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Interactions between dietary oil treatments and genetic variants modulate fatty acid ethanolamides in plasma and body weight composition

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Abstract

Fatty acid ethanolamides (FAE), a group of lipid mediators derived from long-chain fatty acids (FA), mediate biological activities including activation of cannabinoid receptors, stimulation of fat oxidation and regulation of satiety. However, how circulating FAE levels are influenced by FA intake in humans remains unclear. The objective of the present study was to investigate the response of six major circulating FAE to various dietary oil treatments in a five-period, cross-over, randomised, double-blind, clinical study in volunteers with abdominal obesity. The treatment oils (60 g/12 552 kJ per d (60 g/3000 kcal per d)) provided for 30 d were as follows: conventional canola oil, high oleic canola oil, high oleic canola oil enriched with DHA, flax/safflower oil blend and corn/safflower oil blend. Two SNP associated with FAE degradation and synthesis were studied. Post-treatment results showed overall that plasma FAE levels were modulated by dietary FA and were positively correlated with corresponding plasma FA levels; minor allele (A) carriers of SNP rs324420 in gene fatty acid amide hydrolase produced higher circulating oleoylethanolamide (OEA) ($P=0{\cdot}0209$) and docosahexaenoylethanolamide (DHEA) levels ($P=0{\cdot}0002$). In addition, elevated plasma DHEA levels in response to DHA intake tended to be associated with lower plasma OEA levels and an increased gynoid fat mass. In summary, data suggest that the metabolic and physiological responses to dietary FA may be influenced via circulating FAE. Genetic analysis of rs324420 might help identify a sub-population that appears to benefit from increased consumption of DHA and oleic acid.

Key words: Dietary oil treatments: Fatty acid ethanolamides: Intervention trial: Genetic variants: Body weight

Fatty acid ethanolamides (FAE), also referred to as *N*-acylethanolamines, are a group of endogenous ethanolamides of different fatty acids (FA) that were first identified in the late 1970s[1]. *N*-arachidonoylethanolamine (AEA, also called anandamide), the derivative of arachidonic acid (AA), is the first isolated and identified FAE compound[2] and has been characterised in placenta and fetal membranes as well as human plasma, amniotic fluid[3] and the nervous system[4]. AEA serves as an endogenous ligand of cannabinoid receptors (CB)[5]. Oleoylethanolamide (OEA), a derivative of oleic acid (OA), is thought to regulate satiety and body weight[6] by activating PPAR-α, which is responsible for energy expenditure and energy intake through lipolysis in adipocytes[7]. Consequently, administration of OEA may become part of the treatment of eating disorders and body weight maintenance[8]. Other FAE, including palmitoylethanolamide (PEA) and linoleoylethanolamide (LEA) over a range of concentrations are believed to have anti-inflammatory properties[9–11]. Little is known, however, about docosahexaenoylethanolamide (DHEA; derivative

Abbreviations: AA, arachidonic acid; AEA, *N*-arachidonoylethanolamine; ALA, α-linolenic acid; ALEA, α-linolenoylethanolamide; Canola, conventional canola oil; CanolaDHA, DHA-enriched canola oil; CanolaOleic, high oleic canola oil; CornSaff, a blend of corn oil and safflower oil; DHEA, docosahexaenoylethanolamide; FA, fatty acid; FAE, fatty acid ethanolamide; FAAH, fatty acid amide hydrolase; FlaxSaff, a blend of flax oil and safflower oil; LA, linoleic acid; LEA, linoleoylethanolamide; NAPE-PLD, *N*-acylphosphatidylethanolamine-hydrolysing phospholipase D; OA, oleic acid; OEA, oleoylethanolamide; PEA, palmitoylethanolamide.

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of DHA) or α-linolenoylethanolamide (ALEA; derivative of α-linolenic acid (ALA)), although it was observed that a high dietary intake of DHA increased brain DHEA levels in piglets, suggesting a similar metabolism of AEA in the nervous system[12]. Recently, it has been reported that DHEA-dependent pathways may be associated with hippocampal neurodevelopment and synaptic activity[13]. However, the biological importance of *n*-3-derived FAE remains to be resolved.

The endogenous FAE are involved in numerous biological activities and primarily modulated by their biosynthesis and degradation[14]. Generally, the enzymes *N*-acylphosphatidylethanolamine-hydrolysing phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH) appear to be responsible for the synthesis and hydrolysis of FAE, respectively, in turn influencing circulating FAE levels *in vivo*[15,16]. With the growing knowledge of nutrigenomics, the common genetic variants of *NAPE-PLD* and *FAAH* genes may modulate circulating FAE levels in humans. Investigations of common SNP in humans have demonstrated that major allele carriers of SNP rs324420 of *FAAH* are more likely associated with overweight/obesity and metabolic disorders[17,18], although the functional significance of these differences remain unclear over time[19,20]. It was proposed that this polymorphism might impact specific neural mechanisms through CB signalling which, in turn, increase the risk of reward deficiency syndrome that causes obesity[21]. Meanwhile, although little is known about NAPE-PLD, a study has shown that a common haplotype of the *NAPE-PLD* gene may be protective against obesity[22]. Most recently, Geurts *et al.*[23] proposed an important mechanism of adipose tissue NAPE-PLD on whole-body metabolism using NAPE-PLD knockout mice, indicating that NAPE-PLD may play an essential role in regulating energy homoeostasis and be responsive to cold-induced browning. In other words, alterations of the enzyme activity of NAPE-PLD may be beneficial as an anti-obesity treatment.

In addition, evidence has demonstrated that intake of high-MUFA diets elicits weight loss and/or body fat mass reduction compared with consuming high-SFA diets in humans[24–27]. In addition, oral administration of OEA appeared to beneficially affect health, resulting in weight loss in humans[16,28]. Therefore, a knowledge gap exists concerning whether endogenous OEA, converted from dietary OA, plays a role on body fat distribution. In addition, it remains to be determined whether the consumption of FA other than oleic acid can contribute to changes in body fat composition via their corresponding FAE.

Therefore, the purpose of the present study was to answer the following questions; first, how major FAE shift in response to dietary oil treatments in humans; second, whether common genetic variants in *FAAH* and *NAPE-PLD* genes affect plasma FAE levels; third, whether possible diet–gene interactions exist in populations with risk of metabolic syndrome; and, finally, whether associations exist between changes in circulating FAE concentrations, especially OEA, and body fat composition after the dietary interventions. Therefore, we hypothesised that consumption of various dietary oil treatments with differing FA composition can lead to corresponding shifts in plasma FAE levels, and the diet–gene interaction may play an important role on the levels of FAE in response to dietary treatments, resulting in potential changes in body fat composition.

Methods

Clinical design

This study was conducted as part of the Canola Oil Multicenter Intervention Trial (COMIT) study, a dietary intervention in adults with abdominal obesity and at least one criterion for metabolic syndrome[29]. The COMIT study was conducted at the Richardson Centre for Functional Foods and Nutraceuticals (RCFFN) at the University of Manitoba, the Institute of Nutraceuticals and Functional Foods at the Laval University and the Department of Nutritional Sciences at the Pennsylvania State University between September 2010 and March 2012, as previously described[30]. The study was registered at clinicaltrials.gov (NCT01351012). All the participants provided their written informed consent before the study started. The study was reviewed and approved by Institutional Review Boards or Committees at all participating sites.

