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## Spices in the postprandial metabolic regulation of healthy humans

### An integrated physiological and omics approach

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# Spices in the postprandial metabolic regulation of healthy humans

An integrated physiological and omics approach

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Spices in the postprandial metabolic regulation of healthy humans –  
An integrated physiological and omics approach



# Spices in the postprandial metabolic regulation of healthy humans

An integrated physiological and omics approach

Yoghatama Cindya Zanzer



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DOCTORAL DISSERTATION

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<b>Title and subtitle: Spices in the postprandial metabolic regulation of healthy humans – An integrated physiological and omics approach</b>		
<b>Abstract</b> <p>Metabolic syndrome (MetS) is a consequence of obesity and defined as cluster of at least three out of five criteria covering insulin resistance/glucose intolerance, abdominal obesity, hypertension, low HDL-c and elevated TG levels. Imbalance between energy intake and expenditure is known to alter normal physiological function in many aspects, and leads to obesity, which in the long run may turn into type 2 diabetes (T2D) and ultimately cardiovascular disease (CVD). Looking at our everyday meal patterns (breakfast, lunch, dinner and snacks in between), we are constantly being challenged by foods that vary in macronutrient composition. In association with meal patterns, it is well established that the postprandial (occurring a meal) response is importantly associated with cardiovascular risk factors. Exaggerated response in the postprandial glucose and TG levels are known to provoke an atherogenic milieu.</p> <p>Diet and lifestyle interventions are key to alleviating obesity prior to the onset of CVD. Several strategies have been suggested to be beneficial, including the consumption of fibre-rich and whole grains foods, increasing fruit and vegetable intake, lowering fat and processed meat intake, reducing high sugar foods and salt as well as avoiding saturated and trans-fatty acids consumption, features that are also part of current Nordic Nutrition Recommendations. Plants are rich in various bioactive compounds owing pleiotropic health-associated benefits. Many studies have shown that diverse bioactives might beneficially influence several aspects of human metabolism by improving appetite regulation mediated by gut hormones or through their interaction with transient receptor potential (TRP) channels, inhibiting <math>\alpha</math>-amylase and <math>\alpha</math>-glucosidase leading to reduced glucose absorption, affecting lipid metabolism by influencing lipid-associated enzymes as well as modulating the transcription factors of genes associated with metabolic processes. Although in a small amount, spices are one the most commonly consumed plant products, often in the form of sauces. Despite extensive investigation on certain spices such as turmeric and cinnamon, evidences for their beneficial effects in humans are limited, particularly in the postprandial setting and therefore need to be explored.</p> <p>In a meal study performed within this dissertation, spices such as turmeric and cinnamon formulated as beverages, significantly improved early postprandial glycemia when consumed prior to white wheat bread (WWB) as a meal challenge. Not only that, improved appetite modulation was also achieved by turmeric- and star anise-based beverages, with the gut hormone peptide tyrosine-tyrosine (PYY) being increased after turmeric-based beverage. Another study included in this dissertation also showed that a black pepper-based beverage improved overall appetite regulation, but the mechanism behind this response might not be related to gut hormones release. Given prominence to the favourable effect of turmeric on glucose and appetite regulation from the earlier study, another study included in this dissertation showed that turmeric-based beverages significantly improved lipid and oxidative stress markers when consumed prior to medium (MF) or high fat (HF) meals. Furthermore, another study using spice blend included in a high fat meal (HF-Spices) was compared to high fat meal (HF) alone as control. Relative to HF, HF-Spices showed a profound effect in reducing postprandial insulin and increasing insulin sensitivity as well as increasing high density lipoprotein cholesterol (HDL-c). In addition, appetite sensations were also modulated after HF-Spices relative to HF. To a certain extent, cardiometabolic risk markers and appetite sensations were differently affected by gender and metabolic status (i.e. body mass index), which clearly marks the importance of inter-individual variability and supports the concept of personalized nutrition in nutritional research and interventions.</p> <p>Bile acids are known to facilitate lipid digestion and absorption, and are important players in lipid and glucose metabolisms. Turmeric-based beverage consumed prior to MF or HF was shown to modulate the circulating bile acid profiles in postprandial setting, turning it into a possible healthier one.</p> <p>A transcriptomic array covering more than 47000 genes involved in human metabolism revealed possible underlying mechanisms involved in the different responses observed after the intervention. In a study covered within this dissertation, mixed spices included in a high fat meal profoundly dampened inflammatory response indicated by a lower inflammation-associated gene expression of peripheral blood mononuclear cells (PBMCs), such as interleukin (IL)-8, tumour necrosis factor alpha (TNF-<math>\alpha</math>) and prostaglandin-endoperoxide synthase (PTGS)2. The enriched differentially expressed genes were related with pathways and processes involved in inflammation, apoptosis, lipid metabolism and cell adhesion associated with early atherosclerosis process. Furthermore, HF-Spices also appeared to promote cholesterol efflux, possibly mediated by the increase of ATP-binding cassette transporter (ABCA)1 expression in PBMCs accompanied by increased HDL-c levels relative to HF.</p> <p>In conclusion, this dissertation brings novel findings and sheds light on the beneficial effect of spices on the human metabolism. This offers new evidence for advocating the inclusion of bioactive-rich spices in meals in order to mitigate the alteration of cardiometabolic risk-associated markers induced by the diet.</p>		
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# Spices in the postprandial metabolic regulation of healthy humans

An integrated physiological and omics approach

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*To my son, wife and family*

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## Abstract

Metabolic syndrome (MetS) is a consequence of obesity and defined as cluster of at least three out of five criteria covering insulin resistance/glucose intolerance, abdominal obesity, hypertension, low HDL-c and elevated TG levels. Imbalance between energy intake and expenditure is known to alter normal physiological function in many aspects, and leads to obesity, which in the long run may turn into type 2 diabetes (T2D) and ultimately cardiovascular disease (CVD). Looking at our everyday meal patterns (breakfast, lunch, dinner and snacks in between), we are constantly being challenged by foods that vary in macronutrient composition. In association with meal patterns, it is well established that the postprandial (occurring a meal) response is importantly associated with cardiovascular risk factors. Exaggerated response in the postprandial glucose and TG levels are known to provoke an atherogenic milieu.

Diet and lifestyle interventions are key to alleviating obesity prior to the onset of CVD. Several strategies have been suggested to be beneficial, including the consumption of fibre-rich and whole grains foods, increasing fruit and vegetable intake, lowering fat and processed meat intake, reducing high sugar foods and salt as well as avoiding saturated and trans-fatty acids consumption, features that are also part of current Nordic Nutrition Recommendations. Plants, including spices are rich in various bioactive compounds owing pleotropic health-associated benefits. Many studies have shown that diverse bioactives might beneficially influence several aspects of human metabolism by improving appetite regulation mediated by gut hormones or through their interaction with transient receptor potential (TRP) channels, inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase leading to reduced glucose absorption, affecting lipid metabolism by influencing lipid-associated enzymes as well as modulating the transcription factors of genes associated with metabolic processes. Although in a small amount, spices are one the most commonly consumed plant products, often in the form of sauces. Despite extensive investigation on certain spices such as turmeric and cinnamon, evidences for their beneficial effects in humans are limited, particularly in the postprandial setting and therefore need to be explored.

