Title
Plasma endocannabinoid levels in lean, overweight, and obese humans: relationships to intestinal permeability markers, inflammation, and incretin secretion

Permalink
https://escholarship.org/uc/item/63g7s65h

Journal
AMERICAN JOURNAL OF PHYSIOLOGY-ENDOCRINOLOGY AND METABOLISM, 315(4)

ISSN
0193-1849

Authors
Little, TJ
Cvijanovic, N
DiPatrizio, NV
et al.

Publication Date
2018-10-01

DOI
10.1152/ajpendo.00355.2017

License
CC BY-NC-ND 4.0

Peer reviewed
Plasma endocannabinoid concentrations in lean, obese and overweight humans: relationships with intestinal expression of intestinal permeability markers, inflammation and incretin secretion

Tanya J. Little¹,³*, Nada Cvijanovic¹,²,³*, Nicholas V. DiPatrizio⁵, Donovan A. Argueta⁵, Christopher K. Rayner¹,³,⁴, Christine Feinle-Bisset¹,³, Richard L. Young¹,²,³

¹University of Adelaide School of Medicine; ²South Australian Health and Medical Research Institute; ³NHMRC Centre of Research Excellence in Translating Nutritional Science to Good Health, University of Adelaide; ⁴Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, Australia; ⁵Division of Biomedical Sciences, School of Medicine, University of California, Riverside, Riverside, USA

*Equal first authors: TJL and NC contributed equally to the work.

Correspondence: Dr Tanya J. Little, Adelaide Medical School, Adelaide Health and Medical Sciences Building, The University of Adelaide, Corner of North Terrace and George Street, Adelaide SA 5005, Australia. Phone: +61 8 8313 2999, E-mail: tanya.little@adelaide.edu.au

Running title: Endocannabinoids, intestinal permeability and incretin secretion

Keywords: Intestinal fat sensors, anandamide, 2-arachidonylglycerol, n-acylethanolamines, tight junction proteins, inflammation
Clinical trial registration: The trial was registered with the Australia and New Zealand Clinical Trial Registry (www.anzctr.org.au trial number: ACTRN12612000376842).

Funding: TJL was supported by an NHMRC Career Development Fellowship (grant 1022706), CFB by an NHMRC Senior Research Fellowship (grant 1103020, 2016-21). The study was supported by a Royal Adelaide Hospital Research Committee Clinical Project Grant (to TJL, 2015-16), and National Institutes of Health Grant DA034009 to NVD.
ABSTRACT

Introduction/Aims: In rodent studies, high-fat diet-induced obesity impairs intestinal control of endocannabinoid and oleylethanolamide production, contributing to reduced satiety and weight gain. High-fat diets also alter the intestinal microbiome associated with enhanced intestinal permeability and inflammation. The aims of this study were to: (i) evaluate effects of intraduodenal lipid on plasma levels of anandamide (AEA), 2-arachidonyl-sn-glycerol (2-AG) and oleylethanolamide (OEA) in humans, and (ii) to examine relationships with intestinal permeability and inflammation markers and incretin hormone secretion.

Methods: 20 lean, 18 overweight and 19 obese participants underwent intraduodenal Intralipid® infusion (2 kcal/min) for 120 min during which blood samples were collected. Endoscopic duodenal biopsies were collected at baseline, and following 30 min of infusion. Plasma AEA, 2-AG, and OEA concentrations were assessed by HPLC/tandem mass spectrometry, plasma tumour necrosis factor-α (TNF-α), glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinoetric peptide (GIP) by multiplex, and duodenal expression of intestinal permeability (occludin, zona-occludin-1 (ZO-1)) and inflammatory markers (intestinal alkaline phosphatase (IAP), toll-like receptor-4 (TLR4)) by RT-PCR.

Results: Plasma AEA concentrations were increased in obese, when compared with lean and overweight (P<0.05), while there was no effect of BMI group or ID lipid infusion on plasma 2-AG or OEA concentrations. Duodenal expression of IAP and ZO-1 were reduced in obese, when compared with lean, individuals (P<0.05), and these changes were inversely correlated with plasma AEA concentrations (P<0.05). There was a significant relationship between iAUC AEA concentrations with iAUC GIP (r=0.384, P=0.005), but not iAUC GLP-1.

