Unconventional Wisdom about the Obesity Epidemic

Andrew R. Zinn, MD, PhD

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Andrew R. Zinn, M.D., Ph.D.
Associate Dean for the Medical Scientist Training Program
Associate Professor of Internal Medicine
Division of Clinical Genetics
McDermott Center for Human Growth and Development
UT Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, TX 75390-9033
Andrew.Zinn@UTSouthwestern.edu

Dr. Zinn's research interests include genetic obesity, sex chromosome abnormalities, and genomic copy number variation. He and his colleagues discovered that mutations in the *SIM1* gene cause hypothalamic dysfunction and severe, early onset hyperphagic obesity and are studying the pathophysiology of this disorder using mouse models. He also is directs the Medical Scientist (MD/PhD) Training Program at UT Southwestern.

I. Introduction - Etiology of the Obesity Pandemic

"Round up the usual suspects." – Claude Rains as Captain Renault, Casablanca

- Globally, there are more than 1 billion overweight adults, at least 300 million of them obese.
- Obesity and overweight pose a major risk for chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension and stroke, and certain forms of cancer.
- *The key causes are increased consumption of energy-dense foods high in saturated fats and sugars, and reduced physical activity* [emphasis added].
  —World Health Organization report, "Obesity and Overweight", 2009 [1].

The dimensions of the obesity pandemic are well known. Equally familiar are the changes in diet and lifestyle that have resulted in the present "obesigenic" environment. Supported by large diet and exercise industries, the dogma that increased food intake and decreased energy expenditure are the paramount causes of the obesity epidemic fits well with the perception that excess body weight is the result of the cardinal sins of gluttony and sloth. Yet the evidence that changes in diet and physical activity are the principle factors in the obesity epidemic is circumstantial [2], and moralizing the problem of obesity and socially stigmatizing the obese has done nothing to stem the epidemic [3, 4]. While not denying the importance of food intake and energy expenditure in body weight regulation, the intent of these Grand Rounds is to consider other possible etiologic factors in the obesity epidemic.

II. "Do these genes make me look fat?" Original source unknown (9,930 Google hits)

We shall first consider the genetic basis of susceptibility to excessive weight gain. Numerous human and animal studies over the past several decades support the existence of a homeostatic system for maintaining body weight within a relatively narrow range [4]. Shortly after the seminal discovery of leptin, the basic components of an endocrine feedback loop that vigorously defends against weight loss involving the brain, the gut, and adipose tissue were elucidated (Figure 1). However, the degree to which this system defends against weight gain is controversial. Conventional wisdom holds that humans evolved without selective pressure to prevent excessive energy storage because until very recently, most populations were not exposed to an environment of extreme caloric abundance with little effort needed to harvest these calories.

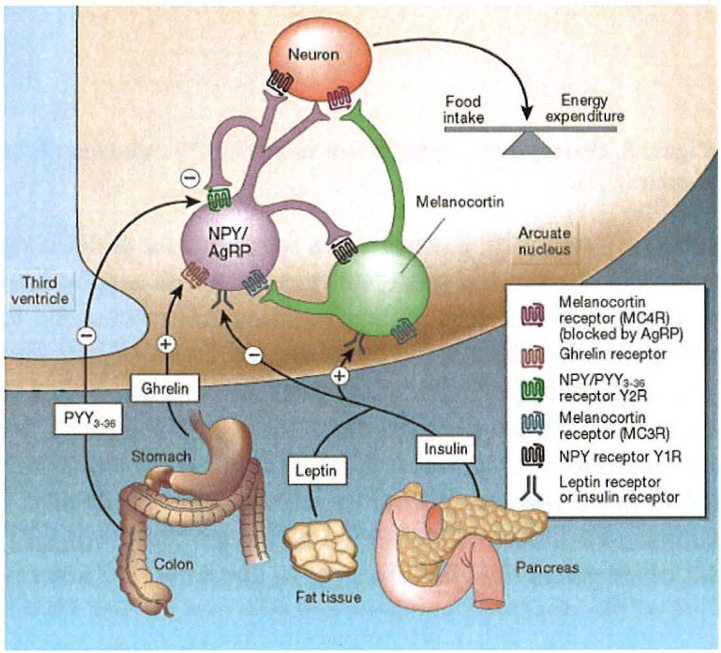

Figure 1. Gut-brain-adipose axis for energy homeostasis [5].

This scenario provides the basis for Neel's 'thrifty gene hypothesis', which states that genes favoring energy storage in the form of fat deposition were favored by natural selection by promoting survival during episodes of famine [6]. The conventional wisdom is that these same genes, interacting with the present environment of caloric abundance, are driving feeding behavior that results in increasing body weight.

What are these 'thrifty' genes? Mutations in a handful of genes, all acting in a hypothalamic pathway that responds to leptin (Figure 2), are associated with severe, early onset obesity [7, 8], and each of these has been suggested as a candidate 'thrifty' gene. For all but one of these genes, clear-cut functional mutations are very rare, leading to the hypothesis that more subtle (and in most cases as yet unidentified) variants may contribute to the 'thrifty' genotype. The exception is *MC4R*, for which loss of function mutations are observed in up to a few percent of morbidly obese individuals [9]. However, the frequency of these mutant *MC4R* alleles in the population is still far too low for them to represent the common 'thrifty' genotype.

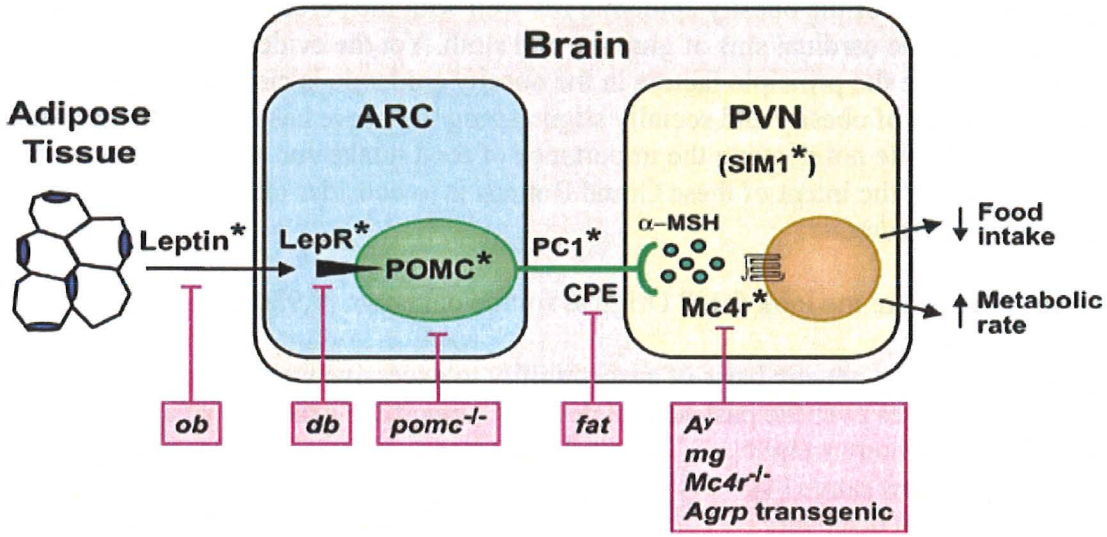

Figure 2. Obesity-causing mutations in human (*) and mouse (pink boxes) genes acting hypothalamic leptin signaling [7].

Until very recently, the evidence for common genetic variants influencing body weight was equivocal. Just in the past year, genomewide association studies achieved sufficient genome coverage and statistical power to detect common gene variants associated with obesity. The first such gene identified is the aptly named Fatso (*FTO*), found in a genomewide association study of type II diabetes [10]. The *FTO* gene has an interesting etymology. It was originally cloned from studies of a mouse deletion mutation, *Ft* (fused toes), that causes partial syndactyly. The gene was named Fatso, or *Fto*, because of its large size, spanning ~250 kb of genomic sequence [11], before there was any hint of its involvement in obesity. More recent studies have shown that common variants in *MC4R* also influence body weight [12]. However, variants in *FTO, MC4R* and other genes detected thus far in genomewide association studies collectively explain only ~1% of the observed variance in body mass index (BMI) [13].

