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Project Title: **Impact of sitagliptin on cardiovascular exercise performance in type 2 diabetes**
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I. **Hypotheses and Specific Aims**: Diabetes is a leading cause of cardiovascular disease (CVD) and mortality (5,6). One factor potentially associated with the excess CVD in diabetes is the well-established impairment in functional exercise capacity in people with type 2 diabetes mellitus (T2D) compared to healthy controls (10,42,72,75,78,80). The causes of the exercise abnormalities in T2D are unknown, although recent work from our group demonstrates associations between insulin resistance, endothelial dysfunction, mitochondrial dysfunction and cardiac dysfunction with decreased exercise capacity in T2D in humans (4,10,42,59,72,75,77,78,80,94). We have also shown in animal work that the normal response to exercise of both vascular mitochondrial function and biogenesis regulators is blunted in CVD and diabetic models (43).

The long-term goal of our laboratory is to understand the mechanisms whereby T2D impairs functional exercise capacity and to develop approaches to improve function. Observations from our group have identified two interventions that improve, but do not normalize, exercise function in T2D. These include: supervised exercise training (10) (and see preliminary data) and more recently, treatment with an insulin sensitizer (74). This latter study illustrated that drug therapy could improve exercise capacity in uncomplicated T2D (74). Despite improvements, neither of these interventions succeeded in normalizing exercise function to equal that of nondiabetic controls. The goal of this proposal is to examine whether sitagliptin, an agent which enhances incretin action, and is already in clinical use for T2D might also improve functional exercise capacity.

Sitagliptin works by inhibiting dipeptidyl peptidase 4 (DPP-4) inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases active incretin and insulin levels, and decreases glucagon levels and postglucose-load glucose excursion. Increasing evidence suggests that sitagliptin and other DPP-4 inhibitors may also provide protection against CV events (39,54,56), although the mechanism for this is not clearly understood. In addition, in a recent study from our lab, saxagliptin restored vascular eNOS and mitochondrial induction after an 8 day exercise intervention in a rodent model of diabetes (41).

Incretins have additional relevant effects that are of particular interest for this proposal including benefiting endothelial and mitochondrial function (22,26), as well as reducing CV risk (51). As has been noted, GLP-1 is a critical factor for healthy metabolism and vasculature and stimulates eNOS (26), (22). Small studies in humans with congestive heart failure show improvements in cardiac function and exercise capacity with GLP-1 administration (82,83). Improvements in endothelial function have also been examined in rodents *in vivo* and *ex vivo* suggesting a direct effect of GLP-1 on endothelial function via eNOS (66-68). In humans, this is manifested in consistently lowered blood pressures in some studies (49,50). Thus overall, increasing evidence in humans and animals suggests that sitagliptin and other incretin regulatory agents may have some cardioprotective effects. Recent observations in rodents and humans suggest that GLP-1 has a beneficial effect on endothelial and cardiac function (two factors associated with exercise function in children and adults with diabetes) (3,48,49,60,63,82,84,87,88). It is plausible therefore, that sitagliptin will improve exercise capacity in this group.

Our primary hypothesis is that sitagliptin will improve functional exercise capacity in people with T2D more than glimepiride. Our secondary hypothesis is that sitagliptin will improve mitochondrial function more than glimepiride.

Specific aims:

1. To test whether sitagliptin will improve functional exercise capacity in persons with T2D compared to glimepiride.

1a. The primary outcome will be peak oxygen consumption (VO_2peak) and oxygen uptake kinetics (VO_2 kinetics).

1b. Secondary outcomes include cardiac function, endothelial function and tissue oxygen saturation (STO_2) as well as health-related quality of life.

Rationale: In this study, we want to compare two approved active drugs used to treat diabetes. GLP-1 improves exercise capacity in persons with heart failure (82,83). In addition, GLP-1 has effects on cardiac and endothelial function in rodents and humans as well as its metabolic effects (3,48,49,60,63,82,84,87,88). Saxagliptin restores vascular eNOS and mitochondrial induction after an 8 day exercise intervention in a rodent model of diabetes (41). Glimepiride is chosen as an active comparator because it is a commonly used oral agent but has no known effect on exercise capacity.

Approach: We will compare exercise capacity in patients with T2D taking sitagliptin plus placebo vs glimepiride plus placebo to determine whether a three month intervention with sitagliptin improves VO_2peak, oxygen uptake kinetics, cardiac function, blood flow, endothelial function and STO_2. These experiments will address the CV and functional impact of sitagliptin on exercise in diabetes.

2. To evaluate the impact of sitagliptin on muscle mitochondrial function

2a. The primary outcome to address this aim will be ^{31}P measurements (phosphocreatine, free Pi, ATP peaks, ADP and pH)

Rationale: It is believed that sitagliptin works by inhibiting the DDP-4 inactivation of incretin hormones (GLP-1, GIP). In general, the GLP-1 receptor acts to stimulate eNOS and ceramide transfer protein (CERT) thereby activating mitochondrial biogenesis and augmenting glucose flux and enhancing oxidative phosphorylation. New data from our group indicates that muscle mitochondrial function as assessed by ^{31}P MRS correlates with functional exercise capacity (41). It is unknown whether this is related to decreased blood flow and substrate delivery or a specific defect in tissue mitochondrial function. Preliminary work suggests that improving oxygen delivery may improve mitochondrial function. GLP-1 has been demonstrated to increase muscle blood flow (25). It is therefore plausible that eNOS stimulation by sitagliptin will improve muscle blood flow, mitochondrial biogenesis and thereby muscle mitochondrial function.

Approach: MRS techniques will be used to evaluate mitochondrial function. These experiments will generate new information on the impact of sitagliptin on mitochondrial function in human skeletal muscle.

Impact: Novel approaches are needed to decrease excess CV morbidity and mortality in diabetes. Diabetes impairs CV fitness and thereby mortality. A demonstration that sitagliptin improves CV fitness, (and possibly mitochondrial function) will provide important new data pertinent to the management of diabetes and pre-diabetes.

II. Background and Significance:

Exercise function is abnormal in people with T2D. Even in the absence of cardiovascular disease (CVD), persons with T2D have an impaired ability to carry out exercise both at maximal and submaximal workloads (10,42,72,75,78,80). The functional significance of decreased exercise performance is that in T2D, poorer exercise capacity is clearly associated with an array of adverse health outcomes, ranging from poorly controlled diabetes to increased CV morbidity and mortality (5,6). In addition, it has been shown in a population study that there is a correlation between all-cause mortality and low levels of fitness in T2D as well as in healthy controls (8,9,93). Therefore, improvements in exercise tolerance and thereby fitness in T2D could potentially have multiple long-reaching benefits for patients.

The underlying causes of the exercise abnormalities in T2D are not well known although there is evidence available implicating several factors. Importantly, the metabolic abnormalities intrinsic to diabetes may play a role in that insulin resistance has been strongly linked to the exercise

impairments observed (10,12,37,40,44,78). There are also inverse associations between habitual physical activity, VO_2peak and insulin resistance (81). Previous studies have reported relationships between higher levels of physical activity/exercise training status and improved insulin sensitivity in nondiabetic persons as well as in persons with T2D (12,37,40,44,75,78) related to impaired exercise capacity in persons with even very recently diagnosed T2D during exercise. Abnormal VO_2 kinetic and muscle O_2 saturation (StO_2) findings support the idea that O_2 delivery is impaired in T2D (4,75) (and see Preliminary data). In addition, impaired endothelial function is likely implicated as a factor associated with reduced exercise performance in T2D in that exercise abnormalities could reflect a deficient endothelial dilator response to exercise-mediated demand in heart and in peripheral skeletal muscle (16,52,76) (see Preliminary Data). Overall, the published experimental evidence suggests that metabolic, cardiac and peripheral circulatory abnormalities as well as endothelial dysfunction are associated with the impaired functional exercise capacity in T2D. In the current proposal we will address functional differences between the proposed interventions upon the above contributing factors and begin to define the relative importance of metabolic abnormalities, endothelial dysfunction, cardiac dysfunction and peripheral circulatory abnormalities for causing exercise impairments. Exercise training has been shown to improve to some degree but not to normalize the metabolic, endothelial, cardiac and peripheral circulatory abnormalities observed. Thus, the role of sitagliptin could be of key significance to improve exercise abnormalities not normalized by exercise training.

Agents that improve functional exercise capacity:

We have previously reported that exercise training and rosiglitazone both improve functional exercise capacity (10,74). In rodents, the AMP kinase agonist AICAR and resveratrol (57) improve exercise endurance (3,88). Animals expressing PPAR delta ligand in the muscle have increased fitness as seen with the "marathon mouse" (92). Treatment with a PPAR delta ligand improved exercise capacity in combination with exercise training. Thus, there is evidence that pharmacologic intervention is a plausible strategy for improving exercise capacity in T2D.

