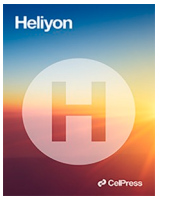
Heliyon 10 (2024) e26968

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

# Investigating the alterations of endocannabinoidome signaling in the human small intestine in the context of obesity and type 2 diabetes

Volatiana Rakotoarivelo [a,b,#], Bénédicte Allam-Ndoul [a,c,#], Cyril Martin [a,b], Laurent Biertho [a], Vincenzo Di Marzo [a,b,c,d,1], Nicolas Flamand [a,b,1], Alain Veilleux [b,c,1,*]

[a] *Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, Département de médecine, Université Laval, Québec City, QC, Canada*
[b] *Canada Excellence Research Chair on the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND), Université Laval, Québec, QC, Canada*
[c] *Centre Nutrition, Santé et Société (NUTRISS), INAF, Québec, QC, Canada*
[d] *Joint International Unit between the CNR of Italy and Université Laval on Chemical and Biomolecular Research on the Microbiome and its Impact on Metabolic Health and Nutrition (UMI-MicroMeNu), Canada*

ARTICLE INFO

*Keywords:*
Obesity
Insulin resistance
Diabetes
Endocannabinoids
Monoacylglycerols (MAGs)
*N*-Acyl-ethanolamines (NAEs)

ABSTRACT

*Background:* Human studies have linked obesity-related diseases, such as type-2 diabetes (T2D), to the modulation of endocannabinoid signaling. Cannabinoid $CB_1$ and $CB_2$ receptor activation by the endocannabinoids (eCBs) 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamine (AEA), both derived from arachidonic acid, play a role in homeostatic regulation. Other long chain fatty acid-derived endocannabinoid-like molecules have extended the metabolic role of this signaling system through other receptors. In this study, we aimed to assess in depth the interactions between the circulating and intestinal tone of this extended eCB system, or endocannabinoidome (eCBome), and their involvement in the pathogenesis of diabetes.
*Methods:* Plasma and ileum samples were collected from subjects with obesity and harboring diverse degrees of insulin resistance or T2D, who underwent bariatric surgery. The levels of eCBome mediators and their congeners were then assessed by liquid chromatography coupled to tandem mass spectrometry, while gene expression was screened with qPCR arrays.
*Findings:* Intestinal and circulating levels of eCBome mediators were higher in subjects with T2D. We found an inverse correlation between the intestinal and circulating levels of monoacylglycerols (MAGs). Additionally, we identified genes known to be implicated in both lipid metabolism and intestinal function that are altered by the context of obesity and glucose homeostasis.
*Interpretation:* Although the impact of glucose metabolism on the eCBome remains poorly understood in subjects with advanced obesity state, our results suggest a strong causative link between altered glucose homeostasis and eCBome signaling in the intestine and the circulation.

* Corresponding author. Centre de Nutrition, Santé et Société (NUTRISS), INAF, École de nutrition, Université Laval, Québec City, QC, Canada.
*E-mail address:* Alain.Veilleux@fsaa.ulaval.ca (A. Veilleux).
[#] Share authorship.
[1] Share senior authorship.

https://doi.org/10.1016/j.heliyon.2024.e26968
Received 30 August 2023; Received in revised form 19 February 2024; Accepted 22 February 2024
Available online 2 March 2024

## Research context

### *Evidence before this study*

The aim of targeting the endocannabinoid (eCB) system to manage the dysfunctional control of energy balance is clinically relevant. Indeed, it was demonstrated in several studies that circulating or tissue levels of 2-AG, AEA, or both, are associated with unbalanced energy acquisition, storage, and expenditure. Furthermore, a strong association between circulating eCB levels and anthropometric parameters such as BMI or visceral obesity were also pinpointed. However, the mechanistic link between altered levels of eCBs and metabolic diseases remains under discussion, and the participation of eCB-related mediators poorly investigated.

### Added value of this study

By analyzing unique and valuable biological samples (ileum and plasma) from people with obesity and eligible for bariatric surgery (BMI >35 kg/m$^2$ with comorbidities, or BMI up to 40 kg/m$^2$), we undertook this comprehensive study to evaluate the circulating and intestinal levels of eCBs and eCB-related mediators that belong to the endocannabinoidome (eCBome). Beyond the correlation analysis between mediators and metabolic parameters, we provide a comprehensive study that also evaluated the expression of genes that play a role in the biosynthesis and inactivation of these mediators. We show that in subjects who exhibit impaired glucose metabolism (type 2 diabetes), the levels of eCBs and congeners are stably higher, as we can observe at intestinal and circulating levels. In addition, differences in the expression of genes associated with eCB metabolism in the gut provide an explanation for this observation. Last but not least, this study offers a global view (through the analysis of plasma), as well as a more specific observation (through analysis of lipid mediators and gene expression) of the tone of the eCBome in obesity and type 2 diabetes.

### *Implications of all the available evidence*

This study in a human cohort suggests that the eCBome, together with other signaling systems, is involved in the metabolic phenotypes associated with obesity. The realization that several biological systems are involved in this condition is of paramount importance to move towards the personalization of obesity therapies and the use of all the available tools and analytical methods to prevent the onset of the metabolic syndrome.

## 1. Introduction

Beyond an excessive storage of energy, obesity is a state in which the abnormal accumulation of fat leads to strong and often irreversible deterioration of health [1]. According to the World Health Organization, there are almost three million deaths associated with obesity every year, underscoring the urgency of finding solutions to treat and predict the emergence of associated comorbidities [2,3]. Constant innovation in pharmaceutical research has recently led to the discovery of new drugs in order to reduce obesity comorbidities such as type 2 diabetes (T2D) [4]. Examples include sodium/glucose cotransporter 2 inhibitors [5], analogs of the incretin glucagon-like peptide-1 [6] and dipeptidyl peptidase-4 inhibitors [7]. Drugs targeting the endocannabinoid (eCB) system were also utilized, notably the inverse $CB_1$ receptor agonist rimonabant [8,9]. Rimonabant was very efficacious at regulating satiety, promoting weight loss and decreasing cardiometabolic risks [10]. However, some central nervous system adverse events, notably depression, led to its withdrawal [11].

The eCB system includes two mediators, 2-arachidonoyl-glycerol (2-AG) and *N*-arachidonoyl-ethanolamine (AEA), which are derived from the long-chain omega-6 polyunsaturated fatty acid arachidonic acid. These eCBs are, by definition, endogenous agonists of the two cannabinoid receptors $CB_1$ and $CB_2$. In parallel, there are other monoacylglycerols (MAGs) and *N*-acyl-ethanolamines (NAEs) that are biosynthesized and metabolized by the same enzymes as eCBs [12]. In fact, an increasing body of evidence is highlighting the role of these eCB-like compounds, such as those derived from oleic acid (OA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in metabolic diseases [13]. For example, the levels of the omega-3 polyunsaturated fatty acid-derived MAGs, eicosapentanoylglycerol (EPG), and docosahexaenoylglycerol (DHG), are associated with a higher intuitive eating score, and hence with healthier feeding behaviors [14]. MAGs and NAEs modulate other receptors, such as transient receptor potential (TRP) channels, peroxisome proliferator-activated receptors (PPAR)-α and γ, as well as some orphan G protein-coupled receptors (GPCRs) [15]. 2-AG, AEA, their congeners from the MAG and NAE families that include a fatty acid containing 14 carbons or more, as well as the receptors they activate, constitute the core of an extended eCB system, known as the endocannabinoidome (eCBome) [15].

