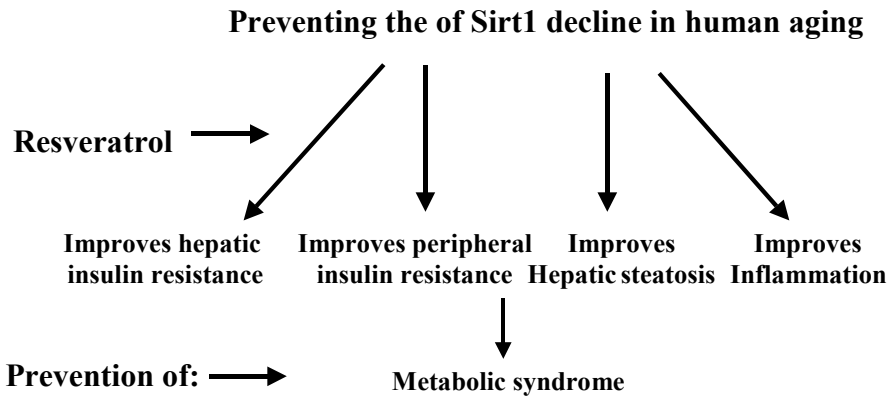
Revised on 6/12/2014

Effect of resveratrol on age-related insulin resistance and inflammation in humans

OVERVIEW

We hypothesize that resveratrol will improve insulin resistance, tissue lipid accumulation, inflammation, and mitochondrial function in middle aged and Type 2 Diabetes subjects. Using state of the art physiological tools for human investigation, we have shown that adipose tissue inflammation and tissue lipid accumulation are enhanced with aging in concert with systemic insulin resistance. We will administer resveratrol to overweight middle aged humans and study its effects on insulin action, tissue lipid accumulation, inflammation, and mitochondrial function. We will perform paired hyperinsulinemic clamps, the „gold standard" measure of insulin action, following both placebo and resveratrol treatment. We will perform adipose tissue and muscle biopsies to assess inflammatory response in adipocytes and adipose tissue macrophages, and to assess mitochondrial biology in muscle. Noninvasive ^{1}H magnetic resonance spectroscopy (MRS) will be used to quantify the effects of resveratrol on tissue lipid accumulation.



SPECIFIC AIMS:

Human aging is associated with increased fat mass, insulin resistance and systemic inflammation. In addition to overall increases in fat mass, accumulation of lipid in such insulin-sensitive tissues as liver and skeletal muscle appears to contribute importantly to insulin resistance. Furthermore, age-related decreases in muscle mitochondrial ATP production rate and mitochondrial DNA copy number are likely to contribute to impaired insulin-mediated glucose disposal, as well as decreased muscular endurance and activity levels. Additionally, systemic inflammation is now believed to contribute to insulin resistance and thus ultimately to many age-related disorders, including diabetes mellitus, Alzheimer"s disease, atherosclerosis and many cancers. Recently, it has been recognized that obesity and aging are both associated with increased macrophage infiltration into adipose tissue, and that these infiltrating macrophages are an important source of systemic cytokine production. We have previously reported that adipose macrophages from older humans display increased activation (and production of pro-inflammatory and pro-atherosclerotic peptides). This heightened reactivity of adipose macrophages in older individuals and in those with Type 2 diabetes is likely to contribute both to insulin resistance and to systemic inflammation.

Mammalian Sirt1 seems to be particularly important in regulating glucose homeostasis, insulin action and nutrient sensing. Collectively, several studies have shown that activation of Sirt1 by pharmacological agents like resveratrol has intracellular effects that are strikingly similar to those observed with caloric restriction. Specifically, resveratrol has been shown to improve mitochondrial function and appears to protect against metabolic disease by activating PGC-1alpha. Activation of PPAR-gamma in macrophages by resveratrol reduces activation of these inflammatory cells, which may be very pertinent to adipose tissue inflammation. It has been proposed that resveratrol may improve insulin resistance, although this has yet to be established *in vivo* in animals or humans.

Moreover, resveratrol has been shown to enhance cognitive function, reduce neural degeneration, and improve motor function in animal models. ***Thus, for this project we hypothesize that resveratrol will mediate important metabolic and anti-inflammatory effects that will improve insulin resistance in middle aged and older humans and in those with Type 2 diabetes.*** Specifically, we will determine whether and how Sirt1 activation with resveratrol will impact systemic glucose metabolism, lipotoxicity, mitochondrial dysfunction, inflammation, and motor and cognitive function in insulin resistant humans.

Aim 1: To study the effects of resveratrol on age and diabetes related insulin resistance. *We hypothesize that treatment of overweight, middle aged human subjects and those with Type 2 diabetes with resveratrol will result in improved insulin sensitivity, as demonstrated by increased insulin-stimulated glucose uptake and enhanced insulin-mediated suppression of glucose production.* Insulin action will be evaluated by stepped hyperinsulinemic euglycemic insulin „clamp" studies in middle aged subjects with normal glucose tolerance, with progressive increases in insulin levels designed to optimally examine both hepatic and peripheral insulin sensitivity.

Aim 2: To examine the effects of resveratrol on systemic inflammation and/or on adipose tissue macrophages. *We hypothesize that treatment of overweight, middle aged human subjects and in those with Type 2 diabetes with resveratrol will lead to reductions in systemic inflammatory markers and reduced content and/or activation of adipose tissue macrophages.* Systemic inflammatory markers to be measured will include TNF-a, IL-6, IL-1b, MCP-1. Adipose tissue macrophages will be isolated from subcutaneous abdominal adipose tissue, and will be quantified by fluorescence activated cell sorting (FACS) analysis. Gene expression of a variety of pro-inflammatory and pro-atherosclerotic factors will be examined by „real-time" rt-PCR, to determine how resveratrol affects activation of adipose macrophages.

Aim 3: To explore the effects of resveratrol on skeletal muscle metabolism and function. *We hypothesize that resveratrol treatment will enhance mitochondrial metabolism and improve strength and contractility.* In collaboration with the Mitochondrial Metabolic Core and Department of Neurology, we will examine the effects of systemic resveratrol on mitochondrial content, signaling molecules involved in mitochondrial biogenesis, DNA and protein oxidative damage, mitochondrial respiration in human skeletal muscle, and motor function and mobility

Aim 4: Assess the effects of reveratrol on cognition. *We hypothesize that resveratrol will enhance cognitive function in middle-aged individuals and in those with Type 2 diabetes.* In collaboration with the Department of Neurology, we will examine cognition, including attention networks of alerting, orienting, and executive attention, as well as perform a clinical neuropsychology battery providing a comprehensive assessment of cognitive function, including measures of: a. intelligence, b. memory, c. language, d. executive function, e. visuospatial function and praxis, f. processing speed, and g. mental status.

