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Original Research

Association between plasma endocannabinoids and appetite in hemodialysis patients: A pilot study

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ABSTRACT

Uremia-associated anorexia may be related to altered levels of long chain n-6 and n-3 polyunsaturated fatty acid (PUFA) derived circulating endocannabinoids (EC) and EC-like compounds that are known to mediate appetite. Our study's hypothesis was that such molecules are associated with appetite in patients with end-stage renal disease. A cross-sectional observational study was performed in 20 chronic hemodialysis patients (9 females, 11 males) and 10 healthy female controls in whom appetite was assessed using the Simplified Nutritional Appetite Questionnaire (SNAQ) and blood drawn in the fasting (and when applicable) pre-dialysis state. Blood levels of PUFA and EC were also measured. Higher blood levels of the long chain n-6 fatty acid 20:4n6 (arachidonic acid) and lower levels of the long chain n-3 fatty acid 20:5n3 (eicosapentaenoic acid) were observed in female hemodialysis patients compared to controls. No differences were observed between male and female patients. In female study participants strong correlations between specific EC-like compounds and total SNAQ scores were noted, including with the n-6 PUFA derived linoleoyl ethanolamide (L-EA; $\rho = -0.60$, $P < .01$) and the n-3 PUFA derived docosahexaenoyl ethanolamide (DH-EA; $\rho = 0.63$, $P < .01$). The L-EA:DH-EA ratio was most strongly associated with the SNAQ score ($\rho = -0.74$, $P \leq .001$), and its questions associated with appetite ($\rho = -0.69$, $P \leq .01$) and satiety ($\rho = -0.81$, $P \leq .001$). These findings support a link between circulating EC and appetite in hemodialysis patients.

1. Introduction

The development of uremia from kidney failure is associated with anorexia and an increased risk of nutritional deficiencies [1–3]. Uremia may lead to loss of appetite by altering levels of circulating molecules known to mediate appetite like leptin, ghrelin, and neuropeptide Y [4]. Whether uremia also contributes to anorexia via the endocannabinoid (EC) system is unknown.

Abbreviations: EC, Endocannabinoids; ECS, Endocannabinoid signaling; PUFA, Polyunsaturated fatty acids; SNAQ, Simplified Nutritional Appetite Questionnaire; FAME, fatty acid methyl esters; FAAH, fatty acid amide hydrolase; L-EA, linoleoyl ethanolamide; DH-EA, docosahexaenoylethanolamine.

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Endocannabinoid signaling (ECS) is a potent mediator of food intake and influences energy metabolism through its regulation of orexigenic and anorectic molecules (including some of those mentioned above) and neural pathways [5–7]. EC mediators oversee complex crosstalk between the central and peripheral nervous system and the gut, muscle, and adipose tissue [8]. Elucidation of this process has led to new drug therapies for appetite-related disorders [9].

Of particular relevance, ECS activation via the brain cannabinoid receptor CB1 leads to stimulation of appetite [10]. Precursor molecules for the most investigated EC ligands of the cannabinoid receptor are derived from the polyunsaturated fatty acid (PUFA) arachidonic acid (20:4n-6), whereas EC-like ligands showing contrary and competing effects [11] are products of various saturated, monounsaturated and polyunsaturated fatty acids that include n-3 PUFA eicosapentaenoic acid (20:5n-3) and docosapentaenoic acid (22:5n-3) [10,12].

Basic information on the physiology and blood content of EC and EC-like metabolites in patients with kidney failure is lacking. We therefore performed a pilot study in hemodialysis patients to characterize EC blood levels and examine their relationship to appetite. The study hypothesis, tested by measuring circulating levels of EC and their fatty acid precursors and comparing them to measurements of appetite [10], was that blood EC levels correlate with appetite.

2. Methods and materials

2.1. Participants

Study participants were recruited from outpatient hemodialysis units affiliated with Indiana University School of Medicine, Indianapolis, IN, and healthy controls from the Indiana University-Purdue University Indianapolis (IUPUI) campus, Indianapolis, IN, in early 2012. The IUPUI campus institutional review board approved the protocol and all participants gave written informed consent after reviewing a written summary of the study plan. Inclusion criteria for the hemodialysis patients were age 18 or older and ability to provide informed consent. Exclusion criterion was hospitalization for any reason within the past 3 months.