Conclusions: Increased plasma levels of AEA, but not 2-AG or OEA, were present in obese, when compared with lean and overweight, individuals. Obese individuals also displayed decreased
baseline (fasting) duodenal expression of ZO-1 and IAP. The correlations between plasma anandamide concentrations with duodenal expression of ZO-1 and IAP, and plasma GIP, suggest that altered endocannabinoid signalling may contribute to changes in intestinal permeability, inflammation and incretins in human obesity.
INTRODUCTION

Obesity is associated with chronic low-grade inflammation, and a cluster of metabolic disturbances, including insulin resistance and type 2 diabetes. One of the major drivers of the obesity epidemic is the consumption of an energy-dense high-fat diet, which promotes hyperphagia via attenuation of the gut-brain signalling mechanisms involved in the control of food intake (20).

In rodents, this high-fat diet-induced hyperphagia has been demonstrated to be driven by alterations in the composition of the gut microbiota with consequent development of low-grade inflammation in the gastrointestinal tract, as determined by increased expression of toll-like receptor 4 (TLR-4, the receptor for the microbial toxin, lipopolysaccharide (LPS or endotoxin)), in the ileum (8), increased systemic levels of LPS and proinflammatory cytokines (2), and altered intestinal permeability characterised by decreased intestinal expression and localisation of tight junction proteins, including zona-occludin 1 (ZO-1) and occludin (3). Furthermore, the intestinal expression of intestinal alkaline phosphatase (IAP), an enzyme that inactivates LPS, is decreased by a high-fat diet in obesity-prone, but not obesity-resistant, rodents (8). Recent studies have also highlighted an important role of interactions between the gut microbiota and the endocannabinoid system in the regulation of energy homeostasis (25).

The endocannabinoids, 2-arachidonyl-sn-glycerol (2-AG) and anandamide (AEA), are lipid-derived mediators involved in many processes, including the control of food intake (1, 10). In the small intestine of rodents, 2-AG and AEA concentrations have been found to increase with fasting (11), after tasting dietary fats (10), and in a model of Western diet-induced obesity (1). Furthermore, they stimulate food intake by activating cannabinoid-1 (CB₁) receptors (13). Human obesity is associated with increased plasma and adipose tissue endocannabinoid concentrations (9), and while now withdrawn from the market due to adverse psychological effects, the drug
Rimonabant, a CB1 receptor antagonist, promoted weight loss in obese humans (19). Interest in the endocannabinoid system as a target for obesity therapy has re-emerged since rodent studies have demonstrated that obesity-associated changes in the gut microbiota activate the endocannabinoid system (via LPS) in the intestine to increase gut permeability, which further enhances plasma LPS levels inducing peripheral inflammation (4, 25). Studies examining the relationships between endocannabinoids, intestinal permeability and inflammation are lacking in humans.

Endocannabinoids also appear to play a role in mediating the secretion of the incretin hormones, glucose-dependent insulino tropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). CB1 receptors have been found to be expressed on enteroendocrine K- and L-cells in rodents (16, 24), and activation of the CB1 receptor by anandamide prior to an oral glucose tolerance test inhibited GIP secretion in rodents (24). In contrast, in humans, acute administration of the CB1 receptor agonist, nabilone, enhanced fasting plasma GIP by ~80%, with no effect on glucose-mediated GIP release, while decreasing the GLP-1 response to oral glucose (5). Therefore, it is possible that changes in incretin levels observed in obesity (Verdich, 2001 #633) are mediated by altered endocannabinoid levels, however, this has not been directly evaluated.

