REVIEW

The gut microbiome, endocannabinoids and metabolic disorders

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Abstract

Two complex systems are emerging as being deeply involved in the control of energy metabolism. The intestinal microbiota, with its warehouse of genes, proteins and small molecules, that is, the gut microbiome; and the endocannabinoid system, with its recent extension to a more complex signalling apparatus including more than 100 lipid mediators and 50 proteins, that is, the endocannabinoidome. Both systems can become perturbed following bad dietary habits and during obesity, thus contributing to exacerbating this latter condition and its consequences in both peripheral organs and the brain. Here, we discuss some of the multifaceted aspects of the regulation and dysregulation of the gut microbiome and endocannabinoidome in energy metabolism and metabolic disorders, with special emphasis on the emerging functional interactions between the two systems. The potential exploitation of this new knowledge for the development of new pharmacological and nutritional approaches against obesity and its consequences is also briefly touched upon.

Key Words

- ▶ microbiota
- ▶ endocannabinoids
- ▶ endocannabinoidome
- ▶ metabolism
- ▶ obesity
- ▶ lipid signals

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The gut microbiota and microbiome and the control of metabolism

Research started several decades ago, but bloomed only since the beginning of the new century, has provided uncontroversial evidence that the complex ecosystem known as the gut microbiota, encompassing bacteria, archaeobacteria, viruses and fungi living in the mammalian gastrointestinal system, plays a fundamental role in the control of the host energy metabolism (Evans *et al.* 2013, Cani 2019, Koh & Bäckhed 2020). Such function is exerted at the same time by: (1) metabolising macro and micronutrients that cannot be otherwise utilised by host cells as a source of energy, hence helping the host to adapt

to new dietary challenges; and (2) producing signalling molecules that can influence all aspects of energy metabolism, including food intake, energy expenditure and lipid accumulation by the adipose tissues and liver, nutrient absorption by the gut, and glucose and lipid anabolism and catabolism (Canfora *et al.* 2019). Indeed, and quite intuitively, the several phyla of microorganisms, selected by genetics and lifelong environmental clues (including, but not limited to, lifestyle habits (Di Marzo & Silvestri 2019)) to live in specific compartments of the gut, can only play their symbiotic (or pathological) role through what

has been recently defined as their ‘theatre of activity’ (Berg *et al.* 2020). This is the molecular warehouse, including gut microbial genes – which largely outnumber those of the host – nucleic acids, proteins (with structural, catalytic and signalling function) and small signalling molecules, best known as the ‘gut microbiome’ (Berg *et al.* 2020).

It was immediately clear that the gut microbiome helps host physiology determine the ideal control of nutrient processing necessary for metabolic health. This concept was supported in particular by laboratory experiments in which a ‘transplant’ of the faecal microbiome of obese individuals (human or mice) to healthy mice could transfer to the latter several features of the metabolic syndrome (Koren *et al.* 2012, Ellekilde *et al.* 2014). Conversely, in a clinical study, transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals with metabolic syndrome (Vrieze *et al.* 2012). As a consequence of these findings, for a decade research has focussed on understanding what would be the ideal relative amounts of the various microbial taxa composing the gut microbiota in order to achieve a healthy metabolic state. Indeed, both human and animal studies have shown that gut microbial composition, from the phyla to the genus level, in individuals with metabolic disorders such as obesity, hyperglycemia and dyslipidemia, and ensuing complications, such as type 2 diabetes (T2D), hepatosteatosis and atherosclerosis, differ from that of metabolically healthy subjects (Allin *et al.* 2018, Cani 2019, Zhong *et al.* 2020). However, the nature and extent of such differences may vary considerably depending on the human cohort investigated and several genetic, developmental, hormonal, lifestyle and environmental factors (e.g. maternal diet during gestation and lactation, type of delivery, sex, age, diet, geographical location, circadian rhythms, underlying pathologies and use of drugs and medications, just to name a few). These factors are known to deeply affect adult gut microbiota composition, often in a time-dependent manner (Hasan & Yang 2019).

For this reason, despite the fact that the lack or overabundance of some bacterial taxa, such as *Akkermansia mucinifila* and *Lactobacillaceae*, respectively, are very often associated with obesity (Cani 2019), previously suggested microbial biomarkers of obesity, such as the *Firmicutes/Bacteroidetes* ratio, are being revisited (Magne *et al.* 2020). In general, it is becoming accepted that it is difficult to find two healthy individuals with the same gut microbial composition, although populations with similar microbiomes do exist. Thus, rather than a specific gut microbiota, it is a certain gut microbiome, with a given

cocktail of biologically relevant molecules – which may derive also from different microbiota compositions – that usually correlates with, and possibly affords, an either healthy or dysmetabolic status.

Among gut microbiota-derived molecules, possibly the best-characterised ones that are known to influence in a beneficial manner several aspects of energy metabolism, from food intake and energy expenditure to insulin sensitivity and fat accumulation, are the short-chain fatty acids (SCFA). These are small metabolites produced from the digestion of complex fibres. Several GPCR targets have been identified for SCFA (Hernández *et al.* 2019). Microbial-derived amino acid derivatives, instead, may produce either negative metabolic effects (as in the case of imidazole-propionate and phenylacetic acid) or again contribute to resolving inflammation and insulin resistance, as in the case of the two tryptophan metabolites, indole-3-acetate and tryptamine, which act at the aryl-hydrocarbon receptor (Delzenne *et al.* 2020). The formation by gut microbiota of secondary bile acids from host cell-derived bile acids seems to mostly have the function of inactivating the latter, which variedly affect metabolism and inflammation (Delzenne *et al.* 2020). Other metabolites may be derived from the diet, such as trimethylamine-*N*-oxide, which is associated with obesity and its cardiovascular consequences (Naghipour *et al.* 2020). Metabolites produced by commensal microorganisms following the dietary intake of certain food components, such as the polyphenols, may differ among individuals with different capabilities of metabolising them, that is, different ‘metabotypes’ (Noerman *et al.* 2020). Despite the fact the much progress has been made towards the understanding of microbiome-mediated chemical communication, the gut microbiota-derived metabolites discovered so far probably represent only the tip of the iceberg.

The endocannabinoid system in the functional and dysfunctional control of metabolism

The endocannabinoid (eCB) system is a signalling apparatus distributed throughout the mammalian body, and present also in non-mammalian vertebrates and in some invertebrates. It is composed of: (1) two main lipid signalling molecules, the endocannabinoids (eCBs) anandamide (*N*-arachidonoyl-ethanolamine, AEA) and 2-arachidonoyl-glycerol (2-AG); (2) several eCB biosynthesising and inactivating enzymes, of which the most studied ones

are *N*-acyl-phosphatidylethanolamine phospholipase D-like esterase (NAPE-PLD) and fatty acid amide hydrolase (FAAH), used for AEA biosynthesis from *N*-arachidonoyl-phosphatidyl-ethanolamine, and AEA degradation to arachidonic acid (AA) and ethanolamine, respectively; and the *sn*-1-selective diacylglycerol lipases α and β (DAGL α and DAGL β) and monoacylglycerol lipase (MAGL), used for 2-AG biosynthesis from *sn*-2-AA-containing diacylglycerols, and 2-AG degradation to AA and glycerol, respectively; and (3) the main eCB receptors, that is, the metabotropic cannabinoid receptor type-1 and -2 (CB1 and CB2) – which are two G protein-coupled receptors (GPCRs) also activated by the psychotropic cannabinoid Δ^9 -tetrahydrocannabinol (THC) (hence the name eCB) – and the ionotropic transient receptor potential vanilloid type-1 (TRPV1) channels (Di Marzo 2018) (Fig. 1).

In the brain, AEA and 2-AG may be released from post-synaptic dendrites and act at presynaptic CB1 receptors and TRPV1 channels to exert modulatory effects on the release of glutamate and GABA from neuronal terminals (Araque *et al.* 2017). The effect of CB1 consists of inhibition of neurotransmitter release, and is due to inhibition of voltage-activated ion channels mediated by activation of the α subunit of the $G_{i/q}$ protein. Instead, TRPV1 activation, by causing Ca^{2+} influx, may either stimulate neurotransmitter release, when the channel is located presynaptically, or inhibit glutamate action by enhancing the reuptake of AMPA receptors, when TRPV1 is post-synaptic (Fig. 1). The two eCBs also act as immune modulators at CB2 receptors expressed mostly in activated microglia (Tanaka *et al.* 2020). CB1 receptors in astrocytes and TRPV1 channels in microglia also participate in the neuromodulatory and

immunomodulatory actions of eCBs, respectively (Araque *et al.* 2017, Marrone *et al.* 2017). A role for mitochondrial CB1 receptors in neurons and astrocytes in the control of the respiratory chain of these cells, and hence of mouse brain function and, subsequently, behaviour is also emerging (Jimenez-Blasco *et al.* 2020). Indeed, one of the major functions of brain CB1 receptors is to control behaviour, including the motivational, homeostatic and sensory aspects of feeding (Lau *et al.* 2017, Tarragon & Moreno 2017, Coccurello & Maccarrone 2018). These latter functions are usually stimulated by CB1 receptors through a plethora of neural pathways in several brain areas, thus resulting in increased food intake. Accordingly, following food deprivation, the hypothalamic levels of eCBs are transiently increased (Kirkham *et al.* 2002). This is believed to be due, in part, to decreased leptin signalling, which normally reduce eCB levels. Hypothalamic and peripheral eCB levels are also increased during obesity (Di Marzo *et al.* 2001, Cristino *et al.* 2013, Morello *et al.* 2016), often in association with dysfunction of leptin, insulin and glucocorticoid signalling, thus contributing to hyperphagia, gut-brain axis dysfunction and inflammation (Balsevich *et al.* 2017, Forte *et al.* 2020).

Indeed, the eCB system is now well recognised to participate in all peripheral aspects of glucose and lipid metabolism. This function occurs through CB1 receptor-mediated control of several metabolically relevant tissues, via either the sympathetic nervous system or direct actions on hepatocytes, white and brown adipocytes, skeletal muscle cells, enteroendocrine epithelial cells and pancreatic β -cells. The eCB system becomes dysregulated in these tissues and cells during obesity, and thus participates,

Figure 1

Endocannabinoid (eCB) signalling in the brain: neuromodulatory and immune modulatory function, receptors and major biosynthetic pathways and enzymes. The type of G-proteins mostly involved in CB1 and CB2 receptor actions are also shown. Pointed arrows denote movement, transformation or activation. Blunted arrows denote inhibition. Abbreviations are defined in the main text, except for: AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; EtOH, ethanol; NAT, *N*-acyl-transferase; PLC, phospholipase C.

also through dysfunctional hormone-mediated and/or inflammatory pathways, in the exacerbation of this condition and its metabolic consequences (Silvestri & Di Marzo 2013, Ruiz de Azua & Lutz 2019). In particular, the following effects have been associated, in animal studies, with CB1 receptor activation in physiological conditions, or CB1 overstimulation in obesity, in the following organs, tissues and cells: (1) *in the gastrointestinal tract* – decreased satiety, gastrointestinal motility and gastric acid secretion, increased fat-induced cephalic responses, and dysregulation of gut microbiota function (see subsequently); (2) *in the liver* – increased de novo lipogenesis and insulin resistance, hepatic steatosis and dyslipidemia; (3) *in the white adipose tissue* – increased energy storage capacity, adipogenesis, lipogenesis, insulin resistance and leptin release, decreased sympathetic innervation and browning and pro-inflammatory macrophage polarisation; (4) *in the brown adipose tissue* – decreased sympathetic innervation and adaptive thermogenesis; (5) *in the skeletal muscle* – decreased insulin-mediated glucose uptake and mitochondrial biogenesis, and decreased myotube formation and muscular function; and (6) *in pancreatic β -cells* – increased basal and glucose-dependent insulin secretion and trafficking and release of insulin granules (Ruiz de Azua & Lutz 2019).

The fact that, in the periphery, as much as in brain areas deputed to food intake, CB1 signalling, in terms of eCB tissue concentrations, may become malfunctioning during obesity in rodents (Silvestri & Di Marzo 2013, Ruiz de Azua & Lutz 2019), possibly explains why alterations in circulating (Blüher *et al.* 2006, Côté *et al.* 2007, Martins *et al.* 2015, Fanelli *et al.* 2017, 2018) or salivary (Matias *et al.* 2012) eCB levels often have been associated with human obesity, and even more with the dysmetabolic consequences of visceral obesity (Di Marzo *et al.* 2009, Abdulnour *et al.* 2014). Such alterations may be normalised following weight loss-inducing interventions, such as caloric restriction and exercise (Di Marzo *et al.* 2009) or bariatric surgery (Azar *et al.* 2019). The presence of polymorphisms in the genes encoding for eCB receptors and anabolic or catabolic enzymes (Martins *et al.* 2015, Doris *et al.* 2019, Thethi *et al.* 2020) may also underlie eCB system dysregulation in obesity.

The endocannabinoidome and its role in energy metabolism control

The link between the eCB system and the control of metabolism became even stronger when it was realised that several congeners of AEA and 2-AG, that is, the long-chain

N-acylethanolamines (NAEs) and 2-monoacylglycerols (MAGs) (Hansen & Vana 2019) (Fig. 2), respectively, also play a role in chemical signalling. While these molecules are in most cases weakly active at CB1 and CB2 receptors, they interact with either alternative eCB receptors, such as TRPV1 channels (Movahed *et al.* 2005, Zygmunt *et al.* 2013), the role in the metabolism of which is becoming increasingly acknowledged (Christie *et al.* 2018), or non-eCB receptors. The latter receptors include peroxisome proliferator-activated receptors α (PPAR α , for *N*-palmitoyl-ethanolamine (PEA), and *N*-oleoyl-ethanolamine (OEA)) and γ (PPAR γ) (for AEA at high micromolar concentrations), or orphan GPCRs, such as GPR55 (for PEA and, possibly, AEA and 2-AG) and GPR119 (for MAGs such as mono-oleoyl- and -linoleoyl-glycerol, and for OEA). PPARs and GPR55/119 are, respectively, either already established or emerging players in glucose and lipid metabolism (Poursharifi *et al.* 2017, Laleh *et al.* 2019, Ramírez-Orozco *et al.* 2019). Importantly, AEA and 2-AG share with their congeners the same biosynthetic and anabolic pathways and enzymes (Di Marzo 2008).

Beyond NAEs and MAGs, several other families of eCB-like mediators have been discovered that share with AEA either catabolic enzymes or molecular targets, or both (Fig. 2). These families include: (1) the primary fatty acid amides, such as the sleep-inducing factor, oleamide, a FAAH substrate that was suggested to activate CB1 (Leggett *et al.* 2004), and, like some unsaturated NAEs, to antagonise the transient receptor potential vanilloid type-2 (TRPV2) channel (Schiano Moriello *et al.* 2018), an emerging regulator of brown adipocyte differentiation and function (Sun *et al.* 2017); (2) the *N*-acylated aminoacids, or lipoaminoacids, such as *N*-oleoyl- and *N*-arachidonoyl-glycine, which are also inactivated by FAAH, and have been suggested to act at GPR18 or PPAR α (Burstein 2018, Donvito *et al.* 2019); *N*-oleoylglycine has also been suggested to produce its effects, among which hyperphagia (Wu *et al.* 2017), by activating CB1, perhaps via inhibition of FAAH and elevation of endogenous AEA levels (Donvito *et al.*, 2019); (3) the *N*-acylated taurines, such as *N*-oleoyl- and *N*-arachidonoyl-aurine, which have been suggested to activate TRPV1 and TRPV4 channels as well as GPR119 (Saghatelian *et al.* 2004, Grevengeod *et al.* 2019); and (4) some *N*-acylated neurotransmitters, such as *N*-oleoyl- and *N*-arachidonoyl-serotonin, which antagonise TRPV1 and/or inhibit FAAH (Ortar *et al.* 2007, Verhoeckx *et al.* 2011), or *N*-oleoyl- and *N*-arachidonoyl-dopamine, which instead activate CB1 and/or TRPV1 (Bisogno *et al.* 2000, Chu *et al.* 2003), and were recently shown to also act as inverse agonists at GPR6 (Shrader & Song 2020). Additionally, the

Figure 2

Synthesis, inactivation, receptors and main metabolic functions of endocannabinoidome mediators. Thick arrows denote the biochemical reactions and functional connections underlying endocannabinoidome mediator action. Not shown, for the sake of clarity, are the negative effects exerted by almost all unsaturated *N*-acyl-amides tested so far on *T*-type (Ca_v3) Ca^{2+} channels. Blunted arrows denote inhibition. 2-AG, 2-arachidonoylglycerol; 2-LG, 2-linoleoylglycerol; 2-OG, 2-oleoylglycerol; 5/12/15-LOX, 5/12/15-lipoxygenase; 15 HAEA, 15(S)-HETE Ethanolamine; AA, arachidonic acid; ABH4/6/12, $\alpha\beta$ -hydrolase 4/6/12; AEA, anandamide; CB1/2, cannabinoid receptor 1/2; COX2, cyclooxygenase 2; DAG, diacylglycerols; DHEA, *N*-docosahexaenoyl-ethanolamine; EET-EA, epoxyeicosatrienoic acid ethanolamine; FA, fatty acid; FAAH, fatty acid amide hydrolase; FA, free fatty acids; GDE1, glycerophosphodiester phosphodiesterase 1; GPR, G-protein-coupled receptor; LEA, *N*-linoleoyl-ethanolamine; MAGL, monoacylglycerol lipase; MAG, monoacylglycerols NAAA, *N*-acylethanolamine-hydrolysing acid amidase, NAPE, *N*-acyl-phosphatidylethanolamine; NAPEPLD, *N*-acyl-phosphatidylethanolamine-specific phospholipase D; OEA, *N*-oleoyl-ethanolamine; P450, cytochrome p450 oxygenases; PEA, *N*-palmitoyl-ethanolamine; PG-Gs, prostaglandin glycerol esters; PLC β , phospholipase C β ; PPAR γ , peroxisome proliferator-activated receptor- γ ; PPAR α , peroxisome proliferator-activated receptor- α ; SEA, *N*-stearoyl-ethanolamine; TRPV1, transient receptor potential vanilloid type-1 channel; TRPV2, transient receptor potential vanilloid type-2 channel.

enzymatic oxidation of polyunsaturated eCB congeners and eCB-like amides by lipoxygenases (LOXs), cytochrome p450 oxygenases and cyclooxygenase-2 (COX-2), may also lead to bioactive lipids. The molecular targets of these oxidation products are largely unknown but, at least for the LOX and cytochrome p450 oxygenase metabolites, still seem to include CB1 and CB2. Conversely, the COX-2 derivatives of AEA and 2-AG, known as prostamides and prostaglandin glycerol esters, respectively, act on non-cannabinoid, non-prostanoid receptors (Rouzer & Marnett 2011, Urquhart *et al.* 2015). The ensemble of these eCB-like mediators, their receptors and metabolic enzymes, together with the eCB system, is now referred to as the 'expanded eCB system' or 'endocannabinoidome' (eCBome).

Much in the same way as the signalling lipids produced by the gut microbiome, also the molecular armamentarium represented by eCBs and eCB-like multi-target mediators,

through their metabolically relevant receptors, may produce complex and site/time-dependent actions on lipid and glucose metabolism. In fact, unlike CB1, most other eCBome receptors negatively affect energy balance and play beneficial roles during metabolic disorders (Fig. 2). In particular: (1) CB2 seems to reduce insulin resistance and hence glucose intolerance, possibly also via peripheral anti-inflammatory effects in peripheral tissue-penetrating macrophages (Kumawat & Kaur 2019, Zibolka *et al.* 2020); (2) TRPV1 inhibits food intake, seemingly via actions at the level of the vagus nerve (Kentish & Page 2015), reduces adipogenesis, ameliorates insulin sensitivity and stimulates white adipocyte browning and brown adipocyte heat production (Baskaran *et al.* 2016, Christie *et al.* 2018) – studies using mice in which the *Trpv1* gene was inactivated have confirmed the beneficial role of this channel against insulin resistance and body weight gain following a high-fat diet

Figure 3

The gut microbiome, the endocannabinoidome (eCBome), their role in metabolic control, dysmetabolism and its consequences, and their cross-talk. Grey arrows denote the best-established mechanisms through which the adipose tissue and the gut eCBomes affect the brain (mostly the hypothalamus) and the brain eCBome affects the gut. Pink arrows denote bi-directional effects of the gut microbiome and the eCBome in various organs on energy metabolism and its disruption following high-fat or Western-type diets or dysbiosis, and vice versa; blue arrows denote where the gut microbiome has been suggested so far to affect the eCBome; green arrows denote from where the eCBome has been suggested so far to affect the gut microbiome; and red arrows denote where eCBome-like mediators and other gut microbiota-derived molecules may affect the host. The general chemical structure of eCBome mediators is also shown, with R denoting a variously modified long chain fatty acyl chain, X an NH or O group, and R1 an H, or an ethanol, glycerol, amino acid or neurotransmitter group. HPA, hypothalamus-pituitary-adrenal; T2D, type 2 diabetes.

(Page *et al.* 2019), with some occasional contrasting result (Mottet & Ahern 2008); however, such studies have not clarified whether all the metabolic effects of TRPV1 agonists such as capsaicin are due to increased activity of the channel or to its desensitisation, which immediately follows its activation; (3) PPAR α activation is well established to induce satiety and reduce fat intake at the level of the small intestine and to counteract hepatic lipogenesis and stimulate fatty acid β -oxidation (Hong *et al.* 2019), whereas PPAR γ activation, whilst necessary for full white adipocyte differentiation, counteracts insulin resistance (Wang *et al.* 2017) – both these nuclear receptors have been suggested to reduce the low grade chronic inflammation that follows obesity (Silva & Peixoto 2018); (4) GPR55 plays a beneficial role at enhancing insulin sensitivity and reducing adiposity, as shown by studies in *Gpr55*^{-/-} mice (Meadows *et al.* 2016, Lipina *et al.* 2019, Ramirez-Orozco *et al.* 2019); (5) GPR119 has well established incretin actions by enhancing glucagon-like peptide-1 secretion from intestinal enteroendocrine cells, thereby potentially enhancing insulin release and reducing food intake (Laufer *et al.* 2009); (6) GPR18 has been proposed to mediate the reduction of body weight and the enhancement of glucose tolerance of the docosahexaenoic acid derivative, resolvin RvD2 (Pascoal *et al.* 2017), possibly in synergy with the proposed anti-inflammatory effect of this compound; hence, GPR18 activation by *N*-acyl-glycines may produce similar effects; and (7) Ca_v3 (T-type) calcium channels,

which are inhibited by several types of long-chain fatty acid amides (Chemin *et al.* 2014), are much less studied than other eCBome receptors in terms of energy metabolism control, but were proposed to participate in weight gain following a high-fat diet in a recent study carried out with Ca_v3.1^{-/-} mice (Rosenstand *et al.* 2020).

In summary, the eCBome, through its various receptors, can affect, in multiple ways and organs, energy metabolism during both physiological and dysmetabolic conditions. In turn, eCBome mediators such as NAEs and MAGs are altered in mouse (Lacroix *et al.* 2019) and human (Pastor *et al.* 2016, Fanelli *et al.* 2018, van Eyk *et al.* 2018) obesity, as well as in overweight volunteers with high visceral adiposity (Castonguay-Paradis *et al.* 2020), although not necessarily in the same manner as AEA and 2-AG. In fact, several pathways control the biosynthesis and degradation (Fig. 2) of NAEs and MAGs, and different members of either class of mediators may be regulated by alternative routes in different tissues (Hansen & Vana 2019), as well as by the different availability of dietary fatty acid precursors (Castonguay-Paradis *et al.* 2020).

The gut microbiome-endocannabinoidome axis in metabolic function and dysfunction

As discussed in the previous sections, through their several molecular signals, both the gut microbiome and

eCBome have many ways to influence energy metabolism in mammals. However, as it is often the case with systems controlling similar functions, it is now emerging that the gut microbiome and the eCBome may communicate and influence each other while performing their role in nutrient processing (Cani *et al.* 2016) (Fig. 3).

Pathological perturbations of gut microbial composition from a 'healthy' steady-state, often collectively defined as dysbiosis, which were caused by high-fat diet-induced obesity or chronic treatment with antibiotics, were found to be accompanied by changes in eCB and eCBome signalling in the gut (Muccioli *et al.* 2010, Guida *et al.* 2018). Such changes, in turn, participate in mediating the negative effects of dysbiosis, as suggested by the fact that partial correction of the latter condition, with either pre- or pro-biotics, resulted in the counteraction of the pathological consequences of dysbiosis and the restoration of 'normal' eCBome signalling. Administration of a metabolically beneficial bacterial species, *Akkermansia mucinifila*, ameliorates high-fat-diet-induced glucose intolerance and insulin resistance in mice and, at the same time, elevates the intestinal levels of 2-AG and some of its MAG congeners (Everard *et al.* 2013), as does the 'protective' knockout of MyD88, a protein mediating the increase in intestinal permeability and systemic inflammation typical of high-fat diet-induced dysbiosis (Everard *et al.* 2014). Thus, one may hypothesise that these MAGs, which potentially ameliorate dysmetabolism via TRPV1, GPR119 and CB2 receptor activation, may mediate in part the beneficial effects of these two interventions.

Perhaps the most convincing evidence that the presence or absence of the gut microbiota directly affects the host eCBome came from recent studies using germ-free mice. These are mice born and raised in completely sterile conditions, and hence deprived of their microbiota, and in which a functionally active intestinal microbial ecosystem can be reintroduced by means of the procedure known as 'faecal microbiota transfer' (FMT). A recent study showed that germ-free mice of both 4 and 13 weeks of age exhibit profound alterations of eCBome signalling in the gut and, particularly, the small intestine. Here, the mRNA expression of some eCBome receptors, namely CB1 and PPAR α , was increased, whereas that of GPR18 and GPR55, was decreased. Importantly, these alterations were reversed in adult male mice after 1 week from a successful FMT procedure (Manca *et al.* 2020a). Interestingly, the brain of juvenile and adult germ-free mice presented with alterations in eCBome mediator concentrations, namely a decrease of 2-AG, MAGs and *N*-arachidonoyl-glycine

and, in females, an increase in AEA and other NAEs. The changes in adult male mice, where the FMT experiment was carried out, were no longer statistically significant 1 week after reintroduction of the gut microbiota (Manca *et al.* 2020b). Whether or not eCBome signalling alterations in germ-free mice account for some of the metabolic features of these animals, which are characterised, among others, by higher energy expenditure and lower intestinal inflammation and immunity (Wolf 2006, Rogala *et al.* 2020), as well by higher HPA axis activity (Farzi *et al.* 2018), remains to be investigated.

If the gut microbiota modulates the eCBome, pharmacological or genetic manipulation of eCBome signalling can modify the composition of gut microbiota, and hence its molecular signalling apparatus, especially following high-fat diet-induced obesity. This can be used to partly prevent the negative metabolic effects and systemic inflammation induced by dysbiosis, as is the case of CB1 receptor antagonists (Muccioli *et al.* 2010, Mehrpouya-Bahrami *et al.* 2017) (or of chronic treatment with THC, which desensitises CB1 receptors (Cluny *et al.* 2015)). TRPV1 agonists, such as capsaicin (Kang *et al.* 2017, Song *et al.* 2017, Hui *et al.* 2020), produce similar effects. The strong modification of gut microbiota composition observed following administration of exogenous NAEs, such OEA and PEA, were suggested to underlie the reduction of obesity and dysmetabolism typically produced by the former compound (Cristiano *et al.* 2018, Laleh *et al.* 2019), and the analgesia and amelioration of behavioural deficits in models of chronic pain and autism, exerted by PEA (Di Paola *et al.* 2018, Guida *et al.* 2020). Given the lack of eCBome receptors in bacteria, it is likely that these effects are indirect, that is, mediated by actions on, for example, host intestinal cells. However, one should not rule out the possibility that gut microorganisms, much in the same way as they do with several drugs (Weersma *et al.* 2020), respond to eCBome mediators, and/or metabolise them. Indeed, many of these lipids have a much older evolutionary history than their receptors (Elphick 2012), and have been found, for example, also in yeasts (Merkel *et al.* 2005). Accordingly, a recent study showed that incubation of bacterial cultures obtained from human faecal microbiota samples with a cocktail of NAEs (namely AEA, OEA, PEA and LEA, admittedly at high micromolar concentrations), deeply affects *ex vivo* gut microbial composition, leading to an increase in *Proteobacteria* and a decrease in *Bacteroidetes*, and, at the family level, to *Enterococcaceae*, *Veillonellaceae* and *Enterobacteriaceae* blooming at the expense of *Streptococcaceae* (Fornelos *et al.* 2020). Previous evidence

of non-eCBome receptor-mediated effects of AEA, NAEs and 2-lauroyl-glycerol in either yeasts, protists or bacteria, which often express orthologues of eCBome metabolising enzymes, is also available (Anagnostopoulos *et al.* 2010, Schlievert *et al.* 2018, Feldman *et al.* 2020, Sionov *et al.* 2020). Emblematic of the target promiscuity of eCBs and their congeners, a very recent study showed that 2-AG inhibits the induction of virulence of enteric pathogens by antagonising the bacterial receptor QseC, a histidine kinase found in *Enterobacteriaceae* and promoting the activation of pathogen-associated type three secretion systems (Ellermann *et al.* 2020).

As mentioned previously, genetic deletion of enzymes that biosynthesise or inactivate eCBome mediators is also being used to see whether a cause–effect relationship exists between eCBome signalling and gut microbiota composition. Targeted adipocyte or intestinal epithelial cell deletion of NAPE-PLD, the most studied NAE biosynthetic enzyme, was accompanied by disruption of different gut microbiota taxa depending on the cell type targeted (Geurts *et al.* 2015, Everard *et al.* 2019). In both cases, the mutant mice exhibited lower levels of NAEs in the tissues where the enzyme had been genetically inactivated, although with some differences: whilst in the adipocyte-specific *Napepld* knockout mice AEA levels were unaltered, and those of OEA and PEA were reduced, in the intestinal epithelial cell-specific knockouts the concentrations of all the measured NAEs, including AEA, were reduced, as were, unexpectedly, those of 2-AG, although to a lesser extent. These latter differences became less marked following a high-fat diet, with only AEA and LEA levels being significantly reduced. Both types of genetically modified mice exhibited dramatically worse metabolic profiles after high-fat diet-induced obesity, in terms of, for example, enhanced fat accumulation or hyperphagia, reduced energy expenditure and insulin sensitivity or enhanced fatty liver. This suggested that the effect of the reduction in metabolically beneficial NAE (i.e. OEA, LEA) signalling at targets such as TRPV1, PPAR α or GPR119 prevailed on the effect of reduced CB1 activation by AEA or 2-AG. Furthermore, both genotypic modifications were accompanied by profound changes in gut bacterial composition, suggesting that NAEs influence the microbiota not only, as it would be expected, from the intestine, but also from the adipose tissue. However, gut microbiome alterations were clearly shown to contribute to dysmetabolism only in adipocyte-specific *Napepld* knockout mice, since the dysmetabolic phenotype of these mice was partly reversed by antibiotics or transferred to germ-free mice by FMT. On the other hand, in intestinal

epithelial cell-specific *Napepld*^{−/−} mice, administration of *A. muciniphila* produced the same beneficial metabolic effects as in WT mice, suggesting that such effects of this bacterial species do not depend on intestinal NAEs (Geurts *et al.* 2015, Everard *et al.* 2019). Nevertheless, increasing evidence indicates that NAEs such as OEA and PEA, similar to oleamide and *N*-arachidonoyl-dopamine, and opposite to AEA and 2-AG, counteract, via TRPV1 or PPAR α -mediated mechanisms, the increased permeability in the intestinal epithelial cell barrier (also known as ‘leaky gut’) that is often a consequence of dysbiosis (Karwad *et al.* 2019).

A very recent study investigated if enhanced MAG levels in mice lacking an active MAGL (*Mgll*^{−/−} mice), which present with decreased fat preference, adiposity and steatosis and improved insulin sensitivity after a high-fat diet, due to both CB1-dependent and independent mechanisms (Yoshida *et al.* 2019), also exhibit an altered faecal microbiota. This was indeed found to be the case, with differences becoming stronger and more statistically significant with increasing durations of an obesogenic high-fat diet. Interestingly, some bacterial families, including species previously described to be either metabolically beneficial or detrimental to high-fat diet-induced obesity and glucose intolerance, were differentially altered in WT and *Mgll*^{−/−} mice, in a manner to suggest their involvement in the obesity-prone or -resistant phenotype of the two genotypes (Dione *et al.*, in press). These alterations, as well as the metabolically healthy phenotype of *Mgll*^{−/−} mice, may have been due to either overstimulation and desensitisation of CB1 receptors by high 2-AG levels (Imperatore *et al.* 2015), or to modulation of non-CB1 receptors by the elevated concentrations of the other MAGs. However, one pitfall of studies using genetically modified mice with different propensity for obesity is that it is difficult to understand to what extent their gut microbiome alterations are the direct effect of altered eCBome signalling rather than of the obesity-associated dysmetabolism. Therefore, the authors of this study also performed ‘culturomics’ experiments with WT mouse faecal samples. These experiments consisted of incubating, under several different culturing conditions, faecal microbiota-containing samples with high micromolar concentrations of 2-AG or MAGs, in order to mimic *ex vivo* the situation occurring in the *Mgll*^{−/−} mouse intestine. The authors found that, under certain culturing conditions, some of the changes in the faecal microbiota composition could indeed be recapitulated, supporting the hypothesis that the higher concentrations of MAGs in *Mgll*^{−/−} mice, rather than, or in addition to,

the metabolic conditions of these animals, could be the direct cause of some of such changes also *in vivo* (Dione *et al.*, in press).

Two studies were recently carried out in mice and human volunteers with the aim of correlating diet-induced changes in gut microbiota composition with changes in eCBome mediators and proteins. In mice, a 56-day administration of a high fat–high sucrose diet caused a gradual perturbation of the gut microbiota composition, ileal and plasma eCBome mediator concentrations and ileal eCBome enzyme and receptor mRNA expression, along with enhanced body weight and early glucose intolerance. Importantly, weight-independent and time-dependent correlations were found between the relative abundances of, among others, the metabolically relevant genera *Barnesiella*, *Eubacterium*, *Adlercreutzia*, *Parasutterella*, *Propionibacterium*, *Enterococcus*, and *Methylobacterium* and the concentrations of AEA or the anti-inflammatory NAE, *N*-docosahexaenoyl-ethanolamine (DHEA). These findings highlight the potential functional interaction between the gut microbiota and the eCBome during the metabolic adaptation to prolonged high-fat and high-sucrose feeding (Lacroix *et al.* 2019). In a study carried out in a cohort of 195 healthy volunteers from the Québec province, circulating levels of all MAGs and NAEs other than OEA correlated with body fat mass and visceral adiposity. Additionally, the self-reported fat dietary intakes of specific fatty acids were positively associated with the plasma levels of 2-AG and omega-3-fatty acid-derived NAEs and MAGs, irrespective of the body fat distribution. In a subset of the individuals, a 2-day Mediterranean diet intervention increased circulating levels of NAEs and MAGs according to similar changes in the intake of the corresponding fatty acids. Importantly, some metabolically relevant gut bacterial families (e.g. *Veillonellaceae*, *Peptostreptococcaceae* and *Akkermansiaceae*) were associated with most NAEs or omega-3-fatty acid-derived MAGs, independently of body fat distribution and dietary FA intake (Castonguay-Paradis *et al.*, 2020). In particular, the positive correlation between *Veillonellaceae* and NAEs was in agreement with the aforementioned *ex vivo* study showing a stimulatory effect of these eCBome mediators on this family of commensal bacteria (Fornelos *et al.* 2020).

Finally, evidence exists that commensal bacteria may influence the eCBome in its function also by producing eCB-like molecules that are capable of binding the same receptors as their host counterparts. The first such compound to be identified was *N*-acyl-3-hydroxypalmitoyl-glycine (commendamide), an agonist

for GPR132 (Cohen *et al.* 2015). This is a GPCR that, in an independent study, was later found to be activated by ‘mammalian’ *N*-acyl-glycines, including *N*-palmitoyl-, *N*-linoleoyl- and *N*-oleoyl-glycine, and also by the primary amide, linoleamide (Foster *et al.* 2019), but for which no role in energy metabolism has been described to date. Subsequently, by looking for *N*-acyl amide synthase genes enriched in various human microbiota species, the same group identified a series of additional *N*-acyl-amides capable of activating other mammalian GPCRs. Among these compounds, *N*-oleoylserinol was found also in human faecal samples and shown to activate GPR119, thus potentially participating in the beneficial actions of some commensal bacteria on insulin sensitivity (Cohen *et al.* 2017). Interestingly, GPR132 is also a receptor for oxidised fatty acids, such as 9-hydroxy-octadecanoic acid, which are sensors of lipid overload and oxidative stress, and are involved in atherosclerosis, one of the consequences of visceral obesity (Vangaveti *et al.* 2010). Oxidised fatty acids can also be produced by commensal bacteria, as in the case of 10-oxo-12(Z)-octadecenoic acid, another linoleic acid metabolite produced by lactic acid bacteria, which enhances energy metabolism by activation of TRPV1 (Kim *et al.* 2017). Since, as mentioned previously, also di- and polyunsaturated eCBome lipids can be oxidised to bioactive compounds, it will be interesting to investigate the possibility that gut bacteria produce these metabolites using intestinal epithelial cell-originating eCB-like mediators, such as LEA, as precursors.

In summary, several recent studies seem to support the concept that the gut microbiome and eCBome signalling communicate for the fine-tuning of lipid and glucose metabolism under both physiological and dysmetabolic conditions, and in gastrointestinal tissues as well as in the adipose tissue and brain (Fig. 3). The molecular mechanisms and functional importance of such communication, however, still need to be fully elucidated. Additionally, since, as discussed previously, several metabolites typically produced by commensal bacteria (e.g. SCFA, indole derivatives, etc.) profoundly affect metabolism, it would not be surprising to find that these molecules also indirectly affect eCBome signalling, and studies in this direction need to be fostered.

Future perspectives for the treatment of metabolic and obesity-related disorders

From the literature data discussed in this article, it is possible to take a few messages useful to conceive and

design new therapeutic approaches for the treatment of obesity and related co-morbidities. First, it is clear that both the gut microbiome and the eCB system, particularly with its expansion to the eCBome, should be considered as targets for new pharmacological and nutritional therapies against the metabolic syndrome and its hepatic, renal and cardiovascular consequences. In particular, interventions aimed at favouring those commensal species that, by mostly producing metabolically beneficial metabolites like the SCFAs and/or eCB-like mediators, acting at insulin-sensitising and weight-reducing molecular targets, should be sought after. These interventions potentially include high fibre diets, prebiotics and probiotics, but, in the future, may also encompass 'post-biotics', that is, metabolites and proteins isolated from beneficial commensal microorganisms and produced by the latter following interactions with the host and/or administration of certain micro and macronutrients. A typical example of research in this direction is represented by recent studies with *A. mucinifila* and one of its proteins (Plovier *et al.* 2017). On the other hand, pharmacological targeting of previously known molecular targets that are increasingly becoming considered as eCBome receptors beyond CB1 and CB2, that is, GPR55, GPR18, GPR119 and PPAR α and γ , should continue to be pursued. New eCBome targets, such as GPR110 and GPR132, known to play a homeostatic role in inflammation (Kern *et al.* 2018, Park *et al.* 2019), should start to be investigated also in the context of metabolic control and obesity. Conversely, beyond its well established anti-inflammatory function and potential exploitation against obesity-related atherogenesis, steatosis and heart and kidney dysfunction (Gruden *et al.* 2016, Pacher *et al.* 2018), the potential beneficial role of CB2 in obesity and insulin sensitivity should be clarified. Also in the case of therapeutic eCBome targeting, nutritional approaches, such as those providing high intake of oleic and omega-3 fatty acids, should be studied more in depth, with a closer eye on the extent to which they may lead to increased beneficial eCBome (i.e. mediated by OEA, PEA, oleoyl- and linoleoyl-glycerol and DHEA) signalling at the expense of dysmetabolic eCB-mediated CB1 signalling (Castonguay-Paradis *et al.* 2020). This should be done without diminishing too much the otherwise pro-homeostatic role of the two cannabinoid receptors (Di Marzo 2018).

In fact, all these strategies should always keep into account the fact that eCBs and eCB-like molecules, similar to many gut microbiota-derived molecules, are multi-target mediators, and hence manipulation of their levels might cause unpredictable results. Additionally, strategies aiming at targeting the gut microbiome and the eCBome should

take into consideration the high degree of interaction that is emerging between these two 'omes'. These limitations, together with the acquisition of 'big data' from the plethora of ongoing studies on these topics, will require the ever-increasing application of systems biology, machine learning and other bioinformatics methodologies (Camacho *et al.* 2018) to these new therapeutic approaches.

No matter how challenging, these efforts might nevertheless be rewarding, also in consideration of the several 'non-cardiometabolic' co-morbidities that often accompany obesity, T2D and the metabolic syndrome. These include anxiety and depression (Simon *et al.* 2006) and different types of cancer (Lauby-Secretan *et al.* 2016). Once again, such co-morbidities have been associated with both gut microbiome and eCBome dysfunction (Ligresti *et al.* 2016, Di Marzo 2018, Rea *et al.* 2020, Rossi *et al.* 2020). Hence, it is possible that therapies aiming at modulating these two complex systems and restoring their pro-homeostatic role in the control of energy metabolism, may result beneficial also for obesity-related affective disorders and malignancies, even beyond their effect on obesity and the metabolic syndrome.

In summary, the future of the research on the gut microbiome and the emerging eCBome has never looked more challenging and exciting, and will likely bring new solutions for the treatment of a plethora of pathological conditions, both related and not to disrupted control of energy metabolism.

Declaration of interest

The authors declare that there is no conflict of interest could be perceived as prejudicing the impartiality of this review.

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References

- Abdulnour J, Yasari S, Rabasa-Lhoret R, Faraj M, Petrosino S, Piscitelli F, Prud'Homme D & Di Marzo V 2014 Circulating endocannabinoids in insulin sensitive vs. insulin resistant obese postmenopausal women. A MONET group study. *Obesity* **22** 211–216. (<https://doi.org/10.1002/oby.20498>)

- Allin KH, Tremaroli V, Caesar R, Jensen BAH, Damgaard MTF, Bahl MI, Licht TR, Hansen TH, Nielsen T, Dantoft TM, *et al.* 2018 Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* **61** 810–820. (<https://doi.org/10.1007/s00125-018-4550-1>)
- Anagnostopoulou D, Rakiec C, Wood J, Pandarinathan L, Zvonok N, Makriyannis A & Siafaka-Kapadai A 2010 Identification of endocannabinoids and related N-acyl ethanolamines in tetrahymena. A new class of compounds for Tetrahymena. *Protist* **161** 452–465. (<https://doi.org/10.1016/j.protis.2009.12.004>)
- Araque A, Castillo PE, Manzoni OJ & Tonini R 2017 Synaptic functions of endocannabinoid signaling in health and disease. *Neuropharmacology* **124** 13–24. (<https://doi.org/10.1016/j.neuropharm.2017.06.017>)
- Azar S, Sherf-Dagan S, Nemirovski A, Webb M, Raziel A, Keidar A, Goitein D, Sakran N, Shibolet O, Tam J, *et al.* 2019 Circulating endocannabinoids are reduced following bariatric surgery and associated with improved metabolic homeostasis in humans. *Obesity Surgery* **29** 268–276. (<https://doi.org/10.1007/s11695-018-3517-0>)
- Balsevich G, Petrie GN & Hill MN 2017 Endocannabinoids: effectors of glucocorticoid signaling. *Frontiers in Neuroendocrinology* **47** 86–108. (<https://doi.org/10.1016/j.yfrne.2017.07.005>)
- Baskaran P, Krishnan V, Ren J & Thyagarajan B 2016 Capsaicin induces browning of white adipose tissue and counters obesity by activating TRPV1 channel-dependent mechanisms. *British Journal of Pharmacology* **173** 2369–2389. (<https://doi.org/10.1111/bph.13514>)
- Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, Chen X, Coccolin L, Eversole K, Corral GH, *et al.* 2020 Microbiome definition re-visited: old concepts and new challenges. *Microbiome* **8** 103. (<https://doi.org/10.1186/s40168-020-00875-0>)
- Bisogno T, Melck D, Bobrov MYu, Gretskey NM, Bezuglov VV, De Petrocellis L & Di Marzo V 2000 N-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. *Biochemical Journal* **351** 817–824. (<https://doi.org/10.1042/bj3510817>)
- Blüher M, Engeli S, Klötting N, Berndt J, Fasshauer M, Bätke S, Pacher P, Schön MR, Jordan J & Stumvoll M 2006 Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* **55** 3053–3060. (<https://doi.org/10.2337/db06-0812>)
- Burstein SH 2018 N-acyl amino acids (elmiric acids): endogenous signaling molecules with therapeutic potential. *Molecular Pharmacology* **93** 228–238. (<https://doi.org/10.1124/mol.117.110841>)
- Camacho DM, Collins KM, Powers RK, Costello JC & Collins JJ 2018 Next-generation machine learning for biological networks. *Cell* **173** 1581–1592. (<https://doi.org/10.1016/j.cell.2018.05.015>)
- Canfora EE, Meex RCR, Venema K & Blaak EE 2019 Gut microbial metabolites in obesity, NAFLD and T2DM. *Nature Reviews: Endocrinology* **15** 261–273. (<https://doi.org/10.1038/s41574-019-0156-z>)
- Cani PD 2019 Microbiota and metabolites in metabolic diseases. *Nature Reviews: Endocrinology* **15** 69–70. (<https://doi.org/10.1038/s41574-018-0143-9>)
- Cani PD, Plovier H, Van Hul M, Geurts L, Delzenne NM, Druart C & Everard A 2016 Endocannabinoids – at the crossroads between the gut microbiota and host metabolism. *Nature Reviews: Endocrinology* **12** 133–143. (<https://doi.org/10.1038/nrendo.2015.211>)
- Castonguay-Paradis S, Lacroix S, Rochefort G, Parent L, Perron J, Martin C, Lamarche B, Raymond F, Flament N, Di Marzo V, *et al.* 2020 Dietary fatty acid intake and gut microbiota determine circulating endocannabinoidome signaling beyond the effect of body fat. *Scientific Reports* **10** 15975. (<https://doi.org/10.1038/s41598-020-72861-3>)
- Chemin J, Cazade M & Lory P 2014 Modulation of T-type calcium channels by bioactive lipids. *Pflügers Archiv* **466** 689–700. (<https://doi.org/10.1007/s00424-014-1467-5>)
- Christie S, Wittert GA, Li H & Page AJ 2018 Involvement of TRPV1 channels in energy homeostasis. *Frontiers in Endocrinology* **9** 420. (<https://doi.org/10.3389/fendo.2018.00420>)
- Chu CJ, Huang SM, De Petrocellis L, Bisogno T, Ewing SA, Miller JD, Zipkin RE, Daddario N, Appendino G, Di Marzo V, *et al.* 2003 N-oleoyldopamine, a novel endogenous capsaicin-like lipid that produces hyperalgesia. *Journal of Biological Chemistry* **278** 13633–13639. (<https://doi.org/10.1074/jbc.M211231200>)
- Cluny NL, Keenan CM, Reimer RA, Le Foll B & Sharkey KA 2015 Prevention of diet-induced obesity effects on body weight and gut microbiota in mice treated chronically with Δ9-tetrahydrocannabinol. *PLoS ONE* **10** e0144270. (<https://doi.org/10.1371/journal.pone.0144270>)
- Coccarello R & Maccarrone M 2018 Hedonic eating and the 'delicious circle': from lipid-derived mediators to brain dopamine and back. *Frontiers in Neuroscience* **12** 271. (<https://doi.org/10.3389/fnins.2018.00271>)
- Cohen LJ, Kang HS, Chu J, Huang YH, Gordon EA, Reddy BVB, Ternei MA, Craig JW & Brady SF 2015 Functional metagenomic discovery of bacterial effectors in the human microbiome and isolation of commensamide, a GPCR G2A/132 agonist. *PNAS* **112** E4825–E4834. (<https://doi.org/10.1073/pnas.1508737112>)
- Cohen LJ, Esterhazy D, Kim SH, Lemetre C, Aguilar RR, Gordon EA, Pickard AJ, Cross JR, Emiliano AB, Han SM, *et al.* 2017 Commensal bacteria make GPCR ligands that mimic human signalling molecules. *Nature* **549** 48–53. (<https://doi.org/10.1038/nature23874>)
- Côté M, Matias I, Lemieux I, Petrosino S, Almérás N, Després JP & Di Marzo V 2007 Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *International Journal of Obesity* **31** 692–699. (<https://doi.org/10.1038/sj.ijo.0803539>)
- Cristiano C, Pirozzi C, Coretti L, Cavaliere G, Lama A, Russo R, Lembo F, Mollica MP, Meli R, Calignano A, *et al.* 2018 Palmitoylethanolamide counteracts autistic-like behaviours in BTBR T+tf/J mice: contribution of central and peripheral mechanisms. *Brain, Behavior, and Immunity* **74** 166–175. (<https://doi.org/10.1016/j.bbi.2018.09.003>)
- Cristino L, Busetto G, Imperatore R, Ferrandino I, Palomba L, Silvestri C, Petrosino S, Orlando P, Bentivoglio M, Mackie K, *et al.* 2013 Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons. *PNAS* **110** E2229–E2238. (<https://doi.org/10.1073/pnas.1219485110>)
- Delzenne NM, Rodriguez J, Olivares M & Neyrinck AM 2020 Microbiome response to diet: focus on obesity and related diseases. *Reviews in Endocrine and Metabolic Disorders* **21** 369–380. (<https://doi.org/10.1007/s11154-020-09572-7>)
- Di Marzo V 2008 Endocannabinoids: synthesis and degradation. *Reviews of Physiology, Biochemistry and Pharmacology* **160** 1–24. (https://doi.org/10.1007/112_0505)
- Di Marzo V 2018 New approaches and challenges to targeting the endocannabinoid system. *Nature Reviews: Drug Discovery* **17** 623–639. (<https://doi.org/10.1038/nrd.2018.115>)
- Di Marzo V & Silvestri C 2019 Lifestyle and metabolic syndrome: contribution of the Endocannabinoidome. *Nutrients* **11** 1956. (<https://doi.org/10.3390/nu11081956>)
- Di Marzo V, Goparaju SK, Wang L, Liu J, Bätke S, Jári Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, *et al.* 2001 Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* **410** 822–825. (<https://doi.org/10.1038/35071088>)
- Di Marzo V, Verrijken A, Hakkarainen A, Petrosino S, Mertens I, Lundbom N, Piscitelli F, Westerbacka J, Soro-Paavonen A, Matias I, *et al.* 2009 Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. *European Journal of Endocrinology* **161** 715–722. (<https://doi.org/10.1530/EJE-09-0643>)
- Di Paola M, Bonechi E, Provensi G, Costa A, Clarke G, Ballerini C, De Filippo C & Passani MB 2018 Oleoylethanolamide treatment affects gut microbiota composition and the expression of intestinal cytokines in Peyer's patches of mice. *Scientific Reports* **8** 14881. (<https://doi.org/10.1038/s41598-018-32925-x>)
- Donvito G, Piscitelli F, Muldoon P, Jackson A, Vitale RM, D'Aniello E, Giordano C, Ignatowska-Jankowska BM, Mustafa MA, Guida F, *et al.* 2019 N-oleoyl-glycine reduces nicotine reward and withdrawal in

- mice. *Neuropharmacology* **148** 320–331. (<https://doi.org/10.1016/j.neuropharm.2018.03.020>)
- Doris JM, Millar SA, Idris I & O'Sullivan SE 2019 Genetic polymorphisms of the endocannabinoid system in obesity and diabetes. *Diabetes, Obesity and Metabolism* **21** 382–387. (<https://doi.org/10.1111/dom.13504>)
- Ellekilde M, Selfjord E, Larsen CS, Jaksevic M, Rune I, Tranberg B, Vogensen FK, Nielsen DS, Bahl MI, Licht TR, *et al.* 2014 Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. *Scientific Reports* **4** 5922. (<https://doi.org/10.1038/srep05922>)
- Ellermann M, Pacheco AR, Jimenez AG, Russell RM, Cuesta S, Kumar A, Zhu W, Vale G, Martin SA, Raj P, *et al.* 2020 Endocannabinoids inhibit the induction of virulence in enteric pathogens. *Cell* **183** 650.e15–665.e15. (<https://doi.org/10.1016/j.cell.2020.09.022>)
- Elphick MR 2012 The evolution and comparative neurobiology of endocannabinoid signalling. *Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences* **367** 3201–3215. (<https://doi.org/10.1098/rstb.2011.0394>)
- Evans JM, Morris LS & Marchesi JR 2013 The gut microbiome: the role of a virtual organ in the endocrinology of the host. *Journal of Endocrinology* **218** R37–R47. (<https://doi.org/10.1530/JOE-13-0131>)
- Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, *et al.* 2013 Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *PNAS* **110** 9066–9071. (<https://doi.org/10.1073/pnas.1219451110>)
- Everard A, Geurts L, Caesar R, Van Hul M, Matamoros S, Duparc T, Denis RGP, Cochez P, Pierard F, Castel J, *et al.* 2014 Intestinal epithelial MyD88 is a sensor switching host metabolism towards obesity according to nutritional status. *Nature Communications* **5** 5648. (<https://doi.org/10.1038/ncomms6648>)
- Everard A, Plovier H, Rastelli M, Van Hul M, de Wouters d'Oplinter A, Geurts L, Druart C, Robine S, Delzenne NM, Muccioli GG, *et al.* 2019 Intestinal epithelial N-acylphosphatidylethanolamine phospholipase D links dietary fat to metabolic adaptations in obesity and steatosis. *Nature Communications* **10** 457. (<https://doi.org/10.1038/s41467-018-08051-7>)
- Fanelli F, Mezzullo M, Belluomo I, Di Lallo VD, Baccini M, Ibarra Gasparini D, Casadio E, Mastroroberto M, Vicennati V, Gambineri A, *et al.* 2017 Plasma 2-arachidonoylglycerol is a biomarker of age and menopause related insulin resistance and dyslipidemia in lean but not in obese men and women. *Molecular Metabolism* **6** 406–415. (<https://doi.org/10.1016/j.molmet.2017.03.005>)
- Fanelli F, Mezzullo M, Repaci A, Belluomo I, Ibarra Gasparini D, Di Dalmazi G, Mastroroberto M, Vicennati V, Gambineri A, Morselli-Labate AM, *et al.* 2018 Profiling plasma N-acylethanolamine levels and their ratios as a biomarker of obesity and dysmetabolism. *Molecular Metabolism* **14** 82–94. (<https://doi.org/10.1016/j.molmet.2018.06.002>)
- Farzi A, Fröhlich EE & Holzer P 2018 Gut microbiota and the neuroendocrine system. *Neurotherapeutics* **15** 5–22. (<https://doi.org/10.1007/s13311-017-0600-5>)
- Feldman M, Smoum R, Mechoulam R & Steinberg D 2020 Potential combinations of endocannabinoid/endocannabinoid-like compounds and antibiotics against methicillin-resistant *Staphylococcus aureus*. *PLoS ONE* **15** e0231583. (<https://doi.org/10.1371/journal.pone.0231583>)
- Fornelos N, Franzosa EA, Bishai J, Annand JW, Oka A, Lloyd-Price J, Arthur TD, Garner A, Avila-Pacheco J, Haiser HJ, *et al.* 2020 Growth effects of N-acylethanolamines on gut bacteria reflect altered bacterial abundances in inflammatory bowel disease. *Nature Microbiology* **5** 486–497. (<https://doi.org/10.1038/s41564-019-0655-7>)
- Forte N, Fernández-Rilo AC, Palomba L, Di Marzo V & Cristino L 2020 Obesity affects the microbiota-gut-brain axis and the regulation thereof by endocannabinoids and related mediators. *International Journal of Molecular Sciences* **21** 1554. (<https://doi.org/10.3390/ijms21051554>)
- Foster JR, Ueno S, Chen MX, Harvey J, Dowell SJ, Irving AJ & Brown AJ 2019 N-Palmitoylglycine and other N-acylamides activate the lipid receptor G2A/GPR132. *Pharmacology Research and Perspectives* **7** e00542. (<https://doi.org/10.1002/prp2.542>)
- Geurts L, Everard A, Van Hul M, Essaghir A, Duparc T, Matamoros S, Plovier H, Castel J, Denis RGP, Bergiers M, *et al.* 2015 Adipose tissue NAPE-PLD controls fat mass development by altering the browning process and gut microbiota. *Nature Communications* **6** 6495. (<https://doi.org/10.1038/ncomms7495>)
- Grevengoed TJ, Trammell SAJ, McKinney MK, Petersen N, Cardone RL, Svenningsen JS, Ogasawara D, Nexøe-Larsen CC, Knop FK, Schwartz TW, *et al.* 2019 N-acyl taurines are endogenous lipid messengers that improve glucose homeostasis. *PNAS* **116** 24770–24778. (<https://doi.org/10.1073/pnas.1916288116>)
- Gruden G, Barutta F, Kunos G & Pacher P 2016 Role of the endocannabinoid system in diabetes and diabetic complications. *British Journal of Pharmacology* **173** 1116–1127. (<https://doi.org/10.1111/bph.13226>)
- Guida F, Turco F, Iannotta M, De Gregorio D, Palumbo I, Sarnelli G, Furiano A, Napolitano F, Boccella S, Luongo L, *et al.* 2018 Antibiotic-induced microbiota perturbation causes gut endocannabinoidome changes, hippocampal neuroglial reorganization and depression in mice. *Brain, Behavior, and Immunity* **67** 230–245. (<https://doi.org/10.1016/j.bbi.2017.09.001>)
- Guida F, Boccella S, Belardo C, Iannotta M, Piscitelli F, De Filippis F, Paino S, Ricciardi F, Siniscalco D, Marabese I, *et al.* 2020 Altered gut microbiota and endocannabinoid system tone in vitamin D deficiency-mediated chronic pain. *Brain, Behavior, and Immunity* **85** 128–141. (<https://doi.org/10.1016/j.bbi.2019.04.006>)
- Hansen HS & Vana V 2019 Non-endocannabinoid N-acylethanolamines and 2-monoacylglycerols in the intestine. *British Journal of Pharmacology* **176** 1443–1454. (<https://doi.org/10.1111/bph.14175>)
- Hasan N & Yang H 2019 Factors affecting the composition of the gut microbiota, and its modulation. *PeerJ* **7** e7502. (<https://doi.org/10.7717/peerj.7502>)
- Hernández MAG, Canfora EE, Jocken JWE & Blaak EE 2019 The short-chain fatty acid acetate in body weight control and insulin sensitivity. *Nutrients* **11** 1943. (<https://doi.org/10.3390/nu11081943>)
- Hong F, Pan S, Guo Y, Xu P & Zhai Y 2019 PPARs as nuclear receptors for nutrient and energy metabolism. *Molecules* **24** 2545. (<https://doi.org/10.3390/molecules24142545>)
- Hui S, Huang L, Wang X, Zhu X, Zhou M, Chen M, Yi L & Mi M 2020 Capsaicin improves glucose homeostasis by enhancing glucagon-like peptide-1 secretion through the regulation of bile acid metabolism via the remodeling of the gut microbiota in male mice. *FASEB Journal* **34** 8558–8573. (<https://doi.org/10.1096/fj.201902618RR>)
- Imperatore R, Morello G, Luongo L, Taschler U, Romano R, De Gregorio D, Belardo C, Maione S, Di Marzo V & Cristino L 2015 Genetic deletion of monoacylglycerol lipase leads to impaired cannabinoid receptor CB₁R signaling and anxiety-like behavior. *Journal of Neurochemistry* **135** 799–813. (<https://doi.org/10.1111/jnc.13267>)
- Jimenez-Blasco D, Busquets-Garcia A, Hebert-Chatelain E, Serrat R, Vicente-Gutierrez C, Ioannidou C, Gómez-Sotres P, Lopez-Fabuel I, Resch-Beusher M, Resel E, *et al.* 2020 Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* **583** 603–608. (<https://doi.org/10.1038/s41586-020-2470-y>)
- Kang C, Wang B, Kaliannan K, Wang X, Lang H, Hui S, Huang L, Zhang Y, Zhou M, Chen M, *et al.* 2017 Gut microbiota mediates the protective effects of dietary capsaicin against chronic low-grade inflammation and associated obesity induced by high-fat diet. *mBio* **8** e00470-17. (<https://doi.org/10.1128/mBio.00470-17>)
- Karwad MA, Couch DG, Wright KL, Tufarelli C, Larvin M, Lund J & O'Sullivan SE 2019 Endocannabinoids and endocannabinoid-like compounds modulate hypoxia-induced permeability in CaCo-2 cells

- via CB1, TRPV1, and PPAR α . *Biochemical Pharmacology* **168** 465–472. (<https://doi.org/10.1016/j.bcp.2019.07.017>)
- Kentish SJ & Page AJ 2015 The role of gastrointestinal vagal afferent fibres in obesity. *Journal of Physiology* **593** 775–786. (<https://doi.org/10.1113/jphysiol.2014.278226>)
- Kern K, Schäfer SMG, Cohnen J, Pierre S, Osthuys T, Tarighi N, Hohmann S, Ferreiros N, Brüne B, Weigert A, *et al.* 2018 The G2A receptor controls polarization of macrophage by determining their localization within the inflamed tissue. *Frontiers in Immunology* **9** 2261. (<https://doi.org/10.3389/fimmu.2018.02261>)
- Kim M, Furuzono T, Yamakuni K, Li Y, Kim YI, Takahashi H, Ohue-Kitano R, Jheng HF, Takahashi N, Kano Y, *et al.* 2017 10-Oxo-12(Z)-octadecenoic acid, a linoleic acid metabolite produced by gut lactic acid bacteria, enhances energy metabolism by activation of TRPV1. *FASEB Journal* **31** 5036–5048. (<https://doi.org/10.1096/fj.201700151R>)
- Kirkham TC, Williams CM, Fezza F & Di Marzo V 2002 Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *British Journal of Pharmacology* **136** 550–557. (<https://doi.org/10.1038/sj.bjp.0704767>)
- Koh A & Bäckhed F 2020 From association to causality: the role of the gut microbiota and its functional products on host metabolism. *Molecular Cell* **78** 584–596. (<https://doi.org/10.1016/j.molcel.2020.03.005>)
- Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, Gonzalez A, Werner JJ, Angenent LT, Knight R, *et al.* 2012 Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* **150** 470–480. (<https://doi.org/10.1016/j.cell.2012.07.008>)
- Kumawat VS & Kaur G 2019 Therapeutic potential of cannabinoid receptor 2 in the treatment of diabetes mellitus and its complications. *European Journal of Pharmacology* **862** 172628. (<https://doi.org/10.1016/j.ejphar.2019.172628>)
- Lacroix S, Pechereau F, Leblanc N, Boubertakh B, Houde A, Martin C, Flamand N, Silvestri C, Raymond F, Di Marzo V, *et al.* 2019 Rapid and concomitant gut microbiota and endocannabinoidome response to diet-induced obesity in mice. *mSystems* **4** e00407-19. (<https://doi.org/10.1128/mSystems.00407-19>)
- Laleh P, Yaser K & Alireza O 2019 Oleoylethanolamide: a novel pharmaceutical agent in the management of obesity-an updated review. *Journal of Cellular Physiology* **234** 7893–7902. (<https://doi.org/10.1002/jcp.27913>)
- Lau BK, Cota D, Cristino L & Borgland SL 2017 Endocannabinoid modulation of homeostatic and non-homeostatic feeding circuits. *Neuropharmacology* **124** 38–51. (<https://doi.org/10.1016/j.neuropharm.2017.05.033>)
- Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K & International Agency for Research on Cancer Handbook Working Group 2016 Body fatness and cancer – viewpoint of the IARC Working Group. *New England Journal of Medicine* **375** 794–798. (<https://doi.org/10.1056/NEJMs1606602>)
- Lauffer LM, Jakoubov R & Brubaker PL 2009 GPR119 is essential for oleoylethanolamide-induced glucagon-like peptide-1 secretion from the intestinal enteroendocrine L-cell. *Diabetes* **58** 1058–1066. (<https://doi.org/10.2337/db08-1237>)
- Leggett JD, Aspley S, Beckett SRG, D'Antona AM, Kendall DA & Kendall DA 2004 Oleamide is a selective endogenous agonist of rat and human CB1 cannabinoid receptors. *British Journal of Pharmacology* **141** 253–262. (<https://doi.org/10.1038/sj.bjp.0705607>)
- Ligresti A, De Petrocellis L & Di Marzo V 2016 From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiological Reviews* **96** 1593–1659. (<https://doi.org/10.1152/physrev.00002.2016>)
- Lipina C, Walsh SK, Mitchell SE, Speakman JR, Wainwright CL & Hundal HS 2019 GPR55 deficiency is associated with increased adiposity and impaired insulin signaling in peripheral metabolic tissues. *FASEB Journal* **33** 1299–1312. (<https://doi.org/10.1096/fj.201800171R>)
- Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P & Balamurugan R 2020 The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? *Nutrients* **12** 1474. (<https://doi.org/10.3390/nu12051474>)
- Manca C, Boubertakh B, Leblanc N, Deschênes T, Lacroix S, Martin C, Houde A, Veilleux A, Flamand N, Muccioli GG, *et al.* 2020a Germ-free mice exhibit profound gut microbiota-dependent alterations of intestinal endocannabinoidome signaling. *Journal of Lipid Research* **61** 70–85. (<https://doi.org/10.1194/jlr.RA119000424>)
- Manca C, Shen M, Boubertakh B, Martin C, Flamand N, Silvestri C & Di Marzo V 2020b Alterations of brain endocannabinoidome signaling in germ-free mice. *Biochimica et Biophysica Acta: Molecular and Cell Biology of Lipids* **1865** 158786. (<https://doi.org/10.1016/j.bbalip.2020.158786>)
- Marrone MC, Morabito A, Giustizieri M, Chiurchiù V, Leuti A, Mattioli M, Marinelli S, Riganti L, Lombardi M, Murana E, *et al.* 2017 TRPV1 channels are critical brain inflammation detectors and neuropathic pain biomarkers in mice. *Nature Communications* **8** 15292. (<https://doi.org/10.1038/ncomms15292>)
- Martins CJdeM, Genelhu V, Pimentel MMG, Celoria BMJ, Mangia RF, Aveta T, Silvestri C, Di Marzo V & Francischetti EA 2015 Circulating endocannabinoids and the polymorphism 385C>A in fatty acid amide hydrolase (FAAH) gene may identify the obesity phenotype related to cardiometabolic risk: a study conducted in a Brazilian population of complex interethnic admixture. *PLoS ONE* **10** e0142728. (<https://doi.org/10.1371/journal.pone.0142728>)
- Matias I, Gatta-Cherifi B, Tabarin A, Clark S, Leste-Lasserre T, Marsicano G, Piazza PV & Cota D 2012 Endocannabinoids measurement in human saliva as potential biomarker of obesity. *PLoS ONE* **7** e42399. (<https://doi.org/10.1371/journal.pone.0042399>)
- Meadows A, Lee JH, Wu CS, Wei Q, Pradhan G, Yafi M, Lu HC & Sun Y 2016 Deletion of G-protein-coupled receptor 55 promotes obesity by reducing physical activity. *International Journal of Obesity* **40** 417–424. (<https://doi.org/10.1038/ijo.2015.209>)
- Mehrpouya-Bahrami P, Chitralla KN, Ganewatta MS, Tang C, Murphy EA, Enos RT, Velazquez KT, McCellan J, Nagarkatti M & Nagarkatti P 2017 Blockade of CB1 cannabinoid receptor alters gut microbiota and attenuates inflammation and diet-induced obesity. *Scientific Reports* **7** 15645. (<https://doi.org/10.1038/s41598-017-15154-6>)
- Merkel O, Schmid PC, Paltauf F & Schmid HHO 2005 Presence and potential signaling function of N-acyl ethanolamines and their phospholipid precursors in the yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta* **1734** 215–219. (<https://doi.org/10.1016/j.bbalip.2005.03.004>)
- Morello G, Imperatore R, Palomba L, Finelli C, Labruna G, Pasanisi F, Sacchetti L, Buono L, Piscitelli F, Orlando P, *et al.* 2016 Orexin-A represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. *PNAS* **113** 4759–4764. (<https://doi.org/10.1073/pnas.1521304113>)
- Motter AL & Ahern GP 2008 TRPV1-null mice are protected from diet-induced obesity. *FEBS Letters* **582** 2257–2262. (<https://doi.org/10.1016/j.febslet.2008.05.021>)
- Movahed P, Jönsson BAG, Birnir B, Wingstrand JA, Jørgensen TD, Ermund A, Sterner O, Zygmunt PM & Högestätt ED 2005 Endogenous unsaturated C18 N-acyl ethanolamines are vanilloid receptor (TRPV1) agonists. *Journal of Biological Chemistry* **280** 38496–38504. (<https://doi.org/10.1074/jbc.M507429200>)
- Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM & Cani PD 2010 The endocannabinoid system links gut microbiota to adipogenesis. *Molecular Systems Biology* **6** 392. (<https://doi.org/10.1038/msb.2010.46>)
- Naghipour S, Cox AJ, Peart JN, Du Toit EF & Headrick JP 2020 Trimethylamine-N-oxide: heart of the microbiota-cardiovascular

- disease nexus? *Nutrition Research Reviews* 1–22. (<https://doi.org/10.1017/S0954422420000177>)
- Noerman S, Kolehmainen M & Hanhineva K 2020 Profiling of endogenous and gut microbial metabolites to indicate Metabotype-specific dietary responses: a systematic review. *Advances in Nutrition* **11** 1237–1254. (<https://doi.org/10.1093/advances/nmaa031>)
- Ortar G, Cascio MG, De Petrocellis L, Morera E, Rossi F, Schiano-Moriello A, Nalli M, de Novellis V, Woodward DF, Maione S, *et al.* 2007 New N-arachidonoylserotonin analogues with potential 'dual' mechanism of action against pain. *Journal of Medicinal Chemistry* **50** 6554–6569. (<https://doi.org/10.1021/jm070678q>)
- Pacher P, Steffens S, Haskó G, Schindler TH & Kunos G 2018 Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. *Nature Reviews: Cardiology* **15** 151–166. (<https://doi.org/10.1038/nrcardio.2017.130>)
- Page AJ, Hatzinikolas G, Vincent AD, Cavuoto P & Wittert GA 2019 The TRPV1 channel regulates glucose metabolism. *American Journal of Physiology: Endocrinology and Metabolism* **317** E667–E676. (<https://doi.org/10.1152/ajpendo.00102.2019>)
- Park T, Chen H & Kim HY 2019 GPR110 (ADGRF1) mediates anti-inflammatory effects of N-docosahexaenoyl ethanolamine. *Journal of Neuroinflammation* **16** 225. (<https://doi.org/10.1186/s12974-019-1621-2>)
- Pascoal LB, Bombassaro B, Ramalho AF, Coope A, Moura RF, Correa-da-Silva F, Ignacio-Souza L, Razolli D, de Oliveira D, Catharino R, *et al.* 2017 Resolvin RvD2 reduces hypothalamic inflammation and rescues mice from diet-induced obesity. *Journal of Neuroinflammation* **14** 5. (<https://doi.org/10.1186/s12974-016-0777-2>)
- Pastor A, Fernández-Aranda F, Fitó M, Jiménez-Murcia S, Botella C, Fernández-Real JM, Frühbeck G, Tinahones FJ, Fagundo AB, Rodriguez J, *et al.* 2016 A lower olfactory capacity is related to higher circulating concentrations of endocannabinoid 2-arachidonoylglycerol and higher body mass index in women. *PLoS ONE* **11** e0148734. (<https://doi.org/10.1371/journal.pone.0148734>)
- Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, *et al.* 2017 A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nature Medicine* **23** 107–113. (<https://doi.org/10.1038/nm.4236>)
- Poursharifi P, Madiraju SRM & Prentki M 2017 Monoacylglycerol signalling and ABHD6 in health and disease. *Diabetes, Obesity and Metabolism* **19** (Supplement 1) 76–89. (<https://doi.org/10.1111/dom.13008>)
- Ramírez-Orozco RE, García-Ruiz R, Morales P, Villalón CM, Villafán-Bernal JR & Marichal-Cancino BA 2019 Potential metabolic and behavioural roles of the putative endocannabinoid receptors GPR18, GPR55 and GPR119 in feeding. *Current Neuropharmacology* **17** 947–960. (<https://doi.org/10.2174/1570159X17666190118143014>)
- Rea K, Dinan TG & Cryan JF 2020 Gut microbiota: a perspective for psychiatrists. *Neuropsychobiology* **79** 50–62. (<https://doi.org/10.1159/000504495>)
- Rogali AR, Oka A & Sartor RB 2020 Strategies to dissect host-microbial immune interactions that determine mucosal homeostasis vs. intestinal inflammation in gnotobiotic mice. *Frontiers in Immunology* **11** 214. (<https://doi.org/10.3389/fimmu.2020.00214>)
- Rosenstand K, Andersen K, Terp R, Gennemark P, Ellman DG, Reznichenko A, Lambertsen KL, Vanhoutte PM, Hansen PBL & Sørensen P 2020 Deficiency of T-type voltage-gated calcium channels results in attenuated weight gain and improved endothelium-dependent dilatation of resistance vessels induced by a high-fat diet in mice. *Journal of Physiology and Biochemistry* **76** 135–145. (<https://doi.org/10.1007/s13105-020-00728-2>)
- Rossi T, Vergara D, Fanini F, Maffia M, Bravaccini S & Pirini F 2020 Microbiota-derived metabolites in tumor progression and metastasis. *International Journal of Molecular Sciences* **21** 5786. (<https://doi.org/10.3390/ijms21165786>)
- Rouzer CA & Marnett LJ 2011 Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chemical Reviews* **111** 5899–5921. (<https://doi.org/10.1021/cr2002799>)
- Ruiz de Azua I & Lutz B 2019 Multiple endocannabinoid-mediated mechanisms in the regulation of energy homeostasis in brain and peripheral tissues. *Cellular and Molecular Life Sciences* **76** 1341–1363. (<https://doi.org/10.1007/s00018-018-2994-6>)
- Saghatelian A, Trauger SA, Want EJ, Hawkins EG, Siuzdak G & Cravatt BF 2004 Assignment of endogenous substrates to enzymes by global metabolite profiling. *Biochemistry* **43** 14332–14339. (<https://doi.org/10.1021/bi0480335>)
- Schiano Moriello A, López Chinarro S, Novo Fernández O, Eras J, Amodeo P, Canela-Garayoa R, Vitale RM, Di Marzo V & De Petrocellis L 2018 Elongation of the hydrophobic chain as a molecular switch: discovery of capsaicin derivatives and endogenous lipids as potent transient receptor potential vanilloid channel 2 antagonists. *Journal of Medicinal Chemistry* **61** 8255–8281. (<https://doi.org/10.1021/acs.jmedchem.8b00734>)
- Schlievert PM, Kilgore SH, Kaus GM, Ho TD & Ellermeier CD 2018 Glycerol monolaurate (GML) and a nonaqueous five-percent GML gel kill bacillus and clostridium spores. *mSphere* **3** e00597-18. (<https://doi.org/10.1128/mSphereDirect.00597-18>)
- Shrader SH & Song ZH 2020 Discovery of endogenous inverse agonists for G protein-coupled receptor 6. *Biochemical and Biophysical Research Communications* **522** 1041–1045. (<https://doi.org/10.1016/j.bbrc.2019.12.004>)
- Silva AKS & Peixoto CA 2018 Role of peroxisome proliferator-activated receptors in non-alcoholic fatty liver disease inflammation. *Cellular and Molecular Life Sciences* **75** 2951–2961. (<https://doi.org/10.1007/s00018-018-2838-4>)
- Silvestri C & Di Marzo V 2013 The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metabolism* **17** 475–490. (<https://doi.org/10.1016/j.cmet.2013.03.001>)
- Simon GE, Von Korff M, Saunders K, Miglioretti DL, Crane PK, van Belle G & Kessler RC 2006 Association between obesity and psychiatric disorders in the US adult population. *Archives of General Psychiatry* **63** 824–830. (<https://doi.org/10.1001/archpsyc.63.7.824>)
- Sionov RV, Feldman M, Smoum R, Mechoulam R & Steinberg D 2020 Anandamide prevents the adhesion of filamentous *Candida albicans* to cervical epithelial cells. *Scientific Reports* **10** 13728. (<https://doi.org/10.1038/s41598-020-70650-6>)
- Song JX, Ren H, Gao YF, Lee CY, Li SF, Zhang F, Li L & Chen H 2017 Dietary capsaicin improves glucose homeostasis and alters the gut microbiota in obese diabetic ob/ob mice. *Frontiers in Physiology* **8** 602. (<https://doi.org/10.3389/fphys.2017.00602>)
- Sun W, Uchida K & Tominaga M 2017 TRPV2 regulates BAT thermogenesis and differentiation. *Channels* **11** 94–96. (<https://doi.org/10.1080/19336950.2016.1228401>)
- Tanaka M, Sackett S & Zhang Y 2020 Endocannabinoid modulation of microglial phenotypes in neuropathology. *Frontiers in Neurology* **11** 87. (<https://doi.org/10.3389/fneur.2020.00087>)
- Tarragon E & Moreno JJ 2017 Role of endocannabinoids on sweet taste perception, food preference, and obesity-related disorders. *Chemical Senses* **43** 3–16. (<https://doi.org/10.1093/chemse/bjx062>)
- Thethi TK, Sigel A, Japa S, Katalenich B, Liu S, Nguyen T, Larrazolo J, Syu S, Carefoot E, McDuffie R, *et al.* 2020 Racial and sex differences in the polymorphisms of the endocannabinoid receptor genes in obesity. *Journal of Diabetes and its Complications* **34** 107682. (<https://doi.org/10.1016/j.jdiacomp.2020.107682>)
- Urquhart P, Nicolaou A & Woodward DF 2015 Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta* **1851** 366–376. (<https://doi.org/10.1016/j.bbailp.2014.12.015>)

- van Eyk HJ, van Schinkel LD, Kantae V, Dronkers CEA, Westenberg JJM, de Roos A, Lamb HJ, Jukema JW, Harms AC, Hankemeier T, *et al.* 2018 Caloric restriction lowers endocannabinoid tonus and improves cardiac function in type 2 diabetes. *Nutrition and Diabetes* **8** 6. (<https://doi.org/10.1038/s41387-017-0016-7>)
- Vangaveti V, Baune BT & Kennedy RL 2010 Hydroxyoctadecadienoic acids: novel regulators of macrophage differentiation and atherogenesis. *Therapeutic Advances in Endocrinology and Metabolism* **1** 51–60. (<https://doi.org/10.1177/2042018810375656>)
- Verhoeckx KC, Voortman T, Balvers MG, Hendriks HF, M Wortelboer H & Witkamp RF 2011 Presence, formation and putative biological activities of N-acyl serotonins, a novel class of fatty-acid derived mediators, in the intestinal tract. *Biochimica et Biophysica Acta* **1811** 578–586. (<https://doi.org/10.1016/j.bbailip.2011.07.008>)
- Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JFW, Dallinga-Thie GM, Ackermans MT, Serlie MJ, Oozeer R, *et al.* 2012 Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143** 913.e7–916.e7. (<https://doi.org/10.1053/j.gastro.2012.06.031>)
- Wang Q, Imam MU, Yida Z & Wang F 2017 Peroxisome proliferator-activated receptor gamma (PPAR γ) as a target for concurrent management of diabetes and obesity-related cancer. *Current Pharmaceutical Design* **23** 3677–3688. (<https://doi.org/10.2174/1381612823666170704125104>)
- Weersma RK, Zhernakova A & Fu J 2020 Interaction between drugs and the gut microbiome. *Gut* **69** 1510–1519. (<https://doi.org/10.1136/gutjnl-2019-320204>)
- Wolf G 2006 Gut microbiota: a factor in energy regulation. *Nutrition Reviews* **64** 47–50. (<https://doi.org/10.1111/j.1753-4887.2006.tb00173.x>)
- Wu J, Zhu C, Yang L, Wang Z, Wang L, Wang S, Gao P, Zhang Y, Jiang Q, Zhu X, *et al.* 2017 N-oleoylglycine-induced hyperphagia is associated with the activation of agouti-related protein (AgRP) neuron by cannabinoid receptor type 1 (CB1R). *Journal of Agricultural and Food Chemistry* **65** 1051–1057. (<https://doi.org/10.1021/acs.jafc.6b05281>)
- Yoshida K, Kita Y, Tokuoka SM, Hamano F, Yamazaki M, Sakimura K, Kano M & Shimizu T 2019 Monoacylglycerol lipase deficiency affects diet-induced obesity, fat absorption, and feeding behavior in CB1 cannabinoid receptor-deficient mice. *FASEB Journal* **33** 2484–2497. (<https://doi.org/10.1096/fj.201801203R>)
- Zhong X, Harrington JM, Millar SR, Perry IJ, O'Toole PW & Phillips CM 2020 Gut microbiota associations with metabolic health and obesity status in older adults. *Nutrients* **12** 2364. (<https://doi.org/10.3390/nu12082364>)
- Zibolka J, Wolf A, Rieger L, Rothgänger C, Jörns A, Lutz B, Zimmer A, Dehghani F & Bazwinsky-Wutschke I 2020 Influence of cannabinoid receptor deficiency on parameters involved in blood glucose regulation in mice. *International Journal of Molecular Sciences* **21** 3168. (<https://doi.org/10.3390/ijms21093168>)
- Zygmunt PM, Ermund A, Movahed P, Andersson DA, Simonsen C, Jönsson BAG, Blomgren A, Birnir B, Bevan S, Eschalier A, *et al.* 2013 Monoacylglycerols activate TRPV1 – a link between phospholipase C and TRPV1. *PLoS ONE* **8** e81618. (<https://doi.org/10.1371/journal.pone.0081618>)

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