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Combination GLP-1 and Insulin Treatment Fails to Alter Myocardial Fuel Selection Versus Insulin Alone in Type 2 Diabetes

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Context Glucagon-like peptide-1 (GLP-1) and the clinically available GLP-1 agonists have been shown to exert effects on the heart. It is unclear whether these effects occur at clinically used doses in vivo in humans, possibly contributing to CVD risk reduction.
Objective To determine whether liraglutide at clinical dosing augments myocardial glucose uptake alone or in combination with insulin compared to insulin alone in metformin-treated Type 2 diabetes mellitus.
Design Comparison of myocardial fuel utilization after 3 months of treatment with insulin detemir, liraglutide, or combination detemir+liraglutide.
Setting Academic hospital
Participants Type 2 diabetes treated with metformin plus oral agents or basal insulin.
Interventions Insulin detemir, liraglutide, or combination added to background metformin
Main Outcome Measures Myocardial blood flow, fuel selection and rates of fuel utilization evaluated using positron emission tomography, powered to demonstrate large effects.
Results We observed greater myocardial blood flow in the insulin-treated groups (median[25th, 75th percentile]: detemir 0.64[0.50, 0.69], liraglutide 0.52[0.46, 0.58] and detemir+liraglutide 0.75[0.55, 0.77] mL/g/min, p=0.035 comparing 3 groups and p=0.01 comparing detemir groups to liraglutide alone). There were no evident differences between groups in myocardial glucose uptake (detemir 0.040[0.013, 0.049], liraglutide 0.055[0.019, 0.105], detemir+liraglutide 0.037[0.009, 0.046] µmol/g/min, p=0.68 comparing 3 groups). Similarly there were no treatment group differences in measures of myocardial fatty acid uptake or handling, and no differences in total oxidation rate.
Conclusions These observations argue against large effects of GLP-1 agonists on myocardial fuel metabolism as mediators of beneficial treatment effects on myocardial function and ischemia protection.

PET studies showed no effect of liraglutide to alter myocardial fuel selection in metformin-treated T2D. This argues against a myocardial metabolic effect in the CVD benefits of GLP-1 treatments.

Background

Glucagon-like peptide 1 (GLP-1) based treatments have been developed for the management of Type 2 diabetes mellitus (T2D). Shortly after the discovery of GLP-1 and its effects via the pancreas to regulate glucose metabolism, GLP-1 was found to have extrapancreatic effects including effects to modulate myocardial metabolism and function (1). Specifically, GLP-1 exposure can acutely augment myocardial glucose uptake, and this effect has been postulated to contribute to cardiac protection under ischemia and to improvements in cardiac function (2). We

and others have shown that these effects can be replicated in small and large animal models, and in lean non-diabetic humans (3-7). Importantly, however, we have demonstrated an impairment in myocardial metabolic effects of GLP-1 agonism in humans with T2D and in obese non-diabetic swine (3). This is potentially important, as the clinical populations treated with GLP-1 agonists have obesity and/or T2D.

This impairment in GLP-1 metabolic responses in the heart is parallel to reports of impaired myocardial responses to insulin in obesity and in T2D (8). The genesis of myocardial insulin resistance is not fully explained, but it has been attributed in part to impairment of myocardial insulin signaling leading to impaired insulin-stimulated glucose uptake (9) and in part to augmented fatty acid uptake by the heart (10) leading to impaired myocardial metabolic flexibility. GLP-1 appears to exert effects directly on the heart to drive myocardial glucose uptake, via pathways that are independent of myocardial insulin signaling (3,11). This suggests the possibility that GLP-1 and insulin actions on myocardial glucose uptake could converge, and perhaps GLP-1 could help overcome myocardial insulin resistance (12).

The actions of current GLP-1 agonists on the myocardium in people with Type 2 diabetes have not been fully described. It is unknown whether the doses that have been developed and approved for the management of glycemia exert metabolic actions on the heart, and how those actions relate to the effects of insulin on heart metabolism. We hypothesized that GLP-1 mimetic in combination with insulin would provide superior stimulation of myocardial glucose uptake compared to either agent alone. Therefore we undertook a randomized clinical trial using positron emission tomography, powered to demonstrate large effects, to compare the effects of insulin detemir, liraglutide, or the combination, on myocardial fuel metabolism in people with Type 2 diabetes.

Methods

Population

Volunteers were recruited by advertising. Inclusion criteria included T2D of at least 2 years' duration stably treated with metformin >= 1500 mg/day, age 18-50 years, HbA1c 7.0 – 10.0%, and treatment with up to 2 oral antidiabetic agents and/or basal insulin. Key exclusion criteria included exposure within the past 6 months to a DPP4 inhibitor, GLP-1 agonist, or thiazolidinedione class diabetes agent; known coronary artery disease or structural heart disease; known diabetic microvascular disease (urinary albumin/creatinine ratio>30 mg/g, diagnosed retinopathy or neuropathy by self-report, or either of these detected on screening physical examination); and contraindications to radiation exposure with planned positron emission tomography. The flow of participants through the study is presented in Figure 1. All participants gave written informed consent for their participation in the study, and the study was conducted according to the Helsinki principles. This study was registered on ClinicalTrials.gov (NCT01232946)

