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Childhood obesity: a systems medicine approach

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1. ABSTRACT

Childhood obesity and its sequelae are a major public health problem in both the USA and globally. This review will focus on a systems medicine approach to obesity. Systems medicine is an integrative approach utilizing the vast amount of data garnered from “omics” technology and integrating these data with conventional pathophysiology as well as diverse environmental factors such as diet, exercise, community dynamics and the intestinal microbiome. Omics technology includes genomics, epigenomics, metagenomics, metabolomics and proteomics. In addition to unraveling etiology, the goals of a systems medicine approach are to provide actionable and evidenced-based clinical approaches. In the case of childhood obesity, an additional goal is characterizing measureable risk factors/biomarkers for obesity at the earliest possible age and devising age-appropriate optimal intervention strategies. It is also important to establish the age at which interventions could be critical. As discussed below, it is possible that some of the pathophysiological and epigenetic changes resulting from childhood obesity could become more irreversible the longer the obesity remains untreated.

2. INTRODUCTION

2.1. A systems medicine approach to childhood obesity

Although there are a good number of recent publications concerned with systems biology and obesity in adults there has been much less emphasis on childhood obesity (1-3). The comprehensive review by Levian *et al.* (3) provides an excellent summary

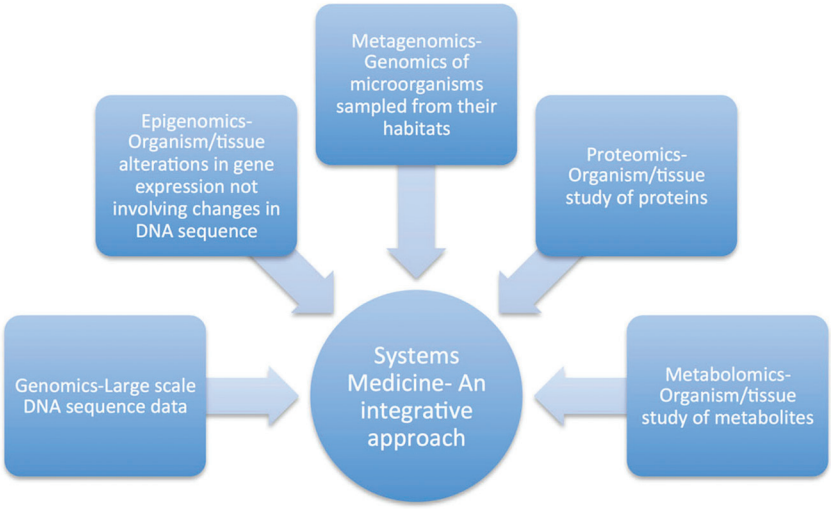

Figure 1. In addition to physiological, biochemical and environmental parameters, systems medicine integrates the large-scale data sets from "omics" technology, i.e., genomics, epigenomics, metagenomics, proteomics and metabolomics. The goal of systems medicine is to improve human health and well being.

of the pathogenesis of adult obesity with an emphasis on genomic data. Skinner and Foster provided a comprehensive review of the application of systems science to childhood obesity emphasizing the importance of community dynamics and the multiple societal levels impinging on obesity (4). System science is especially well suited for helping planners and policy makers evaluate community strategies for dealing with public health threats such as obesity and type 2 diabetes (T2D). Systems biology is more concerned with disease etiology and mechanisms while system medicine is more concerned with clinical utility. Genomics, epigenomics, metagenomics, proteomics and metabolomics are the enabling omics technologies of systems biology and will be described below in more detail (see Figure 1). Proteomics and its application to pediatrics were described in the first article in this Frontiers in Bioscience series (5). Where possible, this review will focus on the systems medicine of childhood/adolescent obesity but will also make reference to the most recent adult "omics" data.

2.2. Childhood obesity is a major public health problem: epidemiology and healthcare costs

The epidemiology of childhood obesity is alarming and presents enormous challenges to both public health and health care costs. Data from the Centers for Disease Control and Prevention (CDC) shows that over the last 30 years, the incidence of obesity in children has doubled and the incidence in adolescents has quadrupled (DOI: healthyyouth/obesity/facts.htm). About one-third of both children and adolescents were obese or overweight in 2012. The definitions provide by the CDC are useful: overweight is having excess body weight for a given height from a combination of fat, muscle, bone and water whereas obesity is defined as having excess body fat. The Duke Global Health Institute (DGHI) recently put the health care costs for childhood obesity in a sobering perspective: over a lifetime the direct costs are estimated to be $19,000 per child (6, 7). For the USA, the direct health care costs for ten year-olds alone are about $14 billion dollars. As noted by Dr. Eric Finkelstein at DGHI, "For the same reasons we don't let kids drink or smoke and force them to go to school, we should also do our best to keep them at a healthy weight"(6). Childhood obesity is a major risk factor for the development of insulin resistance, type 2 diabetes (T2D), hypertension, hyperlipidemia, nonalcoholic fatty liver disease (NAFLD) and renal disease (DOI: article/985333-overview). Moreover, if left untreated childhood obesity is a major contributor to adult obesity, cardiovascular disease and cancer. A recent large-scale study from the United Kingdom suggests that once an adult develops obesity, defined as a BMI between 30.0. and 34.9., the probability of attaining a normal weight is very low, i.e., 1 in 210 for men and 1 in 124 for women (8). These data suggest that current primary care-centered weight loss programs in the UK are not effective and that more emphasis should be placed on preventing children from becoming obese.

