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Relationship Between Endocannabinoids During Pregnancy and Infant Heart Rate and Heart Rate Variability

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Abstract

Maternal factors during pregnancy affect fetal and infant heart rate (HR) and heart rate variability (HRV), clinical indices of early life autonomic development. Although there is an established relationship between maternal n-3 long chain polyunsaturated fatty acid (LCPUFA) intake and early life HR and HRV, the mechanism has not been elucidated. It is plausible that in utero exposure to specific endocannabinoids, dictated in part by maternal nutrition, underlie previous observations. The relationship between circulating endocannabinoids, HR, and HRV at any life or physiological stage has not been assessed. This study explored the relationship between in utero exposure to endocannabinoids and infant HR and HRV. Mother-infant pairs (n = 11) were recruited before 20 gestational weeks. Circulating n-6 and n-3 fatty acid related endocannabinoids were quantified in maternal plasma at 20, 24, 32, and 36 gestational weeks and umbilical cord venous plasma at term delivery. Infant HR and HRV were assessed at 2 weeks, 4 months, and 6 months of age using 24 h continuous ambulatory electrocardiograph monitoring. Regression analyses were used. In general, maternal and umbilical cord n-3 endocannabinoids were inversely related to infant HR and positively related to HRV. When the ratio of n-6 to n-3 endocannabinoids reflected greater presence of the n-6 series, there were positive relationships with infant HR and inverse with HRV. These data indicate, but do not definitively conclude, that in utero exposure to endocannabinoids may contribute to programming the developing autonomic nervous system which is clinically reflected as decreased HR and increased HRV. Future studies focused on autonomic development and function should include an assessment of endocannabinoid status.

Keywords: *Endocannabinoid; Pregnancy; Developmental Programming; Autonomic Nervous System; Docosahexaenoyl Ethanolamide; Heart Rate Variability*

Abbreviations

LCPUFA: Long Chain Polyunsaturated Fatty Acid; HR: Heart Rate; HRV: Heart Rate Variability; SDNN: Standard Deviation of Filtered RR Intervals Over 24-H Period; ASDNN: Mean of Standard Deviations of Filtered RR Intervals for All 5 Min Segments of Analysis; SDANN: Standard Deviation of Means of Filtered RR Intervals for All 5 Min Segments of Analysis; AEA: anandamide; EEA: Eicosanoyl Ethanolamine; EPEA: Eicosapentaenoyl Ethanolamine; DHEA: Docosahexaenoyl Ethanolamine; BPM: Beats Per Minute; MS: Milliseconds

Introduction

The *in utero* environment can influence neonatal outcome with lifelong consequences [1]. Alterations in the fetal environment, including a stimulus or insult during a critical developmental period, can cause structural, physiological, and metabolic adaptations, potentially predisposing the fetus to adverse outcomes in later life [2,3].

The fetal autonomic nervous system can be programmed by maternal factors, including n-3 long chain polyunsaturated fatty acid (LCPUFA) status during pregnancy [4, 5]. This programming effect is apparent as observable differences in fetal and neonatal heart rate (HR) and heart rate variability (HRV), developmental expressions of autonomic activity [6-9]. Similarly, n-3 LCPUFA intake in infancy is related to HR and HRV [10-12]. Overall, these data suggest early life exposure to n-3 LCPUFA decreases infant HR and increases HRV.

Despite recent speculation [13], the mechanism by which n-3 LCPUFA modulate HR and HRV is unknown. It is worth considering if n-3 LCPUFA or their metabolic derivatives are responsible for previously documented HR- and HRV-modulating effects. Endocannabinoids are bioactive fatty acid derivatives [14] and their production is dictated in part by fatty acid intake [14,15-17]. Endocannabinoids modulate autonomic function [18,19], although the relationship between endocannabinoids and HR/HRV has not been assessed. It was recently suggested that the endocannabinoid system is involved in long-distance signaling and underlie developmental programming [20]. It is plausible, then, that fetal/infant autonomic sensitivity to endocannabinoids, altered by maternal n-3 LCPUFA intake or other mechanisms, may underlie previous observations [4,5,10-12] that evidence a HR-lowering and HRV-increasing effect of n-3 LCPUFA. Accordingly, the aim of our study was to assess the relationship between maternal endocannabinoid status during pregnancy and infant HR and HRV during the first 6 months of life.

Materials And Methods

All procedures involving human subjects were approved by the Institutional Review Boards at the Louisiana State University AgCenter, Woman's Hospital, and Pennington Biomedical Research Center.

Study enrollment

Participants enrolled in a larger study (LA Moms and Babies Study [LAMBS] for Nutrition and Growth) were invited to enroll. For LAMBS, women were recruited from an obstetrics and gynecology clinic in Baton Rouge, LA and invited to participate in the study if they were 18 – 35 years of age, had a singleton pregnancy, were 17 – 20 gestational weeks, and had a pre-pregnancy body mass index of 25.0 - 29.9. Exclusion criteria included: history or current diagnosis of high blood pressure, high blood lipids, kidney disease, liver disease, polycystic ovarian syndrome, HIV, or diabetes mellitus (type 1, type 2, or gestational); a first degree relative diagnosed with diabetes mellitus (type 1 or type 2); uncontrolled thyroid disorder; smoking in the past 6 months; parity >5; pre-term birth (≤37 gestational weeks); positive test for group B streptococcus, syphilis, or Hepatitis B; or pregnant or lactating within the previous 6 months. From LAMBS, seventeen women were invited to participate. Informed consent was completed by thirteen women; one mother-infant pair was excluded from the study due to infant diagnosis of congenital heart defects and one mother-infant pair was excluded for lack of compliance. The final *n* was 11. Demographics of our participants are presented in table 1.

