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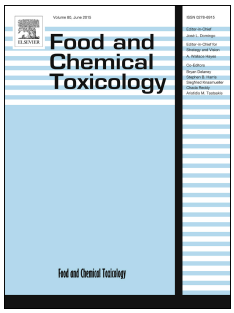

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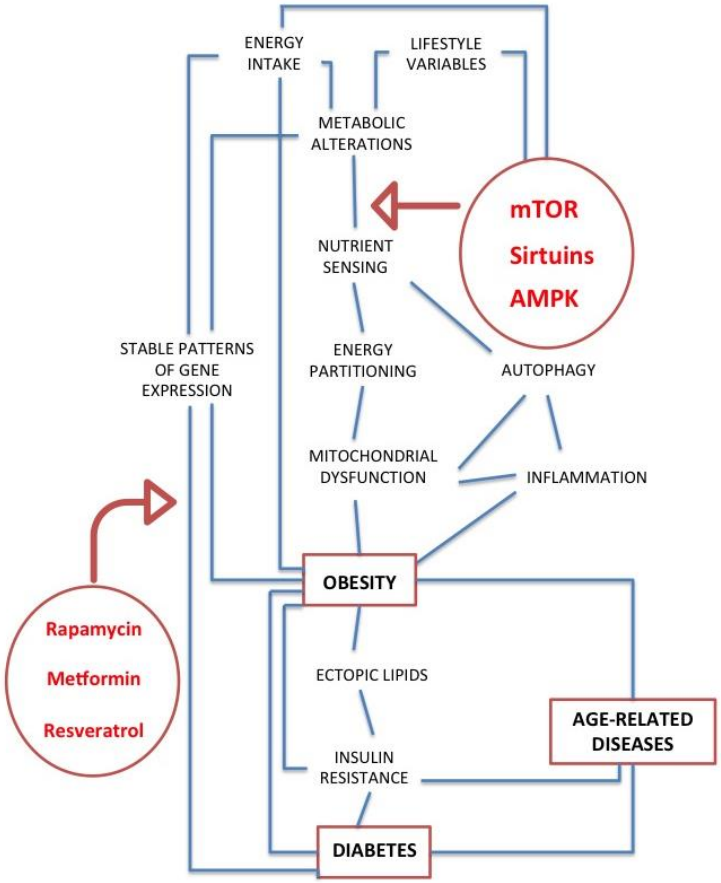
Intrinsic epigenetic changes



Extrinsic epigenetic changes

Epigenetics and nutrition-related epidemics of metabolic diseases: Current perspectives and challenges

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ABSTRACT

We live in a world fascinated by the relationship between disease and nutritional disequilibrium. The subtle and slow effects of chronic nutrient toxicity are a major public health concern. Since food is potentially important for the development of “metabolic memory”, there is a need for more information on the type of nutrients causing adverse or toxic effects. We now know that metabolic alterations produced by excessive intake of some nutrients, drugs and chemicals directly impact epigenetic regulation. We envision that understanding how metabolic pathways are coordinated by environmental and genetic factors will provide novel insights for the treatment of metabolic diseases. New methods will enable the assembly and analysis of large sets of complex molecular and clinical data for understanding how inflammation and mitochondria affect bioenergetics, epigenetics and health. Collectively, the observations we highlight indicate that energy utilization and disease are intimately connected by epigenetics. The challenge is to incorporate metabolo-epigenetic data in better interpretations of disease, to expedite therapeutic targeting of key pathways linking nutritional toxicity and metabolism. An additional concern is that changes in the parental phenotype are detectable in the methylome of subsequent offspring. The effect might create a menace to future generations and preconceptional considerations.

Key words: DNA methylation; diabetes; epigenetics; metabolomics; nutrient toxicity; obesity

1. Introduction

The rise in the prevalence of obesity and diabetes is commonly attributed to changes in dietary patterns, but this view does not explain their epidemic nature and the observed contribution of mitochondrial dysfunction, oxidative stress, inflammation, metabolism and epigenetics. There is a clear need for systematic efforts, methodology and mechanistic support to repurpose data to the concept of nutrient toxicity. The harmful excess of nutrients is not limited to short-term effects and public health concerns are currently focused on the effect of chronic excessive intake (Kristanc and Kreft, 2016). Fatness and the lack of fitness are only part of the problem; the association of obesity and diabetes ("diabesity") with non-communicable diseases such as cardiovascular disease and cancer is also relevant (Camps et al., 2016). Indeed, obesity is not only a "problem" or a "condition", but also a chronic disease. Contrary to what the "weight-loss industry" suggests, permanent weight loss is outwith the reach of the majority of people with obesity. The word here is "permanent". Humans are the only species that become overweight and remain overweight. Losing weight is easy but keeping it off is not. Indeed, the 5-year relapse rate for weight re-gain is consistently greater than 90% and those who do experience (or will experience) the health consequences of obesity deserve better efforts than being simply told to "eat less".

There is a non-genetic transmission of obesity and insulin resistance (IR) (Huypens et al., 2016). It is appealing to consider the hypothesis that a nutrient-associated spillover of energy-related metabolites and inflammatory products from affected cells into circulation provides signaling molecules that regulate gene expression through epigenetic mechanisms. Crosstalk between epigenetic

signals and cellular metabolism in chromatin would represent a sensor and a mechanism to convert metabolic changes into stable patterns of altered gene expression (Katada et al., 2012). But how is gene expression reprogrammed in response to metabolic stimuli?

In addition to their anabolic and catabolic functions, metabolites influence many cellular processes including cell migration and differentiation, and they can interact directly with transcription factors and modulate transmembrane ion channels (Cai et al., 2008; He et al., 2004; Martinez-Outschoorn et al., 2016; Tannahill et al., 2013). These actions are triggered by specific metabolomic patterns that initiate transcriptional regulation in cells (Takahashi and Yamanaka, 2006). Genes do not remain automatically activated or depressed if the metabolic event is not persistent, and the putative regulators need to be continuously present to maintain the state of expression (i.e., specificity and memory are both necessary). Addressing basic questions such as which epigenetic factors are involved in energy metabolism and whether epigenetic mechanisms are causally linked to changes in metabolomic phenotypes, may lead to novel therapeutic opportunities. Future research combining data from several “omics” platforms is therapeutically attractive because diabetes and obesity are potentially reversible through nutritional and/or surgical interventions. These questions require a comprehensive, systems-level understanding of disease mechanisms and molecular alterations. For such a comprehensive topic we found narrative review best suited to summarize primary studies and to draw holistic interpretations contributed by our own experience and existing models. The information was retrieved through PubMed

without restrictions using combined search terms described in **Fig. 1** and explicit criteria for inclusion.

2. Regulating food intake and health

There is a widespread claim that nutritionists are forever changing their advice. This is conceivably due to the majority of data relying on "associations" because of the obvious shortcomings of observational studies and free-living experimental trials (Gorder et al., 1986; Masana et al., 1991; Menotti, 1983). Studies linking food and health should be seen as preliminary, dealing with uncertainties, unknown confounding factors and without proving the whole chain of events. Moreover, presumably through mechanisms evolved to ensure adaptability, the clinical application of dietary changes tend to converge towards non-significance in the long-term and compliance is low (Bravata et al., 2003; Foster et al., 2010; Gardner et al., 2007; Sacks et al., 2009; Shai et al., 2008). These considerations are relevant because the abrupt increase in the prevalence of obesity and type 2 diabetes mellitus (T2DM) is now challenging former hypotheses. The main concerns are the frequent coexistence of both conditions and the undisputed associations with atherosclerosis and cancer (NCD Risk Factor Collaboration, 2016; Twig et al., 2016). These are all age-related diseases entangled with diet and linked to IR or obscure relationships (Joven et al., 2007).

Hippocrates said, "*persons who are naturally very fat are more apt to die suddenly than those who are slender*" (Aphorisms 2:44). This concept and the *Discourses on the sober life* (1558-1562) by Alvise Cornaro were highly influential for centuries to endorse caloric restriction as a means to achieve

longevity and health (Darby, 1990; Howell, 1987). Clearly, these observations rebut the notion that obesity is a recent phenomenon. Indeed, it probably occurs in waves associated with affluence. A correlation between reduction of food intake and extension of lifespan has been demonstrated in a wide range of organisms (Fontana and Partridge, 2015), and it is accepted that excessive food intake, when linked with sedentary behavior, may result in obesity, T2DM and other non-communicable diseases (Hamilton et al., 2007). However, it is not clear whether obesity is truly preventable because there are neither options that work well for most people nor real success in prevention. The question of whether obesity will reverse the life-span gains made over decades is an emerging issue.