These proposed studies will address several unresolved questions about the role of Sirtuins/Sirt1 *in humans*:

1. **on insulin sensitivity in liver and in peripheral tissues (particularly skeletal muscle)**
2. **on several aging-associated determinants of insulin resistance, including:**
    - **tissue lipid accumulation**
    - **adipose tissue and systemic inflammation**
    - **mitochondrial and motor function**
    - **cognitive function**

BACKGROUND AND SIGNIFICANCE

The metabolic syndrome of aging and its clinical consequences. The metabolic syndrome of aging defines a cluster of abnormalities, including insulin resistance, obesity, abnormal glucose tolerance, dyslipidemia, and hypertension (1). Obesity and/or chronic increases in nutrient intake are increasingly recognized to play central roles in the pathogenesis of many age-related diseases, including diabetes mellitus, arteriosclerotic and atherosclerotic vascular diseases, many types of cancer (particularly breast, prostate, colorectal and endometrial), and Alzheimer"s disease (2,3). Together, these conditions contribute importantly to the obesity-related increase in all-cause mortality (4,5). Furthermore, the presence of insulin resistance and the „metabolic syndrome" (impaired glucose tolerance, dyslipidemia, hypertension, visceral obesity, decreased fibrinolysis) is highly correlated with all of these diseases (6,7,8,9,10,11,12).

Role of aging per se in the pathogenesis of metabolic changes. It is particularly important to note that aging itself is associated with increases in inflammatory activity (13). Indeed, changes in the innate immune system have been noted with aging (14). In particular, aging is associated with increased circulating levels of cytokines and acute phase proteins *in vivo,* including TNF-alpha and IL-6 (15,16,17,18). The chronic low-grade inflammation in aging and is epidemiologically related to age-associated disorders (eg, Alzheimer disease, atherosclerosis, type 2 diabetes, etc.) and enhanced mortality risk (19,20,21), and adversely impacts hypothalamic pathways vital to energy balance and glucose homeostasis (22). Among the proposed explanations for this phenomenon, which has been referred to as "inflamm-aging," are continuous exposure to antigenic loads and stress (19).

Furthermore, there is a remarkable similarity in pathologic processes between aging and diabetes mellitus, (23). It is especially noteworthy that nondiabetic older individuals can develop microvascular changes, eg. retinopathy (24,25) and neuropathy (26), that are pathologically indistinguishable from those seen in diabetes mellitus (DM). Indeed, both aging and DM are associated with increased availability of certain substrates such as FFA, as well as increased inflammation and altered body composition.

Effects of aging on mitochondrial function: Mitochondria are known as the “power plants” of eukaryotic cells, generating most of their adenosine triphosphate (ATP) supply through oxidative phosphorylation and electron transfer (27). They are furthermore involved in a number of other important processes, such as apoptosis, cell signaling, and control of cell growth and differentiation. The master regulator of mitochondrial biogenesis and function has been shown to be peroxisome proliferator-activated receptor g-□ coactivatoг1α (PGC-1α). PGC-1α induces mitochondria specific transcription factors, such as nuclear respiratory factors (NRFs). NRFs in turn activate mitochondrial transcription factor A (TFAM) and thereby induce mitochondrial (mtDNA) replication (28,29).

With increasing age there appears to be a decline in mitochondrial function and number (30,31,32). In the elderly, declines in mitochondrial transcription levels (30), muscle mitochondrial protein synthesis (33) and, importantly, decreases in key mitochondrial enzymes and maximal ATP production capacity (MAPR) have been observed (30). Since the transcription of nuclear encoded genes of mitochondrial proteins is regulated by PGC-1α (28), it is possible that the fundamental problem is at the level of nuclear and mitochondrial gene expression. Recent studies have suggested that the underlying cause for mitochondrial dysfunction may be aging-associated reductions in AMP-activated protein kinase (AMPK) activity (34). Activated AMPK phosphorylates and thereby induces PGC-1α gene expression in skeletal muscle (Jaeger et al. 2007). Lower activity levels with increasing age could furthermore present a decreased stimulus for DNA replication, resulting in reduced mtDNA copy

numbers (35). In support of this, various studies have shown a positive effect of exercise on PGC-1α expression and mtDNA copy number (31,34,36,37). In particular, exercise is likely to significantly stimulate AMPK activity, and thus PGC-1α phosphorylation (34).

Mitochondrial function and insulin action: There has been increasing evidence suggesting an association between muscle mitochondrial dysfunction and insulin resistance. Mitochondrial oxidative activity and ATP synthesis are both reduced in states of impaired glucose tolerance and insulin resistance. This was seen in a recent study by Petersen et al. (2003) comparing mitochondrial function between healthy, lean, elderly and healthy, lean, younger volunteers (38). Their results showed a ~40% reduction in mitochondrial activity and oxidative phosphorylation in addition to insulin resistance in the elderly subjects compared to the controls. Another study noted reduced mitochondrial activity and increased skeletal muscle triglyceride storage in young insulin-resistant offspring of diabetic parents compared to insulin sensitive controls (39). However, whether the mitochondrial dysfunction is primary or secondary to the pathogenesis of insulin resistance is currently unknown. A number of studies have proposed that muscle mitochondrial dysfunction may play an etiological role in insulin resistance (32,34,40). Reznick et al. reported a strong relationship between insulin resistance, dysregulated intracellular lipid metabolism and age-related reductions in AMPK-activity (34). They suggested that decreased activation of AMPK, and thus PGC-1α, as well as mitochondrial fatty acid oxidation with aging could lead to mitochondrial dysfunction and increased muscular triglyceride accumulation, thereby predisposing to insulin resistance and type 2 diabetes mellitus (T2DM). Increased intracellular triglyceride storage in liver and skeletal muscle results impairs insulin-stimulated glucose uptake in muscle (41).

An alternative hypothesis is that insulin resistance itself leads to mitochondrial dysfunction with age since insulin resistant states, such as type 2 diabetes mellitus (42), decrease the transcription and translation of mitochondrial proteins (43, 44, 45) and mitochondrial biogenesis in muscle is enhanced by insulin (46). A study by Karakelides et al. recently demonstrated a reduced skeletal muscle mitochondrial ATP production rate (MAPR) and gene transcript levels following insulin deprivation (47). Reduced muscle mitochondrial function may also affect numerous other cellular functions including synthesis of proteins, such as contractile proteins (35,48). Age-related declines in activity levels, ectopic accumulation of fat, and sarcopenia may be related to reduced contractile protein synthesis in combination with decreased ATP production in aging and could result in insulin resistance.

SIRT1 effects on glucose metabolism and insulin action: Recent research has found one of the seven sirtuin genes, SIRT1, to impact mitochondrial function and biogenesis through positive regulation of PGC-1α activity (49,50). SIRT1 is the mammalian homologue of NAD-dependent histone deacetylase Sir2 (silencing information regulator 2) and belongs to the family of proteins collectively known as sirtuins. It is involved in processes such as cell survival, fat metabolism, and insulin and glucose production (51) and has been shown to control the hepatic gluconeogenic/glycolytic pathways in the fasted state through deacetylation of PGC-1α (50,52). One way in which lifespan can be extended in mammals is through caloric restriction. Aging significantly reduced SIRT1 activity in heart of rats (53). Loss of SIRT1 with age was accelerated in mice with accelerated aging but was not observed in long-lived growth hormone-receptor knockout mice (54). Since Sirt1 expression is induced in multiple tissues including the liver, brain and muscle during periods of caloric restriction, it seems likely that Sirt1 may be a potential candidate for enhancing mammalian longevity.