Maternal characteristics	
Age (y)	26.7 ± 3.7[2]
Pre-pregnancy BMI (kg/m^2)	26.9 ± 1.4
Race (*n*)	
African American	7
White	4
Pre-pregnancy parity	
0	4
1	5
3	2
Gestational length (wk)	39.9 ± 1.0
Delivery type (*n*)	
Vaginal	9
Cesarean	2
Infant characteristics	
Sex (*n*)	
Female	9
Male	2
Birth weight (kg)	3.4 ± 0.4
Birth length (cm)	51.1 ± 1.8
Birth head circumference (cm)	13.6 ± 0.6
Apgar, 1 min[3,4]	8 (7 – 9)
Apgar, 5 min[3]	9 (5 – 9)

Table 1: *Characteristics of the study population, n = 11[1].*

[1]Number of subjects in analyses unless otherwise noted; [2]Values are $\bar{x}$ ± SD; 3n = 10; 4Values are $\bar{x}$ (range).

Maternal and cord venous blood collection

Maternal blood (10 mL) was collected at 17-20, 24, 32, and 36 gestational weeks from the antecubital vein into EDTA-containing tubes. Umbilical cord venous blood (5 - 10 mL) was collected at delivery from the umbilical cord vein into EDTA-containing tubes. Plasma was separated from erythrocytes by centrifugation (2600 × *g* at 4°C for 10 min) and stored at -80°C. Erythrocytes were used to determine fatty acid status; those data are reported elsewhere [5].

Plasma analysis for endocannabinoids

A known amount of deuterated internal standard mixture was added to thawed plasma. Plasma proteins were precipitated with chilled acetone and PBS (3:1) and internal standard then centrifuged (14,000 × *g*) at 4°C for 5 min [21,22]. Acetone was evaporated from the supernatant under N. Liquid-liquid phase extraction was performed on the supernatant with 1 part PBS, 1 part methanol, and 2 parts chloroform (by volume). The phases were centrifuged using the above stated conditions and the lower organic layer was quantitatively recovered and evaporated under N until dry. Dried lipid extracts were reconstituted in ethanol, vortexed, sonicated, and centrifuged (14,000 × *g*, 5 min, 4°C).

Multiple reaction monitoring of endocannabinoids and deuterated internal standards was performed with a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron, San Jose, CA) with an Agilent 1100 HPLC on the front-end (Agilent Technologies, Wilmington, DE) [21,22]. Chromatographic separation was achieved with an Agilent Zorbax SB-CN column (2.1 × 50 mm, 5 μm) with gradient elution using 10 mM ammonium acetate and 100% methanol. Eluted peaks were ionized via atmospheric pressure chemical ionization in multiple reaction monitoring mode. Deuterated internal standards were used for the standard curve of each analyte. Concentrations per mL of plasma were determined.

Analysis of infant HR and HRV

Infant HR and HRV were assessed by 24 h continuous ambulatory electrocardiograph monitoring with Holter monitors (DigiTrak XT; Philips, Amsterdam, NL) at 2 weeks, 4 months, and 6 months of age. Diagnostic software that identifies and labels each QRS complex as normal or abnormal (Philips Zymed Holter 1810 Series, Version 2.9.4) was used to analyze recordings. Only complexes with normal morphology were used.

HR was the average of all filtered RR intervals in the 24 h recording period. Three time-domain measures representing HRV were used: SDNN, the standard deviation of filtered RR intervals in the 24 h period; SDANN, the standard deviation of averages of all filtered RR intervals for all 5 min segments in the 24 h period; and 3) ASDNN (also known as SDNN-index), the average of the standard deviations of all filtered RR intervals for all 5 min segments in the 24 h period.

Statistical analyses and calculations

Statistical analyses were conducted with SAS v9.4 (Cary, NC). Level of significance was $P \leq 0.05$ and trends were $P \leq 0.10$. Repeated measures of HR and HRV at the 2 week, 4 month, and 6 month timepoint were analyzed using a randomized block design with factors of infant (blocking) and time. Individual one-tailed t-tests were used to evaluate HR and HRV changes between the 2 week, 4 month, and 6 month assessments.

Simple linear and multiple backward stepwise regression analyses were used to assess relationships between maternal endocannabinoid status during pregnancy and infant HR and HRV. It was often impractical to assess HR and HRV at the exact timepoints intended. Due to this, and the knowledge that HR and HRV rapidly, progressively change with advancing age during infancy [23], we included exact infant age at the time of assessment as an independent variable.

