gastrointestinal disorders

MDPI

Hypothesis

Microbiome–Gut Dissociation: Investigating the Origins of Obesity

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Abstract: The reduction of excessive weight remains a major public health challenge, with control currently limited to a calorie reduction strategy. Currently, attempts are being made at revisiting the fibre hypothesis based on the African studies of Denis Burkitt, that the lack of dietary fibre in the modern diet was responsible for the occurrence of obesity and many of the other non-communicable diseases of what he called "Western civilization". However, the dilemma is that Burkitt himself stressed that other peoples of his day, such as the Maasai, remained healthy without consuming such high fibre diets. Equally, the present obesity epidemic is accompanied by diseases of a malfunctioning immune system and of poor mental health that do not seem to be adequately explained simply by a deficiency of dietary fibre. Though unknown in Burkitt's day, an increasing degradation of a mutualistic intestinal microbiome would offer a better fit to the observed epidemiology, especially if the microbiome is not effectively passed on from mother to child at birth. Taking the broader view, in this article we posit a view of the microbiome as a cofactor of mammalian evolution, in which a maternal microbial inheritance complements the parental genetic inheritance of the animal, both engaging epigenetic processes. As this would require the microbiome to be fully integrated with the animal as it develops into an adult, so we have a meaningful evolutionary role for the microbiome–gut–brain axis. By a failure to correctly establish a microbiome–gut interface, the inhibition of maternal microbial inheritance sets the scene for the future development of non-communicable disease: compromised immune system function on the one hand and dysfunctional gut–brain communication on the other. The basic principle is that the fully functioning, diverse, microbiome achieves interkingdom communication by the generation of messenger chemicals, semiochemicals. It is envisaged that the in situ detection of these as yet ill-defined chemical entities by means of an ingestible sensor would indicate the severity of disease and provide a guide as to its amelioration.

Keywords: Burkitt's fibre hypothesis; epigenetic inheritance; ingestible sensor; interkingdom signalling; fetal origins hypothesis; gut–brain axis; maternal microbial inheritance; non-communicable disease; semiochemicals; twin studies

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1. Introduction

The control of obesity has been a major unmet medical need for more than a century [1]. In the wake of the failure of 20th century behavioural and dietary treatments [2] the economic cost of the on-going obesity epidemic is substantial and is steadily increasing [3]. The aetiology remains unknown but, for whatever the underlying reason, has traditionally been ascribed to either eating too much food or doing too little exercise. The observation that, in Britain at least, average energy intake has substantially declined in the face of escalating levels of obesity would seem to rule out overeating and put the emphasis on to relative inactivity [4]. However, the opposite conclusion was reached following a study by Westerterp and Speakman using heavy isotope labelling of water to confirm that physical activity levels have not changed significantly since the 1980s [5]. The authors point out that energy expenditure levels are comparable among people in all parts of the world and even to those of similar-sized wild mammals. Interestingly, they avoid drawing the conclusion

that excessive eating is to blame, leaving open the option of an unspecified third way to accumulate excessive body mass [5].

In the light of these seemingly contradictory results, an earlier dietary fibre hypothesis by Denis Burkitt [6] has recently been given greater prominence. In essence, a high fibre diet is known to be fermented by intestinal microbes to produce short-chain fatty acids, which have benefits throughout the body [7]. Although he recognised that an environmental influence was at work, significantly he also noted that another people, the Maasai (Masai in his day), remained healthy on a relatively low fibre diet [6]. Burkitt's valuable observations are dealt with in more detail below, but it seems reasonable to suppose that the intestinal microbes are indeed affected by an environmental influence operating throughout the modern world.

It is worth noting that obesity is only one of a number of conditions that have steadily been increasing over many years, a situation summarised in an engaging article in the New England Journal of Medicine, illustrating the change of disease over the period 1812 to 2012 [8]. While the "tidal wave" of pollution over this time should not be forgotten, for example the ancient peril of heavy metal pollution [9], or the more recent danger of microplastics and plasticisers [10], in the years following the publication of this article more information has emerged about the significance of the microbes inhabiting the large intestine. These are referred to either as the microbiota, a collection of individual entities, or as the microbiome—a more flexible concept involving both the individual species and the action of their genes, including any mobile genetic elements that may be taken up from the environment. It seems reasonable to suppose that one outcome of a breakdown of an otherwise optimal mutualistic host-guest relationship, which we term *microbiome–gut dissociation*, is an increase in the likelihood of becoming obese.

While there is little doubt that obesity is fundamentally due to eating too much food that is too easily digested, what constitutes *too much* for each individual is a matter of intense current research, including the twin principles of the "personal fat threshold" [11], and of the microbes inherent within the "personalised nutrition" concept [12]. This present article considers the role of the intestinal microbes in rendering the population as a whole more vulnerable to weight gain, within the context of the other non-communicable diseases that are steadily rising across the modern world [8].

This year marks the tenth anniversary of the death of Professor Lynn Margulis, who introduced the idea of the *holobiont*, that an animal and its attendant mutualistic microbes act down the generations as a single evolutionary unit [13,14]. As these ideas were being debated, however, the world was firmly in the grip of the current obesity crisis. It is the purpose of this article to develop the idea of the breakdown of animal-microbiome mutualism in order to explore the fundamental origins of obesity and other non-communicable diseases of the modern world. In the opinion of the authors the fully functioning microbiome stems from the whole range of microbial life and acts as a *cofactor* for evolution. As such, inheritance must be involved, the phrase "intergenerational" being used recently [15]. In our view, this *maternal microbial inheritance* complements the parental genetic inheritance of the child. In this scheme, the enclosed intestinal microbiome is best understood in terms of its evolutionary role, probably first taking its current form during the Cambrian Period, as a link between environmental microbes and multicellular animals [16].