2.3. Current evidence and recommendations for addressing child and adolescent overweight and obesity

Evidenced-based recommendations for addressing child and adolescent overweight and obesity utilize a stepped-care approach that is typical for treating any chronic disease (9). Current recommendations include: (1) universal screening using BMI in children 2 years of age and older; (2) assessment of risk factors

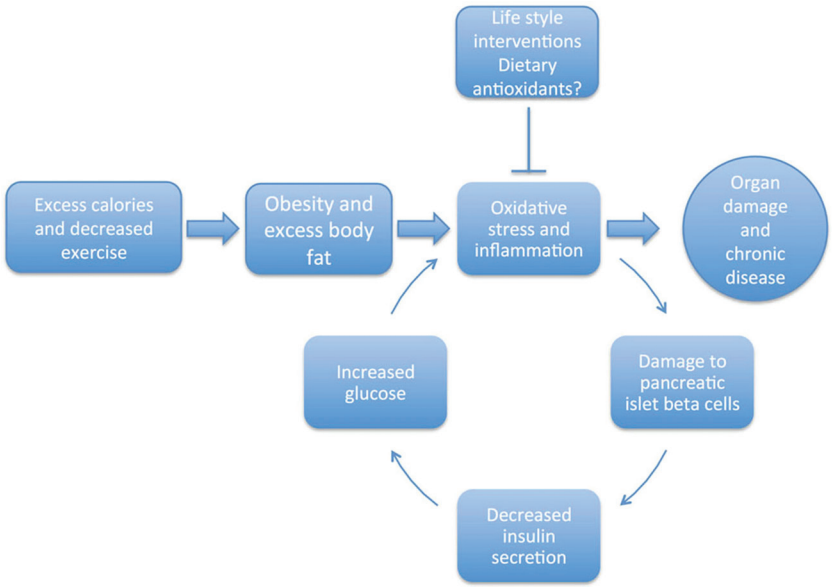

Figure 2. Excess dietary intake of calories, particularly from high fat and high sugar foods, combined with decreased exercise is a major contributor to both childhood and adult obesity and excess body fat. As detailed in the text, obesity and excess body fat contribute to a chronic state of inflammation and oxidative stress. Over time, oxidative stress damages pancreatic islet beta cells leading to decreased insulin secretion and increased blood glucose levels. Increased blood glucose levels give rise to advanced glycation end products (AGEs), which are inflammatory and promote additional oxidative stress. These processes lead to a chronic cycle with ever increasing levels of oxidative stress that can cause ongoing organ damage and chronic diseases such as atherosclerosis. Lifestyle interventions are the key to breaking the chain of events associated with excess caloric intake and lack of exercise. Diets rich in a diverse array of antioxidants may be more effective at decreasing inflammation and oxidative stress than single high dose antioxidants given as a dietary supplements.

(such as parental obesity) and comorbidities, and; (3) use of motivational interviewing, behavioral therapy, and a family-based approach (10). It is recommended to increase frequency of visits, intensity of monitoring, and involvement of a multidisciplinary team if improvement in weight status is not achieved. A 2010 U.S. Preventive Services Task Force meta-analysis concluded that moderate-to-high intensity behavioral therapy is effective in modestly improving weight status in overweight and obese children and adolescents, with pharmacotherapy providing an added benefit among adolescents (11). Low-intensity behavioral treatment, feasible in primary care settings, produced more modest and less consistent improvements. Recent recommendations stress the importance of obesity prevention efforts in clinical and community settings, including during the first two years of life (12). While evidence for behavior targets for obesity prevention is more limited or derived from treatment research, many have multiple health benefits. These behavior targets include breastfeeding, increasing fruit and vegetable consumption, limiting screen time and sedentary behaviors, obtaining healthy amounts of sleep, increasing moderate/vigorous physical activity, and avoiding high-calorie snacks and sugar-sweetened beverages.

3. PATHOPHYSIOLOGY AND ETIOLOGY

We will first review the "conventional" pathophysiology and etiology associated with both adult and childhood obesity since this provides the essential background and framework for integrating the large scale data sets provided by omics technology. The underlying cause of obesity is primarily attributed to a chronic energy imbalance, i.e., more calories consumed than utilized (see Figure 2). The association between childhood obesity and the consumption of sugar-sweetened drinks is particularly striking in this respect (13). It is clear, however, that complex interactions between environmental, behavioral, genetic, epigenetic and metagenomic factors are all important in the development of obesity. As detailed below, it is estimated that 40-70% of inter-variability in BMI can be attributed to genetic factors yet most these factors remain unidentified (14). Epigenetic and metagenomic factors (Figure 1) also play key roles and modern omics technology has made much progress in defining some of the important molecular mechanisms underlying obesity. Most of this research has, however, been limited to adults. Metabolic syndrome is strongly associated with obesity and its pathophysiological consequences. Although primarily considered a clinical problem in adults, metabolic syndrome is now emerging as a major issue in children and adolescents (15).

3.1. Metabolic syndrome is defined differently in children versus adults

Metabolic syndrome is a cluster of signs indicating an enhanced risk of subsequent T2D and

cardiovascular disease. Abdominal obesity in adults is one of the five major risk factors for metabolic syndrome: the other four being elevated blood triglycerides, elevated fasting glucose, low HDL-cholesterol and hypertension. For adults, having at least three of these signs indicates metabolic syndrome (http://www.nhlbi.nih.gov/health/health-topics/topics/ms). Metabolic syndrome in children (ten years or older) is defined as having abdominal obesity (as measured by waist circumference) and at least two or more of the other signs (https://www.idf.org/metabolic-syndrome/children). Obesity in both adults and children is often accompanied by impaired glucose tolerance where fasting glucose is equal to or above 100 mg/dL with insulin resistance as the primary underlying cause (http://www.uptodate.com/contents/comorbidities-and-complications-of-obesity-in-children-and-adolescents). Although there are well defined strategies for dealing with the separate risk factors in children, it is only within the last few years that guidelines have emerged for treating metabolic syndrome in children as a unique entity (16).