Endocannabinoid data include those related to the n-6 (anandamide, AEA) and n-3 (eicosanoyl ethanolamine, EEA; eicosapentaenoyl ethanolamine, EPEA; docosahexaenoyl ethanolamine, DHEA) series. Endocannabinoids related to the n-3 fatty acid family were combined and included in statistical analyses as an independent variable. Analyses for other endocannabinoids (e.g., 2-arachidonylglycerol) were attempted but insufficient data were obtained.

Results

Endocannabinoids in maternal and cord venous plasma

Maternal and cord venous plasma concentrations of n-6 and n-3 fatty acid related endocannabinoids are presented in table 2. The standard curve for each endocannabinoid was linear with regression values ≥ 0.98. Extraction efficiencies, determined by comparing area ratios of each extracted BSA analyte to the unextracted standards in ethanol [21], were ≥ 83%.

	20 GW[2]	24 GW	32 GW	36 GW	Delivery
Endocannabinoids, ng/mL[3]					
AEA	0.24 ± 0.07[5]	0.17 ± 0.09[5]	0.16 ± 0.08	0.30 ± 0.40[4]	0.42 ± 0.19[5]
EEA	0.07 ± 0.03	0.07 ± 0.03	0.10 ± 0.08	0.11 ± 0.14	0.06 ± 0.02[5]
EPEA	0.30 ± 0.20[4]	0.27 ± 0.05[6]	0.35 ± 0.33[5]	0.31 ± 0.18[5]	0.33 ± 0.08[5]
DHEA	0.41 ± 0.15	0.50 ± 0.28[6]	0.59 ± 0.38	0.66 ± 0.59	0.78 ± 0.22[5]

Table 2: *Maternal and cord venous plasma n-6 and n-3 endocannabinoids[1].*

[1]Values are $\bar{x}$ ± SD; n = 11, unless otherwise noted; [2]GW: gestational weeks; Delivery indicates cord venous plasma collected at birth; [3]AEA: anandamide (n-6 related); EEA: eicosanoyl ethanolamine (n-3 related); EPEA: eicosapentaenoyl ethanolamine (n-3 related); DHEA: docosahexaenoyl ethanolamine (n-3 related); [4]n = 8; [5]n = 9; [6]n = 10.

Infant HR and HRV changes with advancing age

Infant HR and HRV at each timepoint and changes between timepoints are presented in figure 1. In brief, HR, SDNN, ASDNN, and SDANN significantly differed ($P \leq 0.05$) among infants. Average HR at 2 weeks was 146 ± 8 beats per minute (bpm), at 4 months was 135 ± 5 bpm, and at 6 months was 128 ± 7 bpm. Average SDNN at 2 weeks was 45 ± 11 milliseconds (ms), at 4 months was 56 ± 7 bpm, and at 6 months was 61 ± 10 ms. Average ASDNN at 2 weeks was 25 ± 6 ms, at 4 months was 27 ± 4 ms, and at 6 months was 29 ± 4 ms. Average SDANN at 2 weeks was 35 ± 10 ms, at 4 months was 47 ± 6 ms, and at 6 months was 53 ± 10 ms. The reduction in HR from 2 weeks to 6 months was 17 bpm ($P \leq 0.01$); the increase in SDNN from 2 weeks to 6 months was 16 ms ($P \leq 0.01$); the increase in ASDNN from 2 weeks to 6 months was 4 ms ($P \leq 0.01$); and the increase in SDANN from 2 weeks to 6 months was 18 ms ($P \leq 0.01$).

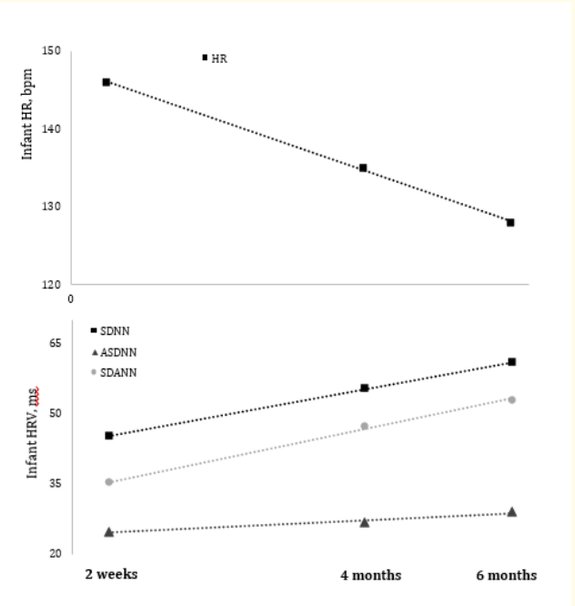

Figure 1: *Infant heart rate (HR) and heart rate variability (HRV) at each assessment, n = 11. SDNN: standard deviation of filtered RR intervals over 24-h period; ms: milliseconds; ASDNN: mean of standard deviations of filtered RR intervals for all 5 min segments of analysis; SDANN: standard deviation of means of filtered RR intervals for all 5 min segments of analysis. Significant difference ($P \leq 0.05$) among participants for HR, SDNN, ASDNN, and SDANN. Change in HR: 2 week to 4 month: $P \leq 0.01$, 4 month to 6 month: $P \leq 0.01$, 2 week to 6 month: $P \leq 0.01$; change in SDNN: 2 week to 4 month: $P \leq 0.01$, 4 month to 6 month: $P = 0.08$, 2 week to 6 month: $P \leq 0.01$; change in ASDNN: 2 week to 4 month: $P = 0.18$, 4 month to 6 month: $P \leq 0.19$, 2 week to 6 month: $P = 0.01$; change in SDANN: 2 week to 4 month: $P \leq 0.01$, 4 month to 6 month: $P = 0.08$, 2 week to 6 month: $P \leq 0.01$.*

