EMERGING SCIENCE OF CARBOHYDRATE RESTRICTION AND NUTRITIONAL KETOSIS

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FOOD INNOVATION CENTER

KetoPet Sanctuary: An Update of the Combination of Standard Care, Ketogenic Diet, and Hyperbaric Oxygen Therapy on Tumor Progression and Survival in Dogs with Cancer

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Cancers exhibit an altered energy metabolism characterized by glycolytic dependency and glucose fermentation in the presence of oxygen (the Warburg effect). Studies suggest this metabolic phenotype can be targeted therapeutically with co-administration of a ketogenic diet (KD) and hyperbaric oxygen therapy (HBOT). To further explore this potential therapeutic regimen, dogs with advanced cancer were entered into a 120-day open-registry trial with a 180-day follow-up period designed to determine if KD+HBOT delivered as an adjuvant to the standard of care would reduce cancer burden and increase survival. All dogs accepted into the study (n=40) were given a 2:1-4:1 (fat: carbohydrate+ protein) raw food KD diet and HBOT (100% O2, 2.0-2.2 ATM, 60 min, 5x/week) for 120 days as well as appropriate standard of care determined by the veterinary team. Blood glucose and ketones were measured 6x/week, tumor burden was measured via PET/CT scans and ultrasound every 60 days, and overall health status and condition of the dog (body score, behavior, blood work) and adverse events were monitored throughout the trial. Adjustments to the diet and medications were made as necessary. All dogs demonstrated reduced blood glucose (66.5 ±3.3 mg/dL) and elevated blood ketones (0.59±0.17 mM) while on the KD. At the end of the 120-day trial, 22 of the 40 dogs completed the program, with eight dogs showing no evidence of disease, four dogs showing decreased evidence of disease, five dogs showing stable disease, and five dogs showing increased evidence of disease. Additionally, six dogs passed away from cancer, two dogs passed away from reasons other than cancer, and ten dogs were unable to complete the program. Two years later, seven dogs still have no evidence of disease, five have active cancer, and ten have passed away (five passed away from cancer, five passed away from other causes). Of the 22 that completed the program, 11 have surpassed the original standard of care prognoses, two did not reach their prognosis, three haven't reached their prognoses yet, and six were unable to determine prognosis due to lack of evidence-based on tumor type. These preliminary findings support the potential use of standard of care in combination with KD and HBOT as adjuvant therapies for dogs and patients with advanced disease.

Association Between Fasting Insulin and High-Sensitivity C-Reactive Protein Among Adults: NHANES 2005-2010

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Hyperglycemia is associated with chronic low-grade inflammation, and thus has become the focus of many screening and treatment recommendations. We hypothesize that insulin may also be associated with inflammation, and thus may be an additional factor to consider in screening and treatment. Independent effects of insulin can be difficult to ascertain in a population with diabetes due to confounding from hyperglycemia, beta cell failure and glucose-lowering medications including exogenous insulin. To ascertain the independent effects of insulin the present study will use a non-diabetic population. The purpose of this study was to examine the association between insulin and high-sensitivity C-reactive protein (hs-CRP), a biomarker for inflammation using data from the National Health and Nutrition Examination Survey 2005-2010. Exclusion criteria were current pregnancy, self-reported diagnosis of diabetes, use of glucose-lowering medications, use of cholesterol-lowering medications and age less than 20 years. The final nationally representative sample included 5,851 participants with an average age of 44 ± 0.36 years. Generalized linear models with Gamma distribution and identity link function was conducted to estimate the associations. After adjusting for age, race, gender, physical activity, and smoking status, fasting insulin level was significantly associated with hs-CRP (β=.0027, p= .0026), independently of waist circumference. Moreover, the associations between fasting insulin and hs-CRP were weakened and reduced by 82.5% when including waist circumference in the model compared to the case without it, indicating some role of waist circumference in modulating the relationship of insulin and hs-CRP. While causal effects cannot be determined utilizing cross-sectional data, the results of this study suggest that further studies are warranted to examine the cause-and-effect relationship between insulin and inflammation and whether this relationship is fully or partially mediated by waist circumference. If future studies confirm a causal association, treatment recommendations will need to be modified. Current treatment recommendations have a singular focus on glycemic goals, and may need to be expanded to include insulin. Decreasing glucose load, for example by implementing a low-carbohydrate diet may be a better approach than stimulating the pancreas to produce more insulin or injecting exogenous insulin to manage hyperglycemia. While both approaches will manage hyperglycemia, only decreasing glucose load will simultaneously decrease insulin levels.

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A Pilot Study of a Low-Carbohydrate Diabetes Prevention Program Among People with Prediabetes

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Comprehensive diet and lifestyle intervention is recommended as first-line treatment for adults with prediabetes (1). The Diabetes Prevention Program (DPP) is an intensive 1-year lifestyle intervention that has been demonstrated to reduce the risk of developing type 2 diabetes by over 50% (2) with durable benefits over time (3) and when administered in community settings (4). The DPP is nationally available and these programs can help participants achieve an average of 4 percent body weight loss utilizing a low-fat, calorie-restricted diet (4). Subsequent research suggests low-carbohydrate diets may be more effective for weight loss and glycemic control among individuals with diabetes (5, 6), suggesting potential efficacy in a prediabetic population. The established DPP structure may represent a potential approach to implementing scalable low-carbohydrate lifestyle interventions.

