Nutrición Hospitalaria

Predictors of obesity: the "power" of the omics

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Abstract

During the entire 20th Century, nutrition research experienced and amazing interest and development fueled by the initial success on the fields of malnutrition and the discovery of vitamins and other essential nutrients. During the second part of the Century, it was realized that most common diseases (i.e., cardiovascular diseases, cancer and obesity) had a strong nutritional component. However, from the public health perspective as well as from the point of view of the individual recommendations, current recommendations for healthy nutrition resemble those provided over one hundred years ago. Therefore, modern nutritional research has a great potential of still contributing to improved health for future generations, assuming that the new developments in research and technologies are applied to nutritional problems. Nutrition research must embrace state of the art epidemiology, objective food assessment tools, genomics, epigenomics, transcriptomics, proteomics, metabolomics, metagenomics, advanced biostatistics, imaging, challenge tests, and integration of all data by bioinformatics, under the umbrella of molecular nutrition research. The ultimate goals of future nutritional research are to understand the detailed mechanisms of action for how nutrients/foods interact with the body and with the individual genomes to further the advance of nutrigenomics, thereby providing new tools for disease prevention and treatment.

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Key words: *Nutrigenomics. Metabolomics. Epigenomics. Chronobiology. Obesity.*

PREDICTORES DE OBESIDAD: EL "PODER" DE LAS OMICAS

Resumen

Durante todo el siglo XX, la investigación en nutrición experimentó un notable interés y desarrollo alentados por el éxito inicial en los campos de la malnutrición y el descubrimiento de las vitaminas y otros nutrientes esenciales. Durante la segunda parte del siglo, se constató que las enfermedades más frecuentes (es decir, las enfermedades cardiovasculares, el cáncer y la obesidad) poseían un fuerte componente nutricional. Sin embargo, desde la perspectiva de la salud pública, así como desde el punto de vista de las recomendaciones individuales, las recomendaciones actuales para una nutrición saludable se parecen a aquellas proporcionadas hace más de 100 años. Por lo tanto, la investigación moderna en nutrición tiene un gran potencial para seguir contribuyendo a mejorar la salud de las generaciones futuras, asumiendo que los nuevos desarrollos en la investigación y las tecnologías se apliquen a los problemas nutricionales. La nutrición en investigación debe incorporar la epidemiología puntera, las herramientas objetivas de evaluación alimentaria, la genómica, la epigenómica, la transcriptómica, la proteómica, la metabolómica, la metagenómica, la bioestadística avanzada, las imagenología, las pruebas de provocación y la integración de todos los datos con la bioinformática bajo el paraguas de la investigación en nutrición molecular. Los objetivos últimos del futuro de la investigación en nutrición deben comprender los mecanismos de acción precisos de cómo interaccionan los nutrientes/alimentos con el organismo y con los genes individuales para avanzar en la nutrigenómica, proporcionando así nuevas herramientas para la prevención y el tratamiento de la enfermedad.

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Palabras clave: *Nutrigenómica. Metabolómica. Epigenómica. Cronobiología. Obesidad.*

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Abbreviations

3-UTR (3'-untranslated region).
Apolipoprotein A2 (APOA2).
Cardiovascular diseases (CVD).
Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE).
Endothelial nitric oxide synthase (eNOS).
Genome-wide association studies (GWAS).
High carbohydrate diets (HCD).
High-fat diets (HFD).
Insulin-like growth factors (IGF).
Lipid storage droplet (LSD).
Lyso-phosphatidylcholine (lysoPC).
Metabolic syndrome (MetS).
microRNAs (miRs).
miR recognition element (MRE) seed sites (MRESS).
Nutrigenomics Organization (NuGO).
Partial least-squares-discriminant analysis (PLS-DA).
Perilipin 1 (PLIN1).
Perilipin 4 (PLIN4).
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α).
Proopiomelanocortin (POMC).
Retinoid X receptor-α (RXRA).
Single nucleotide polymorphisms (SNP).
Suprachiasmatic nucleus (SCN).
Type 2 diabetes (T2D).
United States Department of Agriculture (USDA).

Introduction

Obesity and cardiovascular diseases (CVD) are two dramatic public health burdens that share common underlying mechanisms and afflict most industrialized countries and both are largely influenced by lifestyle, including dietary intake. General recommendations for most CVD —and obesity— related dietary factors are available, but these diseases affect individuals and at-risk subsets (i.e., minorities, elderly) of the population differently. Some of this variability is explained by genetic variation, and in this regard the genetics of CVD, CVD risk factors (e.g. hypertension, dyslipidemia, and diabetes), obesity, or CVD-related phenotypes (e.g., carotid intima media thickness, endothelial function, oxidative stress, and fat distribution) have been explored by several studies, but results are inconsistent and heritability only partially explained. Part of that inconsistency and unexplained heritability could be attributable to complex gene-environment and particularly to gene-diet interactions.[1]