Evidence that GLP-1 improves metabolic, endothelial and cardiac function as well as exercise performance. In addition to enhancing insulin secretion by acting on pancreatic ß cell receptors, GLP-1 also binds to receptor sites in other tissues including brain, gut, adipose tissue and the myocardium. GLP-1 has been shown to benefit glycemic control in that it lowers glucose level (both fasting and postprandial) and HbA_{1C}. (50,55). Studies in rodents and humans show that GLP-1has positive effects on endothelial and cardiac function in T2D (17,35,50). Specific cardiac effects include the *in vitro* finding of increasing the cAMP concentration in cardiac myocytes (91) as well as improved left ventricular function in humans, rodents and dogs (48,50,61-64,82,100). In addition, a key cardiac abnormality associated with exercise in T2D is diastolic dysfunction (likely related to poor metabolism) and this abnormality was not corrected by exercise training although it was improved. In a rodent model of obesity and diabetes (db/db-/- mice), sitagliptin decreased elevated myocardial fatty acid uptake and oxidation in the diabetic heart suggesting a beneficial myocardial metabolic effect of DPP-4 inhibition (45). Of note, GLP-1 null mice develop a cardiomyopathy that strongly resembles the fibrotic changes and diastolic dysfunction observed in other rodent models of diabetic cardiomyopathy indicating a role for GLP-1 in myocardial health (66).

A proof of concept study in humans with congestive heart failure showed improvements in exercise capacity with GLP-1 administration (83). Diabetic and nondiabetic patients with heart failure received GLP-1 infusion over a 5 week period. Primary end points included evaluation of left ventricular function at rest assessed by echocardiography and VO_2peak measured on the treadmill. Left ventricular function improved significantly. VO_2peak improved from 10.8±0.9 to 13.9±0.6 ml/kg/min ($P<0.001$) in the treated vs the control group. In addition, the 6 minute walk test improved significantly from 232±15 to 286±12 m ($P<0.001$). Thus, GLP-1 improved functional exercise capacity over a relatively short period of time. Although the patients we propose to study do not have heart failure, they do have subclinical cardiac abnormalities which correlate with the exercise defects observed and may represent early signs of diabetic cardiomyopathy (77). Thus, the concept of administering GLP-1 to patients with diabetes provides a promising candidate agent to improve exercise function. Improvements in endothelial function have been examined in rodents *in vivo* and

ex vivo suggesting a direct effect of GLP-1 on endothelial function via eNOS (66). In humans, this is manifested in consistent lowering of blood pressure in clinical trials (50).

GLP-1 improves vascular function and mitochondrial adaptation: GLP-1 is an incretin protein secreted by the gut in response to food intake that augments insulin secretion, decreases glucagon, decreases appetite and slows gastric emptying. Analogs of this hormone are currently approved for the management of diabetes (90). Studies in rodents and humans show that GLP-1 has positive effects on endothelial and cardiac function in diabetes (1,2,28,71,90). GLP-1 decreases mitochondrial ROS and improves optimal mitochondrial function in myocardium and beta cells (7,13,22). Direct and indirect vascular effects of GLP-1 include induction of eNOS, decreased expression of VCAM-1, vasodilation, increased cAMP, decreased intimal hyperplasia and blockade of LPS induced vascular permeability (1,20,21,26,28,31,38,58). In diabetic rats, GLP-1 improves endothelial function and stimulates CREB (69,70). In addition, GLP-1 improved hepatic steatosis and increased cytoprotective autophagy in non-alcoholic fatty liver disease and beta cells (30,97,98).

Saxagliptin restores the vascular response to exercise including eNOS induction and mitochondrial biogenesis in diabetes. We recently reported that short term (8 day) exercise intervention increases aortic mitochondrial protein expression in control rats, but not in an obese diabetic model (in press-appendix). Similarly, in Goto-Kakizaki (GK) rats, we recently showed that the response of eNOS, SIRT3, pCREB or mitochondrial protein expression with exercise in diabetes is impaired and that intervention with saxagliptin increased these markers as well as mitochondrial response to exercise in diabetes, restoring normal mitochondrial adaptation to exercise in a diabetes model (41). This is likely the result of the prevention of GLP-1 degradation by saxaglipitin. Therefore, we expect sitagliptin to improve exercise function and propose to evaluate the effect of sitagliptin on mitochondrial function in humans in the present study.

Significance:

Physical activity has a beneficial effect on CV and all-cause mortality. We know that exercise training improves functional exercise capacity in persons with diabetes, but does not close that gap and bring people to normal levels of fitness. Sitagliptin, with its potential impact on cardiac, endothelial and mitochondrial function may restore exercise capacity to non-diabetic levels. Inactivity has adverse effects on the diabetic metabolism and increases CV morbidity and mortality; in contrast, increased physical activity works beneficially in these regards (8,9,19,93). **Thus, from a practical standpoint, a drug which improves exercise capacity has the potential to make the performance of exercise more feasible for the patient with T2D.** Decreased functional exercise capacity in people with T2D is a barrier to physical activity. If sitagliptin, or any agent employed for glycemia, improves exercise capacity, it may beneficially affect the treatment algorithm for persons with T2D. Overall, the findings from this proposal may provide important mechanistic insight into therapies which benefit CV, endothelial and mitochondrial outcomes for persons with T2D via enhanced exercise capacity.

III. **Preliminary Studies/Progress Report:** Below we highlight critical published and preliminary results that support our hypothesis and demonstrate the feasibility of this project in our laboratory.

1.0 Studies from our laboratory that demonstrate that T2D confers exercise dysfunction:

1.0a Diabetes is associated with decreased VO_2peak: It has been consistently observed by our lab and others that VO_2peak is lower in persons with T2D than in nondiabetic controls (4, 10, 42, 72, 75, 78, 80) A difference of approximately 20% in VO_2peak between persons with T2D and controls has been documented in these studies.

1.0b. Diabetes is associated with slowed VO_2 kinetics: Not only VO_2peak, but also submaximal measures of exercise are abnormal in T2D including VO_2 at submaximal workloads (Figure 1) and VO_2 kinetics. VO_2 kinetics are a measure of the rate of adaptation in pulmonary VO_2 following the onset of constant work rate.

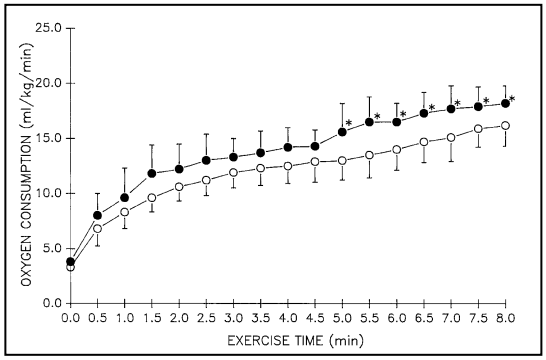


Figure 1. This figure illustrates that VO2 at all submaximal work-loads is reduced in persons with T2D compared to nondiabetic controls of similar age and activity levels during graded exercise testing (78). This is an abnormal response to exercise that has been observed in other disease states with impaired O2 delivery or utilization (4, 10,60,99).

Three phases to the response of VO_2 from rest to moderate constant-load exercise were proposed (95,96). At the onset of exercise, VO_2 from the lungs normally increases abruptly for the first 15 sec (Phase I) as pulmonary blood flow increases. Then the VO_2 increases exponentially in Phase II with a time constant, tau, normally about 30-45 sec representing further increases in blood flow and decreased venous O_2 content. Phase II ends as gas exchange approaches a steady-state. Phase III is steady-state VO_2 below the lactate threshold, but not above the lactate threshold (95,96). We observed that VO_2 kinetics were abnormal in persons with T2D compared to healthy controls (75) as has been observed in other groups with abnormal CV responses to exercise (4, 10) . This finding suggests that persons with T2D have a reduced rate of circulatory adjustment to an increase in work-load. *Improving cardiac or systemic CV function with sitagliptin could correct this defect.*

1.0c Studies characterizing the cardiac contribution to exercise dysfunction in T2D:

Fig 2 addresses likely causes of exercise abnormalities associated with T2D.

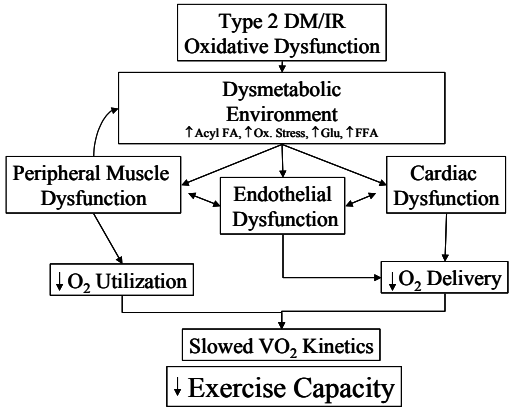


Figure 2. Schema:
Proposed factors contributing to exercise dysfunction in T2D: T2D and prediabetic conditions such as insulin resistance are associated with an abnormal metabolic environment that induces endothelial, cardiac, and peripheral dysfunction. Target organ dysfunction feeds back to exacerbate these defects. Ultimately these factors result in defects in functional exercise capacity.

