J Nutr Health Aging.2022;26(7):657-662
Published online June 13, 2022, https://doi.org/10.1007/s12603-022-1815-7

Original Research

Modestly Increased Incidence of Ketosis in Caloric Restriction Does not Significantly Alter the Effects of Caloric Restriction

B.S. Ferguson[1,2], P. Sahoo[3], E. McGrail[3], A. Francois[3], M.S. Stratton[3]

1. Department of Nutrition, College of Agriculture, Biotechnology & Natural Resources, University of Nevada, Reno, Reno NV, 89557 USA; 2. Center of Biomedical Research Excellence for Molecular and Cellular Signal Transduction in the Cardiovascular System, University of Nevada, Reno, Reno, NV 89557, USA; 3. Department of Physiology & Cell Biology, Davis Heart and Lung Research Institute, Ohio State University Wexner Medical Center, Columbus OH, 43210 USA

Corresponding Author: Matthew S Stratton, Department of Physiology & Cell Biology, Davis Heart and Lung Research Institute, Ohio State University Wexner Medical Center, Columbus OH, 43210 USA, matthew.stratton@osumc.edu

Abstract

OBJECTIVES: Caloric restriction (CR) has been shown to slow the aging processes in a number of preclinical studies and reduces expression of aging-associated biomarkers in human trials. We hypothesized that CR would lead to increased incidence of ketosis and that ketosis in CR individuals would alter the aging-protective effects of CR or biomarkers thereof.

DESIGN/SETTING/PARTICIPANTS: We analyzed data from the "Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE, Phase 2)" Public Use Database available at calerie.duke.edu. In this study, non-obese adults between the ages of 21 and 50 were randomized to 25% CR or control (ad lib) diet groups and extensively monitored for two years. Given our focus on the effect of caloric restriction on ketosis, individuals with detectible ketones during the baseline visit (pre-randomization) and those with missing data for ketone testing were excluded from the analysis, leaving 71 control and 117 CR participants.

MEASUREMENTS: We analyzed the incidence of ketosis as well as ketosis free survival in control and CR participants and assessed the effect of ketosis on a number of clinical lab values, functional assessments, and participant survey data related to aging biology.

RESULTS: We report that CR was associated with modestly increased incidence of ketosis (4.4% in CR vs 1.9% in control), though CR-associated changes in T3, VO2, SUMPT-WT (weight normalized composite strength score - peak torque), physical functioning, and general health did not appear to be altered by the presence or absence of ketosis. Additional observations of interest include: 1) striking patterns of biomarker expression changes (MCP-1, TNFα, TGF-β1, GH) in both the control and CR participants between the baseline visit and the 24-month post-randomization visit and 2) pro-growth/anti-inflammatory baseline (pre-randomization) biomarker expression profile in CR individuals that later test ketone positive relative to other CR individuals.

CONCLUSIONS: CR modestly increases the incidence of ketosis in healthy adults, yet the increase in ketosis in CR patients did not significantly affect the aging-protective effects of CR. However, given the relatively small number of participants who were ketone positive, further investigation in larger study cohorts is still required for definitive conclusions.

Key words: Caloric restriction, ketosis, CALERIE phase 2.

Introduction

Aging is associated with a number of pathologic morphological and functional changes throughout the body. A driving factor in these pathological changes is chronic low-grad inflammation that is observed systemically with advanced age (1). Caloric restriction (CR) has long been known to the slow aging processes in model organisms from yeast to rodents, leading to increased healthspan and lifespan (2). More recent work in humans and non-human primates indicates that CR improves aging-associated morbidities and biomarkers thereof (3-14). It is thought that these benefits of CR are mediated in part, by reducing systemic inflammation (14). The ketogenic diet (KD) has also been associated with increased healthspan and lifespan in model organisms, though the impact of this dietary intervention in the human population is not without controversy. The majority of rodent studies indicate that KD reduces inflammation (e.g. 15-18), however data in human KD subjects is mixed. Several human studies indicate that KD causes increased serum concentrations of molecules classically associated with activation of inflammation, for instance C-reactive protein (19), while many suspect that at least in the presence of an acute disturbance, KD leads to anti-inflammatory effects (e.g. 20-21).

Multiple molecular species of ketone bodies or ketones are generated by the liver when fatty acids are the predominant available fuel source. This can occur during periods of fasting (requiring mobilization of stored fat), with high fat/low carbohydrate diets, or when insulin signaling is impaired and intracellular glucose concentrations are low (diabetes). Ketones are bioactive molecules that can serve as alternate energy substrates and can modulate cellular signaling as receptor ligands (Reviewed in 22). Given that ketones are generated when glycogen stores are depleted, we hypothesized that CR would lead to increased incidence of ketone detection and that biomarker/functional assessments in CR ketone positive individuals might be informative regarding ketone risks and benefits in humans.