3.2. Obesity, glucose toxicity, inflammation and oxidative stress

High plasma levels of glucose, over long periods of time, are a fundamental cause of damage to both small and large blood vesicles (Figure 2). Damage to small blood vesicles, i.e., microvascular diseases, can lead to kidney failure, peripheral neuropathy, limb amputation and diabetic retinopathy. Damage to the large blood vesicles, i.e., macrovascular disease, contributes to cardiovascular problems. High blood glucose is thought to be toxic to both small and large blood vesicles due, in part, to non-enzymatic and non-specific glycation of proteins ultimately leading to the formation of advance glycation end products (AGEs), which are glycotoxins (17, 18). AGEs are linked to a constant low level of systemic inflammation which, when extended over a long time period, results in endothelial dysfunction, metabolic syndrome and increased cardiovascular risk. Type 2 diabetics with tight glucose control have lower incidences of cardiovascular disease (19). A recent study has found (in adults) that serum AGEs were highly elevated in obese subjects with more than one other metabolic syndrome risk factor (20).

Another mechanism for glucose toxicity is via the enhanced production of reactive oxygen species (ROS) as indicated in Figure 2. As reviewed by Robertson (21) high glucose levels, over a long time span, can exert an oxidative stress on pancreatic islet beta cells that can compromise their ability to secret insulin. Moreover, chronic inflammation is usually accompanied by an increase in oxidative stress and it is reasonable to suggest that this deleterious process could be at play in obesity and metabolic syndrome. Not surprisingly, Furukawa *et al.* (22) found that fat accumulation in both humans and an obese mice model was correlated with systemic oxidative stress. In their animal model, ROS production correlated with an enhanced expression of NADPH oxidase, a decreased expression of antioxidant enzymes and a dysregulation of adipocytokines (22). NADPH oxidase is a membrane bound enzyme and a significant source of superoxide radicals. Adipocytokines are bioactive molecules produced by adipose tissue including adiponectin, plasminogen activator inhibitor–1, IL-6, and monocyte chemotactic protein–1. Moreover, the obese mice treated with a NADPH inhibitor had reduced levels of ROS as well as lower plasma glucose, insulin and triglyceride levels (22). As discussed further below, these data suggests that antioxidants could be useful in preventing some of the organ damage due to hyperglycemia. There is abundant evidence that a variety of oxidant mechanisms are active in both adult and childhood obesity (23).

3.3. Insulin resistance, hyperinsulinemia and high blood pressure

Insulin resistance occurs in skeletal muscle and adipose tissue when these tissues do not adequately take up blood glucose in response to pancreatic insulin secretion. This often results in an initial hyperinsulinemia in which higher than normal pancreatic insulin secretion occurs in an attempt to compensate for insulin resistance. Insulin resistance is a key contributor to the development of T2D. Obesity and insulin resistance impair both GLUT4 glucose transport as well as the vasodilator affect of insulin in the microvasculature (24). GLUT4 is the primary glucose transport protein in both skeletal muscle and adipocytes and its activity is regulated by insulin (25). Obese adults with T2D are known to have markedly reduced expression of adipose GLUT4 compared to control obese adults without T2D. Moreover, exercise training increases the expression of adipose GLUT4 in the obese type 2 diabetics. Skeletal muscle GLUT4 expression was not found to be different between the obese and control subjects (26). It is, however, known that insulin-stimulated translocation of skeletal muscle GLUT4 from intracellular pools to the surface cell surface is diminished in T2D diabetics (27). Insulin-stimulated glucose transport in skeletal muscle is also diminished in T2 diabetics and obese subjects compared to normal weight non-diabetics and this is a likely a consequence of diminished insulin-stimulated GLUT4 transport to the cell surface (28).

Insulin is a potent vasodilator that normally enhances blood flow to the microvasculature, which also helps in promoting glucose uptake. A very recent clinical study in adults shows that the vasodilator effect of insulin is impaired in subjects with metabolic syndrome and this effect is linked with inflammation (29). The authors suggest that the impaired vasodilator effect of insulin in metabolic syndrome is a likely contributor to increased blood pressure and decreased insulin dependent glucose uptake. Quite encouragingly, a six month “lifestyle intervention” program reversed both

the impaired vasodilator effect of insulin and the levels of inflammatory markers (29). The lifestyle intervention included both dietary changes and exercise (Figure 2).

3.4. Islet amyloid polypeptide, T2D and the potential for irreversible pathophysiology

The islet beta-cells of the pancreas normally respond to plasma glucose spikes by secreting insulin. With insulin resistance, the islet beta-cells initially compensate by increasing insulin secretion. T2D only develops when there is an inability of the beta-cells to maintain insulin hyper-excretion (30). There is increasing evidence that misfolded islet amyloid protein could be a culprit underlying progressive islet beta-cell failure. Pro-islet amyloid protein (proIAPP) is a protein normally secreted by the islet beta-cells of the pancreas where it functions by blocking insulin and glucagon secretion. There are also binding sites for proIAPP in the brain, where it may act to control appetite and inhibit how quickly food leaves the stomach (31). Like the amyloid occurring in the brains of Alzheimer's disease, proIAPP can form insoluble fibril deposits (IAPP) in the islet beta-cells and these cytotoxic deposits are quite common in subjects with T2D (31). Jurgens *et al.* (32) have shown that increased IAPP deposition is associated with decreased beta-cell mass and increased beta-cell apoptosis. These data support the view that IAPP is a causative factor contributing to the development of T2D by contributing to beta-cell loss via apoptosis.