2. Cardiac Abnormalities with Exercise in T2D: Is cardiac function impaired during exercise in T2D? Ten premenopausal women with uncomplicated T2D (average diagnosed duration of T2D, 3.6 years) and 10 healthy nondiabetic women of similar age, weight and activity levels were studied and data have been reported (77). Participants performed a maximal exercise test while instrumented with an indwelling pulmonary artery catheter. On separate days, Tc-99m sestamibi myocardial perfusion (SPECT) imaging with rest and exercise was performed in a subgroup of diabetic and control patients. VO_2peak, measured during catheterization was lower in T2D than control subjects (18.7±2.3 vs 22.3±4.2 ml/kg/min, $P<0.05$). Pulmonary capillary wedge pressure (PCWP) (a marker of cardiac stiffness) rose more with exercise persons with T2D than controls (146% vs 114% increase, $P<0.01$). (Figure 3, Table 1).VO_2peak was inversely correlated with

maximal PCWP (r=-0.62, P<0.05). Blood flow, as assessed by total normalized myocardial perfusion index (MPI) from SPECT was lower in persons with T2D than controls. (11.0±3.5xE-9 vs 17.5±8.1E-9 respectively, p<0.05) and was inversely related to PCWP (R=-0.56). Since resting systolic function by echo was normal, findings are possibly consistent with diastolic dysfunction in T2D. However, cardiac output was not reduced in T2D as might have been expected. Thus, there is clear evidence of cardiac dysfunction and reduced perfusion in T2D (4) and the impaired cardiac function is correlated with impaired exercise capacity. *The component of these early cardiac abnormalities in T2D related to decreased perfusion could be responsive to sitagliptin.*

Table 1.	**Controls (N=10)**	**T2DM (N=10)**
Age	39.3±6.6	42.5±6.3
Body Mass Index (kg/m^2)	28.3±3.9	31.9±4.3
HbA_1C (%)	5.0±0.4	6.9±2.3*
VO_2 max (ml/kg/min)	22.3±4.2	18.7±2.3*
Peak Respiratory Exchange Ratio	1.14±0.05	1.18±0.05
Peak Cardiac Output (Fick) (L/min)	13.1±2.8	12.6±1.6
Peak Cardiac Index (Fick)	7.3±1.4	6.8±0.5
Peak A-V oxygen difference	12.8±2.2	12.3±1.3
Peak Pulmonary Capillary Wedge Pressure (mmHg)	16.7±3.7	23.6±3.9*

VO_2 max= maximal oxygen consumption; HR, Heart rate; A-V, arteriovenous *P<0.05 difference between Controls and T2DM; $^+$P=0.06 difference between controls and T2DM. Cardiac index=cardiac output/body surface area; Max, maximal; Data are given as mean±SD (11a,b- paper under review)

2.0a Cardiac abnormalities are observed by echocardiography in adults and adolescents with T2D. Having first established the presence of cardiac abnormalities using invasive techniques, we next validated and are now using noninvasive stress echocardiographic techniques to assess cardiac function in a more recent exercise training study. We have preliminary data that markers of diastolic function are abnormal in T2D such as the E:A ratio, showing that cardiac diastolic function was abnormal in people with T2D (E:A 1.33±0.42 controls and 1.11±0.26 T2D (n=5 per group). Similarly, indexed left ventricular mass was already abnormal in 13 adolescent children with T2D compared to13 obese controls (59). Table 2 demonstrates a mild improvement in E:A ratios with exercise training that does not return to healthy norms. *Recent unpublished work using the speckle tracking methods proposed reveal additional abnormalities not detected by traditional echocardiography*

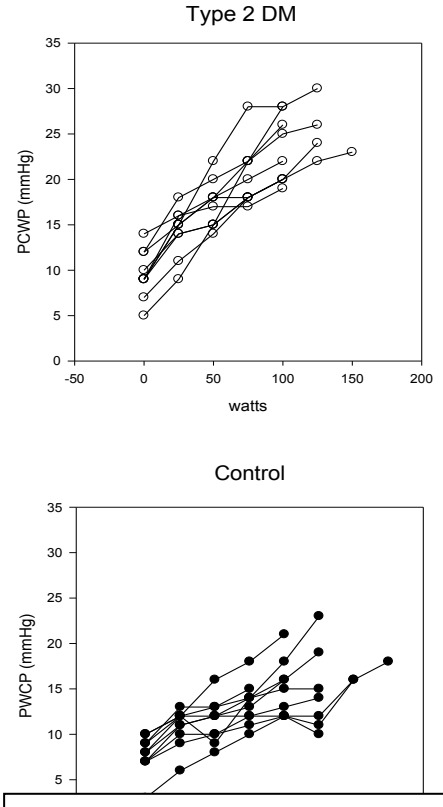


Figure 3. Pulmonary capillary wedge pressure during graded exercise to VO2peak (77)

Interpretation of cardiac findings: These findings suggest that cardiac function is abnormal in relatively recently diagnosed and otherwise healthy children and adults with T2D during exercise even in the absence of clinically evident occlusive coronary artery disease and that this abnormality is related to impaired exercise capacity. *The component of these early cardiac abnormalities in T2D related to decreased perfusion could be responsive to sitagliptin.*

2.0b Endothelial Dysfunction correlates with exercise impairment in T2D: We and others have reported that T2D is associated with impaired endothelial function (53, 65, 76, 89).We measured endothelial function using brachial artery ultrasound in 10 premenopausal women with type 2 diabetic and 10 healthy women. T2D participants had smaller post-ischemic hyperemic increases in both brachial artery diameter and forearm blood flow than controls. Participants with T2D had lesser hyperemic brachial artery dilator (change in diabetics=0.028 ± 0.006 cm vs change in control =0.056 ± 0.008

cm, p<0.05) and hyperemic blood flow (change in diabetics= 16 ml 100 ml/ min vs change in control= 26 ml 100 ml/ min, p<0.05) responses.

Is there a relationship between flow mediated dilation and exercise capacity in T2D? Using data from a subsequent study (data analysis in process), we performed a correlation on the relationship between VO_2peak and change in FMD from rest to hyperemic flow in persons with T2D. The correlation was r=0.65, P<0.05, suggesting a relationship between endothelial function and exercise capacity in this group. *Endothelial function is abnormal in T2D in association with exercise abnormalities and improved by sitagliptin.*

2.0c Skeletal muscle oxygen delivery is slowed in T2D. Cardiac perfusion and function during exercise in T2D are abnormal but cannot account for all of the exercise impairment in T2D. One key factor associated with the exercise intolerance in T2D is abnormally slowed pulmonary VO2 kinetics during submaximal exercise as we have previously demonstrated (10,75). The mechanisms of this maladaptation during exercise are unclear but likely relate to impairments in skeletal muscle blood flow. We conducted a study to compare skeletal muscle deoxygenation ([HHb]) responses (indicating local muscle O_2 extraction) and estimated microvascular blood flow (Qm) kinetics in T2D and healthy subjects following the onset of moderate exercise (4). Pulmonary VO_2 kinetics and [HHb] (using near infrared spectroscopy) were measured in 11 T2D and 11 healthy subjects during exercise transitions from unloaded to moderate cycling exercise. The increase in Qm were calculated using VO_2 kinetics and [HHb] responses via rearrangement of the Fick principle. We found that VO_2 kinetics were slowed in T2D compared with controls (43.8±9.6s vs. 34.2±8.2s, P<0.05), and the initial [HHb] response following the onset of exercise transiently exceeded the steady state level of local muscle O_2 extraction in T2D compared with controls. This response was consistent with a greater dependence on O_2 extraction early in exercise that was not observed in healthy muscle. Concordantly, the mean response time of Qm increase was prolonged in T2D compared with healthy subjects (47.7±14.3s vs. 35.8±10.7s, P<0.05). Thus, skeletal muscle in T2D demonstrated a transient imbalance of muscle O_2 delivery relative to O_2 uptake following exercise onset, suggesting a slowed Qm increase in T2D muscle. This response could not be explained by a central defect in cardiac output as heart rate responses to this low level of exercise were similar between T2D and controls indicating the observed muscle blood flow response were likely related to events occurring local to the exercising muscle. Thus, impaired vasodilatation secondary to vascular dysfunction in T2D during exercise may contribute to this observation, potentially linking this finding to impaired vascular reactivity and endothelial dysfunction. *These data support the hypothesis that muscle blood flow kinetics (and hence O_2 delivery) following the onset of exercise are impaired in T2D; a response that could be improved with sitagliptin.*

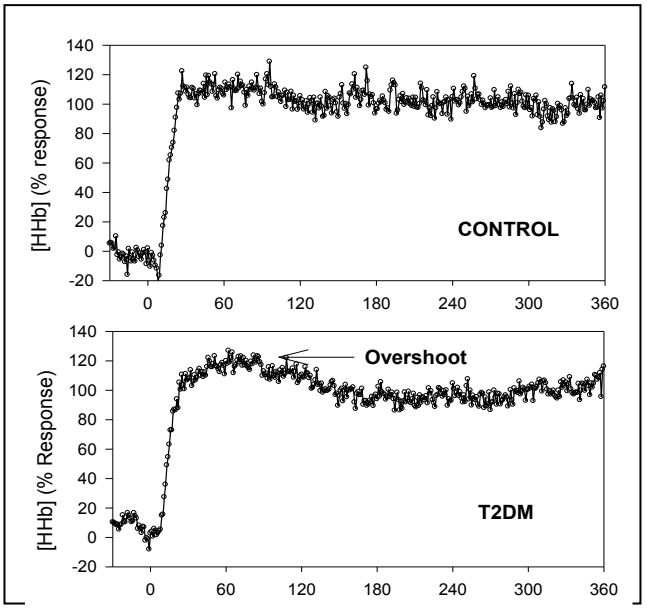


Figure 4. Muscle deoxyhemoglobin ([HHb]) concentration kinetics in a representative healthy subject and a subject with T2D at rest and following the onset of moderate exercise. Exercise begins at time = 0. Note pronounced overshoot of HHb in the T2D subject early in the exercise transition. This response indicates a greater reliance upon local muscle oxygen extraction (e.g. due to slowed increase in microvascular blood flow) early in exercise that is not observed in healthy muscle. (4)

