GDF15 Restricts Energy Intake on a Ketogenic Diet in Mice

Claire D. Gleason, Cody M. Cousineau, Detrick S. Snyder, Molly C. Mulcahy, JeAnna R. Redd, Randy J. Seeley and Dave Bridges

# Abstract

The ketogenic diet (KD), consisting of very-low-carbohydrate and high-fat content, is an emerging and effective method for weight loss and glucose control. In a previous study, mice were placed on a KD or matched controls (CD) for four weeks. Mice on a KD gained less fat mass and lean mass compared to those on CD despite consuming more calories. The satiety hormone, GDF15, was increased by the KD in both liver mRNA and blood. To further investigate the effects of GDF15 on appetite in relation to a KD, we used a GDF15-knockout model where mice do not have the ability to produce GDF15. We hypothesize that the GDF15-KO mice will consume more calories on a KD compared to their wildtype (WT) counterparts and gain more body mass on the CD. Another aspect of carbohydrate restriction is the activation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in glucocorticoid elevation. We compared cortisol levels and GDF15 levels in human patients and found that these were tightly correlated. To understand the relationship between the HPA axis and GDF15 we investigated serum and liver mRNA expression from mice treated with the synthetic glucocorticoid dexamethasone and found elevations in GDF15. To look at this response *in vitro* we exposed liver hepatoma cells, either AML12 or Hepa1c, to the glucocorticoid dexamethasone and found downregulation of GDF15 indicating that the *in vivo* activation may not be cell autonomous. Our study aims to elucidate some of the mechanisms by which GDF15 affects appetite and metabolic regulation of weight loss.

# 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, when GDF15 causes a specific reduction in lipid consumption, 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. Animals were euthanized via cervical dislocation under isoflurane anesthesia. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee.

## Food Preference Test

Food preference was measured by

## Ketone Body and GDF15 Determination

Total ketone bodies were determined using the Wako Autokit Total Ketone Bodies: (Cat#'s 415-73301, 411-73401 and 412-7379) using mouse serum. Rates of changes in absorbance were determined using a Synergy HTX plate reader (Biotek). GDF15 levels were determined using a Quantikine ELISA Assay (Cat# MGD150) following the manufacturer’s instructions.

## Mouse Weight 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). Briefly cells were treated with fresh DMEM/FBS or DMEM without glucose or serum, but supplemented with 50 M WY-14643 to activate PPAR and 2 mM sodium octanoate to supply lipids for conversion to ketones. After 48h cells were lysed and RNA was collected.

## Insulin Tolerance Tests

Mice were fasted for 6h starting at approximately ZT2 and blood was drawn via tail vein. Glucose was determined using an Accuchek glucometer. Insulin (Humulin HR from Lily) was injected at 0.75 mU/g of lean mass (as determined via echoMRI). Blood glucose was determined at 15 minute intervals.

## Identification of Potentially Secreted Proteins

Sequences annotated as having a signal peptide via SignalP (18) were downloaded from Ensembl via the biomart tool (19). These were further filtered removing genes annotated with the gene ontology term for cellular compartment – membrane (GO: 0016020). The transcriptomic dataset is published as GSE84312 and described in (20).

## Statistics

Statistical significance for this study was set at p=0.05. All statistical analyses were performed using R version 3.6.2 (21). For experiments using both sexes, a modifying effect of sex was tested for all outcomes and reported where significant based on the interaction from a 2x2 ANOVA. All raw data and analysis scripts can be found at <http://bridgeslab.github.io/TissueSpecificTscKnockouts/>.

# Results

## GDF15 Produced in Muscle Alters Preference Towards Fatty Foods

Our previous work, consistent with the work of others noted that activation of mTORC1 via tissue-specific *Tsc1* ablation results in lean mice that are resistant to diet-induced obesity (20,22,23). To identify potential factors secreted from muscle that may drive changes in systemic physiology we used transcriptomic data filtered to identify putatively secreted proteins. This identified 1817 potentially secreted gene products, of which 253 were significantly differentially expressed in *Tsc1* knockout muscles (Figure 1A, see Supplementary Table 1 for complete list). Among the most significantly altered genes was GDF15, which was upregulated 46 fold at the mRNA level (adjusted p-value 8.2 x 10-12). To test if this had an impact on circulating levels we performed ELISA assays on the blood from muscle *Tsc1* knockout animals and found a 8.7 fold increase in serum GDF15 (Figure 1B, p=4.7 x 10-5).