In a meal study performed within this dissertation, spices such as turmeric and cinnamon formulated as beverages, significantly improved early postprandial glycemia when consumed prior to white wheat bread (WWB) as a meal challenge. Not only that, improved appetite modulation was also achieved by turmeric- and star anise-based beverages, with the gut hormone peptide tyrosine-tyrosine (PYY) being increased after turmeric-based beverage. Another study included in this dissertation also showed that a black pepper-based beverage improved overall appetite regulation, but the mechanism behind this response might not be related to

gut hormones release. Given prominence to the favourable effect of turmeric on glucose and appetite regulation from the earlier study, another study included in this dissertation showed that turmeric-based beverages significantly improved lipid and oxidative stress markers when consumed prior to medium (MF) or high fat (HF) meals. Furthermore, another study using spice blend included in a high fat meal (HF-Spices) was compared to high fat meal (HF) alone as control. Relative to HF, HF-Spices showed a profound effect in reducing postprandial insulin and increasing insulin sensitivity as well as increasing high density lipoprotein cholesterol (HDL-c). In addition, appetite sensations were also modulated after HF-Spices relative to HF. To a certain extent, cardiometabolic risk markers and appetite sensations were differently affected by gender and metabolic status (*i.e.* body mass index), which clearly marks the importance of inter-individual variability and supports the concept of personalized nutrition in nutritional research and interventions.

Bile acids are known to facilitate lipid digestion and absorption, and are important players in lipid and glucose metabolisms. Turmeric-based beverage consumed prior to MF or HF was shown to modulate the circulating bile acid profiles in postprandial setting, turning it into a possible healthier one.

A transcriptomic array covering more than 47000 genes involved in human metabolism revealed possible underlying mechanisms involved in the different responses observed after the intervention. In a study covered within this dissertation, mixed spices included in a high fat meal profoundly dampened inflammatory response indicated by a lower inflammation-associated gene expression of peripheral blood mononuclear cells (PBMCs), such as interleukin (IL)-8, tumour necrosis factor alpha (TNF- $\alpha$ ) and prostaglandin-endoperoxide synthase (PTGS)2. The enriched differentially expressed genes were related with pathways and processes involved in inflammation, apoptosis, lipid metabolism and cell adhesion associated with early atherosclerosis process. Furthermore, HF-Spices also appeared to promote cholesterol efflux, possibly mediated by the increase of ATP-binding cassette transporter (ABCA)1 expression in PBMCs accompanied by increased HDL-c levels relative to HF.

In conclusion, this dissertation brings novel findings and sheds light on the beneficial effect of spices on the human metabolism. This offers new evidence for advocating the inclusion of bioactive-rich spices in meals in order to mitigate the alteration of cardiometabolic risk-associated markers induced by the diet.

## Popular science summary

Foods contain different macronutrients, as sources of energy, and other minor compounds (*i.e.* vitamins and minerals) which are needed in lower amount but are essential for the normal functioning orchestrated human physiology. In the condition of energy shortage, signals will emanate and the nerve cells in the hypothalamus of the brain will be activated urging the body to eat. When we are sufficiently full and feel satisfied from foods, signals are then sent to the brain prompting us to stop eating. The orchestrated system of energy balance is coordinated by hypothalamus with the help of hormones as chemical messengers. In the everyday life, with three meal times and snacks in between, we are constantly being challenged by foods that vary in macronutrient compositions. Eating too much will result in a positive energy balance and the excess of energy consequently will be stored as fat in the body. In the long run, positive energy balance together with a sedentary lifestyle lead to a condition termed as obesity, and poor management of obesity will eventually lead to type 2 diabetes (T2D) and ultimately cardiovascular disease (CVD). In general, the orchestrated human physiological system is strictly coordinated, resulting in a homeostatic or a 'back to balance' condition when the body is challenged by stressors such as over-consumption of energy-dense fatty foods. However, resilience to the exaggerated responses caused by constant consumption of energy-dense fatty foods will eventually disturb the system. Various bioactive compounds in plants have generally shown to exert beneficial effects in mitigating cardiometabolic risk factors, such as improved blood glucose and lipid concentrations. Having a continuous increase in the total volume of trade over the last few years, spices play an important role not only for global market but also in the food and culinary world. However, current science-based evidence on the potentially favourable effects of spices in association with reducing cardiometabolic risk factors are limited and therefore need to be explored.

Within this context, the work presented in this dissertation was aimed to broaden our knowledge on the beneficial effects of spice consumption as one among other strategies in the prevention of CVD by targeting different risk factors such as those associated with blood glucose and lipid regulation. Other factors related to the energy balance, which include appetite and appetite-related gut hormone responses, were also explored. Other molecules involved in glucose and lipid regulation, such as bile acids which are classically known to promote lipid digestion, were also investigated. Importantly, in order to gain a better insight into possible underlying mechanisms on the beneficial effects that spices may exert when consumed with fatty meals, transcriptomics using a human metabolism gene expression array was also explored. By combining clinical markers associated with cardiometabolic risk factors with the high-throughput gene expression allowed us to get a comprehensive insight into the possible underlying mechanisms.



In one of the studies included in this dissertation, turmeric and cinnamon-based beverages showed an early reduction in the blood glucose response following a carbohydrate-containing meal (white wheat bread/WWB). In addition, turmeric- and black pepper-based beverages were shown to promote higher satiety when consumed prior to WWB. Gaining an interest on the favourable effects of turmeric in blood glucose and appetite regulation, another study included in this dissertation showed that a turmeric-based beverage significantly improved lipid and oxidative stress markers as one of several cardiometabolic risk markers when consumed immediately before medium (MF) or high fat (HF) meals. Another study included in this dissertation showed that mixed spices incorporated into HF had a beneficial effect on blood glucose and lipid regulation, appetite and inflammation caused by bacterial toxin (metabolic endotoxemia). To a certain extent, this dissertation also showed that cardiometabolic and associated risk markers were differently affected by gender and metabolic status.

Bile acids are molecules produced in the liver that are responsible for facilitating fat digestion and absorption, and they also play a role in the regulation of cholesterol metabolism. In this dissertation, a turmeric-based beverage, when consumed prior to MF or HF, significantly modified circulating bile acid profiles turning into a possible healthier one.

Current technological advances enable us to see which genes in cells are significantly expressed after a particular condition and enable us to predict the underlying molecular mechanisms of their relevance in disease-related processes. In order to see which processes are involved in the beneficial effects pertained from an earlier study with mixed spices, high-throughput gene expression analysis was performed. This analysis showed that enriched differentially expressed genes were related with processes involved in inflammation, apoptosis and cell adhesion associated with early atherosclerosis process as well as lipid metabolism. A high fat meal enriched with mixed spices significantly lowered the inflammatory response genes such as interleukin (IL)-8, tumour necrosis factor alpha (TNF- $\alpha$ ) and prostaglandin-endoperoxide synthase (PTGS)2 and promoted athero-protective effects shown by increased high density lipoprotein cholesterol (HDL-c) levels accompanied with the activation of the receptor-mediator involved in the cholesterol efflux mechanism named ATP-binding cassette sub-family A member (ABCA)1.

In conclusion, this dissertation brings novel findings and sheds light on the beneficial effect of spices in the human metabolism. This offers new evidence for advocating the inclusion of bioactive-rich spices in meals in order to mitigate the alteration of cardiometabolic risk and associated markers induced by the diet.