2.1 Studies demonstrating the ability of our laboratory to augment exercise function in T2D:

2.1a Does exercise training improve CV exercise performance in T2D? Men and women with T2D and nondiabetic controls entered into a three month program of exercise training following baseline measures to assess effects of exercise training on endothelial and cardiac function (10) and in preparation for publication). Participants had 36 sessions of exercise training. During each training session, heart rate was kept between 70-85% of bicycle-ergometer measured VO_2peak with work load being increased regularly to ensure that the training effect was continued. Resting measures of cardiac function (determined by echocardiography and tissue Doppler) made before exercise training revealed that both women and men with T2D had significant impairment in cardiac function. Exercise training improved exercise capacity, metabolic control, endothelial function and cardiac function but without normalizing any of these variables. Other studies have also described improvements in exercise capacity in T2D with exercise training (23,79,80)

Table 2: Effects of exercise training on exercise capacity, endothelial function and diastolic function in T2D

		VO2peak ml/kg/min	Respiratory exchange ratio	Flow mediated dilation (Δ)	Tissue Doppler E:A Lateral	Tissue Doppler E:A Septal
Women	Pre	18.6±2.3	1.17±0.07	0.17±0.06	1.09±0.20	0.99±0.22
	Post	20.4±4.3*	1.18±0.11	0.27±0.23*	1.18±0.41+	1.08±0.36
Men	Pre	25.0±6.0	1.22±0.10	0.17±0.14	1.19±0.32	1.14±0.36
	Post	28.0±5.3*	1.22±0.10	0.28±0.16+*	1.25±0.27+	1.08±0.36

Pre, Pre exercise training; Post, Post exercise training; VO2peak, maximal VO_2; RER, respiratory exchange, ** P<0.05 difference pre and post exercise training; +tendency towards difference pre and post exercise training (9,23)

Role of VO_2 kinetics. Previously, we have demonstrated slowed pulmonary VO_2 kinetic time constants in uncomplicated T2D compared with nondiabetics (10,75). The magnitude of slowed VO_2 kinetics in T2D was correlated with the functional impairment in VO_2peak. We have also shown that **exercise training** significantly increases VO_2peak and improves VO_2 kinetics (e.g. faster time constants) in women with T2D but this does not fully normalize the exercise defect (10). *Because we observed an attenuated oxygen pulse response with exercise in T2D in the absence of treatment, the slowed VO_2 kinetics may be attributed to a central cardiac defect in O_2 delivery which could potentially be improved by sitagliptin.*

2.1b Can a pharmacological intervention increase VO_2peak in people with T2D?

Based on our observation that defects in functional exercise capacity correlated with decreased insulin sensitivity and decreased endothelial function, we hypothesized that a thiazolidinedione would improve exercise function (74). 20 patients with uncomplicated T2D were randomized in a double-blinded study to receive either 4 mg/day of rosiglitazone or matching placebo after baseline measurements which included endothelial function (by brachial ultrasound), VO_2peak and insulin sensitivity with reassessment after 4 months of treatment. The two groups of patients did not differ at baseline in any measure. Patients treated with rosiglitazone had significantly improved VO_2peak (by 7%)(Table 3) as well as improved brachial artery diameter (BAD) and insulin sensitivity (74). Rosiglitazone increased the BAD response to cuff inflation by 83% over the baseline response (from 0.024±0.03 cm to 0.044±0.03 cm, P<0.05) while no significant increase seen in the response of placebo-treated controls (from 0.010±0.02 cm to 0.017±0.02 cm, P=NS). Change in VO_2peak correlated with changes in BAD and insulin resistance in the rosiglitazone treated group only. *These data demonstrate that an agent that improves insulin sensitivity can improve exercise function in T2D.*

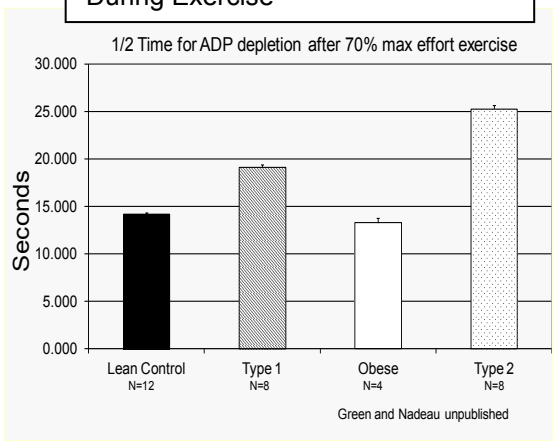
Table 3. Rosiglitazone Effects on Exercise Capacity in T2D (*P<0.05 difference within group)

	Placebo (N=10)	RosiglitazoneTreated (N=10)

VO_2peak (ml/kg/min) Pre **Post**	 19.4±5.2 18.1±5.3	 19.8±5.3 21.2±5.1*
Oxygen pulse (VO_2/HR) Pre Post	 11.3±3.7 11.1±3.6	 13.3±3.7 14.8±4.1*

2.2 Mitochondrial function-Mitochondrial respiration using MRS techniques in human subject correlates with functional exercise capacity (a pilot study from our lab): Following a controlled study diet, in 11 adolescents (14.8±2.2 years), including n=5 T1D, n=1 T2D, n=3 lean and n=2 obese controls, were assessed in the fasting state for VO_{2peak} via a bicycle exercise test, lean body weight (kg) via DEXA, and metabolite recovery time (phosphocreatine (PCr), inorganic phosphate (P_i), and ADP) via ^{31}P magnetic resonance spectroscopy. The soleus and gastrocnemius muscles were studied during isolated static plantar flexion at 70% maximum contraction. Measurements of PCr, P_i, and ADP were made at rest, during 90 seconds of contraction, and for 15 minutes following the exercise bout. A significant negative correlation was found between VO_{2peak} (43.3±7.4 mL/min/kg) and post-exercise P_i recovery half time (23.9±6.4 secs) (r=-0.625, p=0.04). A significant negative correlation was found between VO_{2peak} (29.7±8.3 mL/kg/min) and post-exercise ADP recovery half time (17.8±5.1 secs) (r=-0.644, p=0.03). Thus, metabolic recovery, regarding ADP and P_i recovery time, was slower in adolescents with decreased exercise capacity as determined by VO_{2peak}. Impaired ATP and PCr re-synthesis could minimize energy production and thereby reduce exercise capacity. *We anticipate that sitagliptin will improve mitochondrial function by enhancing substrate delivery.*

Figure 5. Half-Time for ADP Depletion After 70% Max Effort During Exercise



3.0 What is the possible role of Sitagliptin?

3.0a Can DPP-4 inhibitors improve exercise capacity?: No previous reports have investigated the impact of DPP-4 inhibitors on exercise capacity in human subjects. However, the impact of GLP-1 and DPP-4 inhibitor on endothelial function, capillary recruitment, and eNOs activity would be predicted to improve exercise function by correcting the exercise defects we have identified. In human subjects, GLP-1 improves insulin dependent increases in forearm blood flow (86). In a study that parallels our work on kinetics, GLP-1 increased glucose clearance into the myocardium in subjects with T2D via early increases in myocardial blood flow (25).

Rodent studies demonstrate that sitagliptin improves endothelial function through eNOS in a GLP-1 dependent fashion in the renal arteries (46) and recruits capillaries to increase microvascular endothelial surface area thereby improving insulin delivery and action in an NO dependent fashion (18). In other studies, sitagliptin improved angiogenesis in ischemic limbs by enhancing the function of endothelial progenitor cells and stimulation of eNOS (36). We have demonstrated that the diabetic vasculature fails to induce eNOS or mitochondrial biogenesis in diabetic rats (41). Saxagliptin restores the induction of eNOS and vascular mitochondrial protein expression in response to an 8 day exercise exposure. *Taken together, the background and preliminary data support the plausibility of the hypothesis that sitagliptin will improve cardiac and muscle blood flow and increase both VO_2 kinetics and VO2 peak in people with T2D.*

3.0b. Effects of sitaglipin on mitochondrial function. As noted above, in GK rats, we recently showed that the response of eNOS, SIRT3, pCREB or mitochondrial protein expression with exercise in diabetes is impaired and that intervention with saxagliptin increased these markers as well as the

mitochondrial response to exercise in diabetes, restoring normal mitochondrial adaptation to exercise in a diabetes model (41). This is likely the result of the prevention of GLP-1 degradation by saxaglipitin and enhanced activation of vascular GLP-1 receptors. *Therefore, we propose to evaluate the effect of sitagliptin on mitochondrial function in humans in the present study.*

Overall Interpretation: This body of evidence suggests that impairments in metabolic, endothelial, cardiac, peripheral circulatory function and mitochondrial function may each contribute to a portion (but not all) of the exercise defect in T2D. Thus, evaluation of the impact of sitagliptin on each of these factors contributing to the exercise impairment in diabetes is proposed.

Summary and Interpretation of Preliminary Data: Taken together, the above observations show that persons with T2D have substantially impaired exercise performance and identifies some key areas which may be targets for therapeutic intervention. It is notable that although all patients in the prior studies had uncomplicated diabetes and had only had disease for a relatively short period (<10 yrs), the functional impairment is very substantial. Because the lower VO_2peak has been associated with a reduced ability to carry out normal activities as well as excess mortality, any improvement of functional status is of great clinical significance. Endothelial dysfunction is present in T2D and also correlates with exercise impairment (76). Associated abnormalities of cardiac perfusion/function (77) and skeletal muscle oxygenation with exercise (4) suggest local abnormalities in O_2 delivery within the exercising muscles (4). The proposed studies will examine the impact of sitagliptin on exercise capacity and cardiac and vascular function in T2D using a comprehensive assessment plan.

