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Plasma fatty acid ethanolamides are associated with postprandial triglycerides, ApoCIII and ApoE in humans consuming high fructose corn syrup (HFCS)-sweetened beverage.

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Abstract

Epidemiological and clinical research studies have provided ample evidence demonstrating that consumption of sugar-sweetened beverages (SSB) increases risk factors involved in the development of obesity, type 2 diabetes (T2D), and cardiovascular disease (CVD). Our previous study demonstrated that when compared to aspartame (Asp), two weeks of high-fructose corn syrup (HFCS)-sweetened beverages provided at 25% of daily energy requirement (Ereq) was associated with increased body weight, postprandial (pp) triglycerides (TG), and fasting and pp CVD risk factors in young adults. The fatty acid ethanolamide, anandamide (AEA), and the monoacylglycerol, 2-arachidonoyl-*sn*-glycerol (2-AG), are two primary endocannabinoids (ECs) that play a role in regulating food intake, increasing adipose storage, and regulating lipid metabolism. Therefore, we measured plasma concentrations of ECs and their analogs, oleoylethanolamide (OEA), docosahexaenoyl ethanolamide (DHEA), and docosahexaenoyl glycerol (DHG), in participants from our previous study who consumed HFCS- or Asp-sweetened beverages to determine associations with weight gain and CVD risk factors. Two-week exposure to either HFCS- or Asp-sweetened beverages resulted in significant differences in the changes in fasting levels of OEA and DHEA between groups after the testing period. Subjects who consumed Asp, but not HFCS, displayed a reduction in AEA, OEA and DHEA after the testing period. In contrast, there were significant positive relationships between AEA, OEA, and DHEA versus ppTG, ppApoCIII and ppApoE in those consuming HFCS, but not in those consuming Asp. Our findings reveal previously unknown associations between circulating ECs and EC-related molecules with markers of lipid metabolism and CVD risk after HFCS-consumption.

Keywords: High-fructose corn syrup, anandamide (AEA), oleoylethanolamide (OEA), ApoCIII, ApoE, triglycerides (TG)

Introduction

High consumption of sugar-sweetened beverages (SSB) is a leading contributing factor to the obesity epidemic, type 2 diabetes (T2D), and cardiovascular disease (CVD) (9, 20, 24 , 25, 38). Consumption of fructose-containing beverages is associated with increases in body weight and CVD lipid markers, and decreases insulin sensitivity (1, 36, 40). Sugar-sweetened beverages in the U.S. commonly contain high fructose corn syrup (HFCS) with fructose content ranging from 47 to 65% (43). We previously reported that subjects consuming 0, 10, 17.5 and 25% Ereq as HFCS-sweetened beverages exhibited a dose-dependent increase in body weight in two weeks (39), and large dose-dependent increases in postprandial (pp)triglycerides (TG), fasting and pp low-density lipoprotein (ppLDL), pp ApoliproteinB (ppApoB), ApolipoproteinCIII (ppApoCIII), and uric acid. Therefore, fructose-containing beverages may contribute to increased metabolic risk via both weight gain and upregulation of hepatic lipid production (38); however, specific roles for the endocannabinoid system in these processes are largely unknown. Nonetheless, a small number of studies suggest that endocannabinoids (ECs) – which are signaling molecules known to regulate both food intake (4, 10, 11, 17, 29) and lipid metabolism (30, 37) (8, 32) – may play a role in metabolic dysregulation induced by fructose-containing beverages. (17, 23).

Two primary ECs, the fatty acid ethanolamide anandamide (AEA) and the monoacylglycerol 2-arachidonoyl-*sn*-glycerol (2-AG), act through cannabinoid type 1 receptors (CB_1Rs) to stimulate palatable food intake (4, 5, 13, 14, 22). This is in contrast to oleoylethanolamide (OEA), a related fatty acid ethanolamide analog of AEA that does not interact with the CB receptors and plays a role in suppressing food intake (11, 12, 18, 19, 34). Other less-studied analogs of ECs including the fatty acid ethanolamide, docosahexaenoyl ethanolamide (DHEA), and the monoacylglycerol, docosahexaenoyl glycerol (DHG), may stimulate glucose uptake *in* vitro (21) and have anti-inflammatory properties (15, 33); however, a comprehensive understanding of their physiological roles is lacking.

