NICHD Human Subjects Research Protocol

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To: Karim Calis, PharmD, Chair, IRB, NICHD

Recommended by: _____, Branch Chief, PEMG, NICHD

_____, Clinical Director, NICHD

_____, Clinical Director, NHLBI

_____, Clinical Director, NIDDK

_____, Clinical Director, CC

Subject: Protocol Amendment:

Pilot study of the effects of colchicine in non-diabetic adults with metabolic syndrome

Identifying words:

- *Disease(s):* Metabolic syndrome, obesity, dyslipidemia, inflammation
- *Population*: Non-diabetic adults with Metabolic Syndrome
- *Other*: colchicine, NLRP3, cryopyrin, NALP3

Principal Investigator: *Jack A. Yanovski, MD, PhD SGO/DTM/NICHD

Lead Associate Investigator: *Andrew Demidowich, MD OCD/NICHD

Accountable Investigator: Jack A. Yanovski, MD, PhD SGO/DTM/NICHD

*May obtain consent from participants

Study type (check all that apply):
_____Archived biological specimens/medical information
_____Natural history; definition of phenotype, genotype/phenotype correlation
___x___Prospective linkage/gene identification, NOT providing information to participants
_____Prospective linkage/gene identification, providing information provided to participants
_____Social science; assessments of knowledge, attitudes and behavior
_____Genetic counseling
___x___Drugs or devices
_____Gene transfer
_____Other interventions

Estimated Duration of study: 3 years

Subjects of Study:

Description	**Number**	**Sex**	**Age**
Adults screened for protocol	250	M, F	≥ 18
- Screened but not enrolled	163		
- Non-diabetic obese adults with metabolic syndrome randomized to colchicine or placebo	40		
- Studied, but not randomized (Evaluation-only)	40		
- Open-Label T2DM arm	7		

Project uses ionizing radiation: Yes
Medically indicated or research? Research

Project involves use of durable power of attorney: No

Offsite project: No

Research participants to be seen at:
___x___NIH only*
_____Off-site only
_____Both NIH* and off-site
**Includes participants who physically come to the NIH Clinical Center and/or for whom specimens/data are analyzed by Clinical Center departments. If participants will be seen at NIH, a Medical Advisory Investigator must be indicated on the 1195 form, unless this is a social science project with no clinical interventions.*

Collaborative Project: No.

Précis:

Obesity affects one-third of the adult U.S. population and is a major risk factor for the development of type 2 diabetes and cardiovascular disease. Mouse models and human data suggest that obesity-induced chronic inflammation is one mechanism promoting obesity-associated comorbid conditions. In obesity, innate immunity is activated by circulating molecules such as fatty acids and cholesterol crystals bind to nucleotide-binding oligomerization (NOD)-like receptor family, pyrin domain containing 3 (NLRP3) receptors in adipocyte tissue macrophages (ATMs). This binding stimulates NLRP3 oligomerization, inflammasome formation, and proinflammatory cytokine activation. The resultant inflammatory cascade leads to insulin resistance and decreased pancreatic beta-cell reserve. It has been proposed that the suppression of this chronic low-level inflammatory state may impede the onset of diabetes and cardiovascular disease.

Recent studies have shown colchicine, a potent microtubule inhibitor commonly used for the treatment of gout and some rare inflammatory conditions, disrupts intracellular localization of NLRP3, thereby blocking inflammasome assembly. As there are limited medical therapies proven effective to improve obesity-related metabolic dysregulation, we propose to determine the efficacy of colchicine 0.6 mg twice daily in non-diabetic obese adults with metabolic syndrome. We will conduct a randomized, double-blinded, placebo-controlled pilot trial of colchicine in forty subjects. We will study changes in insulin resistance, beta-cell reserve, and systemic inflammation. Using adipose tissue obtained from biopsies, we will also study colchicine's local effects on inflammation and insulin resistance. Should results prove promising, this pilot study will allow determination of the sample size needed for an adequately powered study of the effects of colchicine in obese adults with metabolic syndrome.

Seven patients with type 2 diabetes will be given open-label colchicine and followed as described above. We also plan to perform baseline evaluations on 40 subjects who are not eligible for the treatment protocol. This group will consist of non-obese adults, obese adults who are not insulin-resistant, and adults with diet-controlled type 2 diabetes.

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Introduction:

Obesity is a serious disorder that affects approximately one-third of the adult U.S. population and is a major risk factor in the development of type 2 diabetes (T2DM), hypertension, dyslipidemia, and cardiovascular disease.[1-3] The pathophysiology underlying the relationship between obesity, insulin resistance, and cardiovascular disease is not fully understood, but numerous studies give evidence that inflammation plays a prominent role.

Mouse models of diet-induced and genetically-induced obesity show increased levels of inflammatory cytokines, including interleukin (IL)-1β, and IL-18.[4] Further, injecting wild-type mice with IL-1β decreases insulin sensitivity, whereas IL-1β null mice are protected from insulin resistance after a 12 week high fat diet (HFD)[5].

Cross-sectional human studies also implicate inflammation in the development of insulin resistance and cardiovascular disease. Data from NHANES III indicate that inflammation is positively correlated with BMI[6] and the presence of metabolic syndrome (MetS). Individuals with MetS had more inflammation than those without MetS. Higher C-reactive protein (CRP) levels were seen with increasing components of MetS. Inflammatory cytokine levels, such as IL-1β, IL-6, and IL-18, were also positively correlated with BMI.[7] In the Women's Health Initiative trial, elevated levels of hsCRP and IL-6 were associated with increased risk for T2DM and vascular events in apparently healthy postmenopausal women.[8,9] Conversely a reduction in body weight, either via lifestyle modification or bariatric surgery, lead to decreased levels of inflammation and insulin resistance.[10,11] Other large cross-sectional studies have also confirmed a relationship between inflammation and metabolic abnormalities[12,13] and the development of coronary artery disease.[14-17]

The sources of the chronic low-grade inflammation of obesity arise from multiple organs, but it appears that adipose tissue (AT) itself is a large contributor to the inflammatory process. AT consists of adipocytes and the stromal vascular fraction (SVF), which includes fibroblasts, vascular tissue, and adipose tissue macrophages (ATM). In lean individuals, macrophages comprise less than 10% of the cells in AT.[18] The fat cells themselves are smaller in volume and secrete higher levels of adiponectin and omega-3 fatty acids, which predispose the macrophages to differentiate to an anti-inflammatory M2 state. M2 macrophages release immunosuppressive cytokines, such as IL-10 and TGFβ, which act on fat and muscle tissue to enhance insulin sensitivity.[19] However, in obese individuals, adipocytes are both greater in volume and number, leading to inefficient triglyceride storage and increased levels of circulating fatty acids and ceramides.[20] These molecules attach to the pattern recognition receptors in ATMs, which stimulate differentiation to the pro-inflammatory M1 phenotype.[21] Chronic inflammation ensues, with an increased release of chemokines and cytokines including TNFα, IL-1β, IL-6, IL-18, plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), and reactive oxygen species (ROS).[21-23] These

factors further augment adipose tissue inflammation by recruiting additional ATMs as well as B and T cells.[24]

The resultant inflammation is thought to lead to insulin resistance through several concomitant pathways. GLUT4 mediates glucose uptake in adipocytes and skeletal muscle and is integral in glucose homeostasis. In insulin resistant (IR) states, GLUT4 expression in AT is downregulated, and insulin-stimulated GLUT4 transport to the plasma membrane is impaired.[25] Additionally, the chronic inflammatory state leads to serine phosphorylation and deactivation of insulin receptor substrate-1 (IRS-1), an important downstream mediator of the insulin receptor, by IKK-β.[26] IRS-1 expression is downregulated as well. Interestingly, Lumeng et al observed that in co-cultured adipocyte and macrophage cells this phenomenon was partially reversed when treated with anti-inflammatory TNFα neutralizing antibodies.[25]

Moreover, low-grade systemic inflammation can impair pancreatic islet cell functioning and insulin secretion. In early stages of β-cell dysfunction, inflammatory cytokines disrupt the proper intracellular calcium storage and flux necessary for adequate insulin secretion.[27,28] Chronically, inflammatory signaling via the NF-kB and mitogen-activated protein kinase (MAPK) pathways leads to mitochondrial stress, reactive oxygen species (ROS) formation, and eventual β-cell apoptosis.[29,30]

NLRP3 mediates inflammatory activation in obesity

Leukocytes express pattern recognition receptors on their cell surface which allow for induction of a rapid inflammatory response to pathogen-associated molecular patterns. These components of the innate immune system, allow for rapid activation of cell-signaling pathways and production of a pro-inflammatory cytokine cascade in response to infection. Similarly, members of the NOD-like receptor (NLR) family can recognize endogenous molecules (e.g. free fatty acids, ceramides, urate crystals, ATP) released during cell damage and stimulate a robust inflammatory response.[31]

A member of the NLR family, the NLRP3 (Nod-like receptor family, pyrin domain containing 3) inflammasome has been implicated as the major source of chronic inflammation seen in obesity. NLRP3 (also known as cryopyrin or NALP3) is primarily expressed in monocytes and macrophages, with little production seen in adipocytes or other leukocytes.[11,32] Upon binding with a "danger signal," NLRP3 receptors oligomerize in the cytosol with procaspase-1 via the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain; also known as PYCARD). The formation of this inflammasome autocatalytically cleaves the cysteine protease procaspase-1 to its active form, caspase-1, which in turn cleaves inactive pro-IL-1 and pro-IL-18 into their active forms.[33] IL-1β initiates the acute phase response and triggers the production and secretion of other pro-inflammatory cytokines, such as TNFα,

IL-6, and MCP-1, resulting in a progressively amplified cytokine network.[34] IL-18 also induces the production of a number of cytokines, chemokines, and cell-adhesion molecules.[35]

Obesity and T2DM are associated with elevated circulating levels of danger-associated molecular patterns (DAMPs), such as free fatty acids and ceramides.[36,37] When human macrophages are cultured with saturated fatty acids, IL-1β and IL-18 production increases, but such increases can be abrogated in macrophages knocked out for NLRP3, ASC, or caspase-1. However, knocking out a different Nod-like receptor, NLRC4, has no effect.[5] Likewise, ceramides, derived from the metabolism of long-chain saturated fatty acids, also activate caspase-1 and IL-1β production in human wild-type macrophages, but not in NLRP3-knockouts.[11]

In vivo mouse models demonstrated similar results. *Nlrp3* and *IL1b* expression in AT is positively correlated with body weight and adiposity in mice, and food restriction results in significant decreases in their expression.[11] Knocking out components of the NLRP3 inflammasome (e.g. *Nlrp3*$^{-/-}$, *ASC*$^{-/-}$, *caspase-1*$^{-/-}$ and *lL1b*$^{-/-}$ mice) does not affect baseline glucose metabolism and insulin sensitivity, but prevents the development of insulin resistance and inflammatory cytokine expression during a protracted HFD state.[4,5,11,38] Additionally, ablation of the NLPR3 inflammasome results in markedly smaller adipocytes, elevated levels of adipose GLUT4 and adiponectin, with decreased inhibition of IRS-1.[11,39] Caspase-1 knockouts also demonstrate increased fat oxidation and gain significantly less bodyweight than wild type mice during a 10-week HFD regimen, despite similar food intake.[4] Interestingly no changes in triglyceride or cholesterol levels are seen in obese *Nlrp3* knockout mice.[11]

In humans, metabolically unhealthy obese individuals have greater levels of NLRP3 activity, IL-1β expression, AT macrophages, and regulatory T cells (T_{regs}) in their visceral adipose tissue (VAT) than either metabolically healthy obese or lean individuals. Weight loss in obese subjects with T2DM leads to enhanced insulin sensitivity and decreased mRNA expression of *IL1B* and *NLRP3* in subcutaneous adipose tissue (SAT).[11]

Other circulating molecules seen in models of diet-induced obesity have been found to be potent NLRP3 activators. Oxidized low-density lipoprotein (LDL) and cholesterol crystals increase NLRP3 inflammasome formation in mice, leading to macrophage activation and IL-1β secretion. Conversely, ablation of the inflammasome reduces early atherosclerosis in high cholesterol diet-fed mice.[40] Monosodium urate (MSU) crystals, seen in gout and obesity, can also act as a danger signal by stimulating the NLRP3 inflammasome.[41,42]

The progression from insulin resistance to T2DM involves pancreatic inflammation, destruction, and reduced pancreatic β-cell reserve.[43,44] In a study by Youm, et al, chronically DIO mice had greater levels of inflammation, islet fibrosis, and β-cell death than their lean chow-fed littermates, whereas DIO *Nlrp3*$^{-/-}$ mice were protected from these untoward effects. Consistent with this finding, the DIO *Nlrp3*$^{-/-}$mice had lower

fasting glucose and higher fasting insulin levels than their WT counterparts, suggesting that NLRP3 plays an important role in both early (i.e. peripheral insulin resistance) and late (i.e. pancreatic beta cell destruction) stages of metabolic dysfunction.[38]

Anti-inflammatory agents in obesity

If inflammation is the root cause of obesity-related metabolic dysregulation, then anti-inflammatory agents should show benefit. This has been the case for some mouse models of obesity. HFD mice treated with a neutralizing IL-1β antibody demonstrate significant improvement of glycemic control and beta cell function,[29] with no effect on weight gain or food intake.[45] Given prophylactically, IL-1β antibody treatment helps prevent the onset of fasting hyperglycemia and insulin resistance in HFD mice. IL-1 receptor antagonist (IL-1Ra) treatment of DIO mice leads to similar results, with decreased β-cell apoptosis, increased β-cell proliferation, and improved glucose-stimulated insulin secretion.[45,46] Studies inducing blockade of other inflammatory cytokines or pathways in mice, including TNFα[47], IL-6[48], MCP-1[49], and NF-kB[50] have also demonstrated favorable metabolic results from reduction of inflammatory processes.

However, such robust results have not been replicated in human clinical trials. Blockade of TNFα had no effect on metabolic parameters T2DM participants[47] and only marginal impact in non-diabetic insulin resistant subjects.[51] Similar findings were seen when using aspirin or the IL-1β antibody canakinumab.[52-54] A different monoclonal IL-1β antibody, gevokizumab, has led to improvements in HbA1c, but not beta-cell secretory function or insulin sensitivity in preliminary trials.[55] Only anakinra, a recombinant human IL-1Ra, has demonstrated any significant benefit, with an improvement in beta-cell secretory function and decrease in Hemoglobin A1c in diabetic subjects,[56] but not in subjects with impaired glucose tolerance.[57] Furthermore, anakinra did not affect insulin sensitivity as measured by insulin clamp or HOMA-IR in either study.

While these results are disappointing, they are not wholly unexpected. The machinery involved in obesity-induced chronic inflammation is complex. Cytokines may play complementary, if not redundant, roles in promoting inflammation. Chemokines and endothelial adhesion molecules play integral roles in the sustained inflammatory response by facilitating chemoattraction, diapedesis, and migration of additional leukocytes to adipose and pancreatic tissues. For this reason, eliminating a single cytokine may not be sufficient to demonstrate significant changes in glucose homeostasis.

Colchicine in preclinical trials

Colchicine remains an intriguing treatment for obesity-associated chronic inflammation because it mitigates inflammation through several mechanisms. Colchicine is a tricyclic alkaloid, extracted from the

flowering plants *Colchicum autumnale* and *Gloriosa superba*.[58] It has been used to treat gout for over 2000 years, although the purified form was brought to prominence at the end of the 19th century.[59] Colchicine exerts its effects by binding in a poorly reversible manner to tubulin, thereby interfering with microtubule formation and elongation. At higher concentrations colchicine promotes depolymerization of microtubules.[60]

Classically, colchicine was thought to exert its anti-inflammatory actions by inhibiting neutrophil chemotaxis and diminishing release of lysosomal enzymes during phagocytosis. Colchicine also decreases leukocyte recruitment by blocking the production and/or release of chemotactic molecules leukotriene B4 and crystal-derived chemotactic factor (CCF).[61] Leukocyte recruitment is further impaired by the inhibition of adhesion molecules, such as E-selectin and P-selectins on endothelial cells, L-selectin on leukocytes, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1)[61,62]. Surface expression of the TNFα receptor in macrophages is also reduced with colchicine, as is neutrophil superoxide anion production.[63,64] Recent studies also suggest that colchicine may modulate expression of genes involved in inflammation or cell migration.[65,66]

Notably, **oligomerization and activation of the NLRP3 inflammasome is disrupted by colchicine**. Microtubules mediate NLRP3 inflammasome formation by transporting ASC and NLRP3 into close proximity to each other in the cell, and inhibition of microtubule formation by colchicine has been shown to significantly inhibit NLRP3 activation in macrophages both in vitro and in vivo.[41,67]

Colchicine in human studies

Colchicine is currently FDA-approved for the treatment of gout and Familial Mediterranean Fever,[68] and has shown efficacy in other inflammatory conditions.[69-71] As inflammation has been hypothesized to play a role in the development of cardiovascular disease, several clinical studies have recently examined the efficacy of colchicine in cardiovascular-related conditions. Colchicine 0.5 mg twice daily decreased hsCRP levels after one month of treatment in subjects with stable coronary artery disease already on a statin and aspirin,[72] although this was not seen in patients with acute coronary syndrome or acute stroke.[73] Subjects with paroxysmal atrial fibrillation, randomized to colchicine after radiofrequency ablation, had decreased atrial fibrillation recurrence at 3 months and more substantial post-treatment decrements in hsCRP and IL-6 levels than those randomized to placebo.[74] Colchicine has also demonstrated considerable benefit in reducing incidence of recurrent pericarditis[75-77] and post-pericardectomy syndrome.[78,79]

Notably, colchicine has great promise preventing acute coronary syndrome in high risk individuals. Among the 532 subjects with stable coronary artery disease on a statin and aspirin and/or clopidogrel, 30% of whom had Type 2 diabetes mellitus (T2DM), those randomized to colchicine demonstrated a significantly decreased primary composite endpoint of acute coronary syndrome, out-of-hospital cardiac arrest or stroke at

3 years as compared to placebo.[80] Another study examined restenosis rate in patients with T2DM undergoing percutaneous coronary intervention with bare metal stent placement. Those randomized to colchicine had significantly fewer restenotic events and less neointimal hyperplasia as compared to subjects taking placebo.[81] Subjects in these studies, many of whom had features of the metabolic syndrome, tolerated colchicine well, with the most common side effect being diarrhea/gastrointestinal disturbances (7 – 16%).