Sitagliptin is a promising treatment for exercise abnormalities given its roles in improving cardiac, endothelial and mitochondrial function. Thus, this may be a powerful tool to get persons with T2D able to maximize their ability to exercise and thus improve their metabolic state and CV fitness.

IV. Research Methods

A. Outcome Measure(s):

Subjects: This study will randomize subjects with T2D into one of 2 groups (with 28 patients enrolled in total). The intervention will be: Group 1: sitagliptin 100 mg/d plus placebo. Group 2: glimepiride 2 mg/day plus placebo. Patients on metformin monotherapy or those who are metformin-intolerant will be sought for this study (see inclusion and exclusion criteria). Drugs will dispensed by the University Research Outpatient Pharmacy.

SA#1a. Overview of experimental design: We will compare exercise capacity in patients with T2D taking sitagliptin plus placebo vs patients on glimepiride plus placebo to determine whether a three month intervention with sitagliptin improves VO_2peak and oxygen uptake kinetics.

SA#1b. Overview of experimental design: Experiments under this aim will determine the effect of sitagliptin vs glimepiride upon established measures of cardiac function, endothelial function and peripheral circulatory function. Stress echocardiography with speckle tracking will be used to determine cardiac function. Endothelial function will be assessed by changes in brachial artery diameter in response to cuff occlusion. Vascular stiffness will be assessed using the Sphygmocor. Muscle tissue O_2 saturation will be measured during exercise using near–infrared monitors. We will also assess the relationship between improvements in glycemic control and FFA and changes in exercise function. In addition to testing the stated hypothesis, this aim will also be hypothesis-generating in that the proposed studies will reveal which measures are improved most by sitagliptin. This new information will help to design further grant proposals to the ADA and NIH to examine the cellular and molecular mechanisms by which sitagliptin has benefit for persons with T2D.

SA#2: Overview of experimental design: Experiments under this aim will utilize MRS technology to determine whether sitagliptin normalizes mitochondrial function in human diabetic participants given sitagliptin plus placebo vs those given glimepiride plus placebo.

B. Description of Population to be Enrolled:

Twenty-eight sedentary persons with uncomplicated T2D between the ages of 22 and 70 will be randomized. Subjects must be taking metformin only (500-2000 mg/day) or not on diabetes medication, if intolerant of metformin, to treat diabetes.

Inclusion Criteria:

1. Female subjects may be pre, peri or post-menopausal.
2. People who do not participate in a regular exercise program ($\geq$ one bout of exercise per week).
3. Presence of T2D will be documented by chart review that will confirm the diagnosis as well as the presence of treatment for diabetes.
4. Persons with T2D will be accepted for study only if they have total glycosylated hemoglobin levels (HbA_1C) between 7.0 and 9.5% (adequate control) on therapy.
5. Persons who are taking metformin 500-2000 mg/day only to control their T2D, but are not taking any other diabetes medication in addition to or instead of metformin.
6. Persons not taking medication to control diabetes.

Exclusion Criteria:

1. Females of childbearing potential who are pregnant, planning to become pregnant or breastfeeding.
2. Persons will be excluded if they have evidence of ischemic heart disease by history or abnormal resting or exercise electrocardiogram (EKG) ($\geq$ 1 mm ST segment depression), regional wall motion abnormalities, LV systolic dysfunction or significant valvular disease.
3. Persons with angina or any other cardiac or pulmonary symptoms potentially limiting exercise performance.
4. Presence of systolic blood pressure >160 at rest or >250 with exercise or diastolic pressure >95 at rest or >115 with exercise.
5. Subjects who have peripheral arterial disease.
6. Subjects with proteinuria (urine protein >200 mg/dl) or a creatinine $\geq$ 2 mg/dl, suggestive of renal disease.
7. Persons with liver function impairment defined as elevated liver function tests three times the upper limit.
8. Persons with a history of pancreatitis.
9. Subjects more than 140% of ideal body weight.
10. Patients on insulin therapy will not be included.
11. Current smokers will not be accepted for study since smoking can impair CV exercise performance but people who have quit smoking for at least 1year will be accepted for study.
12. Persons with autonomic dysfunction (>20 mm fall in upright BP without a change in heart rate) will be excluded.
13. Diabetic persons with clinically evident distal symmetrical neuropathy will be excluded from further study, because of possible effects on exercise performance, by evaluation of symptoms (numbness, paresthesia) and signs (elicited by vibration, pinprick, light touch, ankle jerks).
14. Persons with diabetic ketoacidosis.
15. Persons with a serious hypersensitivity to sitagliptin, sulfonylureas or sulfonamides.
16. Inability to walk or ride a bike unassisted for a continuous 5 minutes.
17. Subjects will be excluded if they have any implanted metal in their body.
18. Subjects currently being treated with Digoxin.

C. Study Design and Research Methods

Study Protocol. Subjects will come for a total of nine testing visits during which evaluations will take place. Visits are structured as follows:

1. After subjects review the study and give consent for study participation, a history and physical exam will be performed. Blood drawn for measurement of HbA1C, fasting glucose, fasting insulin, and microalbuminuria, CRP, IL-6, adiponectin, and creatinine. These measures will be covariates in the analyses. Markers of inflammation including CRP, IL-6 and adiponectin are used to assess whether inflammation is present. In this way, we can determine if exercise capacity is affected by markers of inflammation. The glucose and insulin are measured to assess glycemic status. Additional screening labs include CBC, FSH, urine protein and a lipid panel to assess whether women are pre- or post-menopausal (FSH), and overall health (CBC, lipids and urine protein). If participants opt not to fast for visit 1, we will postpone the blood draw to visit 2. Ankle brachial index, ANS function tests, the LoPAR questionnaire and vital signs will be performed.
2. A dietary survey will be administered for food preferences for the two day study diet administered prior to visits 3 and 7 and a study meal and snack prior to visits 4 and 8. DEXA and body composition tests will be done to ensure that groups are weight similar (using fat-free mass). A pulmonary function test, resting EKG and familiarization bicycle test will be performed.
3. Subjects will be given an option of washing out from exclusionary medications or if their hemoglobin A1C results are below the threshold for inclusion. Specific washout periods for medications are as follows:
    a. Metformin – 8 week washout.
    b. Thiazoladinediones (e.g., pioglitazone, rosiglitazone) – 8 week washout
    c. DPP-4 inhibitors (e.g., sitagliptin, saxagliptin, etc.) – 30 day washout
    d. Sulfonylureas (e.g., glipizide, glyburide, glimepiride) – 30 day washout

    If subjects elect to perform a medication washout, approval from their primary care physician will be obtained and they will be closely monitored throughout the washout period and continuing until they are randomized to the study medication.
4. Subjects will receive a two day study diet prior to visit 3. A resting and exercise EKG will be performed on the day of the visit. A graded exercise test will be done to determine the VO_2peak. Patients will have measures of cardiac function and endothelial function on visit 3 by plethysmography and cardiac echo. Vital signs and arterial stiffness/endothelial function will be non-invasively measured by the Sphygmocor system will be taken at rest. If the subject participated in a medication washout, fasting glucose and HbA1C screening labs will be repeated.
5. Subjects will receive a study meal and snack prior to visit 4. Calf muscle MRS will be performed on a 3.0 T whole–body MRI scanner.
6. During visit 5, subjects will perform three constant-load tests to measure VO_2 kinetics where StO_2 will be measured during exercise. A resting and exercise EKG and vital signs will be performed during the visit. Subjects will be randomized to taking sitagliptin plus placebo or glimepiride plus placebo and all must be taking metformin (500-2000 mg /d) for 3 months (except those who are metformin-intolerant). Sitagliptin and its placebo will be administered 100 mg/d. Glimepiride and its placebo will be administered 2 mg/day. During the treatment phase subjects will be given a log to keep track of their blood glucose each day. Study coordinators will contact each subject weekly to obtain these values which will be checked by the study doctors and shared with the subject's primary care physician if adjustments in other medications need to be made.
7. Visit 6 will consist of a physical exam with a clinician as well as a blood draw and check of vital signs during sitagliptin or glimepiride treatment.
8. After 3 months of sitagliptin or glimepiride administration, Visit 3 will be repeated. Additional testing to be performed during visit 7 will include a physical exam performed by a study physician, blood work for covariate lab tests listed in Visit 2 and the LoPAR questionnaire.
9. During visit 8, visit 4 procedures will be repeated.

10. During visit 9, the testing performed during visit 5 will be repeated.

Visit Schedule:

Visit Timeline	Screening Visit 1	Screening Visit 2	Visit 3	Visit 4	Randomization Visit 5	Visit 6 Week 2	Visit 7 Week 12	Visit 8 Week 13	Visit 9 Week 14
Informed Consent and HIPPA Auth.	X								
History & Physical	X					X	X		
LoPAR	X						X		
ANS Function Tests	X								
Ankle Brachial Index	X								
Pulmonary Function Test		X							
Blood Draw and Urinalysis	X					X	X		
Vital Signs		X	X		X	X	X		X
Electrocardiogram		X	X		X	X	X		X
Plethysmography, Cardiac Echo, FMD			X				X		
DEXA		X							
GXT		X	X				X		
Calf MRS				X				X	
Kinetics					X				X
Near-infrared spectroscopy					X				X
Diet Instruction		X							
Study diet			X	X			X	X	
Arterial Stiffness Measurement			X				X		
Study drug dispension					X				
Length of Visit	2.5 hrs	2.5 hrs	2.5 hrs	1.5 hrs	2.5 hrs	1 hr	3 hrs	1.5 hrs	2hrs

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Procedures:

Body Composition and DEXA. These measures will be performed according to standard methods (47).