An alternative hypothesis is that selection may have favored genes that limit body weight, rather than genes that promote energy storage. According to this hypothesis, our present obesigenic

genetic makeup is the result of the accumulation of mutations through the relaxation of selection, i.e., genetic drift. A major advantage of this 'drifty' gene hypothesis over the thrifty gene hypothesis is that genetic drift can account for the ~30% of the US population that remain lean in the face of the present environment: the genes of these lean individuals simply have not drifted. By contrast, a thrifty genotype that confer a selective advantage during episodes of famine should have rapidly become fixed in the population and would therefore be present in nearly all individuals, not just the ~70% of the population susceptible to becoming overweight or obese.

What type of selective pressure would favor limiting body weight? Certainly type 2 diabetes could be one such pressure. This is illustrated dramatically by the epidemic of diabetes affecting the Micronesian population on the Pacific atoll of Nauru [14]. This remote island, 21 square kilometers in size with an estimated population of 13,770, consists mostly of high-quality phosphate rock that can be used for fertilizer. Native islanders became rich from phosphate royalty payments that began in the 1920's. They abandoned agriculture and adopted a sedentary lifestyle, relying upon store-bought food, with an ensuing explosion of obesity. The first case of diabetes mellitus in Nauru was noted in 1925. Following World War II, the prevalence of diabetes rose precipitously, reaching the point by the 1970's where a third of all Nauruans over the age of 20 and two thirds over the age of 55 were diabetic. However, the prevalence of diabetes has recently declined markedly, most likely not because of reduced obesity or increased physical activity, but because of selection for individuals who are less prone to diabetes when obese [15]. Similarly, the increased prevalence of diabetes in African Americans versus European Americans for a given degree of obesity may reflect evolutionary adaption to increased caloric abundance from the success of European agriculture [16].

The predation release hypothesis. "Devouring time, blunt the lion's paws." —Shakespeare, Sonnet 19

A second proposed selective pressure is the need to maintain a lean body weight in order to avoid predators [17, 18]. Increased body weight may directly impair the ability to run away from predators or escape into narrow refuges or indirectly increase susceptibility to predation because of the need to spend more time foraging for food [17-19]. During the Pliocene era, between 6 and 2 million years ago, large predatory animals were far more abundant than they are today, and 6-10% of early hominid fossil bones show signs of predation [20]. About 2-1.8 million years ago, early prehumans began to evolve social behavior. Around the same time, fire and tools were discovered. The collective effect of social organization, fire, and tools was to effectively eliminate predation and thereby relax the selective pressure to escape from predation.

Speakman [18] has recently proposed another hypothesis by which genetic drift results in an obesity-prone genotype. According to this scenario, the key environmental factor is exposure to high levels of dietary fat during recent decades. In the absence of dietary fat, selection against mutations that impair fat oxidation might be relaxed, resulting in the accumulation of variation in the fat oxidation capacity of individuals. This genetic variation might be phenotypically silent until the population is exposed to high levels of fat in the diet. In support of this hypothesis, Speakman cites evidence ([18], references 70-78) that variation in the rate of basal fat oxidation predicts susceptibility to obesity in humans and animal models.

Illius [19] has categorized costs of foraging and food intake as extrinsic or intrinsic. Extrinsic costs are those associated with the increased time spend foraging, and include not only greater exposure to predators, as previously mentioned, but also reduced opportunity to mate, reduced rest and sleep, reduced time to defend territory, and greater exposure to weather. Intrinsic costs are directly associated with the food source and include not only the aforementioned reduced ability to escape predators, but also increased ingestion of toxins, exposure to parasites, dental wear, and oxidative cellular damage.

It is too soon to know from genetic studies which hypothesis – thrifty or drifty gene – better fits the genetic architecture of obesity in present day societies, and the precise selective pressures acting on genes regulating body weight remain uncertain. Nevertheless, the global obesity epidemic has developed so swiftly that it is almost certainly not due to a very recent change in our genetic makeup, and we must look elsewhere to understand the proximate causes of the epidemic.

III. "Infectobesity"

Viruses

The term "infectobesity" was coined in 2001 by Nikhil Dhurandhar, then at Wayne State University in Detroit and now at the Pennington Biomedical Research Center at Louisiana State University [21]. It refers to the idea that human obesity may have an infectious etiology. Dr. Dhurandhar is listed on most papers linking viruses and human obesity. Before dismissing this notion offhand, recall that Marshall and Warren's proposal that *Helicobacter pylori* infection causes peptic ulcer disease was considered heretical just a few decades ago.

The first virus linked to obesity was canine distemper virus (CDV), a paramyxovirus related to measles that infects dogs and other carnivores but not humans. Lyons *et al.* [22] showed that obesity developed in 26% of mice that survived CNS infection with CDV. The magnitude of obesity was comparable to that reported for genetically obese mice or for mice with hypothalamic lesions. The mechanism of post-CDV obesity is thought to involve viral hypothalamic damage [23, 24] and decreased leptin receptor expression [25]. Another CNS virus that can result in post-infection obesity in laboratory rodents is the Borna disease virus, an RNA virus that infects horses and sheep. Rats infected with the BDV-obese strain of Borna disease virus develop inflammation of the septum, hippocampus, amygdala, and ventromedial hypothalamus (VMH) [26]. The last two regions, especially the VMH, are associated with body weight regulation. One other infectious CNS agent, scrapie, a transmissible spongiform encephalopathy of sheep due to an abnormally folded infectious protein, has been associated with obesity in experimental infection of laboratory mice [27].

Another virus that can cause obesity in animals is Rous associated virus type 7 (RAV-7), an avian leucosis retrovirus that induces B-cell lymphomas and other myeloproliferative disorders. Infection of 10-day old chick embryos with RAV-7 resulted in stunted growth, obesity, and hyperlipidemia within 3 weeks after hatching [28]. The mechanism was thought to involve viral-induced thyroiditis and hypothyroidism [29]. Antibodies to avian leucosis virus can be detected in commercial chickens, a proportion of which carry infectious virus [30]. Thus humans are widely exposed to these viruses, which fortunately are not able to replicate in mammals but are able to infect and transform mammalian cells *in vitro*. There are reports of avian leucosis viruses in

measles and mumps vaccines derived from chicken embryonic fibroblasts [31, 32] but no evidence of transmission of these viruses to vaccine recipients [31].
A connection between viruses and human obesity was first proposed after a highly infectious avian adenovirus, SMAM-1, was reported to be responsible for an increased death rate in commercial chicken farms in India [33]. After infection with adenovirus, chickens gained more weight than uninfected controls, with increased adiposity but not hyperphagia. Although avian adenoviruses were not known to infect humans, Dhurandhar *et al.* [34] reported in subsequent studies that 10 out of 52 obese residents of Bombay, India had antibodies to SMAM-1 and were significantly more obese than seronegative subjects (mean BMI 35.3 vs. 30.7). These studies have not been independently replicated.

Dhurandhar and colleagues then turned their attention to human adenoviruses. Using experimental infection of chickens as a model system, they reported that two human adenoviruses, Ad36 and Ad37, were able to cause obesity [35-37]. They also showed that Ad36 infection also increased body weight in mice [35] and marmosets [38]. Cultured cell studies suggest that Ad36 infection enhances differentiation of preadipocytes to adipocytes [39]. *Ex vivo* studies of primary adipocytes from Ad-36-infected rats vs. controls indicates that Ad36 infection reduces leptin secretion and increases glucose uptake in response to insulin [40]. These effects appear to be mediated by the adenoviral E4 orf-1 protein, which contains a PDZ-binding domain [41].

