Investigator Studies Program Review Committee (MISP-RC) IIS Clinical Concept Form

All fields are required, an incomplete form will be returned to the submitter. If a field is not completed, please note the reason.

Proposed Study Title	
Study Title:	**The effect of glucagon on rates of hepatic mitochondrial oxidation and pyruvate carboxylase flux in man assessed by Positional Isotopomer NMR Tracer Analysis (PINTA)**
Request Date:	**July 1, 2019**
Principal Investigator Contact Information	
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Contracting Information (if applicable)	
Name:	**N/A**
Study Information	
Indication	**N/A**
Phase:	**N/A**
Number of Subjects:	**12**

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Background and Rationale

- Provide background on unanswered question(s) the study is attempting to answer (do not exceed one page)

While it is well-established that alterations in the portal vein insulin/glucagon ratio play a major role in causing dysregulated hepatic glucose metabolism in type 2 diabetes (T2D), the cellular and molecular mechanisms by which glucagon promotes alterations in hepatic glucose production and mitochondrial oxidation remain poorly understood. This is borne out by the fact that both glucagon agonists and glucagon antagonists are being developed to treat T2D, with unclear mechanisms of action.

Glucagon has long been considered to be one of the major drivers of hyperglycemia in T2D, and glucagon-blocking therapies have been pursued as adjuncts to therapy for both type 1 and type 2 diabetes. Glucagon antagonism using either antibodies against glucagon or its receptor, small molecule antagonists of the glucagon receptor, and antisense oligonucleotides to knock down expression of the glucagon receptor have all shown promising glucose-lowering effects in humans and in animal models of T2D. However, concerns have been raised regarding the potential of these agents to increase liver enzymes by an unknown mechanism. Conversely, a dual glucagon-like peptide-1/glucagon receptor agonist has recently been shown in rodents and non-human primates to lower blood glucose concentrations, associated with increased energy expenditure and weight loss. Taken together, these data suggest a potential role for glucagon to promote hepatic mitochondrial fat oxidation and suggest that a lack of glucagon action may suppress hepatic fat oxidation and predispose to ectopic hepatic lipid accumulation and non alcoholic fatty liver disease (NAFLD).

The impact of glucagon on hepatic mitochondrial oxidative metabolism in humans is unknown but previous studies have suggested a capacity for glucagon to increase liver fatty acid metabolism *in vitro*, and to stimulate hepatic fat oxidation in awake dogs. As predicted by these data, multiple investigators have observed increases in CO_2 generation, O_2 consumption, and activity of the respiratory transport chain in response to glucagon stimulation, consistent with increased mitochondrial tricarboxylic acid (TCA) cycle flux; however, the mechanism by which glucagon stimulate hepatic mitochondrial oxidation and its impact on hepatic mitochondrial oxidation *in vivo* are unknown. In addition we will assess the effects of a physiological increase in plasma glucagon concentrations on rates of hepatic ketogenesis assessed by an infusion of $^{13}C_4$ β–OHB (Perry et al. **Cell Metabolism** 2017). We have recently shown that allosteric regulation of pyruvate carboxylase by changes in hepatic acetyl-CoA is a key mechanism by which insulin (Perry et al. **Cell** 2015) and glucagon (unpublished results) regulate hepatic gluconeogenesis and that rates of β–OHB turnover are an excellent non invasive surrogate for hepatic acetyl-CoA content (Perry et al. **Cell Metabolism** 2017).

This proposal will directly assess the effect of glucagon on rates of hepatic mitochondrial oxidation and pyruvate carboxylase flux in humans for the first time using Positional Isotopomer NMR Tracer Analysis (PINTA) (Perry et al. **Nature Communications** 2017). The results of these studies have important implications for the possibility of intervening in the pathogenesis of NAFLD-associated hepatic insulin resistance and T2D via chronic dual glucagon-like peptide-1/glucagon receptor agonism and provide an important rationale for why a dual agonist might be more efficacious for treating NAFLD/NASH/T2D than GLP-1 alone.

