Chapter 6

Conclusions and Perspectives

CONCLUSIONS

The clinical nutrition practitioner, such as a registered dietitian, seeks to optimize nutritional therapies for the individual. Such experts rely on clinical practice guidelines translated from scientific publications as realistic recommendations for nutritional diagnosis and therapeutic recommendations. For example, the Academy of Nutrition and Dietetics in the U.S. has over 100 guidelines for common conditions (e.g., nursing) to specific diseases such as emergency medicine, allergy and immunology, obstetrics and gynecology, podiatry, or urology [1]. However, much of the scientific literature in the area of nutrition, and indeed, biomedical research, relies on statistical averages of selected groups (e.g., cases versus controls) which by definition cannot represent larger, more varied populations or an individual. These averages are used to supplement disease diagnostic practices; and translating this corpus of literature results to personalized nutrition recommendations evidence is currently not possible.

Two broad strategies are needed to overcome the challenges of improving population as well as personal health. The first tactic is to develop n-of-1 research strategies that are capable not only of deeply analyzing individuals [2-4] but also aggregating data to expand recommendations beyond the studied population [4]. The second approach expands the reductionism currently used to analyze data from drug or nutrition studies to a more holistic analysis and interpretation of multiple biological and environmental scales (e.g., data from within the body plus the environment of individuals [5]. Systems biology [6, 7] approaches that produce data from the microscales within the body, such as metabolomic, proteomic, and network pathway analyses, hold the potential to assist the clinical nutritionist in developing a more personalized approach. However, the application of systems biology to nutrition in practice is still in its infancy. To date, these approaches rely on aggregating individual reductionistic analysis since the systems view of the whole metabolite-profile has not yet been elucidated. The integration of a systems view of nutrition with a systems view of metabolomics, and a systems view of proteomics, genomics, clinical, and laboratory metabolomics data sets has not yet been attained. Interpreting these systems integrations in the individual's environmental, social, and psychological contexts is at the fore-front of healthcare but has not yet been initiated in academic, government, and all but a few isolated examples.

The challenge remains…how do we integrate the various –omics technologies that are increasingly available from academic and industry research with nutrient measurements and nutrition interventions to generate the statistical evidence

necessary to help people become healthier and prevent disease? This thesis has touched on just a few elements of this challenge, using metabolomics and proteomics to characterize subgroups and diet response.

In **Chapter 2,** we demonstrated it is possible to use a short-term healthy vegan diet to challenge metabolism and produce a metabolic signature conducive to optimal blood sugar, insulin and lipid control after only 48 hours. Our tightly controlled diet intervention resulted in strong correlations between dietary nutrients and plasma metabolites, supporting the notion food intake was closely linked to metabolites measured. Finally, we observed that nutritional biochemistries and the metabolite results, insulin and branched chain amino acids, were impacted by gender dimorphism.

In **Chapter 3,** we continued to build the story begun with **Chapter 2**. We evaluated postprandial responses with glycemic, lipid and related metabolites on day three of the vegan and animal diets and demonstrated both diet types can have health advantages with flexitarian modifications. The vegan diet breakfast resulted in a less optimal metabolic signature despite apparently healthful food choices. However, the fiber content of the vegan diet may have reduced metabolite peaks and promoted bile acid concentrations that have positive health implications. The animal diet produced undesirable insulin and glucose peaks after lunch but a more favorable fatty acid profile from both mealtimes. We concluded that liberalization of the vegan meal plan to vegetarian; and the animal meal plan to a Nordic-based diet with increased focus on vegetable-based foods could result in improved metabolic signatures for both diet strategies. Insulin, triglyceride, amino acid and bile acid results showed gender dimorphic responses in these analyses.

In **Chapter 4**, we further explored the influence of sexual dimorphism plasma profiles using aptamer-based proteomics combined with network analysis in a healthy cohort of women and men. Twenty eight percent of the total proteins analyzed were differentially expressed in a sexually dimorphic manner. These results were then successfully replicated in a larger cohort. The top eight most significant proteins elevated in females had known associations with sex hormone metabolism and each protein was involved in at least one diet- related metabolic disease. These proteins were all involved in glucose and insulin metabolism, metabolic rate, carbohydrate intake and salt sensitive hypertension. Of the top proteins more highly expressed in males, some were also involved in sex hormone metabolism with a focus on such areas as blood coagulation, inflammation and iron metabolism and overload and cardiovascular disease risk. Iron, total lipids, monounsaturated fatty acids, omega 3

polyunsaturated fatty acids, and vitamins K and A are known to play key roles in the proteins found to be highly expressed in males.

