Hormonal regulation of ketone bodies in prolonged-fasting northern elephant seal pups

Background and Rationale: Prolonged food deprivation in mammals is typically associated with a downregulation in plasma insulin and glucose concentrations. As a result, prolonged fasting mammals experience an upregulation in circulating plasma non-esterified fatty acids (NEFA), which are oxidized to meet energetic demands. Plasma concentration of ketone bodies then increases to generate extra metabolic fuel. Over time, increased production of ketone bodies decreases blood pH, which leads to ketoacidosis that may impair organ function.

One month after birth, northern elephant seal (NES) pups undergo a 2-3 month prolonged, post-weaning fast characterized by a reliance on NEFA oxidation for 95% of their metabolic rate (RQ=0.73).[1] Despite this reliance on lipid oxidation, levels of ketone bodies such as β-hydroxybutyrate (β-HBA) are low in NES pups, with plasma β-HBA levels peaking at 2.0 mM compared to over 10 mM in other fasting mammals.[2] At the end of the fast, NES pups experience a ketone deflection point, a phenomenon where ketone body concentrations in NES pups suddenly decrease. This has not been observed outside of a laboratory setting, and as such, the mechanisms by which NES pups regulate plasma ketone bodies remains unknown. Two possibilities include the inhibition of ketogenesis or an upregulation in ketone body clearance via increased cellular uptake of ketone bodies or ketolysis. However, the elevation of pyruvate cycling and PEPCK in NES pups with fasting duration suggests that ketone body concentration is regulated via increased flux of acetyl-CoA into the tricarboxylic acid (TCA) cycle.[1]

Elevated concentration of insulin—a major regulatory hormone—decreases plasma ketone bodies in mammals via a downregulation of lipolysis and an upregulation of extrahepatic ketone body utilization.[3] In contrast, elevated cortisol in mammals increases plasma ketone bodies through impaired insulin secretion and an upregulation of lipolysis and ketogenesis.[4] NES pups do not experience health detriments such as ketoacidosis, which suggests that they have evolved the proper intracellular mechanisms and hormonal responses required to regulate substrate metabolism during periods of high physiological stress. As such, further studies must be conducted to elucidate the role of insulin and cortisol in ketone body regulation in NES pups. To accomplish this, we propose to identify metabolites implicated in ketone body regulation through metabolomics analyses with two exogenous challenges: adrenocorticotropic hormone (ACTH)—which increases cortisol levels—and insulin. We will also characterize the changes in metabolite concentration with fasting duration. We hypothesize that the concentrations of key metabolites contributing to ketone body regulation in NES pups will change in response to exogenous cortisol and insulin challenges, and that concentrations of these metabolites also change before and after the ketone deflection point. We predict that the mechanism by which ketone bodies are regulated occurs via increased flux of acetyl-CoA through the TCA cycle.

Aim 1: Identification of putative intermediates via metabolomics analysis after ACTH administration. Administration of ACTH results in elevated cortisol, and therefore elevated plasma ketone bodies. Conducting a metabolomics analysis after administration of ACTH will enable us to characterize the biochemical changes of metabolites in response to elevated plasma ketone bodies, and preliminarily determine the putative metabolites that regulate this system.

Aim 2: Comparison of putative intermediates after insulin infusion. Insulin sensitivity decreases with fasting duration. As such, we will conduct a metabolomics study examining concentration changes of the putative intermediates in response to insulin. This analysis will elucidate relative changes in insulin-mediated signaling of ketone bodies with fasting duration.

Methods: To accurately characterize changes before and after the ketone deflection point, NES pups will undergo exogenous challenges at three points in the fast: early (2-3 wks),

mid (4-6 wks), and late (6-8 wks). We will measure the body mass of the pups and collect pre-infusion blood samples. NES pups will be injected intramuscularly with ACTH gel (0.152 IU kg^{-1}) or intravenously infused with insulin (0.065 IU kg^{-1}), and control pups will be infused with saline. Post-infusion blood samples will be collected at the following time points: 5, 10, 15, 30, 45, 60, 90, 120, and 150 minutes post-infusion. To identify significant changes in metabolite concentrations at each time point, the blood samples will be prepared and analyzed via mass spectroscopy. Additionally, the concentration of plasma NEFAs and the glucoregulatory hormones insulin, glucagon, and cortisol will be quantified. Linear mixed-modeling will be employed to consider a relationship between changes in putative intermediates and concentrations of these hormones in response to exogenous challenge.

Anticipated Results: Metabolites that characterize regulatory pathways for ketone bodies will be determined via changes in magnitude of concentration. We will compare these changes within both challenge groups, and also consider hormone-dependent and fasting-dependent changes. Furthermore, we will examine changes in metabolites that are upstream and downstream of the ketogenesis, ketolysis, and TCA cycle pathways to identify the predominant mechanism of ketone body regulation. While it is possible that concentrations will not change significantly with ACTH or insulin challenges, prior studies in seals have demonstrated that ACTH and insulin have induced physiological and biochemical effects.[1,4] Nonetheless, this will not hinder the progression of the project, as investigating changes in metabolite concentrations with fasting duration in the saline-infused pups—before and after the ketone deflection point—will lead to valuable insights about key metabolites used to regulate ketone body concentration.

Intellectual Merit: This study is a robust characterization of the biochemical changes in metabolic pathways involved in the regulation of ketone bodies. The study also has translational aspects: diabetics are highly prone to ketoacidosis, yet NES pups, a model for metabolic syndrome, do not face this health detriment. Understanding the mechanisms by which NES pups regulate ketone bodies in spite of extreme physiological stress may lead to the development of alternative and preventative therapies for diabetic ketoacidosis.

Broader Impacts: Since the long-term aspects of this study have implications for diabetes treatments, I plan to visit local high schools, community colleges, and health clinics which have a large population of communities impacted by diabetes (specifically the Hispanic, Hmong, and African-American populations) to: (1) educate students about physiology as a tool to contribute to the health of their communities, and (2) introduce students to scientific research in order to increase involvement and retention of these populations in science.

Feasibility and Support: My prior experience studying NES pups and my skill with statistical programs such as R will make this project feasible to conduct. Additionally, I will take an advanced spectroscopy class to enhance my proficiency in mass spectroscopy. I will perform these studies with [redacted] and possesses the federal permit to study NES pups. Due to conservation laws, only blood and peripheral tissues such as adipose may be collected for study. Because the species recently suffered an extreme bottleneck, the population is homogenous, which will minimize individual variations and thus allows for the use of smaller sample sizes to detect changes.[5]

(1) Houser et al. American Journal of Physiology–Regulatory, Integrative, and Comparative Physiology. (2012) 303(5), pp.R562–R570. (2) Castellini et al. The American Journal of Physiology. (1990) 259(5 Pt 2), pp.R1086-9. (3) Keller et al. Diabetologia. (1988) 31(1), pp.24-9. (4) Ortiz et al. American Journal of Physiology–Regulatory, Integrative, and Comparative Physiology. (2001) 280(3), pp.R790-5. (5) Hoelzel et al. Journal of Heredity. (1993) 84(6), pp.443–449.