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Objectives

- List the objectives to correspond directly with the listed hypotheses:

To examine the effects of glucagon on hepatic glucose and fat metabolism *in vivo*, we plan to apply a novel Positional Isotopomer NMR Tracer Analysis (PINTA) method to quantify rates of hepatic mitochondrial oxidation (V_{CS}) and pyruvate carboxylase flux (V_{PC}), which has been cross-validated in awake rodents and humans (Perry et al. **Nature Communications** 2017). In preliminary rodent studies we have found that glucagon stimulates intrahepatic lipolysis through an $InsP_3R$-I-dependent process, leading to increases in hepatic acetyl-CoA content, which allosterically activates pyruvate carboxylase activity and flux (V_{PC}), and that this phenomenon explains its acute, transcription-independent effect to acutely stimulate hepatic gluconeogenesis *in vivo* (unpublished results). In addition, using PINTA analysis we found that glucagon stimulates hepatic mitochondrial oxidation (V_{cs}) through $InsP_3R$-I-mediated calcium signaling in awake mice, and that this process can be exploited by short-term continuous glucagon treatment leading to two-fold increases in hepatic mitochondrial fat oxidation (V_{FAO}), which in turn results in large reductions in hepatic steatosis and marked improvements in glucose tolerance through reversal of hepatic insulin resistance in a high fat fed rat model of NAFLD.

Hypothesis

- List the clinical Hypotheses in order of priority:

1. A physiological increase in plasma glucagon concentrations will promote a significant increase in rates of hepatic mitochondrial oxidation (V_{CS}) in healthy humans.
2. A physiological increase in plasma glucagon concentrations will promote a significant increase in rates of hepatic pyruvate carboxylase flux (V_{PC}) in healthy humans.
3. A physiological increase in plasma glucagon concentrations will promote a significant increase in rates of $^{13}C_4$ β-OHB turnover (hepatic ketogenesis) in healthy humans.

Study Design/Clinical Plan

- Provide a concise overview stating the type of experimental design

The effects of a physiological increase in plasma glucagon on rates of hepatic mitochondrial oxidation and pyruvate carboxylase flux will be examined in 12 healthy subjects (ages 21-65) using Positional Isotopomer NMR Tracer Analysis (PINTA) (Perry et al. **Nature Communication** 2017). Briefly rates of hepatic mitochondrial oxidation (V_{CS}) and hepatic pyruvate carboxylase flux (V_{PC}) will be assessed in 12 healthy overnight fasted subjects by PINTA after a three-hour infusion of glucagon or saline. The glucagon infusion [3 ng/(kg-min)] will be designed to increase peripheral and portal vein plasma glucagon concentrations 3-4 fold. The effects of a physiological increase in plasma glucagon on rates of hepatic ketogenesis will also be assessed using an infusion of $^{13}C_4$ β–OHB (Perry et al. **Cell Metabolism** 2017).

Rates of hepatic pyruvate carboxylase flux (V_{PC})/citrate synthase flux (V_{CS}) by PINTA: Subjects (n=12) will be studied by PINTA under 2 conditions: 1) at 0700 following an overnight (12h) fast and a 3h saline infusion (Control), 2) at 0700 following an overnight (12h) fast and a 3h glucagon infusion @ [3 ng/(kg-min)] (Glucagon Rx). Briefly, on the morning of the study an IV line will be inserted into an antecubital vein for infusions and second IV line will be inserted retrogradely into a hand vein for blood collections. The hand will be warmed in a 'hot box' to arterialize the blood. After collection of baseline blood samples a 3h infusion of tracers as described below will be started. Relative rates of V_{PC}/V_{CS} will be assessed using a constant

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infusion of [3-^{13}C] lactate (8.7 µmol/[Kg-min], 99% APE) and rates of glucose production will be measured using a primed/constant infusion of [$^{2}H_7$]glucose (42 mg/m^2 - 0.84 mg/[m^{2}min], 99% APE) (Perry et al. **Nature Communication** 2017). Rates of hepatic ketogenesis will be measured using a constant infusion of [$^{13}C_4$]βOHB [10µg/(Kg-min), 99% APE] as previously described (Perry et al. **Cell Metabolism** 2017). This protocol has been approved the Yale Human Investigation Committee (#20997).

