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

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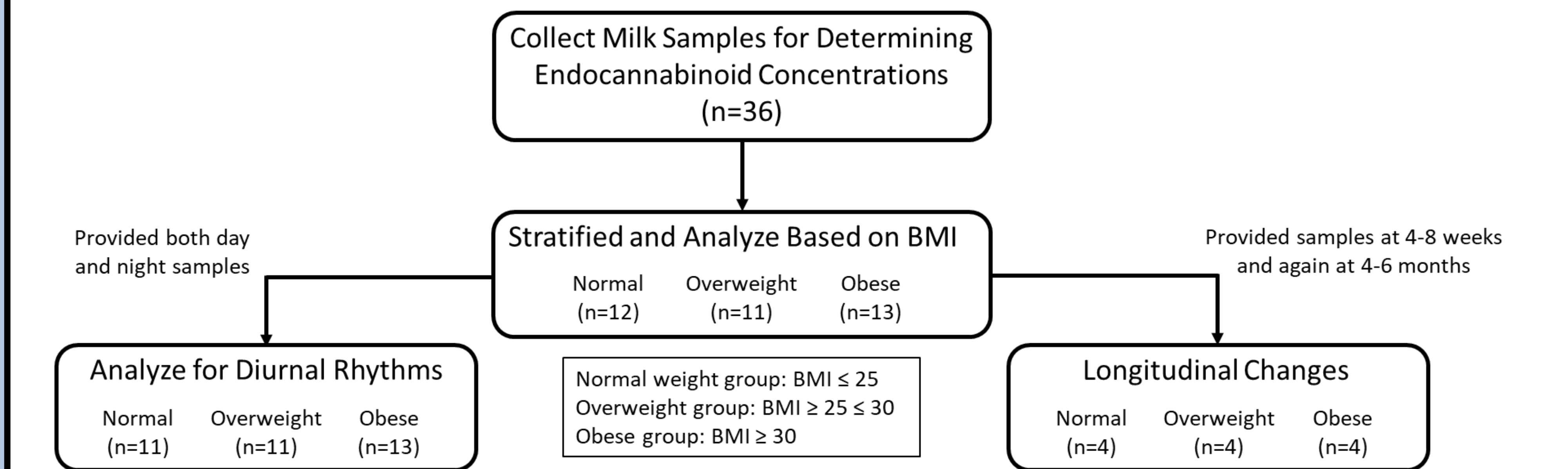
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Background

The endocannabinoid system is involved in regulation of a variety of physiological and cognitive processes[2] 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-arachidonoylethanolamine, 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

Collect Milk Samples for Determining Endocannabinoid Concentrations (n=36)

Provided both day and night samples

Stratified and Analyze Based on BMI
Normal (n=12) Overweight (n=11) Obese (n=13)

Provided samples at 4-8 weeks and again at 4-6 months

Analyze for Diurnal Rhythms
Normal (n=11) Overweight (n=11) Obese (n=13)

Normal weight group: BMI ≤ 25
Overweight group: BMI ≥ 25 ≤ 30
Obese group: BMI ≥ 30

Longitudinal Changes
Normal (n=4) Overweight (n=4) Obese (n=4)

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 Sciex 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

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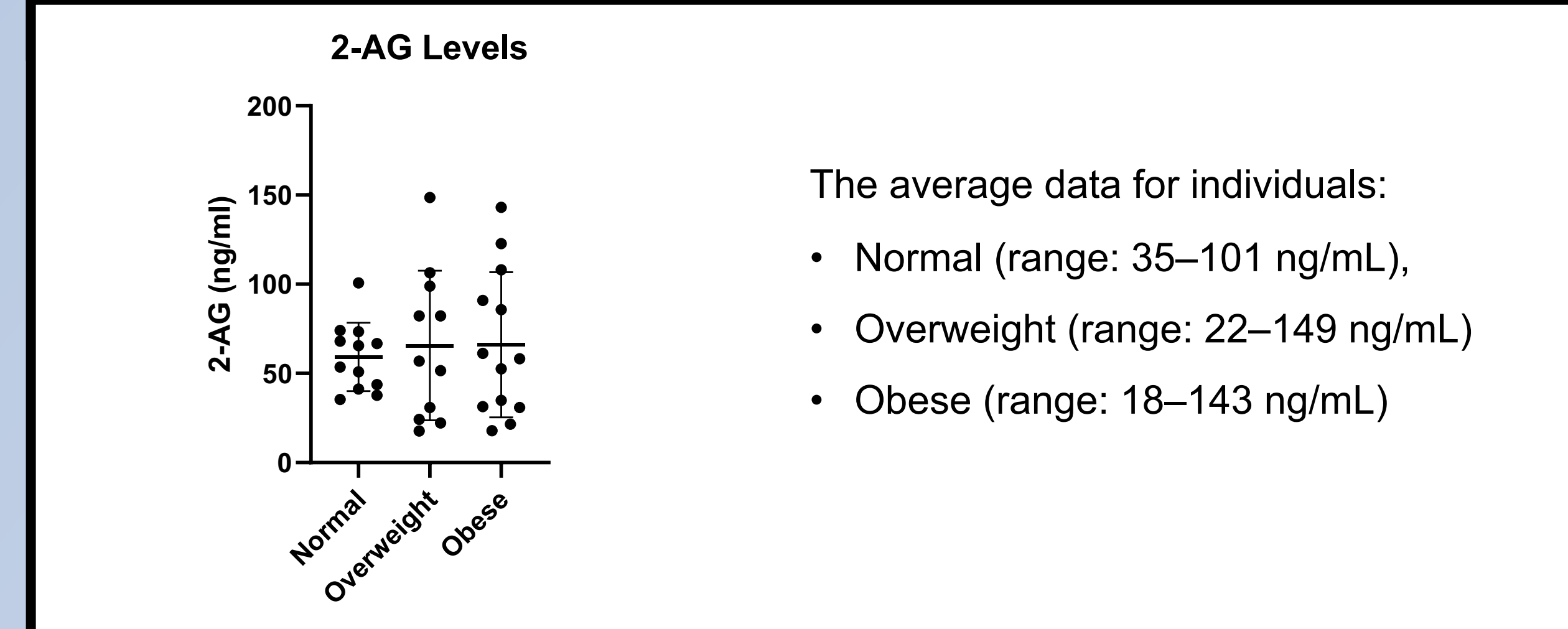

