Epigenetic fleas bite the genome in bone health, muscle strength, the gut, heart health and ketogenesis.

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Big fleas have little fleas upon their backs to bite 'em,

And little fleas have lesser fleas, and so, *ad infinitum*.

And the great fleas, themselves, in turn, have greater fleas to go on;

While these again have greater still, and greater still, *and so on*.

Augustus De Morgan [1]

In the 1970s, I was privileged to work in the research unit of John Waterlow, at the London School of Hygiene and Tropical Medicine. He enthusiastically supported research into the effect of long-term malnutrition in a rat colony (10 to 12 generations) which was then re-fed [2]. This was designed to mimic the situation for children malnourished in utero and at birth in developing countries, a topic which has been extensively investigated by Sally Grantham-McGregor in Jamaica. The human data showed that early stunting led to psychological deficits in later life which were never fully compensated for by nutritional supplementation [3]. What the human studies could never show was intergenerational effects. The 10th or 12th generation offspring of malnourished rats *were "rather small, badly groomed, excitable …. without gross abnormalities"* but it took a couple of generations of adequate feeding for these overall characteristics to disappear [2]. We now know that the offspring were subject to two effects, firstly we would say that intrauterine deprivation (foetal programming) had led to effects in adulthood, but of equal interest was that these effects were an expression of epigenetic inheritance in the offspring. In other words, the inheritance of acquired traits in a Mendelian fashion. At this distance it is hard to understand the revolution this idea has wrought in modern biology. Even the research of distinguished but disgraced and

discarded neo-Lamarckian geneticists like Paul Kammerer (1880-1926) has been reevaluated. His studies on the midwife toad, far from being a fake may be interpreted as the first descriptions of epigenetic inheritance [4]. The concept is simple. Life events (e.g. intrauterine growth retardation, trauma) or environmental factors (e.g. stress, alcohol abuse, smoking) lead to epigenetic changes to the nucleosomal proteins (e.g. acetylation) or to sequences of DNA (e.g. methylation) which lead to silencing or increased expression of specific gene sequences. The trick is that these changes are passed on to the offspring as epigenetic characteristics (Greek epi-, ἐπι- "over, outside of, around" – “on top of” or “in addition to” the traditional genetic basis for inheritance). Subsequent research has shown that other epigenetic effects of malnutrition are now known to be common in subsequent generations as shown in Table 1 [5].

This has meant that in addition to the simpler picture of Mendelian inheritance and modulation of gene expression through transcriptional factors and their complex hinterland of hormones, effectors and single nucleotide polymorphisms, there is an inherited memory effect which will modulate it all with unpredictable consequences. A “healthy diet” for one person may not suit another. Despite the enthusiasm of nutritionists for healthy eating, there is an inexplicable grey area where healthy diet does not seem to have its desired effects. The fleas really do seem to have smaller fleas upon their backs to bite them.

In these 5 reviews, the authors were asked to provide an up to date commentary on the impact of epigenetic modulation on important human organ systems with emphasis, where possible, on how it affects nutrition requirements. Each author has provided an

explanation of the molecular basis of epigenetic effects so that the reader can understand this by reading one or all of the reviews.

In the first review, Lovšin and colleagues have tackled the question of bone health [6]. In brief, osteoporosis represents an uncoupling of the rates of bone deposition and bone resorption [7] so that bone mineral density (BMD) in women declines after menopause. The effect is increased by pre-existing metabolic diseases like types 1 and 2 diabetes and uncoupling also occurs in people with rheumatic disease [8]. Lovšin and colleagues dissect out the role of genetics which accounts for between 50 and 85% of BMD variation [6]. Genome-wide association studies have revealed a huge variety of genes which relate BMD to muscle and adipose tissue remodelling. It has always been thought that exercise can improve BMD and the most recent meta-analyses suggest that this may be so and of clinical significance [9].

In the second review, Ryall and Lynch describe the latest findings in muscle regeneration [10]. This is one of the most significant goals in clinical nutrition support because muscle mass is so easily lost during critical illness and is so slowly regained. As the authors say *"Skeletal muscle contains a population of tissue resident somatic stem cells (muscle stem cells, MuSCs), which give it a high regenerative capacity"* but it has always seemed that nutritional supplementation can only, at best, prevent excess muscle loss whilst regain only occurs after recovery with a vigorous exercise programme. Fiatarone and colleagues showed this convincingly in their old study of exercise or nutrition supplements in frail elderly people [11]. In other words, metabolic supply is dragged along behind the insistent demand for growth which exercise represents. What Ryall and Lynch describe is a different scenario in which MuSCs

exhibit a process of metabolic reprogramming, from fatty-acid oxidation during quiescence to glycolysis during proliferation. What is fascinating is the finding that increased glycolysis shifts the intracellular NAD+/NADH couplet towards NADH and this in turn stimulates histone acetylation (i.e. de-repression) of genes responsible for MuSC activation (via MyoD protein). The matter is complicated by the finding that acetylation is not the only histone modification since propionate, butyrate, 2-hydroxyisobutyrate, succinate, malonate, glutarate, crotonate and β-hydroxybutyrate can all modify histone and activate genes to a variable extent. The modification pattern depends very much on the substrates at hand, which relies on whether the cell is oxidising fatty acids or glucose or is using branched-chain amino acids. It is clear that the “fleas” come in different flavours.