Tissue and circulating levels of eCBome mediators are associated with adiposity and metabolic conditions such as insulin resistance [16]. Moreover, the expression of the eCBome receptors and of the enzymes involved in the biosynthesis and the degradation of MAGs and NAEs is also modulated in several tissues and according to diverse metabolic states [15,17,18]. The small intestine expresses several of these eCBome receptors [19], thus highlighting its sensitivity to variations in the local and circulating eCBome mediator level. The intestine plays an important role in the development of obesity and T2D as the loss of gut barrier function induced by obesity-associated inflammation leads to metabolic endotoxemia [20,21]. It is known that the $CB_1$ receptor can have both a protective role against intestinal inflammation in lean individuals [22,23] and contribute to impairing the intestinal epithelial barrier and contribute to metabolic endotoxemia during obesity [24]. In contrast, the expression of the $CB_2$ receptor remains weakly detected in the human small intestine [25] and, even though this receptor is known to be implicated in regulating inflammation, its role in obesity

remains uncertain [25]. While we have some insight of the impact of obesity and its metabolic complications on other eCBome receptors and enzymes, the precise regulation of the eCBome in the small intestine remains poorly defined in humans.

By taking advantage of intestinal samples obtained during bariatric surgery of individuals with high body mass index (BMI) and varying degrees of glucose homeostasis, we quantified eCBome mediators as well as gene expression of eCBome receptors and metabolic enzymes in the ileum. The aim of the study was to investigate whether there is a correlation between the tone of the eCBome, in the ileum and circulation, with the glucose homeostasis status of individuals with obesity.

## 2. Methods

### 2.1. Subjects and sampling

Tissue specimens were obtained from the Biobank of the *Institut universitaire de cardiologie et de pneumologie de Québec* (Université Laval, Québec City, QC, Canada) in compliance with institutional Review Board-approved management modalities. All participants provided written informed consent. Forty ones severely obese subjects (BMI>40 kg/m$^2$), with or without T2D according to their medical historic were recruited. Most subjects with a diagnosis of T2D were either following specific diet or treated with metformin (40%) to control their glycemia. Additionally, some subjects were treated with insulin (25%) or with other antidiabetic agents. All subjects were candidates for bariatric surgery and undergoing biliopancreatic diversion. Ileum specimens were obtained by anastomosis during the biliopancreatic diversion. The procedure did not enable us to obtain samples from duodenum and jejunum. The ileum samples were immediately washed with cold PBS and stored within 20 min, and the mucosa was removed and frozen in liquid nitrogen and stored at −80C for subsequent lipids and RNA extraction.

### 2.2. Anthropometry and metabolic parameters

BMI, waist circumference and overnight fasting blood samples were drawn on the morning before surgery to assess lipid profile and glucose homeostasis. Glucose, insulin, and triglycerides (TG) were analyzed by the hospital. The homeostatic model assessment for insulin resistance index (HOMA-IR) was calculated using the following formula: fasting insulin (mU/ml) × fasting glucose (mmol/l)/ 22·5 [26].

### 2.3. Analysis of eCBome-related mediators

For the analysis of intestinal eCBome-related mediators, lipids were extracted from tissue samples according to the Bligh and Dyer method [27]. Briefly, about 10 mg of each tissue was homogenized in 0·5 ml of Tris-HCl 50 mM (pH 7·4) containing 0·1 M acetic acid and 5 ng of deuterated standards then mixed with 0·5 ml methanol using a tissue homogenizer. One ml of chloroform was then added to each sample, vortexed for 30 s, and centrifuged at 3000 *g* for 5 min. This was repeated twice for a total addition of 3 ml of chloroform. The organic phases were collected and evaporated under a stream of nitrogen.

For the quantification of eCBome-related mediators in plasma, samples were extracted as described before with slight modification [28]. Briefly, 200 μL of plasma samples were mixed with 300 μL of Tris-HCl 50 mM (pH 7·4). Two ml of Toluene containing 5 ng of deuterated standards was then added to the samples, vortexed for 1 min, centrifuged at 4000*g* for 5 min without brakes. Samples then were placed in an ethanol-dry-ice bath (−80 °C) to freeze the aqueous phase (bottom). The organic phase (top) was then collected and evaporated to dryness under a stream of nitrogen.

For both intestinal and plasma, samples were suspended in 50 μl of mobile phase containing 50% of solvent A (water + 1 mM ammonium acetate + 0·05% acetic acid) and 50% of solvent B [acetonitrile/water (95/5) + 1 mM ammonium acetate + 0·05% acetic acid]. Forty μl of each sample was finally injected onto an HPLC column (Kinetex C8, 150 × 2·1 mm, 2·6 μm; Phenomenex) and eluted at a flow rate of 400 μl/min using a discontinuous gradient of solvent A and solvent B as in Ref. [27]. Quantification of eCBome-related mediators were carried out by HPLC system interfaced with the electrospray source of a Shimadzu 8050 triple quadrupole mass spectrometer and using multiple reaction monitoring in positive ion mode for the compounds and their deuterated homologs.

MAGs are represented as 2-MAGs but represent the combined signals from 2- and 1(3)-isomers as the latter are mostly generated from the former via acyl migration from the *sn*-2 to the *sn*-1(3) position.

### 2.4. Gene expression

Total RNA from intestinal tissues was extracted using the RNeasy lipid tissue extraction kit and on-column DNase treatment, according to the manufacturer's recommendation (Qiagen). Total RNA was used for reversed transcription using High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) and cDNA levels were measured by quantitative PCR as described before [1] or by qPCR-based TaqMan assay open array, ThermoFisher).

### 2.5. Statistical analyses

Statistical analyses were performed using GraphPad Prism 9·5 (GraphPad Software, Inc.; San Diego, CA, USA). For RNA analysis, a total of 220 genes were analyses on the TaqMan open array, ThermoFisher). Messenger RNA expression levels (ΔΔCT) were compared between groups. Data were analyzed using ANOVA or Kruskal-Wallis tests ($p < 0·05$) followed by Dunn's post test for multiple

comparison. Correlation tests were performed by nonparametric Spearman's rank correlation test ($p < 0{\cdot}05$) using *corr.test* function from *pshych* CRAN package and the correlation matrices were generated using the *corrplot* package.

## 3. Results

Plasma and intestinal samples were obtained from Caucasian men (n = 8) and women (n = 32) undergoing bariatric surgery (table 1). Subjects were 44 years old on average and had a mean BMI of 48·3 kg/m$^2$. The subjects with type 2 diabetes (T2D, n = 12) were diagnosed according to the Diabetes Canada Clinical Practice Guidelines Expert Committee [29]. The subjects without T2D (non-T2D) were categorized into two groups according to the HOMA-IR index: 1) insulin-sensitive subjects (IS, n = 16) with an HOMA-IR lower than 5 and 2) insulin-resistant subjects (IR, n = 12) with an HOMA-IR greater than 5. Among the three groups, there was no difference in age, BMI and waist circumference (WC). As expected, subjects with T2D had significantly higher levels of fasting glucose (5·3 ± 0·4 vs. 5·6 ± 0·6 vs. 7·7 ± 1·5 mmol/L), while insulin resistant subjects had higher levels of insulin (102·6 ± 28·3 vs. 237·3 ± 90·9 vs. 267·2 ± 243·0 pmol/L). Plasma triglycerides (TG) levels (1·5 ± 0·6 vs. 1·4 ± 0·6 vs. 1·9 ± 0·8 mmol/L) were comparable between groups (Table 1 and Supplemental fig 1).