Our primary objective of this study was to determine if two weeks of HFCS-sweetened beverage consumption impacts plasma concentrations of ECs or their analogs. We hypothesized that two weeks of HFCS-sweetened beverage consumption would be associated with increases in plasma levels of appetite-stimulating AEA and 2-AG, and decreased levels of appetite-suppressing OEA. A secondary objective was to determine if changes in

AEA, 2-AG, or their analogs are associated with changes in body weight and lipids/lipoproteins in subjects consuming HFCS for two weeks.

Methods

Study participants

Participants in this study are a subgroup from an NIH-funded investigation in which a total of 187 participants assigned to 8 experimental groups were studied, as previously described (39). The current article reports the results from 49 subjects consuming beverages containing either 0% (*n*=21) or 25% (*n*=28) daily energy requirement (Ereq) from high fructose corn syrup (HFCS). The study was conducted in accordance with an experimental protocol that was approved by the UC Davis Institutional Review Board, and participants provided written informed consent.

Participants were recruited through an internet listing (craigslist.com) and local postings of flyers, and underwent telephone and in-person screenings with medical history, completed blood count and serum biochemistry panel to assess eligibility. Inclusion criteria included age 18-40 yrs, BMI 18-35 kg/m^2 with self-report of stable body weight during the prior 6 months. Exclusion criteria included diabetes (fasting glucose >125 mg/dL), evidence of renal or hepatic disease, fasting plasma triglyceride >400 mg/dL, hypertension (>140/90 mm Hg), hemoglobin <8.5 g/dL, and surgery for weight loss. Individuals who smoked, habitually ingested >2 alcoholic beverages/d, exercised >3.5 hr/week at a level more vigorous than walking, or used thyroid, lipid-lowering, glucose-lowering, antihypertensive, antidepressant, or weight loss medications were also excluded. Assignment to experimental groups were not randomized; by design, the experimental groups were matched for sex, BMI, and concentrations of fasting triglyceride, cholesterol, HDL cholesterol and insulin in plasma collected during the in-person interviews.

For the 5 weeks prior to the start of the study, subjects who were scheduled for participation were asked to limit daily consumption of sugar-containing beverages to no more than one 237ml serving of fruit juice and discontinue consumption of any vitamin, mineral, herbal, or dietary supplements, including fish oil. A total of 55 subjects

were enrolled in experimental groups consuming either 0% (aspartame (Asp)) or 25% Ereq-HFCS. Two subjects withdrew from the study before the start of intervention, and four additional subjects withdrew for various reasons previously reported (39). A total of 51 subjects completed the study with 23 subjects in the Asp group and 28 in the HFCS group. Due to the unavailability of samples for two of the Asp subjects, results reported here include a total of 21 subjects in this group.

Study Design

This was a parallel-arm, double-blinded diet intervention study with 3 phases: 1) a 3.5-day inpatient baseline period during which subjects resided at the University of California Davis Clinical and Translational Science Center's Clinical Research Center (CCRC), consumed a standardized baseline diet, and participated in experimental procedures; 2) a 12-day outpatient in intervention period during which subjects consumed their assigned sweetened beverages providing 0% (Asp-sweetened) or 25% Ereq-HFCS along with their usual ad libitum diets; and 3) a 3.5-day inpatient intervention period during which subjects resided at the CCRC and consumed standardized diets that included the sweetened beverages, and all experimental procedures were repeated.

Inpatients diets

During days 2 and 3 of the baseline and intervention inpatient periods, subjects consumed energy-balanced meals consisting of conventional foods. Daily Ereq were calculated by the Mifflin equation (28), with adjustment of 1.3 for activity on the days of the 24-hour serial blood collections and adjustment of 1.5 for the other days. The baseline diet contained 55% Ereq mainly as low-fiber complex carbohydrate (i.e. white bread, white rice, regular pasta), 30% from fat, and 15% from protein. The meals during the inpatient intervention period included that assigned study beverages and were as identical as possible to baseline meals, except for the substitution of the sugar-sweetened beverage in place of isocaloric amounts of complex carbohydrate. The intervention meals contained 19-20 g fiber/2000 kcal fiber, and the baseline meals contained 22 g fiber/2000 kcal. The timing of

inpatient meals and the energy distribution were as follows: breakfast, 0900 (25%); lunch, 1300 (35%); and dinner, 1800 (40%).