In addition to predisposing to cardiovascular disease, inflammation is thought to play a role in insulin resistance. However, colchicine's effect on glucose or insulin in individuals with metabolic dysregulation is unknown. Early studies on colchicine suggested that it worsened glucose control by inhibiting early and late phase insulin secretion. However, these studies were done in healthy adults, and the treatment duration was less than two weeks.[82,83] In subjects with Familial Mediterranean Fever chronically on colchicine for 2-13 years, data from frequently-sampled intravenous glucose tolerance testing (FSIVGTT) and oral glucose tolerance testing did not demonstrate any differences in fasting insulin or glucose levels, early-phase insulin release, or glucose dynamics, as compared with age-matched healthy volunteers.[84] Additionally, neither a Cochrane Database review nor the Colcrys prescriber information insert show hyperglycemia as a potential adverse effect of colchicine use.[68,85]

By blocking NLRP3 inflammasome formation and cytokine production, a plausible mechanism exists by which colchicine could improve glucose homeostasis and insulin resistance in obese individuals who have evidence for inflammation. Improvement in metabolic dysregulation may also improve cardiovascular risk factors in individuals with MetS, although this has not yet been formally evaluated using colchicine. Consequently, colchicine is an intriguing treatment option for metabolic dysregulation and warrants investigation to determine its potential health benefits in obese insulin-resistant individuals. The results of such a study also have the potential to advance our knowledge regarding the interplay of inflammation and metabolic dysregulation in man.

Adipocyte Ex-Vivo Studies

Whole body insulin-mediated glucose uptake depends primarily on glucose uptake in three organ systems: liver, adipose, and muscle. Upon binding to the insulin receptors in peripheral tissues, insulin signaling increases the number of insulin-stimulated glucose transporters (GLUT4) at the cell surface and allows augmented glucose translocation across adipocyte and muscle plasma membranes. However, most research on the kinetics of insulin signaling pathways and the studies that examine how inflammation perturbs these pathways have been performed largely in either non-human models[86-88] or cultured 3T3-L1 mouse cells.[89-91] Preliminary studies have shown that these kinetic models are inadequate to capture insulin dynamics at the level of the *human* adipocyte because they do not recapitulate the multiple phases of GLUT4 trafficking. While *in vivo* human studies would serve as the most appropriate representation of insulin

kinetics, the logistics of these measurements make them unrealistic. Therefore, *ex vivo* experiments may instead serve as a more reasonable portrayal of the kinetics of the adipocyte's response to insulin within obese insulin-resistant subjects with chronic inflammation.

Thus, collaborating with the NICHD Section on Membrane and Cellular Biophysics as a part of the NICHD Director's Investigator Award, we have initiated investigations of the effects of colchicine on insulin sensitivity locally in the subcutaneous adipose tissue. Dr. Chad McCormick, a member of this collaboration, is examining insulin kinetics in coded adipocyte biopsy samples obtained from our subjects by creating a novel reporter system in which we transfect subjects' adipocytes with a HA-pHTomato-GLUT4-mEGFP plasmid to quantify GLUT4 translocation to the cell surface after maximal insulin stimulation in culture.

Additionally, it has been recently hypothesized that individual adipocytes do not exhibit variable degrees of insulin sensitivity along a spectrum, but rather manifest a binary insulin-sensitive or insulin-resistant state.[92] Consequently, insulin-resistant subjects have some insulin-sensitive adipocytes that respond to insulin in a similar fashion as those of lean, healthy individuals, but the percentage of responsive cells in insulin-resistant individuals is much lower than that of lean, healthy individuals.[92] Dr. McCormick is utilizing quantitative immunofluorescence microscopy to investigate whether local SAT inflammation promotes IR states by changing insulin-sensitive adipocytes into insulin-refractory adipocytes [Figure 1]. As Akt is phosphorylated upon insulin binding to the insulin receptor, we are able to visualize and quantify phospho-Akt (phosphorylation at Thr308; pThr308). Ultimately this allows us to evaluate insulin sensitivity at the cellular level. By also staining the tissue with anti-CD11c antibody to identify M1 pro-inflammatory macrophages as well as CD68, a universal macrophage marker, we are able to assess whether adipocytes in close proximity to activated macrophages lack phospho-Akt (pThr308) and therefore exhibit suppressed insulin signaling.

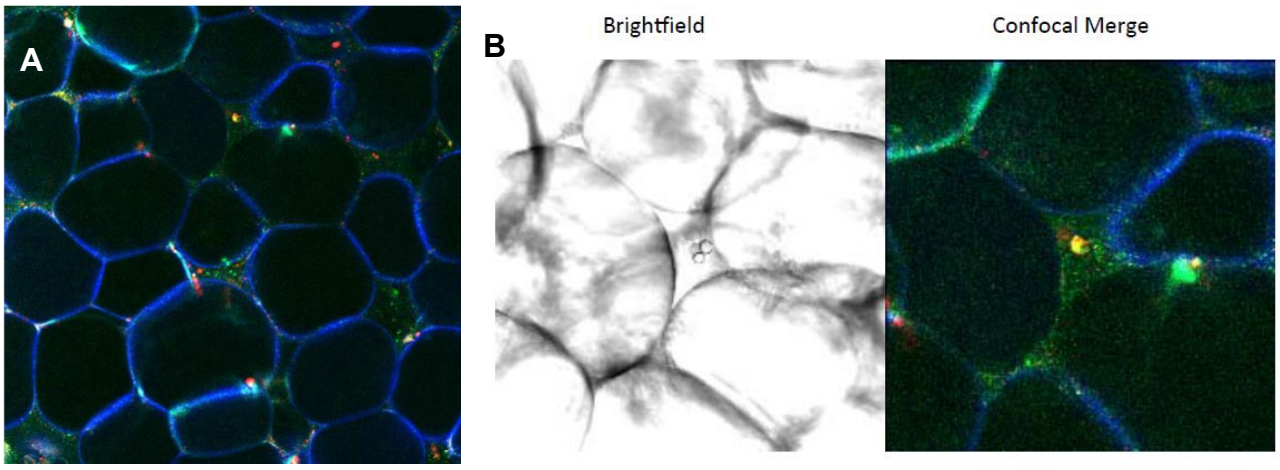


Figure 1. (A) Immunofluorescence imaging of a 3μm Z-slice of subcutaneous adipose tissue from a subject. Blue staining represents AKT p308 (insulin sensitivity), red represents CD68 (macrophages), and green represents CD11c (M1 pro-inflammatory macrophages). Most adipocytes have Akt p308 staining, but some are notably absent. (B) Zoomed-in images of activated M1 macrophages on Bright field and confocal merge immunofluorescent microscopy. Many of the adipocytes surrounding the M1 macrophages lack Akt p308 staining and are insulin-refractory.

The subjects evaluated by the aforementioned methods in our protocol thus far consist only of obese insulin-resistant non-diabetics. Because of the potentially significant findings and novel strategies to evaluate local insulin sensitivity in human SAT, it would be worthwhile to investigate how these results compare with healthy non-insulin resistant volunteers and subjects with T2DM.

II. Study Objectives:

We propose a pilot study of the effects of colchicine in obese non-diabetic adults with metabolic syndrome to determine if colchicine treatment can improve metabolic and inflammatory parameters, both locally and systemically, independent of weight loss. This pilot study will determine the sample size needed for an adequately powered larger study in the future.

We also propose enrolling additional subjects who do not qualify for the protocol to evaluation only. This group will consist of non-obese and obese adults without significant insulin resistance and/or inflammation as well as subjects with diet-controlled T2DM. They will not be randomized or receive study medication. The results from these controls will allow us to determine insulin kinetics in SAT over the entire spectrum of insulin sensitivity.

Subjects with T2DM have more severe metabolic abnormalities and typically higher inflammatory levels, but their pancreatic function may possibly be past the point of reversibility. Therefore, it is unclear whether such subjects would be the most likely or least likely group to derive benefit, if any, from colchicine. We propose to enroll seven diet-controlled subjects with T2DM to an open-label colchicine arm. Results

from this arm will allow us to determine the sample size that might be needed if a future study for the effects of colchicine in T2DM were to be carried out.

III. Specific Aims and Hypotheses:

Through this randomized, double-blind, placebo-controlled clinical trial, we will obtain pilot data for the following aims and hypotheses:

1. **Aim 1:** To explore changes in insulin sensitivity, as measured by FSIVGTT, among obese non-diabetic adults following long-term colchicine administration.
   Hypothesis 1: We hypothesize that obese non-diabetic adults on colchicine 0.6mg bid for 3 months will exhibit significant improvements in insulin sensitivity compared with placebo, independent of weight loss. We also hypothesize that reductions in systemic inflammation, as measured by hsCRP, will moderate this relationship.
2. **Aim 2:** To explore changes in other parameters of glucose homeostasis, among obese non-diabetic adults following long-term colchicine administration.
   Hypothesis 2: Using data from the FSIVGTT, we hypothesize that obese non-diabetic adults on colchicine will exhibit significant improvements in acute pancreatic reserve (as measured by acute insulin response to glucose (AIRg)) and glucose homeostasis (as measured by the Disposition Index). We also hypothesize that insulin resistance as measured by HOMA-IR will demonstrate improvement in colchicine-treated subjects.
3. **Aim 3:** To explore changes in cardiovascular risk factors among obese non-diabetic adults following long-term colchicine administration.
   Hypothesis 3: We hypothesize that colchicine-treated subjects will demonstrate significant improvements in cardiovascular risk factors and other lipid parameters, including blood pressure, LDL, HDL, Lp(a), fatty acid levels, uric acid, hemoglobin A1c, and fasting plasma glucose as compared with placebo-treated subjects.
4. **Aim 4:** To explore changes in inflammation among obese non-diabetic adults following long-term colchicine administration.
   Hypothesis 4a: We hypothesize that colchicine-treated subjects will demonstrate significant improvements in systemic inflammatory markers as well as leukocyte/endothelial adhesion markers.
   Hypothesis 4b: We hypothesize that colchicine-treated subjects will demonstrate significant improvements in local adipose tissue inflammation.

5. **Aim 5:** To explore insulin-dependent GLUT4 dynamics locally in human adipocytes across the spectrum of insulin sensitivity.

   Hypothesis 5a: We hypothesize that colchicine-treated subjects will demonstrate significant improvements in adipose insulin-dependent GLUT4 kinetics in vitro.

IV. Study Design and Methods:

A. Subject Eligibility: All adults who meet the inclusion and exclusion criteria as listed below and who can attend all needed study visits at the NIH Clinical Center will be eligible:

I. Inclusion Criteria for subjects randomized to colchicine or placebo:

Subjects will qualify for randomization to colchicine or placebo if they meet the following criteria:

1. Good general health. In general subjects should take no medications. However, individuals taking medications for obesity-related co-morbid conditions, who have not had changes in dosage for more than 3 months, may be included, at the discretion of the principal investigator.
2. Obesity, defined as a body mass index (BMI) $\geq$ 30 kg/m^2, but weight less than 450 lbs in order for subjects to undergo Dual-Energy X-ray Absorptiometry (DXA) scanning.
3. Age 18-100 years.
4. Metabolic Syndrome[93]

   (Any 3 of the following 5):

   a. FPG $\geq$ 100 mg/dl or Impaired Glucose Tolerance (Glucose $\geq$ 140 mg/dl at 2 hours of OGTT)
   b. Triglycerides $\geq$ 150 mg/dl, or on treatment
   c. Waist Circumference: Men $\geq$ 40 in ($\geq$ 102 cm); Women $\geq$ 35 in ($\geq$ 88 cm)
   d. Reduced HDL-C: Men < 40 mg/dl; Women < 50 mg/dl, or on treatment
   e. Hypertension: $\geq$130 mmHg systolic, or $\geq$85 mmHg diastolic, or on treatment
5. HOMA-IR $\geq$ 2.6. Our goal is to enroll participants who have pre-existing insulin resistance.[94]
6. hsCRP $\geq$ 2.0 mg/L. We aim to recruit participants with increased baseline level of inflammation. Individuals with hsCRP above 2.0 mg/L have been shown to have an increased risk for cardiovascular events.[16]

II. Exclusion Criteria for subjects randomized to colchicine or placebo:

1. Type 2 diabetes mellitus, as determined by either having:
   a. "clear clinical diagnosis" of diabetes, such as "a patient in a hyperglycemic crisis or classic symptoms of hyperglycemia and a random plasma glucose ≥200 mg/dL"
   b. two of the following three:
      i. fasting plasma glucose ≥ 126 mg/dL
      ii. Hemoglobin A1c ≥ 6.5%
      iii. An oral glucose tolerance test glucose concentration of ≥ 200 mg/dL at 2 hours.
   c. one of the above three criteria (bi.-biii.) meeting the T2DM cutoff on two different days.

   If only one of the above three criteria (bi.-biii.) meet the T2DM threshold during the Screening Visit, that test will be repeated on another day to determine if the subject has T2DM or not. As per ADA guidelines, "The diagnosis [of T2DM] is made on the basis of the confirmed test."[95]

   Moreover, because HbA1c has been shown to be higher in African Americans (AA) as compared to other races for the same glycemia, non-diabetic AA may be unfairly excluded by their HbA1c alone.[96-98] Therefore, for AA subjects, if their 2 hour OGTT and fasting glucoses are in the non-diabetic range, and the HbA1c is < 7.0%, we will consider them non-diabetic.
2. Presence of a significant active or chronic illness likely to limit life span and/or increase risk of intervention, including renal (GFR ≤ 60 ml/min/1.73m^2), cardiovascular, hepatic (other than obesity-related steatosis), gastrointestinal, immunologic, endocrinologic (e.g. Cushing syndrome), pulmonary (other than either asthma not requiring continuous medication or sleep apnea-related disorders), or other disorders at the discretion of the investigators.
3. Recent use of colchicine or anorexiant medications in the last 3 months.
4. Known allergy to colchicine.
5. Previous history of agranulocytosis, gout, or significant myositis.
6. Females who are pregnant, planning to become pregnant, currently nursing an infant, or have irregular menses, defined as cycles less than 21 days or greater than 45 days.[99]
7. Individuals who have current substance abuse or a psychiatric disorder or any other condition that in the opinion of the investigators would impede competence, compliance, or participation in the study.
8. Subjects who regularly use prescription medications unrelated to the complications of obesity, especially those known to affect enzymes involved in colchicine metabolism, such

as CYP3A4 or P-glycoprotein (P-gp) (*see Appendix B1*). Oral contraceptive use will be permitted, provided the contraceptive has been used for at least two months before starting study medication. The use of over-the-counter and prescription medications will be reviewed on a case-by-case basis; depending on the medication, subjects who have continued to take prescription medication or have stopped taking an exclusionary medication for at least 3 months prior to study entry may be eligible (*see Appendix B2*).

9. Participation in a formal weight loss program (e.g. Weight Watchers) or recent weight change of more than 3% of body weight in the past two months.
10. Use of anti-inflammatory medications (e.g. prednisone, NSAIDs) chronically or in the last 7 days prior to fat biopsy.
11. History of keloid formation.
12. Current users of tobacco or nicotine products (e.g. nicotine patch, e-cigarettes).

III. Inclusion Criteria for subjects who are evaluated but not eligible for randomization:

Subjects will qualify for the Evaluation-only arm if they meet the following criteria:

1. Good general health. In general subjects should take no medications. The use of over-the-counter and prescription medications will be reviewed on a case-by-case basis; depending on the medication, subjects who have continued to take prescription medication or have stopped taking an exclusionary medication for at least 3 months prior to study entry may be eligible (*see Appendix B2*).
2. Weight less than 450 lbs in order for subjects to undergo Dual-Energy X-ray Absorptiometry (DXA) scanning.
3. Age 18-100 years.

IV. Exclusion Criteria for subjects who are evaluated but not eligible for randomization:

1. Type 2 diabetes mellitus that is not well controlled with diet alone: subjects taking an anti-diabetic medication (e.g. metformin, insulin, sulfonylureas, etc.) or having a Hemoglobin A1c > 9.0%
2. Presence of a significant active or chronic illness likely to limit life span and/or increase risk of intervention, including renal (GFR $\leq$ 60 ml/min/1.73m^2), cardiovascular, hepatic (other than obesity-related steatosis), gastrointestinal, immunologic, endocrinologic (e.g. Cushing syndrome), pulmonary (other than either asthma not requiring continuous medication or sleep apnea-related disorders), or other disorders at the discretion of the investigators.
3. Recent use of colchicine or anorexiant medications in the last 3 months.