### 3.1. Circulating and intestinal MAGs and NAEs levels according to glucose homeostasis status

Circulating levels of MAGs and NAEs in the IR and T2D groups were compared to those of the IS group. We computed an overall mean using the standard score method (Z-score) of MAGs and NAEs, integrating the contribution of each congener identified by our method, in both the circulation and the intestine (Fig. 1). The circulating MAGs Z-score was significantly higher in the T2D group *vs.* the non-T2D groups (Fig. 1 a). When assessed individually, the circulating levels of all MAG congeners are increased in T2D, and these differences are significant for 2-AG, and 2-DPG (Fig. 1 a). In contrast, the NAE Z-score was slightly decreased in the IR and T2D groups while individual NAE congeners were not modulated (Fig. 1 b). In the ileum, both the overall Z-score of MAGs and individual 2-AG congeners are comparable between groups (Fig. 1 c). As for the NAEs, the ileal levels of NAEs levels were significantly increased in the T2D group (Fig. 1 d).

### 3.2. Circulating and intestinal eCBome association with metabolic states

Circulating and intestinal levels of MAG and NAE congeners were not correlated with BMI, at least within the range of severe obesity of bariatric surgery candidates included in this cohort (Fig. 2). Nevertheless, waist circumference tends to be negatively

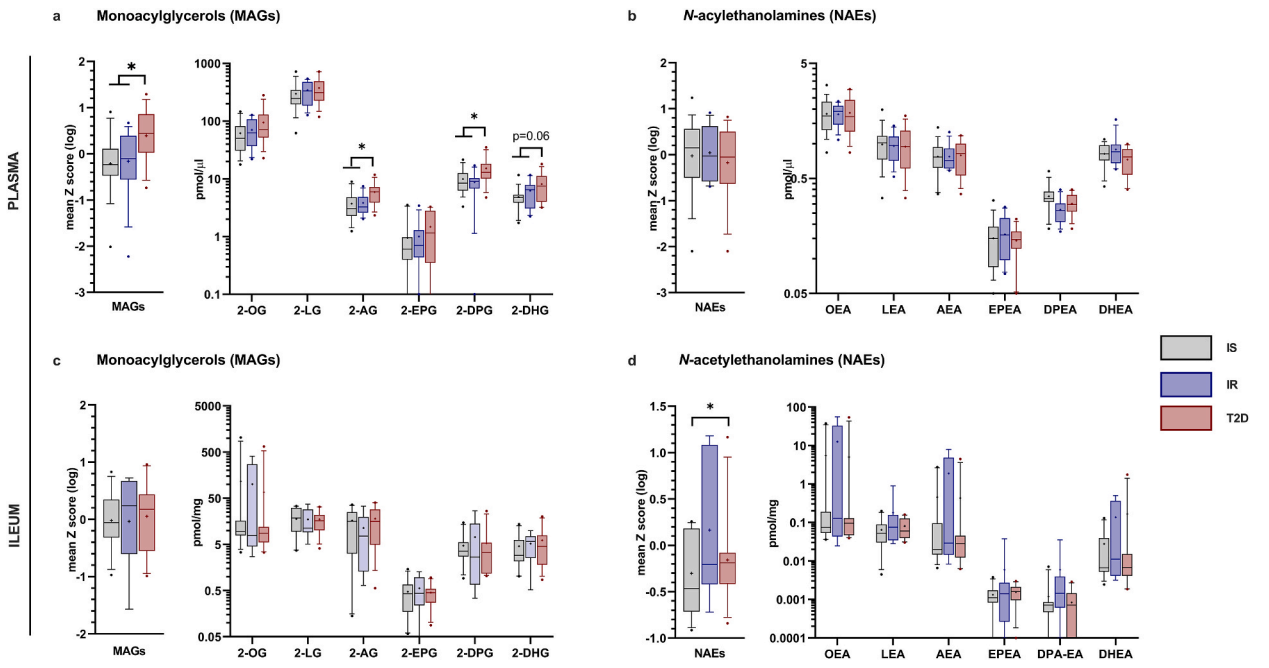



**Fig. 1. Circulating and intestinal MAGs and NAEs according to glucose homeostasis status.** Circulating (a and b) and intestinal (c and d) levels of MAGs and NAEs in the insulin-sensitive (IS, N = 16), insulin-resistant (IR, N = 12) and type-2-diabetes (T2D, N = 12) groups. For each mediator, the Z-score was calculated by subtracting the mean of each MAG or NAE, then dividing the value by the standard deviation ((x-μ)/σ). The mean Z-score values of all MAGs or NAEs, as well as each MAG and NAE congeners were represented as boxplot with 10–90 percentiles. 2-monoacylglycerols data represent the combined signals from 2- and 1(3)-isomers. P values were obtained from Mann-Whitney tests (*P < 0·05) comparing non-T2D group to the T2D group; or from Kruskal-Wallis followed by Dunn's multiple comparisons test comparing the IR and the T2D groups to the IS group.

correlated with the circulating levels of MAGs and was positively correlated with circulating levels of NAEs (Fig. 2 a). Circulating levels of MAGs tended to be positively correlated with fasting glycemia, while circulating levels of total NAEs were negatively correlated this parameter. These associations were not observed for fasting insulin levels and the HOMA-IR (Fig. 2 a). Interestingly, we found that fasting TG levels were positively correlated with the circulating levels of most MAGs congeners and negatively correlated with the circulating levels of most NAEs (Fig. 2 a). These observations suggest that circulating eCBome mediators were more closely related to metabolic parameters than anthropometric measures. In the small intestine, there was no significant association between metabolic parameters and the investigated eCBome-related lipids (Fig. 2 b).

### 3.3. Association between circulating and intestinal levels of eCBome mediators

Next, we investigated whether the associations between MAGs and NAEs, as well as whether the circulating levels of eCBome mediators, were linked to those found in the ileum. First, we noted that the circulating levels of most MAG congeners correlated together. A similar pattern was also observed for the circulating NAE congeners (Fig. 3). Interestingly, the levels of most MAG congeners negatively correlated with those of NAEs in the circulation. In the small intestine, while we observed the same trends as in the circulation, we found a limited number of significant correlations between the levels of MAG and NAE congeners. In addition, we found that the circulating levels of MAGs and NAEs poorly correlated with those found in the ileum. Altogether, circulating and ileum MAG levels do not really correlate with each other and when they do, the correlation was negative. A trend towards a positive correlation pattern between the levels of circulating and ileum NAEs was noted but did not reach statistical significance (Fig. 3).

We then performed an analysis on circulating and ileum levels of eCBome mediators, according to the median of the circulating MAGs Z-score median (i.e., Low and High). The stratification of the cohorts based on the MAGs Z-score was confirmed by higher levels of MAGs in circulation in the High group compared to the Low group (Fig. 4 a). The Low group was mostly represented in the IS group (50%) than the IR (30%) and the T2D (20%), while the High group was mostly represented in the T2D (50%) than the IS (25%) and the IR (25%). Anthropometric and metabolic measures remained comparable between the Low and the High groups (Data not shown). This analysis highlighted the existence of an inverse correlation between circulating MAGs and NAEs. Indeed, plasma AEA and EPEA levels were significantly higher in the Low group than the High group (Fig. 4 b). Interestingly, we observed that ileum levels of MAGs, i.e., 2-OG, 2-LG, 2-DPG, and 2-DHG, were significantly lower in the High group than the Low group (Fig. 4 c). In contrast, intestinal levels of NAEs were not different between the Low and the High groups (Fig. 4 d). These observations suggest that high levels of MAGs in

