SPRING/SUMMER 2024

Gillingham Lab

RESEARCH NEWSLETTER

EMMANUEL SHAPIRA AWARD RECIPIENT

We are pleased to share that Dr. Gillingham and fellow co-authors received the 2023 Emmanuel Shapira Award for their paper, titled ***Resting and total energy expenditure of patients with long-chain fatty acid oxidation disorders (LC-FAODs).*** The award is given by the Society for Inherited Metabolic Disorders (SIMD) and recognizes the best paper in the field of biochemical genetics and metabolism published in the previous year in Molecular Genetics and Metabolism by a SIMD member or their trainee. The Shapira Award was established in 2003 and is named after Emmanuel Shapira, MD, PhD, a founder and supporter of SIMD.

RECENT PUBLICATIONS

A review of fatty acid oxidation disorder mouse models.
Article link: https://doi.org/10.1016/j.ymgme.2024.108351

Optical coherence tomography angiography of choroidal neovascularization in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD)
Article link: https://doi.org/10.1016/j.ajoc.2023.101958

A proposal for an updated staging system for LCHADD retinopathy
Article link: https://doi.org/10.1080/13816810.2024.2303682

Cardiac phenotype in adolescents and young adults with long-chain 3-hydroxyacyl CoA dehydrogenase (LCHAD) deficiency
Article link: https://doi.org/10.1016/j.gim.2024.101123

Early diagnosis and treatment by newborn screening (NBS) or family history is associated with improved visual outcomes for Long-chain 3-hydroxyacylCoA dehydrogenase deficiency (LCHADD) chorioretinopathy
Article link: https://doi.org/10.1002/jimd.12738

KETONE SUPPLEMENTATION IN LONG-CHAIN FATTY ACID OXIDATION DISORDERS

We recently completed two studies, one in humans and the other in mice, on ketone supplementation and long-chain fatty acid oxidation disorders (LC-FAODs). Data analysis and write-up of the mouse study results is still ongoing, but here we present our findings of our study in humans with LC-FAODs. Ketones are produced from the breakdown of fats in the liver and are an important alternative energy source during periods of fasting or with longer bouts of moderate-intensity exercise. Because of the block breaking down fats via fat oxidation, patients with LC-FAODs have impaired ability to make ketones. This leads to low energy availability and increased use of sugar, and may be related to the exercise-induced rhabdomyolysis patients experience. Eating carbohydrates before exercise can help prevent exercise-induced rhabdomyolysis, but it doesn't help to raise blood ketone levels. Alternatively, taking MCT can increase blood ketones by bypassing the defects in the fat oxidation pathway, but MCT can cause significant gastrointestinal (GI) symptoms. Consuming ketones before exercise is being explored as an alternative supplement as it can raise blood ketones with possibly less GI distress.

In our preliminary study of ketone supplementation in LC-FAOD patients, we teamed up with Nestle Health Sciences, the study funder, to study 5 patients with CPT2, VLCAD or LCHAD deficiency and asked them to come to OHSU to complete two 40-minute, moderate-intensity treadmill exercise tests after drinking either a Nestle-formulated ketone beverage or a carbohydrate (CHO) beverage 2 days apart. The drinks were formulated so that they appeared and tasted similar and the number of calories in each was the same. The visit order participants received each beverage was randomized and neither the participants nor investigators knew which drink the participants received before each exercise test (double-blind study). The goal of the study was to test whether the ketone beverage was safe and well-tolerated, to measure if the ketone drink increased blood ketone levels to similar levels observed after exercise and in healthy participants after ingestion of a similar ketone beverage, and to explore its effects on blood metabolites.

