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Chapter 9: General Discussion
In **Chapter 8**, the current state of Genome-Wide association studies of metabolite profiles was reviewed and this served as a discussion of **Chapters 2 and 3**. This chapter provides further perspectives on **Chapters 4, 5, 6, and 7**.

In **Chapter 4** we report that the downregulation of acetyl-CoA metabolic network is an important feature in the pathophysiology of obese type-2 diabetes patients. This work is in line with the network medicine paradigm that aims to address the problem that a disease is rarely caused by malfunction of one individual gene product, but instead depends on multiple gene products that interact in a complex network [1–3]. Through a network-based bioinformatics analysis, we argue that acetyl-CoA metabolic network is the unifying principle behind previously implicated pathways such as branched-chain amino acid degradation, fatty acid oxidation and citrate cycle dysregulation, in the pathophysiology of type-2 diabetes. The vicinity of acetyl-coA metabolism represents a hotspot where abnormalities in individual genes potentially accumulate and upon reaching a certain risk threshold, lead to the manifestation of type-2 diabetes. Furthermore, disease heterogeneity may arise when affected individuals contribute different genes in this network topography, or variants within these genes, to the manifestation of the phenotype.

Type-2 diabetes is currently believed to be a multifactorial, complex disease. While patients may all exhibit similar clinical manifestations like hyperglycemia and insulin resistance, the underlying etiology is heterogeneous [4]. However, the current disease classification paradigm overgeneralizes pathophenotypes, and does not consider individualized nuances in disease expression [3]. More importantly, these patients are often treated similarly, with little consideration of individual characteristics that might affect clinical outcome and therapeutic response. Therefore, there is growing recognition that personalized approaches to treating type-2 diabetes might bring substantial benefits for the patient as well as the pharmaceutical companies. The application of network medicine to pharmacology and clinical trials paves the way for individualized or “precision” medicine. In the context of our research, the fact that a central pathway in energy metabolism is dysregulated as a whole indicates that pharmacological treatment of individual targets in this metabolic pathway is unlikely to succeed. Chen and Butte [5] point out that the best approach to
treating complex disorders such as type-2 diabetes may be to “modulate the disease network by targeting multiple components using a designed polypharmacological ligand or a combination of drugs” rather than using single target drugs. Furthermore, taking individual nuances in the network into consideration in the design of such compounds should pave the way for better and effective drugs. Further research into the causal role of downregulation of the acetyl-CoA network in type 2 diabetes should indicate whether direct intervention in the acetyl-CoA network will provide novel therapeutic approaches.

Chapter 5 explores differential Allele-Specific Expression (ASE) with the aim of identifying genetic variants that are associated with or affect gene expression and contribute to the functional differences observed in visceral and subcutaneous adipose tissues. ASE studies help to understand the cis-regulatory basis of variation in gene expression. The analysis of ASE allows for the analysis of the genetic component of gene expression in much smaller numbers of individuals than in traditional expression quantitative trait loci (eQTL) studies, where the genetic variation is usually only a minor contributor to the total degree of variation in gene expression between individuals [6]. However, it should be realized that allelic imbalance may not be purely genetic, but also caused by epigenetic factors [7, 8]. In general, ASE is a promising technique and has potential for clinical applications. For example, it has been used for tumor type classification [9] and cancer diagnosis [10]. Another application for ASE is in interrogating gene-environment interactions [11]. While environmental factors have been shown to substantially affect human disease risk, this interaction has not been well characterized in genome wide studies owing to small genetic effect sizes and the steep multiple testing burden. By associating risk factors such as diet, exercise, lipid levels, drug usage etc with an individual’s allele-specific expression, it will be possible to understand and treat type-2 diabetes more effectively.