While most environmental factors are discretionary and transitory (e.g. smoking and exercise), nourishment is a necessary, lifelong and universal environmental factor. Gene-diet interactions reflect the fact that genetic variations can predispose individuals to disease while diet can decrease or exacerbate this risk. Nutrigenetics is an emerging discipline that studies the different physiological responses to diet depending on the genotype(s) of each individual. From a nutrition research standpoint, gene-diet interactions likely explain some of the inconsistencies of the diet-disease associations reported in different populations. From a genetic research standpoint, a meaningful gene-diet interaction can neutralize genetic effects (resulting in a null genetic effect). From a public health standpoint, it is critical to distinguish between genetic susceptibility, diet impact, and gene-diet interactions, and to be able to quantify their relative importance as risk factors for morbidity and mortality in an aging population. The proportion of the excess incidence of disease risk that can be reduced by altering the environmental (i.e., dietary) agent can then be estimated and acted upon. A similar discourse applies to the effects, associations and interactions with physical activity. Therefore, nutrigenetics could reveal risks and benefits of specific diets or dietary components to the individual and thus assist the development of personalized dietary recommendations instead of generalized ones. By contributing to the definition of optimal dietary and behavioral (i.e., physical activity and biorhythms) recommendations aimed at preventing disease and promoting optimal health and aging, nutrigenetics offers substantial and prudent direction in the translation of nutrition research into public health recommendations. Accordingly, "personalized nutrition" or "individualized nutrition" approaches are being developed within the USDA (e.g., mypyramid.gov) and already are proposed elsewhere (e.g., Nutrigenomics Organization (NuGO)).

The challenge of accurate and objective dietary assessment

To be effective, the optimal development of evidence-based personalized nutritional guidance for prevention of obesity and other metabolic disorders hinges on an adequate assessment of food intake, nutrient availability, activity, and efficacy. However, assessment of dietary intake has known limitations, including the difficulty of recalling complex food intake patterns over long periods of time. Therefore, the subjective nature of self-reported dietary intake assessment methods presents numerous challenges to obtaining accurate dietary intake and nutritional status. This limitation can be overcome by novel "omic-based" approaches, which can objectively assess dietary consumption (or exposure) without bias of self-reported dietary intake errors.[2] Diet and nutrient biomarkers should provide objective measures of dietary intake and nutritional status, as well as an integrated measure of intake, absorption and metabolism. Thus, the search for an unbiased biomarker of dietary intake and nutritional status is an important aspect of nutritional epidemiology. This also applies to other key environmental factors. Thus, biomarkers of physical fitness are also desired and the need for such developments was addre-

Table I		
Methods in Nutritional "omics"		
Omics	*Technology*	*Measures*
Genomics	Microarrays Next generation sequencing	SNPs mutations
Epigenomics	Microarrays Sequencing	DNA Methylation Histone modification microRNAs
Metabolomics	Nuclear magnetic resonance Liquid chromatography Gas liquid chromatography Mass spectrometry	Metabolites
Proteomics	Protein microarrays Electrophoresis Mass spectrometry	Proteins and protein modifications
Transcriptomics	Microarrays RNA sequencing	mRNA levels
Metagenomics	Sequencing	Microbe species
Bioinformatics	Mathematical models Statistical methods Data mining Network analysis	Data integration and interpretation

ssed by the Institute of Medicine, which recognized the lack of such tools as a knowledge gap requiring future research. In this context, the application of "omics" techniques, especially metabolomics, represents a promising and needed approach to identify new biomarkers in nutrition assessment, through an integrative application of new technologies in human nutritional research.