In **Chapter 5**, we analyzed vitamins, metabolomics and clinical chemistries during the five menstrual cycle phases in women to uncover the sex hormone related metabolic differences, which influence the sexual dimorphism seen in **Chapter 4**. Fifty percent of the metabolites tested showed significant differences in rhythmicity across cycle phases and were enriched in neurotransmitter, glutathione (oxidative stress), urea cycle (nitrogen), vitamin B6 and vitamin D metabolism. Thus, we demonstrated the importance of accounting for menstrual cycle phase and sex hormone concentration differences in performing routine health diagnosis. Additionally, the luteal phase demonstrated the most significant decreases in amino acids and lipids, which may be caused by the anabolic effect of the progesterone peak. The changes in level of these metabolites could be linked to current biochemical and physiologic knowledge on biomarker changes in menstrual conditions such as premenstrual syndrome (PMS) and premenstrual dysphoric disorder (PMDD). Since individuals differ genetically, socially, and in environmental exposures, a larger population sample might identify those susceptible to PMDD and PMS and allow for strategic dietary interventions to alleviate symptoms.

FUTURE PERSPECTIVES

Innovative research designs, such as n-of-1 research [8] approaches that capture individual variability, need to be combined with controlled diet interventions to effectively translate –omics results to diet prescriptions. Challenge studies can be conducted alongside diet interventions to examine short-term changes in phenotypic flexibility.

As a part of this thesis project, **Figure 1** depicts a conceptual experimental framework that was developed to analyze utilization of a combinatorial glucose and fructose (e.g., disaccharides in sugar) tolerance challenge test during different phases of the menstrual cycle. Specifically, this model experiment may determine how a sugar bolus (often consumed in excess in the luteal phase) alters carbohydrate, lipid, and protein metabolic effects, over a 10-hour time period in pre-diabetic and diabetic women. Introducing a protein shake, supplemented with essential fatty acids, may alter metabolism during the luteal phase to compensate for the increased protein and lipid requirements that may be relevant to the cell regeneration and recycling described in **Chapter 5**.

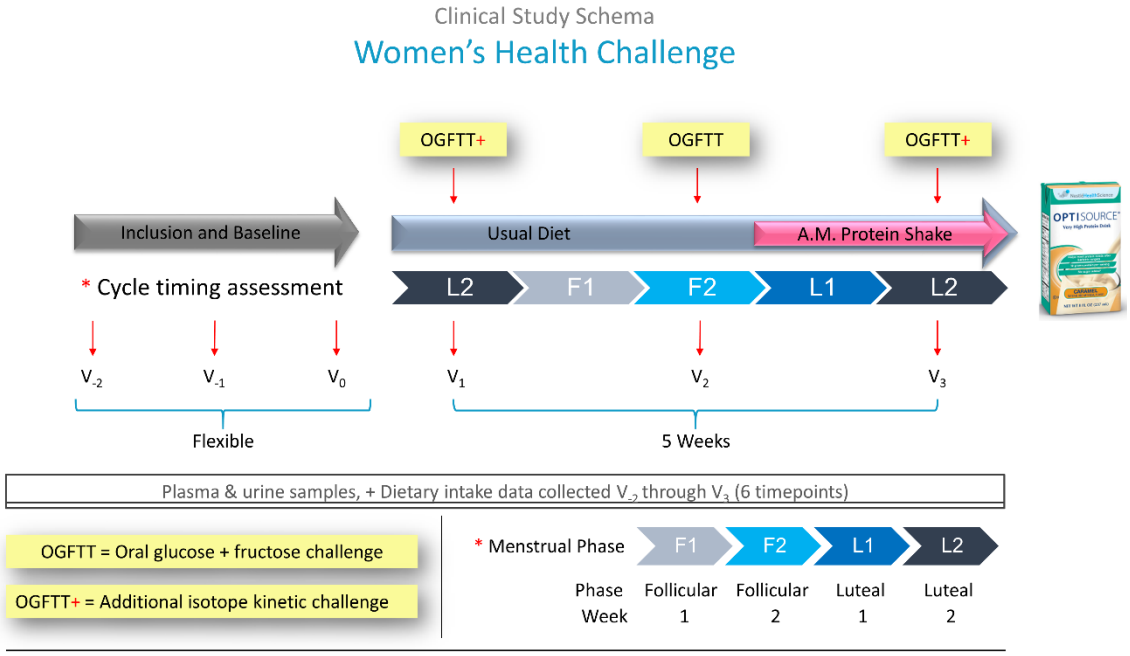

Figure 1: Hypothetical diet challenge study to evaluate the metabolic impact of an increase in protein intake during the luteal phase of the menstrual cycle.

The challenge test would be done during the follicular and luteal phases when the participants are ingesting a usual diet; and then repeated after the daily ingestion of a high protein shake during the luteal phase. The results of the tolerance test would be compared between the follicular phase, which is the more stable phase for blood sugar control, and the luteal phase to examine the differences in the individual's blood sugar control during this more vulnerable timeperiod. Finally, the luteal phase response of the individual to the luteal phase protein shake would be compared to examine the utility of this type of periodic intervention.

Multiple plasma samples would be collected for clinical biomarker and metabolomics assessments. Creatinine clearance from 24-hour urine may be used to evaluate differences in protein utilization during these 3 phase specific timepoints. Dietary intake data would also be collected and evaluated for differences in habitual intake across individuals that are known to influence blood sugar control (**Figure 1**). This type of research strategy may create opportunities to use sex hormone subtyping for diet design that is translatable to practice and deserves further exploration.