In the context of the ongoing interest in the microbiome, specifically with the body and its attendant microbes acting as one unit, we first decided to revisit the earlier account of Denis Burkitt.

2. The Observations of Denis Burkitt

Burkitt worked as a surgeon in Africa in the years following the end of the Second World War and discovered a virally transmissible cancer that is now named after him: Burkitt's lymphoma [17]. However, he also took note of the lifestyle of many of the peoples of Africa and reported that they did not suffer from the diseases of what he called Modern Western Civilization. Essentially these are the ones that we recognise today, primarily due

to the inappropriate deposition of fat; obesity and its related disorders: type 2 diabetes and cardiovascular disease, among others [6]. One of his most striking observations was that many African peoples not only did not suffer from Western-type non-communicable disease but also had a significantly reduced intestinal transit time and produced a much larger faecal volume of at least 3-fold greater than was observed in Western-based populations. Although he blamed the absence of dietary fibre in modern civilisation, he also observed that the Maasai, a cattle-rearing people of the Eastern Africa grasslands, did not consume much fibre and yet remained healthy [6].

A thorough individual, he consulted doctors within developing countries in Africa and Asia, finding the same pattern. In addition, he also noted the rise in such diseases in post Second World War Japan and, especially, in Japanese people who had migrated to Hawaii. In general, he found that people moving from territories of low disease incidence picked up these Western conditions within a generation or so, often with many apparently different conditions being found in the same individual. He surmised an environmental cause [6]. To summarise his findings:

1. People living away from the modern world and free from certain non-communicable diseases also consumed substantial amounts of dietary fibre, exhibited low intestinal transit times, and produced copious faecal matter,
2. That those same people, transferred to a modern environment, developed modern diseases, thus confirming a primarily environmental cause, and,
3. That individuals in the modern environment often suffer from multiple apparently different diseases, a finding backed up by a recent multimorbidity study [18], but,
4. That the Maasai (Masai in his day), who were not in a position to consume significant amounts of fibre, still remained healthy. In addition,
5. To his credit, Burkitt mentioned those immune system complaints present in the modern world that were not adequately explained by his dietary fibre hypothesis, specifically thyrotoxicosis, pernicious anaemia, rheumatoid arthritis, multiple sclerosis, and coeliac disease [6]. Finally,
6. Sadly, but in keeping with the mores of his time, he did not compare rates of poor mental health between peoples living in traditional *v.* modern societies.

Burkitt rationalised the absence of significant amounts of modern disease in the Maasai by suggesting that they had developed a different way to handle cholesterol and that this rendered them immune to the absence of dietary fibre [6]. Of course there were other steppe-dwelling peoples, without access to significant dietary fibre, that have since been subsumed into the modern world. To our knowledge, however, there is no evidence of a substantial subset of individuals that are immune to the development of non-communicable diseases such as obesity. It is important to realise the significance of his observations in the sense that they are a rare case of data obtained by a single individual, presumably using the same criteria, to assess two very different styles of life (high fibre *v.* high dairy diets) at the same time. Burkitt settled on the most natural explanation available to him and married it with his knowledge of low dietary fibre, Western-style, diets, naturally assuming that the Maasai were an exception. Of course, at that time he had no knowledge of the microbiome.

Regardless of its underlying origins, it seems feasible that the proximate cause of obesity is the reduction in faecal output somehow due to an environmental effect stemming from within the modern world. Accordingly:

We hypothesise that the rise in the incidence of obesity is directly related to the reduction in faecal output first noted by Denis Burkitt.

3. The Energy Balance within the Animal/Microbiome Combination

The word "calories", when used within the context of food and drink, primarily refers to the energy released when the C-C and C-H bonds within dried foodstuffs are converted to C-O and H-O bonds by combustion [19]. Since this same process occurs in animal bodies, albeit more slowly, the evolution of carbon dioxide gives a measure of energy "burnt" by the processes of living and moving [5]. Of course not all food is completely

absorbed, and body cells and microbes are ejected within the faeces, along with substantial amounts of water. Over a long enough time period, the input of energy supplied by the food will balance the outflow of energy represented by both carbon dioxide and faeces, regardless of the details of energy flow within the organism itself, including any commensal microorganisms. Naturally this "faecal energy content" has to be measured in the same way as food energy, by drying and combustion. Although purely a practical point, as many carbon atoms have already been taken out via carbon dioxide, so the faeces will contain a higher proportion of nitrogen atoms than the incoming food, and this will complicate the determination of energy content.

Unsurprisingly, perhaps, the people mainly interested in faecal output, albeit in terms of weight and bulk rather than energy, are those employed by sewage disposal facilities. A recent publication compared untreated output per capita per day between high- and low-income countries at about 130 g and 260 g, respectively, the difference being put down to dietary fibre as with Burkitt's suggestions [20]. By contrast, although Burkitt's corresponding figures for the relatively rich countries were about the same, producing "less than" 150 g per day, they were a lot higher for the African peoples that he studied, at 400–500 g [6]. Since obesity can only result from a prolonged imbalance between energy intake and loss, including any microbes, it seems that the steady reduction in average faecal output as a country becomes richer could help to account for the inexorable rise of obesity over the period of a century or more [1,8].

An unexpected recent observation indicates another way to gain weight, as it seems that the previously suspected decrease in body temperature from approximately 37 °C to 36.6 °C over the last 150 years has been confirmed by accurate studies in United States military personnel [21]. Whatever the underlying reason for this potentially significant finding, it represents a reduction in basal metabolic rate that must have contributed a certain extent to the observed weight gain over the same period [8].