Purpose: A pilot trial of a DPP utilizing a low-carbohydrate meal plan (less than 35g/day net carbohydrates) for adults with prediabetes was undertaken to evaluate feasibility, acceptability weight loss, and change in glycated hemoglobin (HbA1c).

Methods: Eligible participants were identified by chart review from a primary care practice with a documented HbA1c between 5.7-6.4% and BMI ≥ 25 kg/m^2 within 6 months. A total of 187 patients were eligible and invited to participate and 22 participants were enrolled. Attendance and body weights were recorded weekly and HbA1c was measured after 6 months. Qualitative interviews were completed after 6 months.

Results: The 22 participants enrolled had a mean BMI of 34.2 kg/m^2, HbA1c of 5.9% and mean age of 59.5 years. At 6 months, average weight loss was 4.4% with 43% of participants achieving 5% body weight loss and 24% achieving 7% body weight loss. HbA1c decreased by an average of 0.1% in all participants and 0.2% in participants who attended more than 9 sessions (n=14).

Qualitative interviews revealed themes of subjective health benefits beyond weight loss and relative ease of learning a low-carbohydrate lifestyle. Barriers included social pressures and concerns about inclusion of high dietary fat intake. There were no significant changes in physical symptoms over the 6-month study period.

Conclusions: A low-carb DPP is an acceptable, feasible and potentially scalable approach to teaching low-carbohydrate lifestyle and reducing progression to type 2 diabetes. Additional insight will be enhanced by 12-month data and interviews and future comparative trials.

References:

1. Final Update Summary: Abnormal Blood Glucose and Type 2 Diabetes Mellitus: Screening. U.S. Preventive Services Task Force. September 2016. https://www.uspreventiveservicestaskforce.org/Page/Document/UpdateSummaryFinal/screening-for-abnormal-blood-glucose-and-type-2-diabetes?ds=1&s=diab

2. The Diabetes Prevention Program (DPP) Research Group. The Diabetes Prevention Program (DPP): Description of lifestyle intervention. *Diabetes care*. 2002;25(12):2165-2171.

3. Diabetes Prevention Program Research Group, Knowler, W. C., Fowler, S. E., Hamman, R. F., Christophi, C. A., Hoffman, H. J., … Nathan, D. M. (2009). 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study. *The Lancet*, *374*(9702), 1677–1686. https://doi.org/10.1016/S0140-6736(09)61457-4.

4. Ackermann, R. T., Finch, E. A., Brizendine, E., Zhou, H., & Marrero, D. G. (2008). Translating the Diabetes Prevention Program into the Community. *American Journal of Preventive Medicine*, *35*(4), 357–363. https://doi.org/10.1016/j.amepre.2008.06.035.

5. Dyson, P. A., Beatty, S., & Matthews, D. R. (2007). A low-carbohydrate diet is more effective in reducing body weight than healthy eating in both diabetic and non-diabetic subjects. *Diabetic Medicine*, *24*(12), 1430–1435. https://doi.org/10.1111/j.1464-5491.2007.02290.x.

6. Feinman, R. D., Pogozelski, W. K., Astrup, A., Bernstein, R. K., Fine, E. J., Westman, E. C., … Worm, N. (2015). Dietary carbohydrate restriction as the first approach in diabetes management: Critical review and evidence base. *Nutrition*, *31*(1), 1–13. https://doi.org/10.1016/j.nut.2014.06.011.

Lipid findings from the Diabetes Education to Lower Insulin, Sugars, and Hunger (Delish) Study

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Purpose: A ketogenic diet can improve glycemic control in type 2 diabetes mellitus (T2DM), however, there have been concerns that the high fat content can result in increased low density lipoprotein (LDL) cholesterol, which has been associated with cardiovascular disease (CVD). We assessed the effects of a ketogenic diet on LDL particle size and number (LDL-P) among individuals with T2DM. Small particle LDL size and number are particularly associated with CVD, thus, these metrics may more accurately predict CVD disease risk than the calculated LDL (LDL-C) obtained on standard lipid tests.

Methods: We recruited 58 adults with T2DM (n = 37 women; baseline HbA1c $\geq$ 6.5) to a group-based ketogenic diet intervention. We delivered the intervention in a classroom format (weekly for the first 3 months, monthly for the latter 3 months; ~n = 10 per class). Although we randomized participants to two different approaches to supporting adherence to the diet (one of which included mindful eating), the nutritional recommendations were identical. Lipid results did not differ between behavioral groups and we combine them in these analyses. We obtained a standard lipid panel (total cholesterol, HDL-C, LDL-C, triglycerides, triglycerides/HDL-C) and used an nuclear magnetic resonance assay (NMR; LabCorp) to obtain lipid particle size and number (LDL size, LDL-P, small LDL-P). All participants provided consent and the UCSF Institutional Review Board (IRB) approved of all study procedures.