Dietary Interviews. Customary macronutrient pattern will be ascertained by food preferences questionnaire. A study diet provided by the clinic will be used prior to certain visits as stipulated in the Visit schedule. The clinic dietician will prescribe a standardized nutrient breakdown including 20% protein, 35% fat and 45% carbohydrate for the two days prior to visits 3 and 7 and stud meal and snack prior to visit 4 and 8. The range of sodium in the CTRC diet will be about <2300 mg/day. In addition, sodium intake guidelines will be followed throughout the study to assure standard hydration and especially with visits where the CTRC diet will be utilized for diets prior to the visits.

Brachial Artery Diameter. Endothelial function as assessed by the brachial artery method will be utilized following the protocol described by Celermajer et al.(14, 15, 85) that measures dilation of the brachial artery by ultrasound in response to hyperemia. The brachial artery diameter will be measured using B-mode ultrasound images, with the use of a GE Vivid 7 ultrasound system and a

10.0-mHz linear-array transducer as previously reported in our laboratory (GE vivid 7 Dimension, Milwaukee WI.). Scans will be obtained with the subject at rest and following reactive hyperemia.

Low Level Physical Activity Recall Questionnaire (LoPAR): This questionnaire has been validated for use in persons with T2DM and peripheral arterial disease as well as in sedentary controls by the Investigator as previously reported and is being used as an outcome measure by Diabetes Prevention Program (44).The LOPAR asks about physical activity level over the previous week. Subjects are asked in a series of questions to itemize their time (reporting specific activities) into work, leisure and housework categories. Questionnaire results are calculated in metabolic equivalents (METS) where one MET equals resting VO_2 (3.5 ml/kg/min). This questionnaire will be used in this study as a screen to ensure that all participants are sedentary.

Skeletal Muscle Oxygenation. (Near-infrared Spectroscopy) Skeletal muscle [HHb] will be assessed by a frequency domain multi-distance NIRS monitor (Optiplex TS, ISS, Champaign, IL, USA) during each CWR exercise test (40). The use and limitations of NIRS have been extensively reviewed (4, 24, 27). The NIRS monitor uses two wavelengths of NIR light (690 and 830nm) and four light source-detector distances at 2.0, 2.5, 3.0 and 3.5 cm. Local muscle O_2 extraction was determined as the change in [HHb] as previously described (5, 10, 42). The NIR data will be sampled continuously at rest and during exercise and recorded at 50Hz. The device probe will be positioned on the distal third of the vastus lateralis of the dominant limb, secured using a Velcro strap, and covered with a cloth bandage to exclude ambient light. The NIRS monitor is calibrated prior to each visit using a calibration phantom of known scattering and optical properties.

Plethysmographic Measurements. Forearm blood flow will be determined in seated participants by venous occlusion strain gauge plethysmography (D.E. Hokanson Inc. Issaquah, WA), using calibrated mercury-in-silastic strain gauges and expressed as ml/100 ml/min (3, 33, 34). The arm will be supported above the heart level. Venous occlusion pressure will average 35 mmHg. Circulation to the hand will be prevented by inflating a wrist cuff to suprasystolic pressure before each forearm blood flow determination. Determination of forearm blood flow will comprise at least five separate measurements performed at 10-15 sec intervals. After a 10-15 min rest period, measurements will be repeated (3-5 times).

Cardiac Echocardiographic Measurements. Standard two dimensional and Doppler echocardiography will be performed using standard methods (32, 73) to exclude the presence of left ventricular systolic dysfunction, regional wall motion abnormalities (suggestive of coronary artery disease), pericardial disease or significant valvular pathology (GE Vivid 7 Dimension, Milwaukee, WI). Chamber sizes, LV end-systolic and diastolic chamber dimensions and wall thickness, fractional shortening and the area-length method for measurement of cardiac volume in order to measure ejection fraction will be quantitated by standard techniques for all individuals.

Cardiac function will be assessed by standard echocardiographic parameters (E and A wave velocities, E:A ratio, E wave deceleration time, pulmonary vein Doppler) as well as tissue Doppler of both the septal and lateral mitral annuli (E' and A' velocities, E':A' ratio, E:E' ratio) and flow propagation determined by color M-mode (32,73). Measurements will be collected at rest and post-exercise (supine). Dr. Jennifer Dorosz , MD, Collaborator who has expertise in echocardiography and these techniques, will be blinded to the diagnostic status of the participant and will supervise acquisition of all of the echocardiographic data as well as perform all of the measurements and interpretation. Echocardiograms will be obtained with a commercially available ultrasound system (GE Vivid 7 Dimension, Milwaukee, WI). Subjects will be examined in the left lateral decubitus position using standard parasternal, short-axis, and apical views. All recordings and measurements will be obtained by the same observer according to the recommendations of the American Society of Echocardiography and will always be performed at the same time of day for each subject to avoid the possible influence of circadian rhythm on left ventricular diastolic function. No subject will have echocardiographically detectable regional wall motion abnormalities, and each subject will have normal left ventricular systolic function. All cardiac valves will be examined to rule out significant valvular disease. Left ventricular mass (LVM) will be calculated using the following formula: LVM (g) = 0.8 x 1.04 $[(LVEDD + IVST + PWT)^3 - (LVEDD)^3] + 0.6$, where LVEDD is left ventricle end diastolic

internal diameter, IVST is interventricular septal thickness, and PWT is left ventricular posterior wall thickness. Measurements made during exercise will include the ratio of mitral inflow E over the ratio of E' from tissue Doppler (29).

Calf MRS. (94) Subjects will be fasting and refrain from physical activity for 3 days prior to the study. Calf muscle MRS will be performed as described in preliminary studies on a 3.0 T whole–body MRI scanner (GE Medical Systems, Waukesha, WI), using a dual frequency $^{31}P/^{1}H$ Flex Coil (Clinical MR Solutions). Scout images of the dominant leg will allow determination of the widest gastrocnemious and soleus cross sectional area. A resistance strap will be affixed to the hips, dominant leg, ankle and foot, on a specialized portable, in-MRI exercise bench to allow isotonic plantar flexion against force. Contractions will be performed at 30% and 60% of the subject's MVC following the calf exercise protocol described below (single leg, plantar flexion exercise tests) monitored by a Single Point Load Cell (Tedea Model 1250), connected via an A/D board to a laptop for analysis on LabView software. After scout images and shimming using the ^{1}H portion of the flex coil, ^{31}P measurements (phosphocreatine, free Pi and ATP peaks are measured while ADP and pH are calculated from JmRUI algorithms) will be made at rest, during calf exercise for five minutes, and for at least 15 min immediately following the exercise bout. Total study time will be 1.5 hours. Phosphocreatine recovery time will be analyzed to determine the rate of oxidative ATP synthesis from ADP using JmRUI (48).

Graded Exercise Test. To determine the VO_2peak and lactate threshold, in all persons, participants, a graded bicycle protocol to exhaustion will be carried out as previously described (10,75,78).

Constant load exercise test. To make VO_2 kinetic measurements, constant load bicycling will performed as previously described at low- to moderate-work-loads at approximately 85% of the lactate threshold (steady state) (10,75,78).

Oxygen Uptake Kinetic Measurements. Using the breath by breath capabilities of our metabolic cart, we will measure kinetics of VO_2 below and above the lactate threshold as we have done in prior studies according to published methods (10,75,78).

Arterial Stiffness Measurement: The Sphygmocor Px/Vx (Actor Medical) will be used to non-invasively assess arterial stiffness in the form of pulse wave velocity (PWV) and augmentation index (AI) during visits 3 and 7. Three electrocardiography leads are placed with removable adhesive on the chest wall, for the purpose of timing systole. A small metal tonometer is sequentially placed on the skin overlying the radial, femoral and carotid arteries. The anterior wall of the artery is flattened, as with palpation of a pulse. The tonometer non-invasively measures the pressure pulse waveform at each of these sites and correlates this with electrocardiographic data and the distance between the femoral artery and carotid artery sites to determine pulse wave velocity. The accuracy and reproducibility of the Sphygmocor Px/Vx have been validated, previously (78).

Autonomic Nervous System Function Testing (ANS): Study participants will undergo supine resting electrocardiogram (ECG) recording for 5 minutes in visit 1 in a quiet room, with a stable room temperature. Participants will be asked to relax but not fall asleep and to breathe normally and remain still without talking during the ECG recording. Following the resting ECG, a second supine ECG will be recorded while study participants take in a deep breath for 10s. The ECG recordings will be transmitted electronically to the Epidemiological Cardiology Research Center (EPICARE) Center (Wake Forest University School of Medicine, Winston-Salem, NC) for analysis, interpretation, and calculation of heart rate variability. The SD of consecutive RR intervals of the entire record in Lead II will be used as a measure of overall HRV. In addition, autoregressive power spectral analysis will be used to determine the spectral power in the following bands: high frequency (HF, 0.15–0.45 Hz, reflects parasympathetic outflow), low frequency (LF, 0.04–0.15 Hz, reflects both sympathetic and parasympathetic outflow), and very low frequency (0.01–0.04 Hz). LF/HF and HF normalized to total power will be analyzed as measures of sympathetic and parasympathetic outflow, respectively.

