Cooperation of Insulin and GLP-1 on Myocardial Glucose Uptake

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A. Study Aim

Extrapancreatic effects of glucagon-like peptide-1 (GLP-1) present novel therapeutic opportunities. In animal studies, and in our preliminary data in humans, GLP-1 can modulate cardiac metabolism by augmenting use of glucose for fuel. Furthermore, animal studies have demonstrated synergistic actions of insulin and GLP-1 to augment myocardial glucose uptake, with net uptake from combined exposure greater than that achieved by either agent individually. This suggests that therapeutic interventions using combinations of insulin and GLP-1 agonism may have important advantages in terms of effects on myocardial fuel metabolism, perhaps providing protection against consequences of diabetes-related changes in myocardial metabolism in ischemia or contractile dysfunction.

Liraglutide is a GLP-1 agonist that has recently been approved in the United States and in Europe for clinical use in the treatment of type 2 diabetes mellitus. There is emerging evidence from animal studies that liraglutide may provide protection against myocardial ischemia, but to our knowledge effects of liraglutide on myocardial glucose uptake, alone or in combination with insulin, have not been explicitly evaluated in any animal model or in humans. Here we propose physiologic studies of the effect of liraglutide to magnify insulin-stimulated myocardial glucose uptake in humans with Type 2 diabetes mellitus, overcoming myocardial insulin resistance and maximizing the shift to carbohydrate fuel utilization.

We will test the hypothesis that effects of liraglutide plus insulin detemir on myocardial fuel selection will be greater than the effects of either agent alone. 27 Type 2 diabetic subjects (HbA1c 7.5 – 9.5%) currently treated with diet and exercise alone or with oral antidiabetic agents will be randomly assigned to one of three treatment groups: insulin detemir, liraglutide, or liraglutide plus detemir (9 subjects per group). All subjects will undergo an initial standardization of background treatment to metformin 2000 mg per day, followed by randomized assignment to 3 months' treatment with liraglutide 1.8 mg/day (once daily each morning, tapering up according to label instructions) and/or detemir (administered twice daily, initially at 10 units per day then titrated to achieve fasting morning glucose readings below 130 mg/dL). PET measurements of myocardial fuel selection will take place at the end of this treatment period, under fasting conditions in the morning following that day's treatment injection(s). The primary study endpoint will be myocardial glucose uptake. This and other metabolic and hemodynamic endpoints measured at steady state of each study condition will be compared across treatment conditions to allow evaluation of additive actions of the combination exposure compared to actions of liraglutide or insulin detemir alone.

The results from these studies will allow objective evaluation of the effects of combining liraglutide with insulin detemir on myocardial fuel selection in humans with type 2 diabetes. These data will provide a rationale for further studies of treatments with liraglutide (with or without insulin) in the setting of myocardial metabolic dysfunction.

B. Significance and Background

B1. Significance

Cardiac disease is currently the second most common cause of mortality in North America, and the obesity epidemic is projected to further increase heart disease incidence and prevalence (1, 2). Current treatments to reduce atherosclerotic heart disease by control of cholesterol, blood pressure, and anti-thrombotics are clearly of benefit, and have contributed to important reductions in heart disease mortality. However, even with the best current treatments, elevated rates of heart disease in obesity and diabetes persist (3, 4). Although it is worthwhile to pursue improvements in therapeutic approaches against these established targets, there is also clear value in pursuing new therapeutic targets.

One emerging target is therapeutic modulation of myocardial metabolism, specifically modulation of fuel selection (5). Control of fuel selection under conditions of ischemia has clear therapeutic potential and is the main focus of the current investigations. More generally there is an emerging understanding of the pathophysiologic connections between abnormalities in cardiac fuel metabolism and abnormalities in cardiac function. Therefore therapies that modulate fuel selection promise to be beneficial in heart disease more broadly, including in patients with chronic angina, congestive heart failure, and diabetic cardiomyopathy. GLP-1 based treatments are particularly promising in targeting myocardial fuel selection because 1) the magnitude of induced changes is large; and 2) these treatments represent a known quantity in terms of toxicity and tolerability, so efforts in pursuit of this therapeutic application are unlikely to be derailed by unexpected toxicities late in development.

Under ischemia, it is conceptually preferable to metabolize glucose over other fuel sources because carbohydrates require fewer moles of oxygen required per mole of ATP derived compared to fats. Also, driving glucose uptake despite stress-induced elevation in circulating free fatty acids helps ensure that carbohydrates are available for anaerobic metabolism, should that be required. Insulin and GLP-1 each help drive myocardial glucose uptake, and this is the presumed mechanism underlying the cardioprotective effect of GLP-1 that has been observed in preclinical studies (presented in detail below). Insulin is difficult to apply therapeutically with the goal of improving myocardial glucose uptake in the setting of myocardial infarction. Two main features are limiting: First, sensitivity to insulin action for systemic glucose disposal varies widely in the population, and is subject to acute changes with physiologic stress. This means that it is difficult to pre-specify a minimum dose that is sufficient to drive myocardial glucose uptake in all individuals. Second, in a given individual the therapeutic window of insulin is fairly narrow. This means that it is difficult to pre-specify a maximum dose that is guaranteed not to produce hypoglycemia in all individuals. This combination of problems means that the correct application of insulin simply cannot be pre-specified, but rather it requires a period of dose adjustment and ongoing close monitoring to ensure that systemic glucose concentrations are maintained within the target range. But even this approach does not address the true goal of driving myocardial glucose uptake; an adequate effect is simply presumed to take place in response to the systemic hyperinsulinemia. The use of

GLP-1 – based treatments instead of insulin, or in combination with insulin, presents an opportunity to maximize the beneficial effects to drive carbohydrate fuel selection and concurrently minimize the risks of treatment-induced hypoglycemia.

B2. Background

We propose studies that will systematically evaluate changes in myocardial fuel selection induced by the GLP-1 agonist liraglutide, alone and in combination with exogenous hyperinsulinemia, in vivo in humans. These studies are predicated on the following key points, briefly summarized in the following text.

1) GLP-1 acts on the heart to drive selection of glucose as a fuel
2) Modulation of myocardial fuel selection is a promising therapeutic target
3) Clinically approved liraglutide dosing is likely to have effects on myocardial fuel selection

B2.1. GLP-1 acts on the heart to drive selection of glucose as a fuel

Molecular Biology of Glucagon-Like Peptide 1 GLP-1 is produced primarily in a subset of enteroendocrine cells (L-cells) that are distributed in the distal small bowel and colon. It is produced from proglucagon, which is cleaved by prohormone convertase-1 into glicentin, GLP-1, GLP-2, and oxyntomodulin (6). There are 2 equipotent naturally occurring active forms of GLP-1, namely GLP-1[7-37] and GLP-1[7-36]amide (7). Both forms are rapidly degraded by circulating dipeptidylpeptidase 4 (DPP-4), and the resulting fragments are cleared by the kidney (6). The GLP-1 receptor is a seven transmembrane segment G-protein coupled receptor that signals via cyclic AMP (8), with wide tissue distribution including expression in the heart and CNS (9, 10). This receptor mediates GLP-1 actions in the pancreas (6, 11). Direct GLP-1 actions on the heart appear to be mediated by cAMP (8) and therefore by this receptor, but evidence also implicates involvement of a putative alternative receptor (12-14). The best-recognized actions of GLP-1 are in the pancreatic islets, where together with GIP it is responsible for amplification of glucose-stimulated insulin release (6). This effect is produced by actions in beta cells (amplifying the intracellular mechanisms linking glucose sensing to insulin secretion) and by actions in alpha cells (suppressing the production of glucagon) (15). Effects of GLP-1 on whole-body metabolism are exerted principally via these islet-derived hormones (6, 15).

Effects of GLP-1 on Cardiac Glucose Metabolism GLP-1 effects on myocardial function and fuel selection have been demonstrated with *in vitro* studies, with studies of isolated hearts, and with *in vivo* animal studies.