## Populärvetenskaplig sammanfattning

Livsmedel innehåller olika näringsämnen som energikällor som åtföljs av andra mindre föreningar som vitaminer och mineraler, vilka behövs i lägre mängd men är väsentliga för normal funktion för den övergripande humanfysiologin. I tillstånd av energibrist, kommer nervcellerna i hjärnans hypotalamus aktiveras och uppmanar kroppen att äta. När vi är tillräckligt mätta och känner oss nöjda med maten skickas även signaler till hjärnan som ber oss att sluta äta. Systemet för energibalans koordineras av hjärnans hypotalamus med hjälp av hormoner som kemiska budbärare. I vardagen, med tre måltider och mellanmål blir vi ständigt utmanade av livsmedel som varierar när det gäller kvalitet och kvantitet. Att äta för mycket kommer att resultera i en positiv energibalans och överflödet av energi kommer följaktligen att lagras som fett i kroppen. På lång sikt leder positiv energibalans tillsammans med en stillasittande livsstil till ett tillstånd som kallas fetma. Fetma i sig är ett tillstånd av förändrad fysiologisk funktion som orsakas av överskott av fettavsättning i organen och kroppen i allmänhet. Dålig hantering av fetma kan så småningom leda till typ 2-diabetes (T2D) och slutligen kardiovaskulär sjukdom (CVD). I allmänhet är det mänskliga fysiologiska systemet strikt samordnat vilket resulterar i ett homeostatiskt eller återställt "balans" tillstånd när kroppen utmanas av stressorer, såsom över- förbrukning av energitäta feta livsmedel. Emellertid kommer motståndskraften mot de överdrivna svaren som orsakas av en konstant konsumtion av energitäta feta livsmedel i slutändan att förskjuta systemet så att det inte är så flexibelt som tidigare.

Olika bioaktiva föreningar i växter har generellt visat sig utöva fördelaktiga effekter för att mildra kardiometabolisk-riskfaktorer, såsom förbättrad blodglukos och lipidkoncentrationer. Med en kontinuerlig ökning av den totala volymen av handel under de senaste åren spelar kryddor en viktig roll, inte bara för den globala marknaden utan även i mat och i den kulinariska världen. Men nuvarande vetenskapsbaserade bevis på de potentiellt gynnsamma effekterna av kryddor i samband med att minska kardiometabolisk-riskfaktorer är begränsade och behöver därför undersökas.

Inom detta sammanhang syftade det arbete som presenteras i denna avhandling till att bredda vår kunskap om kryddkonsumtionens fördelaktiga effekt som en bland andra strategier för förebyggande av CVD genom att undersöka olika riskfaktorer, såsom de som hör samman med blodglukos och lipidreglering. Andra faktorer som är relaterade till energibalansen, som innefattar aptit-och aptitrelaterade tarmhormonrespons, undersöktes också. Andra molekyler som är involverade i glukos- och lipidreglering, benämnda gallsyror som är kända för att främja nedbrytning och upptag av fett i kosten, undersöktes även. Viktigt är att för att få en annan inblick i möjliga underliggande mekanismer om de fördelaktiga effekter som

kryddor kan utöva när de konsumeras med feta måltider, undersöktes genuttryck i blodceller (transkriptomik), som omfattade över 47000 gener som är involverade i mänsklig metabolism. Genom att kombinera kliniska markörer som är associerade med kardiometabolisk-riskfaktorer med denna analys ger genuttrycket oss en omfattande inblick i de möjliga underliggande mekanismerna.

I en av de studier som ingår i denna avhandling, visade gurkmeja och kanelbaserade drycker en tidig minskning av blodsockerresponsen efter en kolhydrathaltig måltid (vitt bröd/WWB). Dessutom främjade gurkmeja- och svartpepparbaserade drycker även högre mättnad när de konsumerades före WWB. Gurkmejans gynnsamma effekt för blodglukos och aptitreglering visade en annan studie som ingår i denna avhandling, där en gurkmejebaserad dryck signifikant förbättrade lipid- och oxidativa stressmarkörer när de konsumerades omedelbart före medium- (MF) eller hög fetthalt- (HF) måltider. En studie som ingår i denna avhandling med blandade kryddor införlivade i HF visade också en positiv effekt i blodglukos och lipidreglering, aptit och inflammation orsakad av bakteriellt toxin (metabolisk endotoxemi). I viss utsträckning visade denna avhandling också att kardiometabolisk-riskrelaterade markörer påverkades på olika sätt av kön och metabolisk status. Gallsyror är molekyler som produceras i levern som är ansvariga för att underlätta fettnedbrytning och absorption, och de spelar också en roll vid reglering av kolesterolmetabolism. I denna avhandling observerades det att en gurkmejebaserad dryck när den konsumeras innan MF eller HF signifikant modifierade gallsyraprofilen till en hälsosammare profil.

Nuvarande tekniska framsteg gör det möjligt för oss att se vilka gener i celler som uttrycks efter ett visst tillstånd och gör att vi kan förutsäga de underliggande molekyllära mekanismerna som är relevanta för sjukdomsrelaterade processer. För att se vilka processer som är inblandade i de fördelaktiga effekter som härrör från tidigare studier med blandade kryddor, genomfördes analys av genuttryck. Denna analys visade att berikade differentiellt uttryckta gener var relaterade till processer involverade i inflammation, celledöd och cellsignaler associerade med tidiga åderförkalkningsprocesser liksom lipidmetabolism. Högfettmjöl som berikats med blandade kryddor sänkte signifikant de inflammatoriska responsgenerna såsom interleukin (IL)-8, tumörnekrosfaktor alfa (TNF- $\alpha$ ) och prostaglandin-endoperoxidsyntas (PTGS)2 och främjade kärl-skyddande effekter, där ökade nivåer av hög densitet lipoproteinkolesterol (HDL-c) nivåer observerades. Sammanfattningsvis ger denna avhandling nya fynd och belyser kryddornas fördelaktiga effekt i människans ämnesomsättning. Detta kan erbjuda nya bevis för näringsrekommendationer att inkludera bioaktiva kryddor i måltider för att mildra det överdrivna svaret hos vissa kardiometabolisk-riskrelaterade markörer som framkallas av kosten.

## List of scientific papers

- Paper I Polyphenol-rich spice-based beverages modulated postprandial early glycaemia, appetite and PYY after breakfast challenge in healthy subjects: A randomized, single blind, crossover study.
- Y. C. Zanzer**, M. Plaza, A. Dougkas, C. Turner, I Björck and E Östman
- Journal of Functional Foods* (2017) 35:574-583.  
doi:10.1016/j.jff.2017.06.016.
- Paper II Black pepper-based beverage induced appetite-suppressing effects without altering postprandial glycaemia, gut and thyroid hormones or gastrointestinal well-being: a randomized crossover study in healthy subjects.
- Y. C. Zanzer**, M. Plaza, A. Dougkas, C. Turner and E Östman
- Food and Function* (2018) 9, 2774-2786. doi:10.1039/c7fo01715d.
- Paper III Inclusion of turmeric-based beverage before high or medium isocaloric meals distinctively modify postprandial lipemia, glycemia, oxidative stress and metabolic endotoxemia in healthy subjects.
- Y. C. Zanzer**, Â. G. Batista, A. Dougkas, J. Tovar, Y. Granfeldt and E. Östman
- Submitted article*
- Paper IV Modulation of postprandial serum bile acids composition by turmeric-based beverage preload consumed prior to isocaloric high- or medium-fat meal challenge in healthy adults: a randomized crossover study.
- T. Ghaffarzadegan, **Y. C. Zanzer**, E. Östman, F. Fåk Hållenius, S. Essén, M. Sandahl and M. Nyman
- Submitted article*

Paper V Distinctive effects of mixed spices-enriched high fat meal on postprandial glucose metabolism, appetite, thyroid hormones and metabolic endotoxemia in healthy subjects.