Study beverages and outpatient diet

HFCS-containing beverages were sweetened with HFCS-55 (Isosweet 5500, 55% fructose, 45% glucose: Skidmore Sales and Distributing), flavored with an unsweetened drink mix (Kool-Aid; Kraft Inc.). A fruit-flavored aspartame drink mix (Market Pantry) was used to prepare the 0% Ereq-HFCS beverages. Participants were blinded to their beverage assignment, as were all CCRC and study personnel who interacted with participants or analyzed samples. Voluntary feedback from participants indicated that they were able to distinguish between beverages containing aspartame (Asp) or HFCS. The amount (grams) of beverage provided was standardized among the 2 groups and based on energy requirements [calculated with the Mifflin equation (28), plus 1.5 activity adjustment]. During the 12-day outpatient phase of the study, participants were instructed to drink one serving of study beverage with each meal, to consume their usual diet, and to not consume other sugar-sweetened beverages, including fruit juice. To monitor compliance of beverage consumption (35, 41), the study beverages contained a biomarker (riboflavin) that was measured fluorimetrically in urine samples collected that times of beverage pickup. Subjects were informed about the biomarker but were not provided information regarding its identify. Fasting urinary riboflavin concentrations following days 9 and 13 of unmonitored beverage consumption were not different from those following one day of monitored beverage consumption at the CCRC, suggesting good and comparable compliance in all groups (39).

Fasting blood collection and lipid analysis

Fasting blood samples reported here were collected at 0800 hr and stored at -80°C for the measurement of triglycerides (TG), apolipoprotein C III (ApoCIII), apolipoprotein E (ApoE), and EC-related outcomes. EC-related outcomes included monoacylglycerols (MAGs) [docosahexaenoyl glycerol (DHG) and 2-arachidonoyl-*sn*-glycerol (2-AG)] and fatty acid ethanolamides (FAEs) [anandamide (AEA), oleoylethanolamide (OEA), and docosahexaenoyl ethanolamide (DHEA)]. Lipid extraction and analysis of MAGs and FAEs were performed as

158 previously described (4). Plasma (0.5 mL) was added to 1.0 mL of methanol solution containing the internal
159 standards, [$^{2}H_{5}$]- 2-AG, [$^{2}H_{4}$]-AEA, and [$^{2}H_{4}$]-OEA (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted
160 with chloroform (2 mL) and washed with 0.9 % saline (0.5 mL). Organic phases were collected and separated by
161 open-bed silica gel column chromatography. Eluate was gently dried under N_2 stream (99.998% pure) and
162 resuspended in 0.1 mL of methanol:chloroform (9:1), with 1 μL injection for ultra-performance liquid
163 chromatography/tandem mass spectrometry (UPLC/MS/MS) analysis.
164 Lipids were analyzed using a Waters Acquity I-Class Ultra Performance Liquid Chromatography system coupled
165 to a Waters TQS-micro Triple Quadrupole Mass Spectrometer. Lipids were separated using an Acquity UPLC
166 BEH C18 column (50 x 2.1 mm; i.d. 1.7 μm), eluted by a gradient of methanol (0.25% acetic acid, 5mM
167 ammonium acetate) in water (0.25% acetic acid, 5mM ammonium acetate) (from 80 to 100% methanol in 2.5
168 min, 100% 2.5-3.0 min, 100-80% 3.0-3.1 min) at a flow rate of 0.4 mL/min. Column temperature was kept at 40°
169 C and samples were maintained in the sample manager at 10° C. Argon was used as collision gas (99.998% pure).
170 2-AG, AEA, OEA, DHG, DHEA, [$^{2}H_{5}$] 2-AG, [$^{2}H_{4}$] AEA, and [$^{2}H_{4}$] OEA were identified in the positive ionization
171 mode based on their retention times and MS^{2} properties. Lipids were quantified using a stable isotope dilution
172 method detecting protonated adducts of the molecular ions $[M+H]^{+}$ in the multiple reaction monitoring (MRM)
173 mode. Extracted ion chromatograms were used to quantify 2-AG (*m/z* = 379.2>287.26), AEA (*m/z* =
174 348.3>62.04), OEA (*m/z* = 326.3>62.08), DHG (*m/z* = 403.3>311.19), DHEA (*m/z* = 372.3>91.02), and [$^{2}H_{5}$] 2-
175 AG (*m/z* = 384.2>93.4), [$^{2}H_{4}$] AEA (*m/z* = 352.3>66.11), and [$^{2}H_{4}$] OEA (*m/z* = 330.3>66.05), which were used as
176 internal standards (Cayman Chemical, Ann Arbor, MI, USA; [$^{2}H_{5}$] 2-AG as internal standard for 2-AG and DHG;
177 [$^{2}H_{4}$] AEA (*m/z* = 352.3>66.11) as internal standard for AEA and DHEA; [$^{2}H_{4}$] OEA as internal standard for OEA).