4. Females who are pregnant, planning to become pregnant, or are currently nursing an infant.
5. Individuals who have current substance abuse or a psychiatric disorder or any other condition that in the opinion of the investigators would impede competence, compliance, or participation in the study.
6. Participation in a formal weight loss program (e.g. Weight Watchers) or recent weight change of more than 3% of body weight in the past two months.
7. Use of anti-inflammatory medications (e.g. prednisone, NSAIDs) chronically or in the last 7 days prior to fat biopsy.
8. History of keloid formation.
9. Current users of tobacco or nicotine products (e.g. nicotine patch, e-cigarettes).

V. Inclusion Criteria for subjects with diet-controlled T2DM

Subjects will qualify for the Open Label arm if they meet the following criteria

1. Diet-controlled T2DM, as determined by having all of the following:
   a A diagnosis of T2DM (as defined above in Section IV.II.1.).
   b Not on any diabetic/hypoglycemic agents
   c Not having an alternate cause of hyperglycemia (e.g. T1DM, glucocorticoid-induced, lipodystrophy, acromegaly, etc.)
   d Hemoglobin A1c $\leq$ 9.0%
2. Good general health. In general subjects should take no medications. The use of over-the-counter and prescription medications will be reviewed on a case-by-case basis; depending on the medication, subjects who have continued to take prescription medication or have stopped taking an exclusionary medication for at least 3 months prior to study entry may be eligible (*see Appendix B2*).
3. Age 18-100 years.
4. Obesity, defined as a body mass index (BMI) $\geq$ 30 kg/m^2, but weight less than 450 lbs in order for subjects to undergo Dual-Energy X-ray Absorptiometry (DXA) scanning.
5. Metabolic Syndrome[93]

   (Any 3 of the following 5):
   a. FPG $\geq$ 100 mg/dl or Impaired Glucose Tolerance (Glucose $\geq$ 140 mg/dl at 2 hours of OGTT)
   b. Triglycerides $\geq$ 150 mg/dl, or on treatment
   c. Waist Circumference: Men $\geq$ 40 in ($\geq$ 102 cm); Women $\geq$ 35 in ($\geq$ 88 cm)
   d. Reduced HDL-C: Men < 40 mg/dl; Women < 50 mg/dl, or on treatment

e. Hypertension: ≥130 mmHg systolic, or ≥85 mmHg diastolic, or on treatment

6. HOMA-IR ≥ 2.6. Our goal is to enroll participants who have pre-existing insulin resistance.[94]
7. hsCRP ≥ 2.0 mg/L. We aim to recruit participants with increased baseline level of inflammation. Individuals with hsCRP above 2.0 mg/L have been shown to have an increased risk for cardiovascular events.[16]

VI. Exclusion Criteria for subjects with diet-controlled T2DM

1. T2DM that is not well controlled with diet alone: subjects will not be eligible if they take an anti-diabetic medication (e.g. metformin, insulin, sulfonylurea, etc.), or have HbA1c > 9%.
2. Presence of a significant active or chronic illness likely to limit life span and/or increase risk of intervention, including renal (GFR ≤ 30 ml/min/1.73m^2), cardiovascular, hepatic (other than obesity-related steatosis), gastrointestinal, immunologic, endocrinologic (e.g. Cushing syndrome), pulmonary (other than either asthma not requiring continuous medication or sleep apnea-related disorders), or other disorders at the discretion of the investigators.
3. Recent use of colchicine or anorexiant medications in the last 3 months.
4. Known allergy to colchicine.
5. Previous history of agranulocytosis, gout, or significant myositis.
6. Females who are pregnant, planning to become pregnant, currently nursing an infant, or have irregular menses, defined as cycles less than 21 days or greater than 45 days.[99]
7. Individuals who have current substance abuse or a psychiatric disorder or any other condition that in the opinion of the investigators would impede competence, compliance, or participation in the study.
8. Subjects who regularly use prescription medications unrelated to the complications of obesity, especially those known to affect enzymes involved in colchicine metabolism, such as CYP3A4 or P-glycoprotein (P-gp) (*see Appendix B1*). Oral contraceptive use will be permitted, provided the contraceptive has been used for at least two months before starting study medication. The use of over-the-counter and prescription medications will be reviewed on a case-by-case basis; depending on the medication, subjects who have continued to take prescription medication or have stopped taking an exclusionary medication for at least 3 months prior to study entry may be eligible (*see Appendix B2*).
9. Participation in a formal weight loss program (e.g. Weight Watchers) or recent weight change of more than 3% of body weight in the past two months.

10. Use of anti-inflammatory medications (e.g. prednisone, NSAIDs) chronically or in the last 7 days prior to fat biopsy.
11. History of keloid formation.
12. Current users of tobacco or nicotine products (e.g. nicotine patch, e-cigarettes).

B. Subject Enrollment

Subjects will be enrolled only at the National Institutes of Health Hatfield Clinical Research Center. Each subject will be registered using the Clinical Research Information System (CRIS), but will be considered enrolled only after the participant signs the study consent form.

C. Clinical evaluation and D. Research Testing, and/or Treatment

Brief summary of study design: Participants who appear qualified when first contacted will undergo an initial screening visit, during which a history and physical examination, initial protocol review, body composition DEXA, and screening laboratory assessment will take place. If after the screening visit, the subject still appears qualified he/she will return for a baseline visit, during which time baseline labs, Hollingshead questionnaire, FSIVGTT, body composition DEXA (if not performed during the Screening Visit), CT abdomen, and SAT biopsy will take place. After assessments are complete, participants who qualify for and agree to randomization will be randomized to either placebo or colchicine and given an eight week supply of medication. Randomized subjects will be called at 1 week, 1 month, and 2 months to assess for adverse events and adherence. Randomized subjects will return for a safety interim visit between 4-8 weeks to assess for adverse events by physical exam and laboratory studies, where they will receive another 8 week supply of study drug. At 3 months, randomized subjects will return for the final visit for repeat physical exam, labs, FSIVGTT, body composition DEXA, CT abdomen, SAT biopsy, and exit questionnaire. For randomized subjects who complete the study, they will be given the option to return for another "washout" visit three months later, which will involve the same testing as the baseline and final visits. All study visits will take place at the NIH Clinical Center, Bethesda, MD. (*Please see Appendices A1-A6)*

Subject who qualify for the Evaluation-Only arm will only undergo the Screening and Baseline visits as described above.

For the Open Label arm, seven subjects with T2DM will be recruited. They will undergo the same visits and testing as in the randomization arm, described above. For the Open Label arm only, pills will be dispensed with a "smart bottle cap" which will be able to record the time and date that

the pill bottle was opened. Specifically, we will purchase the MEMSCaps system (http://www.mwvaardex.com/index.php/our-adherence-solutions-data-collection/memscap-tm). Each bottle supplied by NIH pharmacy will come with a TrackCap®, with the participant's subject ID number assigned to the specific cap. At the end of the three months on study drug, when the subject returns the study drug bottle and cap, we will download the data directly to an SGO lab computer. No PII or medical record number will be associated with the TrackCap, and only the investigators involved in the study (e.g. JY, AD, SB) will have access to the data.

i. Initial Contact: Telephone Screen

For participants who respond to advertisements requesting participants (see below), initial recruitment and eligibility screening will occur by telephone. Recruitment will occur on a continuous basis until study enrollment is complete. Eligible subjects will then be scheduled for an outpatient screening visit. Eligible subjects will be mailed paperwork in advance, including the NIH medical history form, which will then be reviewed in person with the potential subject at the outpatient screening visit.

ii. Screening Evaluation

Complete medical history and physical exam will be performed with weight (in kg) measured while the subject wears light clothes and height (in cm) measured in triplicate. Subjects will rest for 5 minutes in the seated position with the antecubital fossa supported at heart level when blood pressure measurements are made. Body mass index (BMI) will be calculated before protocol is signed and current medications reviewed, so that individuals taking an exclusionary medication, have a disqualifying medical condition, or are weighing more than the maximum weight criterion (450 lbs) can be dismissed before the protocol is reviewed.

Protocol review and signing of consent forms: For those who may be eligible, the relevant consent form (either evaluation-only or randomized study protocol) will be discussed and study risks and benefits explained.

EKG and fasting blood for complete blood count (CBC), acute care, mineral, and hepatic profiles, PT/PTT, TSH, Free T4, Total T3, IGF-1, and creatine kinase (CK) will be obtained to ensure that the subject is in good general health. Fasting blood samples for hsCRP, insulin, and HbA1c will be obtained, and a 2 hour OGTT-GH will be performed to confirm subject eligibility for the study. Additional samples for peripheral blood mononucleated cell (PBMC) analysis and future research and genomic DNA isolation will be obtained.

Urine for urinalysis, urine pregnancy test (female participants only), and urine toxicology screen will be obtained to ensure that the subject is in good general health and meets protocol entry criteria.

If a potential subject is noted to meet any of the exclusion criteria, that individual will be dismissed without further evaluation and referred to their usual physician for any required treatment.

DXA Scan for body composition analysis and fat mass will be obtained. DXA involves passing a collimated X-ray beam of 40 and 70 KeV through the subject and collecting the X-rays that pass through the patient with a standard X-ray detector. Because the delivery and attenuation of the X-rays are measured separately for the two principal energies, the procedure allows the differentiation of substances in their path if the attenuation coefficients of these substances are known. Because the delivery and attenuation of the X-rays are measured separately for the two principal energies, the procedure allows the differentiation of substances in their path if the attenuation coefficients of these substances are known. The manufacturer specifies that the array beam delivers an effective dose of 0.00003rem total body irradiation. Participants will undergo DXA scans in the Metabolic Clinical Research Unit (MCRU) using the Lunar iDXA (GE Healthcare, Madison, WI, USA) machine. The scan takes approximately 20-30 minutes. An external standard, simulating bone, fat, and muscle, is scanned once a week to validate measurements. This standard allows calculation of total body fat, muscle, and bone mass, and percentage of body fat.

iii. Baseline evaluation

An interval history and physical exam will be performed with weight (in kg), height (in cm), and blood pressure measured as above.

Socioeconomic Status will be determined using the Hollingshead Two-Factor Index of Social Position.[100]

Fasting blood samples of hsCRP, insulin, HbA1c, free fatty acids, lipid panel, uric acid, and serum beta HCG (female participants only) will be obtained. Blood samples will also be collected for PBMC analysis, as well as to measure inflammatory cytokines, endothelial adhesion molecules, lipoproteins (e.g. Lp(a), oxidized HDL, NMR lipoprotein particle analysis), leptin, and adiponectin. Additional research blood samples will be drawn for storage in a -80^{o}C freezer for possible future research.

Two stool samples to save for future analyses will be obtained. Subjects will be asked to fill out a paper survey including 24 hour food intake that will be reviewed in-person with an NIH nutritionist (*see Appendix G*).

Urine sample as described previously. In addition to the urine sample collected on admission, we will collect a timed eight hour urine sample, which will be saved for future analyses.[101,102]

FSIVGTT: As described previously,[103-105] two angiocatheters will be placed in the antecubital veins, one for the administration of glucose and insulin and the other for blood sampling from the contralateral arm. A glucose load of 50% dextrose 0.3 g/kg given as a smooth bolus over 2 min is given at time 0 and a bolus of 0.05 U/kg insulin is given just before minute 20. Blood samples are taken at times -15, -10, -5, -1, then at 2, 3, 4, 5, 6, 8, 10, 14, 19, 22, 25, 30, 40, 50, 70, 100, 140, and 180 minutes after glucose injection for the measurement of plasma glucose and serum insulin concentrations.[106,107] Insulin administration is essential for individuals with severe insulin resistance to achieve a valid test.[108-110] Insulin sensitivity and glucose effectiveness will be assessed using Bergman's minimal model,[103] which requires the particular times of blood collection that have been specified above. The principal investigator will be notified immediately of any glucose measurement less than 40 mg/dL. The principal investigator will use his clinical judgment in developing an appropriate, situation-specific management plan.

CT abdomen-adipose: To quantify the fat content of the liver, subcutaneous, and visceral compartments, subjects will undergo a CT of the abdomen, where slices at only three levels will be obtained. CT scanning parameters are 5-10 mm slice thickness, 0.7-0.98 in-plane pixel resolution, and 120 kVp. As previously established liver fat can be reliably quantified from one slice, typically in the T12-L1 region.[111,112]

In accordance with previous studies, subcutaneous and visceral fat compartments can reliably be quantified from slices of the L2-3 and L4-5 levels.[113] Using automated software developed at the NIH, an algorithm consisting of body masking, noise reduction, adipose tissue labeling, VAT and SAT separation, and quantification will be used. The body mask is created by a region-growing algorithm on the image background that segments the low intensity pixels outside the body and in a second pass, removes the CT table. Once the body mask is created, an anisotropic diffusion filter reduces noise and voxels between -274 HU and -49 HU. A contour around the outside of the body, the "external contour", is initialized. Active contour models are then used to iteratively modify the external contour to find the inner boundary of the SAT; this results in a contour along the abdominal wall, specifically the "internal contour". The adipose

tissue volume inside the internal contour contributes to the VAT and the adipose tissue volume between the external contour and internal contour contributes to SAT.

In collaboration with James Vucich, NIH Medical Physicist, it was determined that the CT scan will deliver an effective dose of 0.031 rem total body irradiation per scan. As randomized and open-label participants will undergo three CT scans and three DEXA scans as part of the study, the total effective radiation dose for the study is 0.093 rem. Evaluation-only participants will receive one CT and one DEXA scan (total effective radition dose = .031 rem. For all three protocol arms, this dose is well below the dose guideline of 5 rem per year established by the NIH Radiation Safety Committee for adult research subjects.

Subcutaneous abdominal adipose tissue biopsy: Abdominal subcutaneous adipose tissue samples will be obtained using a mini-liposuction technique (*see Appendix C for checklist*). Subjects will be advised not to use non-steroidal anti-inflammatory drugs (NSAID; e.g. aspirin, ibuprofen, etc.) for at least one week prior to and following the procedure. The biopsies will be performed by an MD or CRNP on the adult outpatient unit. A mixture of 1:1 lactated Ringers or normal saline and lidocaine will be used as a local anesthetic until there is an "orange peel" appearance to the skin. A sterile approach will be used to minimize risk of infection. Using 10 or 20 cc syringes with blunt, fenestrated needles, gentle to-and-fro movements will be used to obtain the samples. The contents of the syringe will be transferred onto a 250 Nylon mesh (Nitex screen cloth, Sefar America, Depew, NY) and rinsed with 5-10ml normal saline to wash away the lidocaine and blood clots as much as possible. Fifteen grams of subcutaneous adipose tissue samples will be used to assess changes in adipocyte size and the expression of proteins involved in inflammation. Subjects will be monitored after the procedure, and post-biopsy care instructions will be provided to the subjects.

Medication Dispensation and Randomization: Subjects eligible for, and willing to undergo randomization will be assigned consecutive study numbers. Investigators, evaluators, and participants will be blinded to assignment to drug or placebo. Assignment of each study number to placebo or colchicine will be performed by the NIH pharmaceutical development section (before 2017) or the NIH Investigational Drug Management Section, with individuals randomized in blocks of four, stratified for sex (male vs. female) and race (black vs. non-black).

Subjects in the Open Label arm will receive purchased brand-name colchicine (Colcrys).

iv. <u>Interim safety evaluations</u>

Randomized and open-label participants will be contacted via telephone to evaluate medication tolerability after 1 week and then monthly until they return for their final evaluation. If a randomized or open-label participant develops any concerning adverse event, the individual will be scheduled for an immediate clinic appointment with a trial investigator for further work up, as necessary. Additionally, randomized and open-label participants will have one interim safety visit at the NIH Clinical Center with one of the investigators (e.g. JY, AD, SB) for a physical examination and blood work (CBC, HbA1c, acute care profile, hepatic profile, lipid panel, insulin, uric acid, hsCRP and CK), between 4-8 weeks. Samples for PBMC analysis and inflammatory cytokines will be collected at this visit. A urine sample will be obtained as described previously. The in-person interim safety visit can take the place of one of the safety telephone calls.

v. Final evaluation

For randomized and open-label subjects, an interval history and physical exam will be performed with weight (in kg), height (in cm), and blood pressure measured as above.

An **exit questionnaire** will be performed (*See Appendix D)*.

Fasting blood for complete blood count (CBC), acute care, mineral, and hepatic profiles, PT/PTT, beta HCG (female participants only), and creatine kinase (CK) will be obtained to monitor for adverse events and to ensure that the patient remains in good clinical health. Additionally, fasting research blood samples, as described in the baseline evaluation for PBMC analysis, inflammatory cytokines, endothelial adhesion molecules, lipoproteins, leptin, adiponectin, and research specimens to store for future studies, will be obtained.

Stool sample for future analyses as described previously.

Urine sample and eight hour urine collection as described previously.

FSIVGTT: As described previously.

DXA Scan as described previously.

CT Abdomen-for liver and intra-abdominal adipose tissue as discussed previously.

Subcutaneous abdominal adipose tissue biopsy as described previously.

vi. Washout evaluation

For randomized and open-label subjects, an interval history and physical exam will be performed, as above.

Fasting blood as described in the Final Visit.

Urine sample and eight hour urine collection as described previously.

Stool sample as described previously.

FSIVGTT as described previously.

DXA Scan as described previously.

