are provided with a sufficient period of time to consider taking part in the research study.

NOTE: It is always a requirement that a prospective subject is given sufficient time to have their questions answered and consider their participation. See "SOP: Informed Consent Process for Research (HRP-090)" Sections 5.5 and 5.6.

Response: participants will be made aware that participating in research is completely voluntary, and they may withdraw at any time with no consequence to their routine clinic care. If the patients requires time to decide and or discuss partaking in a research study, the subject will be given said time.

26.4 Describe any process to ensure ongoing consent, defined as a subject's willingness to continue participation for the duration of the research study.

Response: The research coordinator and study team are available to answer any question or concerns with the patient during the duration of the research trial. At each study visit, the patient is asked a series of questions to ensure they are on task with the study visits and feel comfortable. Upon departing from their study visit, the patients are told of their next visit and given detail instruction for their next visit. If study is revised or amendment or new information becomes available

about drug safety that may affect patients participation, the patient may be reconsented to ensure patient ongoing consent,

26.5 Indicate whether you will be following "SOP: Informed Consent Process for Research (HRP-090)." If not, or if there are any exceptions or additional details to what is covered in the SOP, describe:

- The role of the individuals listed in the application who are involved in the consent process
- The time that will be devoted to the consent discussion
- Steps that will be taken to minimize the possibility of coercion or undue influence
- Steps that will be taken to ensure the subjects' understanding

Response:

☒ We have reviewed and will be following "SOP: Informed Consent Process for Research (HRP-090)."

Non-English Speaking Subjects

☒ **N/A:** This study will not enroll Non-English speaking subjects. (Skip to Section 26.8)

26.6 Indicate which language(s) other than English are likely to be spoken/understood by your prospective study population or their legally authorized representatives.

NOTE: The response to this Section should correspond with your response to Section 6.4 of this protocol.

Response:

26.7 If subjects who do not speak English will be enrolled, describe the process to ensure that the oral and written information provided to those subjects will be in that language. Indicate the language that will be used by those obtaining consent.

NOTE: Guidance is provided on "SOP: Informed Consent Process for Research (HRP-090)."

Response:

Cognitively Impaired Adults

☒ **N/A**: This study will not enroll cognitively impaired adults. (Skip to Section 26.9)

26.8 Describe the process to determine whether an individual is capable of consent.

Response:

Adults Unable to Consent

☒ **N/A**: This study will not enroll adults unable to consent.
(Skip to Section 26.13)

When a person is not capable of consent due to cognitive impairment, a legally authorized representative should be used to provide consent (Sections 26.9 and 26.10) **and, where possible, assent of the individual should also be solicited** (Sections 26.11 and 26.12).

26.9 Describe how you will identify a Legally Authorized Representative (LAR). Indicate that you have reviewed the "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" for research in New York State.

NOTE: Examples of acceptable response includes: verifying the electronic medical record to determine if an LAR is recorded.

Response:

☐ We have reviewed and will be following "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)."

26.10 **For research conducted outside of New York State**, provide information that describes which individuals are authorized under applicable law to consent on behalf of a prospective subject to their participation in the research. One method of obtaining this information is to have a legal counsel or authority review your protocol along with the definition of "legally authorized representative" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)."

Response:

26.11 Describe the process for **assent of the adults**:

- Indicate whether assent will be obtained from all, some, or none of the subjects. If some, indicate which adults will be required to assent and which will not.

Response:

- If assent will not be obtained from some or all subjects, provide an explanation of why not.

Response:

26.12 Describe whether **assent of the adult** subjects will be documented and the process to document assent.

NOTE: The IRB allows the person obtaining assent to document assent on the consent document using the "Template Consent Document (HRP-502)" Signature Block for Assent of Adults who are Legally Unable to Consent.