Based on our recent work, injection of GDF15 reduced the preference of mice to fat, but had little impact on protein or carbohydrate intake (12). We therefore reasoned that GDF15 inductions in muscle *Tsc1* knockout mice may result in an aversion to high fat, low carbohydrate diets relative to low fat high carbohydrate diets. We developed a custom pair of control and ketogenic diets, which were matched for fiber, protein and choline levels (see Table 2). We tested whether muscle *Tsc1* knockout mice preferred the high carbohydrate to the high fat diet when given the choice of both diets. Wild-type mice preferred the fat rich food by 11.7 fold after adjusting for differing caloric content. Muscle *Tsc1* knockout mice had a much lower preference, only consuming 2.6 fold more of the fat rich than the carbohydrate rich food. Together these data show that there is a functionally significant elevation of GDF15 in muscle *Tsc1* knockout mice that results in a reduced preference towards fat rich diets.

## GDF15 Is Induced on Mice Fed a Ketogenic Diet

These mice had XXX changes in fat mass and YYY changes in lean mass, while ZZZZ in food intake (Figures 1A-D). We confirmed elevations of blood ketone body levels after three weeks of ketogenic diet with 11.8 and 10.4 fold induction of total ketone bodies in male and female mice respectively relative to control diets (p<0.001, Figure 1F).

We found an induction of GDF15

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

While GDF15 is likely made in many tissues, due to the key role of the liver in responses to ketogenic diets, we examined liver mRNA expression and found a similar XXX in both male and female mice.

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

While the above studies describe induction of GDF15 under ketogenic conditions, they do not evaluate if this hormone plays a physiological role. To test this we placed male and female wild-type and *Gdf15* knockout mice on normal chow diets, followed by placing mice on KD at 10 weeks of age. We observed XXX changes in body weight and lean mass but an increase in fat mass (Figures 3A-C). Consistent with increases in fat mass, we observed XXX (Figure 3D)

To determine if there was any impact on insulin sensitivity in these mice we performed insulin tolerance tests and monitored changes in blood glucose. After a 6h fast we noted a sex-dependent effect on fasting glucose in Gdf15 knockout mice on a ketogenic diet (pinteraction=0.043). Female mice had a 19% reduction in fasting blood glucose (p=0.037) while male mice had a 3% increase (p=0.62; Figure 3E). After insulin injection, there were no significant effects of Gdf15 knockout in either sex (Figure 3F).

# 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 (8,24–26). This is also the approximate magnitude of exercise-associated elevations in GDF15 (27–31)

There are mixed data on the effects of hypercaloric diets in *Gdf15* or *Gfral* knockout mice with some papers showing hyperphagia and weight gain (32–35), but several others showing no effect (12,36,37) 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

# Acknowledgements

We would like to thank the members of the Bridges, Horowitz and Seeley/Sandoval laboratories for helpful suggestions. This work was supported by the NIH (R01DK107535 and a small grant from P30DK089503) to DB and XXX to RJS, as well as a MCubed Grant to DB, RJS and Dr. Jeffrey Horowitz. We would also like to than Dr. Hyeran Jang at Research Diets for advice on formulating and implementing our diet interventions.

# 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.

# References

1. **International Food Information Council Foundation.** *2018 Food and Health Survey*.; 2018. doi:10.1002/ejoc.201200111.

2. **Meng Y, Bai H, Wang S, Li Z, Wang Q, Chen L.** Efficacy of low carbohydrate diet for type 2 diabetes mellitus management: A systematic review and meta-analysis of randomized controlled trials. *Diabetes Res. Clin. Pract.* 2017;131:124–131.

3. **Huntriss R, Campbell M, Bedwell C.** The interpretation and effect of a low-carbohydrate diet in the management of type 2 diabetes: A systematic review and meta-analysis of randomised controlled trials. *Eur. J. Clin. Nutr.* 2018;72(3):311–325.