Treatment

Volunteers were randomized in a fixed-block design to one of three treatments: insulin, liraglutide, or combination insulin and liraglutide. Antidiabetes agents other than metformin were discontinued, and study treatment was substituted in addition to ongoing metformin; if participants were taking submaximal metformin dosing an attempt was made to increase this to 2000 mg/day prior to randomization. The long-acting insulin detemir was used, with dosing initiated at 5-10 units twice daily (depending on the current blood glucose readings) and titrated twice weekly to achieve fasting glucose values below 120 mg/dL (6.7 mmol/L) without values

below 80 mg/dL (4.4 mmol/L). Liraglutide dosing followed the product labeling, starting with 0.6 mg/day initially and increased weekly to 1.8 mg/day. Participants randomized to combination therapy started first on liraglutide and had insulin added when stable liraglutide dosing was achieved. PET measurements of myocardial metabolism were planned at the end of 12 weeks of study assigned treatment.

Study Measurements

Subjects were admitted to the Indiana University Clinical Translational Sciences Institute's Clinical Research Center before 0700h the morning of the PET study, following an overnight fast (from 2000h the day prior). Anthropometrics, blood pressure and heart rate were measured in a standardized manner. Body composition was measured using dual-energy X-ray absorptiometry. An intravenous catheter was placed in the antecubital vein of one arm for infusates. A second catheter was placed in the contralateral arm for blood sampling.

Liraglutide treated subjects received a subcutaneous injection of liraglutide 1.8 mg at 0800h on the morning of the PET study. Insulin treated subjects took their stable morning insulin detemir dose also at 0800h the morning of the study. The PET measurement protocol began between 1000-1100h.

PET protocol

Positron emission tomography (PET) was used to 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. Blood samples for later measurement of circulating concentrations of glucose, insulin, and non-esterified fatty acids were taken at the beginning and end of the PET measurement sequence, with average values calculated to reflect exposure during the PET measurements.

Conventional data acquisition procedures were used to acquire and reconstruct the PET images. This included a transmission measurement to enable correction for attenuation by body mass. The image data were reconstructed using conventional filtered back-projection algorithms and a Hanning smoothing filter, which produces an image resolution of 1.0 cm full-width at half-maximum. This filter function was selected because it is consistent with the observed resolution degradation observed by the motion of the heart as determined with cardiac gated imaging studies. Custom software was used to identify the entire left ventricle as a single volume of interest and then apply mathematical modeling to the resulting volume of interest. The left ventricle cavity, concurrently identified, served as an arterial blood pool from which the input function was derived. Following acetate infusion, serial timed peripheral blood samples were taken (at minutes 0, 3, 6, 10, 15, 20, 25, and 30), immediately analyzed for circulating concentrations of labeled CO_2 and later used as a correction for the input function (13,14). Timed samples were taken at these same intervals following palmitate infusion and assayed for total and palmitate-specific radioactivity to determine metabolite corrections for this analyte's input function. Tissue perfusion and total oxidative metabolism were quantified from the myocardial time-activity curve following ^{11}C-Acetate injection, using a well-validated two-compartment model (13,15). Myocardial blood flow was measured directly from the acetate influx kinetic parameter K_1 (15,16). Myocardial oxygen consumption was derived from the acetate efflux parameter k_2 using $MVO_2 = 135*k_2 - 0.96$ (17). A 3-compartment, 3-free parameter model including uptake, oxidation and esterification kinetics was used to derive rates of fatty acid utilization from the palmitate time-activity curves (18), with net rates of fatty acid oxidation and esterification calculated according to the method of Bergmann (19). Myocardial glucose uptake was quantified from the time-activity curve following ^{18}FDG injection using a 3-compartment

model, according to the methods of Morita and colleagues (20), with a lumped constant of 1.0 (21,22).

Sample Size Estimation and Statistical Approach

Myocardial glucose uptake was our primary endpoint for analysis; other PET-derived measures were co-secondary endpoints. We were specifically interested in whether the combined exposure produced myocardial metabolic rates greater than exposure to insulin alone. Our pre-specified plan for statistical testing was constructed to first evaluate whether the three treatment groups were different with post-hoc pairwise testing if indicated, and then to compare 2 liraglutide groups against insulin alone, and 2 insulin groups against liraglutide alone. Post-hoc analyses including multivariable analyses with relevant covariates were planned as exploratory analyses; due to unexpected group differences in some baseline factors, post-hoc sensitivity analyses were performed evaluating direct and interaction effects of these factors on the main three-group outcome. A significance p value of <0.05 was applied throughout without adjustment for multiple testing.

Power estimates and sample size calculations were based on the primary endpoint of myocardial glucose uptake. Our prior work suggested that the myocardial glucose uptake in T2D would be ~0.026±0.016 μmol/g/min (mean±SD), and studies by others suggested that diabetes therapies added to background treatments could induce 30-40% increases in myocardial glucose uptake, i.e. effect sizes of ~0.7 SD (23,24). We estimated that a sample size of 9 per group would permit demonstrating between-group differences of ~1.2 SD with alpha=0.05 and beta=0.2 for myocardial glucose uptake. Therefore we were powered to demonstrate ~~moderate to~~ large effects, if present. Working from this glucose uptake-related sample size we estimated demonstrable between-group differences of ~0.5 SD for myocardial blood flow, ~1.4 SD for total myocardial oxidation rates, and ~0.6 SD for myocardial fatty acid oxidation rates.