Regardless of the exact significance of this observation, overall there is little doubt that the current obesity crisis will be ameliorated, if not actually cured, by consuming a higher proportion of vegetable-based dietary fibre, exactly as recommended by Burkitt in the late 20th century. However, this fact should not stop us from further investigation of the underlying environmental origins of Burkitt's "Diseases Characteristic of Modern Western Civilization" [6], in the hope that we can solve more problems than solely obesity.

4. The Microbiome as a Cofactor of Evolution

At this point it is worth noting that human faecal matter contains 25–50% of bacterial biomass by weight [22]. Interestingly, using the Bristol Stool Scale classification as a comparator, Jeroen Raes and his team found that the greater the "richness"—the diversity—of colon bacterial microbiota the shorter the intestinal transit time, but without commenting on the potential significance of energy loss by defecation [23]. In order to allow for a bacteria-friendly environment, adequate amounts of nutrition must be present within the lower intestine. One way in which this may occur is by the presence of indigestible, fermentable, substances in the diet, not only dietary fibre and resistant starches, but also polyphenols and other substances derived from plant matter. However, as outlined below, recent observations suggest that communication across the gut wall may allow the microbes to control the flow of nutrition through the intestine in order to feed themselves [24]. Such an ability may have pertained to the Maasai and other pre-modern steppe-dwelling peoples living on a low-residue diet that allowed them to remain healthy. If so, it would be the loss of this ability due to some Burkitt-like modern-world environmental factor that would be the ultimate origin of obesity and other non-communicable diseases. This paper investigates the idea that such *microbiome–gut dissociation* effectively turns our intestinal microbiota from a mutualistic to a fundamentally parasitic mode of operation.

As obesity spread unchecked around the world, it was only natural to believe that the causative agent was some kind of infection. Accordingly, the report of the transmission of an enhanced metabolic potential from genetically obese mice to germ-free animals by faecal

microbiota transplantation caused a stir [25]. These findings came in the midst of a period of speculation aptly summarised by the term *dysbiosis*, though the absence of an accepted definition eventually made the word essentially worthless [26,27]. In this article we suggest that we are dealing with a *deficiency*, with a lack of some as-yet-undefined communication function of the microbiome, for which we prefer to use the more precise but admittedly more cumbersome term *microbiome-function deficiency disease*, of which obesity is only one component, albeit the most visible.

It is noteworthy that, as a general rule, evolution pares away unnecessary functions. However, it is recognised that the critical point here is the diversity of the microbiome, in other words that the level of disease increases as the range of detectable microbes falls [28]. We suggest the following reasons: diversity of maternal microbial inheritance allows the microbiome of the child to alter with the manifold variations of its own genetic inheritance, as observed for blood group antigens, for example [29]; diversity allows the microbiome to express the full range of mobile genetic elements liberated from external microbes by the action of phage viruses [30]; and redundancy, in the engineering sense of the word, means that the failure of one set of microbes, for whatever reason, allows a second set to take over and perform the same function.

The one thing that comes across clearly is the lack of a single underlying rationale for the existence of the enclosed intestinal microbiome within the Mammalia. For example, a review entitled "The microbiota-gut-brain axis in obesity" (2017) covered a wealth of earlier studies around the various functions of these intestinal microbes, including appetite control and energy balance, but without coming to any specific conclusion except for recommending further studies to elucidate the precise nature of these relationships [31]. The following recent publications show the range of subjects now under consideration, noting that a *cancer* may possess its own, unique, microbiome:

1. Maternal microbiota drives the innate immune system [32]
2. Microbes educating the adaptive immune system from birth [33]
3. Microbes affecting peripheral dopamine and inhibiting natural killer T cells [34]
4. Greater microbiome diversity *within* pancreatic tumours predicts patient survival [35]
5. Parkinson's disease, but without defining a specific causative agent [36]
6. Neuroactive potential of the microbiota in quality of life and depression [37]

A role for the microbiome as a cofactor in evolution implies that it is transmitted from one generation to the next, presumably helping the neonate come to terms with the microbial environment of the parents. There is something inherently satisfying about the idea that the microbiome of the mother "learns" from its environment and, somehow, passes on this information to the child. Indeed, similar observations have already been made [32,33]. Interference with the transfer of microbes from mother to child occurs with caesarean section delivery, for example, a process that can produce pathogen colonisation within the neonate [38]. As noted above, in this article we use the phrase maternal microbial inheritance to emphasise the necessity for effective transfer of microbes from mother to child as soon as possible and note that the post-caesarean procedure known as *vaginal inoculation* is already carried out [39], even though it does not meet with universal approval [40]. Sadly, of course, the logic of the situation suggests that vaginal inoculation would confer no benefits to the child if the microbiome of the mother was already malfunctioning, possibly because she, in her turn, was previously delivered by caesarean section under sterile conditions. To our knowledge, there is no mechanism for the repair of a microbiome once it has become damaged.

In the middle of the 20th century, Waddington suggested that epigenetic information could somehow be inherited along with the genes themselves [41]. More recently, the suggestion has been made that a *paternal* epigenetic effect can somehow be transmitted [42]. However, in the opinion of Horsthemke, the experimental conditions required to confirm the inheritance of epigenetic factors in the presence of ecological and cultural phenomena have still not been achieved [43]. Interestingly, as his critical review was being published, Qin was reporting a review detailing the crosstalk between gut bacteria

and the epigenome [44]. In other words, to complicate the picture still further, one possibility is that parental (mother and, possibly, father) epigenetic transmission may allow for *behavioural* changes such as cigarette smoking, while maternal microbial inheritance somehow provides the necessary epigenetic changes to allow the immune system to cope with the *external* microbial environment.

