eP030

Effect of peanut butter consumption on C26 concentrations in breastmilk – Implications for X-ALD newborn screening

Anna Scott[1] and Irene Chang[1]

[1]*Seattle Children's Hospital*

Introduction: Newborn screening for X-linked Adrenoleukodystrophy (X-ALD) has been expanding across the United States, increasing the frequency of follow-up diagnostic testing in breastfeeding infants. X-ALD is caused by pathogenic variants in the *ABCD1* gene, resulting in toxic accumulation of very long chain fatty acids (VLCFA) in the brain, spinal cord, and adrenal glands. Affected males may present with progressive cerebral disease, adrenomyeloneuropathy, or isolated adrenal insufficiency. The primary biomarkers of X-ALD are elevations of C26 and C26/C22 and C24/C22 ratios in plasma.

Peanut butter contains high amounts of VLCFA, particularly C26, and previous studies have demonstrated that plasma VLCFA elevations following peanut butter ingestion closely resemble the patterns observed in individuals with X-ALD. Peanut proteins such as Ara h 1 and Ara h 2 have been detected in breast milk samples from lactating women within two hours after ingesting 50 grams of dry roasted peanuts. These data suggest peanut proteins and components may cross into plasma and breastmilk.

Peanut butter consumption has previously acted as an interfering compound to give false positive VLCFA results in patients tested clinically at Seattle Children's Biochemical Genetics Laboratory. Clinical VLCFA testing of a breastfeeding infant with multifocal epilepsy showed mild C26 elevations, which normalized after peanut butter was eliminated from the maternal diet. Based on these observations, we hypothesize that maternal peanut butter consumption may increase C26 levels in breastmilk.

Methods: We performed an observational study to analyze VLCFA levels in breastmilk from breastfeeding women after consumption of a one-time, three tablespoons (50 grams) oral dose of peanut butter. Study participants collected timed samples of breastmilk (~1 mL per sample) at 3, 6, 9, and 12 hours after eating peanut butter. Participants served as their own controls, with the baseline breastmilk sample collected after abstaining from peanuts or peanut butter for 24 hours prior to collection. VLCFA were analyzed by gas chromatography-mass spectrometry per standard laboratory procedures.

Results: As of November 2021, twelve study participants provided informed consent and nine sample sets were collected and analyzed. Six of the nine sample sets demonstrate increased levels of C26 between four and nine hours after peanut butter ingestion. Absolute change in C26 varied by individual; the maximum observed increase in C26 was 817% compared to the control sample.

Conclusion: Our study demonstrates peanut butter ingestion by lactating women can increase the concentration of C26 very long chain fats in breastmilk. Whether this transitively causes mild VLCFA elevations in the plasma of breastfeeding infants remains to be elucidated. Maternal diet is known to affect other metabolites detected by newborn screening, leading to false positive results. For example, low free carnitine or mild elevations of C3 (propionylcarnitine) can be seen in newborns secondary to low maternal protein intake or vitamin B12 deficiency. This study has important downstream clinical implications, as false positive VLCFA results in breastfeeding infants due to maternal peanut consumption may lead to unnecessary testing and clinical evaluations.

https://doi.org/10.1016/j.gim.2022.01.068

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Clinical and biochemical characterization of carnitine palmitoyltransferase-2 deficiency and novel case exacerbated by heterozygosity with partial carnitine transporter deficiency

Brian Shayota[1], Bijina Balakrishnan[1], Lorenzo Botto[1], Marzia Pasquali[1], Nicola Longo[1]

[1]*University of Utah*

Introduction: Carnitine palmitoyltransferase 2 (CPT-2) deficiency is a disorder of the carnitine cycle that impairs long-chain fatty-acid oxidation. It can present at birth with a lethal neonatal form, in infancy/childhood with an infantile hepatocardiomuscular form, and later in life with a myopathic form. Patients with the lethal neonatal form have null pathogenic variants in the *CPT2* gene that result in the complete absence of enzyme activity. By contrast, patients with the other forms have at least one copy of a less impactful variant allowing for some residual enzyme activity. It remains unclear why some patients with at least one copy of a milder variant present with a more severe phenotype.

Methods: Subjects were gathered from our database of known CPT2 deficient patients followed within our single institution at the University of Utah. In total, we gathered our experience with 9 patients with diagnosed CPT-2 deficiency. Biochemical and molecular testing was performed on a clinical basis. Genetic testing variants reported using HGVS NM 000098.3.

Results: Our cohort of CPT deficient patients consisted of 7 with the classical later onset myopathic form, 1 with the severe lethal neonatal form, and 1 with infantile hepatocardiomuscular form. The plasma acylcarnitine profile identified increased C16- (0.53±0.35, normal <0.1 μM), C18- (0.26±0.16, normal <0.04 μM), C18:1- (0.6±0.5, normal <0.17 μM), and C18:2- (0.27±0.2, normal <0.08 μM) carnitines in all cases. CPT-2 activity in fibroblasts of 3 patients with the myopathic form was reduced to 13-15% of normal CPT2 enzyme activity compared to controls. DNA sequencing of the *CPT2* gene identified at least one copy of a mild pathogenic variant (c.338C>T/p.S113L) in 7 of the 9 patients along with a second variant. One exception to this was the lethal neonatal case with homozygosity for a severe truncating variant (c.370C>T/p.R124X).

One subject with a suspected mild genotype (c.338C>T/p.S113L; c.534_558 delinsT) had a more severe phenotype with an infantile hepatocardiomuscular form, presenting at 1 year of age with loss of consciousness, severe hypoglycemia, carnitine deficiency, and mildly increased CK. Further investigation revealed a significant carnitine deficiency at time of presentation (free carnitine= 5 μM versus 29.2±4.5 μM in the others), suggesting possible impairment of the carnitine transporter. Carnitine transport was reduced to 35% of normal activity in fibroblast from this subject, which is consistent with carrier status and was not seen in 2 others with the classical myopathic form of CPT2 deficiency. These results suggest that the severity of myopathic CPT2 deficiency at time of presentation may in part be explained by variations in carnitine transporter activity.

Conclusion: In conclusion, this study has further characterized the clinical and biochemical phenotype of CPT2 deficiency. Additionally, we provided the first reported example of the phenotype severity being influenced by variants outside of the *CPT2* gene.