The mechanism(s) for the cytotoxicity of IAPP is an active area of current research but work by Zraika *et al.* (33) points to the induction of oxidative stress. These investigators performed *in vitro* experiments with islet cells isolated from human transgenic IAPP mice. The islet cells were monitored for ROS production, beta cell apoptosis and insulin secretion after being cultured in the presence or absence of N-acetyl-L-cysteine (a water-soluble antioxidant) or an amyloid inhibitor (Congo red). After 144 hours amyloid deposition increased and was accompanied by increased ROS production, increased beta-cell apoptosis as well as decreased insulin content. All of these effects were blocked by the addition of the antioxidant or the amyloid inhibitor (33).

Although not a settled issue, the formation IAPP has been characterized as "apparently irreversible" (34). Given that IAPP deposition is not easily reversed, it is of concern that children with T2D could have an accelerated deposition of IAPP that could irreversibly contribute to a worsening pathology. Unfortunately, there is very little information on IAPP deposition in children or adolescents. The human transgenic IAPP mouse model, with all of its inherent limitations, represents our best hope at addressing many clinically relevant research questions. Meier *et al.* (35) used this model to show that a long term (12 months) high fat diet was needed to induce human IAPP deposition, i.e., non-transgenic mice did not develop IAPP deposits nor did the IAPP transgenic mice fed a low fat diet. Similarly, islets from the high fat diet IAPP transgenic mice showed a high level of inflammation compared to the other experimental groups (35).

There is at least one publication that addresses the question of whether or not childhood obesity irreversibly affects adult cardiovascular health (36). Juonala *et al.* (36) encouragingly found that children with overweight or obesity that achieve a normal weight are not at any greater risk of cardiovascular disease than individuals who never had obesity at any time. In this study childhood obesity was defined using international age-specific and sex-specific BMI cutoff points and for adults a BMI cutoff point of 30.

4. THE SYSTEMS BIOLOGY OF OBESITY IN ADULTS AND CHILDREN

4.1. Genome wide association (GWAS) studies fail to identify most of the genetic contribution to BMI in children or adults

The Department of Integrative Systems Biology at the George Washington School of Medicine and Health Sciences has recently embarked on a unique initiative focused on the systems biology of childhood obesity (http://smhs.gwu.edu/isb/systems-disorders/obesity). A key assertion of this group is that identifying any genomic contribution to obesity is likely to be more straightforward in young obese children than in obese adults where there are additional confounding variables arising from the long-term effects of obesity related disease, e.g., T2D and metabolic syndrome. Nevertheless, most genomic data have been collected only in adults. The most recent results will be briefly reviewed since they also have relevance to childhood obesity. Two very large-scale genome wide association studies (GWAS) have recently been published looking at genetic contributions to adult obesity (14, 37). Both studies used a Metabochip which is a customized genotyping array designed to measure over 200,000 single nucleotide polymorphisms (SNPs) of particular relevance to atherosclerosis and cardiovascular disease (csg.sph.umich.edu/kang/MetaboChip/). SNPs were first noticed when the DNA sequences between many individuals were compared: they are genetic loci at which single-nucleotide substitutions of one base for another occur at a frequency of more than one percent in the general population. The first GWAS study focused on the associations between SNPs and BMI in over 300,000 individuals (14). The authors identified 97 BMI-associated SNPs with 57 being novel (14). It is somewhat discouraging, however, that all the identified SNPs accounted for only about 2.7.% of the variation seen in BMI.

In order to make sense of the 97 SNPs associated with BMI the authors utilized pathway analysis. In general, pathway analysis attempts to find biologically relevant

patterns (e.g., biochemical pathways) and meanings in the large-complex data sets provided by omics technology (38). Pathway analysis for the SNP-obesity associations found strong evidence for the involvement of the central nervous system (CNS). Other important pathways were linked to insulin secretion/action, energy metabolism, lipid biology and adipogenesis (14). This study clearly showed that the predisposition to obesity is due to multiple genes.

Overall, it is estimated that between 40% and 70% of BMI variation is due to genetic factors but most of these factors still remain unknown (14). Most genomic evaluations of obesity have been with adults but this situation is now changing, albeit with much smaller number of cases and controls. A meta-analysis has found that most of SNPs associated with adult variation in BMI are also operative in children and also account for only a small fraction of BMI variation (39). This has led to a quest to find the "the missing heritability in pediatric obesity" (40). Llewellyn *et al.* (40) used a clever method called Genome-wide Complex Trait Analysis (GCTA) that leverages the degree of genetic similarity among the subjects in a population study. This method assumes that the subjects with a high degree of common SNP similarity are also genetically similar and therefore might also have a similar BMI. In essence, this method measures the degree to which BMI is explained by common SNP similarity: it does not measure the degree to which specific SNPs are related to BMI. GCTA analysis (with 1.7. million SNP markers) shows that 37% of the obese heritability can be explained by the additive effects of multiple common SNPs which is at least an order of magnitude more than explained by GWAS (40). While GCTA analysis does not provide predictive information on obesity in an individual, it strongly suggests that there are many hundreds of genetic variants yet to be discovered that have a major additive effect on BMI in children as well as adults.

