

The Epidemiology of the Dysfunctional Microbiome in Animals and in Humans: The Propensity for the Development of Non-Communicable Disease

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Abstract

There is a worldwide increase in the incidence of non-communicable disease associated with obesity, mental health and atopic disease, the latter also observed in animals. In earlier articles we suggested that the microbiome is a single mutualistic microbial community interacting with our body by two overlapping sets of semiochemicals: allomones that enable the microbiota to be fed by effective transfer of nutrition through the gut and kairomones somehow acting to calibrate animal immune systems so as to distinguish the harmless from the potentially harmful, such as pollen from pathogens. Non-communicable diseases arise as the microbiome loses vital functions, a variable state that has been termed dysbiosis and which may theoretically be assessed by measuring the formation of potential allomones such as dopamine and serotonin. This article attempts to shed light on these semiochemical processes by looking at the epidemiology of dysbiosis within both human and animal populations: who does it affect and when? As a possible example, the fate of English populations of the European hedgehog, *Erinaceus europaeus* is briefly considered. These ideas are discussed in the light of a startling new observation: that the mean body temperature of men and women in the United States has been decreasing by 0.03°C per birth decade for at least the last 150 years. Is the nature of our immune system changing from proactive to reactive?

Keywords: Atopic Disease; Body Temperature; Dysbiosis; European hedgehog; Growth Promoters; Gut-Brain Axis; Heavy Metal Poisoning; Holobiont; Ingestible Sensor; Mental Health; Microbiome; Non-Communicable Disease; Obesity; Tsimane

Introduction

Non-communicable disease: lifestyle, genes, or microbiota?

The concept of lifestyle disease, in which health problems associated with excess weight may be solved by having sufficient willpower to diet, is superficially attractive but is steadily coming under fire [1]. Non-communicable diseases come in various disguises, worldwide in extent and increasing in incidence. Diseases associated with obesity [2] are probably most closely associated with lifestyle, however a 2012 Finnish twin study showed that those with sufficient resolve to diet often ended up fatter, apparently regardless of their genetic inheritance [3]. This enlargement is not limited to obesity: a recently reported study of English children showed that 10 year-olds were both wider and heavier in 2014 by comparison with both 2008 and 1998, with no overall change in their BMI but with a significant decrease in muscular fitness. The authors note that a decrease in self-reported physical activity from 2008 to 2014 may explain these observations [4]. Indeed increased physical activity is considered to be complementary to dieting but double-labelled water experiments reported in 2008 showed that energy expenditure has slightly but significantly increased across the world since the 1980s and is comparable to that

observed in wild mammals. The authors conclude that the observed dramatic increase in obesity over this period is not due to decreased energy expenditure [5]. The dilemma of obesity has been pithily described in a recent book, coming to the conclusion that there is no single cause currently known, although it was admitted that there was a lot more to learn about the microbiome [6]. Obesity was also mentioned in earlier work by Denis Burkitt (See section On the edge of the modern world: Denis Burkitt, the Maasai and the Tsimane), who listed what he considered to be “Modern Western Diseases” and compared their incidence in early post Second World War Africa with contemporary Europe and America [7]. He focused on cardiovascular disease and problems such as hernia and varicose veins, which he considered to be due to increased abdominal pressure on defecation due to the relative absence of fibre in the diet [8].

The increasing incidence of mental problems [9] may be linked to nebulous concepts such as stress or social media, but recently a more fundamental association with obesity by degradation of the microbiota-gut-brain axis has been postulated [10].

By contrast, the increase in atopic disease seen during the 20th century [11] cannot easily be linked to lifestyle issues, especially as its incidence is also increasing in animals [12]. A review of the changing nature of human disease is presented in the bicentenary 2012 edition of the *New England Journal of Medicine*, which showed a marked increase in different non-communicable diseases across the last two centuries [13]. Interestingly, a recent publication has revealed that the previously recognised drop in body temperature (actually 0.03°C per birth decade for at least the last 150 years) is real and not as a result of measurement errors, as has been assumed previously [14]. Perhaps the best way to explain this is to say that, for whatever reason, the background level of tissue inflammation has been toning down over recent decades. Although these are early days, the cumulative impression given from these latter publications [11-14] is that the immune system has been changing from proactive to reactive with the consequent danger of overreaction leading to the onset of atopic disease. A corollary of this argument is that many human studies may have to be reinterpreted in the context of a steadily degrading microbiome. For this reason it is important to include the microbiome in studies of peoples such as the Tsimane, existing on the edge of the modern world (See section On the edge of the modern world: Denis Burkitt, the Maasai and the Tsimane), currently being offered medical assistance in return for information on their health [15].