2-AG Levels

The average data for individuals:
- Normal (range: 35–101 ng/mL),
- Overweight (range: 22–149 ng/mL)
- Obese (range: 18–143 ng/mL)

Figure 2: Diurnal Rhythm on Endocannabinoid levels

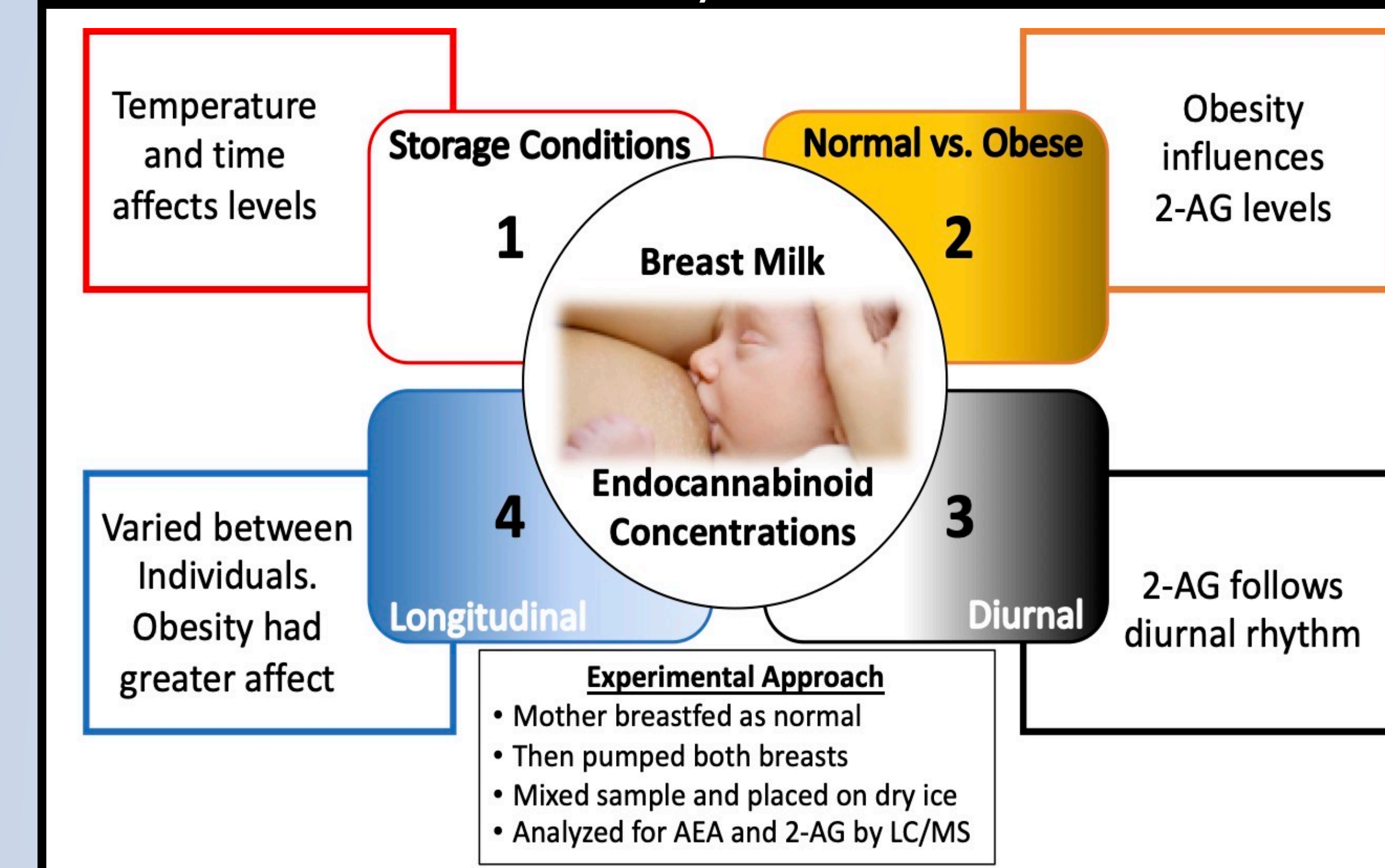A. Normal Weight - Diurnal Pattern
Day = 6AM ~ 10PM   Night = 11PM ~ 5AM
24 Hour
*Dotted line represents SD.