2.2. Appetite questionnaire and clinical information

The Simplified Nutritional Appetite Questionnaire (SNAQ) was developed to assess appetite and predict weight loss. SNAQ has been validated in healthy adults, including elderly adults and those in long-term care residences [13,14] and used in the chronic kidney disease population [15]. SNAQ consists of four questions, individually scored from 1 to 5, in order to give a range of 4–20 points, with lower scores predicting a greater risk of weight loss within six months [13]. Demographic and medical data were obtained from each participant through simple questionnaires. The SNAQ survey was administered at the time of blood draw.

2.3. Measurement of fatty acids and endocannabinoids

A fasting blood draw was obtained immediately before a mid-week dialysis session from the dialysis tubing in each dialysis patient. Control subjects had blood drawn in the fasting state. Blood was collected in EDTA-preserved tubes. PMSF, a fatty acid amide hydrolase (FAAH) inhibitor, was added to a set of samples to prevent breakdown of anandamide (i.e. arachidonoyl ethanolamide (A-EA)). However, results of the analysis with and without PMSF were not significantly different so the data were combined. Plasma fatty acid levels of polar lipids were measured by gas chromatographic analysis as previously described [16]. Fatty acid data reflect fatty acid methyl esters (FAME) as weight % and the values are presented as means ± SD of plasma polar fatty acids. Authentic external standards were used for the peak identification in GC output chromatograms. The sensitivity of the GC detector is at 10 ng/peak [17]. Plasma EC measurements were performed on 250 μL aliquots using ultra-performance liquid chromatography-electrospray tandem mass spectrometry as previously described [18].

2.4. Statistical analyses

Standard descriptive statistics were used. Group differences were evaluated using 2-tailed t-tests after data normalization using imDEV v 1.4.2 [19]. Correlation between EC levels and SNAQ scores were assessed using Pearson correlations calculated in Microsoft Excel. P-values less than 0.05 were regarded as significantly different.

3. Results

Twenty chronic hemodialysis patients and ten healthy female controls were recruited for the study. Mean age and body mass indices were not significantly different between patients and controls, whereas SNAQ scores were depressed in patients in a borderline significant manner ($P = .051$; Table 1). Plasma fatty acids were measured in all subjects and the principal PUFA that act as EC precursors are shown in Table 2. Plasma levels of arachidonic (20:4n-6) and eicosapentaenoic (20:5n-3) acids were higher and lower, respectively, in female hemodialysis patients compared to female controls. No difference was noted for these PUFA between male and female patients.

An array of EC were measured in the plasma of each of the twenty female hemodialysis patients and controls including the acyl ethanolamides of palmitate, stearate, oleate, linoleate, alpha-linoleate, di homo-gamma-linolenate, arachidonate, docosaheptanoate, and docosahexenoate, and the 1- and 2-mono-acylglycerols of oleate, linoleate, and arachidonate (data not shown). The oleoyl ethanolamide (O-EA; 14.8 ± 3.8 vs. 22.6 ± 9.7, $P = .030$) and linoleoyl ethanolamide (L-EA; 6.1 ± 2.1 vs. 9.7 ± 3.7, $P = .016$) were elevated ~1.5 fold in hemodialysis patients. The relationship between EC levels and SNAQ score components were then evaluated (Table 3). The n-6 linoleic acid-derived EC linoleoyl ethanolamide (L-EA) and the n-3 docosahexaenoic acid-derived EC docosahexaenoylethanolamine (DH-EA) were negatively and positively correlated with the SNAQ score, respectively. The correlation between EC and satiety was the strongest among all the four sub-components of the SNAQ score (Table 3). Calculating the ratio of L-EA:DH-EA generated a very strong ($\rho = -0.74$, $P < .001$) metabolic correlate to the SNAQ Score (Fig. 1). The relationship between L-EA and DH-ES and SNAQ are