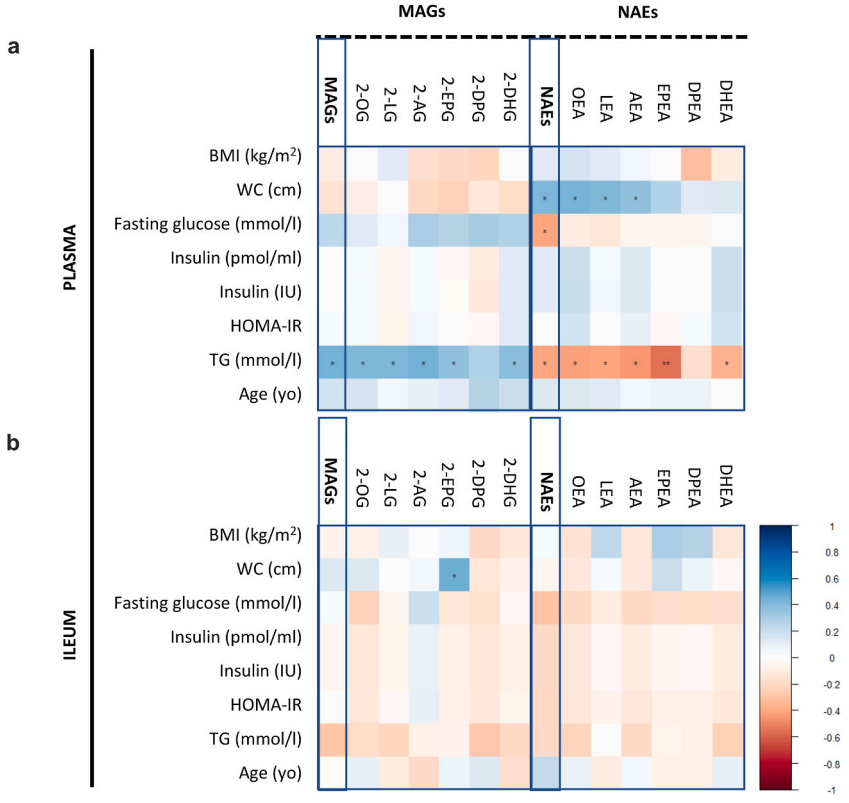



**Fig. 2. Circulating and intestinal eCBome association with metabolic status.** The correlation matrix showed the rho values of the Spearman's rank correlation between circulating (a) and intestinal (b) eCBome mediators and anthropometric/metabolic measured of the subjects. MAGs and NAEs are the mean Z-score values of each congener of MAG or NAE respectively (N = 40, *P < 0·05).

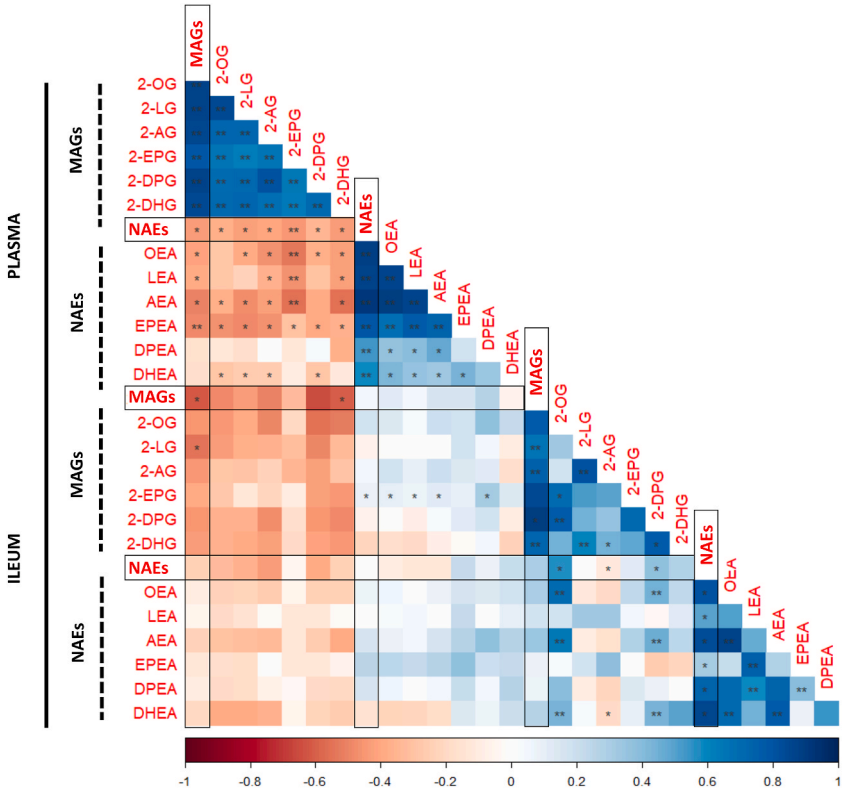



**Fig. 3. Association between circulating and intestinal levels of eCBome mediators.** The correlation matrix showed the rho values of the Spearman's rank correlation between circulating and intestinal eCBome mediators. MAGs and NAEs represent the mean Z-score values of MAGs or NAEs respectively (N = 40, *P < 0·05).

circulation are associated with lower levels of these mediators in the intestine, which confirm the aforementioned negative correlation between the circulating and intestinal mediator levels. Conversely, regardless of their levels in the circulation, intestinal NAEs remain unaffected.

### 3.4. Small intestine gene expression of eCBome metabolic enzymes

Several metabolic enzymes involved in MAGs and NAEs anabolism and catabolism are differently expressed in the small intestine, according to the glucose homeostasis status. In the Supplementary Table 1, we include a list of the genes included in the study. Most of these genes were expressed in the small intestine and suggest this issue has an important metabolic and signaling capacity for diverse eCBome mediators. Compared to the non-T2D group, expression levels of the MAG anabolic gene *DAGLA* and of the MAG catabolic genes *MGLL, CES1* and *DGKE* were increased in the small intestine of the IR group and reduced in the small intestine of the T2D (Fig. 5 a). A similar trend was observed in the small intestine in the High group, which harbor relatively higher circulating MAG levels but relatively lower intestinal MAG levels (Fig. 5 a). Regarding NAE we found that catabolic genes, i.e. *AKR1B1*, *PTGES2* and *FAAH2*, were decreased in presence of T2D. Similarly, reduced expression of several NAE metabolic enzymes was also noted in the High groups, which have relatively lower NAEs levels in the small intestine (Fig. 5 b). In sum, changes in gene expression were mainly associated with the presence of T2D, suggesting that glycemia imbalance reshapes the metabolism of eCBome mediators in the small intestine. These modulations may explain in part the variation observed in intestinal levels of MAGs and NAEs in these subjects.

### 3.5. Small intestine gene expression of eCBome receptors

Beyond the two canonical receptors $CB_1$ and $CB_2$ receptors, we assessed the mRNA expression of 15 other receptors related to the eCBome signaling, including PPARs, TRPs and GPCRs (Supplementary Table 1). Expression of *CNR1* was not impacted by the glucose homeostasis status while the expression of *CNR2* remained undetected using our method (Fig. 5 c). In parallel, *GPR55* expression was relatively higher in the small intestine of subjects with T2D and within the High group. In contrast, the expression of 2 genes encoding for the non-canonical eCB receptors, i.e. TRPA1 and PPARG, was lower in subjects with a diagnosis of T2D (Fig. 5 c). As for the metabolic genes, eCBome receptors were mostly modulated in the T2D group compared to the non-T2D group.