The COMIT study investigated effects of a daily intake of 60 g of dietary oils low in SFA and high in MUFA or PUFA. A randomised cross-over study design was implemented, in which participants were randomly assigned to five novel vegetable oil treatments. Each treatment phase was 30 d in duration and was separated by a 4-week washout period. The diets were designed to maintain body weight, and energy needs for each participant were calculated using the Mifflin equation[31]. With the exception of the treatment oils, an identical 7-d rotating menu in the full-feeding diets was served across all treatments, with a fixed macronutrient composition (35 % fat, 50 % carbohydrate, 15 % protein). The five oil treatments were as follows: canola oil (Canola; 60 % OA, 20 % linoleic acid (LA), 10 % ALA), high oleic acid canola oil (CanolaOleic; 72 % OA, 15 % LA, 2 % ALA), DHA-enriched canola oil (CanolaDHA; 63 % OA, 13 % LA, 6 % DHA (from an algal oil)), a blend of corn oil/safflower oil (CornSaff; 18 % OA, 69 % LA) and a blend of flax oil/safflower oil (FlaxSaff; 18 % OA, 38 % LA, 32 % ALA) (Table 1). Three canola-based diets were rich in MUFA, whereas the two safflower oil blends were high in *n*-3 PUFA or *n*-6 PUFA. Compliance with the feeding protocol was assessed by clinical coordinators who ensured that participants consumed at least one of their two treatment beverages under supervision daily, as well as evaluated the returned meal bags of non-consumed food provided for off-site consumption. Compliance was further confirmed by measuring post-treatment plasma FA profiles.

Subjects

A total of 170 volunteers were recruited using media advertisements. The inclusion criteria required an increased waist circumference (>94 cm for men and >80 cm for women) or at least one of the following criteria: TAG >1·7 mmol/l; HDL-cholesterol <1 mmol/l (men) or <1·3 mmol/l (women); blood pressure ≥130 mmHg (systolic) and/or ≥85 mmHg (diastolic); and glucose ≥5·5 mmol/l. Exclusion criteria included history of thyroid disease, diabetes mellitus, kidney disease and

liver disease, current smokers, consuming more than two alcoholic drinks per week and taking lipid-lowering medications or supplements of any kind in the past 2 weeks.

Blood sample collection

Our intervention study was designed to compare end points after each experimental phase; therefore, the FA baseline composition profiles at each phase were not measured. Our aim was to systematically cross-compare responses across the treatment oil blends that were hypothesised to confer health benefits. After the 4-week washout to maintain their habitual diets and another 4-week treatment period, carry-over effects would be very limited, based on our previous clinical experience. Therefore, on days 29 and 30 of each phase, 12-h fasting blood samples were collected in EDTA-coated tubes and centrifuged at 3000 rpm for 20 min at 4°C. Separated plasma and leucocyte samples were transferred to labelled aliquot tubes and stored at −80°C until further analysed. After the completion of the trial, samples for the present study were shipped to the RCFFN for analysis.

Measurement of plasma fatty acid profiles

Plasma FA concentrations were determined at the end of each experimental phase. Individual plasma FA classes were extracted from plasma-EDTA aliquots using the Folch method[32], followed by methylation using methanolic hydrogen chloride as previously described[30]. FA methyl esters were separated on a DB-225 column (30 m × 250 μm with 0·25-μm film thickness; Agilent Technologies) using an Agilent 6890N gas chromatograph equipped with a flame ionisation detector (GC-FID; Agilent Technologies). The oven was programmed from 70 to 240°C in five temperature steps (70°C for 1 min, rise of 30°C/min to 180°C, rise of 10°C/min to 200°C, rise of 2°C/min to 220°C and hold for 9.5 min, rise of 40°C/min to 240°C). Samples were run with a 20:1 split ratio, and He was used as the carrier gas with a column flow rate of 1 ml/min. Temperatures for the injector and detector were set at 280 and 300°C, respectively. Individual FA were identified by comparison with GLC 461 standard (Nu-Chek Prep). The internal FA standard heptadecanoic acid (C17:0) (Sigma-Aldrich) was introduced into original plasma samples to quantify the amount of each FA in plasma. The proportion of substrate FA converted to FA products was calculated based on the peak area relative to the total area and expressed as the percentage of total FA.

Table 1. Fatty acid composition of the five treatment oils (60 g based on 12 552 kJ diet/d (3000 kcal diet/d))

	Treatments				
Fat type (g)	Canola	CanolaOleic	CanolaDHA	FlaxSaff	CornSaff
SFA	4·33	3·91	5·19	4·87	4·73
*c*16 : 0	2·44	2·20	3·15	2·94	3·52
*c*18 : 0	1·10	1·10	1·02	1·90	1·14
MUFA	37·69	43·19	38·25	10·72	10·60
*c*18 : 1*n*-9	35·17	42·88	37·95	10·72	10·56
PUFA	17·58	10·26	13·97	41·67	41·78
*c*18 : 2*n*-6	11·72	8·84	7·56	22·48	41·61
*c*18 : 3*n*-3	5·86	1·38	1·18	19·19	0·17
*c*20 : 4*n*-6	0	0	0·04	0	0
*c*20 : 5*n*-3	0	0	0·09	0	0
*c*22 : 5*n*-3	0	0	1·42	0	0
*c*22 : 6*n*-3	0	0	3·48	0	0

Canola, conventional canola oil; CanolaOleic, high oleic canola oil; CanolaDHA, DHA-enriched canola oil; FlaxSaff, a blend of flax oil and safflower oil; CornSaff, a blend of corn oil and safflower oil.

Total plasma fatty acid ethanolamide analysis

Analysis of FAE was conducted using a previously described method[33]. The six targeted FAE in plasma samples (PEA, OEA, LEA, AEA, ALEA and DHEA) were quantified according to the ratio of the known deuterated FAE in the internal standard mixture, with an expected relative standard deviation value of <5 %. Standards including PEA, OEA, LEA, AEA, ALEA, DHEA, PEA-d4, OEA-d4, LEA-d4, AEA-d8 and DHEA-d4 were purchased from Cayman Chemicals Company. ALEA-d4 was synthesised in our laboratory using α-linolenic acid chloride (Nu-Chek Prep) dissolved in methylene chloride with ethanolamide-d4 (Cambridge Isotope Laboratories)[2]. Relative ratios of FAE and their deuterated isotopes in the internal standard mixture were generated by serial dilution when the internal standard mixture was adjusted to the optimal concentration.

Aliquots of plasma samples at the end point of each phase were used for FAE extraction using a solid-phase extraction method[34] with some modifications. The stability of FAE in frozen plasma samples has been previously validated in our laboratory[35,36]. In brief, plasma samples that were mixed with internal standards on ice were filtered under gentle vacuum at a low flow rate through an activated Oasis HLB cartridge (Waters Corporation). Extracts were then washed and eluted with acetonitrile, followed by analysis on an ultra-performance liquid chromatography (UPLC) tandem Quattro micro API mass spectrometer (Waters Corporation), as published by Lin *et al.*[33]. A Kinetex XB-C18 column (100 × 2·1 mm, 1·7 μm; Phenomenex) was used for separation at a flow rate of 0·2 ml/min. Data were acquired and processed using MassLynx 4.1 (Waters Corporation).

Measures of body fat mass

Regional changes in body composition of study participants were assessed at both the initiation and termination of each dietary phase by dual-energy X-ray absorptiometry (DEXA) scanning according to the manufacturer's recommendations (GE Healthcare; QDR-4500W; Hologic Corp.). The equipment, room supplies and room set-up were checked on a regular basis. All maintenance work including calibration was performed according to the manufacturer's recommendations. Licensed DEXA technicians and coordinators performed all scans on each participant after each phase. Participants were required to lie down with the same posture each time at the centre of the measurement box from head to toe, face up and keep their arms at their sides during the scans. The regions of interest, including android fat mass, gynoid fat mass and the

ratio of android:gynoid fat, were manually adjusted on the scan images. All data were then automatically calculated using the software Lunar Prodigy Advance enCORE and APEX System.