Maternal and cord venous endocannabinoids and infant HR: simple linear regression

Significant ($P \leq 0.05$) simple linear regression models describing the relationship between maternal and cord venous plasma endocannabinoids and infant HR are presented in table 3. Maternal n-3 endocannabinoid family at 24 gestational weeks was inversely related to infant HR at the 2 week timepoint ($r^2 = 0.38$; $P = 0.05$). There was a positive relationship between infant age and 2 week infant HR ($r^2 = 0.39$; $P = 0.04$). At 24 and 32 gestational weeks and delivery, EPEA was inversely related to infant HR at 4 months of age ($r^2 \geq 0.46$; $P \leq 0.03$). At 32 gestational weeks and delivery, DHEA and n-3 endocannabinoid family were also inversely related to 4 month infant HR ($r^2 \geq 0.67$; $P \leq 0.01$ and $r^2 \geq 0.64$; $P \leq 0.01$, respectively; figure 2). Maternal EPEA at 24 gestational weeks was an inverse predictor of infant HR at the 6 month timepoint ($r^2 = 0.51$; $P = 0.02$). At 32 gestational weeks, maternal EEA was inversely related to infant HR at the 6 month timepoint ($r^2 = 0.35$; $P = 0.05$).

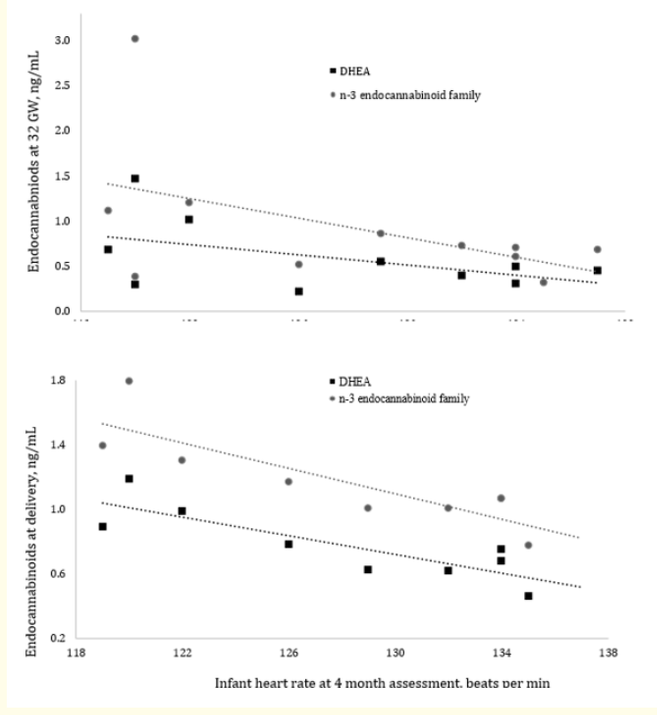

Figure 2: *Endocannabinoids in maternal plasma at 32 gestational weeks (GW) and cord venous plasma at delivery, and infant heart rate at the 4 month assessment. DHEA: docosahexaenoyl ethanolamine; n-3 endocannabinoid family = eicosanoyl ethanolamine + eicosapentaenoyl ethanolamine + DHEA.*

Gestational week		*n*	β ± SE	r^2	*P*
		Infant heart rate, beats per min			
		2 week assessment			
24 weeks					
n-3 family[2]		11	-13.7 ± 5.9	0.38	0.05
Infant age[3]		11	1.4 ± 0.6	0.39	0.04
		4 month assessment			
24 weeks					
EPEA		10	-66.4 ± 25.5	0.46	0.03
32 weeks					
EPEA		9	-11.6 ± 3.6	0.60	0.02
DHEA		10	-11.1 ± 2.7	0.67	≤ 0.01
n-3 family		11	-5.3 ± 1.3	0.64	≤ 0.01

Delivery[4]					
EPEA		9	-44.9 ± 14.4	0.58	0.02
DHEA		9	-18.2 ± 4.7	0.68	≤ 0.01
n-3 family		9	-14.0 ± 3.2	0.73	≤ 0.01
		6 month assessment			
24 weeks					
EPEA		10	-92.5 ± 32.0	0.51	0.02
32 weeks					
EEA		11	-47.9 ± 21.6	0.35	0.05

Table 3: *Maternal and cord venous plasma n-6 and n-3 fatty acid related endocannabinoids and infant heart rate: simple linear regression[1]. [1]Only observations reaching statistical significance (P ≤ 0.05) are reported; [2]n-3 family: eicosanoyl ethanolamine (EEA) + eicosapentaenoyl ethanolamine (EPEA) + docosahexaenoyl ethanolamine (DHEA); [3]Infant age (days) at heart rate assessment; 4Cord venous plasma at delivery*