Changing the nutritional landscape: the power of omics

Nutrigenetics

The concept of nutrigenetics was already introduced above; however, given the pivotal role that this approach will have on the future of nutrition research and practice, we provide in this section a more detailed description as well as some relevant examples. Nutrigenetics refers to the role of DNA sequence variation in the responses to nutrients, whereas nutrigenomics is the study of the role of nutrients in gene expression. This research is predicated on the assumption that there are individual differences in responsiveness to acute or repeated exposures to a given nutrient or combination of nutrients. Throughout human history, diet has affected the expression of genes, resulting in phenotypes that are able to successfully respond to environmental challenges and that allow better exploitation of food resources. These adaptations have been key to human growth and development. Technological advances have made it possible to investigate not only specific genes but also to explore in unbiased designs the whole genome-wide complement of DNA sequence variants or transcriptome. These advances provide an opportunity to establish the foundation for incorporating biological individuality into dietary recommendations, with significant therapeutic potential.
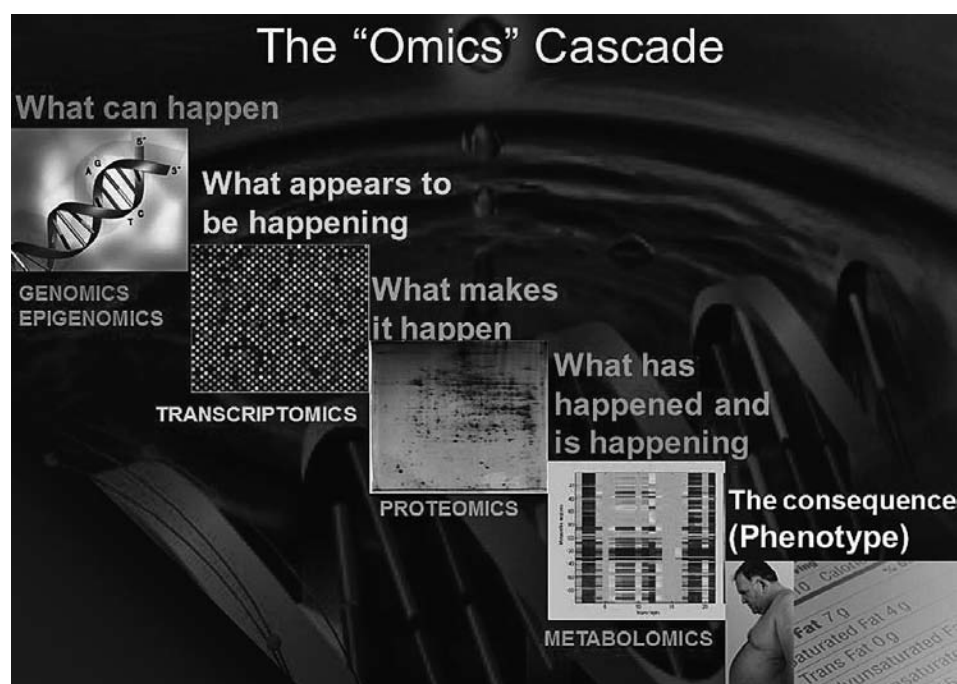

Fig. 1.— Omics cascade showing the specific omic and the biological information provided.

The importance of the genome in nutrition research is self-evident when we consider the multiple processes involved in nutrient handling which need the coordinated working of multiple genes bearing variants that may alter the response to diet. However, the progress of nutrigenetics has been hampered by technological and study design limitations that restricted the number of single nucleotide polymorphisms (SNP) that were examined in past nutrigenetic studies. As part of our research aimed to investigate whether genetic variants found on genes expressed in the adipocyte were associated with obesity risk in human populations and modulated by dietary factors, we have been investigating the perilipin family of genes. Perilipin 1 (PLIN1) is the major protein surrounding lipid droplets in adipocytes and regulates adipocyte metabolism by modulating the activity of enzymes that digest and release the fat in the adipocyte. We have previously identified numerous relationships between PLIN1 gene variants and measures of obesity[3]. In the work described next, we focus on research examining whether dietary macronutrients (e.g. carbohydrates and fats) modulated the associations PLIN1 genetic variants with obesity.[4] For this purpose, we studied a population-based sample of Caribbean-origin Hispanics living in the Boston area and with a high prevalence of obesity and related ailments. In these subjects we found a significant interaction between complex carbohydrate intake and a specific PLIN1 gene variant for waist circumference. When we divided the population into those with low (< 144 grams/day) and high (>/= 144 grams/day) complex carbohydrate intake, we found significantly different effects across PLIN1 genotypes. When complex carbohydrate intake was low, waist circumference was larger in carriers of the PLIN1 polymorphism. Conversely, when complex carbohydrate intake was high, waist and hip circumferences were less in carriers of the polymorphism. These interactions were not found for simple sugars or total carbohydrates. Therefore, we have identified a significant gene-diet interaction associated with obesity at the PLIN1 gene. In subjects with higher complex carbohydrate intake, the gene variant was protective against obesity, whereas in subjects with lower carbohydrate intake, the gene variant was associated with increased obesity. These interactions may be relevant to dietary management of obesity.

A major challenge in the area of gene-diet interactions is the replication of significant findings across different populations. Along these lines, we investigated the role of a functional genetic variant, known as APOA2-265T >C, in the regulation of food intake and body weight. Three independent populations in the United States were examined: the Framingham Offspring Study (1454 whites), the Genetics of Lipid Lowering Drugs and Diet Network Study (1078 whites), and Boston-Puerto Rican Centers on Population Health and Health Disparities Study (930 Hispanics of Caribbean origin)[5]. The results of this study show that people carrying the genetic variant at the APOA2 gene developed obesity only in the presence of a high saturated fat diet. This was true for the three populations and this is the first time that such replication was achieved across populations in research involving gene and diet interactions. This finding will contribute to the identification of individuals susceptible to diet-induced obesity. Moreover, it will guide the implementation of tailored dietary recommendations to specifically quench their increased predisposition to obesity and cardiovascular diseases.