Endocannabinoid-related molecules, such as the fatty acid-derived mediators, n-acyl ethanolamines, in particular, OEA, have also been shown to play a role in energy homeostasis, reducing energy intake (by prolonging the inter-meal interval and reducing meal frequency) via activation of the nuclear receptor, peroxisome-proliferator-activated receptor-α (PPAR-α) (14, 21, 27). OEA is an also endogenous ligand for the fatty acid sensing receptor, GPR119, stimulating GLP-1 secretion (18, 26). Whether OEA levels are altered in human obesity is currently unclear.
We have recently characterised the effects of intraduodenal lipid infusion on the duodenal expression of a range of fat sensing molecules in lean, overweight and obese humans. We reported that human obesity is associated with altered capacity for the sensing of dietary fat, including decreased duodenal expression of free fatty acid receptors (FFAR) 1 and 4, and increased expression of cluster of differentiation 36 (CD36) (6, 7). Using plasma samples and duodenal biopsies collected in these studies, the aim of the current investigation was to (i) evaluate effects of intraduodenal lipid infusion on plasma levels of AEA, 2-AG and OEA in humans, and (ii) to examine relationships between plasma concentrations of endocannabinoid related molecules with BMI, intestinal permeability and inflammation markers and incretin hormone secretion.
MATERIALS AND METHODS

Participants

20 lean (10 males, body mass index (BMI: 22±0.5 kg/m^2, age: 28±2 years), 18 overweight (12 males, BMI: 27±0.3 kg/m^2, age: 32±3 years) and 19 obese (12 males, BMI: 35±1 kg/m^2, age: 30±2 years) participants were enrolled in the study. Participant demographics have been published previously (6, 7). In brief, all participants were weight-stable for at least 3 months prior to inclusion in the study and reported no gastrointestinal (GI) symptoms, had no prior GI surgery, did not take medications or supplements known to affect GI motility or appetite (including fish oil), consumed <20 g of alcohol per week, and were non-smokers. The study protocol was approved by the Royal Adelaide Hospital Research Ethics Committee and carried out in accordance with the Declaration of Helsinki. Each participant provided written informed consent prior to inclusion in the study.

Study design and protocol

**Part A: Endoscopy and collection of duodenal mucosal biopsies**

Participants attended the Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, at 0830 h following a standardized evening meal (400 g beef lasagne (2479 kJ, 20 g fat, 20 g protein, 80 g carbohydrate), McCain Foods, Wendouree, Victoria, Australia) and an overnight fast of 12 h from solids and 10 h from liquids. The protocol for endoscopic collection of mucosal biopsies has been described previously (6). Briefly, a small diameter video endoscope was passed through the nose into the second part of the duodenum and once positioned, 2 duodenal biopsies were collected using standard endoscopic biopsy forceps and placed immediately in Allprotect® Tissue Reagent (Qiagen, Australia). An intravenous cannula was inserted into a forearm vein and a baseline blood sample (10 ml) was collected (t = 0 min). Following this, an intraduodenal infusion of 10% Intralipid® (Fresenius Kabi AB, China; 2 kcal/min, 109 ml/hr) commenced via
the endoscope infusion channel for 30 min. Blood samples, for analysis of plasma TNF-α concentrations, were collected at t= 10, 20 and 30 min, and placed in ice-chilled EDTA-treated and serum tubes, as described previously (6). At the conclusion of the infusion (t = 30 min), two additional biopsies were collected.

Part B: Effect of intraduodenal lipid infusion on plasma concentrations of endocannabinoids and incretins

Participants attended the Discipline of Medicine at 0830 h following a standardized evening meal and overnight fast, as described above. Anesthetic spray and gel was administered into the nasal cavity (as above) prior to insertion of a small-diameter (3.5 mm) catheter (Dentsleeve International, Mui Scientific), which was allowed to pass via peristalsis through the pylorus into the second part of the duodenum. Accurate positioning of the catheter across the pylorus was achieved by monitoring the transmucosal potential difference using a monitoring electrode (Red Dot, 3M Healthcare) placed on the forearm as a reference (17). Once positioned, an intravenous cannula was inserted into a forearm vein and a baseline blood sample (10 ml) was collected (t = 0 min). Intraduodenal infusion of 10% Intralipid® then commenced at a rate of 2 kcal/min for 120 min (t = 0 - 120 min), during which blood samples were collected every 15 min and placed in ice-chilled EDTA-treated and serum tubes, as described previously (6).

Measurements

Analysis of AEA, OEA and 2-AG levels in plasma
Plasma samples collected at t = 0, 30, 60 and 120 min were analysed. Lipid extraction and analysis were performed as previously described (1). Plasma (0.5 mL) was added to 1.0 mL of methanol solution containing the internal standards, [\^2\text{H}_5]- 2-AG, [\^3\text{H}_4]-\text{AEA}, and [\^3\text{H}_4]-\text{OEA} (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted with chloroform (2 mL) and washed with 0.9 % saline (0.5 mL). Organic phases were collected and separated by open-bed silica gel column chromatography. Eluate was gently dried under N\textsubscript{2} stream (99.998% pure) and resuspended in 0.1 mL of methanol:chloroform (9:1), with 1 \muL injection for ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) analysis.