**Y. C. Zanzer**, C. Xue, M. Plaza, A. Dougkas, J. Tovar, F. Fåk Hållenius, Y. Granfeldt and E. Östman

*Submitted article*

Paper VI Acute intake of mixed spices with high fat meal promotes cholesterol efflux metabolism and modulated lipid and inflammation-associated metabolic pathways in healthy subjects

**Y. C. Zanzer**, C. Xue, M. Plaza, M. L. Marina, F. Fåk Hållenius, Y. Granfeldt and E. Östman

*Manuscript article*

## The author's contributions

- Paper I The author designed the study, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research, performed biochemical and statistical analysis and wrote the manuscript.
- Paper II The author designed the study, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research, performed biochemical and statistical analysis and wrote the manuscript.
- Paper III The author conceived the idea of the study and its design, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research as well as responsible for data acquisition, performed biochemical and statistical analysis and wrote the manuscript.
- Paper IV The author performed clinical research, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research, performed statistical analysis in bile acids results and took part in manuscript writing.
- Paper V The author conceived the idea of the study and its design, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research as well as responsible for data acquisition, performed biochemical and data analysis and wrote the manuscript.
- Paper VI The author conceived the idea of the study and its design, wrote the clinical experiment protocol for ethical submission, led and managed the clinical research as well as responsible for data acquisition, performed transcriptomics and bioinformatics data analysis and wrote the manuscript.

## List of abbreviations

AIP	atherogenic index of plasma
Apo-(A1; B)	apolipoprotein-(A1; B)
(i, t, net) AUC	(incremental, total, net) area under the curve
BMI	body mass index
CA	cholic acid
CDCA	chenodeoxycholic acid
CVD	cardiovascular disease
DAD	diode array detector
DCA	deoxycholic acid
ESI	electrospray ionization
FFA	free fatty acids
FP-IS	estimated first-phase insulin secretion
FRAP	Ferric reducing ability of plasma
GLP-1	glucagon-like peptide 1
GAE	gallic acid equivalent
GCA	glyco-cholic acid
GCDCA	glyco-chenodeoxycholic acid
GDCA	glyco-deoxycholic acid
GLCA	glyco-lithocholic acid
GUDCA	glyco-ursodeoxycholic acid
HDCA	hyodeoxycholic acid
HDL-c	high density lipoprotein cholesterol
HF	high fat meal
IGI	insulinogenic index
ISI <sub>est</sub>	estimated insulin sensitivity index
LBP	lipopolysaccharide binding protein
LCA	lithocholic acid
LDL-c	low density lipoprotein cholesterol
LPS	lipopolysaccharide
MCR <sub>est</sub>	estimated metabolic clearance rate of glucose
MDA	malondialdehyde
MetS	metabolic syndrome
MF	medium fat meal
MLI	metabolic load index
MS	mass spectrometry
NEFA	non-esterified fatty acids
PCA	principal component analysis
PYY	peptide tyrosine-tyrosine
QTOF	quadrupole time-of-flight

sCD14	soluble cluster of differentiation 14
SP-IS	estimated second-phase insulin secretion
TBARS	thiobarbituric acid reactive substances
TC	total cholesterol
TCA	tauro-cholic acid
TCDCA	tauro-chenodeoxycholic acid
TDCA	tauro-deoxycholic acid
TLCA	tauro-lithocholic acid
TUDCA	tauro-ursodeoxycholic acid
TEAC	trolox-equivalent antioxidant capacity
T2D	type 2 diabetes mellitus
T3	triiodothyronine
T4	thyroxine
UDCA	ursodeoxycholic acid
UHPLC	ultra-high performance liquid chromatography
VAS	visual analogue scale
WWB	white wheat bread





# Introduction

*“The first wealth is health”* – **Ralph Waldo Emerson**

The prevalence of metabolic syndrome (MetS) is increasing in all parts of the world at alarming rate, costing in terms of both economics and health burden for individuals and society. MetS is a consequence of obesity and defined as cluster of at least three out of five criteria covering insulin resistance/glucose intolerance, abdominal obesity, hypertension, low HDL-c and elevated TG levels (1). Current reports estimate that the prevalence of obesity has doubled in more than 70 countries and this led to the development of type 2 diabetes (T2D), atherosclerosis and ultimately cardiovascular disease (CVD) (2). A consequence of a daily pattern of three meals and inter-meal snacks per day implies that we are in postprandial state during the whole life. Resilience to external stressors, such as exaggerated acute and chronic exposure with high glycemic and fatty meals, leads to the metabolic adaptation of the system. The adaptive response system which could be determined by a series of physiological processes and molecular mechanisms with metabolic and inflammatory processes as a core and embedded into psycho-neuro-endocrine control mechanisms as a whole define the *‘phenotypic flexibility’* in an individual (3). Therefore, integrated mechanisms and processes that maintain this flexibility in an organism as a phenotype is an optimal metabolic health. However, a tight regulation in homeostatic balance might deviate from its *‘flexibility’* which in turn cannot compensate the system itself causing to *‘inflexibility’* of the system leading to pathological condition, in this context associated with MetS. Another factor that might alter the *‘flexibility’* is aging process which known owing altered metabolic characteristic along the life-stage (4). Given the fact that early perturbation observed to the homeostatic balance might be modifiable, therefore a preventive measure aiming to compensate the system into *‘flexibility’* through lifestyle modification with diet and physical activity are pivotal. Owing its distinctive bioactive compounds and health-associated benefits, particularly in association with CVD risk factors– spices are of particular of interest to be explored and as the main focus in this dissertation.



# Background

## *Spices*, spicy stories from Oriental to western world

Spices and herbs have been around for centuries shaping the world face not only in terms of trade, but also through inter-cultural exchange in the culinary and food aspects. Generally, spices are derived from roots, stems, berries, seeds, fruit, flowers and bark, which are usually dried, while leaves of plants which are used in cooking could be referred to as herbs (5). Thyme was reported to be used by Sumerians as early as 5000 BC (6), while early 3000 BC farmers of Mesopotamia were reported to grow garlic (5, 7). Spices such as coriander, fennel, juniper, cumin, garlic and thyme were recorded in Egyptian papyri from 1555 BC (5). In China, Shennong Bencao Jing (Shennong Native Herbs Anthology) is considered as the first Chinese monograph on pharmacology, covering 365 medicinal products including plant, animal and mineral, and a guide on how to differentiate foods from drugs and poisons (8). Recent excavations at Quseir al-Qadim, an ancient port located on the Red Sea coast of Egypt, have provided new evidence for the Islamic and Roman spice trade ca. AD 1-250, known as Myos Hormos, and during ca. AD 1050-1500, known as Kusayr (9). Archeobotanical remains of spices such as turmeric, black pepper, ginger, cardamom, betelnut and black myrobalan have been found in the excavations (9). At the end of middle-ages era where the demand of spices was rising, owing its preventive and curative medical properties (10), European explorers such as Columbus and Magellan set sails to search towards the spice islands, which are now called the Moluccas located in Indonesia. At that time, nutmeg and cloves, which only produced exclusively from the islands, were worth more than gold. The Portuguese wrested control of the spice markets and trade route from seafaring Muslim merchants in 1511. In 1595, Dutch ships led by Cornelius de Houman begin the spices trade monopoly in Indonesia through the establishment of Vereenigde Oost-Indische Compagnie (11). About 350 years ago, Manhattan in the United States, which now has Times Square, was swapped with the spice island, Pulau Run, a tiny volcanic island that is now part of Indonesia under the 1667 Treaty of Breda that ended the second Anglo-Dutch war led to nutmeg monopoly of the Dutch (12). Today, surprisingly, while demand for spices is staggeringly large and growing, we still do not have a good grasp the health-associated beneficial effects spices have on humans (13).