CT Abdomen-for liver and intra-abdominal adipose tissue as discussed previously.

Subcutaneous abdominal adipose tissue biopsy as described previously.

E. Biological Specimens

i. Blood Loss

a. Screening Visit

i.	Acute care, mineral, hepatic panel, CK, TSH, Free T4, T3, insulin, hsCRP, IGF1	8 ml
ii.	CBC/HbA1c	3 x2 = 6 ml
iii.	ESR	1 ml
iv.	PT/PTT	4.5 ml
v.	OGTT 2-hour glucose, GH	2 x3 x3= 18 ml
vi.	OGTT hsCRP (at 2hr time point)	1 mL
vii.	Research (Lavender)	18 ml
viii.	Research (Green)	10x4 = 40 ml
ix.	Research (Red)	20 ml
x.	Research (Red/yellow)	8 ml
xi.	Research (Gut hormones)	3 ml
	Total	127.5 ml

b. Baseline Visit

i.	FSIVGTT	110 ml
ii.	Glucose (Gray)	1 ml
iii.	CBC/HbA1c	3 x2 = 6 ml
iv.	ESR	1 ml
v.	Acute care, lipid panel, insulin, uric acid, CK hsCRP, mineral panel, hepatic panel	8 ml
vi.	Research (Lavender)	10 ml
vii.	Research (Green)	10x4= 40ml
viii.	Research (Red)	10x3= 30 ml
ix.	Research (Red/yellow)	8 ml

x.	Research (for Dr. Sack)	120 ml (optional)
xi.	Research (Gut hormones)	3 ml
	Total	217-337 ml

c. Interim Safety visit (randomized and open-label participants)

i.	CBC/HbA1c	3 ml
ii.	Acute care, lipid and hepatic panels, CK, insulin, uric acid, hsCRP	8 ml
iii.	Research (Red/yellow)	8 ml
iv.	Research (Red)	10 ml
v.	Research (Green)	10 x3 = 30 ml
	Total	59 ml

d. Final visit (randomized and open-label participants)

i.	FSIVGTT	110 ml
ii.	Glucose (GYY)	1 ml
iii.	CBC/HbA1c	3 x2 = 6 ml
iv.	ESR	1 ml
v.	PT/PTT	4.5 ml
vi.	Acute care, mineral, hepatic, lipid panels, insulin, uric acid, CK, hsCRP	8 ml
vii.	Research (Lavender)	10 ml
viii.	Research (Green)	10x4= 40ml
ix.	Research (Red)	10x3 = 30 ml
x.	Research (Red/yellow)	8 ml
xi.	Research (for Dr. Sack)	120 ml (optional)
xii.	Research (Gut hormones)	3 ml
	Total	221.5-341.5 ml

e. Washout Visit (randomized and open-label participants)

i.	FSIVGTT	110 ml
ii.	Glucose (GYY)	1 ml
iii.	CBC/HbA1c	3 x2 = 6 ml
iv.	ESR	1 ml

v.	PT/PTT	4.5 ml
vi.	Acute care, mineral, hepatic, and lipid panels, insulin, uric acid, CK, hsCRP	8 ml
vii.	Research (Lavender)	10 ml
viii.	Research (Green)	10x4=40ml
ix.	Research (Red)	10x3= 30 ml
x.	Research (Red/yellow)	8 ml
xi.	Research (for Dr. Sack)	120 ml (optional)
xii.	Research (Gut hormones)	3 ml
	Total	221.5-341.5 ml

Note: Some research blood samples will be sent to our collaborators for analysis of lipid, inflammatory, and endothelial parameters. Other research blood samples will be saved for storage in a locked -80ºC freezer for possible future research, although there are no specific additional studies planned at this time.

ii. Adipose tissue biopsy

a.	Baseline visit	15 g
b.	Final visit (randomized and open-label participants only)	15 g
c.	Washout visit (randomized and open-label participants only)	15 g

Note: A portion of the adipose samples will be snap frozen and stored in a locked -80ºC freezer for possible future research, although there are no specific additional studies planned at this time.

iii. Urinalysis

a.	Screening visit	10 cc
b.	Baseline visit	10 cc + 8hr collection
c.	Interim visit (randomized and open-label participants)	10 cc
d.	Final visit (randomized and open-label participants)	10 cc + 8hr collection
e.	Washout visit (randomized and open-label participants)	10 cc + 8hr collection

iv. Stool sample

a.	Baseline visit	10 cc

b. Final visit (randomized and open-label participants) 5 cc
c. Washout visit (randomized and open-label participants) 5 cc

F. Molecular Genetic Analysis of DNA: Yes. Samples will be collected and stored for potential whole exome or whole genome sequencing for alleles linked to obesity, metabolic syndrome, or response to colchicine.

G. Approved Drugs:
No experimental studies involve approved drugs. Insulin is administered during the FSIVGTT. Lidocaine is used during adipose tissue biopsies.

H. Unapproved Drugs: Colchicine is FDA-approved for the treatment of gout and Familial Mediterranean Fever. It is unapproved for the amelioration of insulin resistance or metabolic syndrome. In this study we plan to use doses approved and previously proven safe for gout prophylaxis – 0.6mg PO BID.[58,68] From 2014-2016, the NIH Pharmaceutical Development Section used colchicine USP compounding powder to make identical-looking colchicine 0.6 mg and placebo capsules. After this date, Pine Pharmaceuticals will follow the same procedures to produce colchicine and placebo capsules. For the Open Label arm, participants will receive brand-name colchicine (Colcrys). Participants will be instructed to take one capsule twice daily. If participants develop intractable diarrhea, or other mild side effect that does not warrant medication discontinuation and/or study termination, the dose may be reduced to one capsule daily (*see Section V.A.v. and Appendix F)*. If side effects resolve, and the subject remains considerably improved or symptom-free for two weeks, the dose may be increased back to twice daily at the investigator's discretion. All subjects will receive a "Medication Guide" (*See Appendix E*). An IND has been obtained from the FDA (120722).

I. Sharing of Data with Subjects

Specific results that will be given to participants or their health care providers: If participants have concerning results during any of the visits, this will be communicated both to the patient and their designated health care providers. At the conclusion of the entire study, when the randomized assignment of subjects to medication is given to the Investigators and the analysis of the results is complete, the overall results of the investigation will be given to participants and they will be informed of the group to which they were randomized. For the Open Label arm, once all seven

subjects have completed their participation and analysis of the results of the arm has been completed, they will be communicated to the participants of the Open Label arm.

J. Questionnaires/Psychological instruments: The Hollingshead Two Factor Index of Socioeconomic Status[100] will be administered at the Baseline visit. A non-validated exit questionnaire examining the patients' perceptions of their experience in the protocol will be performed at the final visit.

K. Genetic counseling: n/a

L. Follow-up of participants: After the baseline visit, randomized and open-label participants will be contacted by a study investigator by phone at 1 week, 1 month, and 2 months to assess for any adverse outcomes and adherence. If a participant develops any concerning adverse events, the individual will be scheduled for an immediate clinic appointment with a trial investigator for further evaluation, as necessary. Additionally, an interim safety visit at the NIH Clinical Center with one of the investigators (e.g. JY, AD, SB) and laboratory examination (e.g. CBC, acute care profile, hepatic profile, and CK), will be performed for randomized and open-label subjects between 4-8 weeks.

Participants will be contacted by a study investigator in the event that any abnormal laboratory, imaging or EKG findings are found during testing at any of the visits. At the investigator's clinical discretion in conjunction with the subject's wishes, these abnormalities may be worked up further at the NIH Clinical Center as necessary.

M. Conditions for discharge from the protocol (any of the following):
    a. Completion of the protocol. Specifically, for evaluation-only participants, the subject completes the screening visit and baseline visit. For randomized and open-label participants, the subject also completes at least 90 days of investigational medication (colchicine or placebo), and the final visit, as described above.
    b. Development of a significant toxicity, such as myositis, agranulocytosis, acute kidney injury (*see Section V. A. v.*), generally defined as grade II or greater toxicity on the CTCAE 4.03 list (*see Appendix F and attached file for more information)*.[114]
    c. Development of an exclusion criterion, besides T2DM or weight change (*see Section IV. B.*)
    d. Voluntary withdrawal from the trial
    e. Non-compliance with the study procedures at baseline assessment (e.g. unwillingness to take investigational medication, undergo FSIVGTT, etc.)

V. **Analysis of the Study/Oversight**

A. Clinical Description:

i. Study Administration and Data Collection, Management, and Storage:

The study will be conducted by the research team as listed above. Samples and data will be coded. Human biospecimens will be collected using procedures appropriate for the type of biospecimen being collected and will be handled in accordance with the U.S. Occupational Safety and Health Administration's Bloodborne Pathogens Standard for Samples. The principal investigator and the co-investigators will have access to identifiable participant records. Data will be coded by subject number, but will be readily associated with identifiable participant records. Records containing personal identifiers will be maintained consistent with the security measures required by the NIH. Data will either be transferred directly from the electronic medical record into the NICHD Clinical Trials Data Base (CTDB), or for data where direct transfer is unavailable, will be double-entered by hand into CTDB. All data will be kept on secure computers in the SGO office, whose doors will be locked when staff is not present. All paper data will also be kept in locked areas in the SGO office.

Serum and plasma samples not analyzed immediately (as described above) will be stored in locked freezers by the NICHD sample management team supervised by the NICHD Clinical Director and tracked/managed through CTDB. Human biospecimens in storage will have a unique identifier, will be labeled with a printed label and will contain a barcode. Samples will be tracked using a computer-based inventory system that records the location and detailed information of every specimen in the repository.

Sample freezers will be operated using facility environments that include ambient temperature controls, good air circulation, lighting, and security. Systems are in place to allow for local and remote temperature monitoring of freezers. The NIH has an emergency preparedness plan that covers equipment failures and power interruption, including back-up storage capacity and back-up power generators.

Participants give explicit permission regarding sample use in the consent form of the study, indicating if samples may be used for future experimental testing related to colchicine response, body weight and metabolism and specify if additional consent is

required. At the conclusion of the study, samples will be retained for future experimental testing related to colchicine response, body weight and metabolism.

The PI will inform the IRB if obtained samples are lost or destroyed before they are assayed for primary or secondary outcomes. Loss or destruction of samples saved only for tertiary outcomes or future unspecified research will not be routinely reported, unless such loss also entails a breach of the Privacy Act.

Data safety monitoring board (DSMB): A data safety and monitoring board will be assembled as required by NICHD for pharmaceutical studies.

ii. Publication of Research Findings:

On conclusion of the randomized trial, it is hoped that the results of this pilot investigation will be publishable. If so, findings will be submitted to a peer-reviewed journal. Regardless of publishability, results will be deposited on www.clinicaltrials.gov as required by governmental rules.

iii. Statistical Analysis of the Data

Data analyses will be conducted by the investigators. The primary outcome of the randomized study will be change in insulin sensitivity as calculated by the minimal model from data obtained through the FSIVGTT in subjects who are randomized to colchicine versus those given placebo. Secondary outcomes will be changes in metabolic parameters (e.g. AIRg, disposition index, glucose effectiveness, HOMA-IR, HbA1c, lipid panel), and the inflammatory marker hsCRP. Additionally, changes in other inflammatory markers, endothelial cell adhesion markers, PBMC inflammatory parameters, SAT adipocyte size, SAT inflammatory parameters, and adipose tissue compartments will be evaluated as tertiary outcomes.

All analyses will be planned to be performed as intention-to-treat for all randomized subjects. For the primary outcome and the secondary outcomes, we will use an analysis of covariance (ANCOVA) to examine the differences between treatment arms, taking into account age, baseline body fat percentage, change in body fat percentage, and sex. Two-sided significance tests will be performed for these analyses, so that both positive and negative treatment effects may be detected. Spearman rank will be used for correlations.

For the open-label trial of seven patients with T2DM, the mean change (and the standard deviation) for the same variables pre-post treatment will be evaluated to help calculate the power of a potential future RCT.

iv. Monitoring Subjects and Criteria for Withdrawal

Screening evaluations may lead to withdrawal of subjects who are not eligible according to inclusion/exclusion criteria. Noncompliance with study procedures before subjects are started on drug may lead to withdrawal of study participants. Subjects will be followed using an "intention to treat" model once randomized. Phone contacts will be made to review medication tolerance and assess for development of serious adverse events after 1 week, 1 month, and 2 months of treatment. Subjects will be seen if serious adverse events are suspected. If subjects are seen by their own physicians for illnesses, we will request the records for those visits to determine if they are possibly related to the research.

v. Stopping Rules:

Events that will result in discontinuation of treatment include: pregnancy or serious unanticipated complications, including development of any medical problem listed in the exclusion criteria after starting on drug. Subjects will be excluded if they have any verified abnormality in laboratory values or physical status that cannot be explained by a mild intercurrent illness (or by obesity and insulin resistance itself) that in general are considered CTCAE grade 2 (See Appendix F) or greater for gastrointestinal, hematologic, clinical hemorrhage, kidney/bladder, alopecia, pulmonary, cardiac, neurocerebellar, allergy, flu-like symptoms, metabolic, or eye toxicities; and grade 3 or greater for infection, anorexia, circulatory, neurologic, dermatologic, coagulation, endocrine, or performance status toxicities.[115] Problem-specific stopping rules have been defined in the CTCAE v4.03 submitted to the IRB in conjunction with this protocol. Investigator error leading to initiation of treatment in an ineligible subject will lead to treatment discontinuation as soon as the error is uncovered. Should discontinuation be required for any reason, no tapering of colchicine is necessary; subjects will be requested to return all unused medication. Because the randomized study is a pilot intended to identify the sample size needed for a fully-powered trial, there are no formal stopping rules that would halt the study before all participants have been randomized.

B. Molecular Studies

Samples will be stored for future genetic studies of variants associated with body weight and inflammation. Examples of genes that may be examined include obesity-associated genes such as leptin, leptin receptor, melanocortin 4 receptor, melanocortin 3 receptor, brain-derived neurotrophic factor, the beta 3 adrenergic receptor, and SIM1, as well as inflammatory genes such as NLRP3, ASC, procaspase-1, IL-1, and IL-6. The consent form has been prepared to allow participants agree to whole-exome/genome sequencing for identification of genetic changes linked to inflammation, obesity or obesity-linked dysregulation of metabolism.

In some instances, investigators will share coded specimens from this protocol with collaborators who will not be provided with a key to the code. Subcutaneous adipose biopsy specimens will be sent to Dr. Vincent Manganiello's lab in a de-identified manner, where they will be examined for local inflammation. Dr. Joshua Zimmerberg's lab will receive coded samples for analysis of inflammation and metabolic function, such as adipose GLUT4 mobility.

In collaboration with the Center for Human Immunology, Autoimmunity, and Inflammation (CHI) at the NIH, coded samples will be sent for PBMC analysis, including mRNA expression array analysis, flow cytometry and cryopreservation. Samples of PBMC for protein lysates will be analyzed by Dr. Vincent Manganiello's lab via Western Blot analysis to examine colchicine's effects on NLPR3 inflammasome assembly, as well as for its effects on inflammasome-mediated IL-1B production. Dr. Michael Sack, NHLBI, will also receive coded serum samples for analysis of PBMC inflammation.

Dr. James Goedert (NCI) will receive coded samples of blood for analysis of estrogen and androgen markers, as they may change with changes in insulin sensitivity. For collaborators outside the NIH, The PI will establish these collaborations through an OHSRP request for determination and provide a list of all collaborations established in this manner to the IRB at the time of CR.

Stool samples will be obtained for future analyses to be performed by CHI.

Dr. Perry Blackshear, NIEHS, will received coded samples of blood and adipose tissue for analysis.

VI. Determination of Sample Size:

The randomized trial is a pilot study, intended to determine the treatment effect size and standard deviation for colchicine's effects on change in insulin sensitivity, to allow for adequate powering of a larger future study. Nonetheless, we will power it so it might be able to detect a moderate-to-large difference between treatment groups. Based on the data of prior studies,[116,117] the standard deviation (SD) for change in insulin sensitivity as measured by FSIVGTT is about 2 mIU/L/min. If a similar SD is found in the present study, we estimate that a total sample size of 40 subjects (20 colchicine and 20 placebo) is necessary to have 80% power (β) to detect a 60% difference in insulin sensitivity between colchicine and placebo groups, at level of significance <0.05 (2-sided).

VII. Human Subjects Protection:

A. Rationale for Subject Selection: This study seeks to understand how colchicine affects insulin sensitivity, pancreatic beta-cell function, and low-grade inflammation in humans. Because obese participants have a greater level of insulin resistance than non-obese individuals, and participants with MetS have a greater level of inflammation than those without, we selected to test obese individuals with metabolic syndrome who demonstrate both high insulin resistance and evidence for inflammation. Participants with type 2 diabetes mellitus frequently are taking medications that may confound the results of the tests we plan to study. Additionally, diabetics are presumed to have beta-cell destruction which may be too far advanced to demonstrate benefit from an intervention, whereas a disease process in an earlier stage (e.g. insulin resistance and/or prediabetes) may demonstrate benefit. On the other hand, diabetics also have the greatest level of obesity-associated inflammation, so they may demonstrate benefit from colchicine. To investigate this further, we will recruit seven subjects with diet-controlled DM to an Open Label arm.