In one epidemiologic study, Ad36 seropositivity was associated with obesity in 502 individuals of varying body weights from New York City, Madison, Wisconsin, and Naples, Florida [42]. The study also included 89 twin pairs from New York City. Six pairs were concordant for Ad36 seropositivity, 56 pairs were concordant for Ad36 seronegativity, and 28 pairs were discordant for Ad36 serology. Of the discordant twins, the seropositive twins had higher mean BMI than their seronegative cotwins (26.1±9.8 vs. 24.5±9.5). Interestingly, the paper reports the statistical significance for this difference as $P<0.04$, test not specified, whereas an unpaired t-test using the reported mean, standard deviation, and N gives a two-tailed P value of 0.54. Moreover, the first author of this study, Dr. Richard L. Atkinson, is the editor of the journal in which this work appeared as well as cofounder and owner of Obetech, LLC, a company that offers assays for Ad36 (www.obesityvirus.org).

Bacteria
It wasn't long before bacteriologists tried to get a piece of the infectobesity pie. Following the discovery that the stomach secretes the appetite-stimulating hormone ghrelin (the subject of a recent Internal Medicine Grand Rounds by Jeff Zigman), gastroenterologists rounded up their usual suspect, *Helicobacter pylori*, and this time accused its *eradication* of promoting weight gain and obesity. *H. pylori* infection was known to impair secretion of histamine, somatostatin, pepsinogen I, and gastric acid by cells adjacent to ghrelin-producing cells in the glandular corpus [43], and it was therefore not surprising that *H. pylori* might also impair ghrelin secretion. The first supporting evidence came from Nwokolo *et al.* [44], who reported a 75% increase in median integrated plasma ghrelin levels in 10 subjects after cure of *H. pylori* infection (Figure 3). Isomoto *et al.* [45] showed in a group of 68 Japanese subjects, 63% of whom were infected with *H. pylori*, that severity of gastritis was inversely correlated with plasma ghrelin level; this finding was replicated by Osawa *et al.* [46] in a population of 160 Japanese subjects. However, Shiotani *et al.*

[47] found no difference in mean BMI among 801 eighteen-year old Japanese university students who were infected (n=177) or uninfected (n=624) with *H. pylori*, despite the infected students having a significantly lower median plasma ghrelin level (55 pmol/l vs. 103 pmol/l). In the U.S., Cho *et al.* [48] found no significant association in adults between *H. pylori* colonization and BMI in the Third National Health and Nutrition Examination Survey (NHANES, 1988-1994). No prospective studies using obesity as an endpoint have studied the effects of *H. pylori* eradication, nor is it even clear that ghrelin is an important determinant of body weight in humans [49]. Rather than the obese, the population where the lowering effect of *H. pylori* infection on ghrelin secretion may be of most importance could be malnourished children in developing nations, who are nearly universally infected with *H. pylori* and who might benefit from increased ghrelin [49].

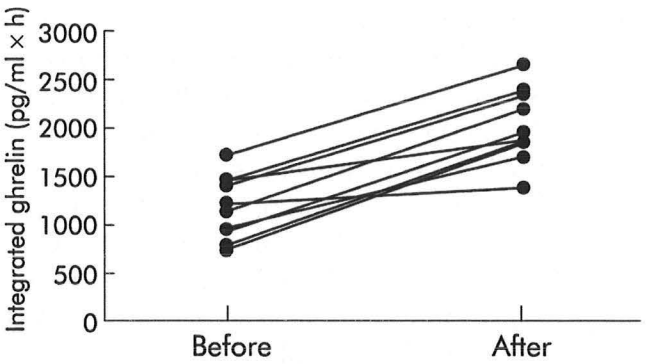

Figure 3. Six hour integrated plasma ghrelin in 10 healthy subjects before and after cure of *H. pylori* [44].

Another wave of interest in a potential infectious origin of obesity has been sparked by microbiomics, the burgeoning field of study of microbial ecosystems enabled by advances in high-throughput DNA sequencing [50]. The human gut is estimated to have 10^{13} to 10^{14} bacterial cells, an order of magnitude or two more than the number of human cells in the body. Prior to microbiomics, our knowledge of the gut microbial flora was limited largely to those organisms that can be cultured, a small subset of the total. With new DNA sequencing techniques, gut bacterial 16S ribosomal RNA molecules can be sequenced *en mass* and the resulting signatures used to identify bacterial species. There are now estimated to be as many as 15,000-36,000 species of gut microorganisms, with more than 90% of these belonging to the phyla Firmicutes or Bacteroidetes [51].

The seminal work in this area comes from Jeffrey Gordon, a gastroenterologist at Washington University in St. Louis. He and his colleagues, including Lora Hooper, now on the UT Southwestern faculty, first showed that germ-free mice had reduced body fat compared with conventionally reared mice, and that colonization of adult germ-free mice with a normal microbiota (known as conventionalization) harvested from the cecum of conventionally raised animals produces a 60% increase in body fat content and insulin resistance within 14 days, despite reduced food intake and no change in energy expenditure [52]. There are several possible mechanisms for the increase in body fat of the host animal in response to gut microbiota (Figure 4). 1. Bacteria promoted absorption of monosaccharides from the GI tract and induced hepatic lipogenesis. 2. Conventionalization suppressed intestinal production of Fasting-induced adipocyte factor (Fiaf), a circulating lipoprotein lipase inhibitor. Increased lipoprotein lipase activity promotes storage of fat in adipocytes. 3. Gordon and colleagues subsequently showed that germ-free mice are resistant to obesity induced by a high-fat, sugar-rich Western diet [54]. Resistance to diet-induced obesity in these mice involves increased fatty acid oxidation, mediated by induction of PPAR-gamma by elevated Fiaf levels, as well as increased activity of adenosine monophosphate-activated protein kinase [54]. 4. Independent work by Cani *et al.* [55] suggests

another piece of the puzzle. Circulating bacterial lipopolysaccharide (LPS) increased 2-3 fold in mice fed a high fat diet, perhaps due to increased LPS-containing gut microbiota. The increased circulating LPS, a state the authors termed "metabolic endotoxemia," was associated with markers of systemic inflammation, known to be associated with obesity and insulin resistance.

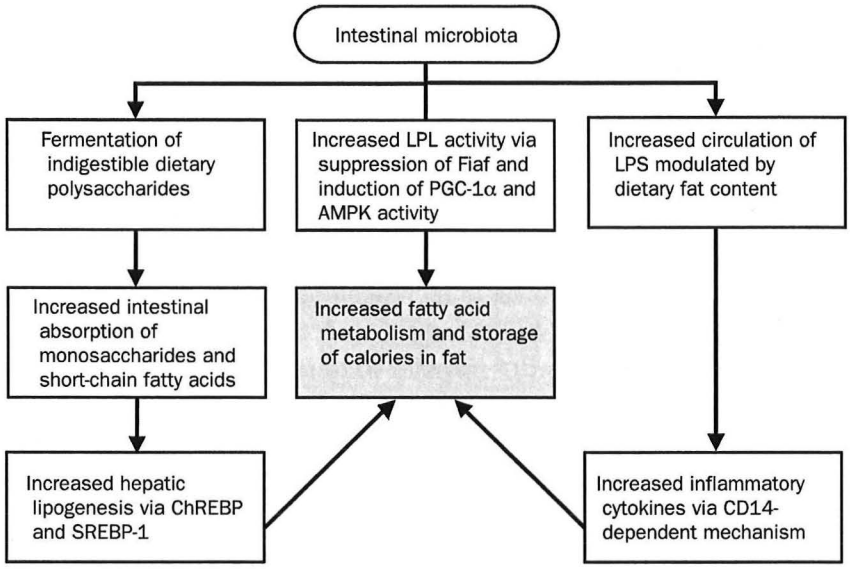

Figure 4. Proposed mechanisms for influence of gut microbiota on propensity to develop obesity [53].