4. **Hall KD, Chung ST.** Low-carbohydrate diets for the treatment of obesity and type 2 diabetes. *Curr. Opin. Clin. Nutr. Metab. Care* 2018;21(4):308–312.

5. **Moberg M, Apró W, Ekblom B, van Hall G, Holmberg H-C, Blomstrand E.** Activation of mTORC1 by leucine is potentiated by branched chain amino acids and even more so by essential amino acids following resistance exercise. *Am. J. Physiol. Cell Physiol.* 2016:ajpcell.00374.2015.

6. **Ebbeling CB, Feldman HA, Klein GL, Wong JMW, Bielak L, Steltz SK, Luoto PK, Wolfe RR, Wong WW, Ludwig DS.** Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. *Bmj* 2018;363:k4583.

7. **Gibson AA, Seimon R V., Lee CMY, Ayre J, Franklin J, Markovic TP, Caterson ID, Sainsbury A.** Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes. Rev.* 2015;16(1):64–76.

8. **Petry CJ, Ong KK, Burling KA, Barker P, Goodburn SF, Perry JRB, Acerini CL, Hughes IA, Painter RC, Afink GB, Dunger DB, O’Rahilly SP.** Associations of vomiting and antiemetic use in pregnancy with levels of circulating GDF15 early in the second trimester: A nested case-control study. *Wellcome Open Res.* 2018;3(0):123.

9. **Fejzo MS, Fasching P, Schneider M, Schwitulla J, Beckmann M, Schwenke E, MacGibbon K, Mullin P.** Analysis of GDF15 and IGFBP7 in Hyperemesis Gravidarum Support Causality. *Geburtshilfe Frauenheilkd.* 2019:382–388.

10. **Johnen H, Lin S, Kuffner T, Brown DA, Tsai VW-W, Bauskin AR, Wu L, Pankhurst G, Jiang L, Junankar S, Hunter M, Fairlie WD, Lee NJ, Enriquez RF, Baldock PA, Corey E, Apple FS, Murakami MM, Lin EJ, Wang C, During MJ, Sainsbury A, Herzog H, Breit SN.** Tumor-induced anorexia and weight loss are mediated by the TGF-β superfamily cytokine MIC-1. *Nat. Med.* 2007;13(11):1333–1340.

11. **Welsh JB, Sapinoso LM, Kern SG, Brown DA, Liu T, Bauskin AR, Ward RL, Hawkins NJ, Quinn DI, Russell PJ, Sutherland RL, Breit SN, Moskaluk CA, Frierson HF, Hampton GM.** Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. *Proc. Natl. Acad. Sci.* 2003;100(6):3410–3415.

12. **Frikke-Schmidt H, Hultman K, Galaske JW, Jørgensen SB, Myers MG, Seeley RJ.** GDF15 acts synergistically with liraglutide but is not necessary for the weight loss induced by bariatric surgery in mice. *Mol. Metab.* 2019;21(xxxx):13–21.

13. **Zhang M, Sun W, Qian J, Tang Y.** Fasting exacerbates hepatic growth differentiation factor 15 to promote fatty acid β-oxidation and ketogenesis via activating XBP1 signaling in liver. *Redox Biol.* 2018;16(February):87–96.

14. **Patel S, Alvarez-Guaita A, Melvin A, Rimmington D, Dattilo A, Miedzybrodzka EL, Cimino I, Maurin A-C, Roberts GP, Meek CL, Virtue S, Sparks LM, Parsons SA, Redman LM, Bray GA, Liou AP, Woods RM, Parry SA, Jeppesen PB, Kolnes AJ, Harding HP, Ron D, Vidal-Puig A, Reimann F, Gribble FM, Hulston CJ, Farooqi IS, Fafournoux P, Smith SR, Jensen J, Breen D, Wu Z, Zhang BB, Coll AP, Savage DB, O’Rahilly S.** GDF15 Provides an Endocrine Signal of Nutritional Stress in Mice and Humans. *Cell Metab.* 2019;29(3):707-718.e8.