Data were collected using an Acquity I Class UPLC system coupled to a Xevo TQ-S Mass Spectrometer (Waters, Milford, MA, USA) with accompanying electrospray ionization (ESI) interface. Lipids were separated on an Acquity UPLC BEH C\textsubscript{18} column (2.1 x 50 mm i.d., 1.7\textmu m, Waters) with inline Acquity guard column (UPLC BEH C\textsubscript{18} VanGuard Pre-column; 2.1 x 5 mm i.d., 1.7\textmu m, Waters), and eluted by a gradient of methanol in water (0.25% acetic acid, 5mM ammonium acetate) according to the following gradient at a flow rate of 0.4 mL per min: 80% methanol 0.5 min, 80% - 100% methanol 0.5-2.5 min, 100% methanol 2.5-3 min, 100% - 80% methanol 3-3.1 min). Column temperature was maintained at 40 °C, and samples were maintained in the sample manager at 10 °C. Argon (99.998%) was used as collision gas. MS detection was in positive ion mode and capillary voltage set at 0.1 kV. Cone voltage and collision energy as follows, respectively: 2-AG = 30v, 12v; [\^2\text{H}_5] 2-AG = 25v, 44v; AEA = 30v, 14v; [\^3\text{H}_4] AEA = 26v, 16v; OEA = 28v, 16v; [\^2\text{H}_4]- OEA = 48v. 14v. Lipids were quantified using a stable isotope dilution method detecting protonated adducts of the molecular ions [M+H]\textsuperscript{+} in the multiple reaction monitoring (MRM) mode. Acyl migration from 2-arachidonylglycerol to 1-arachidonylglycerol is known to occur (28), thus all reported values for 2-AG represent the sum of 2-arachidonylglycerol
and 1-arachidonylglycerol. Tissue processing and LCMS analysis from an individual experiment occurred independently of other experiments. Extracted ion chromatograms were used to quantify 2-AG \((m/z = 379.3 > 287.3)\), AEA \((m/z = 348.3 > 62.0)\), and OEA \((m/z = 326.4 > 62.1)\), and \([^2\text{H}_5]\)2-AG \((m/z = 384.3 > 93.4)\), \([^{2}\text{H}_4]\)-AEA \((m/z = 352.3 > 66.1)\), and \([^{2}\text{H}_4]\)-OEA \((m/z = 330.4 > 66.0)\), which were used as internal standards.

**RNA extraction**

Frozen duodenal biopsies were disrupted using a bead-based tissue homogeniser (TissueLyser LT, Qiagen) and homogenised through Qiashredder columns (Qiagen). Total cellular RNA was isolated using the PureLink™ MicroKit (Invitrogen, Thermo Fisher Scientific), which included an on-column DNase digestion, as per manufacturer’s instructions. RNA quantity was determined using a Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific) and purity assessed using \(A_{260}/A_{280}\) ratio.

**Quantification of duodenal gene expression by relative RT-PCR**

Real-time RT-PCR was performed using the 7500 fast Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific). Taqman® primers (Life Technologies, Thermo Fisher Scientific) were used to determine the expression of TLR4 (Hs00152939_m1), IAP (alkaline phosphatase-1) (ALP1, Hs00357579_g1), occludin (OCLN, Hs00170162_m1), and ZO-1 (tight junction protein-1) (TJP1, Hs01551861_m1) relative to expression of the housekeeper beta-2 microglobulin \((\beta2M)\) (Hs00984230_m1). All targets were assessed in triplicate, according to manufacturer’s instructions.