Results: We observed an increase in HDL-C (M diff (SD) = 4.51 mg/dL (9.43), 95% CI [1.96, 7.06], p=.0008) and decreases in triglycerides (M diff (SD) = -38.07 mg/dL (61.76) 95% CI [-54.77, -21.38], p<.0001) and the triglyceride/HDL-C ratio (M diff (SD) = -1.21 (1.81), 95% CI [-1.70, -0.72], p<.0001). We observed a trend toward increase in LDL-C (M diff (SD) = 7.03 mg/dL (29.93), 95% CI [-1.22, 15.28], p=.0933), but did not observe a significant increase in total cholesterol (M diff (SD) = 5.78 mg/dL (35.25), 95% CI [-3.75, 15.31], p=.2291). We observed a statistically significant decrease in small LDL-P number (M diff (SD) = -116.64 nmol/L (255.70), 95% CI [-185.76, -47.51], p=.0013), but not LDL-P (M diff (SD) = -6.44 nmol/L (359.33), 95% CI [-103.58, 90.70], p=.8948. We observed an increase in LDL size (M diff (SD) = 0.24 nm (0.46), 95% CI [0.12, 0.37], p=.0003). We further examined changes within participants whose LDL-C increased by 5% or greater, and found that of 30 participants whose LDL-C increased, only 7 (23.3%) also demonstrated increases of 5% or greater in small LDL-P.

Conclusions: These results demonstrate that although standard measures of LDL may suggest that a ketogenic diet could increase risk for CVD, measurement of LDL particle size and number suggests that most people do not experience lipid changes likely to increase CVD risk. We observed lipid changes that were consistent with decreased CVD risk in most participants. Additional research is needed to understand why a minority of participants do experience increases of 5% or greater in small LDL-P, and how this should be managed.

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Insulin levels are associated with multi-system alterations in a population-based sample of non-diabetic American adults: NHANES 1999-2016

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Purpose: Even given the obesity epidemic in the United States and globally, the observation that more than half the adults in the United States are diabetic or pre-diabetic is shocking. Epidemiological evidence for a relationship between insulin resistance and cancer is emerging, and insulins' complex actions suggest that its dysregulation may plausibly result in multisystem alterations. Using cross-sectional demographic and laboratory data from the continuous National Health and Nutrition Survey (NHANES) using data from 1999-2014. we surveyed the range of alterations observed in normal (not diabetic or prediabetic) adult subjects in relation to variation in insulin levels. The homeostatic model of insulin resistance (HOMA-IR) was used as the measure of insulin resistance.

Methods: We examined associations with cardiovascular (BP), respiratory (FEV1, FVC), renal (BUN, creatinine), inflammatory (C-reactive protein, fibrinogen), lipid (triglycerides, LDL, HLD), hematologic (WBC, hemoglobin, cell counts), and nutritional (measured vitamin levels) factors in relation to HOMA-IR, C-peptide, Hgb1Ac and fasting glucose levels after adjustment for demographic (age, gender, race, family income, education), anthropometric (BMI), and key exposures (caffeine, alcohol, physical activity, smoking). We examined crude correlations, and base (adjusted for age, gender, ethnicity, height, education, income), moderately (added alcohol, smoking, caffeine), and fully (added: sleep, supplement use, physical activity, season, BMI) adjusted models. Examples are shown (below) of adjusted means of HOMA that take into account: study 'weights', age, gender, ethnicity, smoking, family income, and education.

Results: Key variables in every system exhibited highly significant associations with insulin/glucose measures. Strong correlations ($p < 0.0001$, Pearson, accounting for sample weights) of HOMA-IR were observed with waist size, systolic and diastolic blood pressure, triglycerides, HDL (inverse), LDL and inflammatory markers: fibrinogen, C-reactive protein, ferritin. Measured serum levels of vitamin C, vitamin D, and folate (RBC), were inversely associated with HOMA-IR. Strong associations of HOMA with hematologic parameters were observed including: hemoglobin, WBC, platelets, lymphocyte and neutrophil counts.

Examples of the adjusted (base model) relationship for a few variables, to quartiles of HOMA:

	Quartiles HOMA-IR: adj., mean (95% CI)		
	1st	**4th**	**p trend (4df)**
Ferritin	79.1 (71.1-87.0)	96.2 (82.3-110.2)	< 0.0001
BP (d)	69.7 (68.5-70.9)	73.2 (71.9-74.5)	< 0.0001
HDL	60.8 (59.8-61.8)	51.3 (50.4-52.2)	< 0.0001
WBC	6.37 (6.26-6.47)	6.96 (6.82-7.09)	< 0.0001

Conclusion: In a broad and representative sample of up to 9000 non-diabetic US adults, insulin resistance is associated with adverse changes across diverse classes of markers. Clinicians may want to consider routine evaluation of insulin levels along with more commonly performed glucose screening.

Improvement in Patient-Reported Sleep in Type 2 Diabetes and Prediabetes Participants Receiving a Continuous Care Intervention with Nutritional Ketosis

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Abstract

Purpose: Sleep deprivation and disorders are frequently reported in obesity and type 2 diabetes (T2D). Very limited studies are available on the effectiveness of carbohydrate restriction and ketogenic diet on sleep indexes in these populations. Our recent one-year study demonstrated a digitally supported continuous remote care treatment for T2D including nutritional ketosis significantly improved glycemic control, weight, and cardiovascular disease (CVD) risk factors. Therefore, we assessed the one year effectiveness of this continuous care intervention (CCI) on sleep using a subjective patient-reported sleep measure.