## Appetite regulation

Obesity is characterized by a chronic imbalance between energy intake and decreased energy expenditure fostering the progression of other metabolic associated diseases such as T2D and ultimately CVD. Given the close link between energy intake and appetite regulation, a better understanding of appetite control is essential to combat obesity. Appetite regulation is controlled by a complex system involving psycho-neuro-endocrine control mechanisms. Hunger triggered by a negative energy balance and long term adiposity status generates signals to the hypothalamus in the brain, leading to meal initiation. In the brain, the hypothalamic arcuate nucleus (ARC) will sense peripherally-derived signals via systemic circulation (14) and/or vagal afferent fibres (15, 16). There are two distinct neuronal populations present in ARC considered as first-order neurons on which peripheral signals primarily act, orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP), whereas anorexigenic neurons are comprised of pro-opiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART) (14). There are two categories of peripheral signals involved in appetite regulation; short-term and long-term signals. The short-term signals which are phasically secreted during meals consist of anorexigenic gut-derived hormones (cholecystokinin [CCK], glucagon-like peptide 1 [GLP-1], peptide tyrosine-tyrosine [PYY], oxyntomodulin [OXM]), orexigenic gut-hormone ghrelin and other meal-related hormones/peptides (ApoA-IV, enterostatin, bombesin-family peptides, amylin and glucagon) (17). On the other hand, long-term signals which reflect the adiposity status consist of the adipose tissue hormone leptin and insulin as well as energy signals represented by fatty acid oxidation (18). CCK is secreted from proximal enteroendocrine I-cells and GIP is secreted from enteroendocrine K-cells of the duodenum and jejunum leads to inhibit gastric motility (19), stimulates contraction in the smooth muscle of the gallbladder (19, 20), promote pancreatic enzyme secretion (21), delay gastric emptying (19, 22), and reduce gastric acid secretion (23). On the other hand, GLP-1 is secreted from enteroendocrine L cells located in the distal ileum and colon, which, upon meal ingestion, promotes satiation (24), stimulates insulin secretion (25) and reduces gastric emptying (26). PYY is an anorexic hormones co-secreted with GLP-1 in enteroendocrine L-cells. PYY secretion could affect appetite via a direct central effect and also via its effects on gut motility, acting as an 'ileal brake' leading to a sensation of fullness and satiety (27). There are two more gut-derived hormones, OXM which is co-secreted with GLP-1 and glicentin produced by the enteroendocrine L-cells (28). Although a specific receptor of OXM has not been identified, the action of OXM inhibiting appetite and controlling weight loss was observed (29-31). Ghrelin is the only orexigenic gut-derived hormone which is secreted from oxyntic glands of the gastric fundus and function as a meal-initiation signal in the system of short-term regulation of appetite (32). Another potential

mechanism on appetite modulation is through an activation of transient receptor potential (TRP) channels. The transient receptor potential ankyrin 1 (TRPA1), a member of the large TRP family of ion channels, is a calcium-permeable non-selective cation channel expressed in the gastrointestinal tract and associated with gastric motility, gastric emptying, and food intake. Another member of the TRP family is the transient receptor potential vanilloid 1 (TRPV1) which is partially co-expressed with TRPA1 in sensory nerve endings and associated with metabolism, appetite regulation and energy homeostasis (33).

Thyroxine (T4) and triiodothyronine (T3) which are collectively termed as thyroid hormones are produced and released by the thyroid gland and considered as one of the major determinants of food intake, energy expenditure and basal metabolic rate (34). Dysregulation of the thyroid axis results in altered energy balance as shown in patients with either hyperthyroidism or hypothyroidism. Hyperthyroidism is clinically manifested by excess in both T4 and T3 levels leading to increased food intake, increased metabolic rate and weight loss at the same time due to failure in meeting the exceeding metabolic rate (35). In contrast, hypothyroidism is associated with decreased metabolic rate, increased weight gain and reduced food intake (35). A study showed that individuals with impaired fasting glucose had different thyroid axis activity compared to those with impaired glucose tolerances, while Roux-en-Y gastric bypass was marked with reduced T4 and thyroid-stimulating hormone levels after 6 months of surgery compared to baseline. An earlier report showed that carbohydrate deprivation resulted in reduced T3 levels but increased reverse-T3, T3 uptake and free-T4 (36). In an animal study, a sucrose-rich diet promoted T4 to T3 conversion and increased T3 levels (37). In contrast, intake of a non-caloric sweetener sucralose altered thyroid axis activity by reducing T3 and T4 levels accompanied with increased food intake and body weight gain (38).

There is pre-clinical and clinical evidence highlighting certain beneficial effects of plant-derived bioactive compounds on appetite control. Several plant-based foods such as almonds (39), *Caralluma fimbriata* (40), cacao (41, 42), grapes (43-46), green tea (47), baobab fruit (48), moringa (49), *Phaseolus vulgaris* (50), thylakoids from spinach (51-55) and stevia (49) or certain bioactive compounds *e.g.* (-)-epigallocatechin-3-gallate (56) have been studied for their effects on human appetite regulation. However, there is a substantial lack of evidence based on both clinical and *in vivo* studies with regards to the effect of spices on human appetite regulation. Some limited data exist on caraway (57), cinnamon (58-60) and ginger (61), yet certainly more spices and their bioactive compounds deserve further investigation both on their mechanisms and efficacies on appetite regulation.