The PET data for metabolite rates were right-skewed, and not adequately normalized by standard transformations (logarithmic, square root, inverse). Therefore we applied non-parametric testing, using Kruskal-Wallis tests to compare across 3 treatment groups and Mann-Whitney U tests for pairwise testing. These skewed distributions were best represented in the figures by plotting median and 75th percentile values. Sensitivity analyses evaluating the potential effects of baseline group differences were performed using standard ANOVA with untransformed data excluding extreme outliers, in order to meet the assumptions of the method.

Results

The flow of participants through the study is presented in Figure 1, and the characteristics of the participants who enrolled and completed the study are presented in Table 1. Two participants originally randomized to receive combination insulin detemir plus liraglutide withdrew; one experienced intolerable adverse gastrointestinal effects and another was lost to follow-up without any known reason for withdrawal. One participant originally randomized to the detemir arm died of a narcotic overdose after randomization.

The most notable feature of the baseline characteristics was the unbalanced sex distribution across treatment groups. Despite block randomization, the detemir-only group had proportionally more female participants and fewer male participants than the other two groups (p=0.007). There was also a modest imbalance in race distribution, with proportionally more black participants in the combination treatment group, not achieving significance (p=0.090). Borderline differences were also seen with respect to glucose and HbA1c (lower in the detemir-only group, p=0.084 and 0.060 respectively); body fat measurements were available in 7 detemir participants

(40.6±6.7%), in 5 liraglutide participants (32.6±10.8%) and in 6 combination treatment participants (41.4±3.8%), p=0.08 comparing groups. The groups were well balanced with regard to age, weight, BMI and blood lipid levels (all p>0.40).

The median metformin dose was 2000 mg/d, with only 3 participants taking less; all participants were taking at least 1500 mg/d. All participants who were randomized to receive liraglutide and completed the study achieved stable dosing at 1.8 mg/d. The insulin detemir dose was individualized, guided by fasting blood glucose levels as above. The median daily dose was 15 units, with a range from 5 to 68 units per day.

All three treatment groups experienced significant improvements in glycemia over the course of 3 months' treatment (p=0.04; Table 2), without a statistical difference between groups (p=0.12). Weight fell across all 3 treatment groups (p=0.007); one individual in the liraglutide group lost 15.7 Kg, but otherwise the weight loss was 1.7±2.4 Kg (mean±SD) and did not differ between treatment groups. Heart rate was unchanged across the course of treatment, and not different between groups at the end of treatment (Table 2). Systolic blood pressure fell significantly (p=0.013), not different between groups (p=0.28). Diastolic blood pressure also fell significantly (p=0.006) and this effect differed between groups (p=0.029) owing to larger changes in the detemir-only group.

On the day of the PET studies, the three groups did not differ in hemodynamic parameters (Table 2). Importantly, serum glucose values across the interval of PET scanning did not differ between groups (Figure 2). Non-esterified fatty acid concentrations were numerically but not statistically lower in the detemir+liraglutide group compared to others (Figure 2). Insulin concentrations were statistically higher in the participants randomized to detemir alone or to detemir+liraglutide (Figure 2), reflecting the steady state from prior treatment plus the AM dose on the morning of the study.

There were no observed differences between groups in the PET-derived measure of oxygen consumption (MVO_2; Figure 3). We did observe differences between groups in myocardial perfusion (myocardial blood flow, MBF), with greater rates of blood flow in the two detemir-treated groups compared to the liraglutide-only group. These group differences in blood flow were directionally consistent with the MVO_2 group differences, but the correlation between MBF and MVO_2 was not statistically significant (r=0.25, p=0.22; not shown). The rate-pressure product (mean arterial pressure x heart rate) divided by MVO_2 was calculated as an index of work efficiency (16,25) and was statistically greater in the liraglutide-only group compared to the two groups that received insulin (Figure 3).

We did not observe any differences between groups in the measures of myocardial glucose uptake, fatty acid oxidation or fatty acid esterification (Figure 4). The primary hypothesis for this study was that the combination detemir plus liraglutide group would exhibit a higher myocardial glucose uptake than detemir alone; this was unequivocally not seen, with these two groups showing essentially identical median measures of MGU (detemir 0.040 $\mu mol*g^{-1}*min^{-1}$, detemir+liraglutide 0.037, p=0.65; Figure 4). The liraglutide-only group had a numerically but not statistically higher median MGU (0.055 $\mu mol*g^{-1}*min^{-1}$, p=0.57 against detemir alone). Similarly, the three treatment groups did not differ in the median values of the palmitate-derived measures of fatty acid utilization (MFAO median detemir 0.123 $\mu mol*g^{-1}*min^{-1}$, liraglutide 0.102, detemir+liraglutide 0.099, p=0.60; Figure 4). The measured kinetic rates, distinct from substrate availability, were also not different across treatment groups (e.g. glucose kinetic median detemir $5.5x10^{-3}$ min^{-1}, liraglutide $8.25x10^{-3}$, combination $4.65x10^{-3}$, p=0.69; fatty acid

oxidation kinetic detemir 165.6x10^{-3} min^{-1}, liraglutide 116.4x10^{-3}, combination 139.5x10^{-3}, p=0.80).