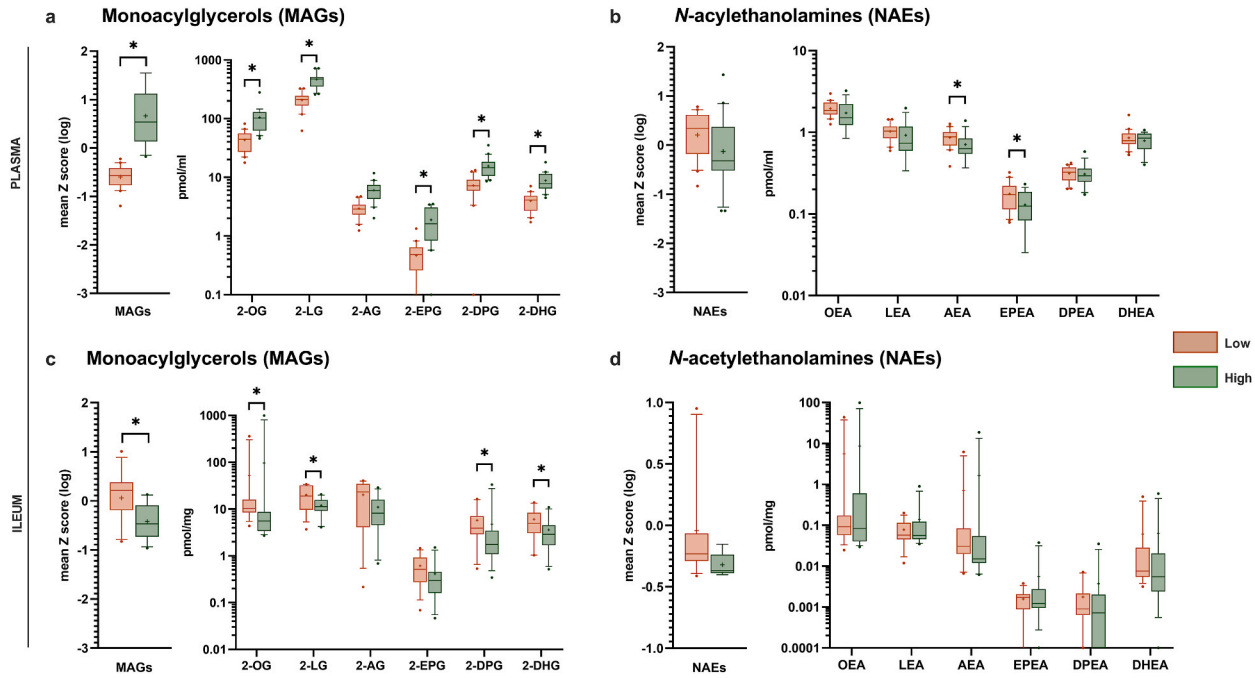



**Fig. 4. Relationship between MAGs and NAEs in plasma and ileum.** The study sample was divided in 2 groups according to the median of the circulating MAGs Z-score (Low: N = 20 and High: N = 20 AAA). 2-monoacylglycerols data represent the combined signals from 2- and 1(3)-isomers. Circulating (a and b) and intestinal (c and d) levels of MAGs and NAEs are shown for each subgroup as mean ± SEM and compared using a Mann-Whitney test (*P < 0·05).

## 4. Discussion

Despite the extensive characterization of the mechanisms by which the eCB system regulates central and peripheral energy metabolism, this effort so far only led to the development of $CB_1$ antagonists against obesity and metabolic complications that had to be withdrawn from the market, or further experimentation, due to the occurrence of CNS unwanted effects [10,30,31]. This indicates that targeting this system needs to take into consideration that it may involve other eCB-like mediators, enzyme, and receptors, namely the eCBome. There is a knowledge gap on the comprehensive mechanisms and the physiological roles of these mediators in the context of obesity and metabolic complications. This lack of knowledge limits our understanding of how this system is involved in the pathophysiology of obesity and in the numerous functional adaptations observed in the small intestine in this disease. We hypothesized that there is a correlation between the tone of the eCBome in the circulation and in the small intestine, and that the glucose homeostasis status influences MAG and NAE eCBome mediators in individuals with obesity. By taking advantage of intestinal samples obtained during bariatric surgery, we characterized eCBome mediators as well as gene expression of eCBome receptors and metabolic enzymes in the ileum. We show that circulating and intestinal levels of MAGs mediators were higher and lower, respectively, in subjects with T2D. Indeed, we found an inverse correlation between circulating and intestinal levels of MAGs, with lower intestinal levels of these metabolites being associated with higher circulating levels. Additionally, we identified genes, known to be implicated in eCBome signaling, with altered expression levels in presence of insulin resistance and/or T2D. Our results highlight the existence of a divergent pathophysiological eCBome response in circulation and the intestine in the context of obesity, insulin resistance and T2D.

Overall, the eCBome is known to be associated with obesity and metabolic complications [16]. Regarding the NAEs, even though these metabolites are known to be associated with obesity, they seem to remain stable once obesity is established, but can be influenced by the glycemic status [32,33]. Several studies conducted by Sipe et al. suggested NAEs as a biomarker that may indicate a risk for severe obesity [34–36]. Here we support this observation by demonstrating a positive correlation between waist circumference and the levels of NAEs, namely, OEA, LEA, and AEA. Cohort studies highlight that circulating levels of eCBs are exacerbated by diabetes status, either in presence [17,37] or not of obesity [38]. In parallel, higher circulating levels of 2-AG were reported in postmenopausal women with obesity and insulin resistance than in those with obesity only [39,40]. Indeed, we observed a positive correlation between circulating MAG levels and fasting glycemia as well as higher 2-AG, 2-DPG, and 2-DHG levels in presence of T2D. Interestingly, levels of AEA, OEA, and PEA are reduced during an hyperinsulinemic-euglycemic clamp in women [39,40]. This agrees with the absence of differences in NAE levels between IS and IR groups in this study composed mostly of women), and suggests that these mediators remain responsive to acute insulin stimulation in the presence of peripheral IR. However, in obese men, insulin infusion decreased AEA levels to an extent that weakly, but significantly, correlated negatively with TGs, liver fat and fasting insulin, and positively with high density lipoprotein cholesterol [41], suggesting the existence of sex-specific effects of IR on circulating NAE levels.

Annuzzi et al. also reported that the presence of T2D along with obesity led to increased levels of certain mediators, namely AEA,