SNP analysis

To investigate the relationship between genetic variants of *FAAH* and *NAPE-PLD* genes and plasma FA and FAE levels, all the subjects were genotyped for two selected SNP (rs324420 for *FAAH* and rs12540583 for *NAPE-PLD*). According to the GWAS and NCBI dbSNP database, the selected SNP both belong to missense mutations with a higher minor allele frequency that may lead to significant functional changes in the corresponding enzymes. Genomic DNA was extracted from leucocyte samples using a commercial Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen Sciences Inc.). The quality and quantity of individual genomic DNA were assessed by Thermo Scientific NanoDrop 2000 micro-volume spectrophotometer (Thermo Fisher Scientific). Amplification and detection of DNA were conducted with the 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies) using optical-grade 96-well plates. Each well contained 5 μl of PCR reaction mixture, containing customised TaqMan SNP Genotyping Assays, TaqMan SNP genotyping Master Mix (Life Technologies Inc.) and pre-diluted DNA samples. Data were acquired by software StepOne 2.1 (Applied Biosystems, Life Technologies).

Statistical analyses

Only participants who completed all five dietary phases were included in the statistical analyses. A per-protocol approach was used to avoid the need for multiple imputations for missing data during the analyses. The power calculation was performed based on previous results on post-treatment plasma OEA levels in the clinical intervention by Jones *et al.*[36], and indicated a power of 100 % ($\alpha = 0{\cdot}05$) to detect significant differences in OEA levels between three MUFA-rich diets and two PUFA-rich diets. Statistical analyses were performed using SAS 9.2 (SAS Inc.). Statistical significance was determined using the adjusted Tukey test for multiple comparisons with $P < 0{\cdot}05$. The results are expressed as least square means with their standard errors, unless noted elsewhere.

The effects of dietary treatment on plasma FA profiles, plasma FAE levels and body composition were analysed using a mixed model with repeated-measures analysis of time. Treatment, age and sex were considered as fixed factors. Sequence of treatments and centre were used as random effects in the model. Tukey-adjusted *P* values were used to examine differences between-treatments. Pearson's correlation analyses were conducted to test for associations between plasma FA and FAE and between plasma FAE and fat mass change.

Effects of different genotypes on FAE levels were analysed using mixed models. Treatment, sex, age, genotype, genotype × sex interaction and treatment × genotype interaction were entered as fixed effects, sequence of treatments were random effects, and clinical sites were selected as random effects. Repeated measures by five dietary phases on subjects were used to investigate the effects of treatments, genotypes and their interactions. Two individual SNP were analysed separately.

Results

Subject characteristics

A total of 130 participants (sixty male; seventy female; 76·5 % of the total randomised subjects) completed the five-phase intervention study. The mean ages of men and women were 46·5 (SD 14·2) and 47·6 (SD 14·5) years, with BMI of 29·8 (SD 4·4) and 29·0 (SD 4·2) kg/m^2, respectively. Baseline characteristics of the study population have been previously published[30] and are shown in Table 2.

Plasma fatty acid profiles. The effects of the diet interventions on plasma FA profiles are summarised in Table 3. Overall, all changes in FA composition agreed with our expectation based on the FA profile of each intervention diet. The Canola and CanolaOleic diets produced the highest levels of total MUFA ($P < 0{\cdot}05$), whereas CornSaff and FlaxSaff rich in *n*-6 and/or *n*-3 PUFA contents resulted in higher ($P < 0{\cdot}05$) levels of PUFA compared with the other three diets. The CornSaff group

Table 2. Baseline characteristics of participants of the dietary intervention (Mean values with their standard errors; *n* 130)

	Male (*n* 60)		Female (*n* 70)		Total (*n* 130)		
Characteristics	Mean	SEM	Mean	SEM	Mean	SEM	*P**
BMI (kg/m^2)	30·7	0·6	29·0	0·5	29·8	4·3	0·0254
Age (years)	45·2	1·8	47·6	1·7	46·5	14·2	0·3308
Total cholesterol (mmol/l)	5·2	0·1	5·4	0·2	5·3	1·1	0·3560
HDL-cholesterol (mmol/l)	1·2	0·0	1·4	0·0	1·3	0·3	<0·0001
LDL-cholesterol (mmol/l)	3·1	0·1	3·3	0·1	3·2	0·9	0·4276
TAG (mmol/l)	2·0	0·2	1·6	0·1	1·8	1·0	0·0119
Glucose (mmol/l)	5·3	0·1	5·4	0·2	5·4	1·1	0·8448
Body weight (kg)	95·1	1·8	76·5	1·4	85·1	12·8	<0·0001
Waist circumference (cm)	106·6	1·3	96·6	1·4	101·2	10·9	<0·0001
Systolic BP (mmHg)	128·9	2·2	120·2	2·0	124·3	16·7	0·0041
Diastolic BP (mmHg)	81·0	1·7	78·6	1·3	79·7	11·8	0·2659

BP, blood pressure.
* ANOVA was used to analyse between-sex differences in continuous variables. Significant difference: $P < 0{\cdot}05$.

Table 3. Selected plasma fatty acid profiles of participants at end points of each dietary phase (g/100 g)*
(Least square mean values with their standard errors; *n* 130)

	Treatments									
	Canola		CanolaOleic		CanolaDHA		FlaxSaff		CornSaff	
Fatty acids	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
*c*16 : 0	27·04[b]	0·20	27·40[b]	0·20	28·10[a]	0·20	27·41[b]	0·20	27·35[b]	0·20
*c*18 : 0	11·83[c]	0·10	11·79[c]	0·10	12·28[b]	0·10	12·51[a]	0·10	12·34[a,b]	0·10
*c*18 : 1*n*-9	14·90[b]	0·19	15·52[a]	0·19	13·36[c]	0·19	12·10[d]	0·18	11·62[d]	0·18
*c*18 : 2*n*-6	22·00[c]	0·23	21·52[c]	0·23	18·68[d]	0·23	25·13[b]	0·23	25·93[a]	0·23
*c*18 : 3*n*-3	0·79[b]	0·03	0·63[c]	0·03	0·57[c,d]	0·03	1·61[a]	0·03	0·49[d]	0·03
*c*20 : 4*n*-6	9·28[b]	0·15	9·67[a]	0·15	9·70[a]	0·15	8·27[c]	0·15	9·59[a,b]	0·15
*c*20 : 5*n*-3	1·09[b]	0·04	0·86[c]	0·04	1·53[a]	0·04	1·45[a]	0·04	0·49[d]	0·04
*c*22 : 5*n*-3	0·81[b]	0·03	0·72[c]	0·03	0·34[e]	0·03	0·97[a]	0·03	0·62[d]	0·03
*c*22 : 6*n*-3	2·84[b]	0·09	2·79[b]	0·09	7·21[a]	0·10	2·59[b]	0·09	2·66[b]	0·09
Total SFA	41·74[c]	0·19	41·92[c]	0·19	43·28[a]	0·19	42·71[a,b]	0·19	42·51[b]	0·19
Total MUFA	18·05[a]	0·22	18·50[a]	0·22	16·20[b]	0·22	14·78[c]	0·22	14·49[c]	0·22
Total PUFA	40·21[b]	0·22	39·54[c]	0·22	40·47[b]	0·22	42·57[a]	0·22	43·04[a]	0·22
Total *n*-6 PUFA	34·67[c]	0·23	34·46[c]	0·23	30·92[d]	0·23	35·92[b]	0·23	38·79[a]	0·23
Total *n*-3 PUFA	5·53[c]	0·10	5·03[d]	0·10	9·62[a]	0·11	6·64[b]	0·10	4·24[e]	0·10

Canola, conventional canola oil; CanolaOleic, high oleic canola oil; CanolaDHA, DHA-enriched canola oil; FlaxSaff, a blend of flax oil and safflower oil; CornSaff, a blend of corn oil and safflower oil.
[a,b,c,d,e] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* Mixed model with repeated measures was used to analyse between-treatment differences in continuous variables.

showed the lowest ($P<0·05$) ALA, EPA and DPA levels compared with the other four treatments. Post-treatment DHA concentrations were higher ($P<0·0001$) after the CanolaDHA treatment than after the other four treatments, whereas no differences were observed across the other four treatments, indicating good dietary compliance across all centres.