# Regulation of postprandial glycemia, lipemia and metabolic endotoxemia

## Postprandial glycemia

The relation between appetite and blood glucose regulation might be explained by the 'glucostatic' hypothesis coined by Mayer (62). A signal of 'hunger' is generated in the state of immediate glucose need by the tissues when carbohydrate intake is limited. Yet, exaggerated postprandial glycemia is pivotal for CVD risk progression. Evidences from large studies have shown that a poor management of postprandial glycemia is considered to be an independent risk factor for CVD (63). In type 1 diabetes (T1D) and T2D individuals, an acute hyperglycemic crisis is followed by an increase of circulating inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 (64). An *in vivo* study showed that repetitive glucose spikes in otherwise healthy conditions significantly promoted atherosclerotic lesion formation (65). Indeed, earlier study showed that repetitive glucose spikes promoted monocytes adherent to the endothelium of thoracic aorta and increased arterial intimal thickening in non-obese T2D Goto-Kakizaki (GK) rats, while adding phloridzin significantly reduced its progression (66). Another *in vivo* study with apolipoprotein-deficient mice (ApoE<sup>-/-</sup>) showed that maltose-induced repetitive glucose spikes exhibited an increase in macrophages adherent to the endothelium after one week and increased the area of fibrotic arteriosclerotic lesions after 5 weeks (67). In addition to the exacerbated endothelial dysfunction following hyperglycemic condition, other deleterious effects, including worsening of the insulin resistance and  $\beta$ -cell function, are evident. The mechanism behinds these effects could partly be related to chronic oxidative stress induced by exaggerated of glucose levels, termed as 'glucotoxicity', which subsequently activates a series of metabolic pathways that lead to inflammation, hypercoagulability and endothelial dysfunction, which could further proceed to diabetes and early signs of CVD (68). Thus, the complexity of postprandial glucose regulation in maintaining metabolic homeostasis is of importance, while the management of glucose homeostasis is critical for health. Certain polyphenols have been acknowledged for their ability to reduce postprandial blood glucose elevations through several mechanisms, such as inhibition of  $\alpha$ -amylase (69) and  $\alpha$ -glucosidase (70). The beneficial effects of certain spices (*i.e.* turmeric and cinnamon) on blood glucose regulation have predominantly been investigated in longer-term interventions and to a limited extent in postprandial setting. While most of previous studies have been performed with capsule-based interventions, the present dissertation will further dissect and offer a real-life approach using meal as interventions in postprandial setting.

## Postprandial lipemia

Although there are controversies related to the type of dietary fats on health-associated outcomes, consumption of fatty meals has been long associated with CVD risk factors and are considered as important factor in CVD incidence. Accumulated human studies have reported that fatty meal consumption results in exacerbation of lipid metabolism and provokes low-grade inflammation irrespective of health status (71) or age (72). Indeed, exaggerated fatty meal consumption irrespective of fat quality could promote an activated state of atherogenic postprandial milieu (71). The balance between reactive oxygen species (ROS) and the body's antioxidant defense system plays an important role to maintain redox homeostasis. Disturbances in redox homeostasis known as oxidative stress, caused by sustained postprandial hyperlipidemia and/or hyperglycemia, are linked to a higher risk for metabolic-associated diseases such as atherosclerosis, diabetes and obesity (73). There are three pathways involved in the lipoprotein metabolism, which can be defined as (a) the exogenous pathway, *i.e.* the postprandial absorption of dietary lipids in the intestine and followed by transport to peripheral tissue and the liver, (b) the endogenous pathway, which is the transport of endogenously synthesized triacylglycerol (TG) and cholesterol from the liver to peripheral tissues via the very-low density lipoprotein (VLDL) – intermediate density lipoprotein (IDL) – low-density lipoprotein (LDL) pathway, and (c) the reverse cholesterol transport in which cholesterol is transported from peripheral tissues back to the liver (74).

### Exogenous pathway

Fatty meals are digested through mechanical and enzymatic reactions– as a result from pancreatic TG lipase hydrolysis, leading to fatty acids and monoacylglycerols formation. Fatty acids and monoacylglycerols then undergo an absorption in the enterocyte. Within enterocytes, apolipoproteins (apoB48 and apoA) will be formed in the endoplasmic reticulum (ER), then TG-rich lipoprotein/TRL will be assembled in the Golgi and becomes a nascent chylomicrons which are then secreted by exocytosis into the intercellular space (75). Nascent chylomicrons then receive apoC and apoE from HDL to become the mature chylomicrons. TRL travel through capillaries in tissues such as muscle and adipose, and their TG are captured and acted upon lipoprotein lipase (LPL), leading to fatty acids and glycerol release from TRLs. Fatty acids will then be used either as sources of energy for extrahepatic tissues such as muscle– or being stored in adipose tissue, while glycerol will be converted into dihydroxyacetonephosphate (DHAP) and subsequently to glyceraldehyde 3-phosphate (GA3P) for rejoining with the glycolysis and gluconeogenesis pathway. Furthermore, TRL also return their apoA and apoC back to the HDL. Upon losing most of their lipid contents as TG, TRL then becomes depleted and what is known as chylomicron remnants (CMR). Circulating CMR



then functioning to return bile cholesterol to the liver by an enterohepatic circulation (76). In addition, CMR might also contribute to the monocyte activation *in vitro* (77), eliciting arterial wall and cellular inflammation (78) and possibly stimulating monocyte migration into vascular smooth muscle cells, which can lead to the formation of atherosclerosis (79). Therefore, a delayed CMR clearance as observed in subjects with heterozygous familial hypercholesterolaemia might compromise the lipid metabolism (80).

#### Endogenous pathway

In the liver, TG and cholesterol esters are generated and packaged into VLDL particles which then are released into the circulation. VLDL is acted upon by LPL, leading to fatty acids and glycerol release. Fatty acids are taken up by muscle cells as a substrate for energy or by adipose cells for storage. After giving their ApoC to HDL, VLDL then becomes VLDL-remnants. The VLDL-remnants are then taken up by the liver via the LDL receptor or become IDL, a smaller, denser lipoprotein than VLDL. IDL can be further hydrolyzed by hepatic lipase to LDL, and retain ApoB as its only apolipoprotein. PCSK9 (proprotein convertase subtilisin kexin type 9) is a liver secretory enzyme that regulates plasma LDL through modulation of LDL receptor (LDLR) density on the surface of hepatocytes, leading to LDLR proteolysis. Thus, inhibition of the PCSK9 will lead to increased LDL clearance in the circulation and consequently improved lipid metabolism. Emerging evidence showed that spices, such as curcumin, promoted LDL uptake, mediated by the PCSK9/LDLR pathway in HepG2 cells (81, 82).

#### Reverse cholesterol transport pathway

Reverse cholesterol transport refers to the process by which cholesterol from peripheral tissues is removed and returned to the liver for further use in bile acid formation. The HDL is the key lipoprotein involved in the reverse cholesterol transport and the transfer of cholesteryl esters between lipoproteins. In this context, macrophages are pivotal for mediating the export of free (unesterified) cholesterol to extracellular HDL. The passive processes include simple diffusion via the aqueous phase and facilitated diffusion mediated by scavenger receptor class B, type 1 (SR-B1) (83). Active pathways are mediated by the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, which are membrane lipid translocases (83). The efflux of cellular phospholipid and free cholesterol to apolipoprotein A-I promoted by ABCA1 is essential for HDL biogenesis.

Some bioactive-rich foods have been shown to have a beneficial effect in mitigating the postprandial course of lipemia. Postprandial lipemia can be improved through the consumption of red wine (84), peanuts (85), sea buckthorn (86) and strawberries. However, there is a lack of evidence concerning the effects of spices in postprandial lipemia, particularly in human studies (87).