Cardiomyocytes GLP-1 receptors are expressed in the heart (9, 10). In isolated rat cardiomyocytes GLP-1 (10^{-8} mol/L) increased intracellular cAMP, implicating signaling via the classical GLP-1 receptor (8). In skeletal muscle strips and cultured myotubes, GLP-1 directly stimulated glucose uptake and subsequent steps of intermediary metabolism ($ED_{50} \sim 10^{-11}$ mol/L), independent of and additive to insulin (16).

Isolated hearts Effects of GLP-1 on isolated perfused rat hearts have been studied using the Langendorff preparation. Importantly, effects observed with this preparation are independent of systemic hormonal changes and neurologic inputs that may contribute to actions observed *in vivo*. GLP-1 (500 pmol/L) acutely and significantly increased glucose consumption by the isolated heart from ~33 to ~81 µmol/min/g (i.e. ~2.5-fold)

(17). Insulin and GLP-1 both increased glucose uptake via changes in glucose transporters, but the GLP-1 effect was independent of the classical insulin signaling cascade (18). These observations and other *in vitro* data also suggest separate and additive effects of insulin and GLP-1 on myocardial glucose uptake (19). These data demonstrate direct effects of GLP-1 on the myocardium to shift fuel selection toward glucose and away from fatty acids, analogous to but independent of actions of insulin.

Isolated rat hearts have also been used to study the effects of GLP-1 on ischemia/reperfusion injury (18, 20-23). GLP-1 perfusion at the time of injury enhanced recovery of contractile function, reduced post-ischemia functional deficit, and reduced infarct size. The GLP-1 receptor *antagonist* Exendin[9-39] blocked these effects, suggesting the effects were mediated at least in part via the classical GLP-1 receptor (21). These observations were confirmed in studies using other GLP-1 agonists including the GLP-1 fragment GLP-1[9-36] (22). These data suggest that GLP-1 may be therapeutically beneficial in ischemia by acting to drive myocardial glucose utilization.

Reference	Animal	Agonist:Dose	Model:Outcome
Kavianipour (24)	Pig	GLP-1: 3 pmol/kg/min for 5 hrs	Acute ischemia: Reduced intramyocardial pyruvate and lactate accumulation under acute ligation ischemia
Nikolaidis (25)	Dog	GLP-1: 1.5 pmol/kg/min for 48 hrs	Pacing-induced cardiomyopathy: Improved contractile function, augmented myocardial glucose uptake, reduced fatty acid uptake.
Nikolaidis (12)	Dog	GLP-1, GLP- 1[9-36]: 1.5 pmol/kg/min for 48 hrs	Pacing-induced cardiomyopathy: Both agonists improved contractile function, augmented basal and insulin-stimulated myocardial glucose uptake.
Bose (20, 21)	Mouse	GLP-1: 4.8 pmol/kg/min	Ligation ischemia/reperfusion model: Reduced infarct size, blocked by GLP-1 receptor antagonism
Dokken (26)	Mouse	GLP-1: 30 pmol/kg/min for 2 hrs	Ligation infarct: Postconditioning approach, reduced infarct size
Sonne (22)	Mouse	Liraglutide: 200 µg/kg/d for 7 days	Ligation infarct: Improved survival, reduced infarct size, reduced rate of myocardial rupture
Noyan- Ashraf (17)	Mouse	Liraglutide: 200 µg/kg/d for 7 days	Ligation infarct: Improved survival, reduced infarct size, reduced rate of myocardial rupture
Kristensen (27)	Pig	Liraglutide 10 µg/kg/d for 3 days	Ligation infarct: No reduction in survival, modest nonsignificant reduction in infarct size.
Timmers (28)	Pig	Exenatide 10 µg twice daily for 3 days	Ligation infarct: Reduced infarct size, preserved contractile function

Table B1. Animal studies of in vivo actions of GLP-1 agonists on the heart

In vivo –Animals Studies of ischemia and pacing-induced dilated cardiomyopathy support the potential for therapeutic benefit from GLP-1 – based treatments *in vivo* under pathophysiologic conditions. The key studies are summarized in **Table B1**. Briefly, in 3 different mammalian models of heart disease, native GLP-1 or GLP-1 agonists provided protection against ischemia-induced cardiac damage, or improved myocardial contractile function (12, 17, 20-22, 24, 26, 29). These beneficial effects were related to actions of the agonists to augment myocardial glucose uptake, which was increased by 1.5 to 3 fold above baseline (e.g. from 5.4 to 7.9 µmol/min in (12) and from ~4 to ~12 µmol/min in (29), both $p<0.05$).

These studies demonstrate that GLP-1 can modulate myocardial fuel selection *in vivo*. The beneficial effects against ischemia were related to effects on myocardial glucose uptake. These effects of GLP-1 on myocardial metabolism may not require insulin since the variant peptide, GLP-1[9-36] was able to recapitulate the actions of native GLP-1[7-36] including augmentation of myocardial insulin sensitivity but specifically without

engendering an insulinotropic response (12, 30). As noted above, *in vitro* observations suggest that actions of insulin and GLP-1 to augment glucose uptake are distinct and additive (18, 19). In the dog studies, separate and combined infusions of insulin and GLP-1 were evaluated (12, 29). With combined exposure myocardial glucose uptake was markedly enhanced (~9-fold above baseline), greater than either agent alone and greater than the simple addition of the individual responses. We propose to undertake studies to specifically explore these effects in humans, using Liraglutide as the GLP-1 agonist.

In vivo – Humans A small number of preliminary clinical studies have been undertaken using GLP-1 to treat heart disease in humans. In one small non-randomized study of 6 diabetic patients with congestive heart failure, GLP-1 at 4.0 pmol/kg/min for 3 days resulted in a nonsignificant trend toward improved contractile function (31). Three proof-of-principle human studies focusing on effects of GLP- 1 on contractile function have been reported (29, 32, 33). These were non-randomized studies of effects of GLP-1 infusions on functional outcomes in patients with cardiac dysfunction. GLP-1 exposures ranged from 1.25 to 2.5 pmol/kg/min for 72 hours to 14 days, differing by study. Overall, significant improvements were noted in multiple echocardiographic measures of contractile function (ejection fraction, global and regional wall motion scores), functional status (distance on a 6 minute walk test and VO_2max), and quality of life indices. Cardiac glucose metabolism was not assessed in any of these studies. Although these are not rigorously controlled clinical trials, they do support the potential for beneficial effects of GLP-1 in heart disease in humans. No human studies of anti-ischemic effects of GLP—based treatments have been reported, and no rigorous studies of the physiologic effects of GLP-1 itself, DPP4 inhibitors, or GLP-1 agonists such as liraglutide on myocardial metabolism in humans have been reported.

B2.2. Modulation of myocardial fuel selection is a promising therapeutic target

Therapeutic modulation of myocardial fuel selection is an increasingly active area of investigation, with burgeoning interest based on the clear potential for development of novel therapeutic interventions (5, 34-37). Perhaps the most classical example of this treatment approach is the application of insulin at the time of infarction. Combined infusions of glucose, insulin, and potassium in the setting of myocardial infarction have reduced mortality in many studies of subjects with and without diabetes (38-41). However, these benefits have not been seen other large studies (42-44), perhaps reflecting a particular benefit among diabetic patients from modulation of fuel selection (45). Other emerging approaches to modulation of fuel selection include inhibition of fatty acid metabolism (using e.g. perhexilene, a carnitine palmitoyl tranferase inhibitor, or trimetazidine, a partial fatty acid oxidation inhibitor). Early clinical studies suggest that these agents are effective treatments for ischemia manifesting as angina (34, 46-48), but alternatives with less toxicity or improved efficacy are needed. GLP-1 agonism with Liraglutide may present a valuable alternative approach, with good effectiveness and minimal toxicity.