Maternal and cord venous endocannabinoids and infant HR: multiple regression

Significant ($P \leq 0.05$) multiple regression models describing relationships between maternal and cord venous plasma endocannabinoids and infant HR are presented in table 4. Infant age was a significant or trending predictor of HR at the 2 week and 6 month timepoint in simple linear regression ($P = 0.04$ and $P = 0.08$, respectively) and, thus, included in multiple regression models for the 2 week and 6 month timepoints. The ratio of maternal AEA:DHEA at 24 gestational weeks and infant age were positively related to infant HR at the 2 week timepoint ($r^2 = 0.89$; $P \leq 0.01$). Maternal EEA at 20 gestational weeks and infant age were inversely related to 6 month infant HR ($r^2 = 0.52$; $P = 0.05$). A model including maternal EEA and EPEA at 24 gestational weeks and infant age was inversely related to infant HR at the 6 month timepoint ($r^2 = 0.82$; $P \leq 0.01$). Maternal n-3 endocannabinoid family at 32 gestational weeks and infant age were inversely related to 6 month infant HR ($r^2 = 0.74$; $P \leq 0.01$). Cord venous EPEA and AEA and infant age were inversely related to infant HR at the 6 month timepoint ($r^2 = 0.87$; $P \leq 0.01$).

	Parameters		**Parameter estimates**			**Model estimates**		
Gestational week			β ± SE	*P*		*n*	r^2	*P*
	Heart rate, beats per min							
	2 week assessment							
24 weeks	AEA:DHEA[2]		20.9 ± 9.6	0.07		9	0.89	≤ 0.01
	Infant age[3]		1.6 ± 0.3	≤ 0.01				
	6 month assessment							
20 weeks	EEA		104.6 ± 54.8	0.09		11	0.52	0.05
	Infant age		-0.8 ± 0.3	0.03				
24 weeks	EEA		-61.7 ± 24.8	0.05		10	0.82	≤ 0.01
	EPEA		-141.5 ± 49.4	0.03				
	Infant age		-0.5 ± 0.2	0.06				
32 weeks	n-3 family		-6.2 ± 1.7	≤ 0.01		11	0.74	≤ 0.01
	Infant age		-0.8 ± 0.2	≤ 0.01				
Delivery[4]	EPEA		-76.1 ± 14.8	≤ 0.01		11	0.87	≤ 0.01
	AEA		-20.5 ± 7.4	0.04				
	Infant age		-0.9 ± 0.2	≤ 0.01				

Table 4: *Maternal and cord venous plasma n-6 and n-3 fatty acid related endocannabinoids in and infant heart rate: multiple regression[1] [1]Only models reaching statistical significance (P ≤ 0.05) are reported; [2]AEA: anandamide (n-6 related); DHEA: docosahexaenoyl ethanolamine; n-3 family: eicosanoyl ethanolamine (EEA) + eicosapentaenoyl ethanolamine (EPEA) + DHEA; [3]Exact infant age (days) at heart rate variability assessment; [4]Cord venous plasma at delivery*

Maternal and cord venous endocannabinoids and infant HRV: simple linear regression

Significant ($P \leq 0.05$) simple linear regression models describing relationships between maternal plasma endocannabinoids and infant HRV at 2 weeks and 6 months are presented in tables 5 and 6, respectively.

Gestational week	*n*	β ± SE	r^2	*P*
	Infant heart rate variability			
	2 week SDNN[2], ms			
24 weeks				
AEA:n-3 family[3]	9	-86.2 ± 37.1	0.44	0.05
36 weeks				
DHEA	11	11.7 ± 5.0	0.37	0.05
n-3 family	11	9.2 ± 3.9	0.38	0.04
	2 week ASDNN, ms			
24 weeks				
AEA:n-3 family	9	-47.6 ± 15.4	0.58	0.02
36 weeks				
EEA	11	27.1 ± 10.3	0.43	0.02
DHEA	11	7.2 ± 2.2	0.54	≤ 0.01
n-3 family	11	5.8 ± 1.7	0.57	≤ 0.01

Table 5: *Maternal plasma n-6 and n-3 fatty acid related endocannabinoids and infant heart rate variability at 2 weeks: simple linear regression[1]. [1]Only observations reaching statistical significance ($P \leq 0.05$) are reported; [2]SDNN: standard deviation of filtered RR intervals over 24-h period; ASDNN: mean of standard deviations of filtered RR intervals for all 5 min segments of analysis[3]AEA: anandamide (n-6 related); n-3 family: eicosanoyl ethanolamine (EEA) + eicosapentaenoyl ethanolamine + docosahexaenoyl ethanolamine (DHEA)*

Gestational week	*n*	β ± SE	r^2	*P*
	Infant heart rate variability			
	6 month SDNN[2], ms			
24 weeks				
AEA[3]	9	-66.0 ± 27.8	0.45	0.05
AEA:DHEA	9	-65.5 ±17.4	0.67	≤ 0.01
AEA:n-3 family	9	-88.8 ± 20.1	0.74	≤ 0.01
32 weeks				
EPEA	9	18.0 ± 6.6	0.52	0.03
36 weeks				
EEA	11	50.6 ± 15.0	0.56	≤ 0.01
EPEA	9	30.6 ± 13.3	0.43	0.05
DHEA	11	13.4 ± 3.0	0.68	≤ 0.01
n-3 family	11	10.7 ± 2.3	0.71	≤ 0.01