SIRT1-induced activation of PGC-1α in cultured liver cells has a differential effect on hepatic glucose production, repressing glycolysis but increasing gluconeogenesis (GNG) (52). By deacetylating forkhead transcription factor FoxO1 in hepatocytes SIRT1 increases the expression of gluconeogenic genes (55). Moreover, the over-expression of pancreatic beta-cell specific SIRT1 in mice has been shown to improve glucose tolerance and increase glucose stimulated insulin secretion, whereas the expression of SIRT1 is decreased in insulin resistant cells and the inhibition of SIRT1 induces insulin

resistance (56,57). In Zucker fa/fa rats, SIRT1 activators were recently shown to improve whole-body glucose homeostasis and insulin sensitivity in adipose tissue, skeletal muscle and liver (58).

SIRT1 exerts numerous additional effects on energy metabolism. It modulates glucose and lipid metabolism through its regulation of the adipocyte-derived hormone adiponectin (51). Adiponectin has been shown to improve insulin sensitivity and may inhibit the development of insulin resistance (59). SIRT1 increases the concentration of adiponectin through deacetylation of FoxO1 (60). In white adipose tissue, SIRT1 is thought to play a role in adipogenesis by promoting fat mobilization through down-regulation of PGC-1α, resulting in decreased triglyceride storage and increased secretion of free fatty acids (FFA) (61). SIRT1 may also contribute to the process of inflammation, considering it inhibits the transcription of nuclear factor-kappa B (NF-kappa B), one of the key regulators of inflammation (51). The pro-inflammatory state is one of the characteristics of obesity and the metabolic syndrome (62). *Thus, as SIRT1 is increasingly being recognized as an important regulator of metabolic homeostasis and mitochondrial function, targeting SIRT1 to ameliorate age-related mitochondrial dysfunction and insulin resistance shows great promise.*

Resveratrol's effects on insulin action and glucose homeostasis: Resveratrol (3,5,4"-trihydroxystilbene) is a plant derived polyphenolic compound mainly well known for its antioxidant and phytoestrogenic properties and is mainly found in the skin of grapes and in red wine (63). Previous studies have also revealed its anti-tumorigenic and anti-inflammatory qualities (51,63). It has been shown to possess antiplatelet (64,65) and cardioprotective, anti-atherosclerotic effects (66,67). Howitz et al. (2003) demonstrated the ability of resveratrol to significantly increase the activity of SIRT1 through allosteric interaction, resulting in increased PGC-1α activity (68). Resveratrol has been shown to produce SIRT1-dependent effects in mammalian cells that improve organismal health and cellular function, such as increased insulin sensitivity, mitochondrial number, and improved motor function (49). In a study by Baur et al. (2006) resveratrol was able to prevent the negative effects of excess calorie intake in mice on a high calorie diet (49). In their studies, the physiology of middle-aged mice on a high-calorie diet shifted towards that of mice on a standard diet when treated with resveratrol, significantly increasing their survival. Resveratrol has also been shown to increase mitochondrial biogenesis and oxidative phosphorylation, as well as reduce diet-induced obesity and insulin resistance in a rodent model (50). Furthermore, in streptozotocin-induced diabetic rats, resveratrol treatment improved hyperglycemia, insulin resistance, hypertriglyceridemia, and common diabetic symptoms such as weight loss, polydipsia and polyphagia (69). In conclusion, given the potential of resveratrol, through activation of SIRT1, to positively impact mitochondrial function and metabolic homeostasis, makes it an optimal instrument of investigation in our proposed studies.

Resveratrol's effects on neural function: Recently, much attention has been paid to the neuroprotective effects of resveratrol. A number of studies have demonstrated protective effects of SIRT1 activation against amyloid-β toxicity in Alzheimer"s disease (70,71). Neurodegeneration in the hippocampus was reduced and learning impairment was prevented in a mouse model of Alzheimer"s disease when intracerebroventricular resvertarol was given (72). Of note, Alzheimer"s disease is associated with impaired mitochondrial energy metabolism (73), and mitochondrial dysfunction in Alzheimer"s disease has been shown to be related to age-dependent interactions of amyloid-β with mitochondrial proteins (74). SIRT1 expression may activate genes responsible for neuronal protection and protect neurological function in disorders such as seizures and brain ischemia (75,76). Resveratrol has also been shown to prevent diabetes-induced learning and memory impairment, modulate cholinergic neurotransmission, and improve cognition in streptozocin-induced diabetic rats (77). Given the neuroprotective properties of resveratrol in rodent models, it would be intriguing to further investigate these effects in the proposed human studies.

Resveratrol in Human Subjects

RATIONALE

Since a causal link between Sirt1 and aging and diabetes related metabolic changes has not yet been established in humans, this protocol is indispensable to establishing the clinical relevance of this program. Sirt1 shows significant potential as a useful therapeutic target that can be used in ameliorating many of the conditions associated with the metabolic decline of aging and in Type 2 diabetes We are particularly interested in investigating the role of Sirt1 in the regulation of glucose homeostasis, insulin action and mitochondria function in humans. Furthermore, demonstrating the ability of resveratrol to reverse the metabolic changes of aging and Type 2 diabetes would be of tremendous therapeutic importance, particularly in light of the feasibility and safety of longterm administration of this orally available agent.

Our decision to study middle aged subjects is primarily due to the importance of timing this intervention to decrease aging-related metabolic changes prior to the presentation of serious age-related diseases. An additional advantage is the feasibility of recruiting and studying subjects of this age group with somewhat arduous insulin clamp studies, biopsies and muscle function tests, as compared to a frailer group of elderly subjects. Our preliminary studies, shown below, indicate that individuals above 40 years of age already have substantial age-related declines in insulin sensitivity that would be appropriate targets of intervention. Additionally, since resveratrol appears to exert more beneficial effects in animal models of caloric excess, overweight subjects will be recruited for these studies. Similarly, subjects with Type 2 diabetes have established insulin resistance and nutrient excess as demonstrated by elevated free fatty acids and are likely to benefit from this intervention.

The proposed *in vivo* studies will permit the study of effects on insulin action in concert with effects on adipose macrophage content and activation and muscle function, as well as circulating inflammatory mediators. Such a comprehensive analysis would be necessary to adequately connect the entire hypothesis in humans.