The third paper describes the genomic changes which occur in the intestine following bariatric surgery [12;12] which can, almost magically, transform the metabolic health of morbidly obese individuals [13] before leading to useful and sustained weight-loss [14]. The cause of this effect is unclear and some argue that it is due to very rapid, post-surgical loss of ectopic fat around the pancreas and liver which had previously interfered with glucose signalling. Certainly, very-low energy diets which yield rapid weight-loss can resolve type 2 diabetes (T2D) when administered in primary care settings [15]. The most effective form of bariatric surgery is called Roux-en-Y, a description which combines the name of the surgeon who first described it (César Roux) and the configuration of the bowel after surgery which is now in the form of the letter “Y”. The “Roux” arm (A) is the proximal segment of stomach joined to the distal small bowel and is also known as the “alimentary arm” since it receives all food after surgery.

The other arm of the “Y” (B) comprises the stomach and proximal small intestine as a “blind” segment which receives and transmits all pancreatobiliary secretions which joins arm A and will aid digestion. The lower part of the Y (the leg?) Is formed by the distal small bowel beyond the anastomosis. One consequence of this is that parts of the bowel will receive substrates (undigested food or pancreatobiliary secretions) to which they are not accustomed. This has been known to lead to marked trophic changes which were previously described in terms of biochemical changes [16] and altered cell turnover kinetics [17]. Two recent findings stand out in this review. Firstly, surgical reduction in gastric volume alone (gastric plication) reduces “hunger” signals because expression of the gene encoding for ghrelin is reduced. Additionally, glucose absorption in the alimentary (A) arm is reduced, partly because of reduced luminal sodium absorption (expression of SGLT1, encoded by SLC5A1 was unchanged) whereas GLUT1 expression was increased to compensate. This may have implications for post-prandial glycaemia. Lastly, the authors present their own recent studies which shed light on the transient vitamin B12 deficiency which follows surgery. Gastric expression of TCN1 (gene encoding transcobalamin 1) and of GIF (gene encoding IF) is reduced but there is a compensatory upregulation of expression of CUBN (gene encoding cubilin) in the small intestine. This acts as a receptor for the IF-B12 complex. In other words, although less vitamin B12 is captured by intrinsic factor in the stomach to form IF-B12, a greater proportion of this IF-B12 is extracted in the small intestine [12].

The nutriepigenetics of cardiovascular disease are covered by Kalea and colleagues in a wide-ranging review [18]. This is such a broad and well-researched area that they do exceedingly well to keep the topic within the narrower compass of the way in which diet

might affect epigenetic modulation with benefit. The point is well made that energy metabolism by mitochondria provides the key substrate of epigenetic change, that is acetate which is a surprisingly active molecule. In the form of acetyl-CoA, its free energy of hydrolysis is equivalent to that of ATP. Furthermore, it is unsurprising that pathophysiological states which impact on the production of acetyl-CoA (or rather its feedstock source) also impact histone acetylation. These factors include adiposity, inflammation and oxidative stress which are covered in this review. I was very interested to read the latest genome wide studies which shed light on the way bioactive dietary compounds (e.g. resveratrol, curcumin or epigallocatechin) which are abundant in the Mediterranean Diet, alter histone acetylation and risk factors [19].

Lastly, we live in the era of social media which has become the arena for gladiatorial combat over diet, specifically the insurgency against national recommendations to reduce fat intake, and especially saturated fat, in favour of mono- and polyunsaturated fats [20]. There is no middle ground in this fight in which one party pins the guilt for T2D firmly on dietary carbohydrate (especially sucrose) which they think should be severely limited to improve health outcomes [21;22]. This is in contrast to the orthodox view that becoming obese is, itself, a major risk factor and that restriction of energy intake by low-carbohydrate or low-fat diets brings benefit, by reducing excess adiposity, in other words “a calorie is a calorie” [15;23]. The biochemical consequence of reducing energy intake dramatically is that carbohydrate intake is reduced and below a certain threshold, the body adapts by producing ketones from hepatic fatty acid β-oxidation, whilst simultaneously switching the source of gluconeogenic precursors from the Cori cycle and glucose-alanine cycle to glycerol released from triacylglycerol hydrolysis. This is a