2.1. Nutrient sensing and the distribution of energy

Changes in food intake may alter metabolic strategies to reset the distribution of energy into different tasks. These changes may or may not be relevant to disease, but, when excessive and continual, they may become toxic and trigger several deleterious events, including chronic inflammatory response, oxidative stress, mitochondrial dysfunction, adiposity, IR in skeletal muscle, and decreased insulin production by pancreatic β cells (Camps et al., 2016).

The perceived links and interactions (**Fig. 1**) can be interpreted as evidence that a growing number of chronic diseases are associated with IR in a vicious cycle. As tissues become unresponsive to insulin, more insulin is secreted by the pancreas and tissues grow ever more resistant. Determining the precise mechanisms of IR is complex because insulin is involved in the most fundamental processes of biology (Fitzgibbons and Czech, 2016).

Investigating the mechanisms of nutrient sensing is important to comprehend how food and metabolism are coupled to disease. In this context, several metabolic sensors have been well characterized. For example, the activated mechanistic target of rapamycin (mTOR) regulates events that modulate protein synthesis, insulin signaling, autophagic flux and mitochondrial function (Albert, 2015). Sirtuins are also controlled by nutrient availability and their activities regulate oxidative phosphorylation, fatty acid oxidation and mitochondrial oxidant production (Barger et al., 2015). Finally, adenosine monophosphate (AMP) activated protein kinase (AMPK) is a critical link between nutrients and health and regulates metabolic pathways that increase energy supplies and reduce energy demand (Hardie et al., 2016). These sensors have been successfully targeted pharmacologically. Notably, metformin has been used for decades to activate AMPK and, if epidemiological evidence is confirmed, has saved more lives from cancer than any other drug in history (Menendez and Joven, 2014). In the overall process of nutrient sensing, it is important to highlight the protective role of autophagy, the controlled degradation and recycling of cellular components. In particular, the specific autophagic targeting of dysfunctional mitochondria (mitophagy) eliminates oxidative stress and mitochondrial damage in obesity and T2DM. Mitophagy appears to be a crucial cellular process for the conversion of functionally mature mitochondria to an immature state and vice versa during reprogramming and differentiation, respectively (Vazquez-Martin et al., 2016). Autophagy also mediates exercise-induced increases in muscle glucose uptake, protects β cells against endoplasmic reticulum stress and promotes adipocyte differentiation. Conversely, decreased autophagic activity is implicated in the

progression of obesity to T2DM (Barlow, 2015; Sarparanta et al., 2016). Given that sirtuins, mTOR and AMPK all regulate autophagy and autophagy activators have demonstrable effects on age-related diseases, the search for activating compounds is an emerging field of investigation (Hubbard and Sinclair, 2014; Imai and Guarente, 2014; Kasznicki et al., 2014; Menendez and Joven, 2014; Menendez et al., 2014).

2.2. Insulin resistance: a multifactorial condition

It is generally accepted that IR is associated with overnutrition and the systemic response of poorly known metabolic feedback loops, but its role in causing disease might be controversial. For some investigators, proposed factors causing IR would work through one or more mechanisms sequentially triggered by excessive food intake: increased inflammation, changes in lipid metabolism, and changes in the gastrointestinal microbiota (Johnson and Olefsky, 2013). To pursue this causal chain may be scientifically sound and the amount of basic and preclinical knowledge supporting this hypothesis is compelling; however, this approach is clinically ineffective.

Pharmacologic therapy based on targets relevant to inflammation-induced IR has marked effects in rodents but has been disappointing in humans. This has been shown for tumor necrosis factor-α blocking agents (Bernstein et al., 2006; Solomon et al., 2011; Stanley et al., 2011), IL-1β inhibitors (van Asseldonk et al., 2011) and aspirin (Goldfine et al., 2010; Raghavan et al., 2014). More targeted anti-inflammatory approaches may improve efficacy in the future, but the role of tissue inflammation in causing IR remains speculative. For example, the usefulness of ligands for peroxisome

proliferator-activated receptors (PPARs) has been curtailed because of potential toxicities, even before the precise mechanisms of action are known (Rull et al., 2014). Lipid accumulation in multiple tissues and the associated metabolic disturbances might be considered a biological marker, but a consequence rather than a contributing factor to IR (Calvo et al., 2015). Similarly, in mice, the gastrointestinal microbiota influences energy metabolism and systemic inflammation (Blumberg and Powrie, 2012; Burcelin et al., 2012; Henao-Mejia et al., 2012), and may produce bioactive metabolites, especially short-chain fatty acids, acetate and bile acids derivatives (Beltran-Debon et al., 2015; Kau et al., 2011; Nicholson et al., 2012). Data from experimental models point to the potential of microbiota to modulate obesity and insulin sensitivity; however, the same data fail to consider that humans live in non-sterile conditions, are genetically heterogeneous, consume a range of different diets and have microbiota that is frequently perturbed by the administration of antibiotics.

Moreover, clinical measurements of insulin sensitivity are challenging and considerable variation exists in healthy individuals and in patients. Methodological flaws are not discarded because laboratory procedures do not measure how individual tissues respond to insulin and most studies are performed with the patient fasting. These measurements are also limited by the observation that IR changes dramatically over the course of a day, from day to day, and in response to exercise and the quality and quantity of food intake, among other factors (Zaccardi et al., 2016). The use of indices combining glucose and insulin levels to predict insulin sensitivity may represent a simplistic assessment of actual glucose metabolism. Some investigators claim a significant (>10%) false-negative rate in assessing IR compared with glucose

tolerance testing. These indices can certainly be used in clinical studies with a secondary interest in glucose metabolism, but considering and balancing possible inaccuracies (Lee et al., 2008; Martinez-Hervas et al., 2011; Pisprasert et al., 2013). Specifically, genetic influence, physical fitness and weight are recognized confounding factors that only partially explain why a significant proportion of severely obese patients are insulin sensitive. Thus, if obesity is not the cause of IR, the search for underlying factor(s) causing both obesity and IR needs to continue.

2.3. Mitochondria are not only providers of energy, but also signaling units

The detrimental effects of IR are associated with liver disturbances, and the deposition of lipids into non-adipose tissues interferes with insulin signaling. Several findings suggest that mitochondrial dysfunction is a cause rather than a consequence of IR. The distinction is important because diabetes and obesity are strong predictors of non-alcoholic fatty liver disease, which is characterized by damaged mitochondria and the progressive inhibition of fatty acid oxidation. Several mechanisms apparently converge to modulate the differential response of energetic and biosynthetic intermediates. The overall picture is unclear, but mitochondrial energetic efficiency, epigenetic signals and nutrient-sensing pathways are necessarily combined (Rull et al., 2009; Fontana and Partridge, 2015) to explain increased glucose oxidation, decreased glucose formation, mitochondrial dysfunction and the accumulation of metabolites that disturb glucose transport activity (Finkel et al., 2015).

In this context, the consideration of obesity and diabetes as likely mitochondrial diseases is clinically relevant. For instance, mitochondrial activity, inflammation and the infiltration of macrophages influence both the extent of atherosclerosis and the pathogenesis of cancer. All of these diseases involve mitochondria and are characterized by a decline in metabolic homeostasis and gene deregulation. This association provides grounds to justify that the detection of an unhealthy metabolic status requires novel, and possibly crucial, testable therapeutic approaches, especially those: 1) modulating the ability of cells to alter their metabolism to different energy requests, 2) therapeutically targeting glycolysis, and 3) directly modulating mitochondrial activity (Suliman and Piantadosi, 2016; Zaccardi et al., 2016a).