Total plasma fatty acid ethanolamides. The end point concentrations of OEA, AEA, PEA, LEA, ALEA and DHEA (*n* 121) were successfully measured and are shown in Fig. 1. Samples from nine participants were excluded from this analysis because of failures in the extraction of samples being run through the cartridges or in the FAE measurement using UPLC. Overall, plasma PEA and OEA were the two major FAE. No differences were observed in plasma PEA levels across the five dietary treatments. Three MUFA-rich treatments – Canola, CanolaDHA and CanolaOleic – resulted in higher ($P<0·05$) OEA levels compared with the two PUFA-rich diets – CornSaff and FlaxSaff. However, despite the similar OA content of the three canola-based diets, the CanolaDHA treatment appeared to lower the OEA levels compared with the CanolaOleic diet ($P<0·05$). The plasma AEA level after the CanolaOleic diet was higher ($P<0·05$) compared with the CanolaDHA, CornSaff and FlaxSaff diets, but no difference was observed between the CanolaOleic and Canola treatments. The two PUFA-rich diets had higher ($P<0·05$) LEA levels compared with the CanolaDHA and CanolaOleic treatments. The FlaxSaff diet had the highest ($P<0·0001$) ALEA levels and the DHEA level was substantially higher ($P<0·0001$) in response to the CanolaDHA diet compared with the other four diets.

Correlation between plasma fatty acid ethanolamides and plasma fatty acids

Pearson's correlation coefficients between plasma FAE and their corresponding FA are presented in Table 4. Overall, positive correlations existed between the five major FAE and their individual precursor FA ($P<0·0001$), whereas DHEA-DHA (r 0·52; $P<0·0001$) and ALEA-ALA (r 0·51; $P<0·0001$) showed the strongest correlations. Only AEA failed to produce a significant association with AA (r 0·04; $P=0·3547$).

Body composition and correlation with plasma fatty acid ethanolamides

A subgroup of twenty-seven (male *n* 7; female *n* 20) volunteers at RCFFN completed all the baseline and end point DEXA measures at each phase, and therefore their data were used for the exploratory analysis on the correlations between body composition and plasma FAE levels. The baseline characteristics of the subjects are presented in online supplementary Table S1. Total fat mass, android fat mass, gynoid fat mass and the ratio of android:gynoid fat mass at baseline, end point and changes in each dietary phase are reported in Table 5. No significant differences were observed except for the end point gynoid fat mass ($P=0·0503$). It was mostly attributed to higher ($P<0·05$) gynoid fat mass for CanolaDHA compared with CanolaOleic. Changes of gynoid fat mass from baseline tended to differ ($P=0·0803$), due to an increase after CanolaDHA and a decrease after CanolaOleic.

Correlations between end point FAE levels and android fat mass change (end point *v.* baseline) were tested in the subset (*n* 27) using Pearson's correlations (Table 6). Although we noticed that this subgroup had an unbalanced sex ratio, no differences on android fat mass change were observed. Overall, negative correlations between plasma OEA (r −0·24; $P=0·0049$) (Fig. 2), AEA (r −0·24; $P=0·0059$) and LEA (r −0·20; $P=0·0225$) levels and android fat mass changes across five diets were observed. Moreover, similar negative correlations were observed between the OEA:DHEA ratio and android fat mass change (r −0·20; $P=0·0187$). No correlations were observed between ALEA, DHEA or PEA and android fat mass change.

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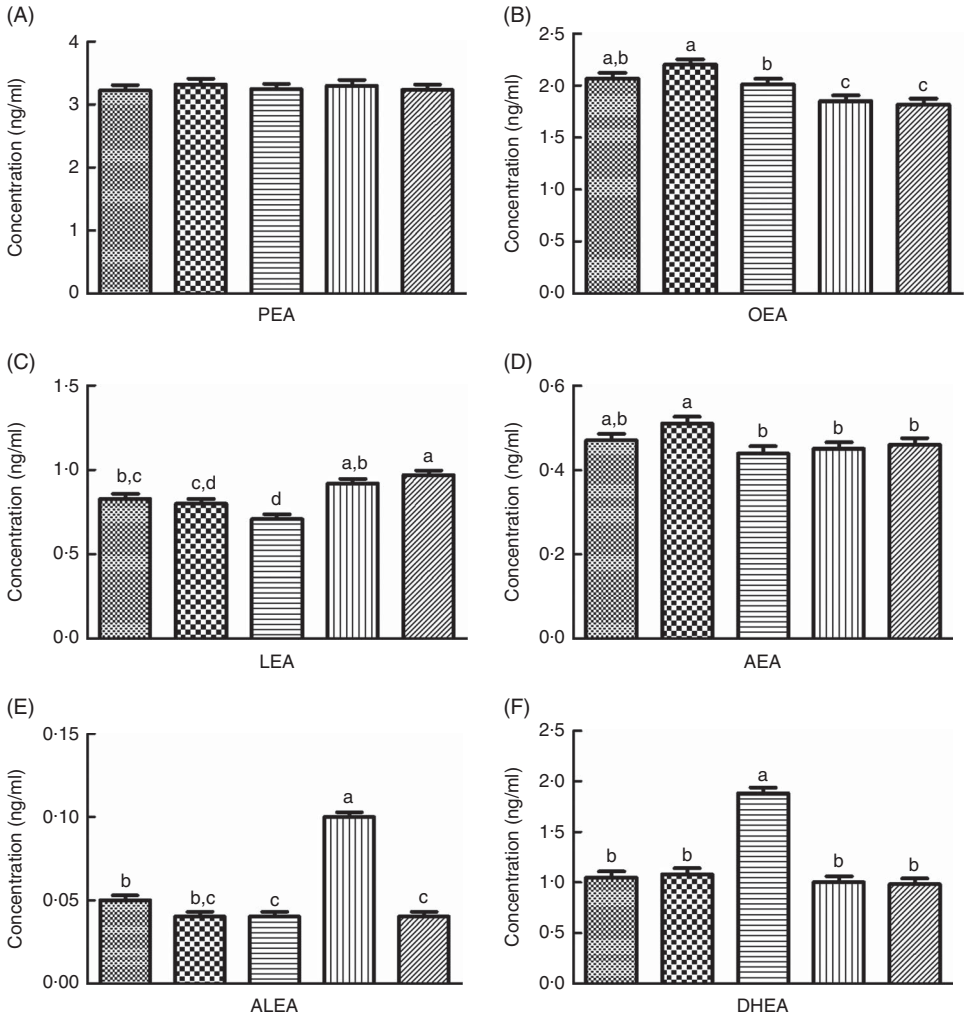

Fig. 1. Total plasma fatty acid ethanolamide levels at the end point of five dietary treatments. Values are presented in ng/ml as least squares means (*n* 121), with their standard errors. [a,b,c] Mean values within each graph indicate significant differences between-treatments ($P < 0{\cdot}05$). (A) Palmitoylethanolamide (PEA); (B) oleoylethanolamide (OEA); (C) linoleoylethanolamide (LEA); (D) arachidonoylethanolamide (AEA); (E) α-linolenoylethanolamide (ALEA); (F) docosahexaenoylethanolamide (DHEA). ▩,Conventional canola oil; ▩, high oleic canola oil; ▤, DHA-enriched canola oil; ▥, a blend of flax oil and safflower oil; ▨, a blend of corn oil and safflower oil.

Table 4. Pearson's correlation between fatty acid ethanolamides (FAE) and their corresponding precursor fatty acids in plasma (*n* 121)

Fatty acids	FAE	Correlation coefficient	*P*
PA	PEA	0·34504	<0·0001
OA	OEA	0·36434	<0·0001
LA	LEA	0·18462	<0·0001
AA	AEA	0·03785	0·3547
ALA	ALEA	0·51068	<0·0001
DHA	DHEA	0·51879	<0·0001

PA, palmitic acid; PEA, palmitoylethanolamide; OA, oleic acid; OEA, oleoylethanolamide; LA, linoleic acid; LEA, linoleoylethanolamide; AA, arachidonic acid; AEA, arachidonoylethanolamine; ALA, α-linolenic acid; ALEA, α-linolenoylethanolamide; DHEA, docosahexaenoylethanolamide.