The CALERIE Phase II trial randomized human participants to ad libitum (control) or 25% CR groups and closely monitored these participants over two years with diet/exercise logs and quarterly clinical visits. These visits included a variety

Received May 2, 2022
Accepted for publication May 20, 2022

of lab tests, functional tests and a battery of patient surveys. The effects of CR in this patient population have been widely published. Notable prior findings include significant weight loss (23, 24), decreased cardiometabolic risk factors (25), improved weight normalized cardiovascular fitness (VO2max/BW) and weight normalized muscle strength (26), and reduced abundance of inflammation biomarkers (14) in the CR group relative to controls. We took advantage of the Public Use Database for this trial to determine if CR was associated with increased ketosis, as measured by standard urinalysis, and if attaining ketosis with CR modified any CR-dependent effects.

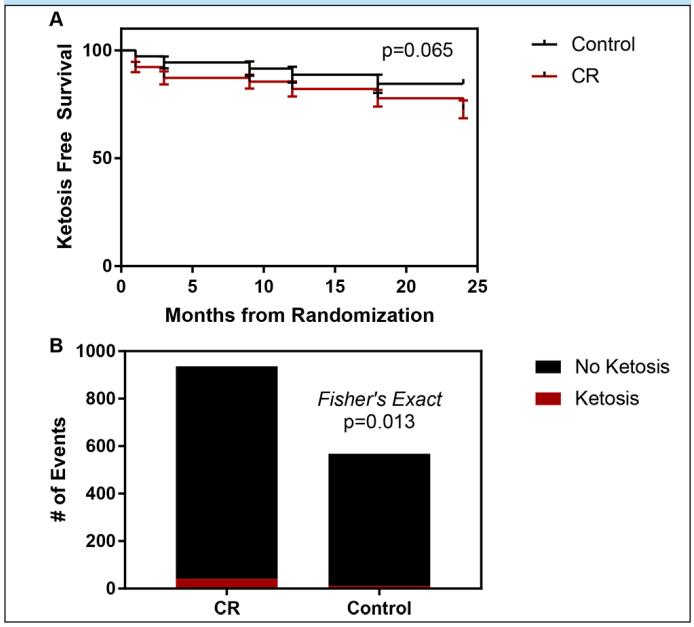
Methods

Clinical lab data: To compare incidence of ketosis and ketone free survival in the CR and control groups, individuals with ketosis detected at baseline and with missing values were removed (leaving N=71 control and N=117 CR). Then the log-rank (Mantel-Cox) test was employed to compare survival curves and chi-square and Fisher's exact tests were used to compare overall incidence. For other analyses, individuals with missing ketone tests were not excluded, though these individuals also tended to have other missing values (i.e. were likely re-excluded because they didn't have data for the measurement of interest). The change of lab values between the end of study and the baseline was defined as the difference between two time points. Levene's test for homogeneity of variance across groups was calculated. The Shapiro-Wilk test of normality was performed to test the normality of the change of lab variables. Non-parametric methods were applied since the lab data didn't pass the normality check. Wilcoxon test was used in testing the effect of ketosis on lab value changes. A non-parametric two-way ANOVA, Scheirer–Ray–Hare test, was performed to test the effects of ketosis status, treatment, and their interaction on lab value changes. A p value of less than 0.05 is considered statistically significant. Functional and survey response data were processed similar to lab variables, where homogeneity of variance, normality and outliers were examined. Wilcoxon test was used in testing the effect of treatment on functional value changes. For visualization, data were loaded into GraphPad Prism and some figures contain more straightforward post-hoc statistical analyses (e.g. t test) when of comparative value and are disclosed as such in the figure legend.

Analyses were conducted on Ketone, GH (growth hormone), TGF-β1 (transforming growth factor beta 1), MCP-1 (monocyte chemoattractant protein 1), Norepi (Norepinephrine), T3 (triiodothyronine/thyroid hormone), TNFα (tumor necrosis factor alpha), and TSH (thyroid stimulating hormone) lab values. Analyzed functional and survey results included mean VO2, mean VE (ventilatory equivalents), weight normalized composite strength score-peak torque, peak force –dominant, perceived stress score, and additional measurements from the SF-36 survey (pfscore, rlphscore, flephscore, efscore, ewbscore, sfscore, pain score, ghscore). Results for analyses not included in the main text can be found in Supplemental Table 1.

Research Subjects: The CALERIE Phase II trial was carried out using an established protocol (available at calerie.duke.edu/files/phase2_protocol.pdf) approved and monitored by an NIA appointed Data and Safety Monitoring Board and multiple institutional review boards. Relevant participant confidentiality, HIPAA considerations, and Informed consent procedures were specifically addressed in the protocol. De-identified data was accessed from calerie.duke.edu following site registration.