In the regulation of metabolism and energy production, mitochondria receive information from other parts of the cell and relay information via retrograde signaling molecules that are not of mitochondrial origin; in particular, reactive oxygen species (ROS), Ca^{2+}, and cytochrome C (Goodwin et al., 2009; Houtkooper et al., 2011; Sethe et al., 2006). Some investigators consider that mitochondrial ROS have evolved as a key communication method between the mitochondria and the cell to regulate homeostasis and normal cellular function (Sena and Chandel, 2012). Moreover, new findings suggest that mitochondria regulate metabolic homeostasis at the cellular and organismal level via peptides encoded within their genome. The mitochondrial transcriptome is a highly complex system and several mitochondria-derived peptides have been discovered. One such peptide, humanin, regulates critical processes such as aging, inflammation, and stress resistance (Guo et al., 2003). A second peptide, derived from mitochondrial 12S rRNA (MOTS-c), is involved in regulating

metabolic homeostasis (Lee et al., 2015). Mitochondria modulate carbohydrate metabolism (Woo and Shadel, 2011) and MOTS-c is proposed as a key endocrine signal that systemically regulates in vivo glucose metabolism and muscle insulin action. MOTS-c has physiological similarities to the anti-diabetic metformin in terms of regulating glucose utilization, mitochondrial and fatty acid metabolism, and body weight (Ferguson et al., 2007) by targeting the folate cycle and one-carbon metabolism (Corominas-Faja et al., 2012; Ducker et al., 2016) and signaling via AMPK (Shaw, 2013). These data support an active role for mitochondria in the regulation of metabolism and weight homeostasis. Moreover, the significance of these peptides in the regulation of obesity, diabetes, exercise, and longevity represents a new frontier in mitochondrial signaling.

The appreciation of mitochondria as signaling organelles is also illustrated by very recent findings (Morton et al., 2016). The authors reasoned counterintuitively that because a substantial and stable proportion of individuals remain non-obese despite modern affluence, there might be genetic mechanisms for resistance to obesity and diabetes or genes that contribute to healthy low adiposity. To address this, the authors used a polygenic lean mouse line generated through selection for low adiposity over 60 generations (Morton et al., 2005) to identify mitochondrial thiosulfate sulfurtransferase as a beneficial regulator of adipocyte mitochondrial function that may have therapeutic significance for individuals with T2DM.

3. Metabolism and epigenetics: insights for an alternative working hypothesis

Systems biology encompasses many different approaches to systematically identify, analyze, control, and design metabolic systems. The convergence of data from these methodologies indicates that metabolites, which are directly related to the visible phenotype of biological systems (Novere, 2015), are organized in genetic- and signaling-regulated metabolic networks.

The relationship between epigenetics and metabolomics may provide immediate clinical applications; however, the extent to which epigenetic information is transmitted and whether the metabolic environment modulates this information are unanswered questions. Exploring how metabolic pathways are coordinated in diabesity might clarify the impact of inflammation, metabolic factors and nutrient excess on epigenetic pathways affecting genomic regulation (Finkel, 2015; Hernandez-Aguilera et al., 2013; Katada et al., 2012). Key metabolites can accumulate in the plasma over time and if this is maintained specific metabolites have the capacity to regulate both epigenetic status and energy supply (Riera-Borrull et al., 2016; Rodriguez-Gallego et al., 2015; Menendez et al., 2016). Precisely how the effects of inflammation and mitochondrial dysfunction collectively work is unknown, but future investigations on chronic diseases should consider the consequence of excessive food intake for the balance of associated cellular pathways and biological mechanisms (Horng and Hotamisligil, 2011; Locasale, 2013; **Fig. 2**). The metabolite-driven changes in epigenetic regulation are mechanistically attractive and are supported by recent concepts that have revolutionized our understanding of chromatin-based epigenetic mechanisms and the relationship with gene regulation in the pathogenesis of human diseases. Nevertheless, interpreting

the biological context and integrating data from metabolite measurements in clinical, epigenetic-guided studies is not an easy task (Dumas, 2012). The challenge is to provide biological explanations in humans before and after therapeutic intervention.

3.1. Intermediates of metabolism influence chromatin structure: mechanisms of epigenetic inheritance

Epigenetic mechanisms control chromatin structure through posttranslational modifications, histone variants, RNA interference and DNA methylation (**Fig. 3**). Several interacting components of chromatin regulation, including enzyme kinases, acetyltransferases and methyltransferases, use cellular metabolites as sources of phosphate, acetyl or methyl groups, respectively. It is conceivable that these enzymes may interpret the metabolic state of a specific cell, but the level of a metabolite is unlikely to be the only determinant of enzymatic activity. Information on intracellular concentration and the dynamic changes in affinity or competition is sparse (Katoh et al., 2011), and diffusion-controlled reactions are unlikely in the viscous medium of nuclei. The concentration of proteins and DNA may reach 200 g/L and the sensitivity to metabolic alterations is not equally distributed in chromatin regions, favoring the heterogeneous occurrence of multiprotein complexes channeling reactions (Wei et al., 2011).

Changes in nutrition can impact gene expression patterns and memory of former metabolic disturbances may be involved in the progression of obesity and metabolic disease as shown in epidemiological studies examining the offspring of extreme nutritional deprivation during the periconceptual period or

during fetal development. This has been extensively studied in cohorts suffering the Dutch Winter Hunger in 1944 (Kaati et al., 2002; Painter et al., 2005; Heijmans etal., 2008). DNA methylation signatures apparently link prenatal famine exposure to growth and metabolism and there are evidences suggesting that epigenetic modulation of pathways by prenatal malnutrition may promote an adverse metabolic phenotype in later life (Tobi et al., 2014). Similar data come from studies of offspring born during the severe Chinese famine in 1958-1961 (Li et al., 2010) but the negative findings obtained during the Siege of Leningrad suggest caution in comparing retrospective analysis with different exposure windows (Stanner et al., 1997). As discussed below (section 4.1) short-term high fat overfeeding may suggest transient epigenetic regulation in humans (Jacobsen et al., 2012).

The mechanisms directing the inheritance of these diseases are unknown but epigenetics is an attractive candidate in animal models. For example, studies in mice carrying the viable yellow allele of agouti (Avy) indicate that a specific mammalian gene can be subjected to germ-line epigenetic change (Cropley et al., 2006). The ablation of key epigenetic enzymes in mice also mimics the heritable effects of metabolic disturbances: mice with an inactive allele of the gene encoding the histone demethylase KDM3a become obese in adulthood and have increased levels of circulating lipids (Tateishi et al., 2009). A defect in the genes encoding metabolic enzymes also directly influences the enzymatic function of epigenetic regulators in cells with major metabolic alterations (Cuyas et al., 2015; Menendez et al., 2016a). Some interesting questions arise as to how plastic is the genome to dietary changes, what magnitude of metabolic stimuli is required to switch between

metabolic states, and whether these metabolic-triggered epigenetic changes are reversible.

During the germline cycle of development, the genetic material is replicated in each round of cell division. Information not replicated in the DNA sequence—epigenetic information—is lost in each generation. At least for epigenetic marks caused by DNA methylation, the information is not completely erased during germline development and may remain in the promoters of protein-coding genes resistant to demethylation (Tang et al., 2015). The evolution of mechanisms conferring long-term epigenetic memory, and that feedback between different epigenetic mechanisms contribute to long-term inheritance, are plausible concepts (Klosin and Lehner, 2016); that is, each epigenetic mechanism alone is unlikely to be used to transmit information reliably for more than one generation. Small RNAs are potential carriers of epigenetic information in animal germlines and their levels vary depending upon parental exposure to high-fat diets (Grandjean et al., 2015). Overexpression of histone demethylases during spermatogenesis alters histone modifications in sperm that impair offspring health transgenerationally (Siklenka et al., 2015). Indeed, the repression of repetitive DNA and transposons is likely the main function of DNA methylation and a barrier for the transmission of information. However, at least in mammals, this mechanism is insufficient and may also influence the expression of neighboring genes through generations (Blewitt et al., 2006). In flies, the specificity in the transcriptional response to low glucose diet is detected in the next generation before the heterochromatin resets. If demonstrated in humans, this mechanism could explain the inheritance of short-term epigenetic effects. In fact, there is evidence in mice and humans that a

high glucose paternal diet can trigger obesity in offspring through deregulation of paternally inherited heterochromatin (Öst et al., 2014). Therefore, germline-transmitted mechanisms are conceivable and future research addressing if and how the diet-induced metabolic perturbations of obesity and diabetes can alter epigenetic information is warranted.

3.2. Energy metabolism and one-carbon metabolism: a targeted metabolomic approach

Several metabolites generated by mitochondrial respiration are implicated in stochastic chromatin remodeling. This is consistent with studies indicating that glucose and body weight homeostasis require an efficient management of energy. For instance, citrate can modulate the global levels of histone acetylation, and other metabolites are obligatory co-substrates (α-ketoglutarate) or potent inhibitors (succinate) of relevant mitochondrial enzymes (Benayoun et al., 2015; Chin et al., 2014; Mentch et al., 2015). Excessive calorie intake leads to mitochondria fragmentation. Mitochondrial dynamics is abnormal in T2DM and the prevention of excessive mitochondrial division ameliorates insulin function. In the obese setting, changes in mitochondrial dynamics control appetite- and diet-regulated signaling pathways in neurons (Roy et al., 2015). Additionally, DNA methylation influences the expression of genes affecting energy homeostasis and is associated with an imbalance in mitochondrial dynamics and IR (Gut and Verdin, 2013).