	6 month ASDNN, ms			
24 weeks				
AEA:n-3 family	9	-28.1 ± 11.1	0.48	0.04
32 weeks				
EPEA	9	10.4 ± 2.2	0.77	≤ 0.01
36 weeks				
EEA	11	22.2 ± 7.0	0.53	≤ 0.01
EPEA	11	19.1 ± 3.8	0.78	≤ 0.01
DHEA	11	6.0 ± 1.4	0.68	≤ 0.01
n-3 family	11	5.1 ± 0.8	0.81	≤ 0.01
Delivery[4]				
EEA	9	142.1 ± 43.8	0.60	≤ 0.01
EPEA	9	44.6 ± 6.6	0.87	≤ 0.01
DHEA	9	12.8 ± 4.7	0.51	0.03
n-3 family	9	11.3 ± 2.7	0.72	≤ 0.01
	6 month SDANN, ms			
24 weeks				
AEA	9	-72.8 ± 27.5	0.50	0.03
AEA:DHEA	9	-68.7 ± 18.0	0.68	≤ 0.01
AEA:n-3 family	9	-89.4 ± 22.9	0.69	≤ 0.01
36 weeks				
DHEA	11	12.9 ± 3.4	0.62	≤ 0.01
n-3 family	11	10.1 ± 2.6	0.62	≤ 0.01

Table 6: *MMaternal and cord venous plasma n-6 and n-3 endocannabinoids and infant heart rate variability at 6 months: simple linear regression[1]. [1]Only observations reaching statistical significance (P ≤ 0.05) are reported; [2]SDNN: standard deviation of filtered RR intervals over 24-h period; ASDNN: mean of standard deviations of filtered RR intervals for all 5 min segments of analysis; SDANN: standard deviation of means of filtered RR intervals for all 5 min segments of analysis; [3]AEA: anandamide (n-6 related); DHEA: docosahexaenoyl ethanolamine; n-3 family: eicosanoyl ethanolamine (EEA) + eicosapentaenoyl ethanolamine (EPEA) + DHEA; 4Cord venous plasma collected at delivery*

At 24 gestational weeks, the ratio of maternal AEA:n-3 endocannabinoid family was inversely related to infant SDNN at the 2 week timepoint (r^2 = 0.44; P = 0.05). Maternal DHEA and n-3 endocannabinoid family at 36 gestational weeks were positive predictors of 2 week infant SDNN ($r^2 \geq 0.37$; $P \leq 0.05$). The ratio of maternal AEA: n-3 endocannabinoid family at 24 gestational weeks was inversely related to infant ASDNN at the 2 week timepoint (r^2 = 0.58; P = 0.02). Maternal EEA, DHEA, and the n-3 endocannabinoid family at 36 gestational weeks were positively related to ASDNN at the 2 week timepoint ($r^2 \geq 0.43$; $P \leq 0.02$).

Maternal AEA, the ratio of AEA:DHEA, and the ratio of AEA:n-3 endocannabinoid family at 24 gestational weeks were independently, inversely related to infant SDNN at the 6 month timepoint ($r^2 \geq 0.45$; $P \leq 0.05$; figure 3). At 32 gestational weeks, maternal EPEA was a positive predictor of 6 month infant SDNN (r^2 = 0.52; $P \leq 0.03$). Maternal EEA, EPEA, DHEA, and the n-3 endocannabinoid family at 36 gestational weeks were independently, positively related to infant SDNN ($r^2 \geq 0.43$; $P \leq 0.05$; figure 4).