B2.3. Liraglutide: Adequacy of Exposure and Side Effects

The affinity of GLP-1 for the classical GLP-1 receptor is on the order of 10^{-10} mol/L (49, 50). The active moiety of liraglutide is a native GLP-1 (7-36) fragment, and despite the modifications that produce a prolonged half-life this agent has similar GLP-1 receptor affinity binding characteristics on classical GLP-1 receptors (51, 52). The circulating concentrations of liraglutide achieved with standard clinical dosing are in the micromolar range following a single subcutaneous injection (53), suggesting that the GLP-1 receptors are fully stimulated for the majority of the duration of exposure. This exceeds the exposure resulting from the physiologic GLP-1 response to meals in humans, which produces GLP-1 concentrations at the low end of the receptor's affinity range(13, 54, 55). Myocardial metabolism is significantly modified with intravenous infusions of native GLP-1 achieving circulating concentrations of ~160 pmol/L (12, 29, 56-58). It is therefore reasonable to predict that clinically used doses of liraglutide will be sufficient to engender GLP-1 mimetic effects on myocardial fuel selection.

Common side effects seen with liraglutide include headache, nausea, diarrhea, respiratory tract infections, and anti-liraglutide antibody formation (59). Between 5 and 10% of patients find the side effects intolerable and withdraw from treatment. GI symptoms are by far the most common, and are dose-related. Up to 41% of patients reported such symptoms in the five major FDA-registration trials (versus 17% of trial subjects treated with comparator agents). These effects are generally transient, lasting only days, and overall are well tolerated (at least 90% of patients continue with treatment). Hypoglycemia is a rare event when native GLP-1 is given alone, and GLP-1 is well tolerated under fasting conditions (57); parallel observations have been made for liraglutide where hypoglycemia rates in liraglutide-treated subjects during the FDA registration trials were 2.6 cases per 1000 patient-years, representing 7 subjects in the entire trial experience (59). Six of these subjects were co-treated with a sulfonylurea type insulin secretagogue. Other rare but important adverse effects include hypersensitivity/urticaria (occurring in 0.8%, versus 0.4% of comparator-treated subjects); pancreatitis (seen in 7 liraglutide-treated subjects versus 1 comparator-treated subject, representing 2.2 vs 0.6 cases per 1000 patient-years); and papillary thyroid carcinoma (6 versus 1 cases, 1.9 versus 0.6 cases per 1000 patient-years). Due principally to data from preclinical development testing of Liraglutide there is a concern about hyperplasia of thyroid calcitonin-secreting (C)-cells potentially leading to medullary carcinoma of the thyroid (MTC) which was observed in rodents. The FDA labeling for Liraglutide contains a boxed warning about this issue, stating that 'it is unknown whether liraglutide causes C-cell tumors in humans, as human relevance could not be demonstrated in clinical or nonclinical studies' (59). Liraglutide is contraindicated for use in patients with genetic predisposition to MTC, such as a personal or family history of medullary thyroid carcinoma or Multiple Endocrine Neoplasia syndrome Type 2 (59) .

B3. Innovation

There is intense interest in extrapancreatic actions of GLP-1, including in particular effects of GLP-1 on the heart (12, 17, 18, 21-25, 31-33, 60-74). Effects of GLP-1 to modulate myocardial fuel selection have been clearly established in preclinical work (presented in detail below) and this promises to result in truly novel applications of GLP-1 – based treatments for heart disease (61, 75). Liraglutide is one such treatment, and

having undergone clinical development testing for use in treating diabetes, the toxicities and averse event profile are already well understood. The studies proposed here are the first to systematically evaluate the physiologic interactions of liraglutide and insulin in the regulation of myocardial fuel selection in humans, and will be of direct relevance to the clinical application of liraglutide as a therapeutic modulator of myocardial fuel selection.

C. Preliminary Data

Insulin and Myocardial Fuel Selection: We have recently completed a set of studies using PET to demonstrating the effects of insulin on myocardial substrate selection. We made PET measurements on 2 consecutive mornings. Day 1 measurements were made under fasting conditions (i.e. fatty acid-dependent) and the Day 2 measurements were made under steady state conditions using a hyperinsulinemic euglycemic clamp (i.e. carbohydrate-dependent conditions; insulin exposure 120 mU/m^2/min). We studied 9 lean males (age [mean±SD] 34.1±9.0 years; body mass index 22.6±2.4 kg/m^2, 17.5±6.6 percent fat) and 8 obese type 2 diabetic males (age 39.1±9.2 years; body mass index 34.2±7.3 kg/m^2*, 37.6±13.2 percent fat* (*p<0.05 vs. lean subjects). Myocardial free fatty acid metabolism was measured using a proprietary tracer (^{18}F-thiapalmitate, FTP; in the current proposal we will use a standard ^{11}C-palmitate fatty acid tracer). In parallel with the current proposal, we also measured total myocardial oxidative metabolism, and myocardial perfusion using ^{11}C-acetate.

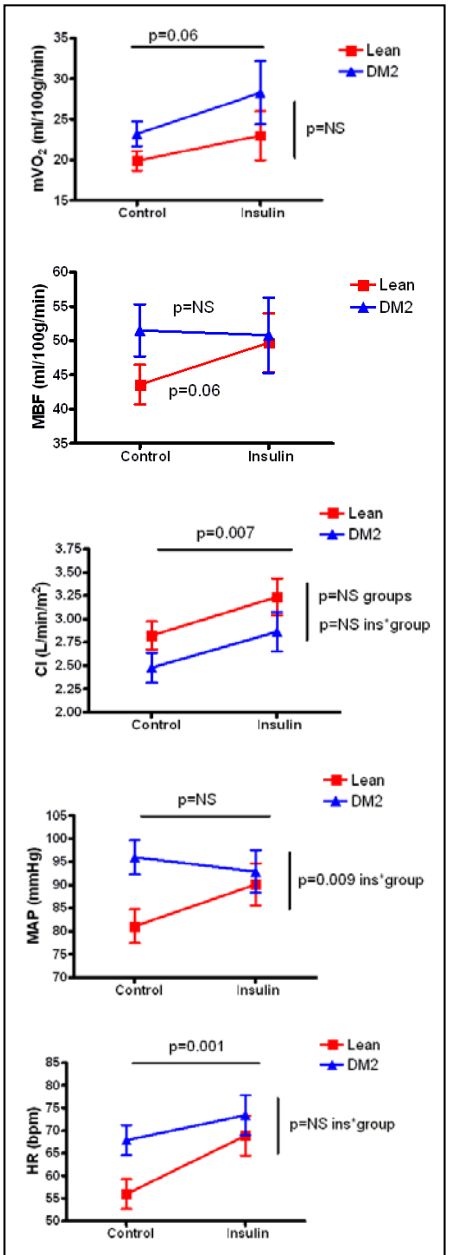

Figure C2. Effects of insulin on myocardial oxygen consumption and blood flow (by PET), and systemic hemodynamics (by impedance cardiography). HR,

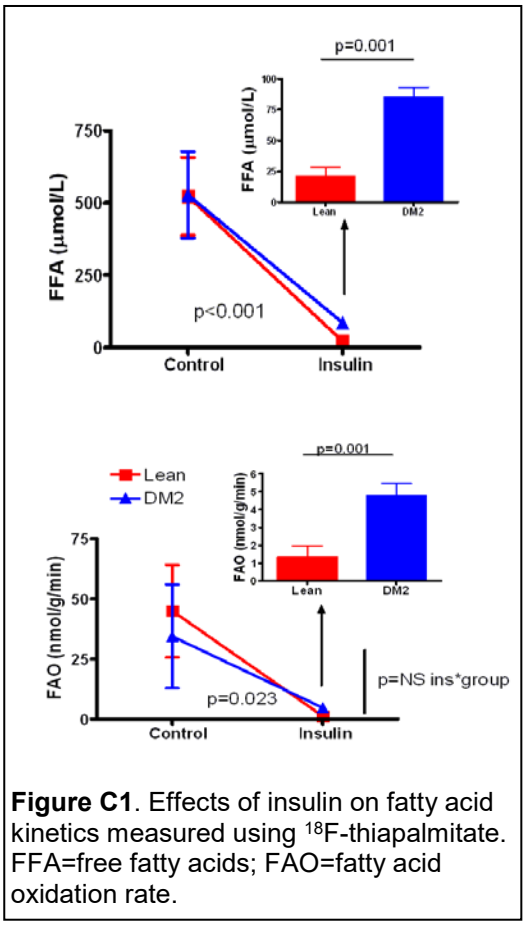

Figure C1. Effects of insulin on fatty acid kinetics measured using ^{18}F-thiapalmitate. FFA=free fatty acids; FAO=fatty acid oxidation rate.