Regardless of the research perspective, be it nutrition research, public health, or personalized diets, a crucial need for robust scientific evidence in support of nutrigenetics remains elusive. Only recently with the advent of genome-wide association studies (GWAS) and huge meta-analysis, we can start examining the interactions between millions of SNPs, dietary factors and the phenotypic trait of interest (i.e., obesity). So far, the number of nutrigenetic studies relying on systematically designed approaches applied to large enough populations has been limited and CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) represents one of the most successful and promising efforts. Although replicating gene-diet interactions is a key step to augment scientific evidence, such interactions must be approached from a more biologically functional angle, integrating higher level interactions that include, genomic and nutritional information, epigenetics, microbiota, and behavioral factors[6] such as physical activity and chronotype. Current gaps in nutrigenomics research may lead to a suboptimal science and partial and fragmented results that delay translation of this science for the benefit of the public. Therefore, in order to move the field forward we need to go beyond genomics and to include in the equation other major "omics". Here we will briefly describe the progress reported around the most promising omics in nutrition research, these being epigenomics and metabolomics.

Epigenomics and Obesity

As stated above, fixed genomic variation explains only a small proportion of the risk of adiposity and epigenetics and epigenomics may help us to understand some of the current gaps in knowledge. Epigenetics refers to the study of mitotically and/or meiotically hereditable changes in gene expression that occur without changes in the DNA sequence. The difference between genetics and epigenetics can be compared to the difference between having all the letters of a text (the DNA sequence) and knowing how to space and punctuate them to provide meaningful sentences (epigenetic modifications). Epigenomics refers to the study of the complete set of epigenetic modifications in a cell or a tissue at a given time. It was Barker and Osmond, using epidemiological observations, who described some of the first links between the impact of environment on fetal development and the subsequent associations with age-related diseases

such as CVD. Results from other studies, such as those coming from the Dutch famine cohort, showed similar findings and provided more mechanistic evidence about the processes involved uncovering connections between epigenetics and environmental factors such as dietary intake.[7]

Work on the three main epigenetic marks and mechanisms (DNA methylation; chromatin organization by histone modifications; and noncoding RNAs) affecting obesity is growing fast and some of the progress is presented here. First, in relation to DNA methylation, this is a major epigenetic mark that involves the addition of a methyl group to a cytosine positioned next to a guanine nucleotide (CpGs) usually in regions with a high presence of CpG dinucleotides. Methylation usually results in the repression of gene expression. Most of the evidence linking epigenetics with obesity has relied on animal studies. In animal models, maternal diet alters offspring body composition, accompanied by epigenetic changes in metabolic control genes.[8]

In humans, and as reviewed by Norheim et al.[6] twin studies have shown that DNA methylation profiles were more divergent in older twins than in infant twin pairs, suggesting that environmental factors may influence the epigenome. Diet-induced weight loss for 8 weeks in obese men altered DNA methylation in peripheral blood mononuclear cells of specific genes. Changes in DNA-methylation levels among humans with metabolic diseases were associated with alterations in expression of genes involved in mitochondrial function, including PGC-1 . Reduced PGC-1 activity is linked with the pathogenesis of metabolic diseases as it increases metabolic and cardiovascular risk and precedes the development of Type 2 diabetes (T2D). Interestingly, whereas palmitate and oleate can acutely induce methylation of the PGC-1α promoter, exercise induces hypomethylation of PGC-1α in skeletal muscle. The hypomethylation of the PGC-1α promoter in response to exercise was paralleled with an increase in PGC-1α mRNA content.

Interestingly, the relation between maternal nutrition, methylation and offspring obesity has been shown quite convincingly in humans by Godfrey et al.[9] revealing some exciting and promising findings. These investigators used Sequenom MassARRAY to measure the methylation status of 68 CpGs 5' from five candidate genes in umbilical cord tissue DNA from healthy neonates. They related methylation status to maternal diet during pregnancy to child's adiposity at age 9 years. The initial findings revealed that retinoid X receptor-α (RXRA) and endothelial nitric oxide synthase (eNOS) methylation had independent associations with sex-adjusted childhood fat mass (P = 0.009 and P < 0.001, respectively) and %fat mass (P = 0.023 and P = 0.002, respectively). Regression analyses including sex and neonatal epigenetic marks explained > 25% of the variance in childhood adiposity. Higher methylation of RXRA, but not of eNOS was associated with lower maternal carbohydrate intake in early pregnancy, pre-

viously linked with higher neonatal adiposity in this population. These investigators sought replication in a second independent cohort, where they found that cord eNOS methylation showed no association with adiposity, but RXRA methylation showed similar associations with fat mass and %fat mass (both P = 0.002). Therefore, these findings support the notion, previously suggested by the Barker hypothesis, that a substantial component of metabolic disease risk has a prenatal developmental basis. Moreover, in addition to genetic testing, perinatal epigenetic analysis may have utility in identifying individual vulnerability to later obesity and metabolic disease.