**Plasma tumour necrosis factor-α (TNF-α) and incretin hormone concentrations**
Multiplex assays were used to determine plasma TNF-α concentrations (pg/mL) \(t = 0, 10, 20, 30\) min) (Milliplex\textsuperscript{®} MAP Human Cytokine/Chemokine Magnetic Bead Panel, HCYTOMAG-60K), and total GLP-1 and GIP concentrations (both pg/ml) \(t = 0, 15, 30, 60, 90, 120\) min) (Milliplex MAP Human Metabolic Hormone Magnetic Bead Panel, HMHEMAG-34K; Millipore Corporation, Temecula, CA, USA) using the Bio-plex\textsuperscript{®} MAGPIX\textsuperscript{TM} Multiplex Reader (Luminex\textsuperscript{®}, Millipore Corporation) and xPONENT\textsuperscript{®} software (Luminex\textsuperscript{®}, Millipore Corporation, version 4.2) according to manufacturer’s instructions. There was negligible antibody cross-reactivity. Intra-assay coefficients of variation (CVs) were \(\leq 10\%\), and inter-assay CVs were \(\leq 15\%\) for all analytes. The minimum detection limits were for TNF-α: 0.7 pg/mL, GLP-1: 2.5 pg/ml, GIP: 0.6 pg/ml.

Data on plasma incretin hormones have been previously published (7).

Data and statistical analyses

Statistical analysis was performed using SPSS\textsuperscript{®} software (SPSS Inc, IBM\textsuperscript{®}, version 24), and all graphs were generated using GraphPad Prism 7 (GraphPad Software Inc). One-way ANOVA with post-hoc Bonferroni testing was used to compare values for all variables across BMI group (i.e. lean, overweight, obese) and time.. Post-hoc paired comparisons, corrected for multiple comparisons using Bonferroni’s correction, were performed if ANOVAs revealed significant effects. Relationships between variables were determined by correlation, with Pearson’s r values presented. Incretin hormone, anandamide, 2-arachidonylglycerol and oleoylethanolamine data were expressed as incremental area under the curve (iAUC) (calculated using the trapezoidal rule from \(t = 0\) min to 120 min (or \(t = 0 – 30\) min for TNF-α)). This iAUC value was divided by the time of last measurement to obtain a final weighted average (AUC) to account for rare times when samples could not be collected (e.g. bathroom breaks) or data could not be obtained from the assays.
Data are expressed as mean ± standard error of the mean (SEM), with statistical significance accepted at $P < 0.05$. 
RESULTS

Plasma 2-AG, AEA and OEA concentrations

There was no effect of BMI group (P=0.77), or ID lipid infusion (P=0.33), on plasma 2-AG concentrations (Figure 1A). There was a significant effect of group (P=0.003), but not lipid infusion (P=0.912), on plasma AEA concentrations (Figure 1B). Plasma AEA was higher in obese when compared with both lean (P=0.005) and overweight (P=0.017), with no difference between lean and overweight (P=1.0). There was no effect of group (p=0.77), or lipid infusion (P=0.332), on plasma OEA concentrations (Figure 1C).

Duodenal expression of TLR4, IAP, occludin, and ZO-1

There was a significant effect of BMI group on baseline duodenal expression of IAP (P=0.018) and TJP-1 (P=0.002), and a trend for TLR-4 (P=0.08) and occludin (P=0.08) (Figure 2). Baseline IAP expression was significantly lower in obese when compared with lean (P<0.05), with no difference between lean and overweight, or obese and overweight. Baseline ZO-1 expression was significantly lower in obese when compared with lean (P=0.002), and in overweight compared with lean (P=0.029), with no difference between overweight and obese. There was no effect of intraduodenal lipid on duodenal expression of TLR-4, IAP, occluding or ZO-1 (data not shown).
Plasma TNF-α concentrations

There was no effect of BMI group (P=0.7) or lipid infusion (P=0.2) on plasma concentrations of TNF-α (Figure 3).
Plasma incretin hormone concentrations

Data are available in the publication from Cvijanovic et al. \cite{Cvijanovic, 2017 #5670}.

Relationships between variables

There was a significant relationship between baseline anandamide, but not 2-arachidonylglycerol or oleoylethanolamine, concentrations with BMI ($r=0.34$, $P=0.014$).

There were inverse relationships between BMI and baseline expression of ALP-1 ($r=-0.329$, $P=0.014$), occludin ($r=-0.28$, $P=0.038$) and TJP-1 ($r=-0.395$, $P=0.003$).