In order to minimize group differences in fuel availability on the non-insulin clamp study day, glycemic management in diabetic subjects was standardized by the PI prior to the PET measurements. Diabetic subjects (DM2) underwent intensification of their diabetes management using basal/bolus insulin for 4 weeks prior to these studies. With this intervention, fasting glucose levels achieved with basal insulin from the preceding night were approximately euglycemic (lean [mean+SD] 90.0±6.9

vs DM2 112.7±19.2 mg/dL, p=NS comparing groups). Insulin suppression of circulating fatty acids with the insulin/glucose clamp was overall comparable between groups (**Figure C1**: lean from 523±134 to 21±4; DM2 from 528±149 to 85±8 μmol/L, p<0.001 for insulin effect and p=NS for group by insulin interaction). However, at steady state insulin infusion the ambient free fatty acid concentrations were significantly different (p=0.001; **Figure C1** upper panel inset). Insulin induced a marked shift in myocardial fuel selection (**Figure C1**), with a >70% reduction in absolute rates of fatty acid oxidation (p=0.02). Comparable magnitudes of the insulin-induced shift in kinetics of fatty acids were seen in the diabetic subjects compared to the lean subjects. However, the residual difference in fatty acid availability contributed to statistically significant differences in fatty acid uptake and oxidation between diabetic and control subjects under steady state insulin exposure (**Figure C1 inset**). Therefore the diabetic subjects exhibited net resistance to insulin's effects to shift myocardial fuel selection. This was accompanied by resistance to insulin's effects on blood pressure and myocardial blood flow as well (**Figure C2**).

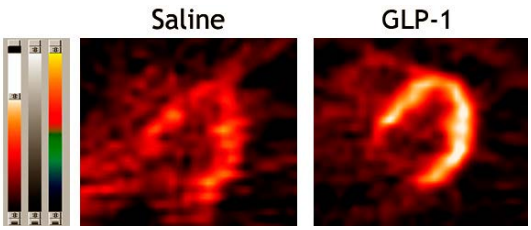

Figure C3 FDG PET images of myocardial glucose uptake under control fasting conditions and following overnight infusion of GLP-1. Images are presented with comparable color gradients

These data demonstrate impairments in insulin's effects on myocardial fuel selection in Type 2 diabetes, and demonstrate impairments in the hemodynamic responses to insulin in this population. They also demonstrate our technical capacity to undertake studies the studies proposed. In secondary analyses under the current proposal we will evaluate whether liraglutide can help overcome these defects in insulin action in Type 2 diabetic subjects.

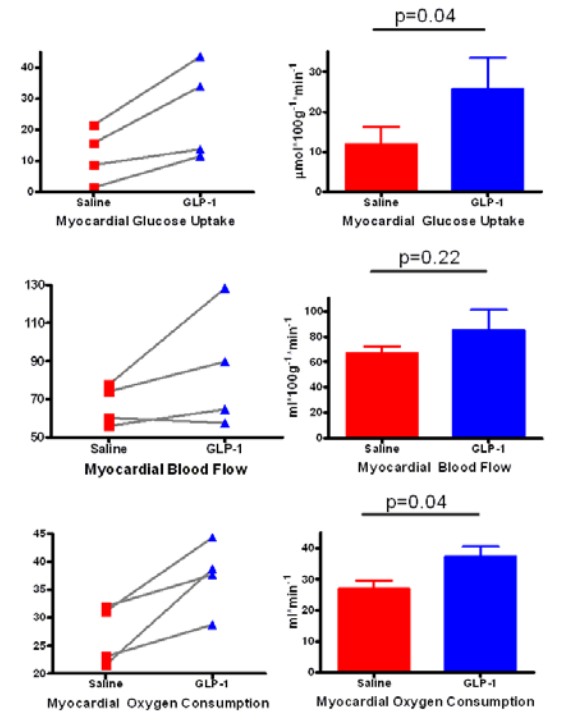

Figure C4 Effects of GLP-1 on myocardial glucose uptake, perfusion, and total oxidation rate. N=4

GLP-1 and Myocardial Fuel Selection: In contrast to the effects of insulin on the heart, very little is known about the effects of GLP-1 on the heart in humans. Under a protocol recently funded by the NIH, we have so far evaluated these effects of native GLP-1 in 4 healthy lean non-diabetic male subjects (age 32±12 yrs, body mass index 23.9±1.6 kg/m2). Subjects underwent PET measurements of rates of myocardial glucose uptake, blood flow, and total oxidation under control conditions, and then again the next day following a 10 hour exposure to GLP-1 (1.5 pmol/kg/min). Representative images demonstrating the effect of GLP-1 on myocardial glucose uptake are presented in **Figure C3**. The quantitative findings for all 4 subjects are presented in **Figure C4**. Kinetic analyses were performed using modeling as currently proposed (details below). Similar to the effect magnitude reported in preclinical studies, exposure to GLP-1 (1.5 pmol/kg/min) resulted in an average **2.1-fold increase in myocardial glucose uptake**

(from 11.9±8.7 mean±SD to 25.7±15.6 µmol/100g/min). In this pilot dataset this change is already statistically significant (p=0.04), clearly demonstrating the sensitivity of this testing methodology to the anticipated effect sizes.

A concurrent nonsignificant increase in myocardial blood flow was seen, accompanied by a significant increase in myocardial oxygen consumption (acetate disappearance, expressed as MvO_2; **Figure C4**). Significant chronotropic or hemodynamic effects of GLP-1 were not evident with this small sample size. (**Table C1**).

	Saline	**GLP-1**
Heart Rate (bpm)	65.0±8.2	61.8±8.2
Mean Arterial Pressure (mmHg)	80.5±6.6	83.8±6.5
Stroke Volume (mL)	110.8±24.2	94.3±17.4
Cardiac Output (L/min)	6.4±1.3	5.8±1.2

Table C1. Hemodynamic effects of GLP-1. N=4, all comparisons p=NS. Measurements were acquired noninvasively using impedance cardiography and an automated blood pressure cuff.

We anticipate that liraglutide will exert similar effects on myocardial fuel selection. Our principal question is whether the effect of combined exposure is greater than the effect of liraglutide alone. We have no data to date evaluating this question.

D. Approach

D1. Protocol Overview We will test the hypothesis that effects of liraglutide plus insulin detemir on myocardial fuel selection will be greater than the effects of either agent alone. We expect to demonstrate that liraglutide and insulin detemir can act in an additive way to affect myocardial fuel selection. We will study subjects exposed to each agent alone and in combination.

PET will be used to measure myocardial perfusion and rates of total oxidation, fatty acid uptake, fatty acid oxidation and glucose uptake. Full details are provided in Section D5 below; in brief, subjects will be randomized at enrollment to one of three treatments (insulin detemir alone, liraglutide alone, or combined insulin detemir and liraglutide). Each subject will be studied once, following a 3 month treatment with the assigned agent(s).