Methods: This was a non-randomized, controlled longitudinal study including 262 T2D and 116 prediabetes patients enrolled in the CCI and 87 separately recruited T2D patients enrolled as the usual care (UC) treatment arm. The patient's subjective sleep measure was assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire. PSQI was administered at baseline, and 1 year in both CCI and UC groups, with an additional time point of 70 days in the CCI group. PSQI cut-off > 5 was used to identify poor sleep quality.

Results: After one year of treatment, we showed that the global sleep quality improved in the CCI T2D ($p<0.001$) and prediabetes ($p<0.001$) patients. No significant changes in the global PSQI score were observed in UC T2D patients. The improvements in the sleep quality were due to reductions in the PSQI subjective sleep quality (component 1), sleep disturbances (component 5) and daytime dysfunction (component 7) in the CCI T2D ($p<0.01$ for components 1 and 5; and $p<0.001$ for component 7) and prediabetes patients ($p<0.001$ for all three components); compared to the UC T2D group. There were 11.8% ($p=0.001$) and 29.2% ($p<0.001$) reductions in the number of patients categorized as "poor sleepers" in CCI T2D and prediabetes participants, respectively. In UC T2D patients, no significant change in the proportion of patients categorized as "poor sleepers" was observed.

Conclusion: This study showed the effectiveness of CCI as a comprehensive treatment option for patients with T2D and prediabetes. The intervention not only improved glycemic control and weight, but also subjective sleep when compared to usual care. Future research should evaluate the effectiveness of this intervention on objective sleep measures.

Continuous Remote Care Model Utilizing Nutritional Ketosis Improves Type 2 Diabetes Risk Factors in Patients with Prediabetes

Prediabetes (preT2D) is a major U.S. public health concern, as over 84 million adults are at greater risk of cardiovascular disease and progression to type 2 diabetes (T2D). Abnormal glucose, BMI, blood pressure, and blood lipids are risk factors for progression to T2D. The purpose of this single-arm prospective longitudinal investigation was to assess change in risk factors after one year of treatment with a continuous remote care model utilizing nutritional ketosis, behavior change, and support from a health coach, medical provider, and peers provided via an online clinic. Patients with preT2D enrolled (n=116); at 1-yr, 95 participants (82%) remained active. Results are reported for participants with measurements completed at 1-yr and baseline; n varied across measures (range: 77-95). Changes from baseline to 1-yr included: weight -13.1±9.5 kg (from 110.6±23.5 kg, $p<0.0001$), BMI -5.0±3.5 kg/m^2 (from 38.9 kg/m^2, $p<0.0001$), HbA1c -0.3±0.3 % (from 5.9±0.2%, $p<0.0001$), fasting glucose -9±14 mg/dL (from 110±15 mg/dL, $p<0.0001$), systolic BP -6±14 mmHg (from 130±13 mmHg, $p=0.0003$), diastolic BP -3±9 mmHg (from 83±8 mmHg, $p=0.0032$), triglycerides -43±55 mg/dL (from 148±67 mg/dL, $p<0.0001$), and HDL-C +7±9 mg/dL (from 52±14 mg/dL, $p<0.0001$). Seventy percent (54/77) of participants lost >7% body weight. At 1-yr, 51.1% (45/88) participants achieved fasting glucose <100 mg/dL (up from 24/94, 25.5% at baseline). No completer progressed to T2D, and 60.7% (54/89) achieved HbA1c <5.7% (up from 14/94, 14.9% at baseline). Most participants (56.3%, 54/96) maintained mean blood beta-hydroxybutyrate concentrations ≥0.5 mmol/L, indicating moderate adherence to nutritional advice. These results demonstrate that patients with preT2D can be supported remotely with this continuous care model to improve risk factors associated with progression of preT2D to T2D. Ongoing research will evaluate the continued sustainability of this intervention and prevention of T2D.
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Nutritional Ketosis Uniquely Enhances Mitochondrial Function in Human Skeletal Muscle During Adaptation to Exercise

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Background: Mitochondria prominently influence health and physical capacity. Exercise-induced changes in mitochondria can be augmented by dietary carbohydrate restriction. However, the characteristics of such augmentation are largely unknown, particularly in humans. Therefore, the objective of this research is to characterize changes in skeletal muscle mitochondrial function induced by nutritional ketosis during adaptation to chronic exercise.

Methods: Twenty-nine participants completed a 10-12-week supervised exercise program while following a ketogenic diet (LC, n=15, males=14) or their habitual high-carbohydrate diet (HC, n=14, males=13). Muscle biopsies were collected from the *Vastus lateralis* pre- and post-intervention. In isolated mitochondria, oxygen consumption and membrane potential were measured with a Clark-type electrode fitted with a tetraphenylphosphonium electrode. H_2O_2 and ATP production were measured using fluorescence (Amplex Ultra Red) and luminescence (luciferase) assays, respectively. Each test was repeated with a carbohydrate- (pyruvate), fat- (palmitoyl-L-carnitine), and ketone-based (β-hydroxybutyrate+acetoacetate) substrate.