SNP characteristics and association with fatty acid ethanolamides

Genomic DNA was genotyped for 129 of 130 subjects (one blood sample failed to provide enough DNA yield). Analyses of the two SNP rs12540583 in *NAPE-PLD* and rs324420 in *FAAH* are summarised in Table 7.

As numbers of rare homozygotes in both polymorphisms were relatively low, the genotypes were grouped into two categories – common homozygous and minor allele carriers. Results of the *NAPE-PLD* polymorphism rs12540583 (Table 8(a)) showed no differences in FAE levels across all treatments, except that the C-allele carriers had a higher ($P = 0{\cdot}0010$) DHEA levels after the CanolaDHEA diet, whereas a strong treatment × genotype interaction for DHEA was also observed ($P = 0{\cdot}0004$). In general, analyses of polymorphism rs324420 in *FAAH* (Table 8(b)) indicated that the A-allele carriers had higher ($P = 0{\cdot}0002$) DHEA levels than the CC genotype carrier. In particular, after the Canola and CanolaDHA treatments, DHEA levels of A-allele carriers were higher ($P < 0{\cdot}05$) than the homozygous C genotype group. In addition, a strong treatment × genotype interaction for DHEA was also observed ($P < 0{\cdot}0001$). DHEA levels after the CanolaDHA diet were observed to be higher in both genotypes compared with other treatments, and were significantly different between two

Table 5. Body fat mass composition at the beginning and the end and changes of each dietary phase*
(Least square mean values with their standard errors; *n* 27 (7, males; 20, females) from Richardson Centre for Functional Foods and Nutraceuticals)

	Canola		CanolaOleic		CanolaDHA		FlaxSaff		CornSaff		
Body fat profile	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Baseline											
Total fat mass (kg)	33·72	2·10	33·35	2·10	33·40	2·10	33·60	2·10	33·49	2·10	0·6231
Android fat mass (kg)	3·42	0·22	3·37	0·22	3·40	0·22	3·41	0·22	3·39	0·22	0·8205
Gynoid fat mass (kg)	5·61	0·43	5·58	0·43	5·56	0·43	5·57	0·43	5·57	0·43	0·9653
Android:gynoid ratio	0·63	0·03	0·62	0·03	0·63	0·03	0·63	0·03	0·63	0·03	0·7659
End point											
Total fat mass (kg)	33·11	2·08	32·81	2·08	33·13	2·08	32·97	2·08	33·28	2·08	0·2496
Android fat mass (kg)	3·39	0·22	3·33	0·22	3·39	0·22	3·36	0·22	3·40	0·22	0·2285
Gynoid fat mass (kg)	5·47	0·41	5·43	0·41	5·63	0·41	5·47	0·41	5·55	0·41	0·0503
Android:gynoid ratio	0·63	0·03	0·63	0·03	0·62	0·03	0·63	0·03	0·63	0·03	0·3273
Changes											
Total fat mass (kg)	−0·42	0·23	−0·15	0·23	−0·05	0·23	−0·49	0·23	−0·23	0·23	0·4703
Android fat mass (kg)	−0·02	0·03	−0·04	0·03	0·01	0·03	−0·07	0·03	−0·01	0·03	0·3848
Gynoid fat mass (kg)	−0·11	0·06	−0·11	0·06	0·06	0·06	0·01	0·06	−0·06	0·06	0·0803
Android:gynoid ratio	0·00	0·01	0·01	0·01	−0·01	0·01	−0·01	0·01	0·00	0·01	0·1464

Canola, conventional canola oil; CanolaOleic, high oleic canola oil; CanolaDHA, DHA-enriched canola oil; FlaxSaff, a blend of flax oil and safflower oil; CornSaff, a blend of corn oil and safflower oil.
* Mixed-model ANOVA corrected by sex and *post hoc* Tucky's test were used to analyse treatment effects on different fat mass profiles. Significant difference: $P<0·05$.

Table 6. Pearson's correlation between end point fatty acid ethanolamide (FAE) levels and android fat mass changes of each dietary treatment (*n* 27).

FAE	Correlation coefficients	*P**
PEA	−0·14	0·1037
OEA	−0·24	0·0049
LEA	−0·20	0·0225
AEA	−0·24	0·0059
ALEA	−0·08	0·3564
DHEA	0·08	0·3564
OEA:DHEA ratio	−0·20	0·0187

PEA, palmitoylethanolamide; OEA, oleoylethanolamide; LEA, linoleoylethanolamide; AEA, arachidonoylethanolamine; ALEA, α-linolenoylethanolamide; DHEA, docosahexaenoylethanolamide.
* Significant correlation: $P<0·05$.

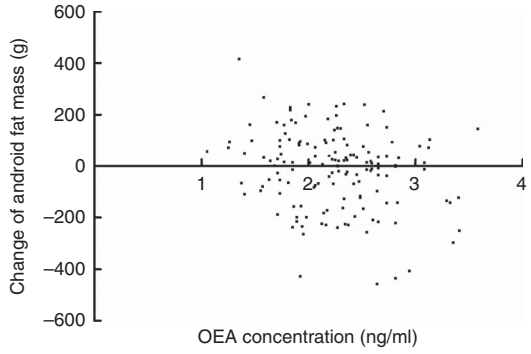

Fig. 2. The correlation between oleoylethanolamide (OEA) and change of android fat mass (end point *v.* baseline) across all five diets (*n* 27). A weak negative but significant correlation was observed (r −0·24; $P=0·0049$).

genotypes. No differences were observed in the corresponding plasma DHA levels between the two genotypes.

Discussion

The present study demonstrated that circulating FAE levels reflected their precursor FA from the dietary intake, under controlled feeding conditions. Polymorphisms of rs324420 (*FAAH*) may play a role in modulating the circulating levels of OEA and DHEA, which might further influence the body fat mass distribution. Given the growing evidence for the importance of FAE in governing energy metabolism, the implications of these trial results could be substantial.

Accumulating evidence suggests that circulating FAE levels are influenced by genetic variants, which account for the FAE synthesis and degradation *in vivo*[37]. In this study, we have extended the genetic investigation by examining the differences among end point plasma FAE in response to various dietary FA. Our results for the polymorphism rs12540583 with a missense mutation in humans also indicated a very limited effect of *NAPE-PLD* mutations on circulating FAE levels, except for the CanolaDHA treatment. To the best of our knowledge, this is the first study that has reported an analysis of the association between this polymorphism and circulating FAE levels. One clinical study reported that a common haplotype in *NAPE-PLD* was protective against obesity[22], but no other human studies have assessed whether NAPE-PLD deficiency influences circulating FAE. In animal models, it has been reported that FAE levels were unaltered in NAPE-PLD-deficient mice[38], suggesting that FAE might be synthesised by both NAPE-PLD-dependent and NAPE-PLD-independent pathways based on the NAPE. Recently, Geurts *et al.*[23] reported approximately 60 % reduction of PEA, OEA and stearoylethanolamine levels in the adipose tissues, but not in the brain, of NAPE-PLD-deleted mice compared with wild-type mice when AEA remained unchanged in both genotypes. The important evidence on research of the *NAPE-PLD* gene suggests the existence of an alternative pathway of FAE synthesis[39]. Interestingly, the lack of positive correlation between circulating AA and AEA in the present study suggests that the similar alternative synthesis pathway of AEA may exist in humans. These data also suggest that plasma AA may not directly reflect the amount in the AEA precursor pool, because AA levels can also be affected by the

Table 7. Characteristics of the selected SNP associated with metabolism of fatty acid ethanolamides (*n* 129).

SNP	Gene	Annotation	Alleles (major/minor)	Genotype			MAF (%)
				MM	Mm	mm	
rs12540583	*NAPE-PLD*	Missense	A/C	103	25	1	10·5
rs324420	*FAAH*	Missense	C/A	85	39	5	19·0

MM, homozygous for the major allele; Mm, heterozygous; mm, homozygous for the minor allele; MAF, minor allele frequency; *NAPE-PLD*, *N*-acyl phosphatidylethanolamine phospholipase D; *FAAH*, fatty acid amide hydrolase.

level of its precursor, LA, and the levels of its derivatives, eicosanoids.