EXPERIMENTAL APPROACH AND METHODS:

STUDY POPULATION AND RECRUITMENT

Overweight, middle aged and type 2 diabetes subjects will be recruited by a variety of approaches, as described below. Subjects will range in age from 21 to 65 years, will be overweight (BMI 25-30 kg/m2) with and without diabetes mellitus. . All subjects with a history of liver disease or liver abnormalities, hyperlipidemia, hypertension, heart disease, cerebrovascular disease, seizures, bleeding disorders, muscle disease, a known history of ovarian, uterine, breast cancer or smoking will be excluded. The following subjects will also be specifically excluded: mentally disabled persons, prisoners, pregnant women, subjects with a history of ethanol or drug or toxin exposure which could be associated with neuropathy, any subject deemed incapable of giving voluntary informed consent, and subjects with a history of a bleeding disorder or with a prolonged PT or PTT. Furthermore, subjects allergic to Novocain, Benzocaine and Lidocaine will also be excluded. Given potential concerns (based on *in vitro* observations) that resveratrol might inhibit various cytochrome P450 enzymes involved in drug metabolism, use of the following drugs will be exclusionary for this study: anti-coagulant and anti-platelet drugs, anti-epileptics, mexilitene, quinidine, cyclorpsorine, tacrolimus, and HIV protease inhibitors (see below under Protection of Human Subjects). NSAIDS should be avoided as far as possible during the study period. Additionally, given confounding effects of extreme exercise on muscle triglyceride content, highly trained or professional athletes will be excluded from the studies.

Women in the child-bearing age-group will be allowed to participate in the studies provided that they have negative pregnancy tests within a week of the studies and that they are not breast-feeding. Children below the age of 18 will be excluded from the current protocol, given the use of labeled isotopes. Prior to their enrollment in the study the purpose, nature, risks and benefits of the study will be explained to all subjects and their voluntary, informed, written consent will be obtained. All subjects will have a screening visit to allow for clinical evaluation, including history, physical examination and consent procedures.

Recruitment and Screening Strategies for Clamp Studies

Recruitment of middle aged subjects will be predominantly by local advertising. This will include posting flyers within Einstein and around the neighborhood and posting advertisements in area newspapers. Dr. Kishores‟ research group has had considerable success with these approaches in the recruitment of many middle aged, nondiabetic subjects to date.

Screening: This visit will include a full history and physical examination, an oral glucose tolerance test and additional laboratory tests to determine eligibility, including serum electrolytes, BUN and creatinine, PT/PTT, liver function tests, CPK, and lipid profile, as well as a screening urinalysis, urine toxicology screening, and blood pressure measurements. Safety laboratories will be repeated during the first and second pancreatic clamp studies and include: CBC, chemistry, liver function tests, CPK, and urinalysis.
All subjects will present to the CRC fasting; consequently, visits will be scheduled in the morning. The oral glucose tolerance tests (only non-diabetic subjects) will consist of a 75g oral glucose challenge in the form of a beverage, with fasting (immediately prior to drinking beverage) and 2 hour venous blood samples for plasma glucose and insulin measurements. Physical examination will include a careful assessment of upper extremity veins, as well as measurement of height and waist circumference and percent body fat (by body impedence absorptiometry). Since public transportation in our local area is limited and many area residents do not drive, we will make arrangements to provide transportation to and from clinic visits, including screening visits, for all participants who require it.

General Experimental Conditions:

On the day of the first pancreatic clamp study the patients will receive a 28 days‟ supply of resveratrol capsules. The pilot study will be an open-labeled study. The study design requires that all 55 subjects participate in all of the proposed studies, as outlined below. Consequently, additional subjects will be recruited to compensate for those subjects who cannot complete all of the required studies.

Prior to each study, subjects will meet with study personnel to discuss dietary recommendations. Each subject will be instructed to follow a standardized diet plan for three days prior to each of the clamp studies (40% fat: 20% saturated, 10% polyunsaturated, 10% monounsaturated; 35% carbohydrate; 25% protein). Subjects will be asked to refrain from vigorous exercise for three days prior to each clamp study.

Aim 1: To study the effects of resveratrol on age-related insulin resistance

We hypothesize that treatment of overweight, middle aged or Type 2 diabetes subjects with resveratrol will result in improved insulin sensitivity, as demonstrated by increased insulin-stimulated glucose uptake and enhanced insulin-mediated suppression of glucose production.

We will study the following subject group:

- **Overweight and either young or middle aged** (BMI 25-30 kg/m2, age 21-65) nondiabetic subjects, n=40 diabetic subjects (BMI=25-35) n=15

Each subject will receive a 28 day course of **resveratrol** (n=55). RevGenetics will supply the resveratrol (500 mg capsules; 2 capsules bid) for our proposed studies. A certificate of analysis has been provided by the manufacturer.

A comprehensive study of whole-body insulin action will be undertaken with specially designed „stepped" euglycemic (~90 mg/dl) -hyperinsulinemic clamp studies, both before and after administration of resveratrol.

Thus, we will compare the following parameters under paired conditions in each subject, on day 0 and day 28 of resveratrol treatment:

a. ***hepatic insulin action:*** this will be determined as the ability of low infusion rates of insulin (20 mU/m2.min above basal requirements) to suppress glucose production during the **"low insulin phase"** of the clamp studies.

b. ***peripheral insulin action:*** this will be determined as the ability of high infusion rates of insulin (80 mU/m2.min above basal requirements) to stimulate glucose uptake during the **"high insulin phase"** of the clamp studies.

In vivo clamp studies – General Conditions

Subjects will be admitted on the day of the study to the General Clinical Research Center (GCRC) study room after an overnight fast. Subjects with type 2 diabetes will be admitted to the Jack Weiler Hospital of the Albert Einstein College of Medicine the night before the study. All anti-diabetic medications, including insulin, will be held from one day to one week prior to the clamp study date depending on the drug they are taking. They will not eat after 10 PM the night before the study (but can drink water). An intravenous catheter will be inserted into their left arm and insulin will be administered overnight to maintain their glucose at normal levels. Blood samples will be collected every hour for glucose values. At 8:00 am 2 intravenous cannulae will be established, one for infusions and a second one to be inserted in a retrograde fashion in a dorsal vein of the opposite arm for blood sampling. To obtain arterialized venous blood samples, this hand will be maintained at 65°C in a thermoregulated plexiglass box.

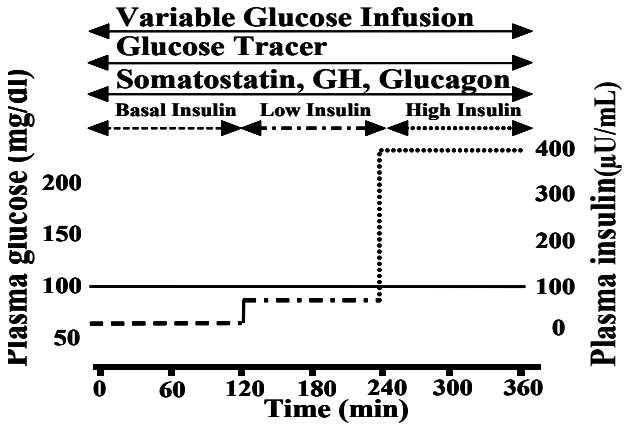
A primed continuous infusion of D2G-glucose will be initiated at t=-120 min (2 hours prior to the onset of the „clamp" study). A bolus of 200 mg/m^2 D2G for the duration of 3 min will be followed by a continuous infusion of 2 mg/min/m^2 D2G for the entire study in all subjects (for total duration of 6 hours) to **quantify glucose turnover**, specifically rates of peripheral glucose disposal and hepatic glucose production.