In relation to histone modifications, the second epigenetic mechanism or mark, it is important to underscore that DNA in cells is packaged as chromatin in a "beads on a string" configuration. The fundamental unit of chromatin is the nucleosome, which consists of 146 bp of DNA wrapped around a histone octamer (made up of two copies of four core histones: H2A, H2B, H3, H4). The N-terminal histone is subjected to a variety of post-translational modifications that include acetylation, methylation, phosphorylation and ubiquination. Gene activation correlates with the hyperacetylation of histones H3 and H4, whereas hypoacetylation correlates with inactive chromatin[8]. The relation between this epigenetic mark and obesity has been less studied, bit some promising findings, in animal models, are already supporting the importance of this epigenetic mechanism in relation to obesity. This is the case of the work by Masuyama and Hiramatsu[10] who investigated in a mouse model the potential epigenetic mechanisms linking a maternal high-fat diet exposure in utero with metabolic syndrome in the offspring. For this purpose, they examined whether the offspring from dams exposed to a high-fat diet during pregnancy (OH mice) exhibited hypertension, insulin resistance, and hyperlipidemia along with epigenetic changes in the expression of adipocytokine, such as leptin and adiponectin. OH mice were significantly heavier than the offspring of dams exposed to a control diet during pregnancy (OC mice). OH mice exhibited higher blood pressure and worse glucose tolerance than the OC mice at 24 wk. Total triglyceride and leptin levels were significantly higher and the adiponectin level was significantly lower in OH compared with OC mice at 12 wk of age. This was associated with changes in leptin and adiponectin expression in white adipose tissue. There were lower acetylation and higher methylation levels of histone H3 at lysine 9 of the promoter of adiponectin in adipose tissues of OH mice at 2 wk of age as well as at 12 and 24 wk of age compared with OC mice. In contrast, methylation of histone 4 at lysine 20 in the leptin promoter was significantly higher in OH compared with OC mice. Thus, exposure to a high-fat diet in utero might cause a metabolic syndrome-like phenomenon through epigenetic modifications of adipocytokine, adiponectin, and leptin gene expression. The fact that these changes were observed in adipose tissue under-

scores the difficulty to carry similar studies in human and the need to rely on experimental models to advance our mechanistic knowledge.

Finally, another important and novel aspect relates to the study of noncoding RNAs or microRNAs (miRs) which are often classified to be part of epigenetics. miRs are small non-coding RNA molecules derived from hairpin precursors, usually between 20 and 30 nucleotides in length. They normally bind to the 3-UTR (3'-untranslated region) of their target mRNA through imperfect base pairing, leading to translation inhibition and/or mRNA degradation. Over 1000 miRs have been found in the human genome, and it has been estimated that they could regulate 74-92% of all protein encoding mRNAs. Considering the complex level of gene expression regulation conferred by miRs, it comes as no surprise that miRs are involved in processes associated with obesity, such as adipocyte differentiation, insulin action and fat metabolism.[6,8]

Our own work provides interesting connections between miRs, genetics, obesity and dietary modulation. This is the case of PLIN4, a member of the PAT family of lipid storage droplet (LSD) proteins.[11] We investigated the associations between seven SNPs at human PLIN4 with obesity related phenotypes. Samples consisted of subjects from two populations of European ancestry. We demonstrated association of one the SNPs (rs8887) with anthropometrics. Meta-analysis demonstrated significant interactions between the rs8887 minor allele with PUFA n3 modulating anthropometrics. rs884164 showed interaction with both n3 and n6 PUFA modulating anthropometric and lipid phenotypes. In silico analysis of the PLIN4 3'UTR sequence surrounding the rs8887 minor A allele predicted a seed site for the human miR-522, suggesting a functional mechanism. Our data showed that a PLIN4 3'UTR luciferase reporter carrying the A allele of rs8887 was reduced in response to miR-522 mimics compared to the G allele. These results suggest variation at the PLIN4 locus, and its interaction with PUFA as a modulator of obesity related phenotypes, acts in part through creation of a miR-522 regulatory site. This is the first example of a genetic variant that creates a miRNA binding site that influences obesity-related traits through a gene-diet interaction. Although further research is necessary, the findings suggest that miRNA activity is a possible target for dietary-based weight-loss therapies for obesity. Therefore we conducted a genome-wide survey for SNPs altering microRNA seed sites identifies functional candidates in GWAS. We focused on functional variants related with the binding of microRNAs (miR), we utilized SNP data, including newly released 1000 Genomes Project data to perform a genome-wide scan of SNPs that abrogate or create miR recognition element (MRE) seed sites (MRESS). We identified 2723 SNPs disrupting, and 22295 SNPs creating MRESSs. We determined that 87 of these MRESS SNPs were listed in GWAS association studies, or in strong LD with a GWAS SNP, and may represent the functional variants

of identified GWAS SNPs. Furthermore, 39 of these have evidence of co-expression of target mRNA and the predicted miR.[12] We also gathered previously published eQTL data supporting a functional role for four of these SNPs shown to associate with disease phenotypes. The potential of miRNA-based therapeutics targeting obesity is high and might lead to breakthroughs in the treatment of obesity.