Apart from synthesis of FAE, the regulation of FAE levels also involves degradation. Enzymatic hydrolysis can degrade FAE to FA and ethanolamine by FAAH in virtually all mammalian tissues[40]. FAAH is an integral membrane enzyme that can catalyse the hydrolysis of all FAE. As such, genetic variants of the *FAAH* gene could alter the functionality of enzyme activity. The genetic missense polymorphism rs324420 in the *FAAH* gene has been extensively studied as an endocannabinoid risk factor in overweight/obesity and for potential cannabinoid antagonist treatment in obese populations[17]. However, the direct association between this SNP and obesity has not been evaluated. One study in overweight/obese subjects with binge eating disorder (BED) or non-BED demonstrated a positive correlation between A-allele carriers of rs324420 with overweight/obesity people, but no differences between BED and non-BED subjects[18]. In addition, it was reported that the polymorphism rs324420 variants may be associated with early-onset but not adult obesity[41]. Our study did not show any direct association between rs324420 and BMI types (data not shown). In the present study, the A-allele carriers of rs324420 in *FAAH* had a higher circulating DHEA level. It suggests that the A-allele carriers may have a lower activity rate for hydrolysing DHEA, resulting in a relatively higher level of DHEA, especially in response to consumption of DHA-enriched diets. Interestingly, such diet–gene interactions were observed only for DHEA and ALEA, indicating this SNP rs324420 may only affect DHEA and ALEA levels. Although little is known about the potential bioactive compounds DHEA and ALEA, studies have reported that the beneficial functions of DHA or DHEA could be attributed to major DHEA derivatives, which both have potent anti-inflammatory and organ-protective properties[42,43]. As such, these findings concerning the SNP rs324420 may be useful for further research designed to examine the beneficial effects of DHA consumption.

On the basis of animal studies, OEA is of pharmaceutical interest because of the effects it has on the endocannabinoid system, which could be a therapy for treating severe obesity by inhibiting appetite. Recently, a study compared the efficacy for anti-obesity treatment using orally administered OEA and rimonabant, an anorectic anti-obesity drug approved for use in Europe[6]. Results indicated that OEA might represent a novel alternative to cannabinoid antagonists for the control of appetite, and rimonabant might also affect food intake and share a similar mechanism. Our results demonstrate a negative correlation between circulating OEA levels and the change in android fat mass from baseline across all five diets. This finding indicates that endogenous OEA from dietary OA might play a role in reducing fat mass. It is possible that the reduction of body weight, especially gynoid fat mass, by consuming diets high in OA is due to OEA: (a) suppression of appetite or (b) increased fat oxidation, resulting in decreased food intake. However, in our study, food intake was controlled and subjects on all diets consumed the same number of energy content across phases[30]. Thus, in the present study, our observations may be due to a different mechanism(s). Nevertheless, a different study conducted by our group showed a trend for increasing food intake and body weight in response to consumption of a DHA-enriched high oleic canola oil compared with corn oil or high oleic canola oil in golden hamsters[35]. In the present study, the CanolaDHA diet did not reduce android fat mass, but increased gynoid fat mass compared with the other two oleic acid-rich diets, resulting in the lowest total fat mass change. Consistent findings about the OEA:DHEA ratio change have been reported in CD1 mice[44], whereas our results reported lower plasma OEA levels in response to the DHA-rich diet. Recent evidence showed that DHEA might be a potent mediator for neurogenic differentiation[45], and plays a role in reduction of headache and psychological distress in humans[46], although we are not aware how such bioactivity of DHEA on neurological system could influence the OEA metabolism. Therefore, our observation provides indirect evidence to support the discovery of the effects of FAE on appetite and satiety that has been reported by Piomelli *et al.*[47]. Moreover, we speculate that the elevation of DHEA in response to dietary DHA interferes with the synthesis and function of OEA to potentially suppress appetite and food intake, which would prevent weight loss.

In the present study, the plasma FA profile reflected the dietary FA in the treatment oils. The good compliance of participants across the three clinical sites has been previously reported[30]. As expected, corresponding elevations in end point FAE levels were also observed after the dietary interventions. Our results indicate that individuals with elevated plasma FA levels tend to have higher corresponding FAE levels. Results of a clinical study by Joosten *et al.*[48] agreed with our findings and further suggest that the NEFA level may be positively correlated with both circulating fasting or non-fasting FAE. Thus, these NEFA molecules from increased membrane phospholipid cleavage become precursors of endogenous FAE as well. It is possible that the influences of NEFA on circulating FAE are based on their mobilisation from adipose tissue and clearance in the fasted state. Nevertheless, no further evidence would support the physiological stimuli involved in the changes of NEFA levels after meal consumption. In addition, as FAE are mostly converted in the tissues and then released into the

Table 8. Selected plasma fatty acid ethanolamides (FAE) at the end point of five dietary treatments, by rs12540583 in *N*-acyl phosphatidylethanolamine phospholipase D (*NAPE-PLD*) and rs324420 in fatty acid amide hydrolase (*FAAH*)*
(Least square mean values; *n* 120)

		Treatments					
FAE	Genotype	Canola	CanolaOleic	CanolaDHA	FlaxSaff	CornSaff	Overall
(a) rs12540583 in *NAPE-PLD*							
PEA (ng/ml)	AA	3·2538	3·3977	3·2641	3·3363	3·2801	3·3064
	AC/CC	3·0356	2·9576	3·1141	3·1249	3·0344	3·0533
	P	0·9867	0·4697	0·9992	0·9892	0·9686	0·1111
	$P_{interaction}$						0·2031
OEA (ng/ml)	AA	2·0240	2·2076	2·0125	1·8198	1·8262	1·9780
	AC/CC	2·1030	2·0432	1·8884	1·8099	1·6429	1·8975
	P	0·9999	0·9764	0·9968	1·0000	0·9507	0·4809
	$P_{interaction}$						0·2144
LEA (ng/ml)	AA	0·7924	0·8027	0·7166	0·8927	0·9557	0·8320
	AC/CC	0·9368	0·7444	0·6671	0·9842	0·9873	0·8640
	P	0·6667	0·9990	0·9997	0·9712	1·0000	0·5756
	$P_{interaction}$						0·0411
AEA (ng/ml)	AA	0·4790	0·5288	0·4522	0·4610	0·4713	0·4785
	AC/CC	0·4450	0·4379	0·4024	0·4189	0·4134	0·4235
	P	0·9960	0·2991	0·9403	0·9808	0·8616	0·0566
	$P_{interaction}$						0·6246
ALEA (ng/ml)	AA	0·0524	0·0442	0·0423	0·0997	0·0408	0·0559
	AC/CC	0·0519	0·0395	0·0363	0·1103	0·0401	0·0556
	P	1·0000	0·9998	0·9978	0·9036	1·0000	0·9543
	$P_{interaction}$						0·3218
DHEA (ng/ml)	AA	1·0434	1·0953	1·7738	1·0146	0·9678	1·1790
	AC/CC	1·1485	1·0234	2·2034	1·0211	1·0457	1·2884
	P	0·9903	0·9995	0·0010	1·0000	0·9989	0·1141
	$P_{interaction}$						0·0004
(b) rs324420 in *FAAH*							
PEA (ng/ml)	CC	3·0678	3·1518	3·1416	3·1820	3·1172	3·1321
	CA/AA	3·5722	3·6652	3·4963	3·5806	3·5045	3·5638
	P	0·2108	0·1865	0·7054	0·5551	0·5930	0·0067
	$P_{interaction}$						0·8483
OEA (ng/ml)	CC	1·9085	2·0534	1·9521	1·7424	1·7156	1·8744
	CA/AA	2·2849	2·3984	2·0372	1·9480	1·9020	2·1141
	P	0·0826	0·1527	0·9996	0·8316	0·8978	0·0209
	$P_{interaction}$						0·0607
LEA (ng/ml)	CC	0·7448	0·7416	0·6877	0·8598	0·9128	0·7893
	CA/AA	0·9668	0·8751	0·7295	0·9981	1·0449	0·9229
	P	0·0346	0·6084	0·9998	0·5640	0·6273	0·0094
	$P_{interaction}$						0·1392
AEA (ng/ml)	CC	0·4447	0·4947	0·4295	0·4325	0·4358	0·4474
	CA/AA	0·5156	0·5273	0·4579	0·4817	0·4966	0·4958
	P	0·5242	0·9937	0·9978	0·9088	0·7308	0·0716
	$P_{interaction}$						0·6260
ALEA (ng/ml)	CC	0·0457	0·0401	0·0403	0·0946	0·0387	0·0519
	CA/AA	0·0647	0·0486	0·0426	0·1162	0·0441	0·0632
	P	0·0832	0·9430	1·0000	0·0257	0·9978	0·0073
	$P_{interaction}$						0·0337
DHEA (ng/ml)	CC	0·9742	1·0427	1·6906	0·9871	0·9401	1·1270
	CA/AA	1·2361	1·1554	2·2018	1·0901	1·0608	1·3488
	P	0·0848	0·9561	<0·0001	0·9761	0·9336	0·0002
	$P_{interaction}$						0·0001

