



## Conference on ‘Inter-individual differences in the nutrition response: from research to recommendations’

### Symposium 2: Sex differences in nutrient availability and health

## Sex matters: a focus on the impact of biological sex on metabolomic profiles and dietary interventions

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The metabolomic profile of a biofluid can be altered by dietary intake, exercise and disease processes and, thus provides an important tool for the study of many physiological processes. However, in addition to perturbation due to disease, the metabolomic profile of urine and plasma has also been shown to vary due to many intrinsic physiological factors such as age, sex, hormonal status and diurnal variation. Characterisation of this normal degree of variation in the metabolomic profiles of human biofluids is a necessary and important step in the development of metabolomics for use in nutrition-related research. The current review focuses on the impact of sex on the metabolomic profile. A number of studies have reported that sex impacts metabolites such as amino acids, lipids, sugars and keto acids. Furthermore, we examine the effect of the menstrual cycle on the metabolomic profile. Responses to dietary interventions can also differ between the sexes and highlighting this is important for the development of the field of precision nutrition.

#### Metabolomics: Sex: Dietary interventions

Recently, there has been growing interest in sexual dimorphism with the full realisation that men and women often respond differently to dietary interventions. Traditionally, many dietary interventions were performed in males only with the knowledge that the menstrual cycle can impact on metabolic measurements. However, in more recent times there has been a shift in interest and now understanding the differences between men and women is viewed as being of paramount importance. Furthermore, studying and understanding these differences will be fundamental to the delivery of personalised healthcare services including personalised nutrition.

As a first step in acknowledging these differences it is necessary to outline the differences between sex and gender. Sex is a biological attribute of cells or organisms which encompasses genetics, physiology, anatomy and hormonal status. As a consequence, differences between men and women extend beyond reproductive differences

and are present in every cell and organ. Gender refers to the dichotomisation of human subjects based on behavioural, psychological and cultural factors. Incorporating sex as a biological variable has been championed by the National Institutes of Health and is viewed as an essential element of all research.

While it is well established that energy requirements differ between men and women it is still common practise to analyse interventions at the group level and not stratify according to sex. With a heightened interest in sex differences it has become increasingly apparent that human metabolism is different between the sexes. Of particular note is the fundamental difference in the regulation and deposition of adipose tissue<sup>(1)</sup>. Women typically present with about 10 % higher body fat compared to men and deposit it in a pattern referred to as the gynoid fat deposition, which is an accumulation of body fat below waist around the hips and thighs<sup>(1,2)</sup>. Men however deposit adipose tissue in a pattern referred to as the android fat

Abbreviation: MedDiet, Mediterranean diet.

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**Table 1.** Examples of metabolites that exhibit sex-specific concentration differences

Metabolite class	Metabolite	Men	Women	Reference
Amino acids	Isoleucine	↑		(7,8)
	Leucine	↑		(6–8)
	Pyroglutamate	↑		(7)
	Valine	↑		(6–8)
	Glycine		↑	(6)
Carbohydrates	Mannose	↑		(7)
	Glucose	↑		(7)
	Arabitol	↑		(7)
Energy related	Phosphate		↑	(7)
	Acetylphosphate		↑	(7)
	Succinylcarnitine	↑		(7)
	Malate	↑		(7)
Phosphatidylcholines	PCaaC32:3		↑	(6)
	PCaeC30:2		↑	(6)
	PCaeC32:2		↑	(8)
Lysophosphatidylcholines	LPCaC20:4	↑		(6,8)
	LPCaC18:2	↑		(6,8)
	LPCaC18:1	↑		(6)
Sphingomyelins	SM(OH)C22:2		↑	(6,8)
	SM C20:2		↑	(6,8)
	SM C18:1		↑	(6,8)

All examples are found in studies examining sex difference in serum or plasma samples. The above is a representation of the significant metabolites and not meant to be an exhaustive list.

deposition which is fat accumulation in the upper body and upper abdominal areas<sup>(1)</sup>. It has been proposed that circulating gonadal steroids determine these sex-specific differences in adipose tissue distribution<sup>(1)</sup>; however other factors including the number of X chromosomes has been shown to affect adipose tissue function in mice<sup>(3)</sup>. These fundamental differences between the sexes highlight the importance of considering sex as a variable in nutrition-related research.