There were inverse relationships between the iAUC of plasma anandamide concentrations with baseline expression of TLR4 ($r=-0.283$, $P=0.04$), IAP ($r=-0.305$, $P=0.032$), occludin ($r=-0.398$, $P=0.001$) and ZO-1 ($r=-0.445$, $P=0.001$). There were positive relationships between baseline TNF-\alpha concentrations with baseline expression of IAP ($r=0.401$, $P=0.002$) and ZO-1 ($r=0.256$, $P=0.05$).
There was a direct relationship between iAUC anandamide concentrations with iAUC GIP (r=0.384, P=0.005), but not iAUC GLP-1.
We demonstrated that fasting plasma levels of the endocannabinoid, AEA, but not 2-AG or the endocannabinoid-related OEA, were higher in obese, when compared with lean and overweight individuals. Obese individuals also displayed lower fasting duodenal expression of ZO-1 and IAP, and a trend towards lower duodenal occludin and TLR4 expression. These changes were inversely correlated with plasma AEA concentrations, suggesting that altered endocannabinoid signalling may contribute to changes in intestinal permeability and inflammation in human obesity. Plasma anandamide levels were also correlated with plasma GIP, indicating that the endocannabinoid system may play an important role in mediating the GIP secretion in humans.

Our finding of increased plasma AEA in obesity is consistent with numerous reports in both animals and humans that peripheral endocannabinoid levels are increased in obesity (1, 9, 22). Mean fasting plasma 2-AG levels also appeared higher in obese compared with lean and overweight, however, the results were more variable between individuals and not significant.

Rodent studies indicate that changes in peripheral endocannabinoid concentrations are induced by a “Western” style high-fat diet (1), and that elevated endocannabinoid levels in plasma and jejunal mucosa acting via peripheral CB1 receptors promote hyperphagia (1). Mammals have an innate preference for dietary fats, and in rats, sham feeding (where food is tasted but does not enter the small intestine) corn oil, or emulsions containing oleic or linoleic acid, rapidly and markedly increases jejunal levels of both 2-AG and AEA (10, 12). Further to this, blockade of the CB1 receptor abolishes the effects of oral fat on sham feeding and preference for fat (12). Hence, changes in peripheral endocannabinoid signalling in obesity may be important mediators of both fat preference and the gut-to-brain signalling mechanisms involved in the regulation of energy intake. Acute intraduodenal infusion of lipid had no effect on plasma endocannabinoid levels. It
is possible that the duration and/or load of lipid exposure was insufficient, or that oral stimulation of fatty-acid sensing receptors (10), such as CD36 and GPR40 and 120, is required to elicit changes in peripheral endocannabinoid levels, thus, further studies are needed to address this question in humans.

Duodenal expression of IAP and the tight-junction protein, ZO-1, was also reduced in obese compared with lean individuals, and there was a trend for reduced expression of TLR4 and occludin. These molecules have been shown in animal studies to be important markers of high-fat diet/obesity-induced changes in the intestinal microbiome (8), associated with increased systemic levels of LPS, termed “metabolic endotoxemia”, which promotes inflammation (2) and enhances intestinal permeability (3). These data suggest that the observed changes in duodenal expression of IAP and ZO-1 in the obese, when compared with the lean, are markers of an altered intestinal microbiome and enhanced intestinal permeability, which may be driven by chronic overconsumption of a high-fat diet. In humans, acute ingestion of fat is associated with an increase in circulating LPS (15), and intestinal permeability, as assessed by lactulose/mannitol tests, has been shown to be increased in obese versus lean individuals (29), although this is not the case in all studies despite increased local and systemic inflammation in obese individuals (30). This may reflect the relative insensitivity of the intestinal permeability tests using detection of urinary markers in humans. In the present study, there was a direct relationship between the duodenal expression of IAP and ZO-1 with plasma levels of the pro-inflammatory cytokine, TNF-α, suggesting an important role for these molecules in mediating the chronic low-grade inflammation associated with obesity.
We also found an inverse relationship between plasma anandamide levels and the duodenal expression of TLR4, IAP, occludin and ZO-1. Rodent studies have provided evidence that the obesity-associated changes in the gut microbiota leads to activation of the endocannabinoid system (via LPS), which in turn drives the alterations in intestinal permeability. Administration of the CB₁ receptor agonist, HU-210, to wild-type mice increased intestinal permeability, and in Caco-2 cells in vitro decreased expression of the ZO-1 and occludin (25). Conversely, in obese mice, pharmacological blockade of the CB₁ receptor restored the distribution and localisation of the tight-junction proteins ZO-1 and occludin, and decreased plasma LPS levels (4, 25). Hence, in human obesity, altered plasma AEA be an important mediator of the effects of obesity on intestinal permeability and inflammation. In rodents, alterations in the composition of the gut microbiota with consequent development of low-grade inflammation in the gastrointestinal tract, have been shown to be responsible for the development of hyperphagia and obesity (8). Further studies in humans are required to understand the relationships between these changes in endocannabinoid system activity, intestinal permeability and food intake.