At the end of the 20th century the epidemiologist David J Barker wrote a paper entitled "the fetal and infant origins of adult disease" [45]. Unfortunately, he emphasised the foetal aspect with his subtitle: "the womb may be more important than the home", later focusing exclusively on in utero effects [46]. Initially, the idea did not gain traction in academic circles as there was no clear mechanism to connect such early life events with subsequent health problems [47], but nevertheless his thesis is successful from an economic perspective [48]. Although Barker's hypothesis incorporates many different factors, the idea of microbiome–gut dissociation during childhood may constitute a valuable addition to the "intergenerational" nature of the debate [15].

In terms of actual action of the microbiome, two basic functions have been identified, the supply of energy and the establishment of interkingdom communication, as illustrated by the following reviews:

1. Bacteria as suppliers of B-group vitamins and short-chain fatty acids [49]
2. Microorganisms releasing small molecules such as dopamine, norephedrine, serotonin, and histamine to mediate the action of the microbiota–gut–brain axis [24].

Within the context of microbiome-body interactions, dopamine and similar neurotransmitters are best thought of as semiochemicals, messenger chemicals, capable of passing information between widely different species [24]. In this way microbes have the potential to feed themselves, balancing the relative needs of body and microbiome in a mutualistic fashion. We have previously described this approach in some detail, stressing the need for microbial diversity to facilitate the expression of mobile genetic elements [50], and tentatively identified chronic heavy metal poisoning in bringing about microbiome–gut dissociation [51]. Although evidence so far is circumstantial, nevertheless:

We hypothesise that the underlying cause of obesity is the loss of the semiochemical-induced ability of the microbiome to feed itself, resulting in a significant increase in intestinal transit time, increased food absorption and decreased faecal output.

It is important to note that microbiome-function deficiency is not a disease in its own right. In order to become obese, it is still necessary to eat more low-residue foodstuffs than the individual microbiome can deal with. Alongside immune system problems, the effect of a degraded microbiome–gut–brain axis is to increase the propensity for the development of non-communicable disease as a child develops into an adult [51].

5. Microbiome Investigation

Studies of the microbiome have focused almost exclusively on bacteria, partly due to their association with infectious disease but also because of the ready availability of the highly conserved 16S rRNA gene as a prokaryote analytical tool [52–55]. The development of the "mycobiome", intestinal fungi, in early childhood has been studied [56] and, although a variety of fungi are known to affect the behaviour of parasitised insect species, perhaps the most interesting unicellular eukaryote in the context of the microbiome is *Toxoplasma gondii* and its ability to control the behaviour of rodent species in the presence of a predator [57]. It is possible to envisage a coordinating role for microbial eukaryotes within the microbiome, and they could therefore represent an interkingdom "missing link", as has been pointed out previously [58].

Of course, the populations that are available for microbiome studies are those very peoples that are already suffering from non-communicable diseases, even if the individuals themselves appear to be disease-free. The danger of this approach is the possibility that key microbes are already missing, and therefore that the picture could be seriously skewed, even if the search does extend to microbial eukaryotes. The Tanzanian Hadza are a people relatively free from such diseases whose microbiome has already been assessed, albeit only

with bacteria in mind [59], while the Bolivian Tsimane have recently been cooperating with medical teams [60]. Investigation of excreta is obviously non-invasive, and any "missing factors" found therein would potentially be very valuable in solving the puzzle of modern disease. Much thought has been expended in providing adequate compensation for the obtention of valuable information from indigenous peoples, for example the "principle of reciprocity" when searching for cancer medicines, and such an approach may be of value here [61].

Although considered in more detail later, the use of an ingestible sensor designed to respond to the presence of semiochemicals [24] would help to connect microbial composition with microbiome function [62]. In principle it should prove possible to track several different semiochemicals in parallel. Such a device has recently been classed as minimally invasive [63].

6. The Gut–Brain Axis: Obesity and Varicose Veins

Although the role of the microbiome–gut–brain axis in mental health will be the subject of a later article, the connection between gut feelings, intuition, and depression has already been reported [64]. Alongside obesity we have the disruption of a smooth mechanism of gut motion as the gut–brain axis is degraded. Clearly unmoderated defecation carries an unacceptable social cost, in human societies at least, and this in turn requires a signal to be passed to from brain to bowel confirming that the time and place are appropriate. When the bowel fails to receive the necessary signal, some straining may be required, in turn leading to the physical effects noted by Burkitt, such as varicose veins, hiatus hernia and haemorrhoids, for example [6]. While the word constipation may refer to a change in normal bowel habit, in an absolute medical sense the word is hard to define, with between 1% and 80% falling into this category [65]. Owing to the variable but extensive nature of microbiome–gut dissociation, perhaps it is no coincidence that absolute definitions of the words *constipation* and *overweight* are both elusive.

7. Probiotics and Missing Microbes

In order to counter the increased absorption of food due to this relatively high transit time, much work is being carried out on so-called prebiotics and probiotics. Prebiotics are substances that cannot easily be absorbed into our body without input from microbial metabolism, including certain oligosaccharides and polyphenol phytochemicals. These substances aim to have the dual action of suppressing appetite and facilitating defecation [66]. Probiotics, by contrast, aim to introduce supposedly beneficial microbes, overwhelmingly bacteria, to supplement the action of our own gut microbes [67]. In a similar fashion, attempts are underway to manipulate the gut–brain axis with the aid of "psychobiotics" [68]. To illustrate the problems inherent within this new field, the bifidobacteria are a class of promising microbes, with attempts being made to cure irritable bowel syndrome by faecal microbiota transplantation [69], or at least to control the pain of this disease, though apparently with limited success [70]. It is interesting to note, however, that the Hadza, a much-studied group of people from Tanzania largely without Burkitt-like modern diseases, are reported not to contain any bifidobacteria at all [59]. On the grounds that the key feature of the microbiome is its diversity, it is hard to see why a single added bacterial species, or even class of bacteria, should make any lasting difference [28].