Randomization: Twenty-eight subjects will be randomized to blinded treatment of sitagliptin 100 mg plus placebo or glimepiride 2 mg plus placebo. This is a 1:1 randomization ratio. The UCH research pharmacy will provide the randomization schema as well as the blinded study medication for each subject. To ensure that our groups are similar for age and sex, we are very careful in our recruitment. We have not found it necessary in any prior study to use a stratification scheme and

we have always been able to successfully attain equal distribution in our groups. We have imposed exclusion criteria that will aid with this process.

Sitagliptin Administration. Participants randomized to the sitagliptin treatment arm will receive 100 mg/d sitagliptin and 2mg/d placebo for 3 months.

Glimepiride Administration. Patients randomized to the glimepiride treatment arm will receive 2 mg/d glimepiride and 100mg/d placebo for 3 months.

Blood and Urine Collection and Preparation. Samples will be collected at baseline for the measurement of glucose, insulin, HbA1C, FSH, creatinine, adiponectin, CBC, urine protein, lipids, CRP, IL-6, and microalbumin. For females of childbearing potential, a pregnancy test will be performed at visits 2,5, 6 & 7. Samples will be collected after two weeks of treatment and after three months of treatment for the measurement of glucose, insulin, HbA1C, creatinine, CBC, urine protein, CRP, IL-6, adiponectin, and microalbumin.

Monitoring for Study Drug Side Effects. The most common side effects of sitagliptin and glimepiride include hypoglycemia and gastrointestinal symptoms. The rare but most severe side effects include eye issues, pancreatitis, and renal impairment. We will be monitoring for all of these potential side effects in the following ways:

1. People who have a history of pancreatitis or kidney disease will be excluded from the study.
2. Subjects will be instructed to check and log their blood glucose twice a day during the treatment period.
3. Subjects will have access to the study team 24/7 via pager.
4. Two to three days after a participant begins the study medication, the study PRA will follow-up by phone call to assess whether the subject is experiencing any symptoms. At that time, the study PRA will also review the participant's blood glucose readings to date and advise the study physician if any readings are below 90 (see paragraph below).
5. Two weeks after a participant begins the study medication, subjects will return to the clinic for a physical exam and blood draw. Blood glucose log will be reviewed and symptoms of study drug side effects will be assessed.
6. The remaining time that subjects are on study drug, the research team will contact subjects weekly to assess possible symptoms.
7. If symptoms are noted, study investigators will assess and treat symptoms appropriately. Subjects will be withdrawn from the study if the pharmacological agents prove harmful to them.

Blood glucose will be monitored at every visit. While the subjects are taking study drug, we will ask them to monitor their blood glucose daily. To ensure subjects can monitor their glucose properly, subjects will be instructed during their randomization visit on proper finger stick blood glucose measuring technique. After randomization, subjects will be asked to monitor blood glucose twice daily; this will include daily monitoring of fasting blood glucose as well as an additional check at varying times (i.e. alternating before lunch, dinner, and before bedtime). Subjects will also be instructed to check their blood glucose PRN if they experience any symptoms of hypoglycemia – these potential hypoglycemia symptoms will be written out and verbally explained to patients. The study team will review these values weekly. Repeated measurements of blood glucose <90 mg/dl will be reported to the study staff so that a determination can be made on whether study medication dose adjustments are warranted to prevent hypoglycemia. Any measurement of blood glucose <70 mg/dl will result in study investigators determining whether the subject needs to be withdrawn from the study or prescribed medication dosages to prevent recurrent hypoglycemia.

Time-line. It is estimated that this study will take two years to complete. Fourteen persons per year will be studied.

Recruitment of Patients. Patients will be recruited by newspaper and radio advertising to the community, by University-wide email recruitment announcements and from the internal medicine and diabetes clinics at the University of Colorado Denver School of Medicine. Dr. Reusch sees patients with T2D and in addition, Drs. Reusch and Regensteiner are frequent speakers at local events targeted to persons with diabetes and will recruit patients at these events.

Risks:

Sitagliptin.

Pancreatitis There have been postmarketing reports of acute pancreatitis, including fatal and non-fatal hemorrhagic or necrotizing pancreatitis, in patients taking sitagliptin. After initiation of SITAGLIPTIN, patients should be observed carefully for signs and symptoms of pancreatitis. If pancreatitis is suspected, sitagliptin should promptly be discontinued and appropriate management should be initiated. It is unknown whether patients with a history of pancreatitis are at increased risk for the development of pancreatitis while using sitagliptin.

Renal Impairment: Assessment of renal function is recommended prior to initiating sitagliptin and periodically thereafter. A dosage adjustment is recommended in patients with moderate or severe renal insufficiency and in patients with ESRD requiring hemodialysis or peritoneal dialysis. Caution should be used to ensure that the correct dose of sitagliptin is prescribed for patients with moderate (creatinine clearance ≥30 to <50 mL/min) or severe (creatinine clearance <30 mL/min) renal impairment.

There have been postmarketing reports of worsening renal function, including acute renal failure, sometimes requiring dialysis. A subset of these reports involved patients with renal insufficiency, some of whom were prescribed inappropriate doses of sitagliptin. A return to baseline levels of renal insufficiency has been observed with supportive treatment and discontinuation of potentially causative agents. Consideration can be given to cautiously reinitiating sitagliptin if another etiology is deemed likely to have precipitated the acute worsening of renal function. Sitagliptin has not been found to be nephrotoxic in preclinical studies at clinically relevant doses, or in clinical trials.

Use with Medications Known to Cause Hypoglycemia: When sitagliptin was used in combination with a sulfonylurea or with insulin, medications known to cause hypoglycemia, the incidence of hypoglycemia was increased over that of placebo used in combination with a sulfonylurea or with insulin. Therefore, a lower dose of sulfonylurea or insulin may be required to reduce the risk of hypoglycemia.

Hypersensitivity Reactions: There have been postmarketing reports of serious hypersensitivity reactions in patients treated with sitagliptin. These reactions include anaphylaxis, angioedema, and exfoliative skin conditions including Stevens-Johnson syndrome. Onset of these reactions occurred within the first 3 months after initiation of treatment with sitagliptin, with some reports occurring after the first dose. If a hypersensitivity reaction is suspected, discontinue sitagliptin, assess for other potential causes for the event, and institute alternative treatment for diabetes.

Angioedema has also been reported with other dipeptidyl peptidase-4 (DPP-4) inhibitors. Use caution in a patient with a history of angioedema with another DPP-4 inhibitor because it is unknown whether such patients will be predisposed to angioedema with sitagliptin.

Glimepiride.

Hypoglycemia: As a result of the blood-sugar-lowering action of glimepiride, hypoglycemia may occur, and may also be prolonged. Possible symptoms of hypoglycemia include headache, ravenous hunger, nausea, vomiting, lassitude, sleepiness, disordered sleep, restlessness, aggressiveness, impaired concentration, alertness and reactions, depression, confusion, difficulty in speaking and even speech loss, visual disorders, tremor, pareses, sensory disturbances, dizziness, helplessness, loss of self-control, delirium, cerebral convulsions, somnolence and loss of consciousness up to and including coma, shallow respiration and slow heart rate (bradycardia). In addition, signs of adrenergic counter-regulation may be present such as sweating, clammy skin, anxiety, rapid heart rate (tachycardia), hypertension, palpitations, angina pectoris, and cardiac arrhythmias.

Eyes: Especially at the start of treatment, temporary visual impairment may occur due to the change in blood sugar levels.

Digestive tract: Occasionally, gastrointestinal symptoms such as the following may occur: nausea, vomiting, sensations of pressure or fullness in the epigastrium, abdominal pain, and diarrhea.

In rare cases, liver enzyme levels may increase. In isolated cases, impairment of liver function (e.g. with cholestasis and jaundice) and hepatitis may develop, possibly leading to liver failure.

Blood: Severe changes in the blood picture may occur: Rarely, thrombopenia and, in isolated cases, leucopenia, hemolytic anemia or, e.g. erythrocytopenia, granulocytopenia, agranulocytosis, and pancytopenia (e.g. due to myelosuppression) may develop.
Other adverse reactions: Occasionally, allergic or pseudoallergic reactions may occur, e.g. in the form of itching, urticaria or rashes. Such reactions may be mild, but also may become more serious and may be accompanied by dyspnea and a fall in blood pressure, sometimes progressing to shock. If urticaria occurs, a physician must be notified immediately. In isolated cases, a decrease in serum sodium, inflammation of blood vessels (allergic vasculitis) and hypersensitivity of the skin to light may occur.
Pregnancy and lactation: To avoid risk of harm to the child, glimepiride must not be taken during pregnancy; a changeover to insulin is necessary. Patients planning a pregnancy must inform their physician, and should change over to insulin. Ingestion of glimepiride with the breast milk may harm the child. Therefore, glimepiride must not be taken by breast-feeding women, and a changeover to insulin or discontinuation of breast-feeding is necessary.
Magnetic Resonance Sonography. The MRI is a non-invasive scan of your abdominal area and calf muscle, respectively, using a magnet. There is no radiation and no risk involved with the MRI. The MRI may be loud, therefore the subject is provided with audio protection and optional television to help increase comfort. Some subjects might feel claustrophobic while having an MRI and the scan will be stopped if it cannot be tolerated. In addition, any subjects with implanted metal cannot have an MRI due to the magnet involved.
DEXA. During DEXA testing the amount of radiation received is less than 25mRem total body equivalent dose per measurement. This is estimated to be less than one-half the amount received from a chest x-ray. The radiation in this study is not expected to greatly increase these risks, but the exact increase in such risk is not known. Women who are or could be pregnant should receive no unnecessary radiation, and should not participate in this study.
Blood Draws. Pain when the needle goes into the vein. A bruise may form at the site.
Plethysmography/FMD (Blood flow measurements). There may be a slight discomfort while the arm cuff is inflated.
GXT (Graded Exercise Test) and Kinetics. The exercise test is a standard procedure. This test is done routinely with hundreds of heart patients every year. The death rate of this test is approximately one in 10,000 tests. The incidence of serious complications including prolonged arrhythmias (irregular heartbeats) or prolonged chest pain is approximately 4 in 10,000 tests. This test will be performed on a Stationary bicycle.
Arterial Stiffness Measurement. There is a very slight chance that fainting or stroke may occur. No actual fainting or stroke has ever been reported. The risk of these happening has been estimated to be less than 1/1,000,000.
Medication washout: Risks of medication washout include high blood glucose levels, and in turn an increase of the hemoglobin A1C of the patient.