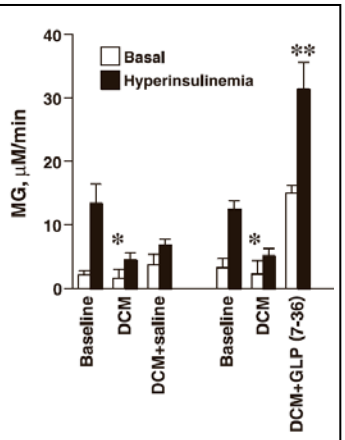

Figure D1. Effects of combination native GLP-1 and insulin (1uM) on myocardial glucose uptake (MG) in the dog model of dilated cardiomyopathy (DCM). From (12)

D2. Rationale Both GLP-1 and insulin exert actions to modulate myocardial fuel selection. The combination of insulin and the GLP-1 agonist liraglutide may be advantageous in maximizing the capacity to therapeutically modulate myocardial glucose uptake, for example in circumstances of acute myocardial ischemia.

Molecular and tissue studies indicate that insulin and GLP-1 act via separate signaling pathways to exert additive effects on glucose transport (16, 65). These observations plus important physiologic differences between the systemic actions of these agents suggest that their effects on fuel selection may arise from distinct and additive mechanisms.

D3. Anticipated Results & Interpretations The dog studies using systemic GLP-1 infusions provide direct guidance as to the expected outcomes. Along with the pacing-induced cardiomyopathy the dogs acquired

myocardial insulin resistance (**Figure D1**). The combination infusion of insulin and native GLP-1 produced a marked augmentation in myocardial glucose uptake, at least 2-fold greater than that seen with insulin alone even prior to the onset of insulin resistance (25) and clearly overcoming this cardiomyopathy-related myocardial insulin resistance.

On the basis of these data and supporting cellular and tissue data as described previously, we predict that the combined exposure to liraglutide and insulin will produce greater effects to stimulate myocardial glucose uptake than either agent alone. A statistically significant difference between combination treatment and the individual treatments will be interpreted as evidence for a significant additive interaction of GLP-1 and insulin on myocardial glucose uptake. Conversely, a lack of difference will be interpreted as evidence against an important additive effect of the combined exposure. The data generated will also be directly informative regarding the individual effects of insulin and liraglutide on myocardial fuel selection, regardless of whether the anticipated additive effect is observed. These studies have the advantage of providing scientifically and clinically meaningful data regardless of whether the hypothesis is proven or disproven.

D4. Troubleshooting/Alternative Approaches In generating the data presented above, we did not encounter any significant technical problems in the execution of study protocols. The prior success of our team with more complicated PET studies of myocardial physiology using insulin infusions and a technically more challenging tracer of fatty acid metabolism (described in detail below) argues that we will be able to readily overcome any technical barriers that arise.

It is possible that our estimated effect sizes and/or predicted measurement variability are incorrect. We plan an interim assessment of observed effect size and variability after 4 subjects have been studied with each exposure, to ensure that our sample size estimates are correct, and to allow modification of study plans if necessary.

There is extensive clinical experience to indicate that the liraglutide will be well tolerated. In order to avoid problems with sudden toxicity on the morning of the study on first exposure to liraglutide, we plan to taper subjects up to the exposure dose over a brief period in advance of the planned PET study (details below). If subjects experience intolerance on the morning of the study despite these efforts, their participation will be discontinued in order to avoid potential confounding effects of the nausea response on our measurements.

Subject recruitment will be by advertizing in local media, and by direct recruitment in the PI's clinic. Over the past 12 years we have been consistently successful with recruitment into physiologic studies such as those proposed, and in this time we have studied over 350 subjects in various CRC-based protocols. Recognizing that recruitment is often the limiting factor for clinical studies of any sort, we have allocated sufficient resources in the budget of this proposal to provide adequate personnel time and resources for recruitment and screening of subjects. We are extremely confident in our ability to recruit subjects for this study.

D5. Protocol Details

We will study only Type 2 diabetic subjects under this protocol. In view of the current widespread use of metformin as a first line therapy in diabetes, we will study the effect of liraglutide and/or insulin detemir on a

background of metformin treatment. After successfully meeting screening criteria (described below) and achieving a stable and well tolerated dose of 2000 mg per day of metformin, subjects will be randomly assigned to added treatment with insulin detemir alone, liraglutide alone, or combined insulin detemir and liraglutide (using a preset randomization schedule ensuring balanced assignment to all 3 groups). Subjects who fail to complete the study will be replaced with the next enrolled subject.

Subject Selection: Subjects will initially be evaluated over the telephone according to the inclusion and exclusion criteria outlined in **Table D1**. Those who appear to qualify will be invited for a formal screening visit. Following a full discussion of the protocol and the nature of the planned studies, subjects will be asked to sign the informed consent document. They will then undergo screening medical history and physical examination, including EKG and blood work to confirm eligibility

Inclusion Criteria:
Age 18-50 yrs
BMI >25 kg/m2. HbA1c 7.0-10.0%. Treated with up to 2 oral agents
Exclusion Criteria:
Chronic illnesses or infections (other than diabetes mellitus).
Known coronary artery disease, structural heart disease or abnormal ECG on screening evaluation.
Treatment with more than 2 antihypertensive agents or blood pressure >140/95 on two occasions during screening evaluations
History of claustrophobia, musculoskeletal or other factors which would result in an inability to comfortably remain within PET scanner gantry for the duration of the imaging protocol
Occupational, investigational or other known radiation exposure which, together with the planned radiologic studies, will result in greater than 500 mrem total exposure in a 12 month period
For female participants, current pregnancy
Treatment with a GLP-1 agonist or DPP4 inhibitor within the past 6 months
Known intolerance to injected GLP-1 agonist
Personal history of pancreatitis, personal or family history of medullary thyroid carcinoma, or other contraindications to Liraglutide treatment
Treatment with a thiazolidinedione class antidiabetic agent within the past 6 months
Recognized microvascular complications (neuropathy, nephropathy, retinopathy)

Table D1. Inclusion/Exclusion Criteria

Subject Preparation: Subjects will be instructed on following an American Diabetes Association-compliant diet (1800 kcal, 30% carbohydrate, 40% fat, 30% protein). Subjects will discontinue their current antidiabetic medications and be switched to metformin on an increasing regimen targeting 2000 mg/day during an initial 2-week run-in period (subjects will be excluded if unable to tolerate at least 1500 mg/day). Metformin treatment will continue throughout the remainder of the study period. Subjects will monitor their blood glucose levels 4 times daily, and report these readings to the study team at regular intervals for safety and efficacy surveillance. Other medications (e.g. ACE inhibitors, angiotensin receptor blockers, HMG Co-A reductase inhibitors) will be withheld the morning of the PET measurements, but will continue otherwise. Subjects will be asked to withhold ASA for 3 days prior to the planned studies. Participants will return to their prior medication regimens immediately following completion of the PET studies (if indicated, the PI may make specific clinical recommendations for improving their treatment at that time).

Subjects assigned to liraglutide treatment will undergo a standard dose-escalation regimen as recommended in the product insert, targeting the maximal approved dose of 1.8 mg per day. Subjects unable to tolerate 1.8 mg/day will be excluded from the study and replaced. Liraglutide will be administered each morning, to align with the planned administration on the morning of the PET study.

Subjects assigned to insulin detemir treatment will begin treatment with an initial dose of 10 units divided as a twice daily injection, and then in concert with the PI will have the dose adjusted to achieve consistent morning (fasting) blood glucose readings below 130 mg/dL (in order to align with the anticipated mean level achieved with the fixed dosing of liraglutide administered). If participants experience blood glucose readings below 70 mg/dL more than twice per week, or any reading below 50 mg/dL, the detemir dosing will be reduced even if this means they no longer achieve the targeted fasting readings. Insulin detemir will be administered once daily in the morning, including the morning of the PET study.