Considering the growing use of omics techniques such as metabolomics in nutrition-related research we examine the impact of sex on the metabolomic profile and the alterations in metabolism throughout the menstrual cycle. Furthermore, we assess evidence for differential responses to nutrition interventions based on sex.

### Impact of sex on the metabolomic profile

Metabolomics is the study of small molecules called metabolites in biological samples<sup>(4)</sup>. Application of metabolomics yields information about alteration of metabolic pathways under different conditions. In the context of nutrition, metabolomics has played a key role in the development of dietary biomarkers and in understanding the mechanism underlying dietary interventions. In recent years, a number of studies have identified metabolites that exhibit sexual dimorphism<sup>(5,6)</sup> (Table 1). One of the most comprehensive studies examined metabolic profiles in 903 women and 853 men<sup>(7)</sup>. Interestingly, one-third of the plasma metabolites displayed sexual dimorphism. Using pathway analysis a series of metabolic pathways were identified as different between men and women. Amongst these were amino acid, carbohydrate and lipid metabolic pathways.

Interestingly, not all lipid pathways were different and there appeared to be some specificity in the differences.

In a population of over 3300 individuals, 102 out of the 131 plasma metabolites measured revealed significant differences between men and women<sup>(6)</sup>. Examples of the significant metabolite classes included amino acids, phosphatidylcholines, sphingomyelins, acylcarnitines and C<sub>6</sub>-sugars. Furthermore, this study performed a sex-specific genome-wide association study and identified different associations with glycine and a specific locus for men and women. More specifically, the carbamoyl-phosphate synthetase 1 locus demonstrated significant associations with glycine; however, once sex-stratified genome wide associations were performed there was a significant difference in the  $\beta$ -estimates between men and women for association with glycine. This study highlights the importance of considering sex-specific genetic variants and their differential relationship with metabolites.

In a study determining the reference metabolome levels for healthy individuals, a number of physiological parameters were examined including age and sex<sup>(8)</sup>. The authors concluded that sex was an important determinant of the human-plasma metabolome with key differences in amino acids, acylcarnitines and phosphatidylcholines.

A number of studies have examined the impact of age on the metabolome in conjunction with sex differences<sup>(9,10)</sup>. Examining 148 subjects with an age range from 30 to 100 years revealed a number of metabolites with clear sexual dimorphism. The metabolites could be mapped to five specific pathways: bile acid metabolism, lysine degradation, fatty acid biosynthesis, pentose phosphate pathway and linoleic acid metabolism. However, despite the sexual dimorphism it was possible to identify biomarkers of ageing shared by both sexes.

Furthermore, a set of metabolites were defined that correlated with age independent of sex. Focusing on lipid molecules Gonzales and colleagues identified a panel of lipids that were associated with longevity in women<sup>(10)</sup>. These lipids include phosphatidylcholines, sphingomyelins and long-chain TAG. Furthermore, several of these lipids were associated with a lower risk of hypertension and diabetes.

While the abovementioned papers have focused their efforts on metabolites in plasma and serum there are also studies that examined the impact of sex on the urinary metabolome. For example, Okemoto and colleagues reported sexual dimorphism in a series of lipid-related urinary molecules<sup>(11)</sup>. Using a range of metabolomic platforms, a series of metabolites in both urine and plasma were found to be discriminatory between men and women<sup>(12)</sup>. Included in these were urinary levels of amino acids, sugars and keto acids. Other studies also support the influence of sex on these urinary metabolites<sup>(13–15)</sup>.

Overall, these studies highlight that sex is an important factor determining the levels of certain metabolites in both plasma and urine. Furthermore, as metabolites are emerging as key potential biomarkers it is imperative that reference values specific to men and women are established and that analysis stratified by sex becomes commonplace.