KETONE DRINK WAS SAFE AND WELL-TOLERATED

To assess whether the ketone beverage was safe and well-tolerated, we asked participants to rate any GI symptoms they experienced within 2 hours after drinking the beverages on a scale of 0-10. We found that the beverages resulted in 12 GI symptoms reported by three of the five patients. Eleven of these were related to the ketone beverage and all were mild ("1") to moderate ("5") in severity. The most common symptom was gastric reflux (Figure 1). Although there were more GI symptoms reported after drinking the ketone beverage compared to the carbohydrate beverage, compared to other sources of ketones such as MCT, the GI symptoms were mild and suggest that it may be a safe alternative.

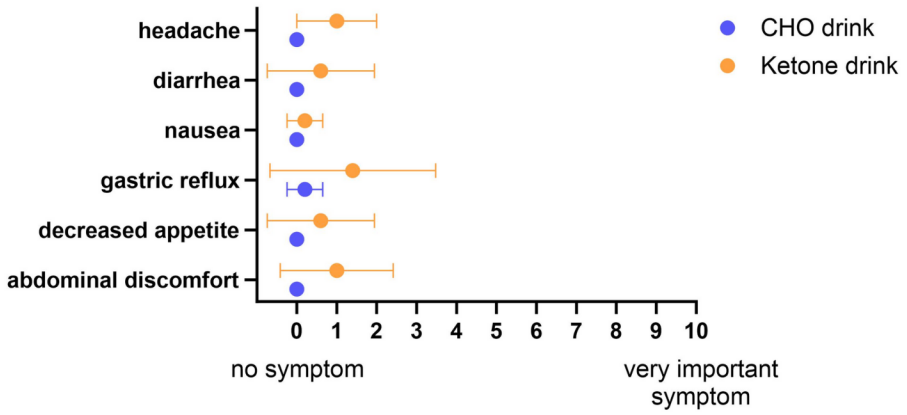

Figure 1. Gastrointestinal symptoms reported by 5 LC-FAOD participants 2-hours after ingestion of carbohydrate or ketone beverage before moderate-intensity exercise. Values are mean ± standard deviation.

KETONE DRINK RAISED BLOOD KETONE LEVELS

We collected blood samples in the morning, when participants were fasted ("baseline"), provided them breakfast, then collected blood again 3 hours later ("pre-beverage"). Participants drank the ketone or CHO beverage and 20 minutes later we collected another blood sample ("pre-exercise"). Subjects then completed the 40-minute treadmill test and we collected blood after the test was completed ("post-exercise") and 20 minutes later ("recovery"). As expected, after ingestion of the ketone drink, blood ketone levels rose significantly compared to levels measured after ingestion of the CHO drink (Figure 2). Post-exercise, blood ketone levels were within a similar range of 0.4-0.6 mM as previously measured in healthy control participants 75-90 minutes after drinking a similar ketone drink[1] and to levels in humans after moderate exercise[2, 3].

Figure 2. Blood ketone levels during the baseline, fasted state and during moderate-intensity exercise, before and after drinking the carbohydrate or ketone beverage. Values shown at baseline are individual data points and the median. Values during exercise are mean ± standard deviation. Significant differences between beverages are denoted: *** p value < .001.

KETONE DRINK SUPPRESSED LIPOLYSIS

Exercise increases free fatty acids (FFA) released from body fat stores into circulation in order to provide the necessary energy to fuel the activity. These FFA travel in blood to the liver to be converted to ketones. We measured a decrease in free fatty acids in circulation with exercise after drinking the ketone drink as compared with the carbohydrate drink (Figure 3A). The release of FFA from body fat stores is called lipolysis. Oral ketones may suppress the release of FFA from fat stores. A decrease in FFA in the blood indicates a decrease in lipolysis.

Figure 3. Blood free fatty acids before and after drinking a carbohydrate or ketone beverage with moderate-intensity exercise. Values shown at baseline are individual data points and the median. Values during exercise are mean ± standard deviation. Significant differences between beverages are denoted: * p value < .05, ** p value < .01.