neat mechanism to ensure that glucose is available for all tissues which require it. This phenomenon has been described elegantly by an isotopic study of exercising athletes who had become adapted to a low-carbohydrate, ketogenic diet [24]. In essence, gluconeogenesis was unaltered but the oxidation of fatty acids directly, or via ketones increased to make up for the reduction in glucose oxidation. So much for the metabolic logic, but can ketosis *per se* be clinically beneficial? Present thinking is quite conflicted. On the one hand, there is little evidence that intravenous ketones can ever substitute the traditional glucose/fat emulsion diad in parenteral nutrition [25] and indeed a constant anxiety has been to prevent ketosis during intravenous MCT infusion in the form of structured MCT/LCT emulsions [26]. However, ketones are often a significant fuel for the body and may have effects over and above those of simple, water-soluble energy substrates for the brain and kidney [27]. They may, for example, have anti-inflammatory effects or alter the epigenome during periods of intermittent-fasting or of consuming a low-carbohydrate diet. In their review, Ruan and Crawford describe the way in which periods of ketosis are associated with covalent modifications to histone protein lysine groups, with acetyl, methyl and hydroxybutyryl groups as the “fleas” to bite the genome. The authors elegantly review latest findings on control of ketogenesis and giving a resumé of how ketogenesis is controlled. It is worthwhile repeating that mitochondrial succinyl-CoA-oxoacid transferase (SCOT) catalyses the fate-committing reaction that converts acetoacetate to acetoacetyl-CoA which will be converted to acetyl-CoA. It is the absence of SCOT in liver mitochondria which condemns that organ to the role of net exporter of ketones during periods of starvation of dietary glucose-deficiency, in other words it does not escape scot-free. The excitement in this field is

that genome-wide lysine β-hydroxybutyrylation (Kbhb) histone marks (or flea-bites) are proportional to the abundance of β-hydroxybutyryl-CoA and may consist of the D-isomer which has arisen by a novel pathway distinct from ketogenesis. Furthermore, low-carbohydrate, ketogenic diets are useful in reducing seizure episodes in patients with intractable epilepsy and may be neuro-protective [28]. It is intriguing that ketosis is associated with succinyl-CoA depletion (vide supra) and that this can only reduce succinylation of histone proteins. Additionally, the signalling roles of ketones may be important in anti-inflammation mediation. This should not be surprising given the known role of other oxo-acids such as the leucine metabolite, β-hydroxymethyl butyrate (HMB) which stimulates the mTOR, pathway which has many anabolic functions. Therapeutically, HMB seems to improve muscle growth and strength [29].

Two things struck me about these reviews. The first was the centrality of intermediary metabolism in providing the "fleas" to bite the genome. By this I mean the availability of metabolites such as acetate, succinate, propionate and β-hydroxybutyrate. The second was the tiny scale of this process and I don't mean the size of DNA itself. What I mean is the number of signalling molecules themselves which are being generated by mitochondria. One of my colleagues, Professor Gordon Stewart attempted to inform our medical students by asking them to *"calculate the number of molecules of oxaloacetate in a mitochondrion, given that the concentration of malate is 0.2 mM, the NAD^+:NADH ratio is 10:1, and the Gibbs free energy change ΔG in the reaction is +7.1 kcal/mol".*

This is the key equilibrium reaction in the Tricarboxylic Acid Cycle in oxaloacetate is engaged:-

Malate + NAD^+ ↔ Oxaloacetate + NADH

The calculation involves the NAD^+/NADH ratio, the equilibrium constant for the reaction, the calculated concentration of metabolites and the volume of an average mitochondrion. According to this, there are approximately 17 molecules of oxaloacetate in an average mitochondrion. Epigenetic changes seem to depend on small numbers of fleas but have large effects!

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Early findings that chronic malnutrition exerts intergenerational effects through epigenetic mechanisms

Insult	**Outcome**	**Generation**
Zinc-deficient pregnant mice	Cell-mediated immunity reduced, reduced circulatory IgM	F0, F1, F2
Nutritionally-deprived young rats	Impaired antibody-forming cells and responses	F0, F1
6 weeks energy restriction in young rats	Impaired humoral immunity	F0, F1, F2
Low protein diet in pregnant rats	Low body weight	F0, F1, F2
Maternal diabetes (streptozotocin) in rats	Glucose intolerance	F0, F1, F2
Chemically-induced thyroid dysfunction	Hormonal disorders	F3, F4, F5