178 *Postprandial lipid measurements*

179 Postprandial measures of total triglycerides (TG), apolipoprotein C III (ApoCIII), and apolipoprotein E (ApoE)
180 were collected at time points 2200, 2300 and 2400 hr because this was the period during which peak ppTG
181 concentrations were observed in our previous study (40). These 3 late-night pp plasma samples were pooled

182 together and multiple aliquots of each pooled sample were stored at -80°C. Lipid and lipoprotein concentrations
183 were measured with a Polychem Chemistry Analyzer (PolyMedCo Inc.) with reagents from MedTest DX.

184 *Statistical analyses*

185 Baseline anthropometric and clinical characteristics were compared between 0% Ereq (Asp) and 25% HFCS
186 groups using a student t-test. The percent change (%Δ) of these measures from 0-wk to 2-wk of intervention was
187 compared using a general linear model (SAS 9.4), with %Δ outcome value at week 2 as the categorical variable,
188 adjusting for sex and change in BMI, as well as sex x group interactions. Secondary analyses of absolute values
189 at 0-wk and 2-wk were analyzed by repeated measures ANCOVA, testing for an interaction between beverage
190 group and time. By group univariate linear regressions were conducted to determine potential relationships
191 between changes in EC-related compounds and fasting or pp-lipids measures. Data presented in Table 1 are
192 means ± SDs; all other data are means ± SEMs.

193

194 **Results**

195 *Baseline characteristics and post-intervention lipid markers of CVD*
196 Baseline anthropometric and metabolic outcomes were not significantly different between groups (**Table 1**).
197 Amongst the ECs at baseline, plasma concentrations of AEA, 2-AG and the related DHG, did not differ; however,
198 OEA and DHEA were significantly higher in the aspartame group (**Table 2**). This difference was driven by 2
199 outliers in the Asp group. Sensitivity analyses and re-analyses of EC-related compounds with removal of these 2
200 outliers revealed that baseline differences between groups were not significant (**Table 2**), and %Δ at intervention
201 was only moderately affected (**Figures 1 and 2**). Therefore, these outliers remained included in analyses;
202 however, we included significance values with and without these outliers in all figures. Body weight, ppApoE,
203 and fasting and pp TG and ApoCIII were all significantly increased in subjects consuming HFCS for two weeks
204 when compared to Asp controls (**Table 3**).
205

Changes in circulating ECs and their analogs after two weeks of SSB

Significant beverage x time interactions were found for OEA (p=0.03) and DHEA (p=0.008), and a trending interaction for AEA (p=0.08) (**Figure 1**). **Figure 2** presents these differences between groups as %Δ from baseline by ANCOVA analyses. There were no differences between groups in the %Δ in 2-AG (p=0.83) or DHG (p=0.74). Including an adjustment for sex revealed a near-significant effect of sex on %Δ in AEA (p=0.06); however, there were no significant sex x beverage interactions on EC-related outcomes. Paired *t*-tests were conducted for within beverage group comparisons of values at baseline versus week 2 of intervention. Consumption of HFCS-sweetened beverage for two weeks did not result in changes in the plasma levels of the ECs and their analogs. Participants consuming Asp, however, exhibited significant reductions in the fatty acid ethanolamides, AEA (p= 0.01), OEA (p= 0.008), and DHEA (p= 0.001) (**Figure 1**).

Differential associations between Asp versus HFCS beverages in their relationships of AEA, OEA, DHEA with CVD lipid markers

Absolute changes in ECs and their analogs did not correlate with change in body weight or BMI in either group, with the exception of OEA, which showed a weak relationship with change in body weight in the HFCS group (r^2=0.11, p=0.02) (data not shown). Linear regression analyses revealed differences between beverage groups in the relationships between changes in lipid and EC-related outcomes. There was no relationship between AEA and fasting lipid outcomes in either group (**Figure 3A-C**). Negative relationships were found between Δ OEA and Δ fasting TG (r^2= 0.20, p= 0.04) in the Asp group; however, the HFCS group trended towards a positive relationship (r^2= 0.10, p= 0.09) (**Figure 3 D**). The relationship between Δ OEA and Δ fasting ApoCIII did not reach significance in either beverage group (Asp r^2= 0.14, p= 0.11; HFCS r^2= 0.18, p= 0.07) (**Figure 3E**). In the Asp group, a trend was observed between Δ OEA and Δ fasting ApoE (r^2= 0.18, p= 0.07) (**Figure 3F**), but no relationship was present within the HFCS group. The strongest relationships under fasting conditions were observed in the Asp group between Δ DHEA and ΔTG (r^2= 0.26, p= 0.005), Δ ApoCIII (r^2= 0.47, p= 0.001), and Δ