The variability observed in human studies is partly due to missing functionality in the microbiome of individual participants. We need to identify accessible microbiologically stable populations in order to provide a “top standard” against which to measure any improvements in human health. Such populations should have a low incidence of non-communicable diseases including, for example, seasonal allergic rhinitis and varicose veins. Consideration should be given as to whether those populations that exhibit a higher than average body temperature in apparently disease-free individuals are more suitable.

Dysbiosis

Microbiome-function deficiency disease

The term microbiome normally refers to the sum total of all the microbial species occupying a specified part of the body. However it is important to note that microbial function is not necessarily the same as microbial species, and we have previously suggested that valuable functions are more readily transferred to and within a microbiome that contains a greater variety of microbial species [16,17]. This lack of diversity, which has been termed dysbiosis [18], is apparently related to non-communicable disease [16]. It is also worth noting that this condition is not an on/off switch but is a variable state in which there are degrees of malfunction with a wide range of potential outcomes.

Both obesity and functional disorders of the gut have been associated with deficits in the microbiome acting via the gut-brain axis [10,19], autoimmune disease acting beyond the gut [20] and type 1 diabetes [21]. Misbehaving microbiota have also been implicated in problems with immunoregulation and mental health [22] and with cancer [23]. It seems that an efficiently functioning microbiome is critical for early life [24]. Although many reports acknowledge that the microbiome is somehow involved in the increasing incidence of non-communicable disease this insight rarely leads to valuable practical applications. There are a few exceptions to this rule: faecal

transplantation can clear up episodes of antibiotic-induced *Clostridium difficile* infection [25]; vaginal inoculation has proved popular in attempting to offset the potential negative consequences of caesarean section [27]; and probiotics aim to improve the outcome for pre-term babies [28,29].

The mutualistic microbiome. Identification friend or foe: the kairomone conundrum

In our previous publications [16,17], we considered that the functioning microbiome may be treated as a single mutualistic community with two parallel allomone/kairomone modes of action. An allomone-like system, probably involving substances such as dopamine and serotonin, strengthens the gut-brain axis and thereby allows a more facile flow of food through the digestive tract to feed the intestinal microbiome. The failure of this system leads to the development of obesity and mental health problems [16] while, in principle, the measurement of such allomones can give an insight into the efficiency of the microbiome itself [17]. Conversely, in this scheme, the microbiome assists us by somehow utilising kairomone-like functions to calibrate the immune system, thus distinguishing harmful from harmless antigens. Failure of these functions allows diseases such as seasonal allergic rhinitis (hayfever), food allergies, asthma, autoimmune, and inflammatory conditions to develop [16]. Many of these present themselves in the same patient, so for example, the term atopic march refers to the development of such diseases as infants progress through childhood [30].

It seems likely that this kairomone-like system acts as the interface between the external microbial world and the host immune system and may correspond to the Old Friends of Rook and coworkers [31]. In the case of mammalian hosts the implication is that the system must have done its job before the neonate was weaned from its mother's milk. As such, dysbiosis within this early stage would render the infant vulnerable to inappropriate responses to environmental changes and potentially initiate immune system malfunction in future years. As mentioned above (Section Introduction. Non-communicable disease: lifestyle, genes, or microbiota?), it is important to note that many human studies (and probably many animal studies) have been carried out on individuals already suffering from variable degrees of dysbiosis. This confounding variable can only be removed when populations are found that are not suffering from significant microbiome-function deficiency disease (See section On the edge of the modern world: Denis Burkitt, the Maasai and the Tsimane).