These **„pancreatic clamp"** experiments will consist of 360 min insulin/somatostatin (250 µg/hr) or insulin/octreotide (30 mg/kg/min) infusions with replacement of glucoregulatory hormones (glucagon 1 ng/kgmin; growth hormone 3 ng/kgmin). Throughout the entire 360 min the plasma glucose concentration will be maintained at basal levels (~90 mg/dl).

Basal phase: From t=0 min to t=120, optimal insulin infusion rates will be selected in each individual by making frequent (~ every 25 mins) adjustments to the insulin infusion rates in order to establish those insulin infusion rates required to maintain euglycemia (90 mg/dl) without the requirement for exogenous glucose infusion.

Low phase: Following establishment of basal insulin requirements during the basal phase, at t=120 the insulin infusion rate will be increased by a fixed increment of 20 mU/m^2/min, and will be maintained at this rate for 2 hours (T=120-240). Plasma glucose will be maintained at euglycemic concentrations (~90mg/dl) by a variable infusion of 20% dextrose for the entire study (78).

High phase: At t=240 min, insulin infusion rate will be increased to 80 mU/m^2/min above basal requirements, and will be maintained at that rate for the final 2 hours of the study (t=240-360).



Plasma measurements: From t=0 to t=360 min., blood samples will be obtained at hourly intervals for determinations of plasma glucose, insulin, glucagon, C-peptide, growth hormone, free fatty acids (FFA), glycerol, and lactate. Additional samples for D2G determinations will also be obtained every 15 min. Plasma glucose will be measured every 5-10 min in duplicate by a Beckman glucose analyzer. Plasma lipids (LDL, HDL, VLDL, total Cholesterol and Triglyerides) will be measured at t=0.

Biopsies: From t=225-240 min, fat and muscle biopsies will be obtained. Participants will have fat biopsies performed in the periumbilical region. A small 0.5 cm cutaneous incision will be performed under local anaesthesia (Lidocaine 1% with Epinephrine) and 1-2 g of adipose tissue will be obtained by aspiration technique (79). A skelatl muscle biopsy will be obtained from vastus lateralis muscle using a cadence muscle biopsy needle following local anaesthesia extending into the muscle area. The total amount of muscle obtained will be ~300 mg. The biopsy specimens will be immediately homogenized in Trizol at the bedside, to inhibit any RNAase activity, and will subsequently be stored at –80 ˚C. Pressure will be applied at the biopsy site for ~10 min following the biopsy. A pressure bandage will be applied and kept on 5-6 hours after the biopsy. Since the biopsies are performed in 4 hours into the clamp and the subject will under direct observation for an additional 3-4 hours. The biopsy site will be examined prior to discharge and a pressure bandage will be reapplied for an additional two hours. Subjects will be able to remove the bandage once they get home.

All infusions will be stopped at t=360 min. The patient will be given a standard meal and plasma glucose levels will be monitored at 15-30 min intervals for the next one hour. Dextrose infusion will be continued for approximately 45 minutes after the study in order to avoid hypoglycemia. The patient will be discharged after about one hour, provided that his or her condition remains stable.

Indirect Calorimetry. A PARVO MEDICS, (TrueOne® 2400 Metabolic Measurements Systems). will be used to estimate rates of carbohydrate and lipid oxidation from T=260-280 in all studies. Twenty minute averaged values for VO_2 and VCO_2 will be corrected for nitrogen excretion as urinary urea nitrogen measured enzymatically by the method of Kjeldahl (80). Net glucose and lipid oxidation rates will be calculated from indirect calorimetry. The equations used to calculate glucose and lipid oxidation from gas exchange and urinary urea nitrogen excretion have been published (81). Under conditions in which RQ> 1.0, the overestimation of glucose oxidation will be corrected as described (81).

Analytical procedures – Plasma glucose will be measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Measurements of plasma insulin, C-peptide, glucagon, and growth hormone are undertaken throughout the studies in order to evaluate the adequacy of the “pancreatic clamp” technique, ie. inhibitory effects of somatostatin on hormone secretion, and uniformity of hormone replacement. All samples will be kept on ice until centrifuged (within 30 min). Plasma samples will then be sorted and handled by the Special Assay Core, and will be stored frozen at -20°C for later assays. Plasma hormone determinations will be performed by radioimmunoassay as previously reported (82), and these determinations will be provided by the Radioimmunoassay Core of the Diabetes Research and Training Center.

Substrate determination- Plasma FFA levels will be determined by using an acyl-CoA oxidase based colorimetric kit (Wako, Osaka, Japan) and glycerol will be measured using colorimetric enzymatic methods (83,84). Plasma lactate will be measured by fluorometric enzyme techniques (85).

Glucose Kinetics- Plasma for labeled glucose radioactivity will be treated as previously described (86). Rates of glucose appearance (Ra) and disappearance (Rd) and other indices of glucose turnover will be estimated by using the steady-state equation of Steele (87). Endogenous glucose production (EGP) will be determined by subtracting the rates of glucose infusion from the tracer-derived rate of appearance of glucose. Data for glucose turnover represent the mean values during the final 60 min of the low insulin period (t=60-120 min), and during the final 60 min of the hyperglycemic period (t=180-240 min).

These techniques are approved by the CCI under Protocols 2000-200 and 2006-414.

Aim 2: To examine the effects of resveratrol on systemic inflammation and/or on adipose tissue macrophages.

This aim will make use of the **adipose biopsy specimens from Aim 1**. We will therefore examine the effects of resveratrol on macrophage content and activation in adipose tissue, and on systemic markers of inflammation, in **the same** n=55 **overweight, middle aged** (age 45-65) nondiabetic and diabetic subjects. We will be utilizing the younger 21-44 year old population to assess whether resveratrol has the same beneficial effects in younger subjects.

We hypothesize that treatment of insulin resistant subjects with resveratrol will lead to reductions in systemic inflammatory markers and reduced content and/or activation of adipose tissue macrophages. Systemic inflammatory markers to be measured will include TNF-a, IL-6, IL-1b, MCP-1. Adipose tissue macrophages will be isolated from subcutaneous abdominal adipose tissue, and will be quantified by fluorescence activated cell sorting (FACS) analysis. Gene expression of a variety of pro-inflammatory and pro-atherosclerotic factors will be examined by „real-time" rt-PCR, to determine how resveratrol affects activation of adipose macrophages.

Following administration of resveratrol (n=55) for 28 days, we will examine:

1. Adipose macrophage content, by fluorescence activated cell sorting (FACS) analysis. These data will additionally be confirmed by immunohistochemistry
2. Activation of adipose macrophages, as assessed by expression and production of a variety of pro-inflammatory and pro-atherosclerotic factors, using „real-time" rt-PCR and immunohistochemistry
3. Plasma levels of cytokines and acute phase reactants

Adipose biopsy specimens- Adipose biopsy specimens will be obtained as described above. Suctioned adipose tissue samples will be suspended in PBS and washed with a 40 μm cell strainer. Approximately 1 gram of tissue will be immediately homogenized in Trizol (Life Technologies, Bethesda, MD) with a Tissumizer homogenizer. This technique will ensure optimized yields of mRNA by the inhibition of RNAses. The homogenate will be frozen on dry ice, transported to the laboratory and stored at -80^0C. Subsequent mRNA extraction and analysis of gene expression will be performed in the Gene Expression Core lab. Remaining fresh adipose biopsy specimens will be immediately processed as described below.