4.2. SNPs and individualized treatment for obesity

Knowing the particular obesity predisposition SNPs in a subject could eventually provide valuable insight into an optimized individual treatment strategy. In this respect it is interesting that some of obesity SNPs related to CNS functioning have also been linked to the mechanism of action of topiramate (14). The US FDA has approved topiramate, in combination with phentermine, for adult weight loss (41). Topiramate has been approved by the US FDA for seizures and migraines in pediatric subjects (42). Topiramate is not approved for weight loss in pediatric subjects but the University of Minnesota has an ongoing clinical trial to test for efficacy in adolescents with severe obesity (https://clinicaltrials.gov/ct2/show/NCT01859013). The side effects of topiramate in children have been well studied and both CNS and anorexia/weight loss events have been noted (43). Ultimately, the application of pharmacogenomics (how genomics affects drug responses) will help in determining which adults and children would be responsive to weight loss with minimal side effects. Knowing the genetics of the relevant pathways may also help in understanding why some obese subjects develop T2D and hyperlipidemia and some do not.

4.3. Genome wide association (GWAS) studies with body fat distribution

The second recent GWAS studied over 220,000 adults and looked for SNPs associated with body fat distribution (37) and used waist-to-hip (WHR) circumference ratio as an estimate of abdominal fat, i.e. a higher WHR suggests more abdominal fat. It is known that abdominal fat deposition in adults (aka visceral obesity) is a risk factor for metabolic and cardiovascular diseases (as well as mortality) that is independent of BMI (37, 44). Shungin *et al.* (37) therefore used statistical methods to adjust for the effects of BMI, i.e., their final statistical associations were independent of BMI. These researchers found 49 SNPs associated with WHR. In contrast to the GWAS results associated with BMI (14), there were marked differences in SNP WHR associations between males and females with 19 SNPs showing a stronger effect in females than males. These results suggest that sex hormones may differentially promote adipocyte growth in a tissue specific fashion. Pathway analyses showed other SNPs to be important in angiogenesis and adipogenesis. Many primary care providers do not quantify waist circumference or WHR. This is problematic since these non-invasive measures could be useful in optimizing individualized strategies for dealing with obesity.

In summary, GWAS holds great promise for predicting some obesity risk factors but there is now a considerable gap between the current "state-of-the-art" and immediately usable clinical information. Genomics is the most static of omics data and the least responsive to environmental influences. We know that environmental factors and their interaction with genetic factors is key to the development of obesity. As discussed below, environmental influences can manifest themselves via both epigenomic and metagenomic mechanisms.

4.4. Epigenomics and obesity

Epigenomics and system biology have been undergoing a serious and coherent merger over the last decade. Epigenomics is focused on alterations in gene expression not involving changes to the DNA sequence, i.e., a phenotypic change without a change in genotype (Figure 1). It has become increasingly clear that environmental factors can affect obesity by epigenetic mechanisms and these are now being characterized but mostly in adults. DNA methylation, histone modifications and microRNAs (miRNAs) are three key epigenetic mechanisms and the set of all such modifications for an organism or

tissue is called the epigenome. Histone modifications modulate the interactions between transcription factors, RNA polymerase and chromatin structure. Transcription factors, in turn, control the rate of mRNA synthesis from the DNA template. DNA methylation is an epigenetic mechanism that can block DNA transcription and involves covalent modification (methylation) of cytosine. "CpG" sites are DNA regions with a cytosine nucleotide occurring next to a guanine nucleotide and the cytosine can be methylated forming 5-methylcytosine. MiRNAs are non-coding RNAs that represent a third category of epigenetic regulation that involves binding to target sites found within the 3'UTR of the targeted mRNA with subsequent suppression of protein synthesis (45).

As detailed above, adipose tissue plays a key role in the pathophysiology of obesity and accumulating research has dramatically changed the view of the adipocyte from that of a passive energy storage depot to that of a more dynamic cell type with endocrine functions and an involvement in inflammatory processes (46). Benton *et al.* (47) looked at DNA methylation patterns in adult female subjects with obesity before and after gastric bypass surgery and the accompanying weight loss. It has long been recognized that differences in the body location of adipose tissue is an important factor in the development of obesity related diseases (see above). Benton *et al.* (47) therefore looked at methylation patterns in 485,577 CpG sites in both subcutaneous and omental adipose tissue. The greater omentum is a layer of adipose tissue attached to the inferior region of the stomach and covers the anterior region of the intestines. The degree of omental adipose tissue (aka "belly fat") is strongly correlated with the development of T2D (44). Benton *et al.* (47) found marked methylation differences between the subcutaneous and omental adipose tissues in the obese adult females and noted more hypermethylation before weight loss (47). Differential methylation was found in genes associated with development, epigenetic regulation, and obesity.

4.5. miRNAs epigenetics and obesity

For the past decade, there has been a nearly exponential growth in investigations looking at the impact of small non-coding RNA species on the expression of key functional cellular proteins. There are now more than 2500 miRNAs identified in humans and there are estimates that more than 60% of mRNAs contain specific miRNA targets in their 3'-untranslated region (3'-UTR). After the mature miRNA complexes with an Argonaute protein, these miRNAs bind to the 3'-UTR target of the mRNAs bound to ribosomal RNA and either deactivate or accelerate degradation of the mRNA, thereby repressing its protein translation (48).