In contrast to the genome, which remains unchanged in most cells, the combination of all chromatin modifications of a given cell type directs a unique gene expression pattern that is shaped by nutrition. Energy metabolism is

important but distinguishing one-carbon metabolism is essential to understand the methylation of nucleic acids (Barth and Imhof, 2010). Accurate measurements of implicated metabolites require targeted metabolomics (**Fig. 4**) to establish the direct effect of one-carbon (and energy) metabolism on the output of a defined methylated state (Mentch et al., 2015).

Methylation of cytosine is the predominant epigenetic modification of DNA in vertebrates and DNA methylation inhibits the binding of transcription factors or recruits proteins with repressive properties (Tate and Bird, 1993; Bell et al., 2011). Methylation status is dependent upon changes in the enzyme activity of methyltransferases and demethylases, and alterations in genes that encode these enzymes are common in dietary-related diseases (Dawson and Kouzarides, 2012). S-adenosylmethionine (SAM) is the universal methyl donor in cells, yielding S-adenosylhomocysteine (SAH), and links metabolism and epigenetic status of cells (Gut and Verdin, 2013). Whether changes in the levels of SAM or SAH are sufficient to alter methyltransferase activity in vitro is controversial, but in mice threonine catabolism affects methylation status (Shyh-Chang et al., 2013) through indirect pathways involving energy production and acetyl-coA metabolism (i.e., pyruvate and glycine metabolism). Moreover, deprivation or restriction of essential amino acids causes profound transcriptional and metabolic responses (Anthony et al., 2013). In particular, dietary restriction of methionine produces responses that improve biomarkers of metabolic health, limit fat accumulation, and even prolong lifespan in rodents (Orentreich et al., 1993; Orgeron et al., 2014).

3.3. Metabolites are signaling molecules

Metabolites and transcriptional regulators are likely connected through as yet undefined mechanisms. Several G protein-coupled receptors (GPCRs) that impact immunity and inflammation are activated by intermediates of metabolism. For example, lactate, produced in the cytoplasm and secreted through the plasma membrane by solute carrier transporters, is recognized as a bioactive molecule with profound effects on immune and stromal cells. Although blood concentration of lactate is around 2 mM, it can reach up to 10 mM in inflammatory sites and up to 30 mM in tumor tissue. Among other effects, lactate is considered the driving force of tumor-associated macrophage development during epithelial-to-mesenchymal transition (Del Barco et al., 2011; Su et al., 2014). Lactate signaling has been also implicated in different features of chronic inflammatory diseases; for example, increased lactate concentration favors its internalization in activated T cells through $CD8^+$ and $CD4^+$ T cell-specific transporters, which causes inhibition of glycolysis and loss of responsiveness to chemokines and partly explains how T cells are entrapped in inflamed tissue (Haas et al., 2015). Acting as a ligand, signaling via lactate modulates insulin-induced reduction of lipolysis by binding to its cognate receptor, Gpr81, which is primarily expressed in adipocytes (Liu et al., 2009). Once considered a consequence of the lack of oxygen, it is now known that lactate is formed continuously in the presence of oxygen as an active part of mitochondrial metabolism (Hashimoto et al., 2006). Over the years, many laboratories have endeavored to identify ligands for orphan GPCRs (i.e., receptors unmatched to known ligands), but to date more than 100 of these receptors remain orphans. For example, Gpr91 is a receptor for succinate and Gpr80/99 is a receptor for alpha-ketoglutarate, revealing that dicarboxylic acids

are active signaling molecules (He et al., 2004; Gonzalez et al., 2004). It is also known that ketone bodies (beta-hydroxybutyrate), produced mainly in the liver as a circulating glucose-sparing energy source, can also serve as signaling molecules in neurons (Shimazu et al., 2013). Other intermediates of glycolysis, the citric acid cycle and undoubtedly products of cellular fatty acid metabolism might also play significant roles in non-metabolic activities (Haas et al, 2016). For example, the polarization of macrophages into M2 cells is important because of their role in wound healing. Two critical pathways regulate this phenomenon: glutamine-related metabolism and the UDP-GlcNAc pathway. These are major connecting hubs between cellular metabolism and signaling. Certain dietary conditions might lead to an interplay between macrophage polarization, metabolism, and mTOR signaling, with the ability to manipulate macrophage function in clinically relevant settings (Wellen and Thompson, 2012; Jha et al., 2015). Similar concepts may be applied to the cancer metabolism program and the responses of healthy tissues during nutritional stress; the limitation of one energy source, glycolysis or mitochondrial metabolism, results in tissue vulnerability to the inhibition of the other energy source (i.e., treatment of metabolic diseases should include both factors).

4. Metabolism and DNA methylation: a search for therapeutic and diagnostic targets in obesity and diabetes

The discovery of endogenous metabolites signaling cell-fate decisions demonstrates the integration of multiple cellular functions. DNA methylation is the only epigenetic mark with strong mechanistic support for both heritability and response to dietary changes (Maddocks et al., 2016; Mentch et al., 2015; Mentch and Locasale, 2016; Rodriguez-gallego et al., 2015), and represents a

metabolo-epigenetic link that needs to be translated into clinical investigations. In particular, the measurement of metabolic states can be correlated with chromatin states and gene expression.

DNA methylation is currently used to construct models for predicting chronological age at a population level because the regulation of the chromatin landscape can alter lifespan (Benayoun et al., 2015). These models have practical implications for studying the role of methylation in age-related diseases, and to explain the association of complex metabolic and inflammatory states with early onset of diseases linked to aging, including atherosclerosis and cancer (Hannum et al., 2013; Horvath, 2013). Human immunodeficiency virus infection is characterized by early onset of age-related diseases (Deeks, 2011; Alonso-Villaverde et al., 2013) that are associated with changes in age-associated methylation sites (Gross et al., 2016). Also, obese patients not undergoing gastric bypass surgery (i.e., no changes in the metabolic state) have a worse long-term survival as they age than among those undergoing surgery, who present dramatic and beneficial metabolic changes (Davidson et al., 2016). These effects have been observed in the complexity of a whole organism, but it is now time to explore how specific metabolic changes may affect chromatin, transcription and consequences in health.

The chromatin landscape is dynamically configured throughout life, and changes in chromatin marks, defined as “epigenetic drift”, occur in response to nutritional, metabolic, environmental or pathological signals. Do changes in diet or in metabolism that are associated with obesity and diabetes lead to epigenetic drift? This has yet to be fully established in humans, but, if confirmed, known dietary manipulation or drugs that regulate methylation might

be used to slow the aging process and influence the onset of age-related diseases. Alternatively, the assessment of DNA methylation might increase the accuracy of biomarkers for evaluating the risk of disease and may provide a mechanistic basis for chronic diseases. More importantly, transmitted molecules beyond DNA can modify human development (e.g., genomic imprinting). To which extent is phenotypic information transmitted? Might diet-induced changes in metabolic or phenotypic traits in one generation affect the next?

4.1. Methodological source of errors: critical reflections

To prove the hypothesis of diet-induced adverse epigenetic drifts is a demanding task. For example, epigenetic processes continuously interpret dietary-induced metabolic alterations and metabolomic studies and epigenome mapping should be concurrent. The choice of when to initiate the analysis is important because chronic diseases evolve through a sequence of metabolic stages over time, from a period when alterations are barely detectable to a stage with complications in multiple tissues. It is also important to consider which tissue to investigate (Rönn and Ling, 2015), but to be worthwhile, markers should be explored in blood where metabolic changes are readily detected and probably affect more rapidly circulating cells. This is plausible in blood cells in the context of DNA methylation involved in insulin secretion (Toperoff et al., 2012) and insulin sensitivity (Nilsson et al., 2014). Blood cells are also practical to explore differential DNA methylations during exposures to high-energy diets (Ling et al., 2007; Jacobsen et al., 2012).