Metabolomics

Another "omic" that is being deeply investigated in relation to obesity is Metabolomics, which refers to the types and concentrations of all metabolites in a biological sample. Biological metabolites are specific products of genomic, transcriptomic and proteomic processes of the host or external organisms. The characteristics and concentrations of all small molecules, provide a potential for measuring flux through all important biological pathways, and thereby allow detailed understanding of how metabolites interact with tissue components of functional importance[6]. Moreover, as indicated above, metabolomics can also be used to identify biomarkers for intake of specific nutrients and health. For example it has recently been shown in an meta-analysis that blood concentrations of carotenoids, a biomarkers for fruit and vegetable intake, are more strongly associated with reduced breast cancer risk than are carotenoids assessed by dietary questionnaires.[6]

Ideally, metabolomics should have the ability to provide a detailed snapshot of biological processes at any particular point in time. In nutritional research, such an approach may provide an opportunity to identify changes in metabolic pathways induced by nutrients or other life-style factors, to explore relationships between environmental factors, health and disease, and to discover novel biomarkers.[6] However, due to the diverse chemical nature of low-molecular metabolites, including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates, the global, untargeted analysis represent a tough challenge. Although development of analytical platforms enables separation, detection, characterization and quantification of a large number of metabolites from only minor amounts of biological samples, targeted metabolomics are most often used.

Targeted analysis, where a pre-defined set of metabolites are monitored, may be used for assessment of single nutrients or metabolites, determination of subsets of metabolites, including lipids, inflammatory markers or oxidative damage.[6] The profiling of lipids has developed into its own field of lipidomics, and as adversely altered lipid metabolism is an underlying factor in a number of human chronic diseases, lipidomics has become an important tool to identify potential novel therapeutic targets.[6]

Although metabolomics gain increased interest in nutrition research, there are still some major limiting

factors. In untargeted metabolomics, there are many unidentified metabolites. The high number of unknown signals makes it often difficult to extract meaningful information. Thus, there is a great need for publically available databases for the identification of metabolites.[6] Furthermore, the use of pattern-recognition techniques is crucial for exploring novel molecules that may serve as biomarkers. Moreover, the data sets based on metabolomics are usually huge and multi-dimensional. The metabolomics data should be compiled along with data on transcriptomics and proteomics, supporting more extensive use of bioinformatics including multivariate analyses.[6]

In the specific case of obesity, there is a great interest in applying metabolomics to examine alterations in the metabolic profile according to weight gain/obesity and identify metabolomic signatures.[13] Using targeted serum metabolomics of 163 metabolites, 12 of them were found significantly related to obesity. Among those, glycine, glutamine and glycero-phosphatidylcholine 42:0 (PCaa 42:0) serum concentrations were higher, whereas PCaa 32:0, PCaa 32:1, and PCaa 40:5 were decreased in obese compared with lean individuals. Likewise, using obese and lean mice fed on high fat or normal diets it was found that liver and serum metabolites analyzed using MS with partial least-squares-discriminant analysis (PLS-DA) of were able to clearly discriminate between obese and lean groups and major metabolites contributing to the discrimination were assigned as lipid metabolites, lipid metabolism intermediates, amino acids, acidic compounds, monosaccharides and serotonin. A high-fat diet increased lipid metabolites, but decreased lipid metabolism intermediates, indicating that abnormal lipid and energy metabolism induced by a high-fat diet resulted in fat accumulation via decreased b-oxidation. It revealed that the levels of many metabolites, including serotonin, betaine, pipecolic acid and uric acid, were positively or negatively related to obesity-associated diseases. These metabolites can be used to better understand obesity and related diseases induced by a hyperlipidic diet. The differences in metabolomic profiling were also investigated between overweight/obese and normal-weight men.[14] Three lyso-phosphatidylcholine (lysoPC) were identified as potential plasma markers and confirmed eight known metabolites for overweight/obesity men. Especially, overweight/obese subjects showed higher levels of lysoPC C14:0 and lysoPC C18:0 and lower levels of lysoPC C18:1 than lean subjects. Results confirmed abnormal metabolism of two branched-chain amino acids, two aromatic amino acids, and fatty acid synthesis and oxidation in overweight/obese men. Furthermore, the level changes of these metabolites can be used to assess the risk of obesity and the therapeutic effect of obesity management.

The growing problem of obesity at younger ages has attracted also the interest of metabolomics as investigation of serum metabolite concentrations in obese children might give new insights into biological mecha-nisms associated with childhood obesity. In this regard, serum samples of obese children were analyzed using a MS-based metabolomics approach targeting 163 metabolites.[15] Fourteen altered metabolites were significantly altered in obese children. The identified metabolite markers are indicative of oxidative stress and of changes in sphingomyelin metabolism, in b-oxidation, and in pathways associated with energy expenditure and might be considered as potential biomarkers on the biological mechanisms behind obesity. High-fat diets (HFD) and high carbohydrate diets (HCD)-induced obesity through different pathways, but the metabolic differences between these diets are not fully understood. In summary, metabolomics approaches have the potential to bring new tools to fight obesity. Among other things, it has the potential to generate novel non-invasive diagnostic tests, based on biomarkers of metabolic dysregulation, which are simple and cost-effective.