The observed effects on glucose uptake included high outlier values in one detemir-only treated participant and one liraglutide-only treated participant. The use of non-parametric statistical testing protects against effects of outliers, but we also performed sensitivity testing excluding these 2 individuals. Doing so did not alter the outcome (p=0.84 for MGU comparing 3 groups). The measures of blood flow, total oxidation and fatty acid handling from these individuals were not outliers, and parallel sensitivity analyses did not alter any of the comparisons between these other PET-derived parameters.

Sex, race, baseline glucose and baseline HbA1c were at least nominally different across treatment groups at baseline (Table 1). However none of these factors was significantly associated with the PET measures of fuel uptake, with the strongest correlation among these relating HbA1c with MFAE (Spearman's rho -0.35, p=0.09) and most r values below 0.10. Sensitivity analyses were performed to further evaluate possible contributions of these imbalances. The results for sex, race, and baseline HbA1c are presented in Supplemental Table 1. Overall these analyses showed no direct or interaction effect of these variables on the PET-derived outcomes; however there were two interesting exceptions. First, myocardial glucose uptake was significantly different by treatment groups after adjustment for sex, higher in the liraglutide treatment arm than the other two groups (p<0.001) and with higher MGU in male participants. Second, myocardial fatty acid oxidation was significantly different across treatment groups after adjustment for baseline HbA1c, lower in the levemir treatment arm than the other two groups (p=0.021) with higher baseline HbA1c relating to higher on-treatment MFAO. This was seen despite equalization of glucose control across the treatment interval prior to PET measures. These observations do not affect the overall conclusion that combination therapy did not augment fuel use compared to detemir or liraglutide alone.

In summary, treatment with combination detemir plus liraglutide did not produce different rates of fuel use or different patterns of fuel selection compared to insulin alone or liraglutide alone.

Discussion

We measured myocardial fuel utilization after 12 weeks of randomized therapy with the long-acting insulin detemir, the long-acting GLP-1 mimetic liraglutide, or the combination of these treatments in humans with Type 2 diabetes (T2D) treated with maximal dose metformin. *In vivo* measurements of myocardial fuel uptake were made using positron emission tomography (PET). Study medications were administered the morning of the PET measurements. We observed expected effects of insulin treatment to increase myocardial blood flow in both groups that received insulin, but otherwise we observed no differences between groups in the rates of fuel metabolism or fuel selection. These data include two distinct novel observations in type 2 diabetes without cardiovascular disease: First, liraglutide alone at clinically used doses does not stimulate myocardial glucose uptake compared to insulin; Second, liraglutide at clinically used doses was not able to augment insulin's effects to drive myocardial glucose uptake.

Our primary endpoint was PET-measured myocardial glucose uptake, and our sample size provided *a priori* power to detect ~1.2xSD differences in this parameter. We were able to demonstrate expected between-group differences in the effect of insulin on myocardial blood flow, demonstrating the viability of the overall approach for showing detectable differences. Nevertheless, the results did not suggest any material difference in myocardial glucose uptake

between groups, with nearly identical group median values and a very high p value comparing the distribution of values. Therefore, although our threshold for demonstrating a change was high, the observed group difference does not suggest that the absence of group differences is a power issue. Similarly, the observed effects on all aspects of fatty acid handling were convincingly equal across groups. These observations ~~clearly~~ disprove the hypothesis that liraglutide alone or in combination with insulin would provide superior stimulation of myocardial glucose uptake compared to insulin alone.

Soon after the discovery of GLP-1, this gut peptide was found to exert hemodynamic and metabolic effects in the heart, including protection from the effects of ischemia in mouse models (1,26-28). Subsequently a growing body of animal studies show cardiac benefits of GLP-1 agonists via a variety of mechanisms including activation of p38alpha MAP-kinase (11), and improving intracellular calcium homeostasis (29), among others. A major hypothesized mechanism of benefit has been a favorable shift in myocardial metabolite selection induced by GLP-1 agonism (2).

It is interesting to note that the MGU rates were not different across the three treatment groups in our study, despite higher insulin concentrations in the insulin-treated participants compared to those receiving liraglutide alone. This implies that exposure to therapeutic levels of liraglutide provides similar stimulation of myocardial glucose uptake as seen with exposure to therapeutically achieved insulinemia. This could reflect similar direct effects, or similar degrees of resistance to the effects. In this regard we note that the observed levels of MGU were objectively low (consistent with low rates described in prior PET studies in obesity or Type 2 diabetes under fasting conditions (3,30,31), which suggests that these observations reflect parallel resistance to the glucose-uptake stimulating effects of insulin and liraglutide in type 2 diabetes.

In large-scale clinical trials, beneficial cardiovascular effects including reduced rates of myocardial infarction and death have been seen with some GLP-1 agonists (liraglutide, semaglutide) (32,33) but not others (lixisenatide, long-acting exenatide) (34,35). The mechanisms underlying these beneficial effects, or the lack thereof, are unknown. As noted above the antecedent preclinical work suggested that changes in fuel selection might contribute to these effects. However, there are very few studies of effects of GLP-1 agonists on myocardial fuel metabolism in humans. In people with congestive heart failure liraglutide improved systemic glucose metabolism but failed to improve myocardial glucose uptake or myocardial blood flow or flow reserve (36). Similarly, albiglutide treatment in people with NYHA II or III class heart failure improved some measures of cardiac function but did not improve myocardial glucose consumption or oxygen consumption (37). Exenatide improved myocardial blood flow without changing myocardial glucose uptake (6), although in that dataset a direct relationship was observed between the change in myocardial glucose uptake and baseline insulin resistance. The current observations are the first in a study cohort that is typical of the general population of Type 2 diabetes patients treated with liraglutide.