The metabolic changes in plasma reflect the metabolic state of all body organs and each metabolite may be a functional intermediate trait or a

correlated biomarker in relation to obesity and diabetes. The choice of analytical platforms and applications is also important and most are currently suitable to modern clinical laboratories. Applications are currently available for nuclear magnetic resonance and gas (GC) and liquid (LC) chromatography coupled to different mass spectrometry (MS) detectors, such as matrix-assisted laser desorption and ionization/time-of-flight (MALDI-TOF), quadrupole time-of-flight (QTOF) and triple quadrupole (QqQ) mass spectrometers. The analytes may be defined in advance to increase quality, but this comes at the cost of missing potentially interesting metabolites (Menendez et al., 2016; Beltran-Debon et al., 2015). We favor an overall design similar to that suggested in Figure 5. Current methods make it economically viable to analyze the metabolic profile of thousands of samples over extended periods of time. The main constraint of this scaled-up process is that samples cannot be run in a single analytical batch. To explore energy metabolism and mitochondrial status and function, GC-EI-QTOF-MS is the method of choice. Conversely, LC-MS/MS methods are preferred for the quantitative analysis of representative metabolites in one-carbon metabolism. Metabolically-related inflammatory stimuli should include secreted cytokines, growth factors and metalloproteinases, but there is no one individual marker that provides sufficient information (Puig-Costa et al., 2014).

The objective of the analysis is to compare changes in metabolism and DNA methylation with chances of error lower than 5% and criteria meeting genome-wide significance after Bonferroni correction for all tested loci and all metabolic traits. All methods should be combined in the quest of the ultimate goal, which is to provide epigenetic associations with a dynamic view of the metabolic phenotype (i.e., capturing the metabolome in its functional

interactions). Longitudinal studies (i.e., stable genetic contribution) are currently favored with respect to other designs. A before-after design adds further power and reliability in patients with diabesity through successful dietary measures and bariatric surgery. Currently, most methods associated with global DNA methylation are performed with protocols from the reagents' manufacturers with minor variations, but some mainly detect variations in repetitive DNA or transposons (Fernandez-Arroyo et al., 2016). There are also available methods to detect changes in individual methylated cytosine guanine dinucleotides (CpGs) and differentially methylated regions (DMRs) through array-based, bisulfite-converted, DNA methylation analysis (Ambatipudi et al., 2016; Chen et al., 2016; Glossop et al., 2016; Louie et al., 2016; van den Dungen et al., 2016).

This technique provides a subset of all potentially methylated sites in the genome. Because it is more selective and because of the limited tissue choices, the CpG–metabotype associations should be likely limited to processes of DNA methylation that are not cell-type specific. However, interpretation requires being unambiguous to distinguish between true functional associations and a mere correlation, and the need for complementary approaches is likely. Full sequencing may be used but the resulting loci will require further validation since polymorphisms in the detected region may provide potentially confounding associations. Typically, PCR products obtained through a Sequenom EpiTyper Assay are pre-treated and analyzed by MALDI-TOF MS. In particular, mechanisms linking the DNA methylation of certain genes and not others have yet to be fully established. Conversely, the replicated methylation sites could be within the proximity of known genes with a possible regulatory role in methylation. It is therefore necessary to compare global methylation

profiling of normal tissue samples from publically available datasets, with DMRs. Finally, to establish correlations between DNA methylation and gene expression changes, confirmation is required at the RNA level. In summary, the combination of epigenetics and metabolomics involves decoding of the genome information, transcriptional status and later phenotypes. Data should be obtained in the complexity of a whole organism and bioinformatic analysis will be required (Cordero et al., 2015; Noureen et al., 2015; Preussner et al., 2015) with the objective of finding mechanistic links between the pathological outcomes and specific chromatin-based mechanisms.

4.2. Transgenerational epigenetic inheritance of obesity and diabetes: current evidence in humans

The reviewed findings raise a crucial question. How do specific nutritional or surgical interventions affect chromatin and transcription and lead to beneficial effects on metabolic health? The role of the epigenome in the development of obesity and diabetes, although plausible, is not yet established. Epigenetics is a comparatively new field of research and the first steps are now being taken to identify potential biomarkers to predict an individual's obesity/diabetes risk before the phenotypes develop. It is also clear that several epigenetic marks are modifiable, which implies that there is the potential for interventions to transform or rescue unfavorable epigenomic profiles (Kirchner et al., 2013; Cheng and Almeida, 2014; de Mello et al., 2014; van Dijk et al., 2015). It should be clarified that in this review we only discuss the transmission of epigenetic alterations that occur in the absence of direct exposure to any specific environmental factor (i.e., the embryo is not exposed during gestation). The

development of nutritional strategies and dedicated pharmaceuticals is plausible. For example, dietary manipulations may contribute to promoter-specific changes in DNA methylation and several clinical trials are investigating the efficacy of epigenetic modifiers already in the marketplace (Cooney et al., 2002; Foulks et al., 2012; Waterland et al., 2006; Weaver et al., 2005). The few studies that have assessed DNA methylation profiles in relation to weight loss interventions indicate substantial variation over time and future research will require establishing the relationships between DNA methylation and metabolomic profile considering potential inter-individual variation.

Interestingly, a recent issue of *Diabetes Care* is mostly devoted to support bariatric surgery as a new treatment option in the management of T2DM. The recommended guidelines endorse interventions initially designed to promote weight loss as an intentional treatment to improve glucose homeostasis, which is more effective than any known pharmaceutical or behavioral approach. Postoperative improvements in metabolic control occur rapidly and are out of proportion to weight loss, yet the physiological and molecular mechanisms underlying these beneficial glycemic effects remain unknown (Cefalu et al., 2016).

Children of obese fathers are at higher risk of developing obesity. Economic status and access to food are not clearly associated and some findings provide insight into how obesity may propagate metabolic dysfunction to the next generation. In particular, changes in metabolism lead to changes in chromatin with the potential of transgenerational inheritance. It has been recently found that whereas spermatozoal histone positioning is unaltered between lean and obese men, DNA methylation patterns are markedly different.

Moreover, the sperm methylome is altered after bariatric Roux-en-Y gastric bypass (RYGB) surgery shortly after the procedure (Donkin et al., 2016). Thus, weight loss-induced changes in methylation are reversible. In this particular study, the consequences for the offspring were not examined, but it is known that children born after maternal bariatric gastrointestinal bypass surgery are less obese and exhibit improved cardiometabolic risk profiles carried into adulthood when compared with siblings born before maternal surgery (Guénard et al., 2013). Both studies indicate that human gametic epigenetic variation can be related to nutritional status and that changes in parental phenotype are detectable in the methylome of subsequent offspring. Interestingly, these methylation patterns are detected in circulating leukocytes.

Patients before and after weight-loss surgery have also been studied to demonstrate that methylation density in the leptin promoter may be a control level for cell type-specific leptin expression, and a main player in the regulation of energy homeostasis (Marchi et al., 2011). A study in obese women before and after RYGB surgery suggests that dynamic changes in DNA methylation may be an early event that orchestrates metabolic gene transcription involved in the regulation of insulin sensitivity in human obesity (Barres et al., 2013). Other studies reporting changes in methylation signatures before and after gastric bypass (i.e., with a substantial modification in metabolic state) provide additional evidence for the role of treatment-induced epigenetic organ remodeling in humans (Ahrens et al., 2013; Horvath et al., 2014; Benton et al., 2015; Dahlman et al., 2015; Nilsson et al., 2015).

Therefore, available data provide evidence that diet-induced metabolic changes might influence preconceptional behavior (Kirchner et al., 2013; Patti,

2013), but mechanistic insights are not sufficient to explain the overall picture. Other environmental stressors for the offspring should also be studied in combination, especially those associated with intra-uterine exposure.

4.3. Genetic predisposition in response to bariatric surgery: changes in the metabolome

It is apparent that there have to be internal underlying causes that influence obesity in addition to the environmental factors and excessive food intake. Genetic factors are known to play a role in weight gain and obesity and genome-wide scans have revealed several genes with altered transcriptional activity and/or epigenetic variations in obesity-related tissues (Levian et al., 2014). It is therefore likely that genetic factors may also be involved in how an individual loses weight following bariatric surgery. This issue, however, is unclear.