Collagenase digestion of adipose tissue and cell separation: The adipose tissue will be digested with collagenase type 1 (Worthington Biochemical, Lakewood, NJ; 0.05gm per 30ml of Hanks Balanced Salt solution with 4% BSA) for 30 minutes at 37°C with intermittent shaking. Adipocytes will be subsequently separated from stromal cells by centrifugation at 2500 rpm for 10 minutes followed by filtration. Macrophages will be separated from stromal cells by CD14+ coated antibody beads

(Dynabeads, Dynal Biotech, Lake Success, NY) by the manufacturer"s method. The separated adipocytes and macrophages will be stored in TRIzol.

Macrophage quantification: It is possible that the 28 day duration of therapy and effects of resveratrol on adipocyte chemoattractant factors could also impact numbers of macrophages in fat (see below). We will therefore quantify adipose tissue macrophages with immunohistochemistry and/or FACS analysis. To measure macrophage content in adipose tissue by **immunohistochemistry**, samples will be fixed and embedded in paraffin (21). Sections of tissue will be stained for expression of F4/80 (macrophage-specific) with a monoclonal antibody. The total number of nuclei and the number of nuclei of F4/80 expressing cells will be counted for each field. We have also developed a technique to count macrophages by **Fluorescence Activated Cell Sorting (FACS)** analysis. After digestion with collagenase, the pellet of stromal cells will be treated with red blood cell lysing buffer for 5 min followed by incubation with saturating amounts of FITC-labeled human CD14+ antibody (BD Pharmingen) in staining buffer (PBS with 1 mg/ml BSA and 12 mM NaN_3, pH 7.2) on ice for 20 min, washed and analyzed immediately by using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA).

Quantitative „Real Time" Reverse Transcriptase Polymerase Chain Reaction (rt-PCR): From the whole fat samples and isolated cells, total RNA will be extracted with TRIzol. cDNA will be made using Superscript First Strand Synthesis System for rt-PCR (Invitrogen Technologies). Gene expression will be quantified using the protocol for the LightCycler instrument (Roche Diagnostics, Indianapolis, IN), using SYBRGreen (Roche Diagnostics, Indianapolis, IN) for fluorescent detection of double-stranded DNA. Plasmid standard curves will be generated to calculate mRNA copy numbers. Gene product specificity will be confirmed by melting curves and by running the PCR products on agarose gels. All reactions will be performed at least three times. The genes of interest will include PAI-1, TNF-α, TGF-β, and interleukins 1-β and 6 both in whole fat and in macrophages. Expression of these inflammatory factors will thus provide a measure of macrophage activation. In skeletal muscle samples we will quantify *Socs*-1/2/3 gene expression, as a documented target of cytokine signaling (44). We will also perform immunohistochemistry and Western blotting to measure *protein levels* of these factors.

Measurement of circulating cytokines and acute phase reactants: Plasma samples will be aliquoted into Eppendorff tubes and stored at -20^0C. Subsequent measurements of plasma leptin, TNF-a, PAI-1, angiotensinogen, IL-1-b, IL-6, resistin, Acrp30 and SAA will be performed by the Special Assays Core.

Aim 3: To explore the effects of resveratrol on skeletal muscle metabolism and function.

We hypothesize that resveratrol treatment will enhance mitochondrial metabolism and improve strength and contractility. We will examine the effects of resveratrol on mitochondrial content, signaling molecules involved in mitochondrial biogenesis, DNA and protein oxidative damage, and mitochondrial respiration in human skeletal muscle. It is anticipated that mitochondrial ATP production and aerobic muscular function will improve in concert with enhanced insulin sensitivity.

This aim will make use of the **muscle biopsy specimens from Aim 1**. We will therefore examine the effects of resveratrol on muscle mitochondrial function and aerobic muscular function in **the same** n=25 **overweight, middle aged** (age 45-65) nondiabetic and diabetic subjects. Again,We will be utilizing the younger 21-44 year old population to assess whether resveratrol has the same beneficial effects in younger subjects.

Following administration of resveratrol (n=55) for 28 days, we will examine:

1. Muscle mitochondrial content
2. Muscle mitochondrial function
3. Muscle strength and mobility

Muscle biopsies: human skeletal muscle biopsies will be obtained as described above, and stored at –80 °C for analysis of the following:

- mitochondrial content
- signaling molecules involved in mitochondrial biogenesis
- DNA and protein oxidative damage, and
- mitochondrial respiration
- skeletal muscle mitochondrial function (citrate synthase and succinate dehydrogenase activity), content, and morphology by electron microscopy.

Muscle strength and mobility tests: will be conducted by our collaborators from the Department of Neurology and assess the following:

- **Mobility measures:** Quantitative gait studies will be conducted using a 28-foot (20 feet recording surface) computerized walkway with embedded pressure sensors (GAITRite, CIR systems). Subjects will walk on the mat at their „normal pace" for two trials in a quiet well-lit room wearing comfortable footwear and without any attached monitoring devices. The walkway includes four unmarked feet at either end to account for initial acceleration and terminal deceleration. Based on footfalls recorded on the walkway, the software automatically computes various gait parameters as the mean of two trials. The GAITRite system is widely used in clinical and research settings, with excellent inter-rater and test-retest reliability (kappa >0.9) (88,89). Our collaborators in the Department of Neurology have experience using this system for the past 8 years in community based studies.

- **Walking while talking test (WWT):** Subjects are asked to walk the course for two trials while reciting alternate letters of the alphabet using protocols developed and validated in our subjects. Number of errors while reciting letters will also be recorded. Subjects are given practice trials as required. The order of the initial letter on WWT is also randomly varied between „A" and „B" to minimize practice effects. The WWT performance has been related to increased attention demands (90) and falls (91).
- **Chair rise:** Time taken to get up from a chair five times unassisted and without pausing is recorded (seconds). This test assesses lower extremity strength and balance, and is a component of the widely used Short Physical Performance Battery (92)**.**
- **Unipedal Stance.** The unipedal stance measures ability to stand on one foot (maximum 30 sec), and is considered a sensitive measure of balance (93).
- **Stair climbing:** Difficulty with climbing up or down stairs is associated with functional limitations.
- **Muscle strength:** Tests of grip and leg strength is commonly used to assess power, and define frailty.

Aim 4: To assess the effects of resveratrol on cognition.
We hypothesize that resveratrol will enhance cognitive function in middle-aged individuals. In collaboration with the Department of Neurology we will therefore examine the effects of resveratrol on cognition in **the same** n=25 **overweight, middle aged** (age 45-65) nondiabetic and diabetic subjects. We will be utilizing the younger 21-44 year old population to assess whether resveratrol has the same beneficial effects in younger subjects.