#### Changes in metabolite levels during the menstrual cycle

For many years, the menstrual cycle represented a complication in nutrition research that many sought to avoid through use of only male subjects. However, in recent years it has become apparent that to truly deliver personalised healthcare one must understand the metabolic alterations occurring through the menstrual cycle. The menstrual cycle is defined by five distinct phases that are characterised by differences in hormone levels. The later part of the cycle, characterised by raised levels of both oestrogen and progesterone, is referred to as the luteal phase. During this phase of the cycle it has been reported that women experience a worsening of disease risk factors; for example women with type-2 diabetes had poor glycaemic control during the luteal phase<sup>(16)</sup>. To fully appreciate female metabolism alterations during the menstrual cycle are crucial to understand. To achieve this, we recruited thirty-four females and collected blood samples once weekly during one menstrual cycle<sup>(17)</sup>. Using a combination of the menstrual calendar and serum hormone levels each sample was assigned into one of the five menstrual phases. Analysis of the plasma NMR metabolomics data revealed significant differences in the profile across the menstrual cycle. More specifically, there were decreased levels of alanine, glutamine, lysine, glycine, serine and creatinine and increased levels of hydroxybutyrate, VLDL CH<sub>2</sub> and acetoacetate during the luteal phase. Considering this and previous literature we concluded that the decreased levels in the luteal phase were reflective of increased utilisation. In a follow-up study we expanded the range of metabolites measured

in these samples and employed a multi-platform approach to capture approximately 400 metabolites and nutrients<sup>(18)</sup>. Examination of the changes across the phases revealed that sixty-seven metabolites related to amino acid, lipid, carbohydrate, energy and vitamin metabolism significantly changed with the majority decreasing in the luteal phase. This consistent decrease in the luteal phase in comparison with the follicular phase is supported by the enhanced lipid, steroid and endometrial biosynthesis during this period. Furthermore, women have higher energy expenditure in the luteal phase supporting the observed decreased in energy metabolism intermediates<sup>(19)</sup>. In a Chinese population similar results were reported; a number of metabolites including amino acids changed significantly across the menstrual cycle reaching the lowest level in the luteal phase<sup>(20)</sup>. Furthermore, a number of free fatty acids increased in the luteal phase.

Collectively, these results help provide the evidence base necessary to develop nutrition advice for women that is specific to the phase of the menstrual cycle. Considering the evidence, developing advice that addresses the higher energy expenditure and lower levels of amino acids in the luteal phase would be an advancement on the ‘one diet fits all’ current approach.

#### Impact of sex on responses to dietary interventions

Examination of the literature with respect to sex responses to dietary interventions revealed that a convincing case existed for the response to the Mediterranean diet (MedDiet). Thirty-eight men and thirty-two premenopausal women with slightly elevated LDL-cholesterol concentrations or total cholesterol:HDL-cholesterol ratio participated in a 4-week isoenergetic controlled experimental diet based on the traditional MedDiet. Bedard and colleagues found cardiometabolic variables differed between men and women in response to the diet<sup>(21)</sup>. Total cholesterol, LDL-cholesterol, ApoB and ApoA-1 plasma concentrations and diastolic blood pressure decreased in both men and women ( $P < 0.05$ ). However, ApoA-2 concentrations and insulin concentrations 2 h after the oral administration of 75 g glucose demonstrated sex  $\times$  time interactions (respectively,  $P = 0.05$  and  $P = 0.03$ ) with only men experiencing a significant decrease<sup>(21)</sup>. In the same cohort, adiponectin concentrations decreased in response to the MedDiet, but this decrease only reached statistical significance in men ( $P < 0.001$ ). Interestingly, leptin response to the MedDiet was comparable in both men and women<sup>(22)</sup>. No sex difference was observed in high-sensitivity C-reactive protein concentrations in response to the MedDiet, with both men and women experiencing no change<sup>(23)</sup>. With respect to lipoprotein particles sex differences were found in response to the MedDiet for the proportion of medium LDL ( $P$  for sex  $\times$  time interaction = 0.01) and small, dense LDL (trend;  $P$  for sex  $\times$  time interaction = 0.06), men experiencing an increase in the proportion of medium LDL with an associated reduction in the proportion of small, dense LDL, while an opposite

trend was observed in women. A sex difference was also noted for estimated cholesterol concentrations among small, dense LDL ( $P$  for sex  $\times$  time interaction = 0.03), with only men experiencing a reduction in response to the MedDiet. Finally, results demonstrated that the more deteriorated LDL particle size features found in men compared with premenopausal women at baseline were no longer present after the short-term consumption of the MedDiet<sup>(24)</sup>. Collectively, these studies examining the MedDiet clearly reveal sex differences in response to the intervention and highlight the importance of considering sex as a biological variable.