Two additional studies from the Gordon lab, published in *Nature* in 2006, garnered much attention. In a previous study, they showed that genetically obese (*ob/ob*) mice with a mutation in the leptin gene had a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes compared to lean controls [56]. In one *Nature* article, they showed that the microbiome of the *ob/ob* mice has an increased capacity to harvest energy from the diet compared to that of the lean mice and that this difference could be transmitted germ-free mice [57]. Mice conventionalized with the *ob/ob* microbiome generated more fermentation end products acetate and butyrate, had decreased fecal residual energy content, and showed a greater increase in body fat than mice conventionalized with the lean microbiome (Figure 5). In an accompanying Brief Communication, they reported that obese humans (n=12) also showed a reduced proportion of Bacteroidetes when compared to lean controls (n=11), and this proportion increased after weight loss on either a fat- or carbohydrate-restricted diet [58].

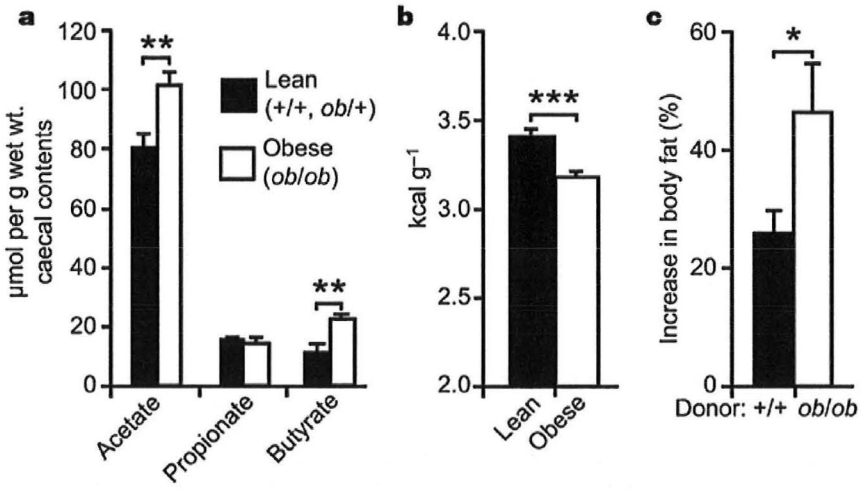

Figure 5. Increased fecal concentration of fermentation products (a), decreased fecal caloric density (b), and increased gain in body fat (c) in mice conventionalized with microbiome from *ob/ob* obese versus lean mice [57].

An accompanying editorial raised several caveats to the interpretation of these findings [59]. First, it is unclear whether the small changes in caloric extraction seen in the mouse study (Figure 5b) can contribute to meaningful differences in body weight. Second, although the 14-day food intake of the mice given the "lean" versus the "obese" microbiota showed "no statistically significant difference," the actual difference (54.0±1.2 g vs. 55.4±2.5 g) was enough to account for the difference in body fat increases (Figure 5c), a fact that is more apparent when these increases are expressed as absolute amounts (0.86±0.1 g vs. 1.3±0.2 g) rather than as percentages. Last, although obese humans and mice showed changes in proportions of Bacteroidetes in the same direction, obese humans have increased leptin levels, whereas the *ob/ob* mice lack leptin entirely. Furthermore, the microbiota from the *ob/ob* mice apparently maintained their 'obese' character for at least two weeks after transfer to germ-free animals, and it is unclear how gut bacteria sense whether their host is obese or lean. The key unanswered question is whether differences in gut flora are a cause or effect of obesity. One study of fecal samples collected prospectively at ages 6 and 12 months suggested that differences in gut microbiota preceded the accumulation of excess body weight at age 7 years [60].

Finally, just a few weeks before these Grand Rounds, a marriage of sorts between hypotheses involving evolutionary and infectious origins of obesity appeared in JAMA and the popular press. Previous work showed that individuals with obese or overweight BMI are at reduced risk of developing active tuberculosis [61], perhaps because of the associated systemic inflammation and immune activation. The authors suggested that increasing BMI could be driving the decline of tuberculosis, which began well before the advent of effective antibiotic treatment. Turning this hypothesis on its end, Jesse Roth at Albert Einstein College of Medicine speculates that tuberculosis could be driving the increase in BMI [62]. He proposed that increased BMI might confer a survival advantage to individuals of reproductive age exposed to tuberculosis, and the 'thrifty' gene selected during human evolution is actually a gene conferring resistance to active tuberculosis. Anticipating a skeptical audience, the JAMA paper [62], aptly titled "Evolutionary speculation about tuberculosis and the metabolic and inflammatory processes of obesity," concluded in an impressively equivocal fashion:

> Speculation that the previous tuberculosis pandemic may have intensified the metabolic syndrome and inflammatory processes associated with obesity suggests a plausible, though hypothetical, evolutionary process. Although these associations might be coincidental, it is important to recognize that theoretical constructs, no matter how logical, may yield conclusions that are not correct.

Manipulating the Gut Flora

The notion that gut flora can cause changes in energy balance provides an intellectual foundation for empiric efforts to therapeutically manipulate of the microbiome. These efforts fall into the alternative medicine categories of prebiotics and probiotics as well as the traditional medical category of antibiotics.

Prebiotics

Prebiotics are indigestible oligosaccharides that enhance the growth of commensal organisms, e.g. *Lactobacillus* species [63]. Studies in rats have shown that addition of the prebiotic oligofructose, a popular dietary supplement in Japan, reduced energy intake and weight gain of animals fed either standard chow or a high-fat diet by modulating endogenous gut peptides such as glucagon-

like peptide 1 and ghrelin [64, 65]. One study in humans found that the nondigestible starches inulin or lupin-kernel fiber can promote short-term satiety and reduced energy intake when used as a fat substitute [66]. A single-blind, crossover study of 10 healthy normal-weight subjects showed that 14 days of oligofructose treatment increased postprandial satiety and reduced total daily energy intake by 5% versus placebo [67]. These findings appear to contradict the mechanism proposed by Jeff Gordon and colleagues whereby fermentation of indigestible dietary polysaccharides by gut bacteria promotes increased energy extraction [52], highlighting the complexity of human/microbial ecology.

Probiotics

Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host"[68]. The most commonly used probiotics are lactic acid bacteria and bifidobacteria. Probiotics have been used for a variety of diseases, particularly diarrheal disorders. Lee *et al.* [69] investigated the use of probiotics for obesity. They fed *Lactobacillus rhamnosus* PL60, a strain, derived from humans, that produces conjugated linoleic acid, to mice with diet-induced obesity After 8 weeks, the mice showed reduced weight without change in energy expenditure. Conjugated linoleic acid is reported to reduce body fat in mice [70] but its efficacy in humans is uncertain [71].

Antibiotics

Antibiotics have been used extensively in agriculture for decades to promote growth and weight gain, and the resulting problem of antibiotic resistance is well known. The mechanism of growth promotion is not well understood but is thought to involve changes in gut microbiota that increase feeding efficiency, the weight gained per unit amount of food consumed. This use of antibiotics generally entails administration of low doses of antibiotics in food or water over long periods of time in large groups of animals. In 2005, the European Union banned the use of antibiotics for growth promotion, but the practice continues in the United States [72]. Surprisingly, hypotheses have only recently been put forward that a similar growth promoting effect may occurring in humans and contributing to the obesity epidemic, either through environmental antibiotic pollution [73] or as a result of antimicrobial therapy for infections [74]. Acute antibiotic treatment of infants has been associated in some instances with abrupt shifts in gastrointestinal microbiota [75], lending plausibility to the latter hypothesis. However, exposure to environmental antibiotics might better represent mimic the chronic antibiotic treatments used in the agricultural setting.

IV. Chemobesity: Did Rachel Carson's Silent Spring Portend Our Fat Fall?

Antibiotics are just one environmental pollutant of potential concern with regard to obesity. A whole set of other synthetic organic and inorganic chemicals appearing in the environment have been associated with weight gain in animal models, leading to the hypothesis which I term "chemobesity" (0 Google hits!) that the human obesity epidemic is due to exposure to these chemicals [76]. One indicator of the degree of interest in this hypothesis is the fact that a session at the 2007 American Association for the Advancement of Science entitled "Obesity: Developmental Origins and Environmental Influences" was devoted to examining whether prenatal chemical exposure may be predisposing some children to a life of obesity [77].