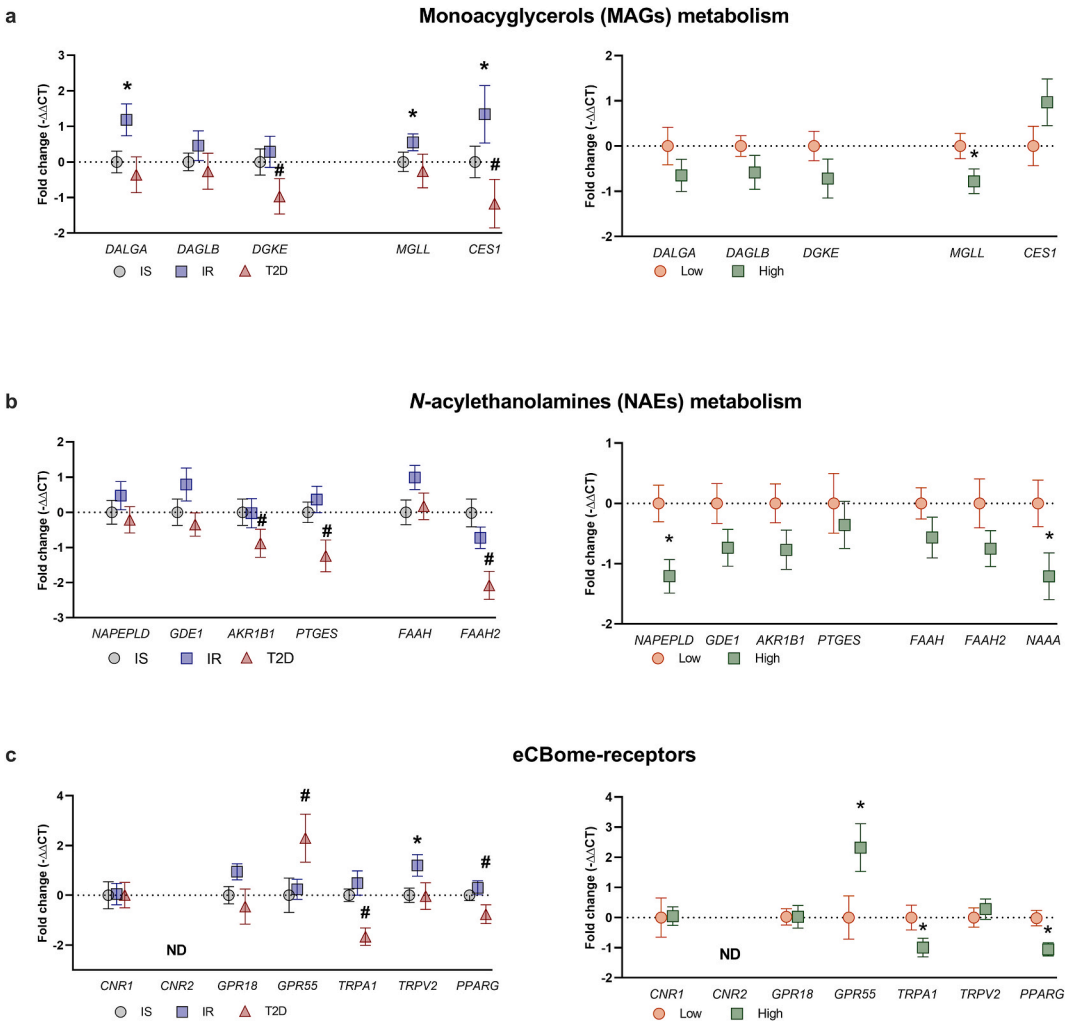



**Fig. 5.** Expression analysis of eCBome genes according to glucose homeostasis. Expression of MAGs and NAEs catabolic and anabolic enzymes and receptors in the ileum was compared by using fold induction analysis (ΔΔCT) method. The expression of selected genes of monoacylglycerols metabolism (a), *N*-acylethanolamines metabolism (b) and endocannabinoidome receptors (c) are represented. Gene expression is compared between glucose homeostasis groups (Left, IS: N = 13, IR: N = 10 and T2D: N = 11) and between Z-score groups (right, Low: N = 13 and High: N = 16) using Mann-Whitney tests and Kruskal-Wallis followed by Dunn's multiple comparisons test (*P < 0·05 vs. IS and #P < 0·05 vs. non-T2D).

OEA, and PEA, in the subcutaneous adipose tissue [42]. In contrast, the levels of 2-AG were decreased in adipose tissue in presence of T2D. In the small intestine, we found that levels of MAGs, including 2-AG, were higher in subjects with obesity and T2D than in subjects with obesity only. These alterations in MAGs and NAEs levels are partly the result of change in expression and activity of catabolic and anabolic enzymes of eCBome metabolism. It was shown that the activity of DAGL and MAGL, respectively MAG anabolic and catabolic enzymes, were increased in visceral and subcutaneous adipose tissue of subjects with obesity [43]. Similarly, FAAH catabolic activity on NAEs in human subcutaneous adipocytes correlates with BMI and waist circumference [44,45]. In the present study, we highlighted the decreased expression of the MAG catabolic genes *MAGL* and *CES1* in the ileum in presence of T2D. This finding associated with the detection of increased circulating, but not intestinal, MAGs. This may suggest that, in presence of T2D, the small intestine may contribute to circulating MAG levels, due to reduced catabolism of these mediators. However, lower intestinal levels of MAG and lower expression levels of their anabolic genes were observed in subjects harboring higher circulating levels of MAGs. This latter finding does not fully support the hypothesis of the small intestine being a reservoir for circulating MAGs, since in this case the biosynthetic enzymes should have been up regulated.

The expression of genes coding for anabolic, i.e. GDE1, and catabolic, i.e. PTGES and AKR1B1, enzymes for NAE was significantly reduced in the T2D group. GDE1 is known to be involved in the biosynthesis of NAEs [46], while PTGES and AKR1B1 catalyze AEA conversion to prostaglandin ethanolamine endoperoxide (i.e., prostamide $H_2$) and to prostaglandin ethanolamides (i.e., prostamides, such as prostamide $E_2$ and F), involved in inflammatory conditions [47,48]. Additionally, *AKR1B1,* encodes aldehyde-ketoreductase, which is also highly implicated in diabetes [48,49]. However, despite these alterations in the expression of these enzymes, NAEs in the

small intestine were not significantly different among the three groups.

Indeed, the outcome of alterations in eCB and eCBome signaling is strongly determined by the tissue levels of the ligands, but also of their receptors. Regarding these latter, the expression of $CB_2$, which plays mostly an anti-inflammatory role, in the human intestine is still under debate. It is not yet clear if its expression is to be attributed to intestinal or immune cells, such as macrophages, which reside in or infiltrate the gastrointestinal tract [25,50]. In this study, several eCBome receptors are expressed to a lesser extent in the T2D compared with the non-T2D group. TRPA1 can be activated and desensitized by AEA [51], and produces both pro- and (when desensitized) anti-inflammatory effects [52]. The expression of this gene was significantly more important in the non-T2D group compared to T2D. PPARγ can be activated by a large number of lipid mediators [53–56], including AEA and 2-AG [57], and its function of regulating lipid metabolism [54], and immune response by inducing an anti-inflammatory response [58] is well described. Moreover, GPR18 is a key player in intestinal inflammation, as this receptor controls the accumulation of CD8 + T cells in the intraepithelial compartment [59]. Thus, all these three receptors share a common protective function in inflammation, which can explain why their expression was lower in the T2D group.

Although eCBs have often been reported to be associated with obesity, here we suggest that these and related mediators of the eCBome play an additional significant role in the establishment of diabetes in obesity, possibly also through their reported regulation of several intestinal functions, such as epithelial barrier integrity and inflammation [60].

Our study focuses on subjects with severe obesity and this design did not allow us to observe a relationship between the eCBome and the development of obesity. Nevertheless, this relationship, i.e., between obesity and the eCB system, has already been investigated in several previous human studies [60]. Of note, the subjects without T2D were not prescribed anti-diabetic treatments as opposed to the subjects with T2D diagnosis. These drugs may be important modulators, either directly or indirectly, of eCBome signaling [61,62]. Previous studies have mostly measured circulating levels of AEA and/or 2-AG and, in only a few cases, these mediators were measured in adipose tissue [16,42] and correlated with adiposity measures [63]. These limitations prevent us from performing a comprehensive comparison with previous studies, including individuals with lower BMI and healthier metabolic profiles [16]. Nevertheless, our results highlight the importance and the complexity of eCBome signaling, in both the plasma and intestine, in the context of human obesity and altered glucose homeostasis. Our data support the existence of important differences in the regulation of circulating and tissue, i.e., intestinal, eCBome signaling in the context of insulin resistance and obesity associated T2D.

## Funding

This work was financially supported by the Canadian Institutes of Health Research (to A.V., DOL342964), the Canada Research Excellence Chair in the Microbiome-Endocannabinoidome Axis in Metabolic Health (CERC-MEND) which is funded by the Tri-Agency of the Canadian Federal Government) (to V.D.), the Fonds de recherche du Québec – Santé (to A.V., 33219), the Institut universitaire de cardiologie et de pneumologie de Québec (to VR), and the FRQS funded Research Network on Cardiometabolism, Diabetes and Obesity CMDO-2021 (to VR).

## CRediT authorship contribution statement

**Volatiana Rakotoarivelo:** Writing – original draft, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Bénédicte Allam-Ndoul:** Writing – review & editing, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. **Cyril Martin:** Writing – review & editing, Methodology, Data curation. **Laurent Biertho:** Writing – review & editing, Resources, Conceptualization. **Vincenzo Di Marzo:** Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Nicolas Flamand:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Alain Veilleux:** Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Alain Veilleux, Vincenzo Di Marzo reports financial support was provided by Canadian Institutes of Health Research.