Leblanc and colleagues also investigated sex differences in response to the MedDiet<sup>(25)</sup>. Sixty-four men and fifty-nine premenopausal women participated in a 12-week nutritional intervention programme promoting adoption of the MedDiet. Changes observed in total cholesterol:HDL-cholesterol ratio, TAG levels and TAG:HDL-cholesterol ratio were more pronounced in men than in women after the intervention as well as at follow-up ( $P \leq 0.03$ )<sup>(25)</sup>.

Sex differences have also been reported in response to low fat diets. A study comparing a diet low in total fat, saturated fat and cholesterol with an average American diet found the low in total fat, saturated fat and cholesterol diet resulted in greater reductions in total cholesterol, plasma ApoB and postprandial TAG concentrations in men than in women ( $P < 0.05$ ). LpAI:AI concentrations were also reduced in men, but not in women ( $P < 0.05$ )<sup>(26)</sup>.

Postprandial lipaemia following a standardised challenge meal was measured in age and BMI matched young healthy men ( $n$  5) and women ( $n$  5) following 2 weeks on the self-selected low fat low cholesterol diet and after another two weeks on the self-selected high fat high cholesterol diet. Postprandial triglyceridaemia did not differ in both sexes on the low fat low cholesterol diet, however women were able to adapt better to the high fat high cholesterol diet as men displayed higher postprandial triglyceridaemia on this diet ( $P < 0.05$ ). The results suggest there may be sex differences in the mechanisms regulating the postprandial lipaemia response to different diets, however this is based on a very small population size and further confirmation in a larger population is warranted<sup>(27)</sup>.

Other studies have also highlighted the importance of considering differential responses to interventions. A randomised crossover study comparing the effects of a lean red-meat diet and a high-dairy diet on insulin sensitivity found fasting insulin was significantly higher after the dairy diet compared to the red-meat diet ( $P < 0.01$ ) with no change in fasting glucose resulting in a decrease in insulin sensitivity after the high-dairy diet ( $P < 0.05$ ) as assessed by homoeostasis model assessment of insulin resistance. However, a *post hoc* analysis revealed a significant interaction between diet and sex ( $P < 0.05$ ), with insulin and homoeostasis model assessment of insulin resistance significantly different between red-meat and dairy diets in women only ( $P < 0.01$ ). Insulin sensitivity, calculated using the Matsuda method, was 14.7% lower in women after the dairy diet compared to the red-

meat diet ( $P < 0.01$ ) with no difference found between diets in men<sup>(28)</sup>.

Collectively, the above studies support the importance of investigating sex-related differences in response to diet. Further studies are needed to acquire a better understanding of sex differences in response to additional diets, which may be useful to provide sex-specific nutritional strategies in the prevention of CVD and other metabolic diseases and further individualise dietary guidelines.

### Precision Nutrition and future outlook

Precision Nutrition is the delivery of dietary advice to an individual's needs<sup>(29)</sup>. Ultimately, in the strive to deliver more precise advice we have to take sex differences into account. This will require a better understanding of responses to interventions and biological requirements of nutrients throughout the menstrual cycle. Considering the fundamental differences between men and women, a first step in the delivery of Precision Nutrition will be establishing the evidence base for the development of sex-specific recommendations incorporating responses to certain dietary regimes. There is also the potential to develop our knowledge further of metabolic changes during the menstrual cycle and to harness these for development of specific dietary advice. With respect to metabolomics it is evident that sex impacts the metabolomic profile and needs to be considered in the analysis of metabolomic data. Furthermore, it is emerging that metabolic responses and regulation are different in men and women and that such differences will have an influence on response to dietary interventions. Examining sex as a biological variable needs to become inherent in the data analysis procedures in nutrition research. By achieving this we will build our knowledge on metabolic differences between the sexes and the subsequent impact on response to dietary interventions.

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### Conflict of Interest

None.

### Authorship

L. B. designed the outline of the paper. L. B. and H. G. drafted the paper.

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