Figure 1. Caloric restriction (CR) modestly increases the incidence of ketosis



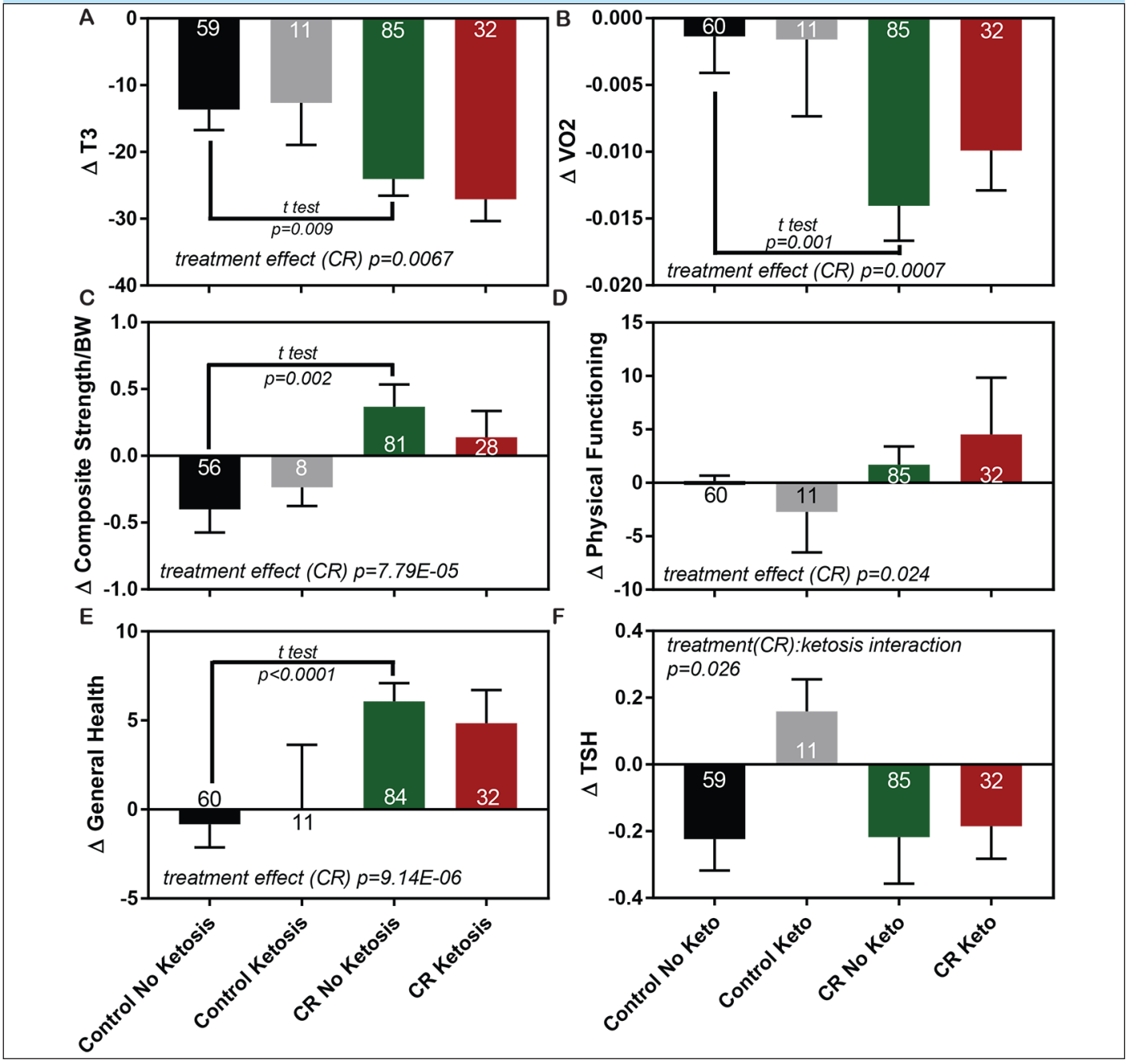
Analysis of ketone free survival in the CALERIE Phase II trial was suggestive of increased ketosis in CR participants (A). Contingency analysis of quarterly ketone urinalysis tests indicated that there was an increased incidence of ketosis in the CR participants (2-sided Fisher's exact test, Relative Risk =2.262, 95% CI 1.188-4.323, B). These analyses were conducted with data from 71 control diet and 117 CR subjects that were not ketone positive at the baseline visit (pre-randomization) and had no missing data for ketone testing.

Results

Ketosis free survival and incidence of ketosis in CR. Based on our initial hypothesis, we conducted an analysis of ketosis free survival in control vs CR participants in the CALERIE Phase II trial (Figure 1). Though not statistically significant (p=0.065), the ketosis free survival analysis suggested a strong trend toward increased ketosis in the CR participants (11 of 71 control and 32 of 117 CR participants had at least one non-baseline visit ketone positive test). We then conducted a contingency analysis by adding all ketosis positive tests relative to total tests for each group. This showed that CR resulted in increased incidence of ketosis relative to controls (4.4% incidence in CR vs 1.9% incidence in controls, p=0.0128).