There are few studies about the effect of single nucleotide polymorphisms in body weight, body composition or weight gain during a follow-up period after bariatric surgery. Among others, variants in fat mass and obesity-associated (FTO) gene, leptin receptor gene, fatty acid amide hydrolase, Bsm1 vitamin D receptor, ghrelin receptor, and melanocortin 4 receptor are known to predispose for response to surgical intervention (de Luis et al., 2010a; de Luis et al., 2010b; Matzko et al., 2012; Hatoum et al., 2013; Mägi et al., 2013; Moore et al., 2014; Alexandrou et al., 2015; Rodrigues et al., 2015; Bandstein et al., 2016). However, these association studies are limited to Caucasians and Roux-en-Y gastric bypass-mediated weight loss. Bariatric surgery may also reverse obesity-related metabolic alterations and changes in

serum metabolites as shown in women undergoing weight loss surgery (Gralka et al., 2015). As expected, these metabolites are mostly implicated in IR. For instance, circulating branched chain amino acids are reduced after bariatric surgery but this is apparently a procedure-dependent effect. (Mutch et al., 2009; Lips et al., 2014; Arora et al., 2015; Lopes et al., 2015; Gralka et al., 2015; Lopes et al., 2016). Future studies should be conducted using metabolite profiling as a means to investigate adaptations associated with bariatric surgery and to identify molecular markers that could be use as surrogate markers of therapeutic response.

5. Concluding remarks

The study of epigenetic inheritance of complex traits characterized by metabolic disturbances, such as diabetes and obesity, is an exciting new frontier. The pivotal regulatory role of energy metabolism in transcriptional deregulation may suggest mechanisms on how toxic nutritional disequilibrium influences gene expression via cell metabolism, and may change the perception and pharmacological treatment of diabetes and obesity. We envision that the use of metabolomics to explore endogenous metabolites will reveal the existence of mechanisms accessible to intervention and will aid in the characterization of molecular mediators in the epigenetic information between generations.

The rising incidence of obesity and T2DM, major risk factors for severe comorbidities, is a major worldwide public health issue. These disorders threaten to reduce the length and quality of life of current and future generations and there is a strong need for safe and effective strategies for prevention and

treatment. To improve such strategies, a better understanding of contributing factors is essential. We emphasize biological evidence indicating that living organisms continuously adapt to fluctuations in the availability of energy substrates. Consequently, the cellular transcriptional machinery and chromatin-associated proteins integrate inputs derived from food to mediate homeostatic epigenetic responses through gene regulation. Therefore, epigenetic mechanisms may exacerbate the epidemic of metabolic diseases by first contributing to the development of obesity and T2DM and second, by passing modifications on to the subsequent generation. Fortunately, epigenetic modifications are not maintained over the lifetime and allow rapid adaptations. The challenge is to incorporate metabolo-epigenetic data in ways that will allow better biological interpretations, to provide clinical tools for diagnosis, prevention and treatment.

Conflict of interest

The authors declare that they have no conflict of interest.

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FIGURE LEGENDS

Figure 1. Food intake participates in the pathogenesis of age-related diseases. Understanding the effects on key pathways linking nutrition, metabolism and disease may lead to preventive and therapeutic approaches. It will be important to determine the ability of insulin resistance and nutrient sensing to modulate gene expression in affected cells. mTOR, mechanistic target of rapamycin; AMPK, adenosine monophosphate activated protein kinase.

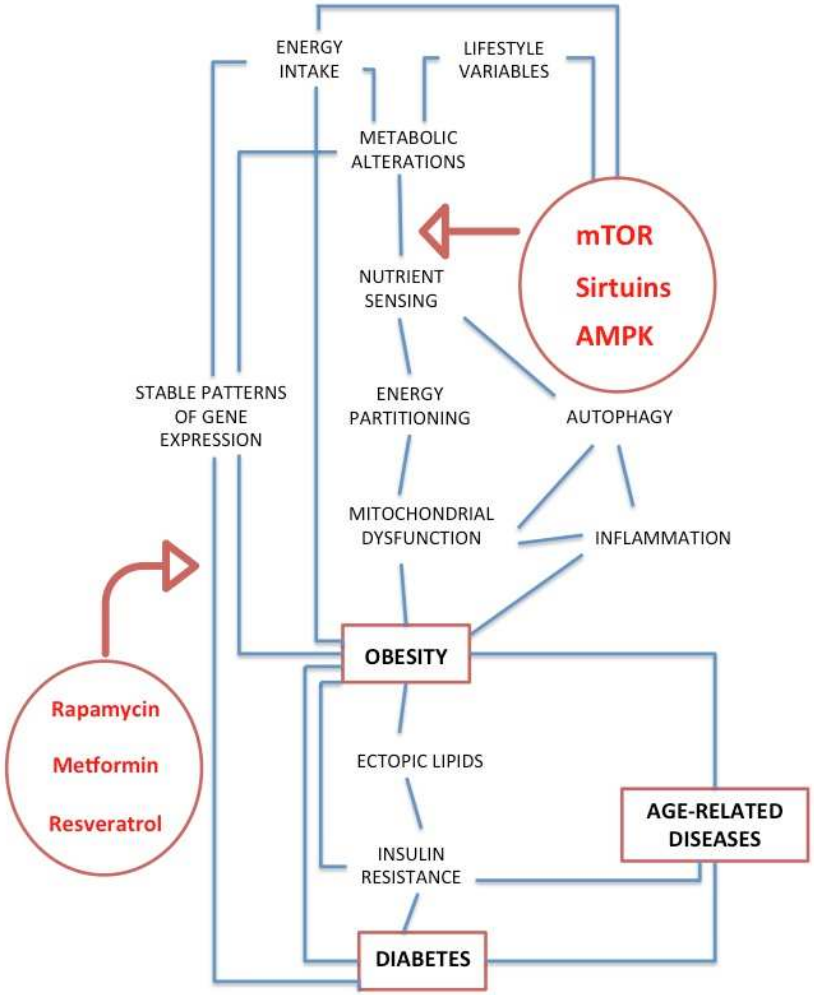
Figure 2. The study of the complex network of cellular pathways and biological mechanisms altered by excessive food intake requires a detailed roadmap. The overall setting of dietary-favored diseases is inflammatory and entails the modulation of mitochondrial function, profound metabolic alterations and changes in epigenetic events. Chronic diseases tend to converge from disturbances in which food is a major contributing factor.

Figure 3. Nutrition may influence epigenetic mechanisms. Epigenetics is the study of heritable changes in gene function not explained by changes in the primary DNA sequence. DNA modifications may include changes across the entire organism or may operate on a tissue-specific level.

Figure 4. Targeted metabolomics may increase the power of associations. Metabolites that regulate chromatin participate in pathways involved in intracellular energy balance (A) or enter one-carbon metabolism generating methyl donors (B). The tricarboxylic acid cycle links catabolic and anabolic pathways; glycolysis and β-oxidation generate acetyl-CoA, whereas removal of acetyl-CoA from mitochondria during glucose excess by the citrate shuttle fuels lipogenesis. Folate enters a cyclic reaction generating methyl donors for DNA methylation. BHMT, betaine—homocysteine S-methyltransferase; DHFR, dihydrofolate reductase; DNMT, DNA methyltransferase; FAD, flavin adenine dinucleotide; GNMT, glycine N-methyltransferase; MAT, aminomethyltransferase; MTHFR, methylenetetrahydrofolate reductase; MTR, Methyltransferase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, Tetrahydrofolate; UDP, uridine diphosphate.

Figure 5. The need for defined tasks in experimental analysis. The expected complexity in the interpretation of the relationships between metabolism and epigenetics requires activities arranged as a workflow. The figure depicts basic steps.