The proposed mechanisms of weight gain due to chemical toxins are manifold and include virtually every element of body weight regulation, but the most extensively studied is the so-called endocrine disruptor effect of chemicals such as polychlorinated biphenyls (PCBs), organochloride pesticides (dichlorodiphenyltrichoroethane or DDT, lindane, etc.), flame retardants (polybrominated diphenyl ether or PDBE), and plasticizers (bisphenol A or BPA, phthalates). These compounds are thought to modulate hormonal action through one or more of the following mechanisms: direct binding to nuclear receptors; nuclear receptor antagonism; inhibition of aromatases; and induction of cytochrome P450 enzymes that metabolize hormones [78].

As one example, BPA, a ubiquitous chemical found in polycarbonate plastic bottles and sealants, has been known since the 1930's to have estrogenic activity [79]. *In vitro* studies show that BPA in combination with insulin can accelerate the differentiation of mouse 3T3-L1 fibroblasts into adipocytes [80]. *In utero* exposure during mid to late gestation of mice to low doses of BPA at concentrations comparable to that found in the environment was associated with increased postnatal weight [81]; other studies of higher BPA doses or early postnatal exposure (reviewed in [78]) showed similar effects.

Epidemiologic studies of the possible link between endocrine disruptors and obesity are sparse, and the findings thus far are inconsistent (reviewed in [78]). However, studies have for the most part examined blood levels of select chemical toxins and various obesity-associated traits such as BMI, waist circumference, and serum lipids. These toxins are generally sequestered in fat, and weight loss has been shown to increase plasma concentrations of lipophilic organochlorine pesticides and PCBs in obese subjects [82].

V. Micronutrients

"I have no truck with lettuce, cabbage, and similar chlorophyll. Any dietitian will tell you that a running foot of apple strudel contains four times the vitamins of a bushel of beans."

—S.J. Perelman

The conventional view of dietary factors in the obesity epidemic focuses on macronutrients: protein, carbohydrate, and fat. Recently, investigators have begun to consider a possible role of minerals and vitamins in energy homeostasis. An appealing hypothesis is that hyperphagia may be a homeostatic response to suboptimal intake of micronutrients, analogous to pica and iron deficiency anemia [83]. An obvious problem with this hypothesis is the lack of evidence for a secular trend of decreasing micronutrient intake coinciding with the increase in obesity.

Nonetheless, there is a body of literature describing possible associations between intake of minerals and vitamins and body weight. In work funded by the National Dairy Council, Zemel *et al.* [84] described a 4.9 kg reduction in body fat over the period of a year in an uncontrolled study of obese African-American men whose daily calcium intake was increased from 400 mg to 1000 mg by daily consumption of 2 cups of yogurt. This finding prompted numerous investigations, culminating in a symposium in December 2006 that produced an eighteen page review with 165 references [85]. Not surprisingly, the conclusions of the symposium are that calcium and dairy food intake "have the potential to increase fat oxidation, decrease fat absorption, promote fat cell apoptosis and increase satiety and decrease food intake...." The details of these studies will not be

reviewed in these Grand Rounds because, as the review also stated, "confounding factors have not been directly addressed and underlying mechanisms are still missing...." Suboptimal intake of vitamins B_6 and B_{12} [86] antioxidant vitamins C and E [87], and trace elements zinc and magnesium [87] have also been suggested to play a role in the obesity epidemic, but the evidence is equally scant.

VI. Psychosocial stress

Although the effect of psychosocial stress on eating behavior is commonly mentioned as a factor in the obesity epidemic, the evidence from human and animal studies that it actually plays a role is surprisingly scant [88]. Acute stress triggers the "fight or flight" response, with activation of the sympathetic nervous system and suppression of food intake. Chronic stress activates the hypothalamic-pituitary-adrenal axis and results in increased cortisol, known to increase appetite and favor abdominal energy storage (e.g., Cushing's disease). In some individuals, chronic stress appears to increase consumption of hedonic, energy dense food, leading to weight gain [89]. However, an alternative explanation may be that stress results in less time for purchase and preparation of foods and increased reliance on energy dense convenience foods [90].

An interesting variation on the theme of stress and feeding involves writing grants. McCann *et al.* [91] studied workers in the University of Washington Grants and Contracts office during high (January, May) or low (Mar-April) workload periods. There were 31.9% more proposals received during the January period and 22.6% more during the May period than during the Mar-April period, and subjects (n=10) reported more perceived stress during the high workload periods. Based on food diaries, subjects (n=10) consumed more calories during the high workload periods (2061±980 vs. 1821±732, $P<0.05$).

Tremblay hypothesized that knowledge-based work especially may predispose to increased food intake [92]. According to this hypothesis, the brain relies on glucose as an energy substrate under normal feeding conditions. Mental activity may lead to decreased plasma glucose, resulting in compensatory increase in feeding. In a bold example of self-experimentation, Tremblay compared

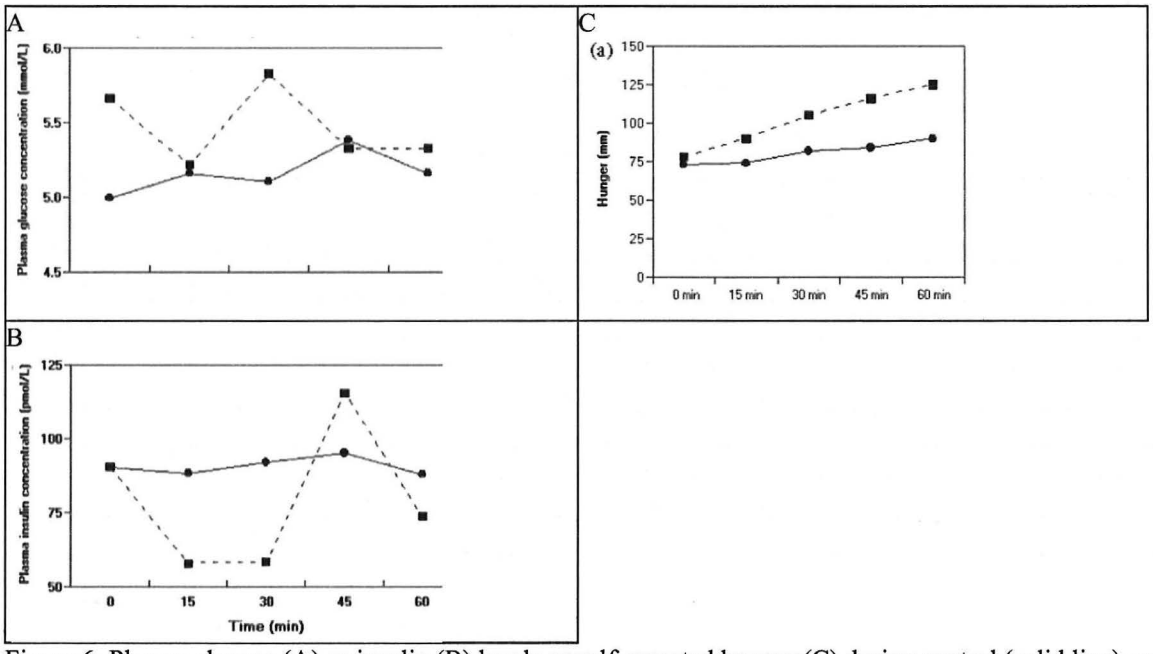

Figure 6. Plasma glucose (A) or insulin (B) levels or self-reported hunger (C) during control (solid line) or grant-writing (dashed line) periods [92].

his plasma glucose and insulin levels, measured at 15 minute intervals, for one hour at rest versus one hour spontaneously dictating the text of a grant application [92]. His plasma glucose and insulin levels were much less stable during grant-writing than during rest (Figure 6A,B). He also noted increased hunger (Figure 6C). The outcome of the grant application was not reported. If this hypothesis is correct, then the recent 'tsunami' of 20,000 NIH Recovery Act grant applications [93] might have inadvertently fueled the obesity epidemic, at least among U.S. biomedical researchers.