Results: Participants were matched by age, gender, and body fat (LC vs HC: 27.4±6.8 vs 24.6±9.0 yrs, 25.6±5.0% vs 22.0±8.6%). Mean daily blood β-hydroxybutyrate concentration for LC was 1.2±0.2 mM. An effect of time was observed for increases in mitochondrial protein (5.56±0.2 to 6.02±0.2 μg/μL, $p<0.00001$) and respiratory control ratio (RCR, 4.22±0.3 to 4.61±0.3, $p<0.01$). Time x diet interactions were observed indicating a lesser increase in H_2O_2 ($p=0.013$) and a relative increase in ATP ($p<0.01$) for LC, as well as a relative increase in uncoupling for HC (based on ATP/O_2, $p<0.001$). With the fat-based substrate, mean RCR and median ATP production increased for LC (4.68±0.3 to 5.62±0.2, $p=0.013$; 15.20 to 30.97 nmol/mg/min, $p=0.027$), but not HC. For all conditions, ATP production with the ketone-based substrate was more than 4-fold lower than with other substrates, indicating that ketones are minimally oxidized in human skeletal muscle.

Conclusions: While the effects of time indicate exercise-induced enhancement of mitochondrial function, the time x diet interactions indicate augmentation of this enhancement by nutritional ketosis, particularly in regard to fat metabolism. When normalized to O_2 consumption, the changes in H_2O_2 and ATP production are in opposite directions for LC, but not HC, indicating that nutritional ketosis induced a shift in metabolic efficiency from carbohydrate to fat, which may be a result of changes in mitochondrial supercomplexes.

Estimated Reduction in Glycemic Control Medication Cost during First Year of a Continuous Care Intervention Including Nutritional Ketosis for Type 2 Diabetes

Authors
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Abstract

Purpose: Results (n=262) from a 1-year prospective longitudinal investigation of a novel continuous care intervention (CCI) suggest that it is possible to improve hemoglobin A1c (HbA1c) levels while simultaneously reducing diabetes medications in patients with type 2 diabetes (T2D). The CCI provided remote guidance from a health coach and physician, education in nutritional ketosis and behavior change, biometric feedback and peer support. At 1-year, 83% (n=218) of patients were retained and 60% of patients with 1-year HbA1c (123/204) achieved HbA1c <6.5%, while taking no diabetes medications or metformin only. At baseline, 121 of 218 were on at least one glycemic control medication other than metformin. A usual care comparator group (n=87 starters, n=78 completers, [90%]) showed an increase in HbA1c and medication use at 1-year. Here we quantified the cost savings associated with reduction in glycemic control medications following 1-year of CCI. Methods: Savings were estimated by tracking daily changes in CCI patients' prescriptions for one year. Medication prices were estimated from goodrx.com, which organizes pricing from over 60,000 U.S. pharmacies. Medications were priced in proportion to: (i) dosage, (ii) number of tablets, or (iii) number of injections. Results: The analysis yielded an estimated baseline average annual cost of $4,438 per patient. Across the intervention group at 1-year, there was a reduction of about 46% in estimated annual cost to $2,394, with most savings from insulin reductions in the first 2-3 months. Among n=62 completing the intervention who were prescribed insulin at baseline, there was a reduction of 40% in estimated annual cost ($10,818 to $6,481 per patient). Conclusions: In patients with T2D, a multifaceted continuous treatment led to a reduction in diabetes medication use and substantial estimated medication cost savings while simultaneously improving glycemic control.

The effects of a carbohydrate-restricted diet on hepatic lipid content and insulin resistance in adolescents with non-alcoholic fatty liver disease: a randomized clinical trial

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Purpose: With increasing prevalence of childhood obesity, non-alcoholic fatty liver disease (NAFLD) has emerged as the most common form of liver disease among children and adolescents in industrialized countries. Presently, caloric restriction and exercise are the mainstay of therapy, but are generally ineffective to reduce liver fat. Results from interventions in adults suggest that reducing intake of carbohydrates (CHO) may be more effective in depleting liver fat. The objective of this study was to determine the effects of a CHO-restricted vs fat-restricted diet in adolescents with overweight/obesity and NAFLD on reduction in hepatic lipid content and insulin resistance.

Design: Twenty-three adolescents (age 9-17) with overweight/obesity (BMI >85th percentile) and NAFLD, confirmed via elevated serum aminotransferase levels or diffusely echogenic liver via ultrasound, were randomized to a CHO-restricted (<10:25:>65% energy from CHO:protein:fat) or fat-restricted diet (55:25:20) for 8 weeks. Caloric intakes were calculated to be weight maintaining. Participants and at least one parent attended bi-weekly counselling sessions with a registered dietitian where they received meal plans, recipes, and food lists. To encourage compliance to the dietary prescriptions, groceries were delivered to participants' families during the first 2 weeks of the study. Enough food was provided to feed a family of four, and groceries corresponded with the meal plans and recipes provided by the dietitian. Both diets included high quality, minimally processed foods with limited added sugar. Change in hepatic lipid content was measured via MRI, body composition via DXA, and insulin resistance via a fasting blood sample.