15. **Garbow JR, Doherty JM, Schugar RC, Travers S, Weber ML, Wentz AE, Ezenwajiaku N, Cotter DG, Brunt EM, Crawford PA, Jr G, Jm D, Rc S, Travers S, Ml W, Ae W, Ezenwajiaku N, Dg C, Em B, Pa C.** Hepatic steatosis, inflammation, and ER stress in mice maintained long term on a very low-carbohydrate ketogenic diet. *Am. J. Physiol. Liver Physiol.* 2011;300(6):G956–G967.

16. **Douris N, Melman T, Pecherer JM, Pissios P, Flier JS, Cantley LC, Locasale JW, Maratos-Flier E.** Adaptive changes in amino acid metabolism permit normal longevity in mice consuming a low-carbohydrate ketogenic diet. *Biochim. Biophys. Acta - Mol. Basis Dis.* 2015;1852(10):2056–2065.

17. **Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM.** MTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010;468(7327):1100–1106.

18. **Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H.** SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat. Biotechnol.* 2019;37(4):420–423.

19. **Durinck S, Spellman PT, Birney E, Huber W.** Mapping identifiers for the integration of genomic datasets with the R/ Bioconductor package biomaRt. *Nat. Protoc.* 2009;4(8):1184–1191.

20. **Stephenson EJ, Redd JR, Snyder DS, Tran QT, Lu B, Peloquin MJ, Mulcahy MC, Harvey I, Fisher K, Han JC, Qi N, Saltiel AR, Bridges D.** Skeletal Muscle mTORC1 Activation Increases Energy Expenditure and Reduces Longevity in Mice. *bioRxiv* 2019. doi:10.1101/720540.

21. **R Core Team.** R: A Language and Environment for Statistical Computing. 2019.

22. **Guridi M, Tintignac LA, Lin S, Kupr B, Castets P, Rüegg MA.** Activation of mTORC1 in skeletal muscle regulates whole-body metabolism through FGF21. *Sci. Signal.* 2015;8(402):ra113–ra113.

23. **Guridi M, Kupr B, Romanino K, Lin S, Falcetta D, Tintignac L, Rüegg MA.** Alterations to mTORC1 signaling in the skeletal muscle differentially affect whole-body metabolism. *Skelet. Muscle* 2016;6(1):13.

24. **Sugulle M, Dechend R, Herse F, Weedon-Fekjaer MS, Johnsen GM, Brosnihan KB, Anton L, Luft FC, Wollert KC, Kempf T, Staff AC.** Circulating and placental growth-differentiation factor 15 in preeclampsia and in pregnancy complicated by diabetes mellitus. *Hypertension* 2009;54(1):106–112.

25. **Tang M, Luo M, Lu W, Wang S, Zhang R, Liang W, Gu J, Yu X, Zhang X, Hu C.** Serum growth differentiation factor 15 is associated with glucose metabolism in the third trimester in Chinese pregnant women. *Diabetes Res. Clin. Pract.* 2019:107823.

26. **Tong S, Marjono B, Brown DA, Mulvey S, Breit SN, Manuelpillai U, Wallace EM.** Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. *Lancet* 2004;363(9403):129–130.

27. **Munk PS, Valborgland T, Butt N, Larsen AI.** Response of growth differentiation factor-15 to percutaneous coronary intervention and regular exercise training. *Scand. Cardiovasc. J.* 2011;45(1):27–32.

28. **Galliera E, Lombardi G, Marazzi MG, Grasso D, Vianello E, Pozzoni R, Banfi G, Corsi Romanelli MM.** Acute exercise in elite rugby players increases the circulating level of the cardiovascular biomarker GDF-15. *Scand. J. Clin. Lab. Invest.* 2014;74(6):492–499.

29. **Joung KH, Kim JM, Yi H-S, Lee JH, Kim KS, Kim HJ, Shong M, Ku BJ.** Effects of exercise program on normal responsiveness of serum GDF15 in middle-aged women. *Diabetes Res. Clin. Pract.* 2016;120:S65–S66.