Impact of ketosis on CR-associated changes. The endpoints analyzed here fall into three categories; standard clinical laboratory test, measured function, and survey response. Of the clinical labs analyzed, only serum triiodothyronine (T3) level was effected by CR (Figure 2A). There was no significant effect of ketosis or interaction between treatment and ketosis. For measured function, CR (treatment) was associated with decreased V02 and increased composite strength score-peak torque when normalized to weight (Figure 2B, 2C). Again, there was no significant effect of ketosis or interaction

Figure 2. No evidence that ketosis impacts the beneficial effects of caloric restriction

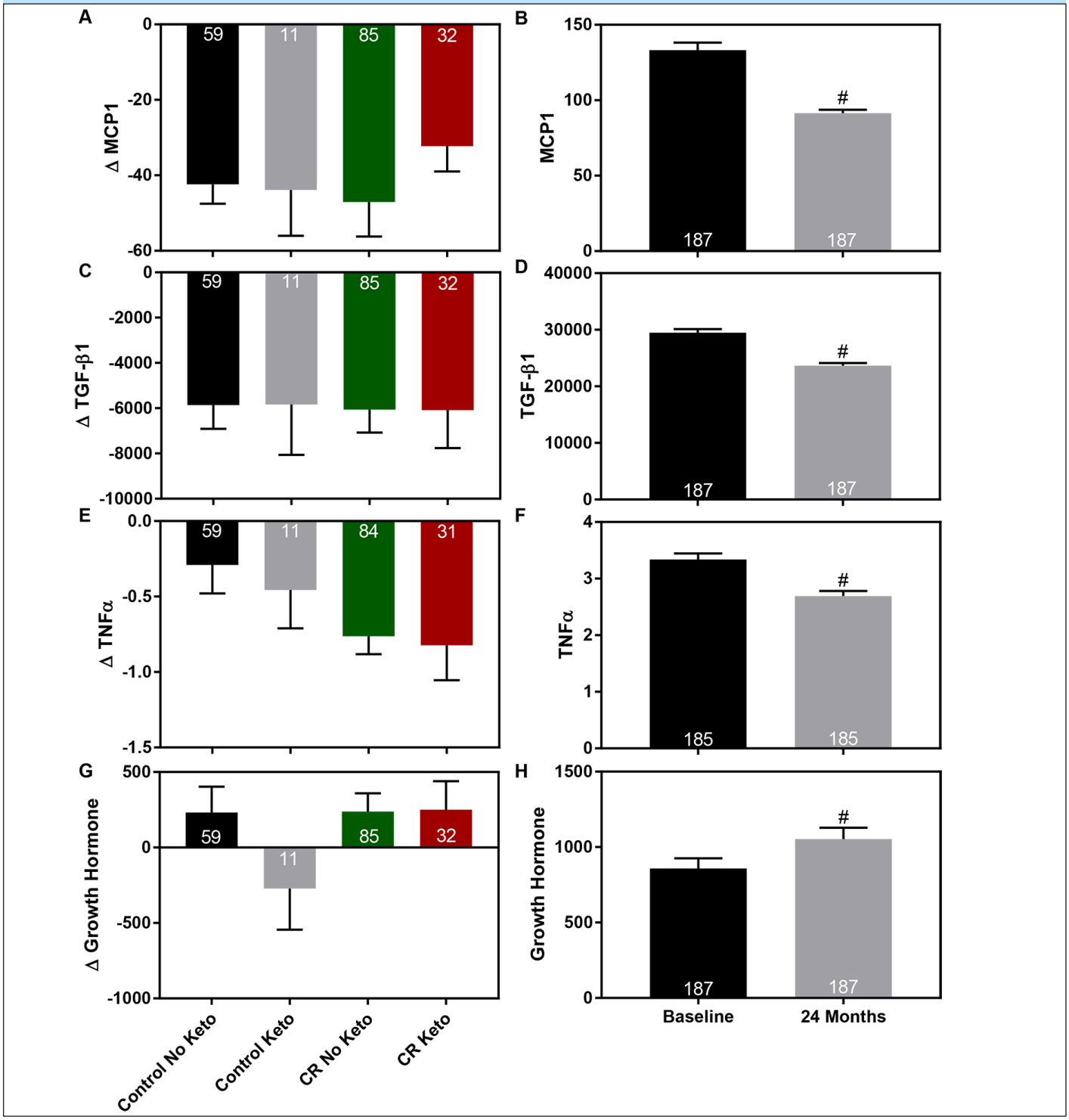


Caloric restriction significantly impacted values for serum T3 (A), mean VO2 (B), Composite Strength:Body Weight (C), Physical Functioning (D) and General Health (E) (24 month post-randomization value minus baseline visit value, significant treatment effect from Scheirer-Ray-Hare test). However, there was no effect of ketosis. TSH showed a significant interaction between ketosis and treatment (F) largely driven by control ketone positive participants, though no posthoc tests could differentiate this group from any others. Number of subjects in each group is depicted within the bars of the bar graph.

between treatment and ketosis. In terms of survey response generated metrics, CR was associated with improved Physical Functioning and General Health scores (Figure 2D, 2E), with no effect of ketosis or ketosis x CR interaction. The only identified significant interaction between Ketosis and treatment (CR) was for TSH. Increased TSH in a limited number of ketone positive control participants drove this result, however no post-hoc tests differentiated this group from any other (Figure 2F).