Figure 1


ENERGY INTAKE
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Sirtuins
AMPK
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ENERGY PARTITIONING
AUTOPHAGY
MITOCHONDRIAL DYSFUNCTION
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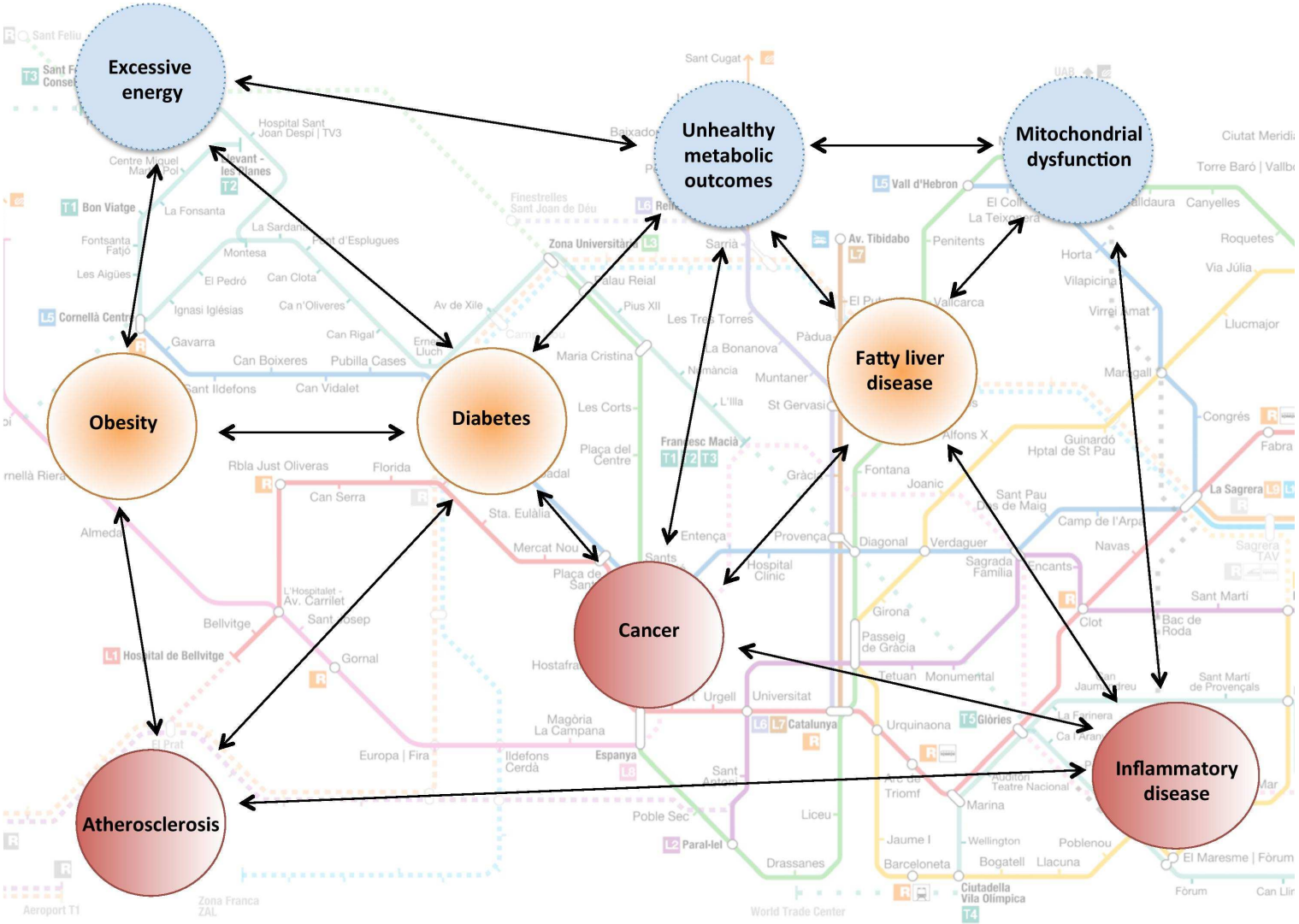
AGE-RELATED DISEASES
INSULIN RESISTANCE
DIABETES

Figure 2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Mitochondrial dysfunction
Obesity
Diabetes
Fatty liver disease
Cancer
Atherosclerosis
Inflammatory disease

Figure 3

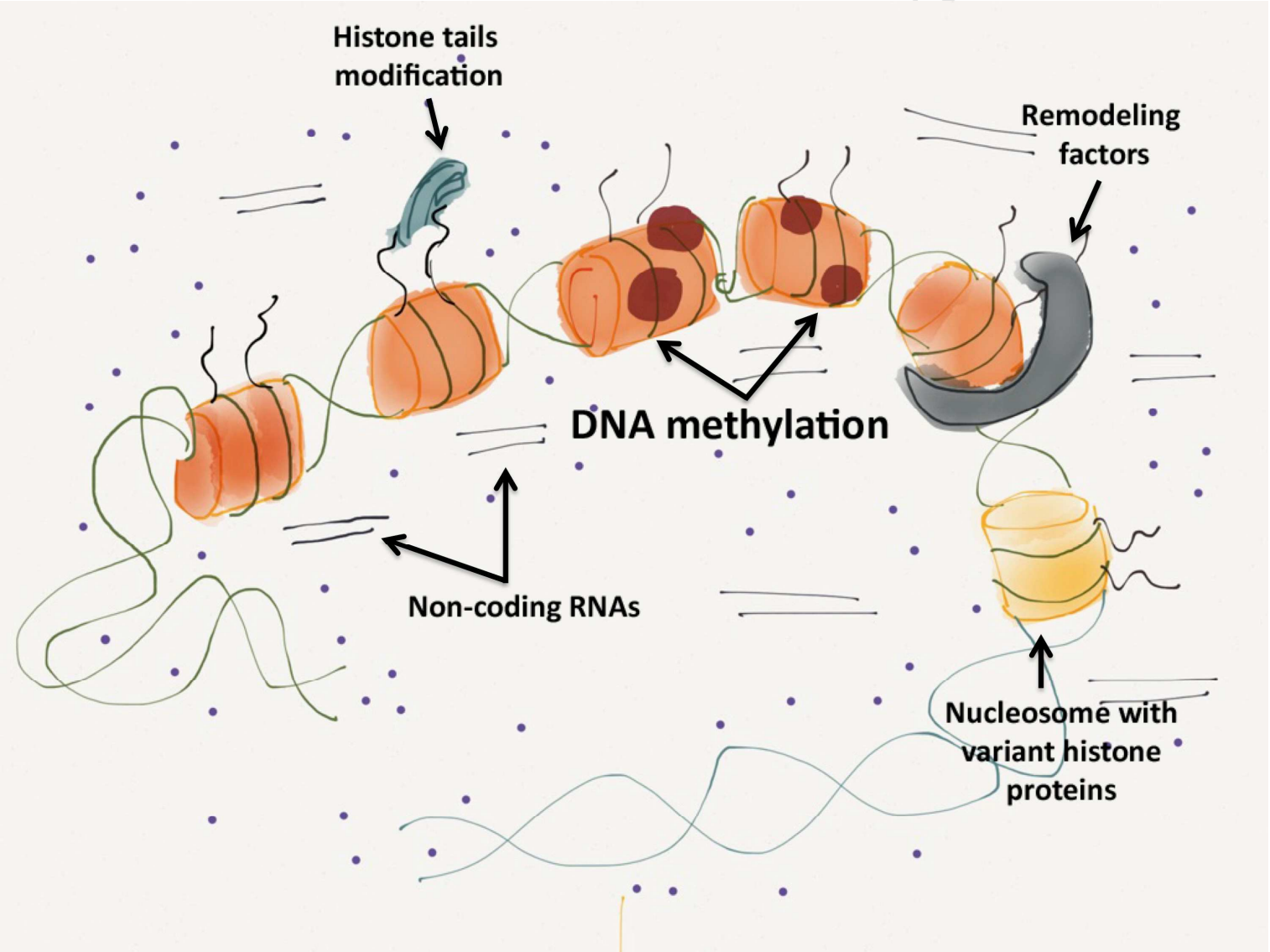

Histone tails modification
Remodeling factors
DNA methylation
Non-coding RNAs
Nucleosome with variant histone proteins