## Acknowledgments

The authors acknowledge the invaluable collaboration of the surgery team, bariatric surgeons, and biobank staff of the IUCPQ.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26968.

## References


[1] P. Dandona, A. Aljada, A. Chaudhuri, P. Mohanty, R. Garg, Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation, Circulation 111 (11) (2005) 1448–1454.
[2] C.M. Apovian, Obesity: definition, comorbidities, causes, and burden, Am. J. Manag. Care 22 (7 Suppl) (2016) s176–s185.
[3] B. Boubertakh, C. Silvestri, V. Di Marzo, Obesity: the fat tissue disease version of cancer, Cells 11 (12) (2022) 1872.
[4] S.Y. Tan, J.L.M. Wong, Y.J. Sim, S.S. Wong, S.A.M. Elhassan, S.H. Tan, et al., Type 1 and 2 diabetes mellitus: a review on current treatment approach and gene therapy as potential intervention, Diabetes Metabol. Syndr.: Clin. Res. Rev. 13 (1) (2019) 364–372.
[5] E. Ferrannini, E. Muscelli, S. Frascerra, S. Baldi, A. Mari, T. Heise, et al., Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients, The Journal of clinical investigation 124 (2) (2014) 499–508.
[6] R. Gentilella, V. Pechtner, A. Corcos, A. Consoli, Glucagon-like peptide-1 receptor agonists in type 2 diabetes treatment: are they all the same? Diabetes/metabolism research and reviews 35 (1) (2019) e3070.
[7] C.F. Deacon, Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus, Nat. Rev. Endocrinol. 16 (11) (2020) 642–653.
[8] J.R. Fernandez, D.B. Allison, Rimonabant Sanofi-Synthélabo, Curr Opin Investig Drugs 5 (4) (2004) 430–435.
[9] M. Gary-Bobo, G. Elachouri, B. Scatton, G. Le Fur, F. Oury-Donat, M. Bensaid, The cannabinoid CB1 receptor antagonist rimonabant (SR141716) Inhibits cell proliferation and increases markers of adipocyte Maturation in Cultured Mouse 3T3 F442A Preadipocytes, Mol. Pharmacol. 69 (2) (2006) 471.
[10] F.X. Pi-Sunyer, L.J. Aronne, H.M. Heshmati, J. Devin, J. Rosenstock, RIO-North America Study Group ft, Effect of rimonabant, a cannabinoid-1 receptor Blocker, on weight and cardiometabolic risk Factors in overweight or obese PatientsRIO-North America: a Randomized Controlled trial, JAMA 295 (7) (2006) 761–775.
[11] F.A. Moreira, J.A.S. Crippa, The psychiatric side-effects of rimonabant, Brazilian Journal of Psychiatry 31 (2) (2009) 145–153.
[12] M. Simard, A.-S. Archambault, J.-P.C. Lavoie, É. Dumais, V. Di Marzo, N. Flamand, Biosynthesis and metabolism of endocannabinoids and their congeners from the monoacylglycerol and N-acyl-ethanolamine families, Biochem. Pharmacol. (2022) 115261.
[13] J.E. Watson, J.S. Kim, A. Das, Emerging class of omega-3 fatty acid endocannabinoids & their derivatives, Prostaglandins & other lipid mediators 143 (2019) 106337.
[14] G. Rochefort, V. Provencher, S. Castonguay-Paradis, J. Perron, S. Lacroix, C. Martin, et al., Intuitive eating is associated with elevated levels of circulating omega-3-polyunsaturated fatty acid-derived endocannabinoidome mediators, Appetite 156 (2021) 104973.
[15] V. Di Marzo, New approaches and challenges to targeting the endocannabinoid system, Nat. Rev. Drug Discov. 17 (9) (2018) 623–639.
[16] V. Rakotoarivelo, J. Sihag, N. Flamand, Role of the endocannabinoid system in the adipose tissue with Focus on energy metabolism, Cells 10 (6) (2021) 1279.
[17] A. Veilleux, V. Di Marzo, C. Silvestri, The expanded endocannabinoid system/endocannabinoidome as a potential target for treating diabetes mellitus, Curr. Diabetes Rep. 19 (11) (2019) 117.
[18] V. Rakotoarivelo, B. Variya, S. Ilangumaran, M.-F. Langlois, S. Ramanathan, Inflammation in human adipose tissues–Shades of gray, rather than white and brown, Cytokine Growth Factor Rev. 44 (2018) 28–37.
[19] G. Galiazzo, F. Giancola, A. Stanzani, F. Fracassi, C. Bernardini, M. Forni, et al., Localization of cannabinoid receptors CB1, CB2, GPR55, and PPARα in the canine gastrointestinal tract, Histochem. Cell Biol. 150 (2) (2018) 187–205.
[20] J.R. Araújo, J. Tomas, C. Brenner, P.J. Sansonetti, Impact of high-fat diet on the intestinal microbiota and small intestinal physiology before and after the onset of obesity, Biochimie 141 (2017) 97–106.
[21] P.D. Cani, S. Possemiers, T. Van de Wiele, Y. Guiot, A. Everard, O. Rottier, et al., Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability, Gut 58 (8) (2009) 1091–1103.
[22] V. Di Marzo, A. Izzo, Endocannabinoid overactivity and intestinal inflammation, Gut 55 (10) (2006) 1373–1376.
[23] A.A. Izzo, M. Camilleri, Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects, Gut 57 (8) (2008) 1140–1155.
[24] P.D. Cani, M. Van Hul, C. Lefort, C. Depommier, M. Rastelli, A. Everard, Microbial regulation of organismal energy homeostasis, Nat. Metab. 1 (1) (2019) 34–46.
[25] K. Wright, N. Rooney, M. Feeney, J. Tate, D. Robertson, M. Welham, et al., Differential expression of cannabinoid receptors in the human Colon: cannabinoids Promote epithelial Wound healing, Gastroenterology 129 (2) (2005) 437–453.
[26] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, R.C. Turner, Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (7) (1985) 412–419.
[27] E. Classics Bligh, W. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[28] F. Fanelli, V.D. Di Lallo, I. Belluomo, R. De Iasio, M. Baccini, E. Casadio, et al., Estimation of reference intervals of five endocannabinoids and endocannabinoid related compounds in human plasma by two dimensional-LC/MS/MS, Journal of lipid research 53 (3) (2012) 481–493.
[29] Z. Punthakee, R. Goldenberg, P. Katz, Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome, Can. J. Diabetes 42 (2018) S10–S15.
[30] B. Poirier, J.P. Bidouard, C. Cadrouvele, X. Marniquet, B. Staels, S. O'connor, et al., The anti-obesity effect of rimonabant is associated with an improved serum lipid profile, Diabetes Obes. Metabol. 7 (1) (2005) 65–72.
[31] P.V. Piazza, M. Vallee, G. Marsicano, F.-X. Felpin, L. Bellocchio, D. Cota, et al., Antagonists of CB1 Receptor, Google Patents, 2018.
[32] M. Pawlak, P. Lefebvre, B. Staels, Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease, Journal of hepatology 62 (3) (2015) 720–733.
[33] A.M. Sharma, B. Staels, Peroxisome proliferator-activated receptor γ and adipose tissue—understanding obesity-related changes in regulation of lipid and glucose metabolism, The Journal of Clinical Endocrinology & Metabolism 92 (2) (2007) 386–395.
[34] J.C. Sipe, K. Chiang, A.L. Gerber, E. Beutler, B.F. Cravatt, A missense mutation in human fatty acid amide hydrolase associated with problem drug use, Proc. Natl. Acad. Sci. USA 99 (12) (2002) 8394–8399.
[35] J.C. Sipe, T.M. Scott, S. Murray, O. Harismendy, G.M. Simon, B.F. Cravatt, et al., Biomarkers of endocannabinoid system activation in severe obesity, PLoS One 5 (1) (2010).
[36] J.C. Sipe, J. Waalen, A. Gerber, E. Beutler, Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH), Int. J. Obes. 29 (7) (2005) 755–759.
[37] M. Rossmeisl, J. Pavlisova, P. Janovska, O. Kuda, K. Bardova, J. Hansikova, et al., Differential modulation of white adipose tissue endocannabinoid levels by n-3 fatty acids in obese mice and type 2 diabetic patients, Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 1863 (7) (2018) 712–725.
[38] T. Jourdan, G. Godlewski, G. Kunos, Endocannabinoid regulation of β-cell functions: implications for glycaemic control and diabetes, Diabetes Obes. Metabol. 18 (6) (2016) 549–557.
[39] J. Abdulnour, S. Yasari, R. Rabasa-Lhoret, M. Faraj, S. Petrosino, F. Piscitelli, et al., Circulating endocannabinoids in insulin sensitive vs. insulin resistant obese postmenopausal women. A MONET group study, Obesity 22 (1) (2014) 211–216.
[40] J. Jull, D. Stacey, S. Beach, A. Dumas, I. Strychar, L.-A. Ufholz, et al., Lifestyle interventions targeting body weight changes during the menopause transition: a systematic review, Journal of obesity 2014 (2014).
[41] V. Di Marzo, A. Verrijken, A. Hakkarainen, S. Petrosino, I. Mertens, N. Lundbom, F. Piscitelli, J. Westerbacka, A. Soro-Paavonen, I. Matias, L. Van Gaal, M. R. Taskinen, Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects, Eur. J. Endocrinol. 161 (5) (2009) 715–722.
[42] G. Annuzzi, F. Piscitelli, L. Di Marino, L. Patti, R. Giacco, G. Costabile, et al., Differential alterations of the concentrations of endocannabinoids and related lipids in the subcutaneous adipose tissue of obese diabetic patients, Lipids Health Dis. 9 (2010) 43.
[43] C. Pagano, C. Pilon, A. Calcagno, R. Urbanet, M. Rossato, G. Milan, et al., The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms, The Journal of Clinical Endocrinology & Metabolism 92 (12) (2007) 4810–4819.
[44] J.C. Cable, G.D. Tan, S.P. Alexander, S.E. O'Sullivan, The activity of the endocannabinoid metabolising enzyme fatty acid amide hydrolase in subcutaneous adipocytes correlates with BMI in metabolically healthy humans, Lipids Health Dis. 10 (1) (2011) 1–9.