PET protocol: The effects of each hormone exposure on myocardial fuel selection will be studied using positron emission tomography (PET). We will measure cardiac perfusion and oxidative metabolism (^{11}C-acetate), fatty acid uptake and oxidation (^{11}C-palmitate) and glucose uptake (^{18}F-deoxyglucose) under resting fasting conditions with hormone exposure (See Figure D1). Blood samples for later measurement of circulating levels of relevant metabolites (glucose, insulin, glucagon, free fatty acid, lactate, liraglutide) will be taken at planned intervals across the study. Noninvasive measurements of systemic hemodynamics will also be taken simultaneously during the PET studies, at similar intervals using surface electrodes placed on the thorax and neck, and an automated blood pressure cuff (impedance cardiograph, BioZ.com, CardioDynamics, San Diego).

Study Day Procedures: Subjects will be admitted to the Indiana University Clinical Translational Sciences Institute's Clinical Research Center before 0700h the morning of Day 1, following an overnight fast (from 2000h the day prior). A 20G X 3cm Teflon coated catheter will be placed in the antecubital vein of one arm, for GLP-1 and other infusates. A second 20G X 3cm Teflon coated catheter will be placed in the contralateral arm for blood sampling.

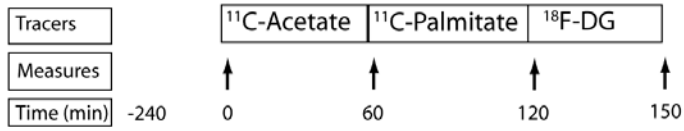

Figure D2. Study schema for the PET imaging protocol. Hormone exposures begin 2 to 4 hours prior to PET measurements, varying by agent, and continue throughout the imaging protocol. PET measures take place with infusion of each tracer, followed by a washout period to allow counts to return to background levels. Arrows indicate time points where hemodynamic measurements and blood sampling will take place. Transmission scanning in advance of the tracer infusion is not depicted.

Subjects will administer their ongoing doses of liraglutide and/or insulin detemir by 0800h. At ~0900h, subjects will be asked to void their bladders prior to being positioned in the scanner and undergoing instrument calibration scans. Active scanning will begin ~1000h with the radiotracer injection and scanning protocol as outlined in **Figure D2**. Immediately after the scanning protocol is completed, subjects will void their bladders again, so as to minimize bladder radiation exposure related to concentration of tracers in the urine. After end-of-protocol blood samples are obtained, subjects will be fed lunch and discharged from the Clinical Research Center. At that time the PI will provide recommendations to each participant regarding their ongoing diabetes management and arrange follow-up if necessary.

D6. Statistical Methods

Study Endpoints The primary study endpoint will be myocardial glucose uptake, measured using PET. Prespecified secondary endpoints of interest will include the other PET kinetic parameters of myocardial fatty

acid uptake and oxidation, total myocardial oxidation, and myocardial blood flow. Other endpoints of interest include rate-pressure product, systemic hemodynamics and changes in circulating levels of metabolic substrates and hormones. The primary endpoint and other fuel kinetics endpoints will be adjusted for changes in demand if significant changes in cardiac work or total oxidation are observed.

PET Endpoints for Analysis Myocardial glucose uptake will be quantified from the time-activity curve following ^{18}FDG injection using a 3-compartment model, according to the methods of Morita and colleagues (76), with a lumped constant of 1.0 (77-79), as was done for the preliminary data above. Tissue perfusion and total oxidative metabolism will be quantified from the myocardial time-activity curve following ^{11}C-Acetate injection, using a well-validated two-compartment model (80, 81). The influx parameter k_1 will be used as the measure of tissue perfusion, and the clearance parameter k_2 will be used as the index of total oxidative metabolism. Fractional tracer retention will be used to calculate fatty acid uptake, and a 3-compartment model for fatty acid kinetics will be used to derive rates of fatty acid uptake and oxidation from the time-activity curves (82-84). Normalizing transformations of these outcome variables will be applied if appropriate, depending on the observed distribution of data collected.

Statistical Approach We are specifically interested in whether the combined exposure produces values greater than exposure to liraglutide alone; therefore the main comparison will be an unpaired t-test across these two treatment groups. One-way ANOVA or mixed modeling will be used to undertake this comparison with adjustment for covariates of interest, recognizing that only a limited set of such analyses is possible with the comparatively small sample size. Parallel analyses will be undertaken to evaluate effects of liraglutide alone versus combination exposure. We also plan to undertake exploratory analyses using multiple linear regression to evaluate relationships of the primary and secondary endpoints with the other measured variables (e.g. between myocardial glucose uptake response and circulating hormone and metabolite concentrations). We have not made adjustments to sample size estimates for these exploratory analyses.

	Baseline rates (mean±SD)	**Stimulated rates (mean±SD)**	**Demonstrable Effect Size n=9, power=0.8**	Demonstrable Effect Size n=9, power=0.9
MGU (µmol/100g/min) [a]	11.9±8.7	**25.7±15.6[a]**	**21.9**	25.4
MBF (mL/100g/min) [a]	67.0±5.3	**85.0±32.1[a]**	**45.2**	52.3
MVO2 (mL/min) [a]	26.9±5.4	**37.4±6.4[a]**	**53.3**	60.9
MFAO (nmol/g/min) [b]	34.4±20.2	**4.8±1.2[b]**	**1.7**	2.0

Table D2. Effect sizes and variability observed in our preliminary studies for the primary and main secondary endpoints. The effect size is estimated for the main comparison of interest, namely the comparison between combination treatment and insulin alone. Effect sizes estimates are calculated for a 2-sided alpha= 0.05. MBF, myocardial blood flow; MFAO, myocardial fatty acid oxidation; MGU, myocardial glucose uptake; MVO2, myocardial oxygen consumption. Superscript **a** indicates these values are calculated based on observed effects of GLP-1. Superscript **b** indicates these values were calculated

Sample Size Considerations

Published rates of basal myocardial glucose uptake measured using ^{18}FDG in control populations under fasting conditions range from 10-60±5-20 µmol/100g/min and insulin-stimulated rates are 100-120 ±30 µmol/100g/min (85, 86) (i.e. overall SD ~1/3 to 1/2 of basal value and ~1/4 of the delta with insulin). In our preliminary studies we observed mean values at the low end of this range, but proportionally comparable variability (**Table D2**). The dog studies reviewed above established that GLP-1 (1.5 pmol/kg/min) can produce a doubling of

myocardial glucose uptake (12, 29), and our preliminary data are concordant with this effect size (**Figure D4** and **Table D3**).

For the other metabolic parameters of interest, overall relative effect sizes and measurement variability are of comparable scale to those of MGU (**Table D2** and **Figure C4**). Therefore sample sizes derived from myocardial glucose uptake measures are likely adequate for simultaneously demonstrating meaningful changes in these secondary endpoints.

For our outcome of interest we need power to demonstrate a between-treatment difference on the order of the effect seen with insulin alone (i.e. at least a doubling of the effect). This is approximately what has been seen in dog studies evaluating interactions of GLP-1 and glucose (25). Using MGU as our endpoint for these calculations, and using a two-group t-test design with pooled variability estimates, we calculate that 9 subjects per group will provide at least 80% power to demonstrate a between-condition difference of 21.9 μmol/100g/min in myocardial glucose uptake with a 2-tailed p value <0.05. This represents a demonstrable increase in MGU of at least ~80% over the effect of insulin alone. With this sample size we predict we will have 90% power to demonstrate the anticipated doubling of effect with combination treatment compared to insulin alone. Similar calculations provide projections of demonstrable effect sizes for the main secondary endpoints (**Table D2**). (Power estimates used online calculators at http://www.stat.uiowa.edu/~rlenth/Power/index.html.) Due to the high cost of the study measurement procedures, we plan to replace subjects who drop out of the study (again applying the randomization scheme) rather than initially study a larger number in anticipation of drop-outs.