Hence, menstrual phase subgroups and sexually dimorphic data, may launch personalized nutrition into future healthcare practice. Metabotypes, which are identified through cluster analysis of individuals based on similar metabolic phenotypes that can be identified using clinical and metabolomic markers [9], provide the conceptual basis to differentiate dietary intervention response.

Metabotype response then translates to targeted diet prescriptions. This type of analysis on the data generated in **Chapters 1** and **2** in response to the vegan and animal test diets would have been appropriate. However, a sample size of 21 was too small to locate significant metabotype clusters.

As one example of how metabotyping works, in a study of 1500 individuals, 3 metabotypes were identified: 1) high HDL (high density lipoprotein) cholesterol, low glucose, low triglycerides; 2) low cholesterol; 3) high trigyclerides, high total cholesterol, low HDL and high insulin resistance. Dietary advice was targeted to metabotypes 1 and 3 to lower the characteristic biomarkers. This simplified targeted approach showed strong agreement with the individualized dietary advice provided by the dietitian (2).

Another study used metabotyping to further analyze metabolic syndrome marker reduction with Vitamin D supplementation. That study analysis demonstrated an increase in Vitamin D concentration but no significant metabolic syndrome marker changes in the responder group.

However, the use of a metabotype clustering approach identified a sub-group of vitamin D responsive patients that demonstrated significant decreases in fasting insulin, homeostatic model assessment score, and C-reactive protein (3). LDL level, fasting glucose, and cholesterol subgroups were also identified based on response to a longer term (6 week) micronutrient intervention in teens and adolescents [4] further buttressing the use of challenges tests to identify metabotypes. This type of approach could be used to analyze response to semi-vegetarian (flexitarian) diets, a concept that emerged from the vegan diet studied in this thesis.

Study approaches need to join the rapid pace of technology development in order to ensure new technologies can help more people sooner. For example, the research results represented in **Chapters 1** and **2** were generated from five years of work; including study design, recruitment, laboratory measurements, data analysis, manuscript generation and publication. The cost was close to 5 million francs. Research takes an average of 17 years to translate evidence into practice [10, 11]. This is too long given the rapid pace of climate change, population growth, food systems changes, and healthcare crises faced by modern society [12].

Self-quantification emerged following the development of smart phones and apps which allow for self-monitoring, data collection, and analysis to track activities, nutrient intakes, and health status. Self-quantifiers reflect on their personal health

data to gain a better understanding of their body, health behavior and interaction with their environment [13]. A worldwide community exists to leverage homemade tools and experiences [14]. For example, smartphone applications, such as Clue [15] are widely used by women to easily track related symptoms and phases of their menstrual cycle, fertility and perimenopausal transition. These types of easy to use applications provide the inputs for individuals to track the results of their own self-experimentation, such as the impact of changing diet strategies on menstrual cycle symptoms. The individual is empowered by the opportunity to test the effectiveness for themselves of an alternate diet strategy, such as the luteal phase protein shake suggested in the Women's Health Challenge future study design (**Figure 1**). This type of self-centered research can be conducted more efficiently than currently accepted standard approaches to human clinical research.

The challenge lies in aggregating this kind of data from a variety of tools collected by individuals in a consistent way so that humanity can benefit. Health information sharing websites, such as PatientsLikeMe [16] empower individuals to share their health conditions, data and unique experiences and connect with other patients like them. Participants have the opportunity to find new ideas and solutions to their unique health challenges that may not be known from published scientific research. Their data is then aggregated and sold to companies that wish to produce new products and services in diagnostics and therapeutics. Other companies, such as Arivale [17], Human Longevity, Inc. [18], and Molecularyou [19] target big data diagnostics analyzing blood, saliva and urine for genomics, metabolomics, microbiome analysis, proteomics, and lifestyle information. Advances in machine learning and artificial intelligence computing are likely to uncover new therapeutic solutions. Health action plans and personalized coaching are provided. Participants pay to provide their samples and receive their analyses. Data are aggregated and research discoveries made on a rolling basis. An example of using public domain data is analysis of 9896 users who self-recorded 587,187 food diary pages of the MyFitnessPal app with machine learning algorithms to discover under and over-reporting of food intake goals [20]. Thus, big data and self-quantification could potentiate translation of valuable nutrition research findings to advance healthcare more quickly by overcoming some of the timeline and cost hurdles associated with the more traditional approaches to clinical trials research.

Challenges with integrating –omics technology and nutrition remain. This thesis has taken some steps in this direction by evaluating healthy women and men to obtain metabolomics and proteomics signatures. Personalized nutrition subtypes were examined using the menstrual cycle phases and gender differences. Short-term diet

challenges were evaluated using metabolomics technology and demonstrated short-term health improvement. Harnessing the power of nutrition by integrating it with new scientific and information technologies will launch us into a new era of preventive healthcare in which we can more effectively use metabolism and nutrition to diagnose and optimize health.

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