B. Diurnal
0.02* ns 0.04*
Day Night
Normal Overweight Obese

2-AG levels in milk significantly higher during the day as compared to night samples
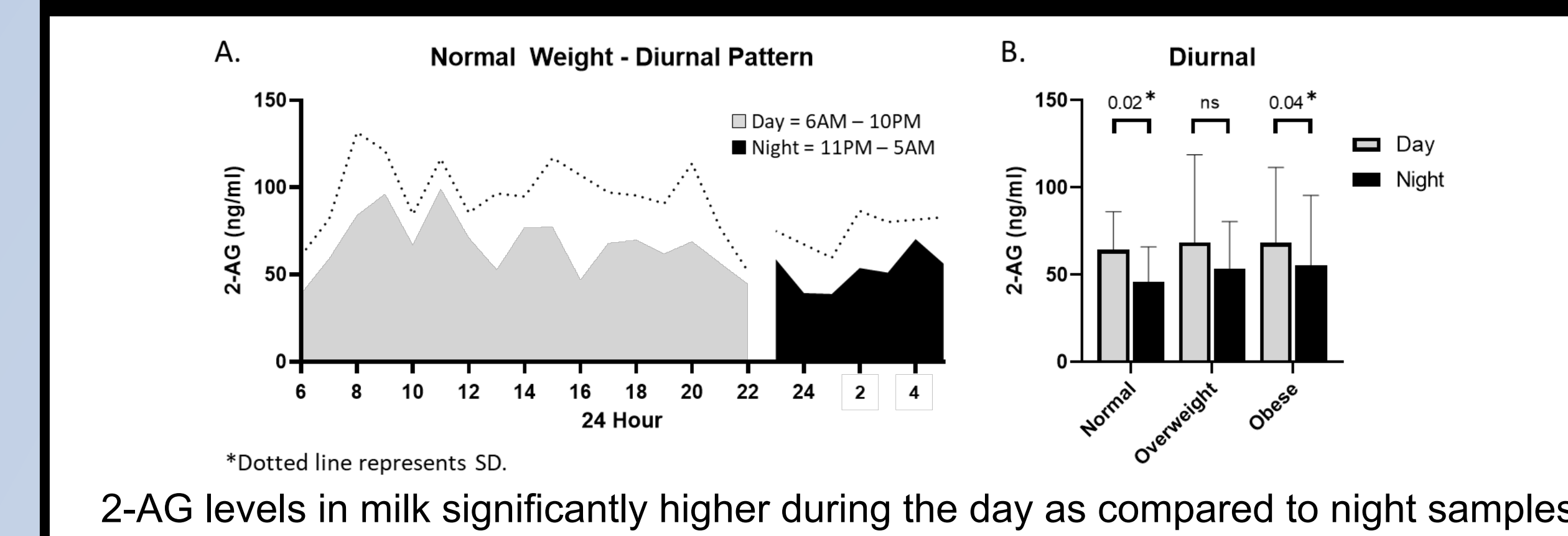
Figure 3. Longitudinal Changes in 2-AG in Milk.

Longitudinal
4-8 Weeks
4-6 Months
Normal Overweight Obese

➢ High attrition rate , only 14 out of 36 women.
➢ No significant difference observed for 2-AG levels overtime.

Summary of Results

Temperature and time affects levels
Storage Conditions 1
Breast Milk
Normal vs. Obese 2
Obesity influences 2-AG levels

Varied between Individuals. Obesity had greater affect
Longitudinal 4
Endocannabinoid Concentrations
Diurnal 3
2-AG follows diurnal rhythm

Experimental Approach
- Mother breastfed as normal
- Then pumped both breasts
- Mixed sample and placed on dry ice
- Analyzed for AEA and 2-AG by LC/MS

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/m^{2} and on an 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 in both normal ($p = 0.02$) 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 contributed milk samples. No significant difference was observed for the average 2-AG levels overtime for the 3 groups (Figure 3)
- Although we were unable to obtain a large number of milk samples for analysis as a function of maternal weight, we were able to see trends develop between 2-AG levels in milk and maternal weight.

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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