Results: After 8 weeks, the CHO-restricted diet group experienced 3-fold greater decrease in hepatic lipid content (-6.0±4.7%, $p<0.001$) than the fat-restricted diet group in which there was no significant change in hepatic lipid. We found significantly lower insulin resistance (HOMA-IR, -1.2±5.1, <0.05), abdominal fat mass (-1.7 ±1.1 kg, $p<0.01$), and body fat mass (-3.1±4.0 kg, $p<0.01$) in response to the CHO-restricted vs. fat-restricted diet.

Conclusion: The CHO-restricted diet approach may be markedly beneficial in improving fatty liver, body composition, and insulin resistance in adolescents with NAFLD even in the absence of intentional caloric restriction. Practitioners should consider recommending this diet approach to effectively improve disease course in this patient population.

Very Low Carbohydrate Nutrition as an Adjuvant Therapy for Type-1 Diabetic Glycemic Management

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Type-1 Diabetes is an autoimmune disorder that results in the destruction of insulin-producing pancreatic beta-cells. Consequently, many Type-1 Diabetics are required to manage their blood glucose levels with medications and technological assistance. However, international analysis of >300,000 Type-1 Diabetics across 19 countries revealed that >70% of Type-1 Diabetic are reported to have a hemoglobin A_{1c} >7.5%, with 31% of Type-1 Diabetics having an hemoglobin A_{1c} >9.0%. This data confirms that the majority of Type-1 Diabetics are not able to achieve glycemic control with the currently available treatment strategies and technologies.

Treatment strategies and technologies include standard practice carbohydrate counting, intensively treated therapy, insulin pumps, continuous glucose monitoring, and closed loop systems, along with experimental approaches such as islet cell transplantation, BCG vaccine, stem cells, etc. Of the standard practice and experimental therapies discussed, only islet-cell transplantation has been able to normalize glycemic control. However, this therapy option has shown mixed results and numerous limitations that prevents its use for most Type-1 Diabetics. As a result, Type-1 Diabetics are left with few options to attenuate known glycemic risk factors such as hemoglobin A1c, blood glucose variability, fasting glucose levels, post-prandial hyperglycemia, total glucose exposure, etc.... Consequently, Type-1 Diabetics are at an elevated risk for all ten of the leading causes of death and a shortened projected life expectancy compared to their non-diabetic peers.

However, emerging evidence indicates that a very-low carbohydrate diet could help normalize glycemic control in several Type-1 Diabetics. This therapeutic option also presents several logistical advantages for glycemic control compared to other management options. Thus, the utility of a very low-carbohydrate diet as an adjuvant therapy for Type-1 Diabetics glycemic management is discussed. Additionally, the efficacy of a flexible high carbohydrate, high protein, low fat diet to a very low carbohydrate, high protein, high fat diet for glycemic control and medication requirements is discussed [HbA1c, continuous glucose monitoring (CGM) mean glucose, CGM standard deviation, insulin requirements].

The Effects of a Ketone-Caffeine Supplement on Physical Performance Testing

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Increasing evidence indicates that elevating ketones through diet and/or supplementation alters metabolism and potentially exercise performance. To date, no studies have evaluated the impact of ingesting ketone salts in keto-adapted individuals. The primary purpose of this study was to determine the performance and metabolic effects of a supplement containing ketone salts and caffeine in adults habitually consuming a mixed diet (keto-naïve; n=12)) and low carbohydrate ketogenic diet (keto-adapted; n=12). The keto-'naïve' (4 female, 8 male; mean ± SD age, 22.9 ±1.9; weight 79.3 ± 16.7 kg; height, 176.6 ± 13.0 cm; VO2max, 41.2 ±5.1 ml/kg/min) and keto-adapted (3 female, 9 male: mean + SD age, 36.1 + 7.5 years; weight, 82.2 + 7.1 kilograms; height, 177.7 + 8.5 cm; VO_2max 40.3 + 10.5 ml/kg/min) groups participated in two experimental sessions in a randomized and balanced order. Subjects consumed either a ketone-salt/caffeine supplement containing 7.2 BHB and 96 g caffeine or water (control condition) 15 min prior to performing a staged cycle ergometer time to exhaustion test followed immediately by a 30 sec Wingate test. Blood ketone concentrations were significantly increased peaking 15 min after ingestion by more than 2-fold and staying elevated throughout 60 min recovery. Compared to the water trial, ingestion of the ketone-caffeine supplement significantly increased time to exhaustion in

both groups (keto-adapted 9.8%; P = 0.003; keto-naïve 8.3%; P=0.009) and increased peak VO_2 during exercise in the keto-adapted group (12%; P = 0.03) but not in the keto-naïve (1.8%; P = 0.15). The keto-naïve group had a significantly higher average power output during the Wingate (432 $\pm$ 155 W vs. 414 $\pm$ 144 W; P = <0.05) whereas the keto-adapted group had no significant difference. There were no significant differences between conditions in peak power output, cognitive performances, or blood glucose responses in either group. These results indicate that a moderate dose of ketone salts and caffeine prior to exercise significantly increases the magnitude of ketosis and improves high-intensity exercise performance in both keto-adapted and keto-naïve individuals.