Interestingly, a recent multi-site study using mice provided by different suppliers gave inconsistent results, tracked down by experiments involving faecal microbiota transplantation to the absence of critical bacteria in one of the two lines of genetically identical mice, the difference presumably being due to feeding routines [71]. Likewise, an experiment in which mice were fed low-quality fast-food over several generations reported a reduction in bacterial diversity reminiscent of modern human microbiomes [72]. In a similar vein, a study in mammals highlighted the influence of both domestication and industrialisation on the observed diversity of gut microbiota, detecting wild-type microbes missing in domesticated animals [73], which suffer from atopic disease in much the same way as

humans [74]. At the very least, these results suggest another reason for caution when the results of animal experiments are being extrapolated to humans.

On farming practice, it has been common to add antibiotics, under the guise of growth promoters, to the feed of mammals such as swine [75], to poultry [76], and even to fish [77]. It seems likely that the resultant animal enlargement is due to the removal of key intestinal microbes. More subtly, however, the ultimate action of antibiotics may be the suppression of the activity of the microbiome, rather than the direct removal of the microbes themselves. Of course, farm animals are slaughtered before they get the chance to become obese, unlike pet animals or, indeed, people.

Worryingly, a recent report documents repeated studies carried out on separate groups of 10-year-old English schoolchildren in the years 1998, 2008, and 2014, showing an increase in mass. Both height and girth increased proportionally, but without a significant rise in overall body mass index. Of greatest concern is the fact that handgrip and sit-up muscle strength showed an accelerating decline over this period [78]. Although it has long been realised that the genetics of human height are complex [79], understanding the involvement of the microbiome in human growth is only at its beginning [15].

8. Weight Gain on Reduction of Faecal Energy Output

The energy balance within the body lies between energy intake from food on the one hand and energy use, and excretion, on the other (Equation 1). While energy use can be measured as the output of carbon dioxide [5], it is composed of both voluntary movement and resting energy: basal metabolic rate, which varies with the size of the individual. Inspection of Equation 1 indicates that, food intake and exercise being essentially unchanged, any decrease in faecal energy output is compensated for by an increase in the basal metabolic rate associated with a larger body: *metabolic scaling* [80]. As indicated in Figure 1, the connection between body size and faecal output is the efficiency of the microbiome: the road to obesity goes through a degraded microbiome.

Equation (1): The balance of energy expenditure over time (and Figure 1)

$$\text{Energy intake (food)} = \text{Energy used (movement and metabolism)} + \text{Energy excreted (faeces)} \quad (1)$$

While it is relatively easy to measure the physical energy output of an individual in the short term, assessing faecal energy output over the longer term is more difficult. Though it may be thought that constipation would provide some information as a physical outcome of reduced faecal output, in reality this condition itself cannot be adequately defined [65]. It is probable that both constipation and weight gain are ill-defined conditions reflecting an inefficient microbiome.

It seems that the health of the population can be assessed through three measures: levels of obesity; degree of microbiome-related non-communicable disease; and throughput via waste disposal systems.

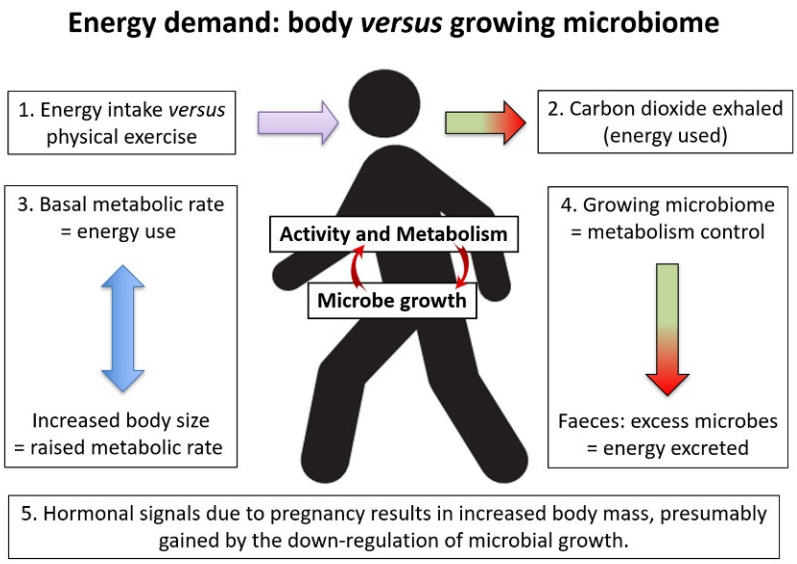

Figure 1. Activity and metabolism *v.* microbiome growth [50,51]. Box 1: Long term average food intake and exercise levels in the healthy adult. Box 2: Output of carbon dioxide reflecting both basal metabolic rate and exercise levels. Box 3: Indicates the relationship between body size and basal metabolic rate. In the presence of a functioning microbiome, eventual size is under genetic control. In its absence, growth will occur until basal metabolic processes balance the reduction in faecal energy output (see Box 5). Box 4: Good health requires a degree of microbial growth (Figure 2) and this leads to eventual energy loss by excretion of faeces, dependant on the health of the microbiome. Box 5: The bodily changes due to pregnancy illustrate the fine control of the allomone/hormone balance exerted through the gut–brain axis (Figure 2). In the absence of extra food or decreased physical exercise the extra energy required can come from a temporary reduction in microbial growth, presumably leading to a corresponding fall in the energy output from excretion. Famine will produce a similar outcome, in which microbial growth will be temporarily placed on hold in order to protect the host, without which the microbiome cannot survive. Feasting in the presence of a functioning microbiome may lead to an increase in faecal energy excretion, morbid obesity being unlikely if not impossible. By contrast, a disabled microbiome cannot compensate for any increased food intake, potentially leading eventually to all the consequences of unrestricted fat accumulation.