Following administration of resveratrol (n=25) for 28 days, we will examine:

- **Executive control:** The Attention Network Test is a combination of the cued reaction time (RT) and the flanker task (94,95). The ANT provides estimates of three separate attention networks of alerting, orienting, and executive attention (95,96). Efficiency of the three attentional networks is assessed by measuring how response times are influenced by alerting cues, spatial cues, and flankers (i.e., congruent or incongruent conditions). The task is relatively short and simple so that reliable estimates of the three attention networks can be obtained within 35 minutes for various healthy and pathological populations.
- **Clinical neuropsychology battery:** provides comprehensive assessment of cognitive function (97,98,99). This battery consists of traditional, standardized measures of: a. intelligence, b. memory, c. language, d. executive function, e. visuospatial function and praxis, f. processing speed, and g. mental status. These tests were used in our previous research to demonstrate informative associations between cognitive and motor function (100,101,102).

Schedule of proposed clinical studies:

Day -1: Subjects visit the Ferkauf School of Psychology at 8 am for motor and cognitive function tests. Subject with type 2 diabetes will present at 2 pm for cognitive and motor testing then be admitted to Weiler Hospital at 5 pm the night before the clamp study.

Day 0: Subjects admitted to GCRC at 8 am following a 10 hour fast,

- first stepped insulin clamp study
- fat and muscle biopsies

Days 1-28: Subjects receive oral resveratrol (last dose on day of final clamp study)

Day 27: Subjects visit the Ferkauf School of Psychology at 8 am for motor and cognitive function tests. Subject with type 2 diabetes will present at 2 pm for cognitive and motor testing then be admitted to Weiler Hospital at 5 pm the night beofer the study.

Day 28: Subjects admitted to GCRC at 8 am following a 10 hour fast

- second stepped insulin clamp study
- fat and muscle biopsies

STATISTICAL ANALYSIS

Data collected from each subject will be edited, entered, verified and entered into a FoxPro data base system in the GCRC. Data quality checking will be performed by computer programs designed to detect errors using accepted ranges, logic checks, and missing values. Outliers will be checked against the original record and correct values will be entered into a final data file. Data from FoxPro files will be transferred into SAS System Version 6.12 (SAS Institute, Cary, NC) for analysis. We shall utilize SAS for all data management and statistical analysis. Descriptive statistics of all the variables at different time points will be derived and reported.

For the specific aims of this study, repeated measurements of several parameters including glucose metabolism and gene expression will be measured in each subject, with the principal endpoint being the rate of whole-body glucose uptake during the final hour of the high insulin clamp. The null hypothesis is that glucose uptake will be equal in each pair of experiments (ie. prior to and following 45 days" treatment with resveratrol or placebo). To determine the changes in glucose uptake between the paired experimental protocols for each subject group, paired Student"s t-tests will be performed. For *between-group* comparisons (ie. between the two subject groups randomized to resveratrol or placebo), the principal endpoint will be the difference in whole-body glucose uptake (ΔGU) between the 2 experimental conditions (eg. Resveratrol vs. placebo), with null hypothesis that the response to the experimental intervention will be the same in all subject groups. To determine the changes in glucose uptake between the paired experimental protocols for each subject group, analysis of

variance (ANOVA) will be performed by using PROC GLM in the SAS statistical package. Within group effects will be derived from the analysis.

All values will be presented as the mean +/- SE.

HUMAN SUBJECTS RESEARCH SECTION

Protection of Human Subjects

1. RISKS TO THE SUBJECTS

Human Subjects Involvement

Subject involvement will include a preliminary screening visit for history, physical exam and lab testing to determine eligibility. Subsequently, each subject will participate in two infusion studies with frequent blood glucose monitoring.

Human Subject Characteristics

The study population will consist of at least 40 nondiabetic subjects and 15 diabetic subjects with the following characteristics: ages 21-65 years, with BMI between 25 and 30 kg/m2. The following subjects will be specifically excluded: children, mentally disabled persons, prisoners, pregnant women, fetuses, subjects with a history of ethanol or drug or toxin exposure which could be associated with neuropathy, any subject deemed incapable of giving voluntary informed consent, and subjects with a history of a bleeding disorder or with a prolonged PT or PTT; a history of uterine, breast, or ovarian cancer, renal disease, and liver disease or impairment. Women in the child-bearing age-group will be allowed to participate, provided they have negative pregnancy tests. The research topic to be studied is not relevant to the involvement of special classes or groups of subjects and therefore no criteria for their involvement are specified.

Table I DEMOGRAPHY OF RECRUITMENT AREA
***Data from United States Census 2000 (http://www.census.gov/)**

	Bronx	Manhattan	Westchester County (Borders the Bronx)
Population	**1, 332,650**	**1,537,195**	**923,459**
Racial Distribution			
White (Non-Hispanic)	**15%**	**50%**	**65%**
Hispanic	**48%**	**24%**	**16%**
African American	**36%**	**14%**	**14%**
Asian	**3%**	**10%**	**5%**
Age Distribution			
>25 years	**40%**	**27%**	**32%**
25-44 years	**31%**	**38%**	**31%**
45-64 years	**19%**	**23%**	**23%**
65+ years	**10%**	**12%**	**14%**

Table II TARGETED/PLANNED ENROLLMENT: Number of Subjects

	Sex/Gender		
	Females	**Males**	**Total**
ETHNIC CATEGORIES			
Hispanic or Latino	8	8	16
Not Hispanic or Latino	12	12	24
Ethnic Category: Total of All Subjects	**20**	**20**	**40**
RACIAL CATEGORIES			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	8	8	16
White	12	12	24
Racial Categories: Total of All Subjects	**20**	**20**	**40**

Sources of Materials/Use of Data:

All data and records obtained in this study will be used for research purposes only. The protocol will be submitted for review and approval by the Institutional Review Board known as the Committee on Clinical Investigations of the Albert Einstein College of Medicine of Yeshiva University (CCI). The CCI is responsible to ensure human subject protections in compliance with institutional policies and federal regulations including the HIPAA privacy law.

Potential Risks:

Potential risks to the subjects include the following:

1. Blood withdrawal: The total amount of blood sampled will not exceed 190 ml per study, less than half of that donated by a blood donor.
2. 20% dextrose: The infusion of 20% dextrose may be associated with local venous irritation. This will be minimized by use of a large-bore antecubital vein in which the glucose will be infused.
3. D2G: is a non-radioactive glucose tracer and is not associated with any known side effects at the doses used.
4. Glucagon infusion in high doses can cause gastrointestinal symptoms such as cramps and nausea, and produce hyperglycemia in persons with glucose intolerance. The doses to be employed here are intended to maintain plasma glucagon at basal levels.
5. Growth Hormone: At higher doses and for prolonged periods of exposure, growth hormone (rhGH) has either insulin-like or insulin-antagonistic effects on carbohydrate and lipid metabolism, however the proposed infusion is for physiological replacement during SRIF infusion and is unlikely to have adverse effects.
6. Hypoglycemia: is unlikely to develop in these subjects because of the desired plasma glucose targets (90 mg/dl during the normoglycemic studies), the low rates of insulin infusion, and the constant blood glucose monitoring.
7. Resveratrol: Resveratrol is present in significant amounts in a variety of foods, including grapes, blueberries, peanuts and red wine and over-the-counter resveratrol supplements are widely used. Formal safety testing is not required for marketing of nutritional supplements, but to date, there have been no published reports of side effects at the commonly used doses (up to 1000 mg /day). A number of *in vitro* studies have demonstrated that resveratrol can be an inhibitor of various cytochrome P450 enzymes that are involved in drug metabolism. The relevance of these *in vitro* observations to *in vivo* use is unclear primarily because oral resveratrol is rapidly metabolized by the liver, free resveratrol levels are quite low and resveratrol metabolites have not been shown to be inducers or inhibitors of CYP enzyme activity. Nonetheless, caution is warranted, especially for drugs with a narrow therapeutic window (anti-epileptics, mexilitene, quinidine, cyclorpsorine, tacrolimus, HIV protease inhibitors) and use of these drugs will be exclusionary for this study. Furthermore, due to the potential estrogenic effects of resveratrol, a medical history of ovarian, uterine, and breast cancer will be exclusionary for the study.
8. Somatostatin: At the proposed physiological replacement doses has no known side effects.
9. Ocreotide: At the proposed physiological replacement doses has no known side effects.