VII. Obesity epidemic 2.0

Another *au courant* hypothesis of the spread of obesity involves social network theory (Figure 7). Using data collected over three decades by the Framingham Heart Study, Nicholas Christakis, a Harvard health care policy analyst, and James Fowler, a political scientist at UCSD, analyzed whether weight gain in a subject was associated with weight gain in his or her spouse, friends, siblings, or neighbors [94]. They found that a person's chance of becoming obese, defined by BMI measurements available for all subjects, increased by 57% if he or she had a friend who became obese, by 40% if a sibling became obese, and by 37% if a spouse became obese. Immediate neighbors did not show this effect, which was not attributable to smoking cessation. They concluded that "obesity may spread in social networks." They claimed that the lack of effect of immediate geographic neighbors and the lack of influence of geographic distance on the strength of the effect with friends or siblings suggested that the clustering effects on obesity were not due to shared environmental exposure.

This article garnered a lot of attention in the lay press, with headlines about obesity being 'contagious' [95] and discussion of attendant concerns such as potential workplace discrimination. It also has already been cited 214 times in PubMed. The social network was incorporated into a new multidimensional 'diseasome' model, along with networks of interconnected diseases and metabolic pathways [96]. Interestingly, the same authors performed similar analyses of the same Framingham subjects and reported social network effects with regard to smoking [97] and happiness [98]. Another group recently proposed that back pain may also be a communicable social network disease [99].

With no end in sight to claims of social network effects on human health and well being, Ethan Cohen-Cole, a financial economist at the Federal Reserve Bank of Boston, and Jason Fletcher, an assistant professor at Yale School of Public Health, performed an interesting control experiment. They analyzed social network effects for acne, height, and headaches [100], using the same methods as Christakis and Fowler. The results showed nominally significant but biologically implausible effects for all three of these conditions. However, after adjustments for confounding environmental factors, all of the effects became smaller and statistically insignificant. They conclude that "researchers [I would add the general public and lay press] should be cautious in attributing correlations in health outcomes of close friends to social network effects, especially when environmental confounders are not adequately controlled for in the analysis." Cohen-Cole and Fletcher went on to publish an analysis of another dataset, the National Longitudinal Study of Adolescent Health (Add Health, www.cpc.unc.edu/projects/addhealth), in which they found that shared environmental factors can cause the appearance of social network effects on obesity [101]. However, they concurred with Cole and Fletcher that even if shared environment rather than

social network effects are the cause of the obesity epidemic, social networks can be exploited for interventions.

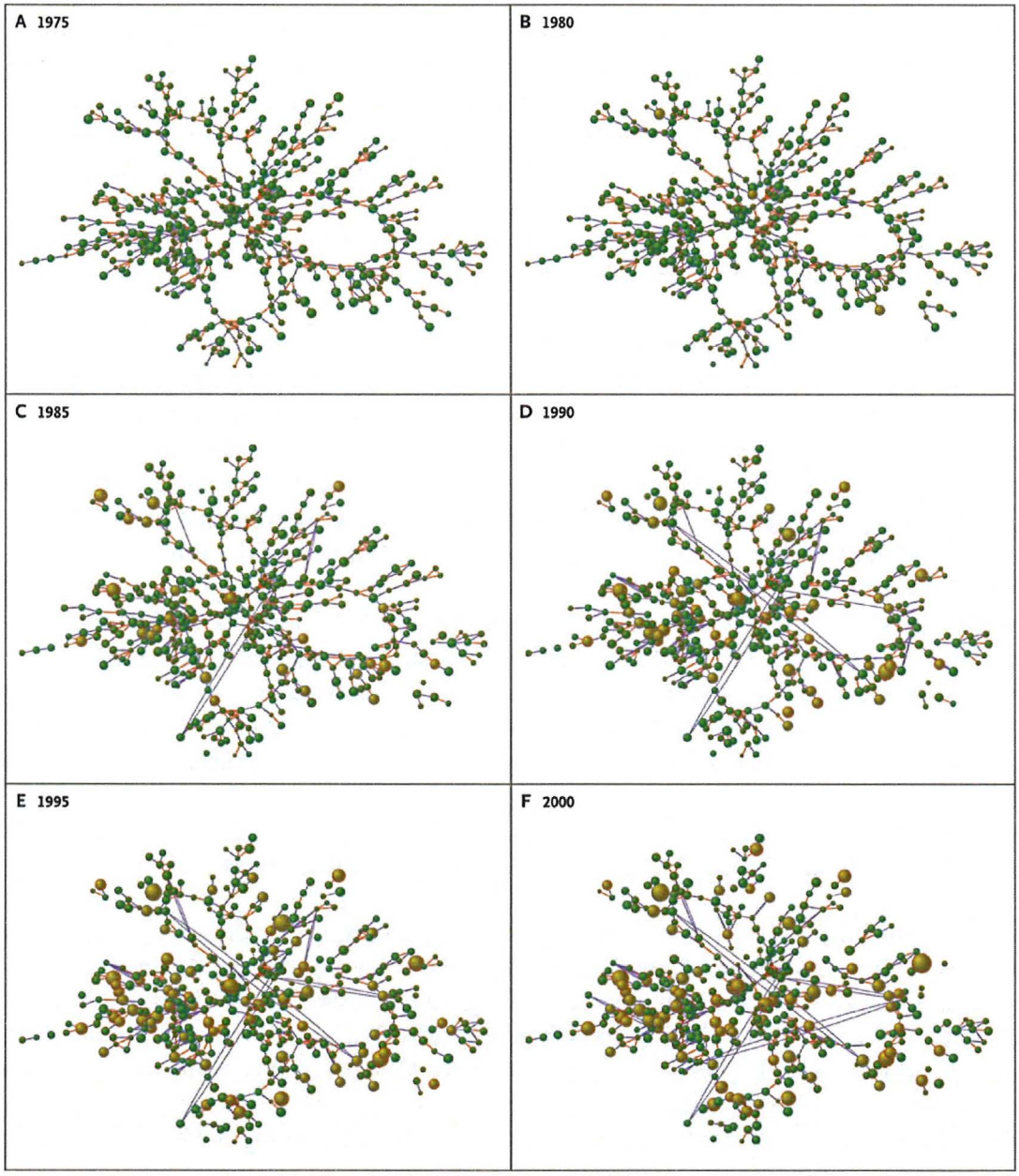

Figure 7. "Part of the Social Network from the Framingham Heart Study with Information about Body-Mass Index According to Year. Each circle (node) represents one person in the data set. Circles with red borders denote women, and circles with blue borders denote men. The size of each circle is proportional to the person's body-mass index. The interior color of the circles indicates the person's obesity status: yellow denotes an obese person (body-mass index, 30) and green denotes a nonobese person. The colors of the ties between the circles indicate the relationship between them: purple denotes a friendship or a marital tie and orange denotes a familial tie. The disappearance of a circle from one year to another indicates the person's death, and the disappearance of a tie between the circles indicates that the relationship between the two persons no longer exists." [94].

VIII. Is it all mom's fault?

The Dutch Famine was a six-month period during World War II from October 1944 until liberation on May 7, 1945. During this time, Allied forces had freed the Netherlands south of the Rhine river, but the portion to the west was still under Nazi occupation. In reprisal for a strike by Dutch railroad workers in response to an appeal by the Dutch government-in-exile in London, the Nazis embargoed all incoming transport, including food. The embargo, exacerbated by a harsh winter, soon resulted in famine. Thanks to meticulous Nazi records, accurate information is available about the average daily calorie ration, which was about 1,800 kcal at the beginning of the occupation, falling to 1,400 kcal the month prior to the embargo. By November during the famine it had fallen to 1,200 kcal, <800 kcal by the new year, and 580 kcal by the end of February, 1945. Prior to the famine, supplements were given to pregnant women and mothers of young infants, but these stopped in the middle of November, 1944.