[45] J.C. Cable, G.D. Tan, S.P.H. Alexander, S.E. O'Sullivan, The effects of obesity, diabetes and metabolic syndrome on the hydrolytic enzymes of the endocannabinoid system in animal and human adipocytes, Lipids Health Dis. 13 (1) (2014) 43.
[46] E.N. Kuipers, V. Kantae, B.C.E. Maarse, S.M. van den Berg, R. van Eenige, K.J. Nahon, et al., High fat diet increases circulating endocannabinoids accompanied by increased synthesis enzymes in adipose tissue, Front. Physiol. 9 (2019) 1913.
[47] M. Murakami, Y. Nakatani, T. Tanioka, I. Kudo, Prostaglandin E synthase, Prostaglandins & other lipid mediators 68 (2002) 383–399.
[48] K.R. Kozak, B.C. Crews, J.D. Morrow, L.-H. Wang, Y.H. Ma, R. Weinander, et al., Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides, J. Biol. Chem. 277 (47) (2002) 44877–44885.
[49] M. Yu, D. Ives, C.S. Ramesha, Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2, J. Biol. Chem. 272 (34) (1997) 21181–21186.
[50] K. Wright, M. Duncan, K. Sharkey, Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation, Br. J. Pharmacol. 153 (2) (2008) 263–270.
[51] L. De Petrocellis, A. Schiano Moriello, R. Imperatore, L. Cristino, K. Starowicz, V. Di Marzo, A re-evaluation of 9-HODE activity at TRPV 1 channels in comparison with anandamide: enantioselectivity and effects at other TRP channels and in sensory neurons, Br. J. Pharmacol. 167 (8) (2012) 1643–1651.
[52] T. Lowin, M. Apitz, S. Anders, R.H. Straub, Anti-inflammatory effects of N-acylethanolamines in rheumatoid arthritis synovial cells are mediated by TRPV1 and TRPA1 in a COX-2 dependent manner, Arthritis Res. Ther. 17 (1) (2015) 321.
[53] F.A. Iannotti, R.M. Vitale, The endocannabinoid system and PPARs: Focus on their signalling crosstalk, action and transcriptional regulation, Cells 10 (3) (2021) 586.
[54] R. Wafer, P. Tandon, Minchin Jen, The role of peroxisome proliferator-activated receptor gamma (PPARG) in Adipogenesis: Applying knowledge from the fish Aquaculture Industry to Biomedical research, Front. Endocrinol. 8 (102) (2017).
[55] Y.-X. Wang, C.-H. Lee, S. Tiep, R.T. Yu, J. Ham, H. Kang, et al., Peroxisome-proliferator-activated receptor δ activates fat metabolism to prevent obesity, Cell 113 (2) (2003) 159–170.
[56] J. Liu, H. Li, S.H. Burstein, R.B. Zurier, J.D. Chen, Activation and binding of peroxisome proliferator-activated receptor γ by synthetic cannabinoid ajulemic acid, Mol. Pharmacol. 63 (5) (2003) 983–992.
[57] C.E. Rockwell, N.T. Snider, J.T. Thompson, J.P.V. Heuvel, N.E. Kaminski, Interleukin-2 suppression by 2-arachidonyl glycerol is mediated through peroxisome proliferator-activated receptor γ independently of cannabinoid receptors 1 and 2, Mol. Pharmacol. 70 (1) (2006) 101–111.
[58] M.A. Bouhlel, B. Derudas, E. Rigamonti, R. Dièvart, J. Brozek, S. Haulon, et al., PPARG activation Primes human Monocytes into Alternative M2 macrophages with anti-inflammatory Properties, Cell Metabol. 6 (2) (2007) 137–143.
[59] X. Wang, H. Sumida, J.G. Cyster, GPR18 is required for a normal CD8αα intestinal intraepithelial lymphocyte compartment, J. Exp. Med. 211 (12) (2014) 2351–2359.
[60] T.J. Little, N. Cvijanovic, N.V. DiPatrizio, D.A. Argueta, C.K. Rayner, C. Feinle-Bisset, et al., Plasma endocannabinoid levels in lean, overweight, and obese humans: relationships to intestinal permeability markers, inflammation, and incretin secretion, American Journal of Physiology-Endocrinology and Metabolism 315 (4) (2018) E489–E495.
[61] A. Pascale, N. Marchesi, S. Govoni, A. Coppola, C. Gazzaruso, The role of gut microbiota in obesity, diabetes mellitus, and effect of metformin: new insights into old diseases, Curr. Opin. Pharmacol. 49 (2019) 1–5.
[62] Y. Saisho, Metformin and inflammation: its potential beyond glucose-lowering effect. Endocrine, metabolic & immune Disorders-drug Targets (formerly current drug Targets-immune, Endocrine & Metabolic Disorders) 15 (3) (2015) 196–205.
[63] M. Blüher, S. Engeli, N. Klöting, J. Berndt, M. Fasshauer, S. Bátkai, et al., Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity, Diabetes 55 (11) (2006) 3053–3060.