Changes found in both control and CR groups. An initial analysis comparing only CR ketone positive subject baseline values vs 24 month diet modification values drove significant excitement within the group as it appeared these participants had dramatic reductions in a number of classically pro-inflammatory and pro-fibrotic molecules (not shown). However, subsequent broader statistical analyses showed these changes were not different from similar reductions seen in the control group. This was the pattern seen for MCP-1, TGF-β1, and TNF-α, where CR and CR ketotic individuals were not different from controls, yet baseline vs 24 month values were significantly reduced across the board (Figure 3). Similarly, growth hormone

Figure 3. Participation in the CALERIE Phase II trial broadly altered biomarker expression profiles in an anti-aging-like pattern



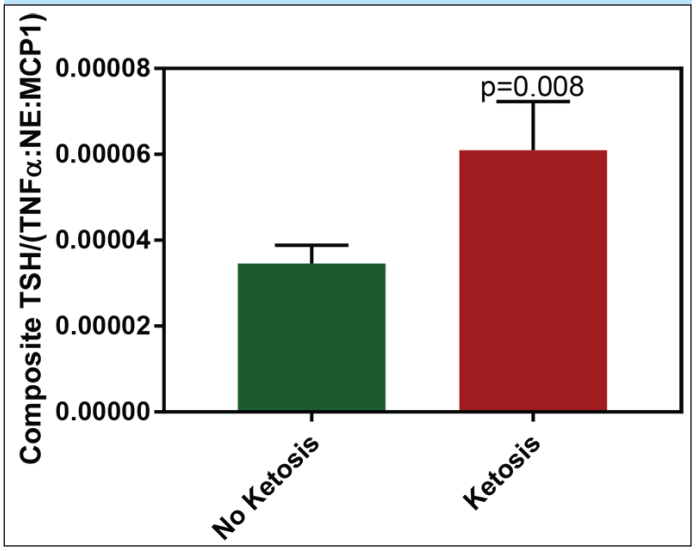
CALERIE Phase II participants randomized to both control diet and CR groups entered a rigorous study with participant education, daily diet and exercise logs, and quarterly clinical appointments. Though no significant treatment effects, ketosis effects or treatment x ketosis interactions were noted for MCP1, TGF-β1, TNFα, or growth hormone concentrations, a clear pattern was seen between baseline and 24-month post randomization values (A, C, E, G). All subjects included in the analysis were analyzed as one group comparing baseline and 24-month post-randomization values by paired two-tailed t test. MCP1, TGF-β1, and TNFα were significantly reduced after 24 months while growth hormone was increased (B, D, F, H, # indicates $p<0.002$).

(GH) concentrations were higher at 24 months vs baseline when considering all groups (Figure 3).

Can we differentiate participants who will and will not become ketone positive? Plotting of baseline lab values by group showed a clear pattern where ketone positive CR participants trended toward having decreased baseline expression of pro-inflammatory and stress-associated markers and increased expression of pro-growth markers (not shown). However, no individual marker was on its own, a significant predictor of ketosis. We then created a composite value

where the growth marker, TSH was divided by the product of stress/inflammation markers TNFα, Norepi, and MCP1. This composite value was almost twice as large in individuals later found to be ketone positive relative to other CR individuals (Figure 4). Of note, Growth Hormone (GH) showed a similar trend to TSH (higher in CR individuals later to be ketone positive) however, the standard deviation of GH concentration was very large. When GH concentration was added to the numerator of the composite score, it introduced sufficient variability to negate statistical significance.

Figure 4. Predicting which CR participants will become ketone positive



Baseline (pre-randomization) lab values were shown to have predictive potential in informing which CR participants would later be ketone positive. A composite score of pro-growth biomarker expression to pro-inflammation/stress biomarker expression was calculated by dividing TSH concentration by the product of TNFα, Norepinephrine, and MCP1 concentration. This score was significantly higher in CR individuals who would later test ketone positive than in CR individuals that were never ketone positive (unpaired, two-tailed t-test).

Discussion

Increased ketone body concentrations in CR participants from this study have been recently reported (27). Detailed nuclear magnetic resonance (NMR) based investigation of specific ketones from overnight fasted participant's blood showed significant between group differences for ketones β-OHB and acetone at either the 24-month or the 12-month post-randomization time points respectively (27). Consistent with this report, our analysis indicates that an increased incidence of ketosis with CR can be found using a standard non-invasive lab urinalysis. The ability to reach statistical significance in the ketosis incidence analysis but not in the ketone free survival analysis indicates that CR individuals testing ketone positive at multiple time points strengthen this difference. These individuals represent a population of interest for further investigation regarding physiological and behavioral determinants of CR leading to ketosis.