Canola, conventional canola oil; CanolaOleic, high oleic canola oil; CanolaDHA, DHA-enriched canola oil; FlaxSaff, a blend of flax oil and safflower oil; CornSaff, a blend of corn oil and safflower oil; PEA, palmitoylethanolamide; OEA, oleoylethanolamide; LEA, linoleoylethanolamide; AEA, arachidonoylethanolamine; ALEA, *α*-linolenoylethanolamide; DHEA, docosahexaenoylethanolamide.
* Significant difference: $P < 0·05$. *P* value shows the differences between two genotypes within the same treatment. $P_{interaction}$ shows the gene-treatment interaction in the entire population.

circulating pool, the enzyme activities of FAE synthesis and degradation are more crucial for circulating FAE levels[15,23]. As such, our important findings demonstrate that the fat types in the diet can positively influence the corresponding FAE levels in humans. These results also provide evidence supporting the conclusion that dietary FA lead to increased circulating FAE in both piglets[12] and humans[36,48], although it remains to be established whether such changes in plasma FAE levels further alter the FAE levels in target tissues such as brain, liver and intestines.

Polyunsaturated long-chain FAE are a relatively minor group compared with saturated and monounsaturated FAE, such as PEA and OEA, which are the major FAE in plasma

and tissues[49]. Evidence shows that feeding a high-SFA diet to mice[50] and rats[51] resulted in lower levels of PEA and OEA. Joosten *et al.*[48] reported that the two most abundant FAE, PEA and OEA levels, were approximately 7-fold higher than AEA levels in a clinical study. Our observation concurs with the findings with similar absolute values for PEA and OEA ranging from 0·1 to 4·0 ng/ml. Owing to the intervention design in our clinical trial, we were unable to demonstrate the effect of dietary SFA on PEA and OEA. However, we noticed that although post-treatment plasma LA, LEA precursor, levels were higher than OA, OEA precursor, levels in our population, the overall plasma LEA levels were lower than OEA. The correlation coefficient between LA and LEA was less significant than that between OA and OEA. It is possible that the endogenous conversion from OA to OEA may be faster compared with LA to LEA. One possible explanation is because of the variations in the positional distribution of FA on TAG molecules. The committed step of FAE synthesis is the exchange of FA at the stereospecific numbering-1 (sn-1) position. Usually, the sn-1 position contains a SFA, whereas the sn-2 position more likely is occupied by an unsaturated FA. Accordingly, PEA levels are overall the highest among the six FAE of interest in our observation as well as in other similar studies. In mammals, the ratio of OA:LA at the sn-1 position is higher than the ratio of LA: OA, which can potentially lead to the higher synthesis rate of OEA compared with LEA. In addition, the negative correlations between android fat mass changes and FAE (OEA, LEA and AEA) indicated that these endogenous FAE levels may be involved in the regulation of food intake and energy balance as suggested by Diep *et al.*[51].

This study has strengths and limitations. First, in contrast to a few previous human studies that relied on small subject numbers on the post-treatment FAE response, this study was powered to examine a larger group of people in North America. Furthermore, to the best of our knowledge, this is the first study ever to show that DHEA may have a suppressive effect on OEA levels, potentially resulting in a failure in body fat loss. Unfortunately, we were unable to assess the baseline FAE contents in plasma, which can establish the initial FAE profiles of the participants for further comparison. Although the cross-over design of our trial clearly showed the treatment effects on different circulating FAE levels, lacking of baseline values restricted our prediction on the association between change of FAE and change of body fat mass in response to different oil treatments. Nonetheless, the correlation analysis was only performed in a small subgroup of twenty-seven subjects with an unbalanced sex ratio due to the limited design of satellite sites in such multicentre trial, and thus our attempt was not able to assess the sex effects, and the question of how the shifts of FAE influence the body fat response requires further investigation.

In summary, our study reports several novel findings that clarify our understanding of the metabolic and physiological responses associated with different dietary FA classes. Our finding on the genetic variants indicates that the polymorphism rs324420 in *FAAH* may influence the beneficial function of FAE in humans by altering the circulating FAE levels. Furthermore, the elevated circulating DHEA in response to dietary DHA may suppress OEA levels and, in turn, interfere with the function of OEA, which may disrupt regulation of body weight.

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The authors' contributions are as follows: S. P. and P. J. H. J. contributed to the design of the study and wrote the manuscript. S. P. and X. L. conducted the data analyses. S. P., P. E. and P. J. H. J. were responsible for interpretation of the results. D. J. A. J., P. W. C., B. L., P. M. E.-K., S. G. W. and P. J. H. J. provided supervision.

None of the authors has any conflicts of interest to declare.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114515005425

References

1. Epps DE, Natarajan V, Schmid PC, *et al.* (1980) Accumulation of *N*-acylethanolamine glycerophospholipids in infarcted myocardium. *Biochim Biophys Acta* **618**, 420–430.
2. Devane WA, Hanus L, Breuer A, *et al.* (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
3. Marczylo TH, Lam PM, Amoako AA, *et al.* (2010) Anandamide levels in human female reproductive tissues: solid-phase extraction and measurement by ultraperformance liquid chromatography tandem mass spectrometry. *Anal Biochem* **400**, 155–162.
4. Pasquariello N, Catanzaro G, Marzano V, *et al.* (2009) Characterization of the endocannabinoid system in human neuronal cells and proteomic analysis of anandamide-induced apoptosis. *J Biol Chem* **284**, 29413–29426.
5. Pacher P, Batkai S & Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* **58**, 389–462.
6. Romano A, Coccurello R, Giacovazzo G, *et al.* (2014) Oleoylethanolamide: a novel potential pharmacological alternative to cannabinoid antagonists for the control of appetite. *Biomed Res Int* **2014**, 203425.
7. Fu J, Gaetani S, Oveisi F, *et al.* (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* **425**, 90–93.
8. Lo Verme J, Gaetani S, Fu J, *et al.* (2005) Regulation of food intake by oleoylethanolamide. *Cell Mol Life Sci* **62**, 708–716.