Response:

Subjects who are not yet Adults (Infants, Children, and Teenagers)

☒ **N/A**: This study will not enroll subjects who are not yet adults. (Skip to Section 27.0)

26.13 Describe the criteria that will be used to determine **whether a prospective subject has not attained the legal age for consent to treatments or procedures involved in the research** under the applicable law of the jurisdiction in which the research will be conducted **(e.g., individuals under the age of 18 years)**. For research conducted in NYS, review "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)" to be aware of which individuals in the state meet the definition of "children."

NOTE: Examples of acceptable responses include: verification via electronic medical record, driver's license or state-issued ID, screening questionnaire.

Response:

26.14 **For research conducted outside of New York State**, provide information that describes which persons have not attained the legal age for consent to treatments or procedures involved the research, under the applicable law of the jurisdiction in which research will be conducted. One method of obtaining this information is to have a legal counsel or authority review your protocol along the definition of "children" in "SOP: Legally Authorized Representatives, Children, and Guardians (HRP-013)."

Response:

26.15 Describe whether parental permission will be obtained from:

Response: N/A

☐ One parent even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.

☐ Both parents unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

☐ Parent permission will not be obtained. A waiver of parent permission is being requested.

NOTE: The requirement for parent permission is a protocol-specific determination made by the IRB based on the risk level of the research. For guidance, review the "CHECKLIST: Children (HRP-416)."

26.16 Describe whether permission will be obtained from individuals **other than parents**, and if so, who will be allowed to provide permission. Describe your procedure for determining an individual's authority to consent to the child's general medical care.

Response:

26.17 Indicate whether assent will be obtained from all, some, or none of the **children**. If assent will be obtained from some children, indicate which children will be required to assent.

Response:

26.18 When assent of children is obtained, describe how it will be documented.

Response:

27.0 Waiver or Alteration of Consent Process

Consent will not be obtained, required information will not be disclosed, or the research involves deception.

☒ **N/A:** A waiver or alteration of consent is not being requested.

27.1 If the research involves a waiver or alteration of the consent process, please review the "CHECKLIST: Waiver or Alteration of Consent Process (HRP410)" to ensure that you have provided sufficient information for the IRB to make the determination that a waiver or alteration can be granted.

NOTE: For records review studies, the first set of criteria on the "CHECKLIST: Waiver or Alteration of Consent Process (HRP-410)" applies.

Response:

27.2 If the research involves a waiver of the consent process for planned emergency research, please review the "CHECKLIST: Waiver of Consent for Emergency Research (HRP-419)" to ensure you have provided sufficient information for the IRB to make these determinations. Provide any additional information necessary here:

Response:

28.0 Process to Document Consent

☐ **N/A:** A Waiver of Consent is being requested.
(Skip to Section 29.0)

28.1 Indicate whether you will be following "SOP: Written Documentation of Consent (HRP-091)." If not or if there are any exceptions, describe whether and how consent of the subject will be obtained including whether or not it will be documented in writing.

NOTE: If your research presents no more than minimal risk of harm to subjects and involves no procedures for which written documentation of consent is normally required outside of the research context, the IRB will generally waive the requirement to obtain written documentation of consent. This is sometimes referred to as 'verbal consent.' Review "CHECKLIST: Waiver of Written Documentation of Consent (HRP-411)" to ensure that you have provided sufficient information.

 If you will document consent in writing, attach a consent document with your submission. You may use "TEMPLATE CONSENT DOCUMENT (HRP-502)". If you will obtain consent, but not document consent in writing, attach the script of the information to be provided orally or in writing (i.e. consent script or Information Sheet).

Response:

☒ We will be following "SOP: Written Documentation of Consent" (HRP-091).

29.0 Multi-Site Research (Multisite/Multicenter Only)

☒ **N/A:** This study is not an investigator-initiated multi-site study. This section does not apply.

29.1 If this is a multi-site study **where you are the lead investigator**, describe the processes to ensure communication among sites, such as:

- All sites have the most current version of the IRB documents, including the protocol, consent document, and HIPAA authorization.
- All required approvals have been obtained at each site (including approval by the site's IRB of record).
- All modifications have been communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.
- All engaged participating sites will safeguard data as required by local information security policies.
- All local site investigators conduct the study appropriately.
- All non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy.

