Human Milk Endocannabinoid Levels as a Function of Obesity and Diurnal Rhythm

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Background
The endocannabinoid system is involved in regulation of a variety of physiological and cognitive processes including fertility, pregnancy, during pre- and postnatal development, appetite, pain-sensation, mood, and memory and in mediating the pharmacological effects of cannabis. The principle endocannabinoids in humans are identified as 2-arachidonoylglycerol (2AG) and anandamide (N-arachidonylethanolamine, AEA), which are lipid-based ligands that bind to cannabinoid receptors. Interestingly, these endocannabinoids have been found in human milk although their role(s) have not yet been fully determined. It has been suggested they initiate infant suckling and may play a role in infant brain and neuronal development. Better understanding of their quantities and potential role in human milk may lead to important discoveries on how endocannabinoids impact the development of the breastfeeding child.

Objective
The purpose of this study was to determine the normal concentration ranges of endocannabinoids (2-AG and AEA) in human milk and also determine how obesity and diurnal rhythm may affect their concentration levels in breast milk.

Experimental Approach
Each sample was analyzed in triplicate and concentrations were averaged. The average data for individuals:

- Normal (range: 35–101 ng/mL)
- Overweight (range: 22–140 ng/mL)
- Obese (range: 18–143 ng/mL)

The optimal chromatographic conditions were selected for the resolution and sensitivity for the endocannabinoids analytes AEA and 2-AG. The analyte and standards were analyzed by AB Scie QTRAP 5500 ultrahigh-performance liquid chromatography (UHPLC/MS/MS) tandem mass spectrometry.

Methodology
- The optimal chromatographic conditions were selected for the resolution and sensitivity for the endocannabinoids analytes AEA and 2-AG.
- The analyte and standards were analyzed by AB Scie QTRAP 5500 ultrahigh-performance liquid chromatography (UHPLC/MS/MS) tandem mass spectrometry.
- Milk sample extraction was accomplished using protein precipitation.
- Each sample was analyzed in triplicate and concentrations were averaged.

Results
- All women participating in the study were between 21 and 39 years of age. Their BMI ranged from 19.6 to 51.6 kg/m² and on average they fed their child approximately 9 times in a 24-hour period of collection.
- In a dataset of 263 milk samples collected for this study, 2-AG concentration levels were quantified and ranged from 13.1 - 221 ng/mL.
- The average levels of 2-AG were compared among the three BMI weight groups with no significant difference determined (Figure 1).
- Behavior and metabolic requirements may be influenced by circadian/diurnal rhythm.
- 2-AG levels in milk were significantly higher during the day as compared to night samples (p = 0.02) and obese (p = 0.04) groups (Figure 2).
- Longitudinal follow up was done 4-6 months postpartum after the initial collection of 4-6 weeks postpartum in order to determine any difference in the 2-AG levels overtime.
- Unfortunately, there was a high attrition rate due to the length of time between collection points for this part of the study with only 14 out of 36 women contributing to the study.
- In a dataset of 263 milk samples collected for this study, 2-AG levels in milk and maternal weight.
- The average levels of 2-AG were compared among the three BMI weight groups with no significant difference observed for 2-AG levels overtime.

Conclusion
Our study demonstrates that appropriate collection and storage of breast milk is critical for determining accurate endocannabinoid concentrations. The findings in this study also provide evidence that 2-AG could play a role in chrononutrition in communicating time of day information for infants. In addition, we found mother’s weight post-partum may influence 2-AG levels in their human milk. Future studies need to be developed to correlate 2-AG in milk and outcome in their infants.

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Figure 1: Concentration of 2-AG in breast milk for different BMI groups

Figure 2: Diurnal Rhythm on Endocannabinoid levels

Figure 3: Longitudinal Changes in 2-AG in Milk.

Summary of Results

- Storage Conditions
  - Normal vs. Obese
  - Breast Milk
  - Temperature and time affects levels

- 2-AG follows diurnal rhythm

- Endocannabinoid Levels
  - Variance between individuals
  - Obesity had greater affect

- 2-AG influences 2-AG levels

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