   In order to expand our knowledge of local insulin action at the level of the adipocyte, we also plan to enroll subjects across a larger spectrum of insulin sensitivity/resistance for testing only at the Screen and Baseline Visits. Subjects who are not eligible to be randomized to study drug but who will be enrolled to the Evaluation Only arm will include those who are not obese, obese but without significant insulin-resistance or evidence of inflammation, and those with diet-controlled type 2 diabetes. Such subjects are excluded, as listed above, only if they are pregnant or nursing, use medications or substances that affect the validity of measures, have a significant medical/psychiatric condition, or weigh too much for required procedures.

   Children are not studied because the nature of the experiment involves a minor increment above minimal risk (adipose tissue biopsies, potentially toxic medication), that can be answered in adult subjects.

Adults of all races and ethnicities will be included. Since metabolic syndrome is identified in large percentages among minority groups including Asian Americans, African American and Hispanic Americans, we anticipate good representation of all major races/ethnicities. It is expected, given our recruitment procedures targeting individuals living in the greater metropolitan Washington D.C. area, that we will recruit rates of minority participation in excess of their representation in the Washington, D.C. metropolitan area: 64.2% Caucasian, 27.2% African-American and 8.6% Hispanic.

B. Participation of Children and Other Vulnerable Populations: n/a

C. Screening Methodology

Subjects who appear eligible when assessed during a phone screening intended to assess general health and body weight/height (to determine BMI) will attend a screening visit at the NIH Clinical Center, during which time eligibility will be determined. History and physical examination will be conducted by a licensed practitioner. Laboratory data from blood draw and pregnancy testing will be reviewed before subjects are scheduled for the Baseline visit and randomization to drug/placebo (or initiation of colchicine in the open-label arm).

Information regarding the key assays used for eligibility criteria (all measured by the NIH Clinical Center Laboratory Medicine Department):

Glucose

@75mg/dL	: intraassay CV 2%	interassay CV 3%
@ 379 mg/dL	: intraassay CV 1%	interassay CV 2%

Sensitivity: 1 mg/dL

Insulin

@ 24.2 µU/mL	: intraassay CV 1.2%	interassay CV 6.0%
@ 82 µU/mL	: intraassay CV 1.0%	interassay CV 2.5%

Sensitivity: 0.2uU/mL

hsCRP

@ 0.239 mg/dL	: intraassay CV 5.2%	interassay 5.2%
@ 0.650 mg/dL	: intraassay CV 5.0%	interassay 5.7%

Sensitivity: 0.016 mg/dL

Triglycerides

@ 68 mg/dL	: intraassay CV 3%	interassay CV 4%
@384 mg/dL	: intraassay CV 2%	interassay CV 2%

Sensitivity: 2 mg/dL

HDL cholesterol

@ 26 mg/dL	: intraassay CV 2.3%	interassay CV 2.7%
@ 47 mg/dL	: intraassay CV 1.6%	interassay CV 2.3%
@ 67 mg /dL	: intraassay CV 1.9%	interassay CV 2.1%

Sensitivity: 3 mg/dL

Hemoglobin A1c

@ 5.1%	: intraassay CV 0.4%	interassay 1.11%
@9.9%	: intraassay CV 0.5%	interassay 0.73%

Sensitivity: 4.2%

D. Risks/Benefits Analysis (including considerations of alternatives to participation)
   1. Potential Benefits, in addition to have the results of all screening testing, (as outlined above) from randomization include the following
      a. **Improving insulin sensitivity**
      b. **Improving pancreatic beta-cell reserve**
      c. **Decreasing inflammation**
      d. **Decreasing cardiovascular risk**
      e. **Benefit to medicine and society**
   2. Risks/Discomforts
      a. **Colchicine (randomized and open-label participants only)**

         i. Adverse Reactions

         Among post-marketing experience in the use of Colcrys (the trade name for colchicine that is available in the US), adverse reactions have been generally reversible upon temporarily interrupting treatment or lowering the dose of colchicine. The following adverse reactions have been reported with colchicine:

         - Neurological: sensory motor neuropathy
         - Dermatological: alopecia, maculopapular rash, purpura, rash

- Digestive: abdominal cramping, abdominal pain, diarrhea, lactose intolerance, nausea, vomiting
- Hematological: leukopenia, granulocytopenia, thrombocytopenia, pancytopenia, aplastic anemia
- Hepatobiliary: elevated AST, elevated ALT
- Musculoskeletal: myopathy, elevated CPK, myotonia, muscle weakness, muscle pain, rhabdomyolysis
- Reproductive: azoospermia, oligospermia

Tolerance, abuse, or dependence with colchicine has not been reported.[68]

In recent clinical trials, the most commonly reported adverse reactions were abdominal symptoms such as diarrhea, cramping, nausea, diarrhea, abdominal pain, and vomiting. For example, in a randomized, placebo-controlled trial investigating colchicine 0.6mg PO bid for 6 months in participants after coronary angioplasty, of the 130 subjects in the intervention group 28% developed diarrhea, whereas other side effects, including nausea or rash, were not significantly different from placebo.[118] Another randomized placebo-controlled study investigating colchicine 0.5mg PO bid for 6 months in DM participants undergoing percutaneous coronary intervention (PCI) with a bare metal stent (BMS), of the 196 subjects 16% developed diarrhea or nausea in the colchicine group vs. 7% in the placebo group.[81]

Subjects who may develop renal or hepatic impairment due to causes independent of participating in the protocol are at increased risk for developing adverse reactions and toxicities as colchicine is metabolized in the liver, and excreted in significant amounts in the bile and urine. (*See Section VII. H.*)

ii. Fatal Overdose

Fatal overdoses, both accidental and intentional, have been reported in adults and children who have ingested colchicine. The exact dose of colchicine that produces significant toxicity is unknown. Fatalities have occurred after ingestion of a dose as low as 7 mg over a 4-day period, while other participants have survived after ingesting more than 60 mg. A review of 150 participants who overdosed on colchicine found that those who ingested less than 0.5 mg/kg survived and tended to have milder toxicities, such as gastrointestinal symptoms, whereas those who took

0.5 to 0.8 mg/kg had more severe reactions, such as myelosuppression. There was 100% mortality in those who ingested more than 0.8 mg/kg.

The first stage of acute colchicine toxicity typically begins within 24 hours of ingestion and includes gastrointestinal symptoms, such as abdominal pain, nausea, vomiting, diarrhea, and significant fluid loss, leading to volume depletion. Peripheral leukocytosis may also be seen. Life-threatening complications typically occur 24 to 72 hours after drug administration, and death may result from respiratory depression and cardiovascular collapse. Individuals who survive a severe overdose may develop rebound leukocytosis and alopecia one week after the initial ingestion. Treatment of colchicine poisoning should begin with gastric lavage and supportive measures. No specific antidote is known, and colchicine is not effectively removed by dialysis.

iii. Drug Interactions

Colchicine is metabolized by P-gp and CYP3A4. Life-threatening and fatal drug interactions have been reported in individuals taking colchicine concurrently with P-gp and/or strong CYP3A4 inhibitors (*see Appendix B1*). If treatment with a P-gp or strong CYP3A4 inhibitor is required in a subject, the investigational medication will be discontinued and the patient will be withdrawn from the protocol.

iv. Pregnancy

Adequate well-controlled studies on the use of colchicine in pregnancy do not exist. Among published reports of pregnant women using colchicine to control their Familial Mediterranean Fever, no increased risk of miscarriage, stillbirth, or teratogenic effects was seen. However, colchicine did demonstrate fetal toxicity and teratogenicity in the offspring of pregnant rats. Subjects currently pregnant, breastfeeding, or planning to become pregnant will be excluded from the study. Enrolled female subjects will be strongly advised to use contraception and condoms if sexually active. As noted above, women who become pregnant while taking study medication will be immediately told to stop medication and will be withdrawn from all study procedures. The study team will follow up with the participant regarding the outcome of the pregnancy.

b. **Physical Examination** by a health care provider involves no known risk, but does take time. A limited physical examination will be performed in a concise but thorough manner with appropriate measures taken to provide the privacy of the subject.

c. **Questionnaires** are also without significant risk, but are inconvenient because of the time required to complete.

d. **DXA scanning** is not painful, but may be inconvenient because of the time needed to complete the study. DXA scanning involves radiation exposure for research purposes only. According to the manufacturer's specifications, DXA scanning for body composition using the array beam delivers an effective dose of 0.00003rem total body radiation. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. This amount of radiation from the three DXA scans to be performed in this study is 0.00009 rem. For comparison, the average person in the U.S. receives a radiation exposure of 0.3 rem/yr from natural background sources, such as from the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil.

e. **Blood withdrawals** will remain well within the NIH guideline for adults of 7 ml/kg every 6 weeks. Blood collection with venipuncture is associated with mild discomfort and the possibility of localized bruising or extravasations. The risk of infection, phlebitis, or fainting is extremely small.

f. **Stool samples** are also without significant risk, but some subjects may find them uncomfortable to collect. The surveys and food records are without significant risk, but may be inconvenient because of the time required to complete.

g. **Oral glucose tolerance test** is also is without significant risk, but is inconvenient because of the time required to complete the study. The only risks from this kind of study are those described for any blood draw, as well as developing nausea from the oral glucose. As we are screening out patients with diabetes mellitus, the risk of developing hyperglycemia exists, but in most cases should be transient and not clinically significant.

h. **Subcutaneous abdominal adipose tissue biopsies.** The risks associated with biopsies include pain, bleeding, bruising, infection, numbness related to nerve injury, and scarring or keloid formation. Lidocaine will be used to diminish pain. A sterile approach will be incorporated to avoid risk of infection, and to our knowledge, there has not yet been a report of infection. After the procedure, sterile dressing will be applied, in addition to an ice pack; the latter will be used to decrease bleeding/hematoma formation. In the immediate post-procedure period (while subjects are observed), the site will be

monitored for immediate complications, and addressed appropriately. Biopsies from abdominal subcutaneous fat will be taken. Subjects will also be instructed on signs of these risks and to observe the site after discharge, and to immediately report any concerns to the clinical team. The physician/nurse practitioner who is in charge of performing the biopsy will fill out a "Surgical/Invasive Procedure Verification Checklist" during the procedure to make sure every prerequisite to ensure safety of the subject is met. If increased occurrences of any of these risks are noted, technique will be adjusted in attempt to reduce the risk.

i. **Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT)** is inconvenient because of the time it takes to complete, the need for two intravenous lines, and the need to be confined to bed. The only risks from this kind of study are those described for any blood draw (see #5), mild arm tenderness while the dextrose is infused, and the possibility of a decreased blood glucose value from the administration of insulin. The standard dose of insulin administered for evaluation of growth hormone secretory capacity, 0.05 U/Kg, will be administered. With mildly low blood sugars (< 50 mg/dL), subjects get hungry and may become drowsy or sweaty. With blood sugars under 30 mg/dL, subjects may be difficult to arouse or have seizures. However, because the obese adults to be studied on this protocol will all have some degree of insulin resistance, significant hypoglycemia is not anticipated. Nevertheless, a physician or nurse practitioner will be present during this test for the first hour of the test (including the first thirty minutes following insulin administration) to monitor for signs of significant hypoglycemia. The principal investigator will be notified immediately of any glucose measurement less than 40 mg/dL. The principal investigator will use his clinical judgment in developing an appropriate, situation-specific management plan. Treatment will take place as needed with dextrose or glucagon according to clinical evaluation.

j. **CT Abdomen for hepatic and intra-abdominal adipose tissue** is not painful, but may be inconvenient because of the time needed to complete the study. Some people feel anxious while inside the CT scanner.

Computed tomography involves radiation exposure for research purposes only. Although each organ will receive a different dose, the amount of radiation exposure subjects will receive per scan is equal to a uniform whole-body exposure of 0.031 rem. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. The total effective radiation dose that patients will be exposed to in the study is 0.093 rem, well within the dose guideline established

by the NIH Radiation Safety Committee for adult research subjects. The guideline is an effective dose of less than 5 rem/yr. **No contrast dye** will be administered as part of this protocol.

One possible effect that could occur at these doses is a slight increase in the risk of cancer. The natural chance of a person getting a fatal cancer during his/her lifetime is about 25 %. The increase in the chance of getting a fatal cancer as a result of the radiation exposure received from this research study is 0.1%. Therefore, the total risk of fatal cancer may be estimated to increase from 25% to 25.1%. This change in risk is small and cannot be measured directly. Compared with other everyday risks, such as flying in an airplane or driving a car, this increase is considered slight.

Subjects will be advised to notify the research staff if they have participated in research studies at the NIH or other institutions that involved the use of radiation so as to make sure that the total radiation dose from all studies is not excessive. Women with childbearing potential must have a negative pregnancy test before undergoing the CT scan.

k. **"Smart" Pill Bottle Cap.** We will use a "MEMS cap" (MWV Switzerland, Ltd) on the top of the medicine bottles to record patient adherence to the study drug. As described above, the cap records the date and time that the bottle is opened, and the data will be downloaded by one of the protocol investigators onto a laboratory computer. This will pose no additional risk to the subjects.

l. **Testing for DNA including exome/whole genome sequencing.** The risks from DNA tests are primarily the risk of discovery of incidental medical information, risks of genetic discrimination and risks related to confidentiality and data availability.

    a. **Incidental medical information.** Genetic risk factors may be found for diseases unrelated to the condition for which the patient is being studied. This risk is inherent in any genetic analysis; however, the risk increases with increased magnitude of data and degree of analysis. This study will uncover incidental information. In some cases this information will provide additional details about diseases already known to be present in a family. For example, identification of a BRCA1 mutation may be new information for a family, but they may already know that breast cancer occurs in their family. Similarly, identification of a Huntington disease mutation is not likely to occur in a family without any prior family history. Clinically significant information could be identified for diseases that were not

previously recognized to be a concern in a given family. This could occur with dominant disorders that show decreased penetrance or with recessive genetic disorders. Exome studies can detect carrier status for recessive disorders such as cystic fibrosis or sickle cell anemia. Determination of carrier status for recessive disorders will not impact the subject's health directly, but this information does have potential reproductive implications for the subject and other members of the family. This information could cause anxiety in some individuals; however, being aware of these conditions provides an opportunity for genetic counseling.

As genetic understanding of genetic risk factors for multifactorial disorders increases, we may detect vast numbers of polymorphisms that slightly increase the risk of a specific disorder such as diabetes or heart disease. The knowledge of these risk factors could provide an opportunity to alter lifestyle factors that contribute to the risk, but at the same time could produce anxiety. Unless felt to be of urgent medical importance, we will not report these results to patients or families. General principles guiding a decision to inform guardians about genetic information obtained from this study will be:

i. The genetic change must be known or predicted to be of urgent clinical significance to either a proband or a first degree relative.
ii. Knowledge of the finding must have a clear direct benefit that would be lost if diagnosis was made at a later time point. Specifically, knowledge of this risk factor will substantially alter the patient's medical care.
iii. The potential benefit of knowing a genetic disorder exists clearly outweighs the potential risks of anxiety and subsequent medical testing that could result from this knowledge.
iv. Autosomal recessive mutations will only be reported if a) the carrier frequency for mutations in that specific gene is greater than 1% (This corresponds to disorders with a disease incidence of more than 1/40,000), b) the syndrome results in significant morbidity, c) early diagnosis and intervention would have significant benefit for an affected individual, and d) early diagnosis is not likely to be made by other means.

The decision to notify or not notify will be made by the study investigators based on the state of genetic knowledge at the time of data analysis. Although not precluded, the database will not be actively screened for changes that in the future are found to be

associated with a specific disease. If a genetic change of clinical significance is detected, subjects will be given the option to have confirmatory testing performed in a CLIA approved laboratory. Confirmatory testing will be performed on new DNA samples obtained from the probands. Identification of genetic disease in a proband could produce anxiety for the parent, siblings or other relatives. Relatives of the proband could learn that they are at risk for a genetic disorder.

b. Genetic Discrimination: If an individual elects to have a genetic finding confirmed, that information will then become part of the medical record at the NIH. This information will be released to third parties if the individual sign a release of information form. An insurance company or an employer could interpret this to be a pre-existing condition to deny benefits or coverage. This however, is largely a theoretical concern. No cases of discrimination are known to have resulted due to participation in a research study. The risk of genetic discrimination in the context of health insurance and employment should also be decreased by Genetic Information Nondiscrimination Act of 2008.

c. Confidentiality and Data availability: Samples and data will be coded. Only individuals involved in consenting and patient care will have access to identifiers. Records containing personal identifiers will be maintained consistent with the security measures required by the NIH. We will follow guidelines developed by the NIH for projects funded by the Federal Government. In lieu of specific guidelines, subjects may opt out of having sequence data deposited in public databases.

Results of genetic tests performed for research will not be placed in the medical record. It is possible that we will send subject's DNA to other scientists working with us on obesity gene studies, but in this case personal identifiers will be removed. If a subject chooses to withdraw from the study, we will keep the DNA for analysis, unless the subject requests that the sample be destroyed.

3. **Assessment of Risk/Benefit Ratio:** Although it is impossible to quantify the exact risk to benefit ratio, we feel that the risks as mentioned above are a minor increment above minimal risk and mitigated whenever possible. The potential benefit to the randomized and open-label subjects can be large with a decrease in cardiovascular risk factors, improvement in metabolic profile, and decrease in chronic inflammation. Additionally, we feel that this

study will contribute significant knowledge to the field of medicine in examining a novel therapeutic approach in humans to improve insulin resistance and metabolic syndrome. To date the effects of disrupting the NLRP3 inflammasome on metabolic parameters and glucose homeostasis have not been studied in humans with metabolic syndrome.