Multiple miRNAs have been implicated in the regulation of adipose tissue development and obesity. Mice with adipocyte-specific loss of the enzyme Dicer, necessary for generation of mature miRNAs, have almost no white adipose tissue, suggesting one or more miRNAs are necessary for adipogenesis (49). Several miRNAs and miRNA clusters have been implicated in adipocyte proliferation, differentiation, and triglyceride accumulation. Pre-adipocyte differentiation in 3T3-L1 cells is increased by miR-143 and decreased by miR-143 inhibition (50). Porcine adipogenesis is associated with increased expression of miR-103 and inhibition of miR-103 prevents adipogenesis (51). Adipogenesis in human adipocytes is stimulated by miR-30c, probably through inhibition of *PAI-1* and *ALK2.* Silencing both of these targets mimics the pro-adipogenic effect of miR-30c (52). There are similar reports in multiple systems implicating these miRNAs and several others in either stimulation or inhibition of adipogenesis and differential expression in obesity (53).

4.6. Histone deacetylase 4 (HDAC4) maybe a key enzyme obesity related epigenetic regulation

It is quite interesting that histone deacetylase 4 (HDAC4) gene was differentially methylated by weight loss since this gene codes for a key protein also involved with epigenetic regulation (47). Histone deacetylases removes an epsilon-N-acetyl group from a histone lysine residue thereby changing the charge on the lysine residue from neutral to positive. The positively charged lysine residues interacts with the negatively charged deoxy-ribose phosphate backbone of DNA making a more compact chromatin structure that blocks transcription. In general histone acetylation is associated with an increased level of gene transcription. HDAC4 is an epigenetic regulator since it can affect DNA function without any alterations in the DNA sequence. Benton *et al.* found that the HDAC4 gene showed two CpG sites that were hypermethylated and one CpG site that was hypomethylated before weight loss (47). As expected, fasting blood glucose levels went down after weight loss surgery and, significantly, this decrease was correlated with differential HDAC4 gene methylation. There are a small number of centers that specialize in pediatric bariatric surgery (e.g., childrensnational.org/departments/bariatric-surgery-program) so the work of these authors could eventually be replicated in children.

Ronn *et al.* (54) have studied the effect of a six month exercise intervention on the methylation pattern of adipose tissue from healthy men. After exercise, there was a global change in DNA methylation patterns as well as alterations in gene expression in the human adipose tissue. This work also identified HDAC4 as a key gene with a differential methylation pattern, i.e., the HDAC4 gene showed increased DNA methylation after exercise and this was accompanied by a decreased expression of HDAC4 mRNA. This makes good molecular sense, i.e., an increased methylation the HDAC4 gene should block its transcription.

In summary, epigenetic changes appear to play a key role in obesity and exercise related metabolic changes. HDAC4 has emerged as a key player in many of these epigenetic studies and this finding has now been confirmed by extensive proteomic studies as detailed below.

4.7. Metagenomics, mode of infant delivery and obesity

The complex factors impinging on obesity and the need to "cast a wide net" are well illustrated by the finding that mode of infant delivery can have an effect on adult BMI and obesity (55). Quite amazingly, a recent meta-analysis has shown that strong association between Caesarean section births and increased baby BMI as well as increased adult obesity (55). The reasons for these associations are not known but it is interesting that the mode of delivery also has an impact on the type of microflora that colonize an infant's intestinal tract (56, 57). The metagenomics of the neonatal gut microbiome has become a topic of intense research (57). Metagenomics is a specialized component of genomics focused on studying and characterizing microbial organisms in their native environments and is based on the ability to analyze microbial DNA extracted directly from these native environments. Quite remarkably, the collective genome of the gut microbiome is about 100 times larger than the human genome (57, 58). The mechanisms by which the gut microbiome could influence obesity are still a "work in progress." A key assertion is that the "obese microbiome" alters energy balance by increasing the absorption/bioavailability of nutrients such as glucose and fat as well as having an influence on appetite and systemic inflammation. This view is, however, strongly supported by animal research showing that antibiotic treatment at or near birth causes mice to develop obesity caused by antibiotic dependent changes in the gut microbiome rather than a direct effect of the antibiotic itself (59).

4.8. Proteomics, obesity, and HDAC4

Proteomic studies in both adults and children with obesity are just now getting underway but with much smaller population sizes compared to genomics studies. Nevertheless, these studies have already proven fruitful. Although limited by only studying an all male adult population, a recent Kuwaiti study focused on the differential protein expression in peripheral blood mononuclear cells (PBMCs) between lean and obese adults and the potential effects of physical exercise (60). This study revealed 47 proteins that showed at least a 1.5. fold difference between lean and obese subjects as measured by BMI. It may well be that the large number of genetic loci influencing obesity converge on a number much smaller number of proteins. Histone deacetylase 4 (HDAC4) was found to be downregulated in obese subjects but upregulated after doing physical exercise for three months. Interestingly, the HDAC4 gene was not identified as having an association with obesity in any of the GWAS studies but did emerge as important in the global DNA methylation studies. As mentioned above, HDAC4 affects the acetylation/deacetylation of DNA bound histones thereby altering chromosome structure and the access of transcription factors to DNA.

Since adipose tissue is central to obesity, Abu-Farha *et al.* (60) also obtained adipose biopsies in a subset of their subjects before and after exercise and looked for the tissue expression of HDAC4 protein by immunohistochemical staining as well as HDAC4 mRNA levels by qRT-PCR. The results paralleled those obtained with PBMCs, i.e., HDAC4 protein and mRNA were lower in the obese subjects and increased after exercise. The authors then established that there was a negative correlation between HDAC4 mRNA levels in individual subjects and BMI, percent body fat and levels of RANTES, an inflammatory cytokine and one of the many cytokine gene targets of NF-kB. They also found a positive correlation between VO_2 Max and HDAC4 mRNA levels. VO_2 Max is good measure of overall aerobic fitness. Although many questions remain, Abu-Farha *et al.* (60) suggest that HDAC4 may play a protective role in obesity and that strategies to increase its expression could be beneficial in subjects with obesity.