## Metabolic endotoxemia

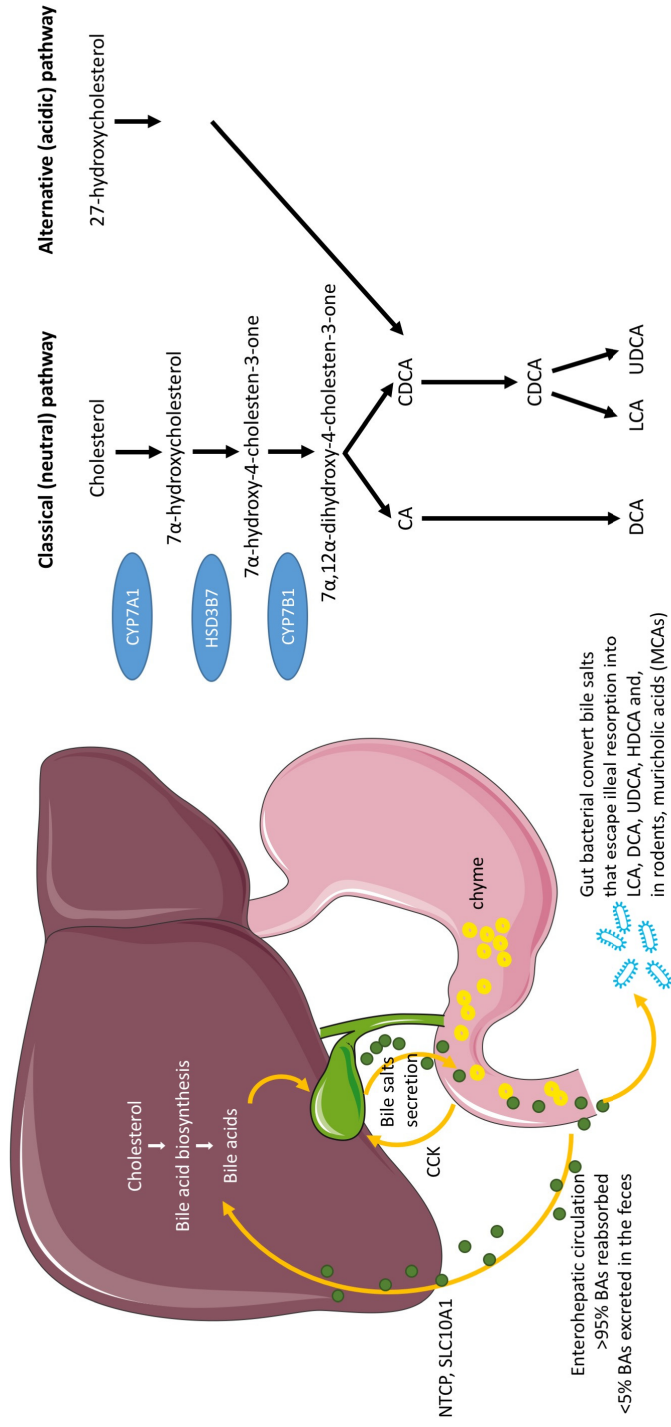
Endotoxemia caused by endotoxins produced by bacterial infections results in an overwhelming production of inflammatory cytokines, which can lead to multiple organ failure and ultimately death (88). As opposed to bacterial-induced endotoxemia, metabolic endotoxemia is a term of metabolic condition characterized by sustained low-level elevation of lipopolysaccharide (LPS), leading to activation of inflammatory signalling cascades (89). In an early study of metabolic endotoxemia, Cani *et al.* described a two- to three-fold increase of plasma LPS concentration relative to control as a defined threshold for metabolic endotoxemia, which was associated with systemic low-grade inflammation leading to body weight gain and diabetes (89). Evidence from animal and human studies highlighted that fatty meals could promote low-grade inflammation, among ailment, through involvement of bacterial lipopolysaccharide (LPS) inflow into circulation (89-91). Different types of fat have been shown to modulate metabolic endotoxemia in postprandial setting. Studies by Lopez-Moreno *et al.* (92) and Lyte *et al.* (93) concluded that a diet rich in saturated fat increases circulating LPS in a postprandial setting. In addition, processing fat, such as emulsification, might as well potentially modulate metabolic endotoxemia as shown by Vors *et al.* (94) and Laugerette *et al.* (95). LPS is a heat stable component of gram-negative bacteria cell walls that, in a human context, may normally reside in the gut (96, 97). LPS can enter the systemic circulation either paracellularly, known as 'leaky gut' (98), or via transcellular transport mediated by chylomicron (99, 100). A recent *in vivo* study reported that a very low-dose of LPS intervention for one month triggered a sustained condition of low-grade inflammation, leading to exacerbation of steatohepatitis in high fat-fed mice (101). Yet, a intravenous endotoxin challenge in human showed a modulation in systemic inflammation characterized by a marked increase in tumour necrosis factor (TNF)- $\alpha$  (102-104), IL-6 (102-104) and IL-8 (103) within 2 hours after intervention. Circulating LPS is recognized by LPS-binding protein (LBP), which is then transferred to soluble cluster differentiation (sCD)14 and further interacts with protein complex toll-like receptors (TLR)4 and myeloid differentiation protein (MD)-2. Activated TLR4 subsequently recruits two major signalling pathways mediated by different adaptor proteins, myeloid differentiation primary response protein (MyD)88 and toll-interleukin-1 receptor-domain-containing adaptor-inducing interferon- $\beta$ (TRIF), leading to the activation of downstream inflammatory signalling cascades. In the MyD88 signalling pathway, signals will terminate in the activation of nuclear factor kappa beta (NF- $\kappa$ B), MAP kinases and IRF5 followed by secretion of inflammatory cytokines, such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 (105) and CCL4 (105). On the other hand, TRIF signalling pathway activates interferon regulatory factor (IRF)3 and IRF7, leading to secretion of type I interferon (IFN) such as IFN- $\alpha/\beta$ .

# Bile acids metabolism

## Formation of bile acids (BAs) from cholesterol

BAs are amphipathic steroid molecules formed from cholesterol in hepatocyte microsomes via classical or alternative pathways as depicted in **Figure 1**. In addition to their known function to facilitate lipids absorption, BAs are increasingly recognized to be involved in glucose and lipid metabolism.

In the '*classical pathway*', cholesterol is converted into  $7\alpha$ -hydroxycholesterol mediated by  $7\alpha$ -hydroxylase (CYP7A1), which is then transformed to  $7\alpha$ -hydroxy-4-cholesten-3-one (C4) by  $3\beta$ -hydroxysteroid hydrogenase (HSD3B7). The C4 is then converted by sterol  $12\alpha$ -hydroxylase (CYP8B1) to  $7\alpha,12\alpha$ -dihydroxy-4-cholesten-3-one which leads to cholic acid (CA) synthesis. In the absence of  $12\alpha$ -hydroxylation, the pathway will result in chenodeoxycholic acid (CDCA) synthesis. At least ~75% of total BA pool originates from the '*classical pathway*'. In rodents,  $\alpha$ - and  $\beta$ -muricholic acids (MCAs) are formed from CDCA and ursodeoxycholic acid (UDCA), respectively by the enzyme CYP2C70. In the '*alternative pathway*', cholesterol is converted into  $27\alpha$ -hydroxycholesterol by  $27$ -hydroxylase (CYP27A1), which is hydroxylated into  $3\beta,7\alpha$ -dihydroxy-5-cholestenoid acid by oxysterol  $7\alpha$ -hydroxylase (CYP7B1) and then converted into CDCA. The '*alternative pathway*' accounts for around 3 - 18% of total BAs formed. The synthesized primary BAs (CA and CDCA) can be conjugated with glycine and taurine by the enzymes BA-CoA synthase and BA-CoA-amino acid N-acetyltransferase. Of note, BA pool size is determined by the availability of CYP7A1, while CYP8B1 is pivotal for BA composition (CA:CDCA or CA:MCA ratios). Bile salt, constituting of conjugated BAs mixed with potassium and sodium ions together with cholesterol, phospholipids, bilirubin and water forming bile, is transported into the gallbladder where it is stored. In the postprandial setting (when the meal digesta reaches the proximal duodenum), cholecystokinin (CCK)-induced gallbladder contractions result in BA release into the duodenal lumen. BAs then promote the absorption of dietary lipids and fat-soluble vitamins (A, D, E and K) in the small intestine. In terminal ileum, about 95% of the BAs undergo resorption which is mediated by a sodium dependent BA transporter (ASBT, SLC10A2) and a basolateral heterodimer organic solute transporter  $\alpha/\beta$  (OST  $\alpha/\beta$ ) reaching the portal blood circulation. BAs are subsequently taken up by the liver from portal circulation via sodium taurocholate cotransporting polypeptides (NTCP, SLC10A1).