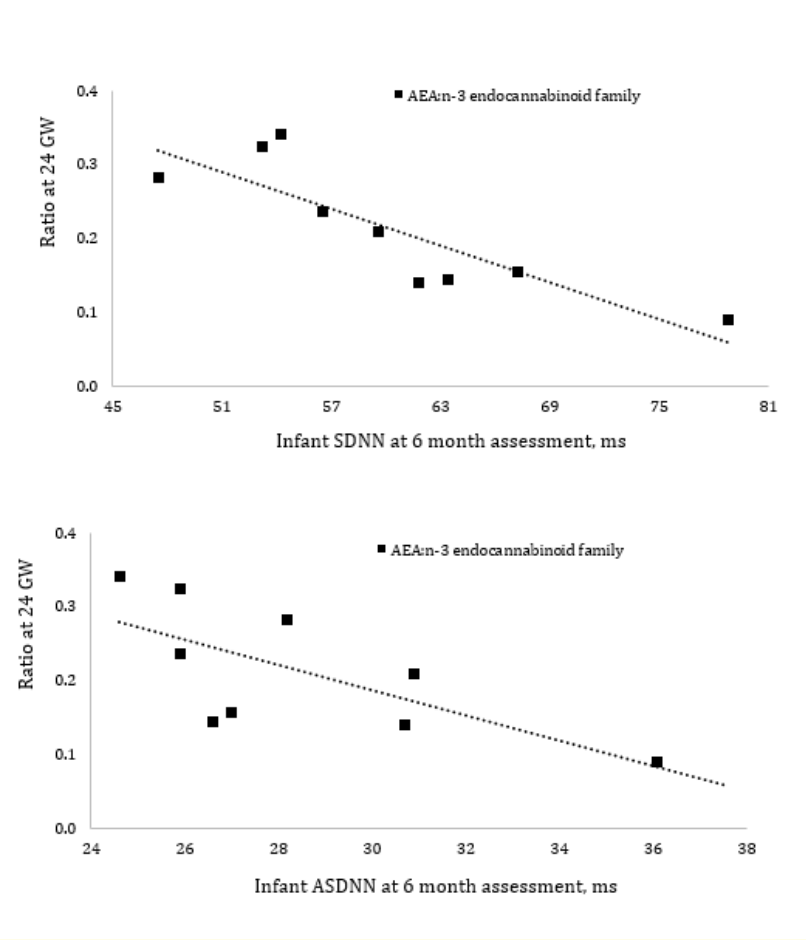

Figure 3: *Endocannabinoids in maternal plasma at 24 gestational weeks (GW) and infant heart rate variability at the 6 month assessment. AEA: anandamide (n-6 related); n-3 endocannabinoid family = eicosanoyl ethanolamine + eicosapentaenoyl ethanolamine + docosahexaenoyl ethanolamine; SDNN: standard deviation of filtered RR intervals over 24-h period; ms: milliseconds; ASDNN: mean of standard deviations of filtered RR intervals for all 5 min segments of analysis; SDANN: standard deviation of means of filtered RR intervals for all 5 min segments of analysis.*

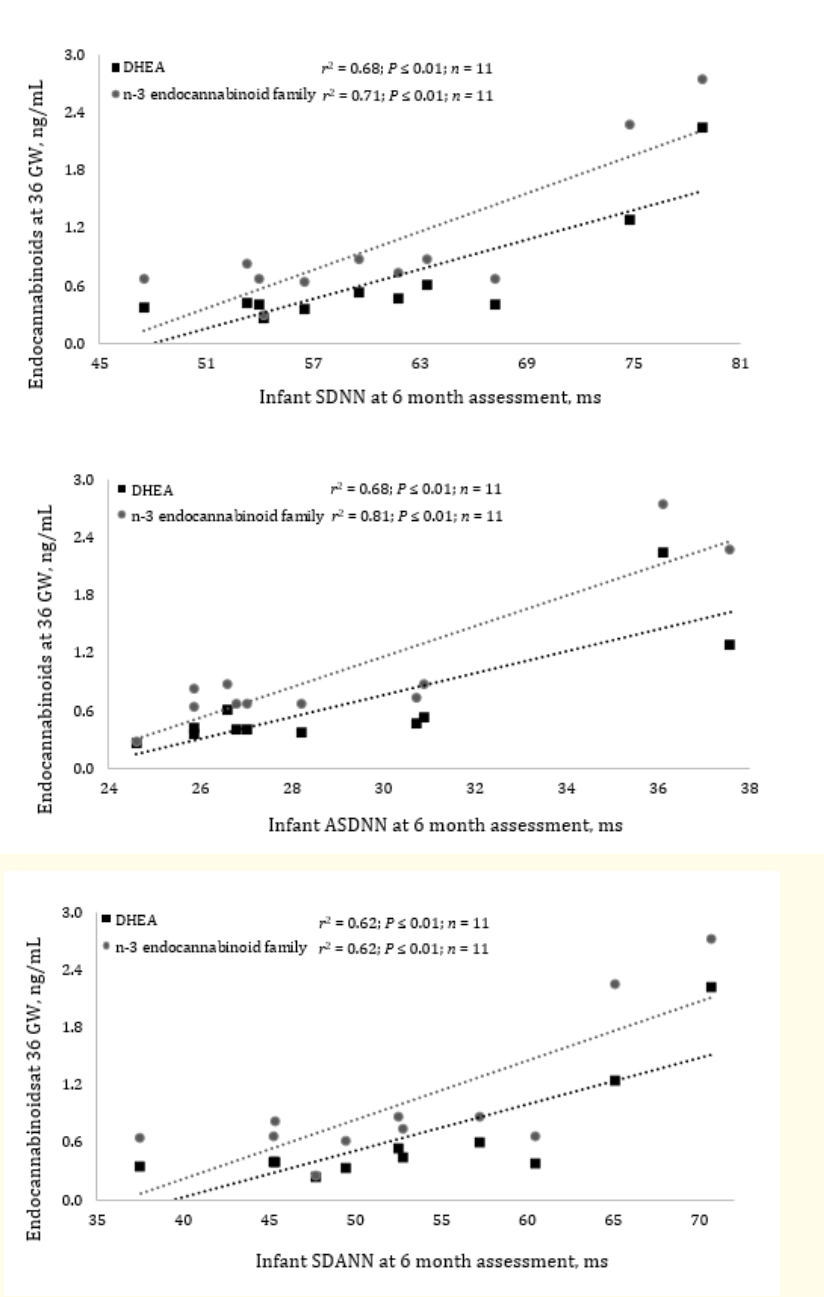

Figure 4: *Endocannabinoids in maternal plasma at 36 gestational weeks and infant heart rate variability at the 6 month assessment. DHEA: docosahexaenoyl ethanolamine; n-3 endocannabinoid family = eicosanoyl ethanolamine + eicosapentaenoyl ethanolamine + DHEA; SDNN: standard deviation of filtered RR intervals over 24-h period; ms: milliseconds; ASDNN: mean of standard deviations of filtered RR intervals for all 5 min segments of analysis; SDANN: standard deviation of means of filtered RR intervals for all 5 min segments of analysis.*

