GDF15 Restricts Energy Intake on a Ketogenic Diet in Mice

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# Abstract

# Introduction

Ketogenic or low carbohydrate diets, often accompanied by an increase in dietary fat are increasingly common in the population with 16% of Americans reporting that they are on a carbohydrate restricted diet (1). Several randomized controlled trials have demonstrated weight loss, improved glycemic control, and reduced energy intake (reviewed in 2–4). For those individuals who lose weight on a LCHF diet, there is broad agreement that much of this effect is due to energy restriction with either modest or insignificant changes in energy expenditure (5,6). A recent meta-analysis showed decreased hunger and increased satiety on LCHF diets, though the hormonal mediators of this increased satiation remain unclear (7).

GDF15 is a hormone and emerging drug target that signals through GFRAL receptors in the hindbrain to reduce food intake. In humans, elevations of this hormone are associated with pregnancy-related nausea and cancer-associated cachexia (8–11). Elevations in GDF15 suppress appetite in a GFRAL-dependent manner. In terms of specific macronutrients, GDF15 causes a reduction in lipid consumption, and not other macronutrients (12). GDF15 is generated in many tissues in response to a variety of stressors but the integrated stress response has emerged as an important pathway controlling GDF15 production (13,14). Prior studies have implicated the hepatic integrated stress response to ketogenic diets (13,15,16). In this study we investigate the role of GDF15 in moderating energy intake, body composition and insulin sensitivity on a ketogenic diet.

# Methods

## Animal Handling and Diets

Animals were either purchased from the Jackson Laboratory (A/J mice; 000646, all resource identifiers are provided in Table 1) or were previously described (Gdf15 null; (12)). Diets were provided by Lab Diet (Normal Chow Diet; NCD, 5L0D) or Research Diets (Control Diet; CD; D1053001 or Ketogenic Diet; KD: D17053002). Mice were weaned on NCD until ten weeks of age and then transferred to CD or KD as described. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

## Ketone Body Determination

## Mouse Weights and Body Composition

## AML12 and Ketogenic Media

AML12 cells were purchased from ATCC (Cat# CRL-2254) and grown in DMEM with 10% FBS and penicillin/streptomycin/glutamine. To treat the cells we followed the protocol described in (17).

# Results

## GDF15 Is Induced on Mice Fed a Ketogenic Diet

To determine how mice respond to a ketogenic diet, we developed a custom ketogenic diet alongside a fiber, choline and protein matched control, rather than using standard mouse chow (see Table 2). We observed elevations of blood ketone levels after four weeks of ketogenic diet (Figure 1A). Upon sacrifice, we measured the levels of GDF15 in the blood and found XXX (Figure 1B). We next examined liver mRNA expression and found a similar XXX in both male and female mice. In a subsequent cohort of male mice, we evaluated GDF15 levels at both one and four weeks of CD or KD treatment and found XXX.

## Induction of Hepatic GDF15 Occurs with Activation of the Integrated Stress Response

To test whether hepatocytes were able to produce GDF15 under ketogenic conditions we treated AML12 cells with control or ketogenic media as described in (17)

## Ablation of GDF15 Results in Weight Gain and Increased Energy Intake on a Ketogenic Diet

# Discussion

In this study, the observed increases in GDF15 are relatively modest, but similar increases in GDF15 in humans are associated with pregnancy-related outcomes such as pre-eclampsia, nausea, gestational diabetes and miscarriage (8,18–20). This is also the approximate magnitude of exercise-associated elevations in GDF15 (21–25)

There are mixed data on the effects of hypercaloric diets in *Gdf15* or *Gfral* knockout mice with some papers showing hyperphagia and weight gain (26–29), but several others showing no effect (12,30,31) potentially representing strain, timing or background differences. As such, it is plausible that GDF15 is only physiologically relevant when elevated, but when signaling is absent (especially from birth) it is either dispensable or made to seem so by other adaptations. It is also plausible that other hormones which affect LCHF-dependent feeding changes may partially or completely compensate in the absence of GDF15.

# Author Contributions

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# Conflict of Interest

﻿RJS receives financial support from Novo Nordisk, Janssen, Zafgen, Kallyope, and Medimune. He has also served as a paid consultant for Novo Nordisk, Janssen, Kallyope, and Scohia. MGM receives research support from Novo Nordisk and MedImmune

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# Figure/Table Legends

Table 1: Composition of diets used in this study.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Control Diet | Ketogenic Diet | Normal Chow Diet |
| Carbohydrate | 75% | 0% | 36% |
| Protein | 15% | 15% | 24% |
| Lipid | 10% | 85% | 5% |

Table 2: Reagent resource identification information.

|  |  |  |
| --- | --- | --- |
| Type | Resource | Identifier |
| Mouse Line | A/J | RRID:IMSR\_JAX:000646 |
| Mouse Line | Gdf15 null |  |
| Diet | NCD |  |
| Diet | CD |  |
| Diet | KD |  |
| Cell Line | AML12 | RRID:CVCL\_0140 |