HDAC4 is a class II deacetylase and *in vitro* work by Weems *et al.* (61) has shown that reducing the expression of these deacetylases increases the expression of GLUT4 mRNA in pre-adipocytes. GLUT4 is an insulin sensitive glucose transporter found in adipocytes and striated muscle and its expression plays a key role in reducing blood glucose levels after carbohydrate consumption. Exercise is a known mechanism for the induction of GLUT4 (62). The data presented by Weems *et al.* (61) suggests that a decreased expression of HDAC4 would be beneficial in obesity since it could increase GLUT4 expression and thereby reduce fasting blood glucose. The DNA methylation data are consistent with this viewpoint: increased DNA HDAC4 gene methylation as a result of exercise results in decreased HDAC4 mRNA and, by inference, could result in deceased HDAC4 protein and increased GLUT4 expression with decreased fasting glucose. This simplistic picture is, however, not consistent with the proteomic data which shows HDAC4 protein levels in PBMCs and subcutaneous adipose tissue to be increased with obesity and decreased after exercise.

The potential reasons for this lack of superficial harmony are beyond the scope of this review but a number of issues are immediately obvious and point to information gaps requiring further studies. HDAC4 does not act in isolation from other cellular events. The cellular localization of HDAC4 protein is controlled, in part, by phosphorylation. Phosphorylation keeps HDAC4 in the cytoplasm and when acted upon by phosphatases it moves to the nucleus where it can perform it deacetylase

function. HDAC4 protein also interacts with MEF2 (monocyte enhance factor 2), which is a transcription factor controlling the expression of muscle fiber types. This is significant since exercise can influence muscle fibers types. In particular, endurance training promotes the formation of slow twitch oxidative muscle fibers. HDAC4 is a potent inhibitor of MEF2.

4.9. Proteomics, obesity in children, low molecular weight haptoglobin and apoAI

Martos-Moreno *et al.* (63) studied the proteomic profile of fasting serum samples from very young obese subjects and lean controls (about 9 years old) using two-dimensional electrophoresis followed by mass spectrometry. Their goal was to identify potential protein biomarkers of metabolic impairment. They noted a decreased expression of apo-A1 isoforms and an increased expression of low molecular weight isoforms of haptoglobin. Apo-A1 is a component of high density lipoprotein (HDL) which is thought to be protective against cardiovascular disease. Quite remarkably, these investigators also found that weight loss in the obese children reversed the decrease in apo-A1 levels (63). In adults it has been found that the ratio of apo-B/apo-A1 ratio is an excellent predictor of cardiovascular disease risk in overweight and obese subjects (64). Apo-B is a protein found in all of the atherogenic human lipoproteins (64). HDL with apo-AI is also a carrier of the antioxidant enzyme paraoxonase (PON1) that can detoxify the lipid hydroperoxides in oxidized-LDL, a particularly atherogenic form of LDL. It is very interesting, therefore, that PON1 activity in obese children is lower than normal weight controls and inversely correlates with leptin levels (65). Leptin is a hormone made by adipose tissue and it levels correlate well with adiposity and other risk factors associated with metabolic syndrome (65). In summary, the work of Martos-Moreno *et al.* (63) shows that apo-A1 serves as useful biomarker for metabolic impairment in very obese children.

4.10. Metabolomics, branched-chain amino acids and androgen metabolites

Metabolomics is the large-scale characterization of metabolites and low molecular molecules and usually employs mass spectrometry or nuclear magnetic resonance. It is the most dynamic of the omics and provides a “snapshot “of an individual’s physiologic state. Moreover, metabolomics is the most responsive of the omics to environmental factors. Dessi *et al.* (66) have provided an outstanding review of clinical metabolomics and its relevance to neonatology, pediatrics and obesity. Metabolic studies in obese/overweight children are increasingly making their way into the literature. Perng *et al.* (67) studied a total of 262 school-aged children and found marked differences the levels of plasma metabolites between obese (high BMI) and lean children. In particular, the obese children had higher plasma levels of branched-chain amino acids (BCAA) and their metabolites compared to the lean children. In addition, the obese children had higher levels of androgen metabolites. The mechanisms responsible for the association of high BMI with BCAAs are not fully understood and could involve differences in the gut microbiome or an increased degradation of skeletal muscle in the obese children due to insulin resistance (67). The increased androgen metabolites in obese children were attributed to an increased androgen synthesis. Work by McCormack *et al.* (68) also showed elevated levels of plasma BCAAs in obese children and adolescents. Furthermore, these researchers found a positive association between baseline plasma BCAAs levels and the future development of insulin resistance (68). While these results are very interesting, their pathophysiological and mechanistic significance needs more study.