We did not observe significant reductions in clinical lab values previously reported to be decreased with CR relative to controls, for instance, TNFα (14). We attribute this to lost statistical power when removing individuals with baseline visit ketone detection and spreading variation across 4 groups as opposed to only two. Another potential interpretation, however, could be that the individuals removed due to baseline ketone detection (potentially indicative of undiagnosed metabolic dysfunction or diabetes) were the strongest responders to CR. Regardless, ketosis does not appear to worsen inflammation in this population.

The significant increase in GH and reductions in MCP-1, TGF-β1, and TNFα in participants, regardless of treatment or control group, is noteworthy for at least two reasons. First, it indicates that participation in a rigorous lifestyle modification clinical trial, even as a control participant can result in a biomarker expression profile consistent with improved health. This is clearly the case with reduced MCP-1, TGF-β1, and TNFα expression, which are pro-inflammatory (28) or pro-fibrotic (29) molecules. Interpretation of increased GH as potentially beneficial is more controversial. Long-term reduction in GH can result in increased healthspan/lifespan in pre-clinical models, though some aging associated pathologies are at least partially attributed to waning GH concentration with advanced age (30). Second, this finding highlights the need for inclusion of robust control groups in clinical trials assessing the effects of lifestyle modifications, and we applaud the CALERIE team for doing so. As average annual health care expenses are over $12,000 per person in the United States (https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/NationalHealthExpendData/NationalHealthAccountsHistorical), these results suggest a potentially attractive cost/benefit analysis for enrolling and reimbursing every American as a control participant in lifestyle modification clinical trials. An alternate interpretation, however, could be that these all-group findings are reflective of reduced "white-coat-syndrome" responses through training/habituation (31). Additional investigation is required to test these possibilities.

Control diet participants who were ketone positive were an interesting, though very small cohort in the study. Albeit hampered by insufficient power (only 11 subjects), this cohort appears to be very different from the others in terms of growth signaling. These participants showed increased TSH concentrations (Figure 2) and decreased growth hormone concentrations at the 24 month visit (Figure 3), while all other groups showed the opposite response to study participation.

Limitations: While our data demonstrate that ketosis linked to CR did not result in any negative impacts on human health, we note the limitations within our study. First, this study was a post hoc analysis and not a prospective trial. Second, participants were not blinded to whether or not they were calorie restricted, leading to potential bias in survey reporting as well as improvements in their overall diet that could impact functional and molecular parameters. Third, our analysis did not take into account individual calorie intake or energy expenditure, nor did our analysis of VO2 take into account participant body mass. This is important, as others have reported that absolute VO2 decreased in CR participants, while relative VO2 (i.e. normalized to body mass) actually increased (26), however fat mass has been shown not to significantly impact VO2. Lastly, we were unable to consider sex as a

variable due to our reduced statistical power. However, others have reported that the beneficial effects of CR are stronger in men than women (27), suggesting that further investigation into how sex differences could drive functional and molecular changes between CR and CR with ketosis patients is still needed.

Acknowledgements: MS was supported by NIH AG056848, AHA 857280 and a local CCTS Voucher from CTSA RUL1TR02733 to fund biostatistics analysis by the OSU Center for Biostatistics. USDA NIFA Hatch-NEV00727, NEV00767, the Dennis Meiss & Janet Ralston Fund for Nutri-epigenetic Research, NIH P20 GM130459 and R15 HL143496, and NSF EPSCOR Track II (OIA-1826801) supported BSF.

Conflicts of Interest: The authors declare that no financial conflicts of interest exist.

Ethics Standards: The work in this manuscript complies with the current laws of the USA.