ApoE (r^2=0.24, p= 0.03) (**Figure 3 G-I**). Change in DHEA did not correlate to any of the lipid outcomes in the HFCS group under fasting conditions.

Under pp conditions, relationships between lipids and ECs were only present in those consuming HFCS. In the HFCS group, Δ AEA correlated positively with Δ ppTG (r^2=0.26, 0.006) and Δ ppApoCIII (r^2=0.29, p=0.020) (**Figure 4A and B**). Changes in OEA were positively related to Δ ppTG, Δ ppApoCIII and Δ ppApoE in those consuming HFCS, with the strongest relationship being with the change in Δ ppApoCIII (r^2=0.53, p<0.0001) (**Figure 4D-F**). This differed from changes in DHEA, which only showed a weak relationship with changes in Δ ppApoCIII (r^2=0.17, p=0.03) and no relationship with either Δ ppTG or Δ ppApoE (**Figure 4G-H**). There we no associations between pp lipids and the lipid-derived EC analogs in subjects consuming Asp beverage (**Figure 4)**.

Discussion

This is the first study in humans to demonstrate an association between the EC system and increased CVD risk factors in response to HFCS consumption. We hypothesized that two week consumption of HFCS-sweetened beverage, when compared to Asp-sweetened beverage, would be associated with increased plasma levels of appetite-stimulating AEA and 2-AG, and decreased appetite-suppressing OEA. Contrary to our hypotheses, HFCS beverage in normal-weight adults was not associated with any significant changes in ECs and their analogs; however, subjects consuming Asp beverage displayed decreases in levels of the fatty acid ethanolamides, AEA, OEA, and DHEA. Furthermore, plasma levels of AEA, OEA, and DHEA were positively associated with changes in ppTG, ppApoCIII and ppApoE in participants consuming HFCS, but not in those consuming Asp beverage. These findings demonstrate an association between ECs and their analogs with markers of lipid metabolism and CVD in response to sugar-sweetened beverage consumption.

Despite significant increases in body weight following the HFCS beverage intervention, ECs and their analogs were not strongly associated with weight gain, with the exception of a weak relationship between Δ OEA and body

weight. This effect is possibly due to the short-term intervention resulting in modest weight gain rather than longer-term interventions resulting in more clinically-significant weight gain. Nonetheless, the weak positive correlation is in line with findings of higher plasma OEA concentrations in obese compared to lean individuals (2, 26).. Furthermore, Matias and colleagues (26) demonstrated that salivary OEA and AEA correlated with BMI, body weight, and waist circumference in obese individuals.

In rodents, high-fat diet-induced obesity is associated with greater expression of hepatic CB_1 receptors through which ECs may stimulate hepatic DNL (31), and high-fructose or -sucrose diets result in greater hypothalamic synthesis of ECs and CB_1 receptor activity (17, 23). No associations were found when comparing plasma levels of the ECs with TG, ApoCIII and ApoE in the fasted state in subjects consuming HFCS. In the pp state, however, significant positive relationships were found between AEA and OEA versus TG and ApoCIII, and OEA versus ApoE. This finding may suggest that ECs and related molecules did not affect TG and ApoCIII production, but rather HFCS-induced increases in ppTG, ppApoCIII, and to a lesser extent ppApoE, affected plasma levels of AEA, OEA and DHEA. This result may be a threshold effect, however, because increases in ECs and their analogs were observed mainly in the subjects who exhibited higher increases in ppTG, ppApoCIII and ppApoE. HFCS-induced increases in TG, ApoCIII and ApoE were approximately twice as high in the pp state than the fasting state,; thus, a positive relationship between ECs and TG/lipoproteins was apparent only in the pp state.

Consumption of asp-sweetened beverages was associated with reduced fasting concentrations of plasma AEA, OEA, and DHEA. Whether reductions in appetite-stimulating AEA in the absence of changes in body weight is a result of the presence of Asp, or in contrast, the absence of SSB, requires further study. Understanding this relationship could have implications for interventions aimed at reducing food intake and body weight since. Indeed, Asp beverage consumption has been shown to lower caloric intake and reduce desire for highly palatable foods (3), and reductions in salivary AEA were found following a three-month weight loss intervention (16, 26).