4. **Alternatives to participation:** The major alternative is not to participate in this experiment, as there are no inflammasome-reducing medications approved for patients with metabolic syndrome. Participants could also consider starting a formal lifestyle modification program, including improved diet and exercise. Established medical options for obesity and metabolic syndrome include undergoing bariatric surgery or starting weight-loss medications (e.g. lorcaserin or phentermine/topiramate). Participants can look up research alternatives for interventions for obesity or metabolic syndrome on www.clinicaltrials.gov.

E. Privacy and Confidentiality Provisions

We will follow all relevant NIH policies and HRPP SOPs. Samples and data will be coded. Only individuals involved in consenting and patient care will have access to identifiers. Records containing personal identifiers will be maintained consistent with the security measures required by the NIH. Access to the code will be limited to the study investigators (e.g. JY, AD, TC, and SB). Electronic data will be password protected and accessible only by authorized study personnel. Electronic data will be backed up daily and stored at a secure server. All hardcopy data will be locked in Dr. Yanovski's storage cabinets in research offices at the NIH Clinical Research Center and will be accessible only by authorized study personnel. All data will be collected specifically for the purpose of the current proposed research project. Any email communications that involve PII will be sent using encryption or the secure email system of the NIH. Since even when precautions are taken to ensure participant confidentiality, data collection in any study is accompanied by threat to privacy and confidentiality, in the unlikely event of a potential breach of confidentiality, the PI will immediately inform the IRB. The hsCRP and other clinical chemistry results are done by NIH Clinical Pathology and will be in the medical record. Results of the serum cytokine profile, endothelial adhesion profile, lipoprotein NMR, DXA, DNA, and adipose tissue studies will not be placed in the medical record.

In the Clinical Trials Database, clinical and demographic information will include age; sex; race/ethnicity; family history of diabetes, hypertension, or hyperlipidemia and results entered from each case report form for visits and phone conversations.

F. Compensation

We will use NIH guidelines for payment of research volunteer. The remuneration is based on time and inconvenience during the stay. Payment will be as follows:

[For randomized or open-label subjects]

1. Outpatient screening visit:	$100
2. Baseline evaluation visit:	$300
3. Interim Safety Visit:	$50
4. Final Visit:	$300
5. Washout Visit:	$300
Total=	$1050

[For evaluation-only subjects]

1. Outpatient screening visit:	$100
2. Baseline evaluation visit:	$300
Total=	$400

G. Consent Procedures

Each subject will receive a written explanation of the purposes, procedures, and potential hazards of the study (*See Consent Form*). The purpose of the project, all testing procedures, and study components will be described in detail. Potential participants will also be informed of the possible risks and inconveniences of the study. Consent will be obtained only by the principal and associate investigators and Key Research Personnel designated on the first page of the protocol (e.g. JY, AD, SB), in person at the beginning of the first Screening Visit. Communication of this information and of the subject's consent will be documented in the medical record. All subjects will be informed of their right to withdraw from the study at any point of time during the study.

Non-English–Speaking Participants

If a non-English speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non-English Speaking Research Participants in the participant's native language and a verbal explanation of the purpose,

procedures and risks of the study as described in MAS Policy M77-2, NIH HRPP SOP 12 and 45 CFR 46.117(b)(2). The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will interpret all oral communications (English to target language and conversely) about the IRB-approved English consent form, facilitate discussion between the participant and investigator, and ensure understanding.
The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home. The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter.

We request prospective IRB approval of the use of the short form for up to a maximum of 5 separate encounters in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and that we will have that consent document translated into the given inherent language.

H. Pharmaceutical Information

Colchicine is an alkaloid chemically described as (S)N- (5,6,7,9-tetrahydro- 1,2,3,10-tetramethoxy-9-oxobenzo [alpha] heptalen-7-yl) acetamide with a molecular formula of C22H25NO6 and a molecular weight of 399.4.

Pharmacokinetics

Absorption: "In healthy adults, colchicine is absorbed when given orally, reaching a mean C_{max} of 2.5 ng/mL (range 1.1 to 4.4 ng/mL) in 1 to 2 hours (range 0.5 to 3 hours) after a single dose of 0.6 mg administered under fasting conditions. After 10 days on a regimen of 0.6 mg twice daily, peak concentrations are 3.1 to 3.6 ng/mL (range 1.6 to 6.0 ng/mL), occurring 1.3 to 1.4 hours post-dose (range 0.5 to 3.0 hours). In some subjects, secondary colchicine peaks are seen, occurring between 3 and 36 hours post-dose and ranging from 39% to 155% of the height of the

initial peak. These observations are attributed to intestinal secretion and reabsorption and/or biliary recirculation. Absolute bioavailability is reported to be approximately 45%. Administration with food has no effect on the rate of absorption, but does decrease the extent of colchicine by approximately 15%, which is likely clinically insignificant."[68]

Distribution

"The mean apparent volume of distribution in healthy young volunteers was approximately 5 to 8 L/kg. Colchicine binding to serum protein is low, 39 ± 5%, primarily to albumin regardless of concentration"[68]. Accumulation into neutrophils and macrophages is slow, only reaching peak concentration at 48 hours.[119]

Colchicine crosses the placenta (plasma levels in the fetus are reported to be approximately 15% of the maternal concentration). Colchicine also distributes into breast milk at concentrations similar to those found in the maternal serum.[68]

Metabolism

Colchicine is demethylated to two primary metabolites, 2-O-demethylcolchicine and 3-O-demethylcolchicine (2- and 3-DMC, respectively), and one minor metabolite, 10-O-demethylcolchicine (also known as colchiceine), primarily through CYP3A4. Plasma levels of these metabolites are minimal.[68]

Elimination/Excretion

"In healthy volunteers (n=12) 40 – 65% of 1 mg orally administered colchicine was recovered unchanged in urine. Enterohepatic recirculation and biliary excretion are also postulated to play a role in colchicine elimination. Following multiple oral doses (0.6 mg twice daily), the mean elimination half-lives in young healthy volunteers (mean age 25 to 28 years of age) is 26.6 to 31.2 hours."[68]

Preparation of Investigational Colchicine by Pharmaceutical Development Section or Pine Pharmaceuticals

Colchicine USP powder, purchased from Spectrum Chemical, will be dispensed. Each capsule will contain 600 micrograms of colchicine. Matching placebo capsule will be produced that only contain the non-colchicine ingredients. Colchicine will be verified to be chemically pure and to

have acceptable stability using standard approaches by the NIH Pharmaceutical Development Section (before 2017), the NIH IDMS subsequently, or Pine Pharmaceuticals.

I. Recruitment Strategies

Participants will be recruited from Washington, DC and local suburbs in Maryland and Virginia. Recruitment efforts will aim to enroll subjects in proportion to their representation in the population of Maryland. Subjects of all racial, ethnic and socio-economic backgrounds will be invited to participate in this study. We will contact by mail or email, adult obese subjects who have previously indicated interest in research and/or have already participated in protocols run by the Section on Growth and Obesity (SGO), to inform them of this new study and assess their potential interest. These potential subjects will be provided with a telephone number and email address to call or write if they are interested to request more information. Participants will be also be recruited by means of flyers posted in the NIH Clinical Center, in other buildings at the NIH campus in Bethesda, MD, and in surrounding local businesses. If necessary, further subjects will be recruited through newspaper advertisements or letter mailings. Advertisements will be submitted to the IRB before use.

J. Existing data: n/a

K. Description of criteria for withdrawal from study

The types, frequency, and duration of tests and visits are outlined on the flow diagrams (Appendix A1-6). Subjects will be examined at each of their visits. If unanticipated reactions are uncovered during the study, we will report these promptly to the IRB. The CRC has ample medical resources should any unanticipated event occur. Additionally, study staff will seek evidence of emotional or physical distress or discomfort, and will be instructed to terminate the protocol if a participant demonstrates distress.

Study staff will emphasize to participants that they are able to withdraw from the study at any time, without penalty. Noncompliance with study procedures may lead to withdrawal of study subjects – for example, unwillingness to take the medication or inability to complete required testing (e.g. FSIVGTT). Other events that will result in discontinuation of treatment include:

1. Pregnancy.

2. Any verified abnormalities in laboratory values or physical status that cannot be explained by an intercurrent unrelated illness (or by obesity itself) that are considered CTCAE grade 2 or greater for gastrointestinal, hematologic, clinical hemorrhage, kidney/bladder, alopecia, pulmonary, cardiac, neurocerebellar, allergy, flu-like symptoms, metabolic, or eye toxicities; and grade 3 or greater for infection, anorexia, circulatory, neurologic, dermatologic, coagulation, endocrine, or performance status toxicities.[115] Problem-specific stopping rules have been defined in the CTCAE v4.03 submitted to the IRB in conjunction with this protocol.

Unblinding Procedure

In the randomized portion of the trial, all participants, Study Site staff, and pathology and laboratory personnel are blinded to the individual assignment of the order in which colchicine and placebo are administered. Participants will learn their individual assignments (i.e., be unblinded) when the trial is complete. In rare instances, it may be necessary to unblind a participant's order of assignment before completion of the trial, to a physician, to the Study Site, or to the participant.

In general, however, participants should not be unblinded. Anytime a staff member learns the treatment assignment of a participant, the staff member is at risk of being influenced by that information. For example, if the unblinded participant was known to have taken the active agent and reported a toxicity, the staff member might make inferences about the effects of colchicine on that participant, and possibly on other participants as well. The management of future participants may be influenced by this information. Maintaining the double-blind design offers the best protection against this potential bias.

In the unlikely event that a Serious and Unexpected Suspected Adverse Reaction (SUSAR) occurs and a physician must be unblinded to the treatment group to which an affected trial subject belongs, the Investigational Drug Management Section (IDMS) will be contacted by a study investigator (usually by the Principal Investigator). The study investigator will request that IDMS release the needed information and specify the individual to receive the information. IDMS will normally supply unblinding information only to a physician who is not a co-investigator on the study and make clear that group assignment must not be given to study investigators. This procedure is intended to ensure that the identity of the investigational medicinal product is revealed only insofar as is necessary. As much as possible, the blinding should be maintained for the investigators and for anyone responsible for data analysis or interpretation. A subject’s treatment group should not be revealed to a study investigator except in a medical emergency, i.e. when this appears necessary to ensure the subject’s safety and

would be instrumental in further treatment decisions. If a subject's treatment is unblinded, this must be documented by IDMS in their written records. In all cases, the reasons and rationale for unblinding will be also documented in writing and maintained in the study file.

Unblinded data are also available to the Data Safety Monitoring Committee (DSMC). In the case of a SUSAR, the DSMC is informed and will thus have the opportunity to review subject-level data and make any changes to the experimental plan that are required. In the case of a SUSAR, the DSMC may elect to reveal the treatment group to which a subject belongs, request additional safeguards, or stop the study. The DSMC will assist investigators in deciding if a SUSAR requires any of the study investigators to become unblinded so that the FDA can be informed of subject assignment because of a SUSAR.

L. Re-evaluation of Study: Not Applicable for this pilot study.

M. Collection, monitoring, analysis and reporting of adverse events

Adverse events, protocol deviations, unanticipated problems (UP), serious adverse events, sponsor and serious, are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations."). All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and CD as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

The PI and AIs will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs and report them to the Sponsor according to the timetable for reporting specified in the protocol (21 CFR 312.64(b)).

a. Waiver of Reporting to the IRB of anticipated minor protocol deviations and minor adverse events (AE)

The following anticipated minor deviations in the conduct of the protocol will not be reported to the IRB unless they occur at a rate greater than that which is anticipated to occur:

Problem	Expected Frequency
Rescheduled appointment for protocol visit	50%
Unable to be contacted for follow up phone call	20%
Inability to obtain blood sample after 2 attempts	25%
Venous sample at a time point not obtained	25%
Bruising, fainting or lightheadedness after a blood draw	10%
Hemolysis or other issue preventing analysis of a blood or urine sample	25%
Stool sample not obtained or obtained on a different day	25%
Adequate subcutaneous adipose tissue sample not obtained	25%
Subject needs to make an additional visit for labwork (e.g. arrives too late for bloodwork, specimen result hemolyzed/equivocal, etc.)	30%
CT at time point not obtained	15%
Loss of investigational medications by subject	15%
Inadvertent loss of samples or data	10%
Appointment conducted >2 weeks beyond anticipated date	10%
Extra samples of blood drawn	10%
Inadvertent collection of additional data (e.g. extra survey, food record, stool sample, etc.)	10%
Becoming physically ill (e.g. abdominal pain, nausea, etc.) from consuming NIH CC food	10%
Sustaining minor bodily injury (e.g. twisted ankle) while participating in protocol	5%
Subject missed at least one dose but took at least 50% of study medication prescribed	100%
Subject took fewer than 50% of study medication prescribed	15%
Subject took an extra dose of study medication	5%
Subject forgot to return study medications at subsequent visit	20%

Loss of, or forgot to bring back, MEMSCap	20%
MEMSCap not dispensed with open-label colchicine	20%

The following anticipated non-UP adverse events will not be reported to the IRB unless they occur at a rate greater than that known to occur in this population:

i. Colchicine administration (events detailed in the FDA-approved package insert for colchicine): We anticipate that up to 30% of subjects will experience gastrointestinal side effects (e.g. diarrhea, abdominal pain, nausea, vomiting),[68] which may require dose reductions to 0.6mg once daily. If this dose adjustment does not improve symptoms, it will be decided by patient and physician, on a case by case basis, whether to discontinue medication before the study is completed. Other anticipated adverse events from colchicine include: fatigue (4%), pharyngolaryngeal pain (3%), and headache (2%). Rare events (< 1%) may include alopecia, blood dyscrasias, hepatotoxicity, hypersensitivity reaction, neuromuscular toxicity (e.g. myalgia, myasthenia, myopathy, neuropathy, dizziness, rhabdomyolysis), and rash.[68]
If the rate or severity of these events exceeds the rate or severity anticipated in the protocol or FDA-approved package insert, the events will be classified and reported as though they are Unanticipated Problems.

Because we anticipate that other mild symptoms may occur in the course of participation in the protocol unrelated to study drug or participation, we will not submit Unanticipated Problems to mild signs/symptoms (CTCAE Grade ≤ 2), such as rhinorrhea, nasal congestion, watery eyes, cough, lightheadedness, mild wheezing, menstrual cramps, muscle weakness, fever blister, mild urinary tract infection, etc., that occur infrequently (≤ 10%).

ii. OGTT: Infection (<5%), bruising (<30%), phlebitis (10%), nausea (10%), vomiting (10%), lightheadedness (10%).
iii. FSIVGTT: Infection (<5%), bruising (<30%), phlebitis (10%), nausea (10%), vomiting (10%), hypoglycemia (10%), lightheadedness (10%), hunger (10%).
iv. Fat Biopsy: Infection (<5%), bruising (<30%), bleeding (10%), nausea (10%), lightheadedness (10%), soreness (50%), local skin reaction (10%).
v. Other: Skin reaction to EKG electrodes <15%

If the rate of these events exceeds the rate specified in the protocol, the events will be classified and reported as though they are Unanticipated Problems.

b. Plan to monitor and report adverse events

As outlined in Appendix A, for randomized and open-label subjects, adverse events will be monitored via telephone at one week, one month, and two months after the Baseline Visit using structured case report forms. If any participant develops any concerning adverse events, the individual will be scheduled for an immediate clinic appointment with a trial investigator for further work up, as necessary. Adverse events will also be assessed during the final visit via interim history and physical, as well as laboratory studies as described in Section IV C/D.

Once 20 subjects have been enrolled into the randomized study, an interim analysis examining the rate of unanticipated problems and adverse events will be performed to evaluate whether they are occurring at a frequency greater than anticipated.

c. DSMB

A DSMB will be assembled for oversight of the randomized phase of this protocol by the Clinical Director, NICHD, with membership and scope determined by applicable institutional guidelines. The DSMB will meet to evaluate all serious adverse events, and will at a minimum convene after at least 20 subjects have been enrolled. The DSMB will examine whether there is evidence of safety and efficacy for colchicine. The primary outcome measure that we propose the DSMB examine for efficacy is change in insulin sensitivity as determined from the FSIVGTT using the minimal model calculation. For safety, the DSMB will review adverse event questionnaire data, and reports of side effects from all visits. These reports will also be supplemented by a manual review by the investigators, who will add any other events not captured by these systems, and data files of laboratory values for CBC, acute care, hepatic, and mineral panels. Results will be presented as an A vs. B analysis prepared by the data manager. The DSMB will be asked to prepare a detailed report to supply to the IRB that describes any adverse events discussed, and that explains the DSMB's vote to allow the study to continue or to end the study.

N. IND Monitoring Plan

1. Purpose

The purpose of this Monitoring Plan is to describe the rationale and process for the collection, recording, and verification of data for NICHD Protocol, titled: Pilot study of the effects of colchicine in non-diabetic adults with metabolic syndrome.