Ravelli *et al.* [102] obtained weight and height data from 300,000 nineteen-year old Dutch men born between January 1, 1944 and December 31, 1947, collected at the time of military induction. The subjects were grouped according to where and when they were born. Famine-exposed subjects were compared with geographic controls (subjects born contemporaneously in liberated regions of the country) and temporal controls (subjects born in the same location before or after the famine). Famine cohorts were further divided according to the time of exposure to severe undernutrition: first-second trimester or third trimester-early infancy, and the prevalence of obesity among the various cohorts compared. The results of this "experiment of human nature" were that men exposed to famine during the first two trimesters (n=4,300) showed an increase in the prevalence of obesity at age 19 (2.77% vs. 1.45%, $P<0.0005$) compared to geographic controls (n=15,900), whereas men exposed to famine during the third trimester or early infancy (n=6,200) showed a decrease in the prevalence of obesity at age 19 (0.82% vs. 1.32%, $P<0.005$) compared to geographic controls (n=11,200). Comparisons to temporal controls showed similar effects. This study prompted the "developmental origins of obesity" hypothesis, supported by many subsequent animal studies (reviewed in [103]). The "developmental origins" hypothesis has been generalized to many other adult diseases, notably cardiovascular disease [104].

The molecular basis for the lasting effect of prenatal/perinatal famine on adult body weight is not known. One possibility is epigenetic changes, e.g., altered DNA methylation. A recent investigation compared methylation of the insulin-like-growth factor II (*IGF2*) gene, a key regulator of human growth, in white blood cells from 60 individuals exposed to the Dutch Famine to their same-sex sibling controls [105]. The results (Figure 8) showed that periconception famine exposure was associated six decades later with a 5.2% decrease in *IGF2* DNA methylation. By contrast, subjects exposed to famine during late gestation did not show any difference in *IGF2* DNA methylation in later life. It is interesting to consider that altered DNA methylation has been observed in cloned mammals, which often show an obese phenotype [106], with implications for *in vitro* fertilization (IVF) outcomes.

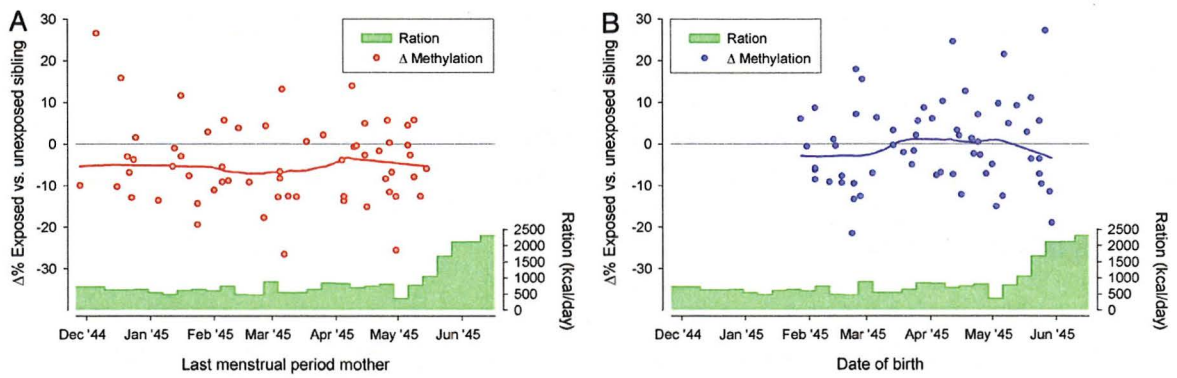

Figure 8. Difference in IGF2 DMR methylation between individuals prenatally exposed to famine and their same-sex sibling [105].

Absent severe famines that might account for the recent epidemic of obesity in Western societies, the focus of studies of the developmental origins hypothesis has shifted to possible effects of fetal overnutrition on the subsequent risk of obesity in the child. Gestational diabetes represents a scenario where there is extreme fetal overnutrition. In his 1980 Banting lecture entitled "Of Pregnancy and Progeny," Freinkel proposed that changes in fetal fuel economy from gestational diabetes may result not only in teratogenic organogenesis effects but also "long-range effects upon behavioral, anthropometric, and metabolic functions" [107]. The association between gestational diabetes and large-for-gestational age babies is well known; studies also suggest a U- or J-shaped relationship between birthweight and later obesity [108-110]. One recent study reported that maternal hyperglycemia strongly predicted BMI in offspring at 5-7 years of age, after adjustment for maternal weight gain and birth weight [111]. If maternal obesity indeed predisposes the child to later obesity, we could be entering a vicious generational cycle predicting an accelerating obesity epidemic, independent of further genetic or environmental factors [112, 113].

A Mendelian randomization study in 2008 suggests that this may not be the case. As this type of genetic epidemiology study is beginning to appear more often in the general medical literature, it is worth discussing the methodology. To understand the concept of Mendelian randomization, consider the following example: Low cholesterol levels are known to be associated with cancer, and it has been hypothesized that low cholesterol levels are in fact carcinogenic. We can test this hypothesis if we can identify a common genetic variation that affects cholesterol levels but does not otherwise cause or prevent cancer. Mendelian inheritance of this genetic variation can then serve as a surrogate for a randomized controlled trial of an agent that lowers cholesterol. In this hypothetical example, the E2 allele of apoE is associated with lower cholesterol levels but is not otherwise implicated in cancer. If low cholesterol levels cause cancer, there should be a higher frequency of ApoE E2 alleles in cancer cases versus controls. A real example of a Mendelian randomization study in the news recently cast doubt on a causal role of C-reactive protein in cardiovascular disease [114].

The Mendelian randomization study of the developmental origins of obesity [115] took advantage of the association of a common allele of the *FTO* "obesity" gene, discussed previously, with obesity. 4,091 trios (father, mother, child) in the U.K. were examined. The authors first showed that offspring fat mass correlated more strongly with maternal than paternal BMI, consistent with the developmental origins hypothesis. However, after controlling for the children's FTO

genotype, which would itself affect their fat mass, the Mendelian randomization analysis did not show a statistically significant association between the presence of the obesity-associated *FTO* allele in mothers and offspring fat mass at age 9-11 years, measured by dual energy X-ray absorptiometry (DEXA). However, the study was not adequately powered to detect a small effect, and the adjustment for the *FTO* genotype of the offspring introduced methodological issues [116].

IX. Lose weight while you sleep!

Numerous studies have showed an association between short sleep duration and increased BMI (Kripke 2002). At first blush this is paradoxical, since sleep is the quintessential sedentary behavior. Until recently it has been assumed that obesity is the cause and lack of sleep, e.g., sleep apnea, the effect. The converse hypothesis, that lack of sleep causes obesity, had been gaining popularity in recent years. Although rigorous peer-reviewed studies are hard to come by, the National Sleep Foundation (www.sleepfoundation.org) states that sleep duration has been steadily decreasing over the past century. One Canadian study found that subjects who slept 5-6 hours per night gained an average of 4.4 pounds more over the course of six years than subjects who slept 7-8 hours per night [117]. A 2004 study of 1,024 participants in the population-based Wisconsin Sleep Cohort Study found an inverse correlation between sleep duration and BMI in individuals sleeping less than 8 hours per night [118]. Simple explanations include nocturnal snacking or the notion that people who don't sleep enough feel too tired to exercise. However, evidence is accumulating for a more fundamental neurohormonal link between sleep and appetite. The same 2004 Wisconsin study found that short sleepers had 15% higher serum levels of the orexigenic gut peptide ghrelin and 16% lower levels of leptin, findings that agree with the results of experimental studies of sleep reduction in animals (reviewed in [119]). A small study of experimental sleep deprivation in young healthy male volunteers found similar effects [120].