Reductions in DHEA, AEA, and OEA in Asp-consuming subjects may also reflect a decrease in inflammatory responses (27). DHEA, AEA, and possibly OEA have been implicated in anti-inflammatory responses (7, 42, 44), and share common fatty acid ethanolamide biosynthetic and degradative pathways (12, 18, 19, 34). The EC, 2-AG,

is a monoacylglycerol (30) that is also synthesized from AA (similar to AEA) and plays a role in inflammation, but our results suggests that only fatty acid ethanolamides are associated with Asp consumption. Further studies are needed to better understand the biological relevance of the Asp-associated reduction in fatty acid ethanolamides in the context of, both, appetite regulation and anti-inflammatory responses.

To our knowledge, this is the first study to examine the effects of HFCS beverage consumption on circulating ECs, and importantly, in healthy, non-obese individuals. Nonetheless, this study has several limitations. Participants consumed ad libitum diets during the 12-day outpatient period, thus we did not control for precise quantities of sugars consumed. Nonetheless, our study participants were instructed to abstain from consuming outside beverages containing added sugar, but were not instructed to abstain from naturally-occurring sugars such as those found in fruits, which also contain antioxidants and polyphenols. Unlike natural sources of sugar, added sugars consumed as sweetened beverages provide little to no nutritional value. Therefore, we did not feel it necessary to restrict participants from consuming natural, nutritional food items and did not have prior evidence to suggest that this would impact the outcomes of this study. Similarly, participants were instructed to cease intake of fish oil supplements five weeks prior to and during the study, as these supplements have been shown to alter levels of DHEA, DHA, and ECs (6). Although participants were not prohibited from consuming fish during the study, it is unlikely that fish intake was greater in one group over the other as there is no prior evidence to suggest that Asp or HFCS influences the desire to eat foods high in omega fatty acids. Another limitation to our study is that ECs and their analogs were measured only in fasting plasma. Future studies that include postprandial EC measures will provide valuable insight into the heterogeneous functions (e.g. regulation of appetite signaling and lipid metabolism) of AEA, OEA, and DHEA in response to a HFCS beverage. In addition, saliva measures of ECs would better assess whether or not increases in ECs can explain taste-related links between SSB, hedonic feeding behavior, and weight-gain in humans.

Conclusion

This is the first study to demonstrate the effects of Asp- and HFCS-sweetened beverage consumption on circulating ECs in humans. The unexpected absence of an effect of HFCS on the EC system in this study should

be further investigated under longer-term exposure to HFCS and in response to a meal. On the contrary, observed effects of Asp on circulating ECs raise questions regarding the potential effects of artificial sweeteners on food-reward pathways and should be further explored. Lastly, our study shows differential effects of beverage type on circulating EC compounds in relationship to lipid risk factors of CVD in the fasted and postprandial states. Future studies are needed to further understand the possible implications this may have on metabolic functions.

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Figure Legends

Figure 1: Baseline and 2-wk EC and EC-related compound concentrations in Asp- or HFCS-sweetened beverage groups. Between-group comparisons were conducted by repeated measures analysis, and within group differences from baseline were conducted by *t*-test. Significance at $p < 0.05$. p-values in parentheses reflect significance after removal of 2 outliers in Asp group.

Figure 2: The percent change of endocannabinoids and their analogs in Asp and HFCS groups from 0-wk to 2-wk intervention. ANCOVA with adjustment for change in BMI; significance at $p < 0.05$. p-values in parentheses reflect significance after removal of 2 outliers in Asp group.

Figure 3: Linear regressions by beverage group comparing changes in AEA, OEA, DHEA versus changes in fasting TG, ApoCIII and ApoE. A through C, comparisons with ΔAEA; D through F, comparisons with ΔOEA; G through I, comparisons with ΔDHEA. ■ and solid line= HFCS; ▲ and dotted line=Asp.

Figure 4: Linear regressions by beverage group comparing changes in AEA, OEA, DHEA versus changes in postprandial TG, ApoCIII and ApoE. A through C, comparisons with ΔAEA; D through F, comparisons with ΔOEA; G through I, comparisons with ΔDHEA. ■ and solid line= HFCS; ▲ and dotted line=Asp.