9. Microbial vs. Genetic Inheritance: A New Angle on an Old Debate

As obesity and its complications took hold throughout the 20th century, the question became the relative importance of genetic and environmental factors: innate versus learned behaviour. When dealing with people, experimental constraints are severe, requiring sufficient subjects and time for statistically significant information to emerge. In principle studies involving monozygotic twins will allow control of at least some of the variables. Accordingly, in the 1980s a study was performed in Quebec, Canada, by Claude Bouchard and his co-workers, in which 12 pairs of genetically identical male twins were overfed by 1000 kcal per day for 6 days a week over a period of 100 days, with limited exercise, after an initial 14-day period to determine normal eating patterns [81].

The volunteers were young (19 to 27 years), were initially lean, had normal blood chemistry and were basically sedentary. Although confined during the study they had previously been living at their homes with their parents, who were themselves confirmed to be free from obesity or lipid-related disease. Although the twin pairs all gained weight during the study, the amount gained, the proportion of fat versus lean tissue and the distribution of fat around the body varied substantially *between* different pairs rather than *within* them. Based on the fact that each twin pair were genetically identical the authors' conclusion was that the accumulation and distribution of fat was strongly dependent on genetic factors. Furthermore, Bouchard and his team also stated that "the various determinants of the resting expenditure of energy" were also under genetic control [81].

However, unlike with the experiment of Westerterp and Speakman mentioned earlier, in such a long-term study it was not possible to estimate energy expenditure by measurement of carbon dioxide production [5]. Accordingly, in line with Burkitt's observations of the decrease in faecal output within Western-based civilisation [6], it is possible to assign a radically different explanation of Bouchard's results: as, of necessity, each twin pair were born of the same mother at the same time then, alongside their genetic inheritance there may exist a more powerful determinant of overall health: *maternal microbial inheritance*.

Although Burkitt's 1960s work had led people to reassess the significance of dietary fibre, by the time of Bouchard's study in the 1990s it seemed that his approach was not going to solve the obesity crisis. By contrast, throughout the latter half of the 20th century there was a belief that the sequencing of the human genome was within reach and, therefore, the excitement that disease as we know it may have been on the point of being conquered. Accordingly, it is not surprising that Burkitt's observation about the diminution of faecal volume in Western civilisation was overlooked. As, in principle, Bouchard's study could have included faecal collection it seems that an opportunity to investigate alternative causes of obesity were missed.

At this point it is important to mention another determinant of human behaviour: a feeling of disgust. As researchers are not immune to this sensation, this may be another reason why Burkitt's original observations on faecal volume have not been enthusiastically pursued.

10. The Microbiome as a Mutualistic Entity: Allomones and Kairomones

In a separate article we make the point that the microbiome, a diverse array of single-celled microbes *behaving as a single entity* and cooperating with a multicellular host, is likely to have been intimately involved in the evolution of animals at least since the Ediacaran Period, taking its current form following the development of the gut–brain axis in vertebrates and in mammals [16]. In previous documents, we postulated a rationale for the degradation of the microbiome as a driver for the appearance of non-communicable disease in humans and in domesticated animals [50], including a suggestion of microbiome-specific heavy metal poisoning probably related to the historical use of colourful toxic salts as cosmetics [51]. Rather than considering individual components of the microbiome we treated it as if it were a single mutualistic community of variable membership but with a single major function: to direct the immune system of the host from the moment of birth. It follows that the microbiome must remain viable in the adult in order to be transferred directly to the neonate at the critical time [50,51].

In principle, the situation is reminiscent of the relationship between a flower and a bee. From the point of view of the plant the purpose of the bee is to transfer pollen to another flower, but to do this successfully the bee must first be brought to the right place and then supplied with adequate nutrition. The scent acts as a *semiochemical* (signalling molecule), specifically an *allomone*, designed to attract a member of a different species, in this case the bee, to the flower as a source of honey. As a *quid pro quo* to this benefit, the bee physically transfers the flower's pollen and, therefore, the genetic inheritance of the plant. Noting that mutualism between a multicellular host and its microbial community likewise requires two complementary activities, we suggested chemical communication between these nominally separate entities for which we have slightly modified the semiochemical terms *allomone* and *kairomone*.

Allomones represent chemicals that a member of one species releases to affect the behaviour of another to the benefit of the originator but not the receiver. Microbes are known to produce agents such as dopamine in the gut lumen [82], and there is a suggestion that this ability was initially derived from bacteria by horizontal gene transfer [83]. These agents are small, potentially time-limited, and rapidly diffusible: ideal allomone-like semiochemicals for inter-species communication [24]. We have suggested that such chemicals act alongside hormones to facilitate gut movement thus ensuring that the microbiome receives adequate nutrition, allowing for events such as pregnancy or famine. In principle

these allomones may be monitored by means of ingestible sensors and thereby provide information about the state of microbiome effectiveness [62].