We indirectly addressed whether GLP-1 added to insulin could overcome myocardial insulin resistance. There are some data to suggest this is possible. In lean dogs, GLP-1 co-treatment was sufficient to restore insulin-mediated myocardial glucose uptake in the setting of chronic heart failure (12). In an observational clinical study, adding liraglutide to ongoing diabetes treatments in Type 2 diabetes for 6 months was associated with improved echocardiographic markers of diastolic function (38). In patients with Type 2 diabetes and known coronary artery disease studied in a prospectively randomized design, echocardiographically-measured cardiac function

was unaffected by insulin infusion alone but improved with co-infusion of GLP-1 (significantly improved strain and systolic tissue velocity, but unchanged ejection fraction) (39). One study has reported reductions in major atherosclerotic cardiovascular events with adding liraglutide to insulin (40). Collectively these observations suggest that combination therapy does not detract from hemodynamic or metabolic benefits of individual components, although our current observations suggest that effects on myocardial fuel selection do not explain the observed benefits.

Strengths of this work include the use of typical clinical dosing in the usual target population, randomized assignment to treatment, and the use of high-quality, high-sensitivity measurements of myocardial fuel utilization with PET. The choice to include metformin as a background treatment condition improves the ability to translate this work to the general circumstance of clinical application of liraglutide, but also raises the possibility that our negative results could be due to an unexpected effect of metformin to dominate the apparent treatment response; this seems unlikely given the low absolute rates of MGU overall. Limitations of this work include the relatively low total insulin exposure, which was determined by each individual's clinical need based on achieved fasting blood glucose levels. We were powered only for demonstrating large between-group differences in our main outcome measure, MGU, but the approximately equal median and distribution across groups argues against a power problem in this finding. It would have been ideal to perform paired measurements of myocardial fuel selection before and after therapy but this was not possible within the available resources. We performed studies under resting conditions; it is possible that differential responses could be seen under circumstances of physiologic or pathophysiologic demand (i.e. exercise or ischemia). We were not powered a priori for post-hoc analyses, so the results of these evaluations need to be considered exploratory. The sex imbalance across treatment groups at baseline might have resulted in imbalanced measures of myocardial fatty acid handling, as women and men differ in this aspect of cardiac fuel selection (41,42); the sensitivity analysis suggests the possibility of a difference in response across treatments but does not alter the overall conclusion that combination therapy failed to augment myocardial glucose uptake or other measures of fuel selection compared to individual treatments.

Conclusions

These observations suggest that liraglutide alone in clinically used dosing in patients with Type 2 diabetes does not induce alterations in resting, fasting myocardial fuel uptake or fuel selection, and that liraglutide was not able to augment the effects of insulin alone on myocardial fuel uptake or selection under these conditions. These observations argue against large effects of GLP-1 agonists on myocardial fuel metabolism as mediators of beneficial treatment effects on myocardial function and ischemia protection.

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We have no other dualities of interest to declare pertaining to the current study.

K.J.M. and G.D.H conceived the study. K.J.M. drafted the initial manuscript, reviewed and revised the manuscript and approved the final manuscript as submitted. A.G., C.M., W.T. and J.D.T. contributed to the data analysis and approved the final manuscript as submitted. R.V.C., L.H., N.A.P., M.G. and G.D.H. collected data, contributed to the discussion, reviewed the manuscript, and approved the final manuscript as submitted. K.J.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Disclosure Summary:
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References

1. Nauck MA, Meier JJ, Cavender MA, Abd El Aziz M, Drucker DJ. Cardiovascular Actions and Clinical Outcomes With Glucagon-Like Peptide-1 Receptor Agonists and Dipeptidyl Peptidase-4 Inhibitors. Circulation 2017; 136:849-870

2. Aravindhan K, Bao W, Harpel MR, Willette RN, Lepore JJ, Jucker BM. Cardioprotection Resulting from Glucagon-Like Peptide-1 Administration Involves Shifting Metabolic Substrate Utilization to Increase Energy Efficiency in the Rat Heart. PLoS One 2015; 10:e0130894

3. Moberly SP, Mather KJ, Berwick ZC, Owen MK, Goodwill AG, Casalini ED, Hutchins GD, Green MA, Ng Y, Considine RV, Perry KM, Chisholm RL, Tune JD. Impaired cardiometabolic responses to glucagon-like peptide 1 in obesity and type 2 diabetes mellitus. Basic Res Cardiol 2013; 108:365

4. Sassoon DJ, Tune JD, Mather KJ, Noblet JN, Eagleson MA, Conteh AM, Sturek JT, Goodwill AG. Glucagon-Like Peptide 1 Receptor Activation Augments Cardiac Output and Improves Cardiac Efficiency in Obese Swine After Myocardial Infarction. Diabetes 2017; 66:2230-2240

5. Gejl M, Lerche S, Mengel A, Moller N, Bibby BM, Smidt K, Brock B, Sondergaard H, Botker HE, Gjedde A, Holst JJ, Hansen SB, Rungby J. Influence of GLP-1 on myocardial glucose metabolism in healthy men during normo- or hypoglycemia. PLoS One 2014; 9:e83758