Metabolic flexibility, chronobiology and obesity

Some of the health disparities reported above relate to the impaired adaptation of an ancestral genome to a new environment, similar to what in the sociological field is known as lack of acculturation. The fact is both, in the short term (i.e., day to day variation in food intake or physical activity) and over the long term (i.e., human migrations) we are exposed to a constantly changing environment to which our physiology has to adapt. This adaptive capacity requires a metabolic flexibility that is key to maintaining overall homeostasis and thus, to a healthy life in old age. An individual's capacity to respond to environmental challenges has a strong genetic component, and our long-term translational objective aims to uncover the optimal environment (i.e., diet, physical activity, biorhythms) that will maintain sufficient metabolic flexibility for an individual's genetic architecture. In this regard, the circadian clock governs a large array of such physiological functions that maintain our metabolic flexibility, and current studies suggest that interruption of the circadian system may contribute to metabolic syndrome (MetS) and obesity-related complications.[16] Moreover, metabolic processes are aligned with the periodic environmental changes and behavioral cycles, such as the sleep/wake and fasting/feeding cycles. Thus, a precise estimation of an individual's internal body time may be a key component to facilitate metabolic homeostasis. Relative to dietary intervention, the timing of food intake may contribute to weight gain and metabolic disease because energy homeostasis and circadian rhythms are molecularly and physiologically interconnected.[16] Consequently, altering the timing of food intake can alleviate or exacerbate diet-induced obesity.

The role of this circadian system, including such central components as CLOCK, BMAL1, PER2 and CRY1/CRY2, in human obesity has been demonstrated by clock genes variants in association with obesity and

metabolomic outcomes.[17-19] Moreover, although the circadian systems are controlled by the master pacemaker located in the suprachiasmatic nucleus (SCN), other endogenous oscillators are found essentially in every cell in the body, and mounting evidence suggests that these local oscillators regulate critical functions in most organs. In this regard, our observation that the basal expression of clock genes in human adipose tissue is associated with abdominal fat content and cardiovascular risk factors is significant.[20-24]

Recently, miRs (microRNAs) have emerged as significant players in circadian clock timing, thus implicating clock-controlled miRs and/or miRs controlling clock genes as contributors to disorders related to the circadian system. Hence, miRs present novel therapeutic targets for disorders of the circadian clock. Examples include miRs both regulated by the circadian system and regulating circadian period length and clock resetting,[25] miRs that show rhythmic fluctuations in circulating levels, and miRs under circadian control with roles in hepatic lipid metabolism. Chronodisruption, a dysregulation of the finely tuned central/peripheral synchrony, impairs the response to time-based signals, and the resulting disruption in peripheral clocks (e.g., skeletal muscle, adipose) impairs homeostasis for energy, glucose, and immunity leading to obesity, MetS and systemic inflammation. Therefore, understanding of chronodisruption, its molecular basis, its responsiveness to timing and quality of the diet, and potential therapies provides an exciting entry point for unraveling and ameliorating the diseases of unhealthy aging. To this end, we have been emphasizing obesity and its effects on age-related diseases; however, another hallmark of unhealthy aging is sarcopenia, in which skeletal muscle loss is accompanied by muscle weakness and adipose accumulation. We propose that chronodisruption may medicate sarcopenia. Suggestively, chronodisrupted animals exhibit a premature aging phenotype, complete with sarcopenia, systemic inflammation, and circadian behavioral disturbance and loss of the characteristically rhythmic gene expression patterns in peripheral circadian targets. Although chronodisruption appears to mediate unhealthy aging, mechanistic understanding of central-peripheral clock cross-talk and its dysregulation is limited, especially in humans. Equally critical, but unidentified, are remedies that will rescue the chronodisrupted phenotype. Light and feeding regimes synchronize and reset the clocks to some extent, but the role of physical activity remains unexplored. Through its targeting of peripheral clocks in relevant tissues (i.e., muscle and adipose), the feedback provided by physical activity potentiates strengthening of homeostatic circadian regulation.

Building networks

In the pursuit of health, considerable effort has been placed in defining and cataloging diseases and we have made significant advances. However, the ability to precisely define health has met with less success. "Optimal health", from a metabolic and physiological point of view, can be defined as an organism's ability to maintain or regain homeostasis in an ever-changing environment, and especially in response to a wide range of stressors ("buffering capacity"). For an organism to remain stable (healthy) even in the presence of unpredictable changes, it must be able to adjust molecular parameters within cells and organs to match the situation. This can be achieved by appropriate changes in protein functions, either by direct modifications or indirectly via gene expression. Thus, the organism is in fact continuously changing its phenotype (defined as the observable properties of an organism as produced by the interaction of the genotype and the environment). The efficiency and timeliness of these phenotypic adjustments to new situations will determine its health and healthy aging. The individual's capacity to adapt in time and location to alterations in external conditions is called "phenotypic flexibility".