Kairomone systems represent interactions initiated by a member of one "species" (the microbial community) to confer an advantage on the receiver (the multicellular host) but with no direct benefit to the originator. As the function of the kairomone system is to ensure the viability of the host in its pathogen-rich environment so it ensures the eventual survival of the microbial community itself [50]. It is possible that such unicellular eukaryotes represent precursors of antigen-presenting cells such as dendritic cells [84]. Interestingly, such a physical transfer process would be equivalent to the physical transfer of pollen by a bee, in principle another kairomone-like system.

As illustrated by the left-hand side of Figure 2, the fully functioning microbiome stimulates the flow of nutrition using allomones so as to facilitate its growth, albeit modified by hormonal input. Excess microbial growth is eliminated but it is worth noting that the faeces are still active in the sense that they can usefully repopulate the intestines of people suffering from conditions such as overgrowth of *Clostridioides difficile* by faecal microbiota transplantation [85]. Alongside the nutrients will come fragments of external microbes, mobile genetic elements, and phage virions. These interact with the existing microbiome, somehow setting up the kairomone function for future use. We suggest that pregnancy sends a hormonal signal to the microbiome [86], which moderates its demand for nutrition and thereby allows greater fat reserves to build up in the woman. As illustrated on the right-hand side of Figure 2, the microbes that are transferred to the baby during the process of natural birth constitute the *microbial inheritance* of the child. The kairomone system then operates as the child grows to be an adult, probably by epigenetic modification of its genetic inheritance [44].

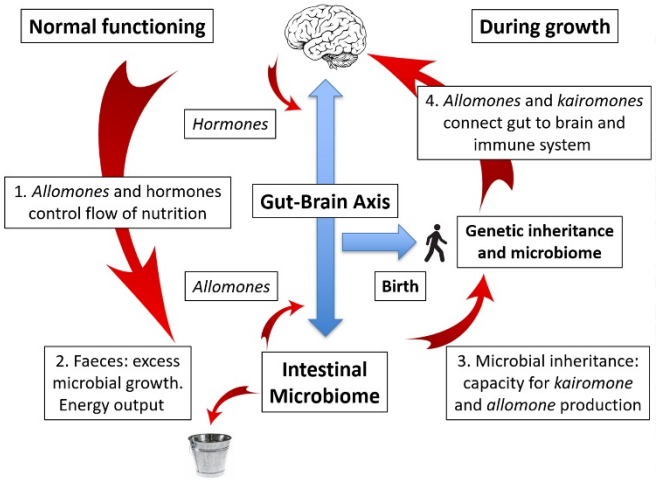

Figure 2. The gut–brain axis [50,51]. Left hand side: Normal functioning with a healthy microbiome already in place. Box 1: Illustrates the operation of the gut–brain axis following input from both brain (hormones) and growing microbiome (allomones). The result is the partition of nutrition to satisfy the needs of both body and growing microbes. Box 2: As allomones are produced during active microbial growth excess is ejected at intervals, along with waste products. In contrast, a poorly growing microbiome will produce a correspondingly lower volume of waste products. Allomone production will be lower and more nutrition will be retained within the body (Figure 1). Right hand side: Processes occurring during the growth phase with a functioning microbiome. Box 3: As the child embodies the genetic inheritance of its parents, so the microbiome of the mother is transferred to the child, with its potential for future development. Box 4: The kairomone system operates throughout the growth process, directing the immune system to tolerate its environment. A poorly growing microbiome may result in problems with the immune system. Allomones and hormones act in concert so as to strengthen the gut–brain axis. A failure at this stage allows the potential for poor mental health.

11. The Road to Disease: Microbiome–Gut Dissociation

The consequences of microbiome failure can be estimated by inspection of Figure 2. An absence of allomones has the effect of slowing down the flow of nutrition, allowing more time for digestion and the accumulation of body fat. Although the effect is similar to pregnancy, the lack of overall allomone/hormone control may account for the appearance of fat around the internal organs and in the bloodstream. Equally, a poor microbiome leads to a compromised immune system and poor mental health. Such problems tend to build up in a way we have previously described as a *vicious circle* [50].

The increase in immune disorders such as hay fever noted by Strachan [87] has multiplied in recent years [88] and is replicated in domestic animals [74]. It is possible that there is a double effect on cancer. If an inefficient immune system allows precancerous cells to escape detection and the presence of excess nutrition allows faster growth, then the fact that more malignancies are detected becomes explicable. Note that this would be true regardless of the apparent cause [89]. The likelihood that the gut–brain axis has a central coordinating function likewise implies involvement in another increasingly common ailment: poor mental health, including anxiety and depression [90].

While the overuse of antibiotics would seem to be an obvious reason for the degradation of the microbiome [91], nevertheless many of the characteristic features of non-communicable disease substantially predate the invention of modern-day antibiotics. Though obesity has been known for many years [1,8], John Bostock described a novel condition that he called *catarrhus aestivus*, or summer catarrh, in 1819 [92]. While this was clearly hay fever, he could find only 28 cases, all from the richest strata of society, in spite of extensive searching over several years [93]. As stated above, in a recent publication we suggested that poisoning due to toxic heavy metals in early cosmetic preparations may be to blame [51]. Another way in which the microbiome has undergone widespread damage is by the extensive use of sterile caesarean section in recent years [94], which both changes the nature of the intestinal microbiota and also provides opportunities for pathogen introduction [38].

Regardless of the precise way in which the damage first arose, in the apparent absence of a natural repair mechanism microbial inheritance ensures that the situation can only get worse across affected populations [51].