Very low calorie diets (VLCD) with and without exercise programs lead to major metabolic improvements in obese T2DM patients. However, the biological mechanisms underlying these improvements have so far not been elucidated fully. Chapter 6 describes the effects of VLCD with or without exercise in obese T2DM patients through proteomic analysis of plasma obtained from these subjects as lean controls. This study shows that proteomic analysis reveals many proteins that exhibit significantly different levels in type 2 diabetes patients versus controls and before and after a VLCD. Although this gives us an insight into the proteins affected by obesity, insulin
resistance, T2DM and diet-induced weight loss, further studies are needed to establish if these proteins are causally related to these conditions or the success of the intervention. Dense longitudinal sampling could potentially resolve the issue of causality by providing mechanistic insight into the changes occurring in these subjects as a result of VLCD. A recent study [12] showed that a differential metabolic adaptation of mice to a high fat diet is associated with striking differences in gene expression patterns. After an initial state where high fat feeding induces coherent changes in gene expression in liver in all mice tested, there is subsequent modification of gene expression towards patterns characteristic of each phenotype (obese and diabetic, lean and diabetic, lean and non-diabetic). This shows that there is a phased response to an intervention and that mechanisms causing insulin resistance may vary over time. High fat diet-induced obesity/diabetes in the mouse is considered a good model for the pathogenesis of the human conditions. Therefore, future studies should consider integrative, longitudinal “omic” assessments to monitor intervention specific adaptations [13].

Prolonged niacin treatment elicits beneficial effects on the plasma lipid and lipoprotein profiles that are associated with a beneficial cardiovascular disease (CVD) risk profile. However, niacin also elicits unwanted effects which include a severe flushing response. In Chapter 7 we explore the prolonged effects of niacin on lipid metabolism in adipocytes of a hyperlipidemic mouse model. Prolonged niacin treatment resulted in upregulation of the biosynthesis of unsaturated fatty acids pathway in gonadal white adipose tissue (gWAT), increased n-3 PUFA secretion from the adipocytes, and an increased plasma level of n-3 PUFAs and their anti-inflammatory oxylipins, which together point towards an atheroprotective plasma profile. Niacin (also known as nicotinic acid or vitamin B3) has been widely used in the prevention of cardiovascular disease. However, the majority of patients experience the aforementioned flushing response that is characterized by severe reddening of the skin, itching, and tingling. Studies have shown that the flushing response is due to the vasodilatory effects of prostaglandin D2 (PGD2) and prostaglandin E2 (PGE2) which are formed by the enzymatic action of cyclooxygenase (COX) on arachidonic acid (AA) that is released from membrane phospholipids as a result of niacin action. Interestingly, schizophrenia is associated with a blunted flush response to niacin and there is evidence for the relevance of n-6 PUFA pathway to the phenotype in these subjects [14]. Interestingly, both niacin and omega-3 PUFA have shown clinical potential for the treatment of psychosis in schizophrenia patients [15,
Therefore, a clear mechanistic understanding of the biochemical response to these supplements can benefit both CVD and schizophrenia.

It should be noted that PUFA supplementation as a preventive strategy for CVD has shown mixed results. While a few studies suggested improvements of risk factors [17], a more systematic clinical trial showed no improvements for CVD endpoints [18]. A recent article reported that the Inuit population in Greenland evolved unique genetic adaptations for metabolizing omega-3 and other fatty acids [19]. This discovery raises questions about whether omega-3 fats are really good for everyone despite the recommended guidelines that have been in place for several decades. Alternatively, endogenous induction of PUFA or modulation of PUFA derived oxylipin profile may be explored as therapeutic strategies. However, outside the context of metabolic disease, increased PUFA biosynthesis might be harmful due to their potential oxidation to lipoperoxide inflammatory triggers. Similarly, modulating PUFA conversion to specific oxylipins may have unintended consequences for the inflammatory pathways that play an important role in cancer progression. Therefore these therapeutic strategies must be explored with caution while taking into account the many functions of PUFA metabolites. Future studies should explore novel bioinformatics and systems biology approaches to build a network model that predicts phenotypic traits and outcomes for various perturbations such as niacin and omega-3 PUFA. Furthermore, this model must incorporate individual variation in response, to better understand the genetic underpinnings of these complex pathways.

References


11. Allele-specific expression reveals interactions between genetic variation and environment [http://biorxiv.org/content/early/2015/09/13/025874]