6. Gejl M, Sondergaard HM, Stecher C, Bibby BM, Moller N, Botker HE, Hansen SB, Gjedde A, Rungby J, Brock B. Exenatide alters myocardial glucose transport and uptake depending on insulin resistance and increases myocardial blood flow in patients with type 2 diabetes. J Clin Endocrinol Metab 2012; 97:E1165-1169

7. Moberly SP, Berwick ZC, Kohr M, Svendsen M, Mather KJ, Tune JD. Intracoronary glucagon-like peptide 1 preferentially augments glucose uptake in ischemic myocardium independent of changes in coronary flow. Exp Biol Med (Maywood) 2012; 237:334-342
8. Iozzo P, Chareonthaitawee P, Dutka D, Betteridge DJ, Ferrannini E, Camici PG. Independent association of type 2 diabetes and coronary artery disease with myocardial insulin resistance. Diabetes 2002; 51:3020-3024
9. Bertrand L, Horman S, Beauloye C, Vanoverschelde JL. Insulin signalling in the heart. Cardiovasc Res 2008; 79:238-248
10. Mazumder PK, O'Neill BT, Roberts MW, Buchanan J, Yun UJ, Cooksey RC, Boudina S, Abel ED. Impaired cardiac efficiency and increased fatty acid oxidation in insulin-resistant ob/ob mouse hearts. Diabetes 2004; 53:2366-2374
11. Bhashyam S, Fields AV, Patterson B, Testani JM, Chen L, Shen YT, Shannon RP. Glucagon-like peptide-1 increases myocardial glucose uptake via p38alpha MAP kinase-mediated, nitric oxide-dependent mechanisms in conscious dogs with dilated cardiomyopathy. Circ Heart Fail 2010; 3:512-521
12. Chen M, Angeli FS, Shen YT, Shannon RP. GLP-1 (7-36) amide restores myocardial insulin sensitivity and prevents the progression of heart failure in senescent beagles. Cardiovasc Diabetol 2014; 13:115
13. Buck A, Wolpers HG, Hutchins GD, Savas V, Mangner TJ, Nguyen N, Schwaiger M. Effect of carbon-11-acetate recirculation on estimates of myocardial oxygen consumption by PET. J Nucl Med 1991; 32:1950-1957
14. Ng Y, Moberly SP, Mather KJ, Brown-Proctor C, Hutchins GD, Green MA. Equivalence of arterial and venous blood for [11C]CO2-metabolite analysis following intravenous administration of 1-[11C]acetate and 1-[11C]palmitate. Nucl Med Biol 2013; 40:361-365
15. Hutchins GD, Chen T, Carlson KA, Fain RL, Winkle W, Vavrek T, Mock BH, Zipes DP. PET imaging of oxidative metabolism abnormalities in sympathetically denervated canine myocardium. J Nucl Med 1999; 40:846-853
16. Mather KJ, Hutchins GD, Perry K, Territo W, Chisholm R, Acton A, Glick-Wilson B, Considine RV, Moberly S, DeGrado TR. Assessment of myocardial metabolic flexibility and work efficiency in human type 2 diabetes using 16-[18F]fluoro-4-thiapalmitate, a novel PET fatty acid tracer. Am J Physiol Endocrinol Metab 2016; 310:E452-460
17. Sun KT, Yeatman LA, Buxton DB, Chen K, Johnson JA, Huang SC, Kofoed KF, Weismueller S, Czernin J, Phelps ME, Schelbert HR. Simultaneous measurement of myocardial oxygen consumption and blood flow using [1-carbon-11]acetate. J Nucl Med 1998; 39:272-280
18. de Jong HW, Rijzewijk LJ, Lubberink M, van der Meer RW, Lamb HJ, Smit JW, Diamant M, Lammertsma AA. Kinetic models for analysing myocardial [(11)C]palmitate data. Eur J Nucl Med Mol Imaging 2009; 36:966-978
19. Bergmann SR, Weinheimer CJ, Markham J, Herrero P. Quantitation of myocardial fatty acid metabolism using PET. J Nucl Med 1996; 37:1723-1730
20. Morita K, Katoh C, Yoshinaga K, Noriyasu K, Mabuchi M, Tsukamoto T, Kageyama H, Shiga T, Kuge Y, Tamaki N. Quantitative analysis of myocardial glucose utilization in patients with left ventricular dysfunction by means of 18F-FDG dynamic positron tomography and three-compartment analysis. Eur J Nucl Med Mol Imaging 2005; 32:806-812
21. Botker HE, Bottcher M, Schmitz O, Gee A, Hansen SB, Cold GE, Nielsen TT, Gjedde A. Glucose uptake and lumped constant variability in normal human hearts determined with [18F]fluorodeoxyglucose. J Nucl Cardiol 1997; 4:125-132