It is envisaged that the role of a kairomone function would be to probe suspect particles to see if they were friend or foe, a harmless pollen grain or a harmful bacterium, for example. If such a particle turned out to be poisonous, such as a toxic heavy metal, then a sufficient concentration could render the animal immune system effectively blind. This idea is explored further in the light of the causes of dysbiosis.

The causes of dysbiosis

In order to understand the cause of disease it is necessary to know both its temporal and spatial spread, as well as any overlap with different species [32]. Unfortunately, the idea of disease depends on the social mores of the day, especially so for non-communicable conditions, and can be hard to track over the centuries [13]. Of the three major manifestations of dysbiosis (diseases related to obesity; poor mental health and problems related to immune system malfunction) the one that is historically the most well documented is perhaps the most mysterious: hayfever.

Immune system malfunction: John Bostock and his hayfever

There is only one unambiguous historical reference to hayfever prior to the mid 19th century: Dr. John Bostock who, in 1819, reported himself as the first example of this condition (seasonal allergic rhinitis). In 1828 he reported that he had found a further 28 cases of the condition he called "*Catarrhus aestivus*" (summer catarrh), specifically stating that there is a socio-economic element to the aetiology: "it is remarkable that all cases are of the upper and middle classes, some of high rank" and that he "has not heard of a single unequivocal case amongst the poor" [35].

Compare with today: a questionnaire issued to schools in 2004 showed that 51% of 15/16 year-old students self-reported having hay-fever symptoms, of which 21% were confirmed by nurse or doctor, the symptoms being trivialised by both health professionals and the patients themselves [36]. Nevertheless, the symptoms manifested in 1819 are the same as today with the key differences being the vastly increased incidence and the lack of previously described class selectivity. It seems that these differences are the hallmark of the change of dysbiosis from endemic to epidemic status and must somehow be reflected in the cause of this condition.

There have been scattered reports of allergies throughout history, perhaps going back to the death of King Menses of Egypt after being stung by a wasp in the 4th millennium BCE. Of course, history is written by and on behalf of rich people. What was going on in the silent populations at the same time? Equally, although animals don't have much of a voice they too are suffering from similar atopic diseases, as a recent comparative review shows [12].

Obesity and heart disease: Endemic to epidemic

While obesity and concomitant heart disease have always been with us, they have mostly been associated with the "upper and middle classes" (to paraphrase Dr. Bostock), if only because they were the ones that could afford sufficient obesogenic food. The situation has changed recently, however, with both obesity and atherosclerotic heart disease becoming much more widespread, roughly paralleling the rise in hayfever [2,13].

In addition, the deliberate induction of accelerated growth in farmed animals by adding "growth promoters", including antibiotics, is widespread but without any clear understanding of their mechanism of action, for example in pigs [38]. Accordingly, the pressure to reduce antibiotic use has led to a belated drive for such mechanistic understanding [39,40]. Fish are also subject to the action of antimicrobial growth promoters, suggesting a similar mechanism of action, whatever that may be [41]. It is worth noting that the microbes mentioned in these articles are all considered to be competing with the animal in some way. The possibility that we could be dealing with a vertebrate mutualistic microbiome has not yet been reported. The parallel with the recently reported increase in size of children [4] is striking and suggests that antibiotic use in humans also drives growth promotion. It may be that we should aim to encourage antibiotic use to drive animal growth while introducing methods such as phage therapy for use in humans (See section The causes of dysbiosis: selective loss of microbiota).

Wild animals may also be affected: there have been newspaper reports of English suburban populations of the *European hedgehog*, *Erinaceus europaeus*, developing obesity after what was described as inappropriate feeding by householders. As this prevented them adopting the normal defence of curling up into a tight ball it would only be possible in a sheltered environment and would normally result in death by predation. This behaviour, so obviously reminiscent of humanity, strongly suggests a population struggling in the grip of dysbiosis. Although the measurement of wild animal populations is difficult there is ample evidence that hedgehog populations in heavily industrialised England have declined substantially since the mid 20th century.