30. **Kleinert M, Clemmensen C, Sjøberg KA, Carl CS, Jeppesen JF, Wojtaszewski JFP, Kiens B, Richter EA.** Exercise increases circulating GDF15 in humans. *Mol. Metab.* 2018;9(January):187–191.

31. **Zhang H, Fealy CE, Kirwan JP.** Exercise Training Promotes a GDF15 Associated Reduction in Fat Mass in Older Adults with Obesity. *Am. J. Physiol. Metab.* 2019:ajpendo.00439.2018.

32. **Mullican SE, Lin-Schmidt X, Chin C-N, Chavez JA, Furman JL, Armstrong AA, Beck SC, South VJ, Dinh TQ, Cash-Mason TD, Cavanaugh CR, Nelson S, Huang C, Hunter MJ, Rangwala SM.** GFRAL is the receptor for GDF15 and the ligand promotes weight loss in mice and nonhuman primates. *Nat. Med.* 2017;23(10):1150–1157.

33. **Hsu JY, Crawley S, Chen M, Ayupova DA, Lindhout DA, Higbee J, Kutach A, Joo W, Gao Z, Fu D, To C, Mondal K, Li B, Kekatpure A, Wang M, Laird T, Horner G, Chan J, Mcentee M, Lopez M, Lakshminarasimhan D, White A, Wang SP, Yao J, Yie J, Matern H, Solloway M, Haldankar R, Parsons T, Tang J, Shen WD, Chen YA, Tian H, Allan BB.** Non-homeostatic body weight regulation through a brainstem-restricted receptor for GDF15. *Nature* 2017;550(7675):255–259.

34. **Tran T, Yang J, Gardner J, Xiong Y.** GDF15 deficiency promotes high fat diet-induced obesity in mice. Peterson JM, ed. *PLoS One* 2018;13(8):e0201584.

35. **Tsai VW-W, Zhang HP, Manandhar R, Schofield P, Christ D, Lee-Ng KKM, Lebhar H, Marquis CP, Husaini Y, Brown DA, Breit SN.** GDF15 mediates adiposity resistance through actions on GFRAL neurons in the hindbrain AP/NTS. *Int. J. Obes.* 2019. doi:10.1038/s41366-019-0365-5.

36. **Emmerson PJ, Wang F, Du Y, Liu Q, Pickard RT, Gonciarz MD, Coskun T, Hamang MJ, Sindelar DK, Ballman KK, Foltz LA, Muppidi A, Alsina-Fernandez J, Barnard GC, Tang JX, Liu X, Mao X, Siegel R, Sloan JH, Mitchell PJ, Zhang BB, Gimeno RE, Shan B, Wu X.** The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat. Med.* 2017;23(10):1215–1219.

37. **Yang L, Chang C-C, Sun Z, Madsen D, Zhu H, Padkjær SB, Wu X, Huang T, Hultman K, Paulsen SJ, Wang J, Bugge A, Frantzen JB, Nørgaard P, Jeppesen JF, Yang Z, Secher A, Chen H, Li X, John LM, Shan B, He Z, Gao X, Su J, Hansen KT, Yang W, Jørgensen SB.** GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nat. Med.* 2017;23(10):1158–1166.

# 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: 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 |
| Resource | SignalP | RRID:SCR\_015644 |
| Resource | Biomart | RRID:SCR\_010714 |
| Resource | Gene Expression Omnibus | RRID:SCR\_005012 |

**Figure 1: Muscle *Tsc1* knockout mice have elevated GDF15 and a lowered preference wto high fat foods.** A) Volcano plot of predicted secreted proteins, dark shading indicates statistical significance. B) GDF15 levels in male muscle *Tsc1* knockout mice. C) Preference of fat rich ketogenic diet relative to control diet in male muscle *Tsc1* knockout mice. Dashed line indicates equal caloric intake from both diets (n=6,9). Asterisks indicate p<0.05

**Figure 2: GDF15 is induced upon feeding A/J mice a ketogenic diet.** A) Body weight of male and female mice on a control or ketogenic diet. B) Total fat mass and C) Lean mass from A). D) Energy intake during KD feeding. E) Ketone body levels at 3 weeks of age from fed serum (n=7-8/group). F) GDF15 levels at four weeks of age.