22. Botker HE, Goodwin GW, Holden JE, Doenst T, Gjedde A, Taegtmeyer H. Myocardial glucose uptake measured with fluorodeoxyglucose: a proposed method to account for variable lumped constants. J Nucl Med 1999; 40:1186-1196
23. van der Meer RW, Rijzewijk LJ, de Jong HW, Lamb HJ, Lubberink M, Romijn JA, Bax JJ, de Roos A, Kamp O, Paulus WJ, Heine RJ, Lammertsma AA, Smit JW, Diamant M. Pioglitazone improves cardiac function and alters myocardial substrate metabolism without affecting cardiac triglyceride accumulation and high-energy phosphate metabolism in patients with well-controlled type 2 diabetes mellitus. Circulation 2009; 119:2069-2077
24. Hallsten K, Virtanen KA, Lonnqvist F, Janatuinen T, Turiceanu M, Ronnemaa T, Viikari J, Lehtimaki T, Knuuti J, Nuutila P. Enhancement of insulin-stimulated myocardial glucose uptake in patients with Type 2 diabetes treated with rosiglitazone. Diabet Med 2004; 21:1280-1287
25. Hattori N, Tamaki N, Kudoh T, Masuda I, Magata Y, Kitano H, Inubushi M, Tadamura E, Nakao K, Konishi J. Abnormality of myocardial oxidative metabolism in noninsulin-dependent diabetes mellitus. J Nucl Med 1998; 39:1835-1840
26. Zhao T, Parikh P, Bhashyam S, Bolukoglu H, Poornima I, Shen YT, Shannon RP. Direct effects of glucagon-like peptide-1 on myocardial contractility and glucose uptake in normal and postischemic isolated rat hearts. J Pharmacol Exp Ther 2006; 317:1106-1113
27. Bose AK, Mocanu MM, Carr RD, Brand CL, Yellon DM. Glucagon-like peptide 1 can directly protect the heart against ischemia/reperfusion injury. Diabetes 2005; 54:146-151
28. Noyan-Ashraf MH, Momen MA, Ban K, Sadi AM, Zhou YQ, Riazi AM, Baggio LL, Henkelman RM, Husain M, Drucker DJ. The GLP-1R Agonist Liraglutide Activates Cytoprotective Pathways and Improves Outcomes Following Experimental Myocardial Infarction in Mice. Diabetes 2009;
29. Hu SY, Zhang Y, Zhu PJ, Zhou H, Chen YD. Liraglutide directly protects cardiomyocytes against reperfusion injury possibly via modulation of intracellular calcium homeostasis. J Geriatr Cardiol 2017; 14:57-66
30. Botker HE, Moller N, Schmitz O, Bagger JP, Nielsen TT. Myocardial insulin resistance in patients with syndrome X. J Clin Invest 1997; 100:1919-1927
31. Ohtake T, Yokoyama I, Watanabe T, Momose T, Serezawa T, Nishikawa J, Sasaki Y. Myocardial glucose metabolism in noninsulin-dependent diabetes mellitus patients evaluated by FDG-PET. J Nucl Med 1995; 36:456-463
32. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, Nissen SE, Pocock S, Poulter NR, Ravn LS, Steinberg WM, Stockner M, Zinman B, Bergenstal RM, Buse JB, Committee LS, Investigators LT. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2016; 375:311-322
33. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jodar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsboll T, Investigators. Semaglutide and Cardiovascular Outcomes in Patients with Type 2 Diabetes. N Engl J Med 2016; 375:1834-1844
34. Derosa G, Maffioli P. Lixisenatide in Type 2 Diabetes and Acute Coronary Syndrome. N Engl J Med 2016; 374:1095
35. Holman RR, Bethel MA, Mentz RJ, Thompson VP, Lokhnygina Y, Buse JB, Chan JC, Choi J, Gustavson SM, Iqbal N, Maggioni AP, Marso SP, Ohman P, Pagidipati NJ, Poulter N, Ramachandran A, Zinman B, Hernandez AF, Group ES. Effects of Once-Weekly Exenatide on Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2017; 377:1228-1239

36. Nielsen R, Jorsal A, Iversen P, Tolbod LP, Bouchelouche K, Sorensen J, Harms HJ, Flyvbjerg A, Tarnow L, Kistorp C, Gustafsson I, Botker HE, Wiggers H. Effect of liraglutide on myocardial glucose uptake and blood flow in stable chronic heart failure patients: A double-blind, randomized, placebo-controlled LIVE sub-study. J Nucl Cardiol 2017;
37. Lepore JJ, Olson E, Demopoulos L, Haws T, Fang Z, Barbour AM, Fossler M, Davila-Roman VG, Russell SD, Gropler RJ. Effects of the Novel Long-Acting GLP-1 Agonist, Albiglutide, on Cardiac Function, Cardiac Metabolism, and Exercise Capacity in Patients With Chronic Heart Failure and Reduced Ejection Fraction. JACC Heart Fail 2016;
38. Saponaro F, Sonaglioni A, Rossi A, Montefusco L, Lombardo M, Adda G, Arosio M. Improved diastolic function in type 2 diabetes after a six month liraglutide treatment. Diabetes Res Clin Pract 2016; 118:21-28
39. McCormick LM, Heck PM, Ring LS, Kydd AC, Clarke SJ, Hoole SP, Dutka DP. Glucagon-like peptide-1 protects against ischemic left ventricular dysfunction during hyperglycemia in patients with coronary artery disease and type 2 diabetes mellitus. Cardiovasc Diabetol 2015; 14:102
40. Anyanwagu U, Mamza J, Donnelly R, Idris I. Effect of adding GLP-1RA on mortality, cardiovascular events, and metabolic outcomes among insulin-treated patients with type 2 diabetes: A large retrospective UK cohort study. Am Heart J 2018; 196:18-27
41. Kadkhodayan A, Lin CH, Coggan AR, Kisrieva-Ware Z, Schechtman KB, Novak E, Joseph SM, Davila-Roman VG, Gropler RJ, Dence C, Peterson LR. Sex affects myocardial blood flow and fatty acid substrate metabolism in humans with nonischemic heart failure. J Nucl Cardiol 2017; 24:1226-1235
42. Peterson LR, Soto PF, Herrero P, Mohammed BS, Avidan MS, Schechtman KB, Dence C, Gropler RJ. Impact of gender on the myocardial metabolic response to obesity. JACC Cardiovasc Imaging 2008; 1:424-433

Figure 1. Flow of study participants. One participant in the detemir-only arm died of non-study related causes during randomized treatment.