2. Objectives

a. To establish a monitoring plan to ensure the protocol data are in compliance with Good Clinical Practice (GCP), NICHD Institutional Review Board (IRB) and Federal regulations.
b. To ensure the validity, accuracy and integrity of the data

3. Study Staff Responsibilities

Jack A. Yanovski, MD, PhD (the principal investigator) is responsible for all aspects of the study. Some responsibilities may be delegated to the current associate investigator Andrew Demidowich, MD:

Delegation of responsibility will be documented on a study staff *Signature and Delegation of Responsibility Log.*

STUDY STAFF NAME	TITLE	*DELEGATED RESPONSIBILITIES
Jack Yanovski, MD, PhD	Principal-Investigator	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Andrew Demidowich, MD	Lead Associate Investigator	1, 2, 3, 4, 5, 6, 7, 8, 11, 12
Jordan Levine, BS	Co-investigator	1, 6, 7, 8, 11

*****Delegated Study Tasks:		
1. Obtain Informed Consent	5. Prescribe Study Drug/device	9. Maintain Regulatory Docs
2. Obtain Medical History	6. CRF Completion	10. Maintain IRB documents
3. Perform Physical Exam	7. CRF Queries	11. Data Monitoring
4. Assess Eligibility Criteria	8. Query completion	12. Safety Monitoring

4. Source Documentation and Case Report Forms

Jack A. Yanovski, MD, PhD and Andrew Demidowich, MD are responsible for coordinating data collection and will review the data for accuracy and completeness within seven days of each subject visit.

The PI (*Jack A. Yanovski, MD, PhD)* along with study co-investigator *Andrew Demidowich, MD* will conduct initial monitoring. Primary documents, Case Report Forms, and the Clinical Trials Database will also be audited per NICHD guidelines by Amarex. LLC. Patient consent documents, primary outcome and safety laboratory results, and diagnostic test results will be monitored for accuracy, correct dating, and agreement between case report forms and source documents. As case report forms are entered electronically into the NICHD Clinical Trials Database, the computer system contains logs indicating changes made and the circumstances leading to these changes.

FDA regulatory requirements (annual reports, adverse events reporting, etc.) related to IND # 120722 (Colchicine) will also be monitored. The medical records of active subjects (defined as subjects receiving study medication) will be monitored quarterly or more frequently as required. The FDA issues will be monitored at least annually. Any major findings will be summarized in writing and reported to the NICHD Institutional Review Board and DSMC, if indicated. Investigator credentials, training records, and the delegation of responsibility log will also be reviewed on an annual basis.

The NICHD Data Safety Monitoring Board will also conduct monitoring per NICHD guidelines. They will conduct A versus B analyses on data, but may also see unblinded data from the trial for their review of adverse events.

5. IRB and DSMC Documentation

All IRB documentation can be found in PTMS. The Principal Investigator, Jack A. Yanovski, MD, PhD, is responsible for maintaining IRB and DSMC correspondence related to this protocol, including records of all reviews of the study and submissions to the IRB and DSMC.

6. FDA Documentation

Jack A. Yanovski, MD, PhD is responsible for maintaining FDA correspondence, including forms 1571 and 1572 and other correspondence (e.g., annual reports, amendments, safety reports) in the electronic binder for IND # 120722 (Colchicine).

Copies of all such correspondence are also maintained in PTMS as part of the NICHD protocol record.

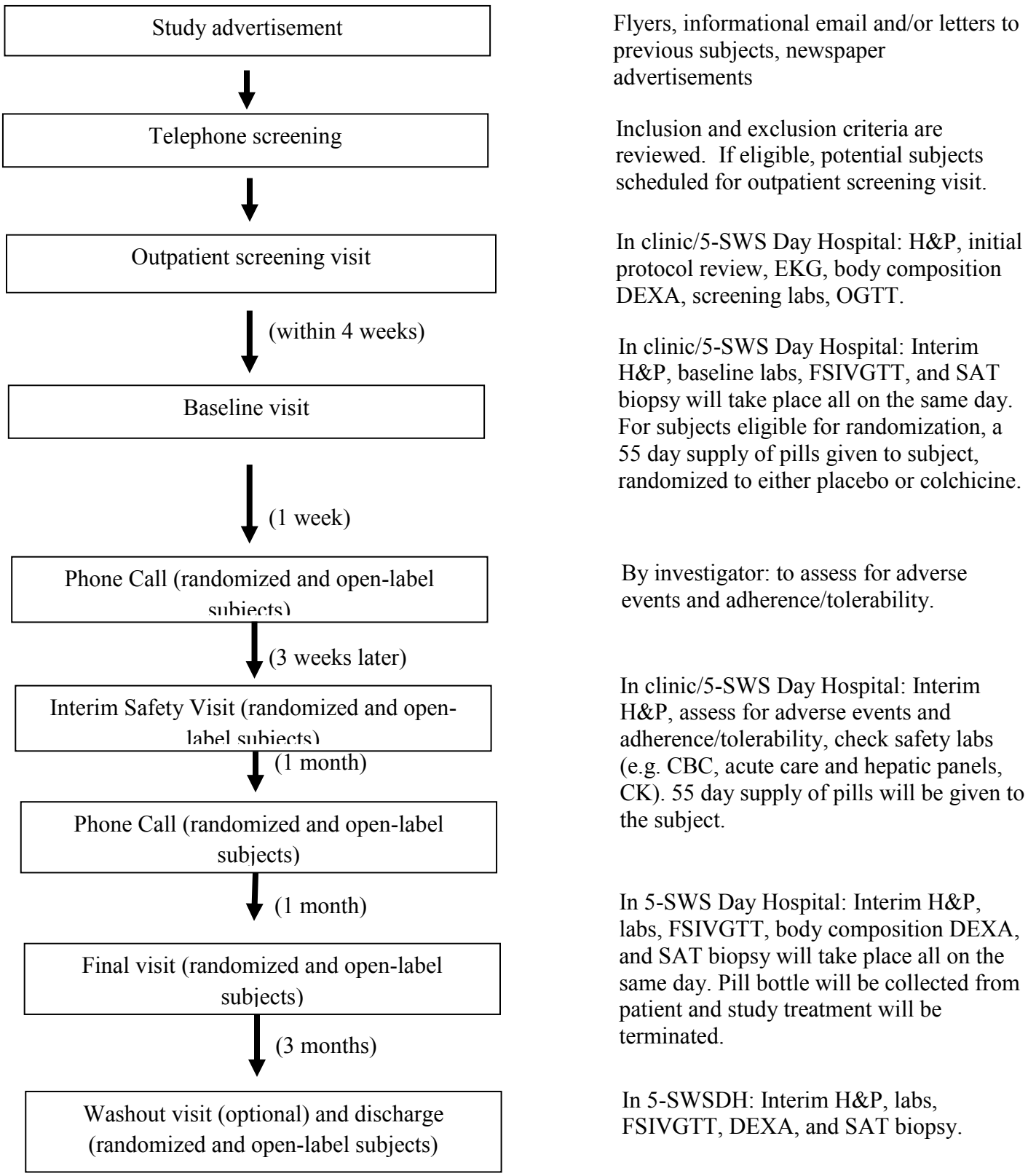
7. Adverse Event Procedures and Documentation

Please refer to Section M of the protocol.

8. Study Completion

Upon completion of the study *Jack A. Yanovski, MD, PhD* will retain possession of the electronic IND binder and electronic Protocol Binder in a secure location. FDA requires records be retained for at least 2 years after study completion. HHS regulations require that subjects' records be maintained for at least three years after completion of a study.

Appendix A1– Study Design



Appendix A2 – Screening Visit Flowsheet

Event	Procedure
Arrive to NIH 5-SWS Day Hospital by 8AM	Page Protocol team on arrival of patient. Accurate height x3 and weight. H&P performed.
Protocol Review	Protocol is reviewed by research team, and consent is signed formally with witness present.
EKG	Performed in Day Hospital
DXA Scan	Performed on 7th floor, Metabolic Unit
Fasting blood draw	Performed at Time 0 of the 2 hour OGTT.
2 hour oral glucose tolerance test	Performed in Day Hospital.
Discharge from NIH	Schedule baseline visit if subject qualifies

Appendix A3 – Baseline Visit Flowsheet

Event	Procedure
Arrive to NIH 5-SWS Day Hospital by 8AM	Page Protocol team on arrival of patient. Accurate height x3 and weight. Interval H&P performed.
Stool sample and Hollingshead Questionnaire	Performed in Day Hospital. Stool sample collected in Day Hospital if subject did not bring sample from home.
Fasting blood draw	Performed at Time -15 min of the FSIVGTT
FSIVGTT	Performed in Day Hospital.
Subcutaneous Adipose Tissue Biopsy	Performed by a study investigator.
CT Scan	Performed in Radiology
Discharge from NIH	

Appendix A4 – Interim Safety Visit Flowsheet (randomized and open-label only)

Event	Procedure
Arrive to NIH 5-SWS Day Hospital by 8AM	Page Protocol team on arrival of patient. Accurate height x3 and weight. Interval H&P performed.
Fasting blood draw	Performed in Day Hospital.
Discharge from NIH	

Appendix A5 – Final Visit Flowsheet (randomized and open-label only)

Event	Procedure
Arrive to NIH 5-SWS Day Hospital by 8AM	Page Protocol team on arrival of patient. Accurate height x3 and weight. Interval H&P performed.
Stool sample	Collected in Day Hospital if patient did not bring sample from home.
Fasting blood draw	Performed at Time -15 min of the FSIVGTT
FSIVGTT	Performed in Day Hospital.
Subcutaneous Adipose Tissue Biopsy	Performed by a study investigator.
DXA Scan	Performed on 7th floor, Metabolic Unit
CT Scan	Performed in Radiology
Exit Questionnaire	Performed in Day Hospital.
Discharge from NIH	

Appendix A6 – Optional Washout Visit Flowsheet (randomized and open-label only)

Event	Procedure
Arrive to NIH 5-SWS Day Hospital by 8AM	Page Protocol team on arrival of patient. Accurate height x3 and weight. Interval H&P performed.
Stool sample	Collected in Day Hospital if patient did not bring sample from home.
Fasting blood draw	Performed at Time -15 min of the FSIVGTT
FSIVGTT	Performed in Day Hospital.
Subcutaneous Adipose Tissue Biopsy	Performed by a study investigator.
DXA Scan	Performed on 7th floor, Metabolic Unit
CT Scan	Performed in Radiology
Discharge from NIH	

Appendix B1

Potentially Significant Drug Interactions with Colchicine

Drug	Noted or Anticipated Outcome
Strong CYP3A4 Inhibitors	
Atazanavir Clarithromycin Cimetidine Cobicistat Darunavir/Ritonavir Indinavir Itraconazole Ketoconazole Lopinavir/Ritonavir Nefazodone Nelfinavir Ritonavir Saquinavir Telaprevir Telithromycin Tipranavir/Ritonavir Tolbutamide	Significant increase in colchicine plasma levels; fatal colchicine toxicity has been reported with clarithromycin, a strong CYP3A4 inhibitor. Similarly, significant increase in colchicine plasma levels is anticipated with other strong CYP3A4 inhibitors.
Moderate CYP3A4 Inhibitors	
Amprenavir Aprepitant Azithromycin Diltiazem Erythromycin Fluconazole Fosamprenavir Grapefruit Juice Verapamil	Significant increase in colchicine plasma concentration is anticipated. Neuromuscular toxicity has been reported with diltiazem and verapamil interactions.
P-gp Inhibitors Cyclosporine Ranolazine	Significant increase in colchicine plasma levels; fatal colchicine toxicity has been reported with cyclosporine, a P-gp inhibitor. Similarly, significant increase in colchicine plasma levels is anticipated with other P-gp inhibitors.
HMG-Co A Reductase Inhibitors	
Atorvastatin Fluvastatin Lovastatin Pitavastatin Pravastatin Rosuvastatin Simvastatin	The addition of one drug to a stable long-term regimen of the other has resulted in myopathy and rhabdomyolysis (including a fatality).
Other Lipid Lowering Drugs Fibrates Gemfibrozil	The addition of one drug to a stable long-term regimen of the other has resulted in myopathy and rhabdomyolysis (including a fatality).
Digitalis Glycosides	
Digoxin	Rhabdomyolysis has been reported.

Other	
Dasatinib	Combination with colchicine may increase CYP3A4 substrates.
Fusiric Acid (systemic)	
Ivacaftor	
Mifepristone	
Other (con't)	
Aripiprazole	Colchicine may cause a decrease in serum concentrations.
Axitinib	
Cyanocobalamin	
Saxagliptin	

Appendix B2

Medications that may confound the assessment for diabetes

Thiazide diuretics at a dose greater than 25mg/day
Systemic non-cardioselective beta-blockers - Carvedilol - Labetalol - Nadolol - Penbutolol Sulfate - Pindolol - Propanolol - Timolol
Niacin
Systemic glucocorticoids
Prescription or over-the-counter weight loss or weight gain medications/supplements

Appendix C- Surgical/Invasive Procedure Verification Checklist

Date: Time:
Procedure (PER CONSENT):
Responsible Physician(s)/LIP(s):
Surgical Site(s) Marked:
INSTRUCTIONS: Check the boxes and sign in the appropriate boxes below to indicate the steps that have been completed. Do not continue with the process unless you are able to verify that each step has been completed as appropriate.

Step	Verification Process			Signature of Care Team Member Documenting the Information
Pre-Procedure Area:				
1)	**Identification of Correct:**	**Yes**	**N/A**	
	Patient			
	Procedure			
	Side, site, and level (if appropriate)			
2)	Surgical Site and/or Side Marked			
3)	Appropriate documents confirming the procedure side, site, and level where appropriate are present (for example, history and physical, nursing assessment, and pre-anesthesia assessment)			
	Accurately completed, and signed, procedure consent form			
	Correct diagnostic and radiology test results(for example, radiology images and scans, or pathology and biopsy reports) are properly labeled and available			
	Required blood products, implants, devices, and/or special equipment for the procedure are available			
Immediately Prior to the Start of the Surgery/Invasive Procedure:				
4)	**"Time Out" – Prior to the procedure in the operating room/procedure area, ALL TEAM MEMBERS participate together in a time out to verbally verify:**	**Yes**	**N/A**	
	Correct patient identity			
	Correct side, site, and level are marked as appropriate			
	Accurately completed, and signed, procedure consent form			
	Agreement on the procedure to be performed			
	Correct patient position			
	Availability of appropriately labeled relevant images and tests			
	The need for antibiotics or fluids for irrigation purposes			
	Safety precautions based on patient history or medication use			

***COMPLETE THIS SECTION ONLY IF UNABLE TO COMPLETE A STEP IN THE VERIFICATION PROCESS ABOVE – EXPLAIN WHY**

Step	Explanation	Signature of Care Team Member Documenting the Information	Date

Appendix D

3-Month Visit (Final Visit) Exit Questionnaire

Date _____/_____/_____

Dear Study Participant,

You recently completed a research study where half of the study participants took colchicine and half took placebo capsules for three months. We would like to ask you some questions about your feelings and attitudes about the study. With your help we hope to be able to improve our studies in the future. Please answer all questions as honestly as possible.

1. Which capsule (colchicine or placebo) do you think you were taking?
   Check one

   _____ colchicine _____ placebo

   Why do you think so? _____

2. How sure or unsure are you about which capsules you were taking?
   Circle one:

1	2	3	4	5
Very unsure	Somewhat unsure	Undecided	Somewhat sure	Very sure

3. When you agreed to join the study, do you think that you fully understood the study and everything you would need to do? Check one:

   _____ yes _____ no

   If no, what didn't you understand? _____

4. Knowing what you know now, if you could do it all over again, would you join this study?
   Check one:

   _____ yes _____ no

5. What would you tell a friend who was thinking of entering the study? Check one:

   _____ join the study _____ don't join the study

6. Suppose we knew for sure that taking colchicine could help you lose one pound per year, or at least keep you from gaining a pound or two. Would it be worth the effort of taking the colchicine?

_____ yes _____ no

7. Do you feel your overall health has improved due to being in the study?

_____yes _____no

8. What effect do you think the capsules had on your body weight?

_____ no effect _____ lost weight _____ gained weight

9. If you lost weight, what do you think was the reason?
Check all that apply

_____ study capsules _____ changed eating habits

_____ exercise other (please list) _____

10. If you tried any special diets or weight loss supplements during the three month period please describe (for example, Atkins, low fat, low carb, meal bars):

_____

11. Do you feel the study capsules improved any of the following in you? (check all that apply):

_____ joint pain _____ sense of taste

_____ mobility _____ energy

If yes, please explain: _____

12. Do you think the study capsules had any side effects?

_____ yes _____ no

If yes, please list: _____

_____

13. Did you find the alarm clock useful to help remind you to take your medication? (check one):

_____ yes _____ no

If no, why not? _____

_____

14. Why did you decide to be part of the study?
Rank in order of importance (1 for first choice, 2 for second choice, etc.)
Leave blank if the reason does not apply to you at all:

_____ wanted to improve my health
_____ make a contribution to science / help other people
_____ try to lose weight
_____ financial compensation
_____ opportunity for medical care at no cost
_____ wanted to take colchicine
_____ other: _____

15. What was the worst part of the study?
Rank in order of importance (1 for first choice, 2 for second choice, etc.)
Leave blank if item does not apply to you:

_____ coming to NIH (traveling, security checks)
_____ taking the capsules
_____ blood draws
_____ FSIVGTT (3 hour IV glucose test)
_____ Fat biopsy
_____ DEXA (body fat) scan
_____ Collecting the stool sample
_____ physical exams and body measurements
_____ appointment times/having to take time off from work
_____ filling out questionnaires
_____ didn't get the results I was expecting from the study capsules
_____ other: _____

16. We know that for most people it is difficult to take every capsule on time every day and you probably missed some doses. What do you think might have helped you to be better at taking the capsules? (check all that apply):

_____if I only had to take one capsule a day instead of two
_____if I could have taken the two capsules together, once a day
_____if the capsules were smaller
_____if the capsules were easier to swallow
_____if it were easier to remember to take the capsules
_____ other: _____

Please tell us anything else that may have helped you to do a better job of taking your capsules

_____

17. Please write in any other comments or suggestions for the study.

_____

THANK YOU FOR BEING A PART OF THE STUDY

3-Month Visit (Final Visit) Exit Questionnaire for T2DM Open Label Arm

Date _____/_____/_____

Dear Study Participant,

You recently completed a research study where study participants took colchicine capsules for three months. We would like to ask you some questions about your feelings and attitudes about the study. With your help we hope to be able to improve our studies in the future. Please answer all questions as honestly as possible.