Figure 4

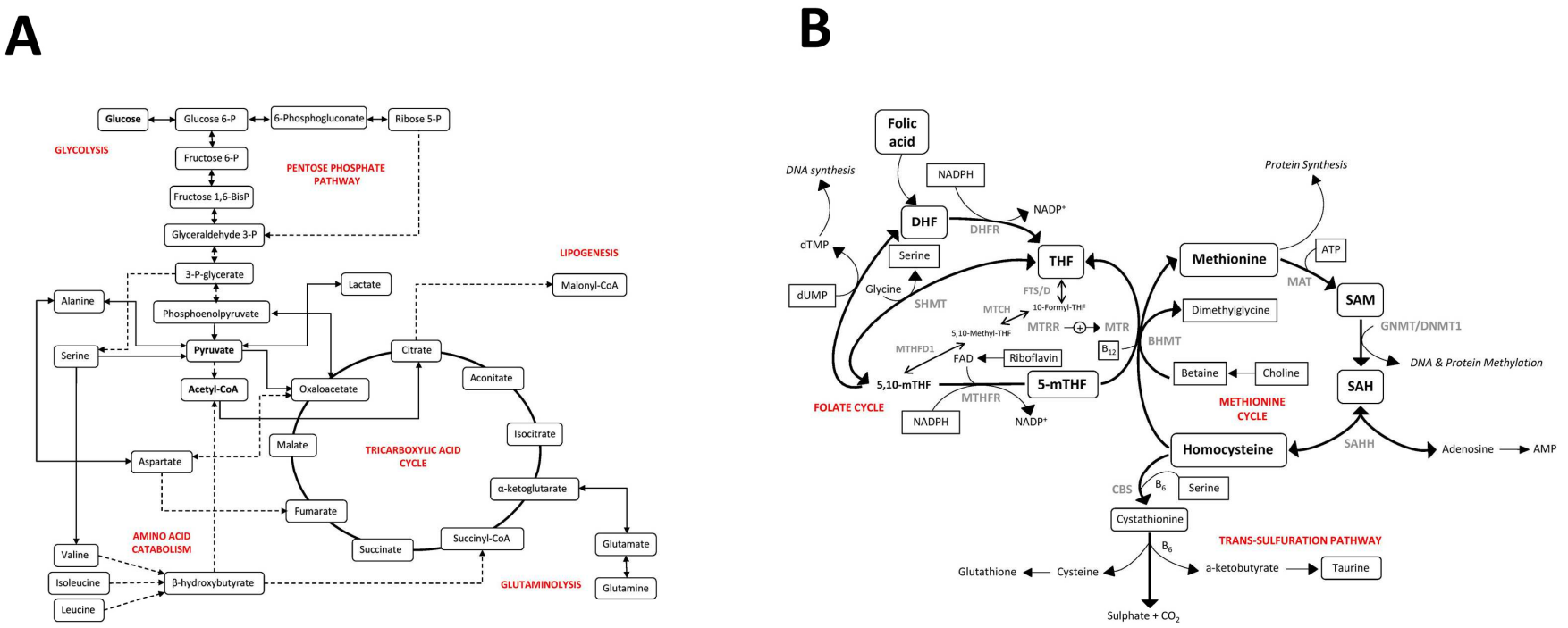
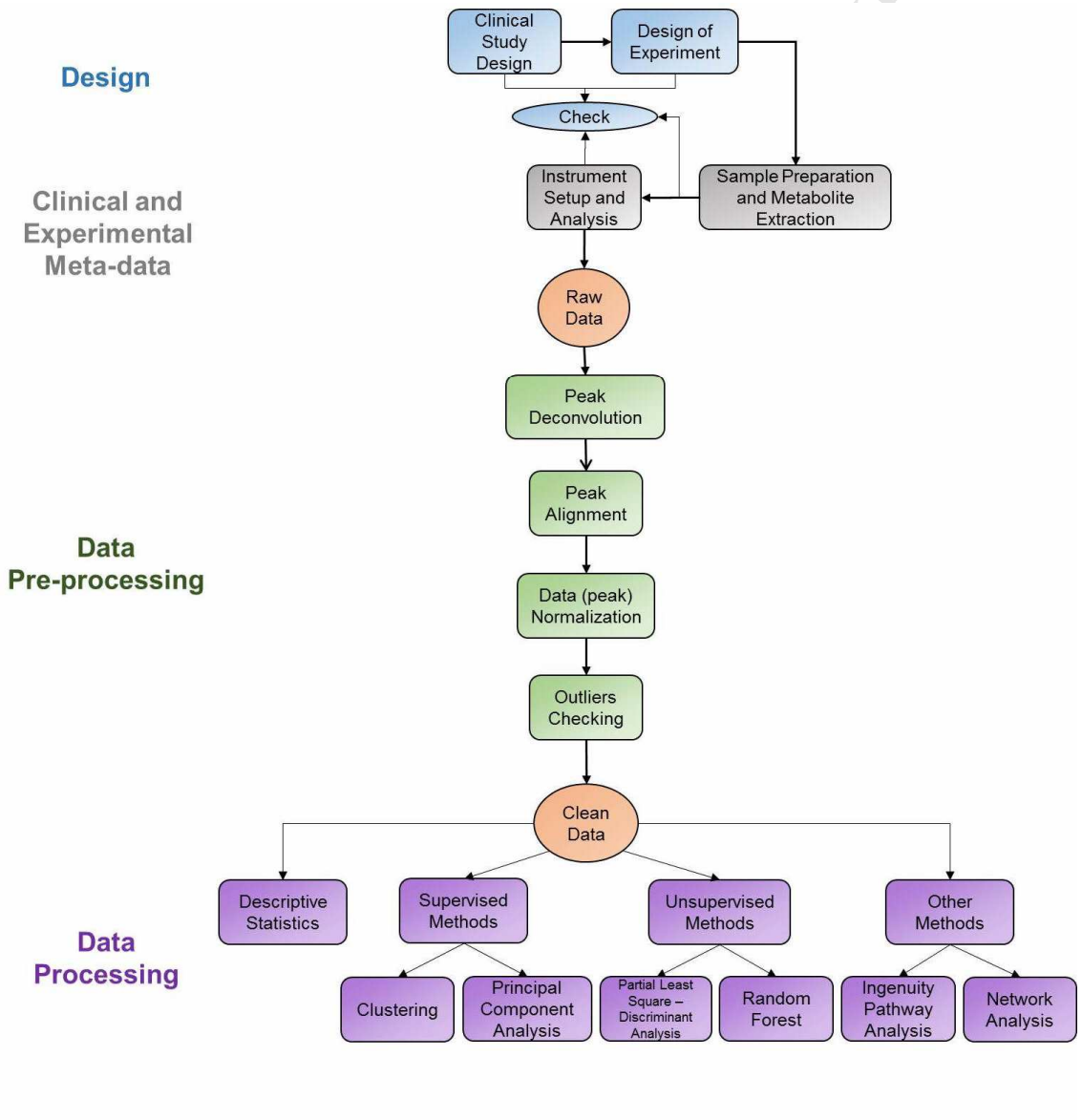

A
Glucose
Glucose 6-P
6-Phosphogluconate
Ribose 5-P
GLYCOLYSIS
PENTOSE PHOSPHATE PATHWAY
Fructose 6-P
Fructose 1,6-BisP
Glyceraldehyde 3-P
LIPOGENESIS
3-P-glycerate
Lactate
Malonyl-CoA
Alanine
Phosphoenolpyruvate
Serine
Pyruvate
Citrate
Aconitate
Acetyl-CoA
Oxaloacetate
Isocitrate
Malate
TRICARBOXYLIC ACID CYCLE
Aspartate
α-ketoglutarate
Fumarate
AMINO ACID CATABOLISM
Succinyl-CoA
Succinate
Glutamate
Glutamine
Valine
Isoleucine
Leucine
β-hydroxybutyrate
GLUTAMINOLYSIS
B
Folic acid
DNA synthesis
NADPH
NADP+
DHF
DHFR
dTMP
Serine
THF
dUMP
Glycine
SHMT
FTS/D
MTCH
10-Formyl-THF
5,10-Methyl-THF
MTRR
MTR
MTHFD1
FAD
Riboflavin
B12
5,10-mTHF
5-mTHF
MTHFR
NADPH
NADP+
FOLATE CYCLE
Protein Synthesis
Methionine
ATP
MAT
SAM
Dimethylglycine
BHMT
GNMT/DNMT1
DNA & Protein Methylation
Betaine
Choline
SAH
METHIONINE CYCLE
Homocysteine
SAHH
Adenosine
AMP
CBS
B6
Serine
Cystathionine
TRANS-SULFURATION PATHWAY
B6
Glutathione
Cysteine
a-ketobutyrate
Taurine
Sulphate + CO2

Figure 5


Design
Clinical and Experimental Meta-data
Data Pre-processing
Data Processing
Clinical Study Design
Design of Experiment
Check
Instrument Setup and Analysis
Sample Preparation and Metabolite Extraction
Raw Data
Peak Deconvolution
Peak Alignment
Data (peak) Normalization
Outliers Checking
Clean Data
Descriptive Statistics
Supervised Methods
Unsupervised Methods
Other Methods
Clustering
Principal Component Analysis
Partial Least Square – Discriminant Analysis
Random Forest
Ingenuity Pathway Analysis
Network Analysis

Highlights

- Nutrient sensing plays a determinant role in energy partition
- Intermediates of metabolism are signaling molecules and influence chromatin structure
- Epigenetic mechanisms are causally linked to metabolic diseases
- Current nutrition-related diseases may reverse the lifespan gains
- Transgenerational epigenetic inheritance of metabolic diseases may cause concern