Poor mental health: Sigmund Freud and the placebo effect

Poor mental health likewise seems to be ubiquitous in its various guises. Perhaps the most significant early worker was Sigmund Freud [44], who found a calling among people from the "middle and upper classes", to coin a phrase of Dr. Bostock's [35], either because the "poorer classes" did not suffer from similar symptoms or because they were simply ignored and/or committed to asylums. More recently, and in line with the rise of hayfever and obesity there has been a corresponding increase in the incidence of poor mental health across all classes, though in this case changes in diagnostic criteria complicate the situation [9].

These conditions may be related to the degradation of the microbiota-gut-brain axis as microbiome-function deficiency disease takes effect. It is possible that the interesting theories of Dr Freud and the early psychoanalysts, along with the personal attention lavished

on the patient, were effective in that the stimulation of the brain side of the gut-brain axis temporarily made up for deficiencies in the function of the microbiota themselves [10]. What is now known as the placebo effect is ubiquitous and has “spoilt” many an optimistic clinical trial. Of course, the effect is itself hard to study, partly owing to the inevitable absence of an appropriate placebo (!) but also due to a plethora of different biases [45]. Interestingly, deep brain stimulation of structures related to the corpus striatum in humans led to dopamine release and was shown to increase hepatic and peripheral insulin sensitivity [46], consistent with the increasing evidence for the central regulation of glucose metabolism [47]. While this was initially an accidental observation made while investigating obsessive-compulsive disorder, it may thereby cast light on the relationship between the microbiota-gut-brain axis and obesity [10].

In principle poor mental health should also be observed in animals suffering from dysbiosis. Unnatural and repetitive behaviour such as hair pulling in laboratory animals is reminiscent of obsessive-compulsive disorder and is ascribed to being forced to live in a non-enriched environment [48]. It is possible that a degraded microbiome may partly be responsible. It is possible that similar symptoms may arise by degradation of either terminus of the microbiota-gut-brain axis [10].

On the edge of the modern world: Denis Burkitt, the Maasai and the Tsimane

Burkitt travelled throughout post-Second World War Africa noting that their primarily rural communities ate a higher proportion of dietary fibre and passed stools of three to five times greater volume than their Western contemporaries. He also noted the absence of what he called “characteristic modern Western diseases” in these communities [7]. He popularised the theory, not unreasonable from a mid-20th century perspective, that the lack of fibre caused these diseases simply by increasing intra-abdominal pressure upon defecation [8]. Unfortunately, he underestimated the importance of the Maasai, who subsisted largely on meat and dairy, ate relatively little fibre and yet stayed healthy [49,50]. In our recent publications we have reinterpreted the findings of Burkitt in the light of the modern understanding of the microbiome, coming to the conclusion that dietary fibre is of little use itself unless it is accompanied by an efficient working microbiome [16,17].

In contrast to the majority of the world, people living outside the reach of modern life do not exhibit these diseases. So, for example, the Tsimane, living in the upper amazon basin, have been reported to be free from Western levels of obesity and heart disease, though they do have high levels of inflammation and therefore, probably, raised body temperature [15]. Sadly, this study did not report any data on either their microbiome or their faecal volume, indicating that the lessons of Burkitt have been forgotten. It is likely that antibiotic use will have been documented, so it should be possible to obtain some retrospective data on microbiome alterations. This opportunity should not be lost.

Interestingly, analysis of preserved faecal matter from a small number of sites around the ancient world shows that the high diversity of their microbiome more closely resembles that of peoples peripheral to the modern world [51].

Dysbiosis: The holobiont and reproductive success (Figure 1)

In summary, although acknowledging the inevitable huge gaps in the data, it seems that there were two phases to the development of these parallel dysbiosis conditions: an initial endemic phase mostly affecting well-off people followed by an on-going epidemic potentially involving everybody. At some point domesticated animals began to exhibit symptoms [12], of which one, increased growth, was actually a desirable outcome [38]. Although it is hard to be sure, it is possible that the collapse of English populations of the European hedgehog is caused by dysbiosis [43]. As indicated above (Section The mutualistic microbiome. Identification friend or foe: the kairomone conundrum), it is possible that dysbiosis is brought on by heavy metal poisoning (Section Poisoning the microbiome in the pre-antibiotic age: cosmetics).