1. When you agreed to join the study, do you think that you fully understood the study and everything you would need to do? Check one:

   _____ yes _____ no

   If no, what didn't you understand? _____

2. Knowing what you know now, if you could do it all over again, would you join this study? Check one:

   _____ yes _____ no

3. What would you tell a friend who was thinking of entering the study? Check one:

   _____ join the study _____ don't join the study

4. Suppose we knew for sure that taking colchicine could help you lose one pound per year, or at least keep you from gaining a pound or two. Would it be worth the effort of taking the colchicine?

   _____ yes _____ no

7. Do you feel your overall health has improved due to being in the study?

   _____yes _____no

8. What effect do you think the capsules had on your body weight?

   _____ no effect _____ lost weight _____ gained weight

9. If you lost weight, what do you think was the reason?
Check all that apply

_____ study capsules _____ changed eating habits

_____ exercise other (please list) _____

10. If you tried any special diets or weight loss supplements during the three month period please describe (for example, Atkins, low fat, low carb, meal bars):

_____

11. Do you feel the study capsules improved any of the following in you? (check all that apply):

_____ joint pain _____ sense of taste

_____ mobility _____ energy

If yes, please explain: _____

12. Do you think the study capsules had any side effects?

_____ yes _____ no

If yes, please list: _____

_____

13. Did you find the alarm clock useful to help remind you to take your medication?
(check one):

_____ yes _____ no

If no, why not? _____

_____

14. Why did you decide to be part of the study?
Rank in order of importance (1 for first choice, 2 for second choice, etc.)
Leave blank if the reason does not apply to you at all:

_____ wanted to improve my health
_____ make a contribution to science / help other people
_____ try to lose weight
_____ financial compensation
_____ opportunity for medical care at no cost
_____ wanted to take colchicine
_____ other: _____

15. What was the worst part of the study?
Rank in order of importance (1 for first choice, 2 for second choice, etc.)
Leave blank if item does not apply to you:

_____ coming to NIH (traveling, security checks)
_____ taking the capsules
_____ blood draws
_____ FSIVGTT (3 hour IV glucose test)
_____ Fat biopsy
_____ DEXA (body fat) scan
_____ Collecting the stool sample
_____ physical exams and body measurements
_____ appointment times/having to take time off from work
_____ filling out questionnaires
_____ didn't get the results I was expecting from the study capsules
_____ other: _____

16. We know that for most people it is difficult to take every capsule on time every day and you probably missed some doses. What do you think might have helped you to be better at taking the capsules? (check all that apply):

_____if I only had to take one capsule a day instead of two
_____if I could have taken the two capsules together, once a day
_____if the capsules were smaller
_____if the capsules were easier to swallow
_____if it were easier to remember to take the capsules
_____ other: _____

Please tell us anything else that may have helped you to do a better job of taking your capsules

_____

17. Please write in any other comments or suggestions for the study.

_____

THANK YOU FOR BEING A PART OF THE STUDY

Appendix E

MEDICATION GUIDE

Congratulations on starting your involvement with our research protocol! Participants in the Randomization Arm of this study have been randomized to receive either the research medication (colchicine) 0.6 mg capsules twice a day or a placebo. Neither the study subjects nor the investigators know which "version" of the research medication you are receiving. Participants in the Open-Label Arm of the study will be taking colchicine 0.6 mg capsules twice a day

Read this Medication Guide before you start taking the medication. This Guide does not take the place of talking to your healthcare provider or the NIH research team about your medical conditions or treatment. You should let your healthcare provider know that you may be taking the research medication as part of a research protocol.

What is the most important information I should know about the research medication?
The research medication can cause serious side effects or death if levels of the research medication are too high in your body.

- Taking certain medicines with the research medication can cause your level of the research medication to be too high, especially if you have kidney or liver problems.
- Tell the NIH research team about all your medical conditions, including if you have kidney or liver problems. Your dose of the research medication may need to be changed.
- Tell the NIH research team about all the medicines you take, including prescription and non-prescription medicines, vitamins and herbal supplements.
- Even medicines that you take for a short period of time, such as antibiotics, can interact with the research medication and cause serious side effects or death.
- Talk to the NIH research team, your healthcare provider, and pharmacist before taking any new medicine.
- Especially tell the NIH research team if you take:
  - atazanavir sulfate (Reyataz®)
  - cyclosporine (Neoral®, Gengraf®, Sandimmune®)
  - fosamprenavir (Lexiva®) with ritonavir
  - indinavir (Crixivan®)
  - ketoconazole (Nizoral®)
  - nefazodone (Serzone®)
  - ritonavir (Norvir®)
  - telithromycin (Ketek®)
  - clarithromycin (Biaxin®)
  - darunavir (Prezista®)
  - fosamprenavir (Lexiva®)
  - itraconazole (Sporanox®)
  - lopinavir/ritonavir (Kaletra®)
  - nelfinavir mesylate (Viracept®)
  - saquinavir mesylate (Invirase®)
  - tipranavir (Aptivus®)

Ask the NIH research team, your healthcare provider, or pharmacist if you are not sure if you take any of the medicines listed above. This is not a complete list of all the medicines that can interact with the research medication.

- Know the medicines you take. Keep a list of them and show it to your healthcare provider and pharmacist when you get a new medicine.

- Keep the research medication out of the reach of children.

Who should not take the research medication?
Do not take the research medication if you have liver or kidney problems and you take certain other medicines. Serious side effects, including death, have been reported in these patients even when taken as directed. See "What is the most important information I should know about the research medication?"

What should I tell the NIH research team before starting the research medication?
See "What is the most important information I should know about the research medication?"

Before you take the research medication tell the NIH research team about all of your medical conditions including if you:
- have liver or kidney problems
- are pregnant or plan to become pregnant. It is not known if the research medication will harm your unborn baby. Please tell the NIH research team immediately if you are pregnant or plan to become pregnant.
- are breast-feeding or plan to breast-feed. The research medication passes into your breast milk. Due to the possible risks to the child, you are not eligible for this study if you are nursing.

Using the research medication with certain other medicines, such as cholesterol-lowering medications or digoxin, can affect each other causing serious side effects. Talk to the NIH research team and your healthcare provider about whether the medications you are taking might interact with the research medication, and what side effects to look for.

How should I take the research medication?
- Take the research medication exactly as the NIH research team instructs you to take it. **If you are not sure about your dosing**, call the NIH research team.
- The medication can be taken with or without food.
- If you take too much the research medication go to the nearest hospital emergency room right away.
- If you miss a dose, then take it as soon as you remember. If it is almost time for your next dose, just skip the missed dose. Take the next dose at your regular time. Do not take 2 doses at the same time.

What should I avoid while taking the research medication?
Avoid eating grapefruit or drinking grapefruit juice while taking the research medication. It can increase your chances of getting serious side effects.

What are the possible side effects of the research medication?
The research medication can cause serious side effects or even cause death. See "What is the most important information I should know about the research medication?"

Get medical help right away, if you have:
- Muscle weakness or pain
- Numbness or tingling in your fingers or toes
- Unusual bleeding or bruising
- Increased infections
- Feel weak or tired
- Have significant dizziness
- Pale or gray color to your lips, tongue, or palms of your hands
- Severe diarrhea or vomiting

The most common side effects of the research medication are abdominal pain, diarrhea, nausea and vomiting.

Tell the NIH research team if you have any side effect that bothers you or that does not go away.

These are not all of the possible side effects of the research medication. For more information, ask the NIH research team, your healthcare provider, or pharmacist.

How should I store the research medication?
- Store at room temperature between 68° and 77°F (20° to 25°C).
- Keep tightly closed in the provided container.
- Keep out of the light.

Keep the research medication and all medicines out of the reach of children.

Appendix F

(Adapted from Common Terminology Criteria for Adverse Events v4.0 (CTCAE)
Publish Date: May 28, 2009)

Grades

Grade refers to the severity of the adverse event (AE). The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Appendix G

You will be completing Self Sampling at home (or at the NIH) for up to 4 visits as part of the colchicine study:

- ☐ Screening Visit
- ☐ Medication Initiation Visit
- ☐ 3 month Final (Medication Completed) Visit
- ☐ 6 month Washout Visit (Optional)

Supply list for Stool Collection:

1. Fecocainter stool container with toilet "frame"
2. Brown Top Stool Collection Vial (x3)
3. Permanent Marker
4. Clear Bag for stool collection vial (biohazard bag)
5. Disposable gloves
6. Stool sampling instructions
7. Food Dietary record + instructions

For Questions Contact:

Jordan Levine, Research Coordinator [Phone Number Written Here]

jordan.levine@nih.gov

Ginika Onyekaba, Research Coordinator [Phone Number Written Here]

ginikanwa.onyekaba@nih.gov

Andrew Demidowich, MD, Lead Associate Investigator [Phone Number Written Here]

andrew.demidowich@nih.gov

STOOL SPECIMEN COLLECTION:

1. Wash your hands.
2. Label your brown-lid container with the date and time of collection using the permanent marker provided. The inside of the container is sterile until opened.

3. To minimize urine collection, **first empty your bladder** before performing the next steps.

4. Place the stool-collecting frame and Fecocontainer into the toilet, securely under the seat.

5. Take a bowel movement in the bowl.

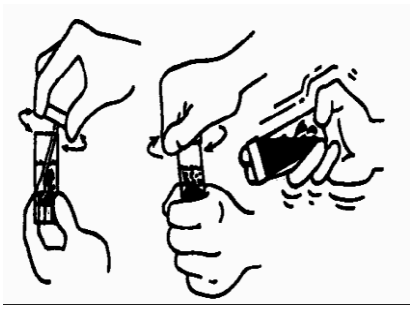
6. Put on gloves.

7. As shown in Figure A, unscrew the cap carefully to avoid spilling fluid. Use the small spoon attachment on the lid to scoop up a "pea size" sample. Place the shovel with the sample into the specimen container, and secure the lid tightly and shake vial for 5 seconds. Try not to spill any of the liquid inside the vial (it helps preserve the stool).

A

B

C



8. Repeat two more times for a total of **three** stool vials. **Uncap and work with only ONE vial at a time**.
9. Place all three stool vials into the biospecimen bag provided.

10. Dispose of remaining feces into toilet and flush (optional). Close Fecocontainer with the lid provided and throw away into a garbage bag. Throw the gloves away in the same bag. Wash Hands.

11. Return stool samples during your scheduled clinic visit. Because the vials have the special preservative, **they do not** need to be refrigerated or frozen. However, you put the bag in the freezer if you wish.

Colchicine Study (14-CH-0119)
Keeping Food Records

To better understand what you usually eat and drink when you are not in the hospital, please write down everything you eat and drink for a typical day.

When should the food records be kept? Write down all foods and beverages you consume for 24 hours before you come to the Clinical Center for your appointment. This day should represent a fairly typical day for you.

Eat normally on this day. Don't change your food choices, methods of food preparation, or where you eat just because you are recording your intake. There is no right or wrong way to eat for this evaluation.

How and what should be recorded? Use a new form each day. It is best to record what you eat *immediately* after each meal and snack.

You should write down:

- the time you begin eating each meal and snack
- all foods and beverages, except for plain water.
- all condiments (such as ketchup, margarine, mayonnaise, salad dressing, sauces, gravy, sugar, etc). You *do not* need to record salt, pepper, herbs and spices.
- all medicines, vitamins, minerals, and/or other supplements taken.

For each food and beverage that you list, include an amount and a description. To guide you, refer to the sample food record and the hints for recording amounts and description information on the next few pages.

What do I do with the forms after they are filled out? Bring them back with you to the NIH when you come next time.

If you have any questions or concerns, please contact me by e-mail or by phone.

Dietitian's name: Amber Courville
Phone number: 301-594-8051
E-mail: courvillea@nih.cc.gov

Hints for Completing the *Amount* Column

A. Measure foods *after* preparation and cooking is completed.

B. Measurements can be listed in 4 ways:

1. The number of items.

Examples:	saltines	6
	white bread	2 slices
	grapes	12
	ketchup	2 packets

2. In household measures, using standard level measuring cups and spoons.

Examples:	applesauce	1/3 cup
	popcorn	3 cups
	jelly	1 ½ TB [tablespoons]
	2% milk	1.5 CP [cup]

(Do not use non-standard measures like "handful" or "serving")

3. By weight or by volume, as listed on a package (or by using a kitchen scale if you have one).

Examples:	ginger ale	12 FL OZ [fluid ounces]
	yogurt	4.5 oz [ounces]
	Almond Joy	49 gm [grams]
	roast beef	2 ½ oz
	pretzels	1/3 of 6.5 oz bag

4. By dimensions, using a ruler.

Examples:	pancake	5" diameter
	meatball	1 ¼" diameter
	lasagna	3 ½" x 4" x 1½" cube
	pizza	1/8 of 14" diameter [or draw it with dimensions]

- Remember not all food that is served is eaten, and at other times, you may go back for seconds. You may need to adjust portion sizes to reflect the amount you actually ate.

Hints for Completing the ***Description*** Column

A. Describe foods completely.

Examples:

sirloin steak, *fat partially trimmed*
80% lean ground beef (or 80/20 ground beef)
chicken drumstick, *skin removed before cooking*
reduced fat or *2%* milk
baked potato, *skin eaten*
carrot cake *with cream cheese frosting*

B. Include brand names whenever possible. Also include terms like *calcium-fortified, light*, and *reduced calorie* if listed on the label.

Examples:

Country Crock Light spread tub margarine
Oreo reduced-fat cookies
Miller Lite draft beer
Hellmann's fat-free cholesterol-free mayonnaise
Dannon lowfat fruited yogurt
sliced peaches in *light* syrup

C. Include information about preparation and cooking methods.

Examples:

skinless chicken breast	*floured and pan-fried in corn oil*
canned corn	*tub margarine and sugar added*
Campbell's tomato soup	*made with water*
mac and cheese	*from mix, made with whole milk and Parkay stick margarine*

D. For mixed dishes and recipe items, you only need to list major ingredients. You do not need to write down the entire recipe.

Examples:

potato salad	*made with potatoes, eggs, regular mayo*
chocolate chip cookies	*homemade with real butter, walnuts added*
meatloaf	*made with 85/15 ground beef, oatmeal, ketchup, egg*

E. For fast food items from major chains, you only need to name the item. No description is necessary, unless you "special order" an item. Also, note if all of it wasn't eaten.

Examples:

Biggie Fries (Wendy's)
Quarter Pounder with Cheese (McDonald's) - didn't eat pickles
Meat-lovers Pan Pizza (Pizza Hut)

F. Remember to record any additions made at the table, such as margarine, sugar, ketchup, mustard, sauces, mayonnaise. List them separately, and include amounts.

NAME: Mary A. Smith

Day of Week: Tuesday

Date: 3/5/05

Is this a typical day? ✓yes __no

If no, give reason:

Fill out the following on the 1st day only:

Home phone: ( )

Daytime phone: ( )

Date of birth: / /

See space on back to write additional info, recipes, etc

Patient number: _____

Intake: T M L

Info: R I U

Visit # ___

PLACE	TIME	FOODS AND BEVERAGES	AMOUNT	COMPLETE DESCRIPTION	REVIEWER'S COMMENTS
Home	6:45	Honey Nut Cheerios	1¼ c		
		2% milk	¾ c		
		eggs	2	scrambled in sausage drippings	
		sausage	2 links	3" long microwaved	
		bagel	1	cinn. raisin Giant bakery 3½" dia	
		margarine	2 tsp	Shedds spread tub	
		coffee	12 oz	+ 1 tsp sugar + 1 tsp Cremora powder	
		orange juice	6 oz	Tropicana with calcium	
Work	9:30	donut	1	glazed 3" dia Krispy Creme	
Work Cafeteria	12:30	cheeseburger	1	quarter pounder size, on bun w/ lettuce, tomato, onion, Ketchup	
		fries	15 ea	reg size, deep fried	
		Ketchup	1 pkt		
		frozen yogurt	¾ cup	vanilla, soft serve	
		Coke	20 oz	bottle	
Sally's house	5:30	Beer	12 oz	Miller Lite	
		Cake	1 rectangle	2"x3"x1" yellow w/ white frosting from grocery bakery	
Home	6:30	Steak, Sirloin	5 oz	raw weight, fat trimmed, grilled	
		Mac + cheese	1 cup	box mix made w/ 2% milk + butter	
		peas	⅓ cup	canned, plain	
		Salad	1½ cup	lettuce, tomato, carrots + 2 TBSP croutons	
		Ranch dressing	2 TBSP	fat free kraft	
		Iced tea	16 oz	includes ice. 2 TS sugar added	
Home	9:30	Ice cream	¾ cup	Breyers light vanilla	
		Pretzel nuggets	½ cup	Honey Mustard Snyders	

SAMPLE

continue on reverse side

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