Figure 2 – Achieved insulin, glucose and fatty acid concentrations at the time of the PET measurements. Bars present median values and whiskers present upper 75th percentile values. Insulin concentrations were significantly different across the 3 groups, with significance in the pairwise comparison of detemir groups versus liraglutide alone. The other measures were not significantly different between groups.

Figure 3 – Myocardial blood flow, oxygen consumption, and work efficiency. Bars present median values and whiskers present upper 75th percentile values. Liraglutide alone showed lower blood flow and greater work efficiency than the other two groups. MBF, myocardial blood flow; MVO2, myocardial oxygen consumption; RPP, rate-pressure product.

Figure 4 – Myocardial fuel consumption. Bars present median values and whiskers present upper 75th percentile values. There were no significant differences across groups in these measures. MGU, myocardial glucose uptake; MFAO, myocardial fatty acid oxidation; MFAE, myocardial fatty acid esterification.

Table 1 – Participant Characteristics

Variable	Detemir	Liraglutide	Detemir+Liraglutide	P value (ANOVA)

Race (B/W)	3/6	3/6	7/2	0.090
Sex (F/M)	7/2	2/7	8/1	0.007
Age (yrs)	52.1±8.6	53.9±5.3	49.2±9.0	0.45
Weight (kg)	94.8±20.0	99.3±17.9	88.7±19.6	0.50
Height (m)	1.64±0.09	1.74±0.06	1.61±0.04	0.002
BMI (kg/m^2)	35.1±7.2	33.4±±6.8	34.2±6.9	0.87
Total Cholesterol (mmol/L)	3.4±1.4	3.8±1.4	3.8±0.8	0.77
LDL Cholesterol (mmol/L)	1.8±1.4	2.2±1.2	2.2±0.9	0.76
HDL Cholesterol (mmol/L)	0.9±0.3	0.8±0.2	1.0±0.2	0.42
Triglycerides (mmol/L)	1.9±1.1	2.0±1.0	1.5±0.9	0.60
Glucose-Screening (mmol/l)	6.5±2.6	9.4±3.2	9.3±3.1	0.084
HbA1c - Randomization (%)	7.00±0.79	7.59±0.96	8.13±0.99	0.060
HbA1c – Randomization (mmol/mol)	53±6	60±8	64±9	0.060

Table 1 Note: Values presented as mean±SD. The P value represents testing for a difference among the 3 groups by analysis of variance. B, black; BMI, body mass index; F, female; M; male; W, white.

Table 2 – Treatment Effects

Variable	Randomization	Month 1	Month 2	Study Day	P value (Time)	P value (Treatment*Time)
Fasting Glucose (mmol/L)					0.04	0.12
Detemir	6.5±2.6	7.2±0.9	6.9±1.4	7.8±2.9		
Liraglutide	9.4±3.2	6.9±1.1	7.2±1.8	7.3±2.4		
Detemir+Liraglutide	9.3±3.1	7.2±1.9	6.2±0.8	6.9±1.4		
Weight (Kg)					0.007	0.69
Detemir	97.4±19.7	97.3±20.9	96.8±7.0	95.7±7.0		
Liraglutide	103.1±7.8	101.8±7.7	100.8±7.5	100.1±7.5		
Detemir+Liraglutide	88.3±8.5	86.9±8.3	86.9±8.1	87.1±8.1		
Heart Rate (bpm)					0.93	0.82
Detemir	69.9±3.3	72.7±3.0	70.9±3.3	69.9±4.0		
Liraglutide	72.3±4.4	70.3±3.9	71.3±4.4	74.0±5.3		
Detemir+Liraglutide	71.3±3.6	70.8±3.2	75.8±3.6	70.8±4.3		
Systolic BP (mmHg)					0.013	0.28
Detemir	149.2±6.8	129.8±4.4	127.8±5.3	122.2±7.1		
Liraglutide	136.0±8.3	135.3±5.3	136.0±6.5	119.5±8.7		
Detemir+Liraglutide	131.2±7.4	126.8±4.8	121.8±5.8	126.0±7.8		
Diastolic BP (mmHg)					0.006	0.029
Detemir	78.4±2.3	71.0±2.0	70.9±2.1	66.1±2.8		
Liraglutide	76.0±2.3	70.4±2.0	76.1±2.1	72.9±5.8		
Detemir+Liraglutide	73.0±2.5	74.0±2.2	73.3±2.3	72.3±3.0		

Table 2 Note: P values are presented evaluating changes over time for all groups combined (Time), and testing whether this difference over time differed among treatment groups (Treatment*Time interaction). BP, blood pressure.