The idea that the reproductive success of an animal is a function of an effective symbiosis between it and its microbiome was stated as far back as 1991 and was expressed as the holobiont [52,53]. Dysbiosis would be the selective disruption of the holobiont by loss of microbiome-related functions. Its negative effects may partly be cancelled by the development of effective medicines, at least in humans and our associated animals. By contrast, susceptible wild animal populations may become unsustainable (Figure 1).

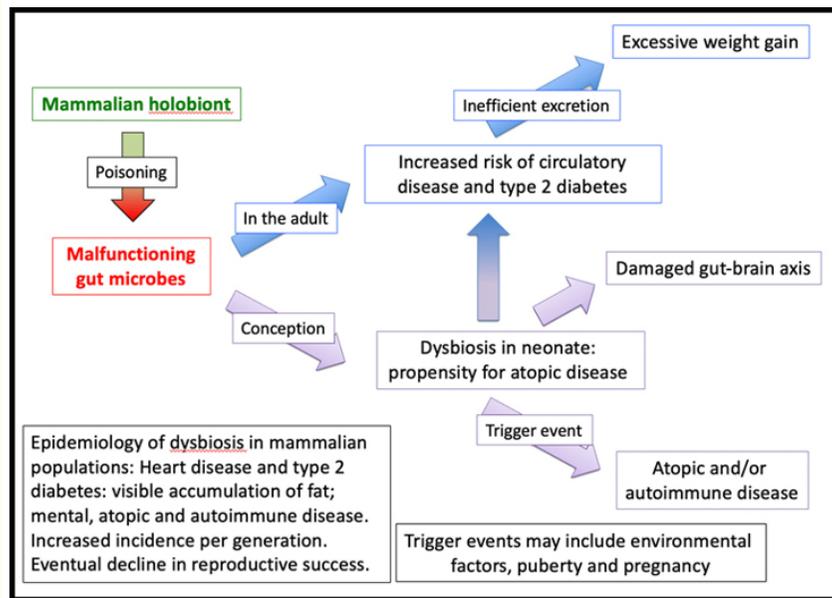


Figure 1: Suggested outcome of holobiont disruption due to microbiome poisoning.

The causes of dysbiosis: selective loss of microbiota

In an attempt to understand the new epidemic of hayfever, Strachan suggested that modern people were exposed to fewer relatively harmless microbes than people living in the past [11]. Although this idea has been taken up and developed into the concept of “Old Friends”: creatures that tone down the immune system, nevertheless it does not seem to be the whole story [54]. Equally, although there is evidence that the microbiome may lose diversity with a poor diet, this is a slow process that takes many generations and is therefore unlikely to be a major driver of the current epidemic [55]. A more likely cause is microbial loss during the process of caesarean section [27], granted that any loss of key microbial species may not be replaced and that such a deficit may be passed on to subsequent generations in a “snowball effect”. Of course non-selective antibiotics are specifically designed to destroy bacteria and can only contribute to the downgrading of the microbiome. Accordingly a model system has been set up in an attempt to assess antibiotics that produce only minimal disturbance [56]. Equally, in principle, phage therapy ought to be more selective for a target pathogen [57].

This correlation also accounts for the absence of “Western disease” observed by Denis Burkitt in his African studies and of the observation of enlargement in farmed and domestic animals owing to veterinary antibiotic use. However, it is harder to understand dysbiosis in pre-antibiotic societies and in wild animal populations.