Maternal ratio of AEA:n-3 family at 24 gestational weeks was inversely related to infant ASDNN at the 6 month timepoint (r^2 = 0.48; P = 0.04; figure 3). Maternal EPEA at 32 gestational weeks was a positive predictor of 6 month infant ASDNN (r^2 = 0.77; $P \leq 0.01$). Maternal EEA, EPEA, DHEA, and the n-3 endocannabinoid family at 36 gestational weeks were independent, positive predictors of infant ASDNN at the 6 month timepoint ($r^2 \geq 0.53$; $P \leq 0.01$). There were independent, positive associations for cord venous EEA, EPEA, DHEA, and the n-3 endocannabinoid family with 6 month infant ASDNN ($r^2 \geq 0.51$; $P \leq 0.03$; figure 4).

Maternal AEA, the ratio of AEA:DHEA, and the ratio of AEA:n-3 endocannabinoid family at 24 gestational weeks were independently, inversely associated with infant SDANN at the 6 month timepoint ($r^2 \geq 0.50$; $P \leq 0.03$; **Figure 3**). Maternal DHEA and the n-3 endocannabinoid family at 36 gestational weeks were independent, positive predictors of 6 month infant SDANN (r^2 = 0.62; $P \leq 0.01$; figure 4).

Discussion

Infant HR and HRV advanced with age

As infant age advanced, HR decreased and HRV increased. We expected this given the developmental stage of our population and previous data [7,23]. In early life, relatively low HR and high HRV indicate accelerated autonomic maturation [7]. Thus, endocannabinoids inversely related to HR and/or positively related to HRV are interpreted to potentially accelerate autonomic development.

Maternal and cord venous endocannabinoids are associated with infant HR and HRV

Endocannabinoids related to the n-6 (AEA) and n-3 (EEA, EPEA, DHEA) fatty acid families were of particular interest as these families are metabolically competitive and the maternal n-6 and n-3 fatty acids were closely related to infant HR and HRV in a previous analysis [5].

Early life HR and HRV are responsive to n-3 LCPUFA fatty acid intake [4, 5,10-12]. As fatty acid intake modulates the endocannabinoid system [14,15-17], it is plausible that *in utero* exposure to specific endocannabinoids is responsible for these responses. We cannot, however, definitively conclude that endocannabinoid status underlies the current observations. Endocannabinoids have several roles *in vivo* and their concentrations reflect those of the respective fatty acid precursors; n-3 LCPUFA intake shifts n-6: n-3 fatty acid balance of membrane lipids, resulting in compensatory increases in n-3 related endocannabinoids and reduced production of n-6 related endocannabinoids [14]. Therefore, our observations may reflect effects mediated by the fatty acids from which endocannabinoids originated. Indeed, a previously published study by our laboratory indicates significant relationships between maternal n-3 and n-6 fatty acid status and infant HR/HRV [5].

The current data suggest that *in utero* exposure to n-3 endocannabinoids reduce HR and increase HRV during the perinatal period. Using regression analyses, we consistently observed an association between relatively elevated n-3 endocannabinoids in maternal and/or cord venous plasma, reduced infant HR, and increased HRV. The ratio of maternal AEA to n-3 family and AEA to DHEA were also predictive; shifts favoring AEA, a n-6 endocannabinoid, were related to increased infant HR and decreased HRV. There was an inverse relationship between cord venous plasma AEA and infant HR at 6 months; cord venous plasma EPEA was also inversely 6 month infant HR. Compared with AEA, an equivalent increase in cord venous plasma EPEA was predicted to result in an approximate 3.7-fold reduction in HR. Therefore, while these data indicate cord venous plasma AEA may be related to decreased infant HR, they also suggest that EPEA is a more potent HR- and HRV-mediating factor.

Study Limitations

This study was limited by sample size (n = 11 mother-infant pairs) which restricted inclusion of factors other than circulating endocannabinoids and infant age in regression models. To address this, there were several criteria for study enrollment, ensuring participants had similar characteristics to minimize the effect of external factors on observations. We do, however, advise our data are interpreted with caution and encourage replication of this work to confirm preliminary findings and bolster hypotheses offered here.

Conclusions

In utero exposure to endocannabinoids may be the mechanism underlying the previously observed effects of nutrition on early life HR and HRV [4,5,10-12]. Our data evidence a relationship between maternal endocannabinoids during pregnancy and infant HR and HRV. Although we cannot definitively conclude our observations are a direct consequence of specific endocannabinoids, we provide a discussion supporting this hypothesis. Further, we encourage future studies to include assessments of endocannabinoid exposure when measuring early life autonomic development.

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Conflict Of Interest

The authors do not declare a conflict of interest.

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