Data are recorded and stored in a custom-designed SQL database system hosted on our institution's server farm. This system meets HIPAA and FDA mandated standards for data security.

D7. Technical Details

PET Imaging A Siemens ECAT EXACT HR+ whole body PET scanner is available in the Department of Radiology for research studies. This system has an intrinsic in-plane image resolution of approximately 4.2 mm FWHM (full width at half maximal). The system simultaneously measures 63 image planes with an axial coverage of 15.5 cm. A Siemens CTI RDS 112 medical cyclotron system is used for the production of radionuclides. The Indiana University radiopharmacy is a member of PETNET, a radiopharmacy network that produces ^{18}F- deoxyglucose commercially for clinical use, with the attendant high-throughput, expertise, and rigorous quality controls this entails (www.petnetsolutions.com). ^{11}C and ^{18}F will also be generated using this cyclotron, and the compounds will be labeled as previously described (80, 87, 88). The labeled chemicals are collected, evaporated to dryness, reformulated in isotonic NaCl solution and filtered through a 0.22μm filter (Millex-GS, Millport, Bedford MA). Radiochemical purity by HPLC, pyrogenicity and sterility tests are performed on each product batch. Conventional data acquisition procedures will be used to acquire and reconstruct the PET images, as previously published (89). The image data will be reconstructed using conventional filtered backprojection algorithms and a Hanning smoothing filter, which produces an image resolution of approximately 1.0 cm FWHM. Computer-based parameter estimation compartmental modeling algorithms are

applied to the serial tracer intensity data, to extract parameters which reflect the kinetics of appearance or disappearance of the radionuclide.

Impedance Cardiography: Systemic hemodynamic parameters will be measured noninvasively using impedance cardiography (BioZ, CardioDynamics, San Diego CA) (89, 90). The change in impedance across the thorax over time (dZ/dt) reflects the cardiac cycle, and the morphology of the waveform allows calculation of stroke volume (time-frequency distribution), peak blood flow rates (dZ/dt max) and contractility (upslope of dZ/dt) (90, 91).

Hormones: Insulin (Humulin R, Lilly, Indianapolis IN) will be diluted in 100 mL NS and delivered at 40 mU/kg/min. Liraglutide will be provided by Novo Nordisk A/S (Copenhagen, Denmark) in clinically available pens allowing injection of 0.6 and 1.8 mg doses.

Substrate and Hormone Measurements: Plasma glucose will be measured by the glucose oxidase method (STAT3000 Yellow Springs Instruments, Yellow Springs OH). Non-esterified fatty acid concentrations will be determined by a colorimetric assay using a kit from Wako (Richmond, VA). Glucagon will be measured using an EIA kit from Millipore (Billerica, MD). Samples for liraglutide and GLP-1 measurements will be collected into blood tubes pre-treated with DPP-4 inhibitor (Linco DPP4-010). GLP-1[7-36]amide will be measured using ELISA (Millipore), detection limit 3 pM and no cross-reactivity to GLP1[9-36], GLP-2 or glucagon. GLP-1[9-36] will be measured using a non-specific GLP- 1 C-terminus assay (Millipore;detecting both 7-36 and 9-36 forms), followed by subtraction of the measured amount of active GLP-1 . Insulin will be measured by ELISA (Alpco, Salem, NH).

D8. Timeline

Studies will be performed in random sequence according to the randomization scheme generated at study inception. We anticipate completing these studies within 12 months.

D9. Investigational uses of study drugs

The proposed studies will apply Insulin Detemir and Liraglutide in a manner outside of the current FDA approval. Specifically, liraglutide is not FDA indicated for use in combination with any form of insulin. Therefore the PI will perform this work under Investigational New Drug surveillance by the FDA, to be obtained by the PI for this project.

E. Human Subjects

E1. Risks to the Subjects

a. Involvement and Characteristics of Subjects

The characteristics of the study populations are described in the section on experimental design and methods. Lean healthy subjects of both genders will comprise the study group for the work proposed. All subjects will be between the ages of 18 and 50 years and healthy. No children under age 18, fetuses, pregnant women, prisoners or mentally disabled individuals will be studied. Study populations will include both genders and all racial groups, although the study design is not directed at detecting gender or racial differences. All data obtained will be for research purposes only.

Study population to be recruited:

Volunteer population of Marion County, IN

	American Indian or Alaskan Native n (%)	Asian or Pacific Islander n (%)	Black, not of Hispanic Origin n (%)	Hispanic n (%)	White, not of Hispanic Origin n (%)	Other or Unknown n (%)	Total n (%)
Female			3 (22.1)	0 (1.05)	10 (78.5)		13 (52.2)
Male			4 (20.1)	0 (0.95)	10 (76.3)		14 (42.8)
Total			7 (21.2)	0 (1.0)	20 (77.3)		27 (100)

Source: Marion County: US Bureau of Census

b. Sources of research material

Lab specimens: Blood and urine will be collected for routine screening chemistries and hematology to rule out disease at the time of screening. At timed intervals during the studies blood will be collected for glucose, insulin, non-esterified fatty acid and GLP-1 concentrations. Extra blood will be stored for possible later biochemical analyses (no genetic material will be extracted or stored).

Hemodynamic measurements: cardiac output, heart rate, blood pressure, systemic vascular resistance, and other parameters derived from combinations of these measures by impedance cardiography

PET measurements: basal and hormone-stimulated myocardial glucose uptake, total oxidation rates, and rates of myocardial blood flow.

Metabolic Measurements: oral glucose tolerance, percent body fat and lean muscle mass.

c. Potential Risks

The potential risks to the subjects associated with insertion of catheters include a slight (~1/1000) risk of bleeding/hematoma and infection. Regarding avoidance of infection, meticulous care will be taken with respect to sterility and technique. The catheters will be kept patent with saline to prevent clotting.

PET scan – Subjects with a history of claustrophobia will be excluded from participation due to the somewhat close nature of the gantry of the PET scanner. There is a routine period of ~30 min for transmission scans prior to the first radionuclide injection; subjects' comfort will be routinely assessed at that point prior to continuing.

The use of radionuclides results in radiation dosing to the research participant. 20 mCi of ^{11}C-acetate will be used. This produces an estimated total body dose of 40 mrem, with the maximal individual organ dose of 1480 mrem in the pancreas, compared to the annual occupational limit of 50,000 mrem for the pancreas. 5 mCi of ^{18}FDG will produce a whole body dose of approximately 340 mrem, with a maximal organ dose of 160 mrem in the bladder, compared to the annual occupational limit of 50,000 mrem for the bladder. 7.5 mCi of ^{11}C-Palmitate will produce a whole body dose of approximately 44 mrem, with a maximal organ dose of 3,850 mrem in the bladder. In total for the complete study subjects will receive approximately 424 mrem. By comparison, a typical radiation dosage from an AP chest film is 30 mrem, and the maximal permissible whole-body dose for the general population in research applications is 500 mrem/year. Radiation exposure confers significant risks to developing fetuses, and therefore all female study subjects with the potential to be pregnant will be specifically tested immediately prior to PET protocols and excluded if pregnant.

Risks from the treatments: Liraglutide is an FDA-approved treatment for diabetes with a well-understood side effect profile. A minority of subjects will be expected to experience some GI discomfort on initiation of therapy, which is expected to abate. The standard dose escalation procedure is designed to help patients tolerate this effect and allow time for them to adjust to receiving the medication. Some subjects will be unable to tolerate the full treatment dose, and will be withdrawn from the study if that is the case. Other common side effects affecting >5% of treated subjects include diarrhea, headache and vomiting. As with the nausea these are expected to abate. In rat models GLP-1 based treatments are associated with medullary carcinoma of the thyroid, a rare form of thyroid cancer. There is insufficient data to conclusively rule out this effect in humans, and therefore subjects with increased risk for this due to a personal or family history of predisposing conditions will be excluded. GLP-1 based treatments have been associated with an increased prevalence of recognized pancreatitis (although there is debate whether this represents a drug treatment effect or a previously unrecognized problem among patients with diabetes). Participants with a known history of pancreatitis will be excluded from participation. Hypoglycemia is a possibility in participants where liraglutide is added to other glucose-lowering agents; the rate of hypoglycemia in subjects treated with liraglutide alone is very low. All subjects will be routinely monitoring their blood glucose and instructed on the appropriate management of hypoglycemia.