9. Hoareau L, Buyse M, Festy F, *et al.* (2009) Anti-inflammatory effect of palmitoylethanolamide on human adipocytes. *Obesity (Silver Spring)* **17**, 431–438.
10. Klein TW (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol* **5**, 400–411.
11. O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* **152**, 576–582.
12. Berger A, Crozier G, Bisogno T, *et al.* (2001) Anandamide and diet: inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding *N*-acylethanolamines in piglets. *Proc Natl Acad Sci U S A* **98**, 6402–6406.
13. Kim HY, Moon HS, Cao D, *et al.* (2011) N-docosahexaenoylethanolamide promotes development of hippocampal neurons. *Biochem J* **435**, 327–336.
14. Rahman IA, Tsuboi K, Uyama T, *et al.* (2014) New players in the fatty acyl ethanolamide metabolism. *Pharmacol Res* **86**, 1–10.
15. Tsuboi K, Ikematsu N, Uyama T, *et al.* (2013) Biosynthetic pathways of bioactive *N*-acylethanolamines in brain. *CNS Neurol Disord Drug Targets* **12**, 7–16.
16. Thabuis C, Tissot-Favre D, Bezelgues JB, *et al.* (2008) Biological functions and metabolism of oleoylethanolamide. *Lipids* **43**, 887–894.
17. Sipe JC, Waalen J, Gerber A, *et al.* (2005) Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). *Int J Obes (Lond)* **29**, 755–759.
18. Monteleone P, Tortorella A, Martiadis V, *et al.* (2008) The cDNA 385C to A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) is associated with overweight/obesity but not with binge eating disorder in overweight/obese women. *Psychoneuroendocrinology* **33**, 546–550.
19. Jensen DP, Andreasen CH, Andersen MK, *et al.* (2007) The functional Pro129Thr variant of the *FAAH* gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5801 whites. *J Mol Med* **85**, 445–449.
20. Monteleone P, Milano W, Petrella C, *et al.* (2010) Endocannabinoid Pro129Thr FAAH functional polymorphism but not 1359G/A CNR1 polymorphism is associated with antipsychotic-induced weight gain. *J Clin Psychopharmacol* **30**, 441–445.
21. Hariri AR, Gorka A, Hyde LW, *et al.* (2009) Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. *Biol Psychiatry* **66**, 9–16.
22. Wangensteen T, Akselsen H, Holmen J, *et al.* (2011) A common haplotype in NAPEPLD is associated with severe obesity in a Norwegian population-based cohort (the HUNT study). *Obesity (Silver Spring)* **19**, 612–617.
23. Geurts L, Everard A, Van Hul M, *et al.* (2015) Adipose tissue NAPE-PLD controls fat mass development by altering the browning process and gut microbiota. *Nat Commun* **6**, 6495.
24. Piers LS, Walker KZ, Stoney RM, *et al.* (2003) Substitution of saturated with monounsaturated fat in a 4-week diet affects body weight and composition of overweight and obese men. *Br J Nutr* **90**, 717–727.
25. Moussavi N, Gavino V & Receveur O (2008) Could the quality of dietary fat, and not just its quantity, be related to risk of obesity? *Obesity (Silver Spring)* **16**, 7–15.
26. Pelkman CL, Fishell VK, Maddox DH, *et al.* (2004) Effects of moderate-fat (from monounsaturated fat) and low-fat weight-loss diets on the serum lipid profile in overweight and obese men and women. *Am J Clin Nutr* **79**, 204–212.
27. Krishnan S & Cooper JA (2014) Effect of dietary fatty acid composition on substrate utilization and body weight maintenance in humans. *Eur J Nutr* **53**, 691–710.
28. Naughton SS, Mathai ML, Hryciw DH, *et al.* (2013) Fatty Acid modulation of the endocannabinoid system and the effect on food intake and metabolism. *Int J Endocrinol* **2013**, 361895.
29. Jones PJ, Senanayake VK, Pu S, *et al.* (2014) DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. *Am J Clin Nutr* **100**, 88–97.
30. Senanayake VK, Pu S, Jenkins DA, *et al.* (2014) Plasma fatty acid changes following consumption of dietary oils containing *n*-3, *n*-6, and *n*-9 fatty acids at different proportions: preliminary findings of the Canola oil Multicenter Intervention Trial (COMIT). *Trials* **15**, 136.
31. Mifflin MD, St Jeor ST, Hill LA, *et al.* (1990) A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* **51**, 241–247.
32. Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
33. Lin L, Yang H & Jones PJ (2012) Quantitative analysis of multiple fatty acid ethanolamides using ultra-performance liquid chromatography-tandem mass spectrometry. *Prostaglandins Leukot Essent Fatty Acids* **87**, 189–195.
34. Marczylo TH, Lam PM, Nallendran V, *et al.* (2009) A solid-phase method for the extraction and measurement of anandamide from multiple human biomatrices. *Anal Biochem* **384**, 106–113.
35. Lin L, Rideout T, Yurkova N, *et al.* (2013) Fatty acid ethanolamides modulate CD36-mRNA through dietary fatty acid manipulation in Syrian Golden hamsters. *Appl Physiol Nutr Metab* **38**, 870–878.
36. Jones PJ, Lin L, Gillingham LG, *et al.* (2014) Modulation of plasma *N*-acylethanolamine levels and physiological parameters by dietary fatty acid composition in humans. *J Lipid Res* **55**, 2655–2664.
37. Pecina M, Martinez-Jauand M, Hodgkinson C, *et al.* (2014) FAAH selectively influences placebo effects. *Mol Psychiatry* **19**, 385–391.
38. Tsuboi K, Okamoto Y, Ikematsu N, *et al.* (2011) Enzymatic formation of *N*-acylethanolamines from *N*-acylethanolamine plasmalogen through *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochim Biophys Acta* **1811**, 565–577.
39. Simon GM & Cravatt BF (2010) Characterization of mice lacking candidate *N*-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production *in vivo*. *Mol Biosyst* **6**, 1411–1418.
40. Blankman JL & Cravatt BF (2013) Chemical probes of endocannabinoid metabolism. *Pharmacol Rev* **65**, 849–871.
41. Muller TD, Bronner G, Wandolski M, *et al.* (2010) Mutation screen and association studies for the fatty acid amide hydrolase (*FAAH*) gene and early onset and adult obesity. *BMC Med Gene* **11**, 2.
42. Yang R, Fredman G, Krishnamoorthy S, *et al.* (2011) Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel bioactive signals. *J Biol Chem* **286**, 31532–31541.
43. Brown I, Cascio MG, Wahle KW, *et al.* (2010) Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. *Carcinogenesis* **31**, 1584–1591.
44. Wood JT, Williams JS, Pandarinathan L, *et al.* (2010) Dietary docosahexaenoic acid supplementation alters select physiological endocannabinoid-system metabolites in brain and plasma. *J Lipid Res* **51**, 1416–1423.

45. Rashid MA, Katakura M, Kharebava G, *et al.* (2013) N-docosahexaenoylethanolamine is a potent neurogenic factor for neural stem cell differentiation. *J Neurochem* **125**, 869–884.
46. Ramsden CE, Zamora D, Makriyannis A, *et al.* (2015) Diet-induced changes in *n*-3- and *n*-6-derived endocannabinoids and reductions in headache pain and psychological distress. *J Pain* **16**, 707–716.
47. Rodriguez de Fonseca F, Navarro M, Gomez R, *et al.* (2001) An anorexic lipid mediator regulated by feeding. *Nature* **414**, 209–212.
48. Joosten MM, Balvers MG, Verhoeckx KC, *et al.* (2010) Plasma anandamide and other *N*-acylethanolamines are correlated with their corresponding free fatty acid levels under both fasting and non-fasting conditions in women. *Nutr Metab (Lond)* **7**, 49.
49. Schmid HH, Schmid PC & Berdyshev EV (2002) Cell signaling by endocannabinoids and their congeners: questions of selectivity and other challenges. *Chem Phys Lipids* **121**, 111–134.
50. Aviello G, Matias I, Capasso R, *et al.* (2008) Inhibitory effect of the anorexic compound oleoylethanolamide on gastric emptying in control and overweight mice. *J Mol Med (Berl)* **86**, 413–422.
51. Diep TA, Madsen AN, Holst B, *et al.* (2011) Dietary fat decreases intestinal levels of the anorectic lipids through a fat sensor. *FASEB J* **25**, 765–774.

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