References

1. Franceschi C, Bonafe` M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci June 2000;908:244e54.
2. Fontana L, Partridge L. Promoting health and longevity through diet: from model organisms to humans. Cell. 2015 Mar 26;161(1):106-18.
3. Fontana L, Villareal DT, Weiss EP, Racette SB, Steger-May K, Klein S, Holloszy JO; Washington University School of Medicine CALERIE Group. Calorie restriction or exercise: effects on coronary heart disease risk factors. A randomized, controlled trial. Am J Physiol Endocrinol Metab. 2007 Jul;293(1):E197–202. doi: 10.1152/ajpendo.00102.2007.
4. Heilbronn LK, de Jonge L, Frisard MI, DeLany JP, Larson-Meyer DE, Rood J, Nguyen T, Martin CK, Volaufova J, Most MM, Greenway FL, Smith SR, Deutsch WA, Williamson DA, Ravussin E; Pennington CALERIE Team. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA. 2006 Apr 5;295(13):1539–48. doi: 10.1001/jama.295.13.1539.
5. Lefevre M, Redman LM, Heilbronn LK, Smith JV, Martin CK, Rood JC, Greenway FL, Williamson DA, Smith SR, Ravussin E; Pennington CALERIE team. Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals. Atherosclerosis. 2009 Mar;203(1):206–13. doi: 10.1016/j.atherosclerosis.2008.05.036.
6. Racette SB, Weiss EP, Villareal DT, Arif H, Steger-May K, Schechtman KB, Fontana L, Klein S, Holloszy JO; Washington University School of Medicine CALERIE Group. One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue. J Gerontol A Biol Sci Med Sci. 2006 Sep;61(9):943–50. doi: 10.1093/gerona/61.9.943.
7. Redman LM, Heilbronn LK, Martin CK, Alfonso A, Smith SR, Ravussin E; Pennington CALERIE Team. Effect of calorie restriction with or without exercise on body composition and fat distribution. J Clin Endocrinol Metab. 2007 Mar;92(3):865–72. doi: 10.1210/jc.2006-2184.
8. Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Holloszy JO; Washington University School of Medicine CALERIE Group. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr. 2006 Nov;84(5):1033–42. doi: 10.1093/ajcn/84.5.1033.
9. Edwards IJ, Rudel LL, Terry JG, Kemnitz JW, Weindruch R, Zaccaro DJ, Cefalu WT. Caloric restriction lowers plasma lipoprotein (a) in male but not female rhesus monkeys. Exp Gerontol. 2001 Aug;36(8):1413–8. doi: 10.1016/s0531-5565(01)00107-3.
10. Gresl TA, Colman RJ, Roecker EB, Havighurst TC, Huang Z, Allison DB, Bergman RN, Kemnitz JW. Dietary restriction and glucose regulation in aging rhesus monkeys: a follow-up report at 8.5 yr. Am J Physiol Endocrinol Metab. 2001 Oct;281(4):E757–65. doi: 10.1152/ajpendo.2001.281.4.E757.
11. Rezzi S, Martin FP, Shanmuganayagam D, Colman RJ, Nicholson JK, Weindruch R. Metabolic shifts due to long-term caloric restriction revealed in nonhuman primates. Exp Gerontol. 2009 May;44(5):356–62. doi: 10.1016/j.exger.2009.02.008.
12. Yamada Y, Colman RJ, Kemnitz JW, Baum ST, Anderson RM, Weindruch R, Schoeller DA. Long-term calorie restriction decreases metabolic cost of movement and prevents decrease of physical activity during aging in rhesus monkeys. Exp Gerontol. 2013 Nov;48(11):1226–35. doi: 10.1016/j.exger.2013.08.002.
13. Mattison JA, Colman RJ, Beasley TM, Allison DB, Kemnitz JW, Roth GS, Ingram DK, Weindruch R, de Cabo R, Anderson RM. Caloric restriction improves health and survival of rhesus monkeys. Nat Commun. 2017 Jan 17;8:14063. doi: 10.1038/ncomms14063.
14. Meydani SN, Das SK, Pieper CF, Lewis MR, Klein S, Dixit VD, Gupta AK, Villareal DT, Bhapkar M, Huang M, Fuss PJ, Roberts SB, Holloszy JO, Fontana L. Long-term moderate calorie restriction inhibits inflammation without impairing cell-mediated immunity: a randomized controlled trial in non-obese humans. Aging (Albany NY). 2016 Jul;8(7):1416-31. doi: 10.18632/aging.100994.
15. Roberts MN, Wallace MA, Tomilov AA, Zhou Z, Marcotte GR, Tran D, Perez G, Gutierrez-Casado E, Koike S, Knotts TA, Imai DM, Griffey SM, Kim K, Hagopian K, McMackin MZ, Haj FG, Baar K, Cortopassi GA, Ramsey JJ, Lopez-Dominguez JA. A Ketogenic Diet Extends Longevity and Healthspan in Adult Mice. Cell Metab. 2017 Sep 5;26(3):539–546.e5. doi: 10.1016/j.cmet.2017.08.005.
16. Newman JC, Covarrubias AJ, Zhao M, Yu X, Gut P, Ng CP, Huang Y, Haldar S, Verdin E. Ketogenic Diet Reduces Midlife Mortality and Improves Memory in Aging Mice. Cell Metab. 2017 Sep 5;26(3):547–557.e8. doi: 10.1016/j.cmet.2017.08.004.
17. Yang X, Cheng B. Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. J Mol Neurosci. 2010 Oct;42(2):145–53. doi: 10.1007/s12031-010-9336-y.