Fat Quality and Source Modulate Blood Lipid Response and Fatty Acid Composition While Restricting Carbohydrates

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Saturated fat consumption from both plant and animal sources has been associated with increased cholesterol. However, the source and composition of the saturated fat can affect the hypercholesterolemic response differently. Macronutrient distribution of the diet, specifically the carbohydrate content, can also impact blood lipids. Hyperlipidemic responses to ketogenic diets have been observed in both clinic and research settings. To date very little, if any, research exists examining fat quality and nutritional ketosis. Therefore, the objective of this research was to determine how altering both fat quality and source, while restricting carbohydrates, impacts lipid profile and fatty acid (FA) composition responses. **Methods:** Twelve (male n=6; female n=6) healthy, normal weight participants completed this controlled feeding study (NCT02922062). Participants completed three, 3-wk eucaloric feeding phases (8% Carbohydrate, 74% Fat, 18% Protein) separated by 2-wk washout periods. During each feeding phase, 40% of fat calories came from canola oil (CO), palm oil (PO), or butter (BU). The first feeding phase acted as a low saturated fat baseline and the following two high saturated fat feeding phases were randomized and balanced for each subject. At the completion of each feeding phase, fasting blood was collected to determine serum cholesterol profiles and plasma phospholipid (PL) FA composition. **Results:** BU resulted in higher levels of total cholesterol ($p=0.011$) and LDL-cholesterol ($p=0.002$) compared to PO. Both saturated fat feeding phases (BU & PO) significantly increased total cholesterol ($p=0.003$, $p=0.02$) and LDL-cholesterol ($p<0.001$, $p=0.005$) compared to CO. Furthermore, BU increased PL myristic acid (C14:0), margaric acid (C17:0), and stearic acid (C18:0) more than PO ($p=0.002$, $p<0.001$, $p=0.001$). PO increased palmitic acid (C16:0) more than the BU ($p=0.002$). Both BU and PO increased total SFA ($p=0.003$, $p=0.006$) and decreased MUFA ($p=0.001$, $p<0.001$) compared to baseline. **Conclusion:** In the context of a very low-carbohydrate diet, emphasizing monounsaturated fat over saturated fat lessens the increase in total and LDL-cholesterol and the proportion of SFA in plasma PL. A diet rich in PO (higher in C16:0) had less of a hypercholesterolemic effect than one rich in BU (higher in short-/medium-chain SFAs and C18:0) suggesting that differences in SFA chain length modulate cholesterol and FA composition responses.

A Eucaloric Carbohydrate-Restricted Diet Significantly Improves VAT Mass and Markers of Metabolic Health in Subjects with Metabolic Syndrome: A Crossover Feeding Trial

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Background/Objective:
Metabolic syndrome (MetS) is an early sign of insulin resistance that increase risk of heart disease, stroke, type II diabetes, and premature morbidity. The most common features that define the metabolic syndrome include central obesity, dyslipidemia (high triglycerides and low HDL-C), hyperglycemia, and hypertension. Previous research has shown consistent benefits of carbohydrate restricted diets on features of MetS. However critics have suggested that beneficial effects of low-carbohydrate diets are primarily due to weight loss rather than a unique effect of carbohydrate restriction per se.

Purpose:
Using a controlled feeding model, we designed eucaloric and isonitrogenous diets that varied in carbohydrate and fat to determine the impact of carbohydrate content of the diet on central adiposity and MetS independent of weight loss.

Methods:
Sixteen adults with MetS participated in this controlled feeding trial (NCT02918422). Following a two-week run-in diet to determine energy expenditure, participants were assigned to one of three eucaloric and isonitrogenous (20% protein) feeding conditions: low-carbohydrate (LCD; 10% Carbohydrate, 70% Fat), moderate-carbohydrate (MCD; 30% Carbohydrate, 50% Fat), and a high-carbohydrate (HCD; 55% Carbohydrate, 25% Fat) diet. A two-week washout period separated each diet. The order of diets was randomized and balanced. At baseline and after each diet phase, participants completed a testing battery that included body composition and visceral adipose tissue (VAT) by dual energy x-ray absorptiometry (DXA) and a fasting blood draw for determination of lipid profiles and glucose.

Results: Body mass remained unchanged across all feeding periods indicating successful implementation of eucaloric feeding. A main effect of diet was determined for VAT, HDL, fasting blood glucose (FBG) and triglycerides (TG). Pairwise comparisons demonstrated significantly less VAT after LCD compared to HCD ($p=0.011$; $\Delta = -5.17\%$, 95% CI: -17.88%, 20.38%) and baseline ($p=0.011$; $\Delta = -9.44\%$, 95% CI: -27.13%, 8.26%). FBG ($p=0.017$) and TG ($p<0.01$) were decreased, while HDL increased ($p<0.01$) following LCD compared to HCD. LCD was the only intervention that had beneficial effects on MetS criteria compared to baseline for both HDL ($p<0.01$) and TG ($p<0.01$). MCD resulted in significantly greater TG

concentrations compared to LCD ($p=0.031$) and was not significantly different from HCD ($p>0.05$).

Conclusion:
The greater decrease in VAT and improved dyslipidemia after a LCD relative to eucaloric moderate- and high-carbohydrate diets in the setting of a controlled feeding environment, provides evidence that carbohydrate-restriction is a control element in the expression of MetS independent of weight loss.