12. The Storage of "Excess" Energy: The Personal Fat Threshold and Cancer

The Quebec experiment mentioned earlier [81] illustrated the range of responses to overfeeding of young adult males probably representing a loss of control under the influence of poor microbial inheritance. Although some put on lean muscle mass, those individuals exhibiting a strong tendency to visceral fat build-up will find themselves at risk of type 2 diabetes even when non-obese. More recently, Roy Taylor has developed the notion of a *personal fat threshold*, beyond which further accumulation begins to affect health, regardless of their body mass index [11]. He found that a strict diet can sometimes reverse the progress of disease, although it does not constitute a cure [95].

Individual responses to the storage of the excess energy gained from restricted microbial growth will vary with the degree of microbiome–gut dissociation and also with their stage of growth [51]. The following seems likely but will need to be confirmed with further studies:

1. That children will grow larger during their growth period, laying down lean muscle, possibly dependent upon sufficient protein being available, otherwise intramuscular fat [78],
2. That adults, and children outside their growth period, will accumulate both subcutaneous and visceral fat dependent on the degree of microbiome–gut dissociation. Excess fat may leach into the circulation, leading to heart disease and stroke, among other conditions, as enumerated by Burkitt [6]; and
3. That the growth of cancer cells will accelerate under the availability of excess energy.

It seems to be important to keep the intake of energy within an individual's threshold [11] so, for example, while a compromised immune system may fail to recognise and eliminate pre-cancerous cells, the resultant tumours may not cause problems until stimulated by excess available energy. It could be that the association of cancer with specific foodstuffs, such as red meat [96], is more to do with the stimulation of growth under the influence of excess energy rather than with carcinogenicity in its own right. Similarly, the response of different people to the different components of their diet is now recognised as microbiota related; and is described by the term "personalised nutrition" [12].

13. Studying the Microbiome–Gut–Brain Axis: A Role for Ingestible Sensors

On the grounds that the microbiome interacts with the endocrine and nervous systems by the production of allomone-like semiochemicals it is likely that representatives of these agents will be excreted in the faeces. Unfortunately, as this will be an accumulation over several hours or days it is unlikely that measurement within this matrix will afford sufficient detailed information to assist in understanding the processes involved. Gross levels of such allomones may provide some information, however.

By contrast, modern miniaturisation technology has given us the opportunity to transmit real-time information from the gut lumen by means of an ingestible sensor, effectively an electronic device containing a sensor attuned to a specific allomone and attached to a radio transmitter, as we have reported earlier [62]. Although such a device is not yet available, in principle it would allow a researcher to monitor the time-dependent presence of a target compound when swallowed alongside test substances. Equally, in principle it may be possible to track several different potential semiochemicals alongside one another.

We considered the most likely allomone to be dopamine, as it and other catecholamines are known to be produced in the gut lumen [24,82] and are ubiquitous throughout brain and body [97]. Note that the action of dopamine as an allomone does not require it to penetrate the brain itself but simply triggers a sequence of events. However, many similar agents are thought to have been passed to animals from bacteria at an early stage so investigations should not be restricted to dopamine alone [83].

14. Conclusions: An Unfolding Disaster

So far, the public health response to the obesity crisis has been to encourage both the food industry to sell less and for people to eat less, in spite of warnings that this should not be treated as a lifestyle disease [98]. Unfortunately, alongside the inherently contradictory nature of this self-limiting message, the focus on eating less has not helped with the parallel epidemic of poor mental health, often associated with an inappropriate attitude to food [90]. Research has tended to focus on the combination of probiotics, usually bacteria [67] and prebiotics, forms of fibre unable to be digested by the body itself [66].

Bearing in mind Burkitt's observation that the cattle owning Maasai of his day ate little fibre, *and yet remained healthy*, in complete contradiction to the majority of the people that he studied, one concern is that individuals may already be missing an essential component of the microbiome that can neither be supplemented by standard bacterial probiotics nor enhanced by dietary fibre. Recently we have suggested that unicellular eukaryotes may be vital for the functioning of the microbiome, possibly as microbial sentinel cells working alongside the dendritic cells of our own immune system [16]. Compare with the spectacular effect of the microeukaryote *Toxoplasma gondii* in hijacking the brain of rodents in the presence of cats [57], for example.

As such microeukaryotes would have deep evolutionary significance, animal studies would be expected to offer suggestions as to the situation in humans, if only we knew what we were looking for. As mentioned above, attention is beginning to be focussed on microbial eukaryotes which have been highlighted as a "missing link" between bacteria and the human gut, their absence perhaps helping to explain the current prevalence of non-communicable disease [58]. Although there are a number of such species, one that has

drawn attention are the organisms known as *Blastocystis*, which, although first thought of as pathogens, are now known to be present in the apparently healthy gut [99]. Interestingly, they swap between different animal hosts with ease [100] and respond to both dietary conditions and the specific types of gut bacteria [101]. However, it could be that the key health-ensuring ingredients are simply no longer present in people suffering from the diseases of "Modern Western Civilization" as described by Denis Burkitt [6].

In conclusion, we believe our hypothesis to be consistent with the facts outlined above: that obesity occurred as faecal output dropped due to the loss of critical microbes during the transition to the modern world.

It is clear that people living on the edges of the modern world, relatively free from pollution (e.g., heavy metals [9] and plastics [10]), and non-communicable disease, provide an opportunity to uncover any missing microbial ingredients. With their agreement, such people could include the Hadza of Tanzania [59], or the Tsimane of Bolivia [60]. It is more likely that we would herein discover what we have lost during the transition to the modern world: the essence of a fully functioning microbiome.

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