18. Dupuis N, Curatolo N, Benoist JF, Auvin S. Ketogenic diet exhibits anti-inflammatory properties. Epilepsia. 2015 Jul;56(7):e95–8. doi: 10.1111/epi.13038.
19. Rosenbaum M, Hall KD, Guo J, Ravussin E, Mayer LS, Reitman ML, Smith SR, Walsh BT, Leibel RL. Glucose and Lipid Homeostasis and Inflammation in Humans Following an Isocaloric Ketogenic Diet. Obesity (Silver Spring). 2019 Jun;27(6):971-981. doi: 10.1002/oby.22468.
20. Yarar-Fisher C, Kulkarni A, Li J, Farley P, Renfro C, Aslam H, Bosarge P, Wilson L, Barnes S. Evaluation of a ketogenic diet for improvement of neurological recovery in individuals with acute spinal cord injury: a pilot, randomized safety and feasibility trial. Spinal Cord Ser Cases. 2018 Sep 21;4:88. doi: 10.1038/s41394-018-0121-4.
21. Poff A, Koutnik AP, Egan KM, Sahebjam S, D'Agostino D, Kumar NB. Targeting the Warburg effect for cancer treatment: Ketogenic diets for management of glioma. Semin Cancer Biol. 2019 Jun;56:135-148. doi: 10.1016/j.semcancer.2017.12.011.
22. Mihaylova MM and Stratton MS (2019) Short chain fatty acids as epigenetic and metabolic regulators of neurocognitive health and disease. In: Ferguson BS (ed) Nutritional Epigenomics, Vol 14 in Translational Epigenetics. 2019. Elsevier Inc, Chapter 23, pp 381-397
23. Ravussin E, Redman LM, Rochon J, Das SK, Fontana L, Kraus WE, Romashkan S, Williamson DA, Meydani SN, Villareal DT, Smith SR, Stein RI, Scott TM, Stewart TM, Saltzman E, Klein S, Bhapkar M, Martin CK, Gilhooly CH, Holloszy JO, Hadley EC, Roberts SB; CALERIE Study Group. A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity. J Gerontol A Biol Sci Med Sci. 2015 Sep;70(9):1097-104. doi: 10.1093/gerona/glv057.
24. Das SK, Roberts SB, Bhapkar MV, Villareal DT, Fontana L, Martin CK, Racette SB, Fuss PJ, Kraus WE, Wong WW, Saltzman E, Pieper CF, Fielding RA, Schwartz AV, Ravussin E, Redman LM; CALERIE-2 Study Group. Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: a 2-y randomized controlled trial of calorie restriction in nonobese humans. Am J Clin Nutr. 2017 Apr;105(4):913-927. doi: 10.3945/ajcn.116.137232.
25. Kraus WE, Bhapkar M, Huffman KM, Pieper CF, Krupa Das S, Redman LM, Villareal DT, Rochon J, Roberts SB, Ravussin E, Holloszy JO, Fontana L; CALERIE Investigators. 2 years of calorie restriction and cardiometabolic risk (CALERIE): exploratory outcomes of a multicentre, phase 2, randomised controlled trial. Lancet Diabetes Endocrinol. 2019 Sep;7(9):673-683. doi: 10.1016/S2213-8587(19)30151-2.
26. Racette SB, Rochon J, Uhrich ML, Villareal DT, Das SK, Fontana L, Bhapkar M, Martin CK, Redman LM, Fuss PJ, Roberts SB. Effects of two years of calorie restriction on aerobic capacity and muscle strength. Medicine and science in sports and exercise. 2017 Nov;49(11):2240.
27. Huffman KM, Parker DC, Bhapkar M, Racette SB, Martin CK, Redman LM, Das SK, Connelly MA, Pieper CF, Orenduff M, Ross LM, Ramaker ME, Dorling JL, Rosen CJ, Shalaurova I, Otvos JD, Kraus VB, Kraus WE; CALERIE™ Investigators. Calorie restriction improves lipid-related emerging cardiometabolic risk factors in healthy adults without obesity: Distinct influences of BMI and sex from CALERIE™ a multicentre, phase 2, randomised controlled trial. EClinicalMedicine. 2022 Jan 3;43:101261. doi: 10.1016/j.eclinm.2021.101261.
28. Apostolopoulos V, De Courten MP, Stojanovska L, Blatch GL, Tangalakis K, De Courten B. The complex immunological and inflammatory network of adipose tissue in obesity. Molecular nutrition & food research. 2016 Jan;60(1):43-57.
29. Hanna A, Frangogiannis NG. The Role of the TGF-β Superfamily in Myocardial Infarction. Front Cardiovasc Med. 2019 Sep 18;6:140. doi: 10.3389/fcvm.2019.00140.
30. Colon G, Saccon T, Schneider A, Cavalcante MB, Huffman DM, Berryman D, List E, Ikeno Y, Musi N, Bartke A, Kopchick J, Kirkland JL, Tchkonia T, Masternak MM. The enigmatic role of growth hormone in age-related diseases, cognition, and longevity. Geroscience. 2019 Dec;41(6):759-774. doi: 10.1007/s11357-019-00096-w.
31. Pioli MR, Ritter AM, de Faria AP, Modolo R. White coat syndrome and its variations: differences and clinical impact. Integr Blood Press Control. 2018 Nov 8;11:73-79. doi: 10.2147/IBPC.S152761.

How to cite this article: B.S. Ferguson, P. Sahoo, E. McGrail, et al. Modestly Increased Incidence of Ketosis in Caloric Restriction Does not Significantly Alter the Effects of Caloric Restriction. J Nutr Health Aging.2022;26(7):657-662; https://doi.org/10.1007/s12603-022-1815-7