As indicated above, nutrition plays a key role in promoting health and healthy aging. In mammals, downregulation of the activity of several nutrient-sensing pathways (e.g. insulin/IGF-1/mTOR) induced by dietary restriction, mutations or chemical inhibitors promotes an increase in health status, lifespan, and preservation of biological functions in more "youthful-like" states. Dietary restricted animals are also more metabolically flexible and resistant to many types of stresses (e.g. surgery, radiation, acute inflammation, exposure to heat, and oxidative stress) as compared to those fed ad-libitum. A prime feature of phenotypic flexibility is that alterations in the availability of the different energy substrates and metabolites can be resolved by the various metabolic compartments involved (plasma, muscle cell, adipocyte, liver) in a time- and magnitude-dependent manner. Phenotypic flexibility thus is a 3-dimensional (time, location and extent) measure for all processes underlying metabolic adaptation. "Metabolic flexibility" is one of the important aspects of phenotypic flexibility and refers to "the capacity of the organism to adapt fuel oxidation to fuel availability." Most tissues undergo rapid changes in the flow of nutrients and metabolites between the fasting and fed states. Major regulators in this rapid adaptation to catabolic and anabolic states are insulin and insulin-like growth factors (IGF) with reciprocal changes in circulating levels of glucagon and catecholamines and glucocorticoids.

Building upon metabolic flexibility and its foundation in numerous inter-related processes, we view obesity and its complications (e.g., CVD and T2D) as multifaceted, involving numerous organs, cell types, biological pathways as well as genetic variation, metabolite levels, circadian status and gene expression activity. Thus, in a manner that takes its cues from cancer genomics, we need to implement state of the art computational biology tools to uncover high-level interactions between genetic, epigenetic and environmental factors responsible for maintaining the balance between health and

disease. Current and future research needs to capitalize on the use of data generated using high-throughput omic techniques (genomics, epigenomics, transcriptomics, and metabolomics), both in ongoing studies of free-living ethnically diverse populations and in the metabolic ward (intervention studies). The primary focus of this effort is to identify new genomic, epigenomic and metabolomic biomarkers to apply individually or in concert as indicators of metabolic health as well as for early detection of metabolic disruption. These indicators will act as predictors of the health consequences derived from an individual's interaction with environmental challenges with special emphasis on cardiovascular health and obesity prevention especially in an aging population. We need to build interaction networks of relevant phenotypes, aging and nutrition in which diet, health/disease biomarkers, genetic variants, biological processes and gene activity are linked in a manner that permits the generation of new, testable hypotheses.

However, these networks will not be complete without the inclusion of the microbiota, an emerging player in obesity. The human gastrointestinal tract is estimated to host up to 10^{14} microorganisms, tenfold the number of human cells, predominately composed of bacteria. Together they make up the gut microbiota, which during normal circumstances live in a commensal or mutualistic relationship with their host. Although humans can live with a bacteria-free intestine the microbes are crucial for human health. For example, the gut microbiota metabolizes indigestible carbohydrates to valuable short-chain FAs; synthesize certain vitamins; degrade oxalates and is essential in recirculation of bile acids.[6]

Traditional *in vitro* cultivation has limited the research on gastrointestinal bacteria because their normal growth environment is complex and difficult to imitate. Thus, the introduction of gene-sequencing has markedly extended the knowledge about their species diversity. Between 500 and 1000 different species occupy a single human gut, whereas the total microbiome in humans include between 10,000 and 40,000 species. However, the majority of microbes within the digestive tract appear to include less than 100 different species.

Development of next-generation sequencing have permitted mapping of the microbial metagenome in humans and 3.3 million non-redundant microbial genes have been annotated with 536,000 prevalent unique genes detected in each individual. Among these are genes involved in the biosynthesis of short-chain fatty acids, amino acids and certain vitamins, which all are molecules suggested to be provided by bacteria to humans.[6]

How nutritional habits interfere with the intestinal microbiota is far from understood. It was traditionally believed that the microbe composition was relatively unchangeable, but DNA sequence analyses have challenged this view. Studies have clearly shown that the composition of gut microbiota adapt during changes from breast milk to solid food and when altering the composition of ingested macromolecules.[26] In terms of obesity, certain bacteria, specifically the archaeon Methanobrevibacter smithii, have enhanced ability to metabolize dietary substrate, thereby increasing host energy intake and weight gain. With weight loss, there is a decrease in the ratio of Firmicutes to Bacteroidetes phyla and the research in this area is booming to identify approaches with pre-, probiotics or more novel approaches, to tilt the balance of the gut microbiota towards a less obesogenic mix.

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