GC/MS Method for Quantifying [$^{13}C_4$]β OHB turnover: Analysis of the 13C-enrichment in β OHB will be performed by GC-MS. Samples (100 µL plasma) will be dried under N_2 gas and derivatized with 200 µL n- butanol 4N HCl, then heated for 60 min at 65°C and dried under N_2 gas. The samples will next be reacted with100 µL of trifluoroacetic acid (Thermo Fisher Scientific)-methylene chloride (Sigma) (1:7). [$^{13}C_4$]βOHB enrichment will be measured by GC-MS (CI mode, m/z 257 [m+0] and 261 [m+4]).

Whole body energy expenditure, VO_2, VCO_2, respiratory quotient assessed by indirect calorimetry: Rates of total-body glucose and lipid oxidation and energy expenditure will be calculated from rates of O_2 consumption and CO_2 production measured during continuous indirect calorimetry.

This trial is registered at CT.gov: NCT 03965130

References

1. Perry RJ, Peng L, Cline GW, Petersen KF, Shulman GI. A non-invasive method to assess hepatic acetyl-CoA in vivo. ***Cell Metab.*** 2017; 25(3) 749-756. PMCID: PMC5342911. PMID: 28111213.
2. Perry RJ, Peng L, Cline GW, Butrico GM, Wang Y, Zhang XM, Rothman DL, Petersen KF, Shulman GI. Non-invasive assessment of hepatic mitochondrial metabolism by positional isotopomer NMR tracer analysis (PINTA). ***Nature Communications***, 2017; 9 (1) 1-9. PMCID: PMC5630596, PMID: 28986525.
3. Perry RJ, Wang Y, Cline GW, Rabin-Court A, Song JD, Dufour S, Zhang XM, Petersen KF, Shulman GI. Leptin mediates a glucose-fatty acid cycle to maintain glucose homeostasis in starvation. ***Cell***. 2018; 172 (1-2) 234-248. PMCID: PMC5766366, PMID: 29307489.

Treatment

- **List the clinical dosage/dosage form, route, and dose regimen:**

Glucagon will be infused intravenously at rate of 3 ng/(kg-min) for three hours.

Collateral Research

- **Include biomarkers, PK, etc.**

Plasma samples will be collected at the end of each infusion study and banked for future metabolomic analyses to determine if any plasma metabolites track with glucagon-stimulated rates of hepatic mitochondrial oxidation and/or pyruvate carboxylase flux.

Statistical Plans

- **Include justification for clinical sample size and primary hypothesis testing:**

A group size of 12 subjects was determined based on power calculations to detect a moderate to large difference in the effects of glucagon on rates of hepatic mitochondrial oxidation (V_{CS}). The key endpoints will be to determine if glucagon treatment results in significant differences in: 1) rates of hepatic mitochondrial oxidation, 2) rates of hepatic pyruvate carboxylase flux (V_{PC}), 3) rates of hepatic glucose production, and 4) rates of hepatic β–OHB turnover. Statistical differences in these

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parameters (within the same individuals) for saline vs. glucagon treatment will assessed by paired t-tests.

Budget Summary

- Please be sure to complete budget template (excel document)

Total Amount Requested: (Include overhead)	$1,295,423 (including 30% Yale overhead)
Additional sources of funding required? (Yes/No) If Yes, please be specific.	No

Timelines and Study Plans

Number of Sites:	1
Site Names:	**Yale Center for Clinical Investigation, Hospital Research Unit**
Study Start Date:	**July 1, 2019**
Study End Date:	**June 30, 2020**
Number of Subjects:	**10**
First Patient In Date:	**July 1, 2019**
Last Patient Out Date:	**May 30, 2020**
Enrollment Period in Months:	**11**

Publication Plan

Where are you planning to submit for publication? (journals, etc):	**Cell Metabolism, Journal of Clinical Investigation, Nature Medicine, PNAS**
Are you planning to present your data at a scientific meeting?	
Please list your target date for submission of publication.	**Approximately 2020**

Drug Supply Information

Drug Supplies Required (Yes/No)?	No
List Drug Supplies and Amount Required:	**Drug Name:** **Amount:**
List Drug Supplies and Amount Required:	**Drug Name:** **Amount:**
Placebo Required (Yes/No)?	No

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Additional Sources of Drug Supply (Yes/No). If Yes, please specify	No

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