Poisoning the microbiome in the pre-antibiotic age: cosmetics (See section The mutualistic microbiome. Identification friend or foe: the kairomone conundrum)

Although there were numerous potentially toxic substances in use since early modern times there is one class of materials that stands out as the most likely candidate for such poisoning: heavy metal salts, especially those of lead [58]. The use of colourful heavy metal salts for cosmetic purposes has been reviewed in book form [59]. It is likely that all civilisations with extensive trading links will have had access to such metals, especially lead and its various salts, with the corresponding risk of both overt poisoning and of selective, covert, poisoning of the microbiome: dysbiosis. The ancient Egyptians used galena (lead sulfide) as an eyeshade and, unlike the less well-connected Tsimane [15], their mummified bodies have been shown to have atherosclerotic plaques in their veins [60]. A little later in history, the extremely well connected Romans made extensive use of lead and probably suffered from similar diseases, so that the skeleton of a young, well-off lady exhibiting the signs of coeliac disease has been uncovered [61]. Investigation of her dental enamel showed traces of both turmeric and ginseng, leading to the suggestion that these agents were an attempt to treat the disease [62]. It seems that endemic dysbiosis is an occupational hazard of technologically advanced civilisations with extensive trading networks using toxic heavy metals.

Contemporaries of Dr. Bostock may have suffered as their mothers' beautified themselves in a, not always successful, attempt to attract powerful husbands. In this way dysbiosis became a chronic disease of the richer classes, leavened only by (not infrequent) occasional input from (younger, more attractive) mothers from less well-off backgrounds. Natural birth ensures the smooth transfer of the gut microbiome while breast-feeding helps its establishment [63,64], if necessary assisted by a wet nurse or milk bank. Unfortunately, dysbiosis in the mother is passed directly on to the child and in this way it gives the impression of being solely a genetic inheritance.

Following the analogy of cosmetic heavy metal poisoning it is possible that microscopic inorganic lead particles emitted from the exhausts of vehicles powered by leaded petrol could have provided enough environmental contamination to affect both humans and hedgehogs. Of course, the poisonous nature of lead was well understood but its removal from petrol remains a slow process [65]. There are many other sources of lead exposure, but it is important to note that, although the dangers of lead are well understood, its effects on the microbiome have not been considered [66]. The crucial point is that maternal transmission of dysbiosis means that it is possible that lead continues to exert its effects long after the original source of the poisoning has been removed.

Summary: Toward a germ theory of health

In many respects the current understanding of the microbiome resembles the years before the formulation of Koch's postulates (or the Koch-Henle postulates [67]). Even when complete, researchers knew that these postulates did not hold all the time, but by formalising the situation they laid the foundations for the now-accepted germ theory of disease [68]. Unfortunately, the very success of this theory has long overshadowed the idea that intestinal microbes could somehow be essential to health. Of course the two approaches are not incompatible, as pathogenic microbes are often found in unexpected places, for example the *Porphyromonas gingivalis* currently being associated with Alzheimer's disease [69], but it could be that this penetration is facilitated by a dysfunctional microbiome.

Since our species first appeared on this planet we have occupied all parts of the world that can possibly sustain life. Our pace was slow, generation-by-generation, at walking pace or by boat, with Rapa Nui (Easter Island), possibly the last place, first occupied well into historical times [70]. Nowadays we can travel by aeroplane, much more quickly, but with the possible downside of traveller's diarrhoea [71]. From a Western-centric point of view, the causative agents of this dramatic condition may be termed pathogens, and yet they do not affect the locals. Presumably it takes time for our immune system to learn to tolerate these sub-pathogens, but nobody knows how long this process takes. It is possible that this internal rearrangement, though uncomfortable at the time, has some long-term intergenerational benefit in the way in which the microbiome interacts with the environment. It is likely that such information transfer is transmitted through these mysterious kairomones, in which the immune system of a developing infant is calibrated to allow for the local environment into which the child is born.

It is likely that the variability of people's response to stimuli such as food and drugs is associated with both the nature of their genome and their degree of dysbiosis. At the moment there are no useful ways to compare levels of this condition, though it may prove that an ingestible sensor would be helpful if it could be calibrated to an indicator semiochemical generated in the gut [17]. Although a higher body temperature may be indicative of a more effective immune system it is clear that more studies will be needed before that can be realised, especially on a per-individual basis [14].

A consequence of this analysis is that we may have to look outside modern Western-based populations for some sort of "top standard" by which the function of the intestinal microbiome may be compared. It is for this reason that studies of the Tsimane are so important [15]. It is unlikely that such studies will be excessively invasive.

Conflicts of Interest

The authors declare no conflict of interest.

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