Studies by UT Southwestern scientists also support the existence of neurohormonal links between appetite and sleep. About 10 years ago, Masashi Yanagisawa isolated a novel hypothalamic peptide which he named orexin on the basis of its ability to stimulate feeding behavior in rats. He subsequently showed that orexin knockout mice exhibit narcolepsy. Most human narcolepsy appears to be due to lack of orexin neurons as a result of autoimmunity [121]. Interestingly, patients with narcolepsy show a small but statistically significant increase in BMI compared to controls [122]. Mice with genetic ablation of orexin neurons become heavier than controls by 10-12 weeks of age, although paradoxically, they eat less than controls and gain weight because of reduced energy expenditure [123]. Teleologically, orexin may serve to maintain awakeness needed for foraging behavior to defend against negative energy balance, a connection known since antiquity:

> CAESAR
> Let me have men about me that are fat;
> Sleek-headed men and such as sleep o' nights:
> Yond Cassius has a lean and hungry look;
> He thinks too much: such men are dangerous.
>
> ANTONY
> Fear him not, Caesar; he's not dangerous;
> He is a noble Roman and well given.

CAESAR
Would he were fatter!
—Shakespeare, Julius Caesar, Act 1, Scene 2

A second line of evidence comes from the study of circadian rhythms. While at Northwestern, Joseph Takahashi, now chairman of the UT Southwestern Department of Neuroscience, discovered and positionally cloned the mouse *Clock* mutation that lengthens the period of circadian locomotor activity [124]. Subsequent investigation showed that homozygous mouse *Clock* mutants are hyperphagic and develop obesity, hyperlipidemia, and hyperglycemia [125]. The mechanism of obesity is still under investigation but likely involves dysregulation of hypothalamic neuropeptides related to energy balance.

An educational and behavioral intervention study is underway, led by Giovanni Cizza at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), to see if obese people (BMI 30-50 kg/m^2) who are chronically sleep-deprived (less than 6.5 hours per night) can manage to sleep an hour longer without taking medication, and whether doing so will result in weight loss. Other study endpoints include prevalence of the metabolic syndrome and circulating levels of ghrelin and leptin. This study was scheduled to begin in 2006 and to last for 12 months but is still listed as active on the NIH web site (http://clinicalstudies.info.nih.gov/detail/A_2006-DK-0036.html), and as yet no results have been published. If the findings are positive, prescribing more sleep in order to lose weight loss would perhaps be met with greater compliance than other lifestyle modifications such as diet and exercise.

X. CONCLUSIONS

The list of proposed causes of the obesity epidemic discussed in these Grand Rounds is by no means exhaustive. Some other proposed etiologic factors for the U.S. obesity epidemic include more widespread use of air conditioning (reducing energy expenditure); reduced smoking (accounting in one CDC study for a fourth to a sixth of the increase from 1978-1990 in the prevalence of overweight men and women, respectively [126]); iatrogenic causes, e.g. psychotropic medications, thiazolidinediones, etc.; changes in the ethnic and racial distribution of the population; increasing gravida age; selection for increased reproductive fitness associated with higher (up to a point) BMI due to biologic and/or socioeconomic factors (very low BMI is associated with infertility in both sexes; obesity in women leads to lower socioeconomic status which in turn leads to more offspring); and assortative mating (the tendency for individuals with similar adiposity to mate, which would tend to increase the skewing of BMI distribution in the population) [2].

It is important to recognize that obesity is defined by an arbitrary threshold, i.e., BMI > 30 kg/m^2. Since BMI is approximately normally distributed, a small shift in the mean population BMI will result in a disproportionate increase in the fraction of the distribution to the right of the threshold (Figure 9). In real terms, the 3-4.5 kg increase in mean body weight of the U.S. population from 1991-2001 resulted in an increase in the prevalence of obesity from 23.3% to 30.9 % [4]. Conversely, a small reduction in the population mean BMI would translate to a large reduction in the prevalence of obesity.

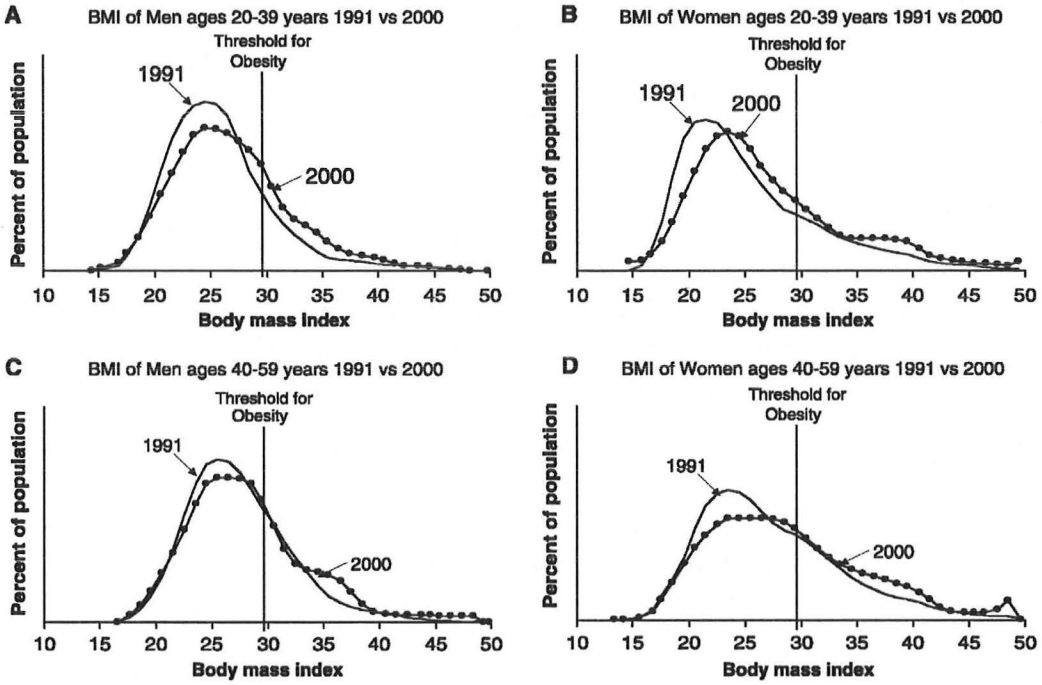

Figure 9. "The smoothed distribution of BMI for men and women in the United States aged 20 to 39 (A and B) and 40 to 59 (C and D) is shown for the years 1991 and 2000. In both cases, the distributions have shifted to the right and become more skewed. For the 20-to-39 age group, the average BMI for males increased from 25.9 to 27.0, and the average BMI for females increased from 25.4 to 27.5. For the 40-to-59 age group, the average BMI for males increased from 27.5 to 28.3, and the average BMI for females increased from 27.6 to 29. In both cases, there was a marked increase in the number of individuals with BMI > 30." [4].

Examples of erroneous causal inferences drawn from epidemiologic associations are legion in the history of medicine. Even the dogma that reduced physical activity is a principle cause of increasing obesity has recently been challenged. A study presented at the European Congress on Obesity in May, 2009 by Swinburn and colleagues [127] calculated how much adults need to eat maintain a stable weight and how much children need to maintain normal growth. The authors then calculated how much Americans actually ate from the 1970's through the early 2000's, using national food supply data. The predicted weight gain, based solely on calories consumed, precisely matched the actual weight gained by children and exceeded the actual weight gained by adults by 4 kg per person, suggesting there may actually have been an increase in physical activity over the past 30 years that blunted the effect of increased caloric intake.

To date, public health interventions have largely focused on diet and exercise, and although there are recent signs that the obesity epidemic among children may be leveling off [128], this plateau still portends a mounting toll of future morbidity and mortality [112]. Although no reasonable person denies the importance of diet in the obesity epidemic, the question becomes, "What is driving increased caloric intake?" When this author was a medical student, it was a 'fact' that peptic ulcer disease was caused by excessive acid secretion (often attributed to stress), to which treatments including surgery and drugs were directed for many years. Of course, we now believe that *H. pylori* infection is the major cause of ulcers. In this spirit, the unconventional ideas about the obesity epidemic presented in this Grand Rounds are offered as food for thought that will perhaps whet the appetite for further research.

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