5. CAN SYSTEMS MEDICINE HELP GUIDE INTERVENTIONS OR PROVIDE INSIGHTS INTO NEW TREATMENT MODALITIES?

Chronic oxidative stress and inflammation appear to be important pathophysiological consequences of obesity (Figure 2)(69). Work by Dennis *et al.* (70) has clearly shown that F2-isoprotanes show a remarkable correlation with the number of metabolic risk factors in overweight children. F2-isoprostanes are an excellent and very sensitive biomarker for oxidative stress (71). It is reasonable to suggest that antioxidants such as vitamin E could be beneficial (21). Vitamin E is not a single organic compound and includes alpha-, beta-, gamma- and delta-tocopherols and four corresponding tocotrienols (72). Most clinical research has been done with synthetic alpha-tocopherol (all-rac-alpha-tocopherol), which is an equimolar mixture of eight stereoisomers with only one eighth being the naturally occurring RRR-alpha-tocopherol. Moreover, dietary vitamin E is primarily gamma-tocopherol, not alpha-tocopherol. While all forms of vitamin E are antioxidants they have distinct biochemical properties and abilities to modulate signal transduction pathways. Devaraj *et al.* (73) looked at the effects of dietary supplementation with alpha-tocopherol alone, gamma-tocopherol alone, and the combination of both on reducing biomarkers of oxidative stress and inflammation in adult subjects with metabolic syndrome. Their results clearly show that the combination supplement with both alpha- and gamma-tocopherol was superior to a supplement with either alpha-tocopherol alone or gamma-tocopherol alone. This is a very positive result and needs to be repeated in a pediatric population with a follow-up time sufficient to also determine if the combined supplement helps in maintaining insulin secretion which could otherwise be compromised by chronic oxidative damage to pancreatic islet beta cells caused by hyperglycemia (21).

Akbar *et al.* (74) have performed a meta-analysis on the use of vitamin E or vitamin C dietary supplements

to treat T2D in adults. Unfortunately, the form of vitamin E used in many of the included studies is not clear but is likely to have been all-rac-alpha-tocopheryl acetate in most cases. Moreover, in most cases un-physiological levels of vitamin E were used, e.g. >200 IU/day. Neither vitamin E nor vitamin C supplementation was found to affect plasma glucose levels or insulin levels. Nevertheless, HbA_{1C} levels were found to be significantly reduced by antioxidant supplementation (74). Data from the Selenium and Vitamin E Cancer Prevention Trial (SELECT) suggests that long-term supplementation with all-rac-alpha-tocopheryl acetate (400 IU/day) increases the risk of developing prostate cancer (75). Given the distinct biochemical properties of the different forms of vitamin E, it is not possible to extrapolate this finding of increased prostate cancer risk with high dose all-rac-alpha-tocopheryl acetate to all forms of vitamin E.

Tocotrienols show much promise for treating metabolic syndrome (76). The GWAS data reviewed above point to the importance of adipocyte angiogenesis as being a key contributor to obesity. In addition to being potent antioxidants, tocotrienols are *in vivo* inhibitors of angiogenesis (77). As reviewed by Weng-Yew and Brown (76), there are multiple mechanisms whereby tocotrienols could be therapeutically useful in treating metabolic syndrome including inhibition of adipogenesis, decreasing both blood glucose and HbA_{1c}, improving lipid profiles, reducing atherosclerotic plaques and lowering blood pressure. Nevertheless, there has not been a long-term clinical trial in the pediatric population.

The biochemical rationale for initially using high dose antioxidant supplements for treating the pathophysiological consequences of oxidative stress accompanying obesity is very compelling as detailed in the review article “Reactive oxygen species, nutrition, hypoxia and diseases: problems solved?” (78). Nevertheless, the lack of strong supportive evidence from clinical trials and safety issues has lessened enthusiasm for this approach (78). There is growing support for the notion that diets rich in a wide array of natural antioxidants might be an optimal approach (78, 79). Indeed, Montonen *et al.* (80) estimated the dietary intake of multiple antioxidants (four tocopherols, four tocotrienols and six carotenoids) from a dietary history interview in a cohort of adult men and women who were initially free of diabetes. During a 23 year follow-up the dietary intake of alpha-, beta-, gamma- and delta-RRR-tocopherols were associated with about a 30% decreased risk of developing T2D. Similarly the risk of T2D was decreased by about 40% by the consumption of beta-cryptoxanthin. These are very encouraging data and should be repeated in a pediatric population.

6. SUMMARY

Given the daunting task of reversing obesity in adults, it is clear that public health efforts should prioritize the goal of minimizing childhood obesity through aggressive lifestyle changes, i.e., increased opportunities for exercise and a low-fat, low-sugar, anti-inflammatory diet rich in a wide-variety of antioxidant rich foods (Figure 2). Primary care providers should treat metabolic syndrome in children as a unique entity and measure WHR as well as BMI. The epigenetic studies that have been done in adults need to be repeated in a pediatric population. The physiological role of BCAAs in childhood obesity needs to be defined as well as its potential association with SNPs.

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Abbreviations: AGEs: advanced glycation end products; apo-A1: apolipoprotein A-I, a component of high density lipoprotein; apo-B: apolipoprotein B**;** BCAA: branched-chain amino acid; BMI: body mass index; CNS: central nervous system; GCTA: genome-wide complex trait analysis; GLUT4: glucose transporter type 4; GWAS: genome-wide association study; $HbA_{1C:}$ hemoglobin A1c, a glycated form of hemoglobin; HDAC4: histone deacetylase 4**;** HDL: high density lipoprotein; IAPP: islet amyloid polypeptide; MEF2: myocyte enhancer factor-2; miRNA: microRNA; NAFLD: non-alcoholic fatty liver disease; PBMC: peripheral blood mononuclear cell**;** PON1: serum paraoxonase/arylesterase 1; pro-IAPP: pro-islet amyloid polypeptide from which IAPP is derived by proteolytic cleavage; ROS: reactive oxygen species; SNP: single nucleotide polymorphism; T2D: type 2 diabetes; WHR: waist-to-hip ratio

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