2. ADEQUACY OF PROTECTION AGAINST RISKS

Recruitment and Informed Consent Procedures:

We will recruit at least 40 overweight, middle aged, nondiabetic subjects and 45 subjects with Type 2 diabetes by local advertising. Consent will be initially obtained during telephone screening performed by a research fellow or the study coordinator. Formal consent procedures which adhere to the Committee on Clinical Investigations of the Albert Einstein College of Medicine will be followed. Specifically, each subject will be verbally informed in layman's language of the purpose, benefits and possible risks of the studies. They will then read the written consent form in the presence of a member of the research team and a physician who will answer any further questions. The subject and

witness shall be asked to sign the consent form which will be kept in the patient"s chart. Each subject's potential participation in related experiments (for example, when each subject is asked to perform repeat studies) will be explicitly stated.

All clinical data on the subjects will be confidential. Data generated in these studies will be considered only relevant for research purposes and will not be included in any clinical databases or the patient"s chart. Since the GCRC databases are coded, the study data will not be available on any clinical database system.

Protection Against Risk:

Procedures employed to protect subjects from undue risks include the following:

1) Blood withdrawal: Blood withdrawal during any single study will be limited to less than 190cc and subsequent studies will be separated by at least, a four week interval. The infusion and withdrawal catheters may produce infection or local hematoma, but strict aseptic technique will be observed by the experienced physician performing the procedure.
2) 20% glucose: Vein irritation with 20% glucose infusion will be prevented by use of a large-bore antecubital vein.
3) Glucagon: Symptoms of mild nausea will be treated with antiemetic medications, if needed.
4) Hypoglycemia: All of these studies will be performed by a physician, hypoglycemia will be prevented by blood glucose sampling every 5-10 minutes and if inadvertent hypoglycemia develops can be rapidly treated by glucose infusion.
5) Resveratrol: Given data from a number of *in vitro* studies demonstrating that resveratrol can be an inhibitor of various cytochrome P450 enzymes involved in drug metabolism, use of the following drugs will be exclusionary for this study: anti-epileptics, mexilitene, quinidine, cyclorpsorine, tacrolimus, HIV protease inhibitors. Dosages of up to 1000 mg/day have been widely used without reports of adverse effects.

3. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

The study will be of no direct benefit to the subject, although subjects may gain a better understanding of their own metabolic processes. The results of the study will contribute to generalizable knowledge about the effects of resveratrol on insulin action, mitochondrial function, muscle strength and neurocognitive function.

4. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED (Risk: Benefit Ratio):

The risks involved are minimal as all procedures are well established. The study may result in a better understanding of the role of resveratrol in determining insulin action in humans. As described above, a number of mechanisms exist to ensure safety of subjects. All studies will be performed by a physician and nurse in attendance in the CRC procedure unit with emergency equipment readily accessible. The PI, coinvestigators, and research team have a combined experience of over 10 years in human investigation, and sufficient care in choice of subjects should minimize the risks. The scientific value of these studies is to develop an understanding of the mechanism of an important clinical and potentially-exciting device for the treatment of diabetes.

INCLUSION OF WOMEN

As mentioned in the human subject characteristics section above, the study will exclude pregnant women because of the use of radioactive tracer. Otherwise, an anticipated 50% of subjects will be female based on preliminary data and the population distribution in the Bronx.

INCLUSION OF MINORITIES

We anticipate that 50% of our study subjects will be from minority communities, based on our preliminary data and the population distribution in the Bronx. Recruitment strategies to encourage minority enrollment include advertisements in ethnic newspapers and magazines.

INCLUSION OF CHILDREN

The purpose of this study is to determine effects of resveratrol on age-related parameters in middle-aged subjects and in those with Type 2 diabetes and hence no children will be studied.

DATA SAFETY AND MONITORING PLAN (DSMP):

All human subject research being conducted at the Einstein GCRC must have a DSMP. The guidelines for Data and Safety Monitoring have been established by the GCRC Research Subject Advocate (RSA), and approved by the Assistant Dean for Compliance who oversees human research subject safety at the Albert Einstein College of Medicine. http://gcrcweb.aecom.yu.edu/gcrc/dsm.htm

Adverse Event Monitoring:

Process: Data and safety monitoring for this study will be performed by the principal investigator on an ongoing basis. All volunteers will be seen by the P.I. or her research staff. Laboratory data will be reviewed by the P.I. within 24 hours of receipt.

Reporting: All adverse events will be compiled, and reported in summary form, on an annual basis to the IRB, and at the conclusion of the study. Unanticipated (non-serious) adverse events will be reported to the IRB within 30 days and serious adverse events will be reported to the IRB within 48 hours by phone, email or fax

Recruitment Monitoring:

Process: The P.I. will assess the recruitment and retention of study subjects on an ongoing basis. The recruitment goal for this protocol is 40 subjects to complete all of the proposed studies.
Reporting: Summary statistics regarding recruitment and retention of study subjects will be reported to the IRB on an annual basis, and at the conclusion of the study.

Early Study Termination:

Process: The P.I. will determine if the study is to be terminated prior the scheduled study conclusion. Early study termination will be considered in the event of an unanticipated serious adverse event determined to be possibly, probably or definitely related to the study. The P.I. may also terminate the study if a positive drug screening test at any time during the study is found. Payment will be reduce to $50 and the subject will be withdrawn from the study
Reporting: The P.I. will report the decision to terminate the study to the IRB within 48 hours of this determination. The P.I. will submit a narrative description of the reasons for early termination of the study within 10 days.

DATA SHARING

We respect that the rights and privacy of people who participate in NIH-sponsored research must be protected at all times. Thus, data intended for broader use will be free of identifiers that would permit

linkages to individual research participants. We and our collaborators will make the data and associated documentation available to users only if they provide: (1) a commitment to using the data only for research purposes and not to identify any individual participant; (2) a commitment to securing the data using appropriate computer technology; and (3) a commitment to destroying or returning the data after analyses are completed. We expect that the results of this study will be presented at national scientific meetings and published in peer reviewed journals.

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