Plasma AEA concentrations were also directly correlated with plasma GIP, but not GLP-1, concentrations. Recent studies have suggested a role of the endocannabinoid system in mediating the secretion of the incretin hormones (5). As discussed, CB₁ receptors are expressed on enteroendocrine K- and L-cells in rodents (16, 24), and AEA enhances the GIP response to oral glucose in rodents (24). In contrast, our data, and a recent study using acute administration of the CB₁ receptor agonist, nabilone (5), suggest that AEA enhances GIP secretion in humans. The reasons for these discrepancies are unclear, however, the endocannabinoid system seems to play an important role in mediating the GIP response to fat in humans.
Plasma OEA concentrations did not differ between lean, overweight and obese humans during fasting, and did not change in response to duodenal fat infusion. In rodents, the presence of fat in the duodenum stimulates the small intestinal conversion of oleic acid, found commonly in the diet, to OEA (27). When administered exogenously to rodents, OEA reduces overall energy intake by prolonging the time interval between meals (27). While our lipid emulsion, Intralipid, is made up of ~19-30% oleic acid, this may not have been sufficient to stimulate OEA mobilisation in the current study. Previous studies in humans have demonstrated a significant increase in plasma OEA concentrations in proportion to the oleic content of the ingested oil (30 g), which was associated with reduced subsequent energy intake (23).

In conclusion, we have demonstrated increased plasma levels of AEA, but not 2-AG or OEA, in obese, when compared with lean and overweight individuals. Obese individuals also displayed decreased fasting duodenal expression of ZO-1 and IAP. Plasma AEA concentrations were inversely correlated to duodenal expression of ZO-1 and IAP, and positively correlated to plasma GIP, suggesting that altered endocannabinoid signalling may contribute to changes in intestinal permeability, inflammation and GIP secretion in human obesity.
ACKNOWLEDGEMENTS

We thank the participants who volunteered their time for this study, and the nursing staff of the Department of Gastroenterology and Hepatology at the Royal Adelaide Hospital for assisting with endoscopy studies. We would like to acknowledge biostatistician, Kylie Lange, for statistical support, and Nicole Isaacs and Penelope Fitzgerald for their assistance with participant recruitment and infusion studies, and Jaspreet Kaur for technical assistance with lipid analysis.

None of the authors have any conflicts of interest, financial or otherwise.
REFERENCES


8. de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, and Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut


FIGURE LEGENDS

**Figure 1:** Plasma 2-arachidonylglycerol (2-AG) (A), anandamide (AEA) (B), and oleoylethanolamine (OEA) (C) concentrations during fasting (0 min) and intraduodenal infusion of Intralipid (2 kcal/min for 120 min) in lean (n=20), overweight (n=18) and obese (n=19) humans. *P<0.05 vs. lean and overweight.

**Figure 2:** Baseline (fasting) duodenal expression of toll-like receptor-4 (TLR4) (A), intestinal alkaline phosphatase (IAP) (B), occludin (OCLN) (C), and zona-occludin 1 (ZO-1) (D) in lean (n=20), overweight (n=18) and obese (n=19) humans. *P<0.05 obese vs. lean, #P<0.05 overweight vs. lean.

**Figure 3:** Plasma tumour-necrosis factor-α (TNF-α) concentrations during fasting (0 min) and intraduodenal infusion of Intralipid (2 kcal/min for 120 min) in lean (n=19), overweight (n=16) and obese (n=17).