Response:

29.2 Describe the method for communicating to engaged participating sites:

- Problems
- Interim results
- Study closure

Response:

29.3 Indicate the total number of subjects that will be enrolled or records that will be reviewed across all sites.

Response:

29.4 If this is a multicenter study for which UB will serve as the IRB of record, and subjects will be recruited by methods not under the control of the local site (e.g., call centers, national advertisements) describe those methods.

Response:

30.0 Banking Data or Specimens for Future Use

☐ **N/A:** This study is not banking data or specimens for future use or research outside the scope of the present protocol. This section does not apply.

30.1 If data or specimens will be banked (stored) for **future use, that is, use or research outside of the scope of the present protocol**, describe where the data/specimens will be stored, how long they will be stored, how the

data/specimens will be accessed, and who will have access to the data/specimens.

NOTE: Your response here must be consistent with your response at the "What happens if I say yes, I want to be in this research?" Section of the Template Consent Document (HRP-502).

Response: The study data/specimens will be stored in a locked closet or 80 freezer at the research facility of the Diabetes and Endocrinology Center of WNY for up to 7 years.

The research staff (study personnel including coordinator) only will be authorized to access data and or specimens

30.2 List the data to be stored or associated with each specimen.

Response: Patient ID number, study visit information and date of collection will be stored with specimen. Other data stored will include record files of all patients participating in the study, including data collection sheets andlab results.

30.3 Describe the procedures to release banked data or specimens for future uses, including: the process to request a release, approvals required for release, who can obtain data or specimens, and the data to be provided with specimens.

Response: The copy of the individual patient data collected during the study period will be provided to these individual patients who can choose to hand carry it to their respective physicians and a copy will be faxed to their respective clinical providers upon verbal request from the patient. The data provided will include the insulin pump or CGM data or any of the lab results obtained during the study period. The results of the completed study will be made available to the patients if requested through published manuscript.

31.0 Drugs or Devices

☐ **N/A:** This study does not involve drugs or devices. This section does not apply.

31.1 If the research involves drugs or devices, list and describe all drugs and devices used in the research, the purpose of their use, and their regulatory approval status.

Response:

Investigational product	**Dosage form and strength**	**Approval status**	**Manufacturer**
Androgel 1% (50grams)	gel	Approved for testosterone treatment	Abbive
Matching placebo for Androgel	gel	N/A	Abbive

31.2 Describe your plans to store, handle, and administer those drugs or devices so that they will be used only on subjects and be used only by authorized investigators.

Response: Drugs will be stored in a locked cabinet and temperature controlled refrigerator at 4C at the research facility of the Diabetes and Endocrinology Center of WNY.

If the drug is investigational (has an IND) or the device has an IDE or a claim of abbreviated IDE (non-significant risk device), include the following information:

31.3 Identify the holder of the IND/IDE/Abbreviated IDE.

Response: IND holder: Dr Paresh Dandona. FDA has issued IND exempt and this is on file with IRB

31.4 Explain procedures followed to comply with FDA sponsor requirements for the following:

	Applicable to:		
FDA Regulation	**IND Studies**	**IDE studies**	**Abbreviated IDE studies**
21 CFR 11	**X**	**X**	
21 CFR 54	**X**	**X**	
21 CFR 210	**X**		
21 CFR 211	**X**		
21 CFR 312	**X**		
21 CFR 812		**X**	**X**
21 CFR 820		**X**	

Response: All FDA sponsor requirements have been reviewed and will be followed during the study procedures.

32.0 Humanitarian Use Devices

☒ **N/A:** This study does not involve humanitarian use devices. This does not apply.

32.1 For Humanitarian Use Device (HUD) uses provide a description of the device, a summary of how you propose to use the device, including a description of any screening procedures, the HUD procedure, and any patient follow-up visits, tests or procedures.

Response:

32.2 For HUD uses provide a description of how the patient will be informed of the potential risks and benefits of the HUD and any procedures associated with its use.

Response: