Old Herborn University Seminar Monograph 29:
Microbial metabolism and mammalian physiology - One integrated system.
Editors: Peter J. Heidt, James Versalovic, Andreas Schwiertz, and Volker Rusch.
Old Herborn University Foundation, Herborn, Germany: 63-70 (2015).

THE GUT MICROBIOTA, SHORT CHAIN FATTY ACIDS AND APPETITE

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INTRODUCTION

Modern science has left little doubt that in terms of evolutionary success, bacteria must be at its pinnacle. Every single corner of this planet, and its inhabitants, has a substantial community of bacteria perfectly adapted to their environment. From the depth of the sea to the inners of our own guts, bacteria have learnt to adapt and survive and in many cases have made themselves essential to the overall capacity of its host. Indeed, given the intrinsic role of mitochondria, with its ancestral bacterial endosymbiotic origin, we can safely say that bacteria have not only survived for billions of years, but have been fundamental to the evolution of complex life (*Lane*, 2014). Moreover, and leaving aside mitochondria, bacteria themselves have evolved to symbiotically co-exist with most organisms, conferring them with unique traits and possibilities. To humans, as with all living animals, bacteria have become a unique set of "friends with benefits".

Currently, it is estimated that there are at least 70 different groups of bacteria in the world, however only four appears to inhabit the human gut, forming what has become known as the microbiome (*Macfarlane* et al., 2004). Most of these bacteria are selected at birth, or soon thereafter (*Palmer* et al., 2007), and their overall population appear to be highly dependent on their ability to interact with intestinal derived IgA, which in turn allows them to penetrate the mucous layer protecting this tissue. Here, the microbiota flourishes and is in continuous interaction with our bodies. In ideal conditions, this interaction leads to clear benefits to both parties; however the mechanisms associated with this interaction, both positive and in some cases negative, are not fully understood. What is clear is that more and more research is showing that the synergetic balance between the microbiota and our bodies can be readily disrupted, in many cases in unforeseen ways, by medicines and diet. This arises mainly from the fact that we currently have not proper handle on the extent and depth at which our gut microbiota interact and modulate our own physiology. Indeed, there is increasing evidence that alteration in the microbiome may lead to many chronic disorders, including obesity, type II diabetes, the metabolic syndrome, autoimmune diseases (*Hansen* et al., 2015) and even some neurological and cognitive conditions (*Wang* and *Kasper*, 2014). It is this close correlation between the microbiome disruption and disease development that is driving current research, all of it aimed at understanding the microbiome-host interaction and how we can use this knowledge to prevent and/or treat many modern non-inheritable diseases.

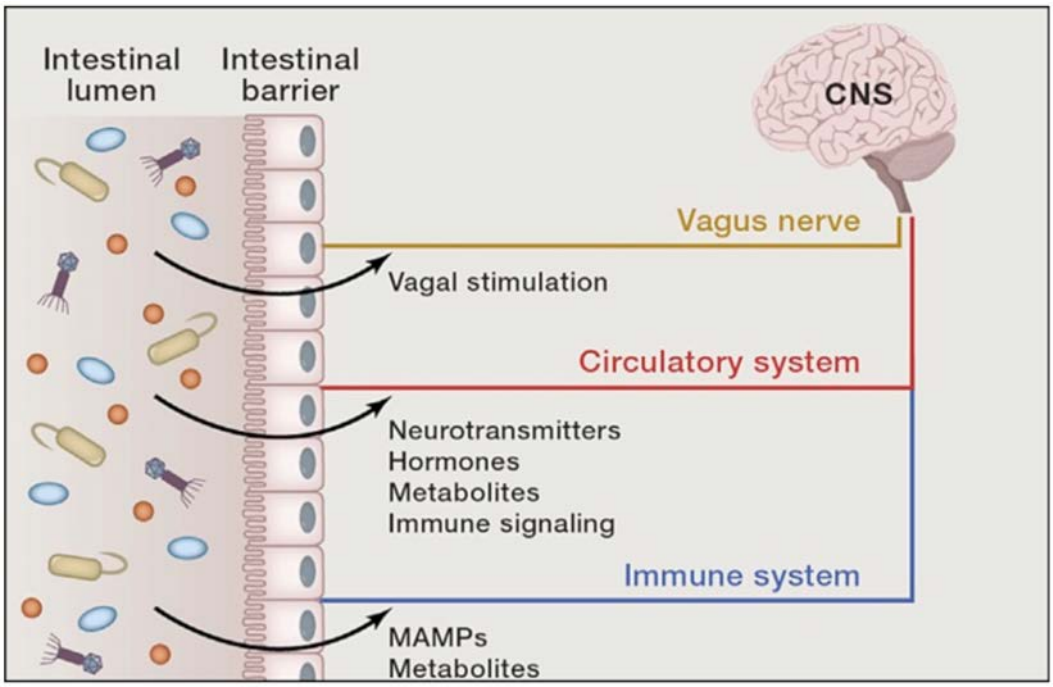

Figure 1: Interaction between the gut microbiota and the brain (Adapted from *Sampson* et al., 2015).

MICROBIOTA-HOST INTERACTION

Broadly speaking, the microbiome interacts with its host in three different, yet linked, manners. Firstly, and at its most basic, the symbiotic relationship between gut microbiota and our bodies leads the former to increase energy availability to our system by digesting food components that would otherwise be lost through the faeces. It is now well accepted that under some dietary conditions the gut microbiota can increase energy supply by well over 20% (*Krajmalnik-Brown* et al., 2012). This energy is principally in the form of short chain fatty acids (SCFAs - mainly acetate, propionate and butyrate), and monosaccharides, together with gases such as CO_2, CH_4, and H_2. Gut microbes have also been shown to produce vitamins, detoxify plant components as well as metabolise medicine in unexpected ways. Secondly, the microbiome can directly interact with our bodies through its modulation of the immune system and the production of neuroactive compounds (*Sekirow* et al., 2010). Indirectly it also modifies levels of many gut peptides which in turn modulate a myriad of anatomical, physiological and metabolic systems, including affecting the blood-brain barrier permeability (*Braniste* et al., 2014). Finally, the gut microbiota derived products e.g. SCFA interact directly with different organs including the brain (*Frost* et al., 2014). Interestingly, yet less understood is the interaction between the gut microbiota and our central nervous system (*Sampson* et al., 2015). This interaction again appears to take three distinctive, yet interconnected paths: directly through the Vagus nerve and indirectly through the circulatory system and the immune system (Figure 1).

In recent years, a number of groups

around the world, including our own, have turned their attention to the modulation of brain function by the gut microbiota through the circulatory system, with especial focus on the production and role of gut-microbiota derived SCFAs in brain function.

SCFA PRODUCTION AND BRAIN METABOLISM

Our gut microbiota is believed to be made up of over 500 bacterial species, with Bacteroidetes and Firmicutes accounting for >90% of the phylotypes (*Eckburg* et al., 2005). A key action of this microbiota population is the production of large amounts of SCFAs. Colonic levels of SCFAs can reach well >100 mM concentration, derived principally from the fermentation of indigestible carbohydrates, unabsorbed sugars, cellulosic/non-cellulosic polysaccharides and some proteins normally found in our diets (*Cummings* et al., 1987). A number of studies with preclinical models and humans have shown that increasing the level of non-digestible carbohydrates in the diet, including inulin, has significant effects on both the colonic microbiota population and the amount of SCFAs produced (*Arora* et al., 2012). As previously mentioned, acetate, propionate and butyrate are the main SCFAs produced in our colon, in a ratio that varies according to our diet of around 40:40:20 to 75:15:10, respectively (*Rubinstein* et al., 1969; *Macfarlane* et al., 2003). In the case of butyrate, this SCFA is mainly utilised by colonocytes, with little or no butyrate being detected in circulation (*Roediger*, 1980). Propionate on the other hand is both utilised by colonocytes, as well as reaching the liver where it serves as substrate for gluconeogenesis and also appears to modulate cholesterol synthesis (*Mortensen* and *Clausen*, 1996). Acetate, the most abundant of SCFAs, is mostly spared by colonocytes and is found in high concentration in plasma (*Cummings* et al., 1987). Indeed, and again depending on the diet, acetate can reach mmol concentration in plasma. Yet, despite its abundance, the overall function of acetate is still unknown, with recent work suggesting that this SCFA may be metabolised in many organs, is associated with energy metabolism (*Kondo* et al., 2009) and appears to be closely linked to histone and protein acetylation (*Soliman* and *Rosenberger*, 2011).

Until recently, SCFAs were believed to play no direct role on brain function, exerting their effects through modulation of inflammatory processes and/or behaved as signalling molecules that interact with the G-protein-coupled receptors, leading to changes in hormones production from L-cells, including the satietogenic hormones glucagon-like-peptide-1 (GLP-1) and peptide-YY (PYY) (*Tolhurst* et al., 2012; *Psichas* et al., 2014). Moreover, propionate and acetate have been shown to affect adipogenesis and through this, leptin production. Thus, through modulation of gut and adipocyte derived hormones, SCFAs was thought to affect brain function, including appetite and satiety (*Xiong* et al., 2004; *Samuel* et al., 2008). However, more recently and through the use of advanced imaging techniques, it has been possible to demonstrate that the SCFA acetate appears to reach many organs in the body, besides the liver. This points towards the fact that this acetate may not only affect brain metabolism indirectly, but may play an important role in the overall function of the central nervous

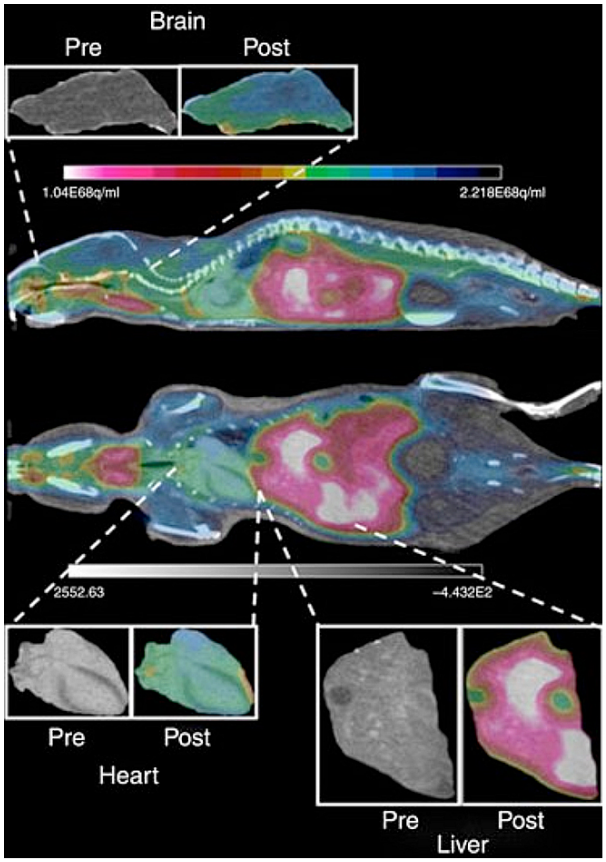

Figure 2: Biodistribution of ^{11}C-acetate in a mouse by PET imaging.
Note that acetate can be detected in most organs, including the brain (Adapted from *Frost* et al., 2014).

system. In the brain, acetate is able to directly affect neuronal function in a number of key brain regions, especially those associated with appetite and satiety control (*Anastasovska* et al., 2012).

ACETATE IMAGING AND BIODISTRIBUTION

The advent of *in vivo* imaging techniques, including magnetic resonance imaging (MRI) and positron emission tomography (PET), has made it possible for researchers to obtain quantitative data on biodistribution, *in vivo*, of many compounds, including SCFAs (*Song* et al., 2009). Carbon-11 labelled acetate was initially produced as a potential PET marker for clinical studies of tumour metabolism (*Vavere* et al., 2008); however it quickly became clear that here was a unique opportunity to get a better understanding of the overall biodistribution of this SCFA. From these studies it became clear that acetate is actively taken up by many organs, including the liver, skeletal muscle, spleen, heart and adipose tissue (Figure 2). More importantly it has been shown to reach the brain in small, yet significant amounts,

independent of route of administration (ip/rectal) or whether it arose directly from dietary components or given in purified form (*Frost* et al., 2014).

However, due to the nature of the PET technique it was not possible to assess the overall metabolism of acetate in different organs (*Grassi* et al., 2012). Carbon-11 has a rather short half-life (c.a. 20 minutes) making longitudinal studies extremely difficult. Moreover, due to the inability of PET to determine the chemical nature of the compound-giving rise to the positron-signal, it is not possible to identify acetate-derived metabolites. These issues could in theory be overcome by the use of hyperpolarised ^{13}C-acetate, in combination with MRI (*Bastiaansen* et al., 2013), yet no such experiments have been carried out so far. This is principally due to the fact that this technique is not widely available to the scientific community, especially those involved in nutritional studies, and also due to the overall cost associated with this methodology.

ACETATE AND APPETITE

Brain plays an important role in the control of appetite and it was first the lesions or surgical transections of a nuclei of the hypothalamus such as the arcuate nucleus (ARC), ventromedial nucleus (VMH) and paraventricular nucleus (PVN) resulting in changes in daily food intake that supported this link (*Dube* et al., 1999; *Penicaud* et al., 1983). More recent work has shown that these nuclei express a number of key neuropeptides receptors that are activated by gut peptides or adipokines and regulate food intake (*Van Den Top* et al., 2004; *Satoh* et al., 1997). Two sets of neurons exists in the ARC; orexigenic neuropeptides Neuropeptide Y (NPY) and Agouti Related Peptide (AgRP) increase food intake and induce obesity, whereas Pro-opiomelanocortin (POMC) and Cocaine and Amphetamine Regulated Transcript (CART) inhibit food intake and these signals from the ARC projects to other nuclei (*Parkinson* et al., 2009).

Manganese-enhanced MRI (MEMRI) is used for functional MRI in animals due to the unique T1 contrast generated by the paramagnetic analogue Mn^{2+} (*Kuo* et al., 2005; *Lin* and *Koretsky*, 1997). Due its ability to mimic Ca^{2+} ions, Mn^{2+} ions can permeate presynaptic voltage-gated calcium channels (VGCC) and induce neurotransmitter release from depolarised nerve terminals (*Narita* et al., 1990). The technique has been implemented successfully to monitor brain activation in the regions controlling appetite (*Kuo* et al., 2007; *Anastasovksa* et al., 2012).

Acetate has been shown to be actively metabolised in neuronal and astrocyte cell culture *in vitro* (*Brand* et al., 1997). Supplementation of fermentable carbohydrates such as inulin has long been shown to reduce appetite in animal studies and this effect has been thought to be through the effect of SCFA on gut peptides (*Cani* et al., 2004) but recently appetite suppressing effect of acetate was shown to be through hypothalamic neuronal activations (*Frost* et al., 2014). Intraperitoneal administration of acetate resulted in reduced food intake. Interestingly when acetate was encapsulated in liposomes limiting its distribution to the brain, no reduction in food intake was observed which would suggest that acetate has a direct neuronal function. Using ^{13}C HR-MAS it was shown that acetate, administered or produced in

the colon, in the hypothalamus incorporates into the glutamate-glutamine transcellular cycle, increasing lactate and GABA labelling, thus supporting hypothalamic glutamatergic or gabaergic neurotransmissions. This increased gabaergic neurotransmission together with increased lactate oxidation will result in increased ATP production in turn inhibiting AMPK activity and activating Acetyl-CoA Carboxylase. The increased ACC activity induces Malonyl-CoA expression which activates POMC neurons and therefore reduces food intake. By using MEMRI hypothalamic activation was also monitored which showed increased activation with acetate administration in hypothalamic brain regions such as arcuate nucleus, which is populated by POMC neurons among others.

SUMMARY

Short chain fatty acids derived from the gut microbiota fermentation of non-digestible carbohydrate, principally, acetate have now been shown to reach the brain where it can affect important function including appetite and satiety. The extent of this effect can be readily observed in the significant decrease in food intake in preclinical models. However, acetate is also metabolised in other organs which themselves may indirectly affect appetite. It is therefore imperative that central and peripheral effects are studied in isolation to determine the extent by which acetate directly affects brain function and behaviour.

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