KETONE DRINK SUPPRESSES RISE IN LONG-CHAIN ACYLCARNITINES

The rate of lipolysis and rise in blood FFA is proportional to the metabolism of fat in the liver for energy. In patients with LC-FAOD, a rise in FFA is coupled with an increase in long-chain acylcarnitines (LC-AC) in blood, markers of partial fat metabolism. We measured a decreased change of LC-ACs C14:1, C16:1 and C18:1 from pre-beverage levels with exercise after the ketone drink (Figure 4). This suggests that the decrease in lipolysis is associated with a decrease in fat metabolism in the liver after the ketone drink, leading to a significantly reduced rise in LC-AC with exercise.

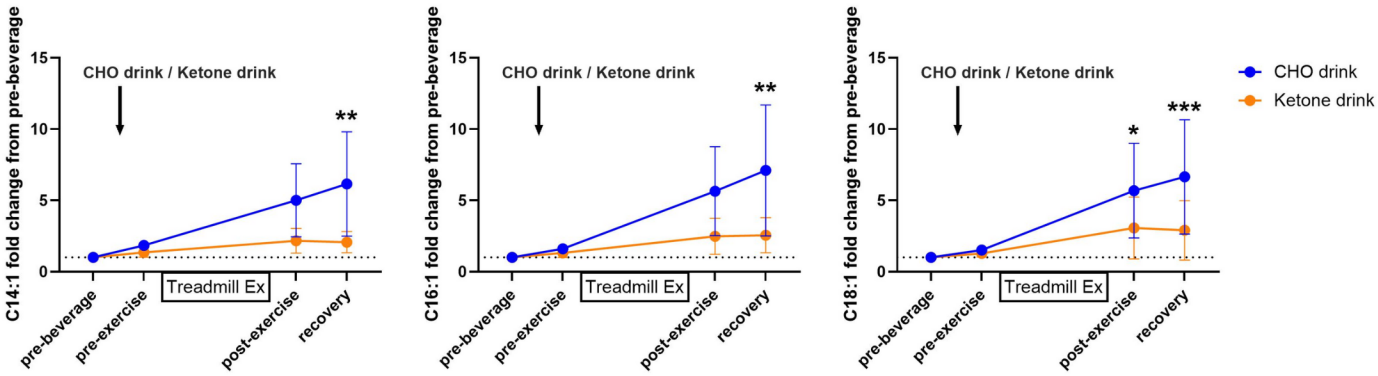

Figure 4. Long-chain acylcarnitine C14:1, C16:1 and C18:1 fold change from pre-beverage before and after drinking a carbohydrate or ketone beverage with moderate-intensity exercise. Values during exercise are mean ± standard deviation. Significant differences between beverages are denoted: ** p value < .01, *** p value < .001.

CONCLUSIONS

Our study showed that ketone supplementation was safe and well-tolerated and resulted in a mild rise in blood ketones in LC-FAOD participants. Ketone supplementation also suppressed lipolysis and the rise in long-chain acylcarnitines normally associated moderate-intensity exercise. Our study had limitations, including the small sample size of participants and the timing of our blood sampling which didn't allow us to capture blood ketone levels at the time of the expected peak around 45 minutes post-drink.

An area of future study may be a dose escalation study where participants with LC-FAODs receive increasing doses of ketones. This would help us to determine the optimal dose to reach peak ketone concentrations closest to healthy controls and stimulate increased ketone utilization for energy without causing adverse health effects. Additionally, examining ketone supplementation in combination with carbohydrates is also of interest.

REFERENCES

1. Cuenoud, B., et al., *Metabolism of Exogenous D-Beta-Hydroxybutyrate, an Energy Substrate Avidly Consumed by the Heart and Kidney*. Front Nutr, 2020. 7: p. 13.
2. Fery, F. and E.O. Balasse, *Response of ketone body metabolism to exercise during transition from postabsorptive to fasted state*. Am J Physiol, 1986. 250(5 Pt 1): p. E495-501.
3. Koeslag, J.H., T.D. Noakes, and A.W. Sloan, *Post-exercise ketosis*. J Physiol, 1980. 301: p. 79-90.