Magnetic Resonance Evaluation of Cardiac Function and Structure following Extended Ketogenic Diet & Training Intervention

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Background/Objective:
Ketones are a preferred fuel for the heart, and emerging evidence indicates they may have beneficial effects on cardiac function. Cardiac magnetic resonance (CMR) measures of cardiovascular function and heart tissue characterization outcomes following keto-adaptation have not been evaluated in healthy humans to our knowledge.

Purpose:
The purpose of this experiment was to utilize advanced exercise stress and non-invasive CMR techniques to evaluate human cardiac function and tissue characterization in response to a ketogenic diet intervention guided by daily blood ketone monitoring in military personnel.

Methods:
A representative sample of 28 healthy adults from various branches of the military self-selected into a control (CON) group that consumed a high-carbohydrate diet, or a ketogenic diet (KD) group that included daily monitoring of blood ketones for ~12 weeks. Both groups performed a supervised mixed training intervention (2x/wk). CMR measure of heart function; ejection fraction (EF), stroke volume (SV), cardiac output (CO), and cardiac index (CI) in addition to tissue characterization were performed before and after the intervention. Cine images depicting dynamic cardiac function were acquired in seven short-axis slices covering the left ventricle from base to apex. Post exercise stress heart rate was recorded during image acquisition at 38.0±10.7sec after cessation of exercise.

Results:
There were no significant differences between KD and CON at baseline in cardiac function measures at rest and following max exercise stress EF, SV, CO, and CI. At post intervention, resting heart rate was not different between groups CON 73.3±10.3bpm vs KD 75.1±14.5bpm, p=0.700. Post exercise stress cardiac output was elevated (p=0.037) in KD participants (15.5 ± 3.5 L/min) compared with CON (13.1 ± 2.0L/min) at post. Cardiac output relative to participant body surface area, cardiac index (CI), was increased (p=0.024) in KD participants (6.6 ± 1.2 L/min/m^2 versus 7.9 ± 1.7L/min/m2) post-intervention. There were no differences in intra-myocardial heart fat between groups at baseline (CON 1.0±1.1% vs KD 1.2±1.3%, p=0.723). Post intervention heart fat percentage in CON (1.9±1.4%) was not different (p=0.082) than KD (1.0±1.1%). Following intervention, T1 values measured in the septum were significantly (p=0.050) lower in KD group (959.9±61.4ms) than CON (997.2±28.9ms) at rest. T1 values

measured in the septum post exercise were also lower ($p=0.005$) in KD (968.5±87.9ms) than CON (1051.8±53.3ms).

Conclusion: Extended adherence to a well-formulated ketogenic diet guided by daily blood ketone measures had positive effects on functional measures of resting and post-exercise cardiac performance (e.g., increased ejection fraction, cardiac output, and cardiac index) compared with a mixed diet in healthy military personnel.

Extended Ketogenic Diet and Physical Training Intervention in Representative Sample of Military Personnel

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Background/Objective:
Training is an important component of military readiness, health, and safety. Likewise, adequate nutrition can work synergistically to maximize training effects. Low carbohydrate and ketogenic dietary paradigms may augment training-induced changes in body composition and performance relevant to the modern combat soldier exposed to chronic multifactorial stressors.

Purpose:
The purpose of this experiment was to evaluate the effects of an extended duration ketogenic diet and physical training intervention on body composition and physical performance of military personnel.

Methods:
Twenty-nine healthy adults from various branches of the military self-selected into a control (CON) group that consumed their habitual high-carbohydrate diet or a ketogenic diet (KD) group that included daily monitoring of blood ketones for ~12 wk. Both groups performed a supervised mixed training intervention (2x/wk) that emphasized strength, power, and high intensity interval exercise. Body composition and physical performance including aerobic capacity and maximum squat and bench press strength were evaluated.

Results:
Blood glucose and ketone concentrations tracked daily in KD participants resulted in a 97% reporting rate. Average daily blood ketone concentration was 1.2 mmol/L in KD participants, indicating high compliance with the diet. The KD group completed 88% of physical training sessions while the CON group completed 85%. There were no significant changes in exercise heart rate between groups at baseline or post intervention. The KD group lost 7.7kg, whereas CON remained weight stable. Body fat percentage decreased -5.1 ± 2.7% in KD versus -0.7 ± 1.9% in CON group ($p < 0.001$) There was an increase in VO_2peak in the KD group (45.1 ± 7.0 to 48.7 ± 6.6 ml/kg/min, $p = 0.039$). KD had a significantly lower peak exercise RER (1.02 ± 0.05) than CON (1.15 ± 0.04, $p < 0.001$) post intervention. Both groups increased relative lower body (22.3 ± 17.3% , $p<0.001$ KD; versus 21.2 ± 17.1%, $p< 0.001$ CON) and upper body strength (9.40 ± 10.1%, $p = 0.004$ KD; 9.18 ± 13.3%, $p < 0.001$ CON) pre to post. There were no differences between relative strength changes for one-repetition squat and bench press between groups.

Conclusion:

This is the first study to test body composition and physical performance response associated with keto-adaptation and physical training intervention in individuals representing a broad range of United States Military personnel. Extended adherence to a ketogenic diet guided by blood ketone measures may present a possible strategy for robust improvements in body composition while maintaining normal training-induced increases in physical performance.