The risks of insulin treatment are well recognized, and no different when using insulin detemir than other insulin formulations. The most common and important problem is hypoglycemia. As above, this will be managed by having subjects monitor their blood glucose levels routinely.

As with all human subject-based medical research, there is a risk of loss of confidentiality owing to disclosure of health information. These disclosures may be mandated by local or national regulatory agencies (i.e. IRB, FDA) or may be unintentional. Safeguards regarding both physical and electronic copies of all subject data are in place, as dictated by the current standards of Indiana University.

E2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent Procedures

All personnel associated with this study have successfully completed specific training in Human Subjects Protection, as required by our local IRB and HHS. All subjects will be recruited directly and by advertisement from the general local population and the various clinics at IU Medical Center and the Indianapolis Veterans Affairs Medical Center which serve the general population of Marion county and surrounding areas. All volunteers will be enrolled irrespective of race, creed and sex, therefore, one would expect that the study population mirror the demographics of the area, i.e., approximately 21% Afro-American, less than 2% Hispanic, American Indian and Asian, and 77% Caucasian with equal gender distribution. When we look at our recruitment statistics over the past few years including all studies (NIH and other funding), we find that we recruit ~40% women, and 49% black, 50% white and 1% Hispanic. Thus, we have successfully met federal mandates for representative inclusion of study participants with our recruiting strategy. The PI is cognizant of the recurrent problem of obtaining equal populations of both genders and therefore we continue to aggressively recruit to meet that goal.

Prior to enrollment in the study, the entire protocol will be reviewed with each volunteer. Subjects will be given ample opportunity to ask questions, and once all concerns are satisfied, formal written consent will be obtained. A signed consent form will then be saved as a permanent record. No studies will be initiated until approval is obtained by the Indiana University-Purdue University at Indianapolis (IUPUI) Institutional Review Board (an OHRP-registered IRB) and Radiation Safety approval. Subjects will be compensated financially for their participation.

b. Protection Against Risks

During these studies there are medically trained personnel at the bedside and the subjects are monitored continuously. In the unlikely event that any of the above mentioned complications occur, immediate medical intervention would be undertaken.

In menstruating females, pregnancy will be ruled out by urine pregnancy test immediately prior to study. Such subjects can only participate with a documented negative test immediately prior to initiation of the study

Risks associated with the treatments will be managed overall through controlled dosing exposure, and through close monitoring of blood glucose across the course of the treatment period. Subjects at particularly elevated risk of the rare complications associated with liraglutide treatment will be excluded at the screening stage.

The risks associated with radiation exposure are managed through limitations of exposure for non-clinical reasons. The limits applied to research subjects are arbitrarily set at 1/10 of those allowed for occupational exposure, namely 500 mrem/calendar year. Subjects' recent prior exposure will be assessed during screening for participation in this protocol, and if their prior exposure plus the planned studies will exceed this limit they will be excluded from participation.

Except for research data, all other information relevant to patients collected in these studies will be kept confidential, including locked storage of all paperwork, and secure digital storage on a database to which only members of our research group have access. The security and integrity of this database are maintained with the direct assistance and oversight of the Department of Medicine's Information Technology support teams, and the database is served centrally from a closely monitored, regularly backed up server. The current HIPAA and FDA standards for research database security are met by these efforts.

E3. Potential Benefits of the Proposed Research to the Subjects and Others

The subjects will not benefit directly from participating in this study except possibly for a physical examination and determination of lean and fat body mass performed prior to the study. In general, the benefit of the PET quantification of heart fuel selection relates to the favorable signal-to-noise ratio, which allows us to undertake studies of human physiology in very small numbers of subjects. Therefore, the benefit to society related to each individual's participation is maximized. The potential benefit to society in general is a better understanding of the role and application of GLP-1 in the modulation of myocardial fuel selection, with the intent to further translate these observations into clinical practice. This has a clear significance to the population as a whole, given the dominant role of cardiovascular disease in morbidity and mortality in North America and the world.

E4. Data Safety Monitoring Board

Composition of the Data and Safety Monitoring Board

The DSMB for this protocol will consist of the PI and the co-investigators, and will be chaired by Dr. A Lteif (Endocrinology/Medicine)(Chair of the DSMB) who is not directly involved in the study.

Frequency of Review

The Principal Investigator will make a report to the DSMB at 6 month intervals, and the DSMB will meet with the PI to review this report.

Features Assessed

Adverse Events Grading and Attribution Adverse events in this protocol are expected to be evident during the hospitalization for the study measurements. Study subjects are encouraged to contact study staff with any

concerns following discharge from hospital, and are assessed on the GCRC at the discretion of the study physician.

The PI, in consultation with the DSMB, will grade adverse events severity as follows (as recommended by the National Cancer Institute):

0-No adverse event or within normal limits

1-Mild adverse event

2-Moderate adverse event

3-Severe adverse event

4-Life-threatening adverse event

5-Fatal adverse event

Grade 4 and 5 events will prompt an immediate report to the DSMB, with expedited reporting to the IRB and the GCRC (see below).

Where investigational agents or devices are used, attribution of the adverse event to this agent or device will be categorized as follows (as recommended by the National Cancer Institute):

Definite – The AE is clearly related to the agent or device

Probable – The AE is likely related to the agent or device

Possible – The AE may be related to the agent or device

Unlikely – The AE is doubtfully related to the agent or device

Unrelated – The AE is clearly NOT related to the agent or device

Protocol Compliance and Data integrity: Any deviations from protocol will be reported to the DSMB. Completeness and accuracy of data records will be assessed by a random check of 5 recent study records.

Data sufficiency/Study completion: Assessments of completeness of data collection for subsections of this project are continuous, and will be reported by the PI at each meeting.

Protocol review: Where appropriate, changes to the protocol suggested either by interim results, adverse events, or external events such as new FDA recommendations will be discussed with the DSMB.

Reporting

Any serious and unexpected adverse events associated with the study intervention that occur on-site will be reported to the IRB and the GCRC within 3 working days of notification of the event. Any serious and unexpected adverse events associated with the study intervention that occur at an external site will be reported to the IRB and the GCRC within 10 working days after receipt from the sponsor (this statement is a generic IUPUI IRB requirements, does not apply to this study). All other adverse events will be reported annually with the continuing review. All expedited reports will also be sent to the GCRC.

Biohazards

Potential hazards related to infusates (GLP-1, insulin) and to radiation exposure (PET scanning, DEXA measurement of body composition) are addressed in detail above under E3c (Potential Risks) and E4b (Protection Against Risks). This project will be performed under FDA Investigational New Drug supervision (IND 105,155).

E5. Women and Minority Inclusion in Clinical Research

Women and minorities will be studied under this proposal, in proportions anticipated to reflect the proportions in our local population (see recruitment population table above).

E6. Inclusion of Children

Recruitment criteria include subjects aged 18 and above who otherwise meet the study criteria. Cardiac fuel selection across the stages of puberty has not been studied, and in particular it is unclear whether sex steroids are potential regulators. Therefore, studying children who are traversing puberty could introduce confounders, and specifically asking this question requires an entirely different study design. Therefore, we plan to include post-pubertal children only, aged 18 and above.

F. Vertebrate Animals

N/A

G. Literature Cited

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