E. Potential Scientific Problems:
One potential pitfall is the use of sitagliptin since we have not evaluated its effects on exercise capacity. However, although we have not used this agent before, others have demonstrated that GLP-1 can improve exercise capacity in some patient groups. Given the finding that there are cardiac, endothelial and mitochondrial abnormalities associated with exercise impairment in T2D and the likely beneficial effects of sitagliptin on the heart, endothelial function and mitochondrial function in humans and animals, the proposed testing of this agent is both logical and innovative. Another potential pitfall is that we have not specifically evaluated the effect of metformin on exercise capacity. However, a recent report in healthy subject suggested that metformin did not improve exercise function; although this study was not in people with diabetes (11). In addition, in our prior studies, about 50% of patients have been on metformin and these patients' results did not differ from the results of T2D patients not on metformin in terms of exercise capacity (or other measures of cardiac or endothelial function). Therefore we do not anticipate that metformin will have effects on exercise capacity in the present study. Other issues that will be investigated

potentially in further studies include the possibility that a different GLP-1 agent may have even stronger benefits or that a different type of agent could have similar or better effects.

F. Data Analysis Plan:

Power Analysis

Study Design:

The proposed trial investigates the impact of sitagliptin on CV exercise capacity in individuals with T2D. Improved exercise capacity could reduce barriers to regular physical activity and improve CV health. We will enroll up to 75 study participants at the first visit. A total of 28 participants with diagnosed T2D will be randomized to receive either sitagliptin plus placebo or glimepiride plus placebo. The sample size was chosen so that the study had 94% power, even with about 15% dropout, and a reduction of the total sample size from 28 to 24. If, in fact, the study randomizes 28 participants, and only 10 are left per group after dropout, for a total sample size of 20, the power of the study will still be high. To detect a mean difference of 3.0 mL/kg3 in change in VO2 peak between the treatment groups, power will be 0.89 with a Type I error rate of 0.05, for 10 participants per treatment group. Thus, the study should have adequate power even if there is a 28% dropout rate.

Peak oxygen consumption (VO_2peak) and oxygen uptake kinetics (VO_2kinetics) will be measured prior to treatment and three months post-treatment. The primary hypothesis is that sitagliptin plus placebo will lead to greater improvement in functional exercise capacity in people with T2D when compared to glimepiride plus placebo. Secondary analyses will investigate the impact of sitagliptin plus placebo on cardiac function, endothelial function and tissue oxygen saturation (STO_2) as well as health-related quality of life.

Proposed statistical analysis: We plan to fit a general linear univariate model with the change in VO_2peak as the outcome, and treatment and baseline VO_2peak as the predictors. We will test the null hypothesis of no difference between the treatment groups on change in VO_2peak, using an F test. We will fit a similar model with VO_2kinetics as the outcome. The power analysis is based on the primary hypothesis of interest: the drug-mediated change in VO_2peak.

To control Type I error rate for the experiment, a possible alpha-spending approach would be to test the hypothesis about VO_2peak at a Type I error rate of 0.02, the hypothesis about VO_2kinetics at 0.02, and the secondary hypothesis at 0.01, for an experiment-wise error rate of 0.05. Because each hypothesis test is independently scientifically meaningful, we plan to test each hypothesis instead at an uncorrected Type I error rate of 0.05. The power analysis reflects this approach.

Power Summary for Grant:

We calculated unconditional power (101) for the F test of no difference in change from baseline for VO_2peak between the two treatment groups, when controlling for baseline VO_2peak. We assumed a standard deviation of 2 mL/kg for the change in VO_2peak, a standard deviation of 5 mL/kg in baseline VO_2peak, and a correlation of -0.17 between the baseline VO_2peak and the change in VO_2peak. Estimates were based on prior studies of the effect of rosiglitazone, a related drug, on exercise capacity in Type 2 diabetics.(2) The power analysis assumed a standard deviation of 2 mL/kg for the change in VO2 peak, a standard deviation of 5 mL/kg in baseline VO2 peak, and a correlation of -0.17 between the baseline VO2 peak and the change in VO2 peak. We expect the change in VO_2peak to be at least 3 mL/kg higher in patients taking sitagliptin plus placebo as compared to patients randomized to glimepiride plus placebo. This effect is clinically meaningful and of a magnitude similar to that observed in the study by Regensteiner et al.(74)

To detect a mean difference of 3.0mL/kg (102) in change in VO_2peak between the treatment groups, power will be 0.89 with a Type I error rate of 0.05, for 10 participants per treatment group. If we plan 12 participants per group, however, the power increases to 0.94. The modest increase in sample size leads to much higher power to detect the treatment effect. To ensure a conclusive study, we will target an effective sample size of 12 participants per group. Over the three months, we expect to lose up to two participants per treatment group. To account for the attrition, we have set an enrollment goal of 28 participants, or 14 participants per treatment group.

Table 1. Power to detect a difference in change in VO2peak (Post–Pre) at a Type I error rate of 0.05

Enrollment Goal Before Attrition	Number of Participants Per Treatment Group After Attrition	Total Participants After Attrition	Detectable Difference(mL/kg)	Power
24	10	20	3.0	0.89
26	11	22	3.0	0.92
28	12	24	3.0	0.94

Data Analysis. Comparisons between exercise performance and endothelial function in persons taking sitagliptin vs persons taking placebo will be made using unpaired t-tests. Comparisons between key variables before and after treatment will be made within each group using paired t-tests. Multivariate regression techniques will be used to evaluate the influences of covariates. Possible covariates (analyzed using ANCOVA) include but are not limited to insulin, fasting and post-prandial glucose and FSH levels. Weight loss will be carefully controlled for since this is a possible result of sitagliptin administration. Relationships between variables (i.e. insulin sensitivity and VO_2peak) will be assessed using the Pearsons product moment correlation coefficient (r). We plan to fit a general linear univariate model with the change in VO2 peak as the outcome, and treatment and baseline VO2 peak as the predictors. We will test the null hypothesis of no difference between the treatment groups on change in VO2 peak, using an F test. We will fit a similar model with VO2kinetics as the outcome.

We will handle dropout using case-wise deletion. Because the power analysis shows that the study will have adequate power even with 28% dropout, this is a conservative approach.

G. Summarize Knowledge to be Gained:

T2D is associated with impairment in functional exercise capacity which may be caused by endothelial and/or cardiovascular dysfunction. Sitagliptin works by inhibiting dipeptidyl peptidase 4 (DPP-4) inactivation of the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). This increases active incretin and insulin levels, and decreases glucagon levels and postglucose-load glucose excursion. Increasing evidence suggests that sitagliptin and other DPP-4 inhibitors may also provide protection against CV events (39,54,56), although the mechanism for this is not clearly understood. In addition, in a recent study from our lab, saxagliptin restored vascular eNOS and mitochondrial induction after an 8 day exercise intervention in a rodent model of diabetes (41).

Incretins have additional relevant effects that are of particular interest for this proposal including benefiting endothelial and mitochondrial function (22,26), as well as reducing CV risk (51). As has been noted, GLP-1 is a critical factor for healthy metabolism and vasculature and stimulates eNOS (26), (22). Small studies in humans with congestive heart failure show improvements in cardiac function and exercise capacity with GLP-1 administration (82,83). Improvements in endothelial function have also been examined in rodents in vivo and ex vivo suggesting a direct effect of GLP-1 on endothelial function via eNOS (66-68). In humans, this is manifested in consistently lowered blood pressures in some studies (49,50). Thus overall, increasing evidence in humans and animals suggests that sitagliptin and other incretin regulatory agents may have some cardioprotective effects. Recent observations in rodents and humans suggest that GLP-1 has a beneficial effect on endothelial and cardiac function (two factors associated with exercise function in children and adults with diabetes) (3,48,49,60,63,82,84,87,88). It is plausible therefore, that sitagliptin will improve exercise capacity in this group.

Previous studies in those with diabetes have shown that this exercise impairment is improved, but not entirely erased through exercise training. If improvements in exercise capacity are seen resulting from sitagliptin, a greater understanding may be gained of the mechanisms by which exercise capacity is reduced in those with diabetes and how they can be reversed.

H. Funding:

This is an investigator-initiated and protocol with funding and study drug provided by Merck & Co., Inc. The protocol was designed, written completely by Drs. Regensteiner and Reusch. The protocol will only be performed at University of Colorado. All data collection and statistical analysis will be performed by Drs. Regensteiner and Reusch through the University of Colorado. Any publications as a result of this research will be submitted by Drs. Regensteiner and Reusch; Merck will have no say in what data are published.

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