by **Y.C. Zanter**  
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**Figure 1**  
 Formation of bile acids from cholesterol

A small part of BAs, which escape from resorption in the terminal ileum arrive in the colon and are converted into deoxycholic acid (DCA), UDCA, hyodeoxycholic acid (HDCA) and lithocholic acid (LCA), as well as the MCAs in mice and rats, through deconjugation and 7  $\alpha/\beta$  dehydroxylation by gut microbiota (106). These secondary BAs are partly reabsorbed by passive diffusion and contribute with BAs in the liver, while approx. 500-600 mg/day are lost via fecal excretion and then replaced by *de novo* BAs synthesis in the liver. Within enterohepatic circulation, BAs are circulated as much as 4 to 12 times per day maintaining a BA pool of around 3 g in humans (107).

### **Bile acids in lipid and glucose metabolisms**

BAs have been acknowledged as important signalling molecules pivotal in the regulation of lipid, glucose and energy homeostasis, hence regulating relevant processes in the context of MetS. The regulatory function of BAs as signalling molecules is mediated by several receptors such as farnesoid X receptor (FXR; NR1H4), Takeda G-protein-coupled BA receptor (TGR5/Gpbar-1), pregnane X receptor (NR1I2), constitutive androstane receptor (NR1I3), vitamin D receptor (NR1I2) and liver X receptor (LXR; NR1H3). A balance condition between production and clearance of TG-rich lipoproteins (TRL) is reflected in plasma TG levels. In addition, FXR can activate ApoC-II and further stimulate lipoprotein lipase (LPL) activity as a key enzyme in lipolysis of lipoproteins into free fatty acids (FFA). Thus, maintaining TG normal concentration is imperative in the management of hypertriglyceridemia, which is considered as major modifiable risk factor of CVD. Previous studies indicated that chenodeoxycholic acid (CDCA), used as a treatment for gallstones, additionally reduced TG level in humans (108-110). The TG-lowering effect of CDCA is initiated by the activation of FXR, which lead to increased short heterodimer partner (SHP) level and ultimately reduce sterol regulatory element-binding protein-1c (SREBP-1c) expression, considered as main regulator of fatty acid synthesis (111). CDCA administration in patients with gallstones is also known to lower proprotein convertase subtilisin-kexin type 9 (PCSK9) levels. PCSK9 is a hepatic protease that attaches and internalizes LDL receptors (LDLR) into lysosomes leading to proteolysis (112). Therefore, inactivating or reducing PCSK9 will prevent LDLR destruction and subsequently leading to reduced LDL-c levels (113, 114). Another study based on CA showed a reduction in TG levels in individuals with hyperlipoproteinemia (115). However, a 6-months intervention with CA did not effectively reduce hepatic or circulating TG levels in individuals with lipodystrophy (116). Another role of BAs, such as tauroursodeoxycholic acid (TUDCA), was reported to modulate adipose tissue formation as indicated by adipogenic marker genes peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) and glycerol-3-phosphate dehydrogenase 1

(GPDH) in human adipose-derived stem cells (hASCs) (117). Surprisingly, UDCA administration in morbidly obese patients for 3 weeks inhibited FXR signalling and increased hepatic TG levels, but otherwise lowered hepatic cholesterol and serum LDL-c, and increased the concentration of less toxic unsaturated fatty acids in the liver (118). A connection between BAs and glucose metabolism is indicated by TGR5 activation which induces the incretin hormone glucagon-like peptide 1 (GLP-1) secretion, leading to improved glycemia. In addition, TGR5 activation in peripheral tissues could induce type 2 deiodinase activation and thus lead to increased thyroid hormone and increase energy expenditure (119).



# Objectives

The general objective of this dissertation is to understand whether intake of single (cinnamon, turmeric, ginger, star anise or black pepper) or mixed spices (blend of cinnamon, turmeric, black pepper, ginger, star anise and cloves) can contribute to modulate circulating cardiometabolic-related risk markers; modify appetite sensations, BA profile and peripheral blood mononuclear cells (PBMCs) gene expressions induced by meal challenge perturbation (high glycemic or fatty meals) in postprandial setting. The investigation was carried out in three meal studies in healthy, normal to overweight subjects with the following aims and study designs:

- To examine the effect of single spices, formulated as extract-based beverages, consumed either prior to a white wheat bread (WWB), isocaloric medium (MF) or high (HF) fat meals, or as a mixed spices incorporated in HF meal challenges in postprandial appetite sensations, circulating gut and thyroid hormones levels in healthy subjects.
- To evaluate the effect of single spices, formulated as extract-based beverages, consumed either prior to WWB (in healthy normal weight subjects), isocaloric MF or HF (in healthy normal to overweight subjects), or mixed spices (in healthy normal weight participants) incorporated in HF meal challenges, on postprandial glucose metabolism.
- To investigate whether a turmeric-based beverage consumed either prior to isocaloric MF or HF meal challenges, or mixed spices incorporated into HF meal challenge could modify postprandial lipid metabolism, metabolic endotoxemia and other cardiometabolic risk-associated markers in healthy normal to overweight subjects.
- To explore whether a turmeric-based beverage, when consumed prior to isocaloric MF or HF meal challenges has an impact in postprandial BA profile of healthy normal to overweight subjects.
- To assess whether a spice blend incorporated into a HF meal challenge could influence PBMCs gene expression, pathways and processes associated with cardiometabolic risk in postprandial setting in healthy normal to overweight subjects.
- To ascertain whether inter-individual differences between the observed markers are present, based on metabolic status (BMI) and gender.





# Material and Methods

## Test and reference products

### Raw materials

Turmeric (*Curcuma longa*), cinnamon (*Cinnamomum burmannii*), star anise (*Illicium verum*), ginger (*Zingiber officinale*), black pepper (*Piper nigrum*) and cloves (*Syzygium aromaticum*) were used either individually (**Papers I, II, III, and IV**) or in combination (**Papers V and VI**) in human meal studies as presented in **Table 1**. All the spices were donated from the same batch for each respective study by Santa Maria AB, Sweden.

**Table 1**  
List of spices used in each of scientific paper of this dissertation.

Spices	Paper I	Paper II	Paper III	Paper IV	Paper V	Paper VI
Cinnamon	✓				✓	✓
Clove					✓	✓
Ginger	✓				✓	✓
Black pepper		✓			✓	✓
Star anise	✓				✓	✓
Turmeric	✓		✓	✓	✓	✓

### Spice-based beverages

Tumeric, star anise, ginger, cinnamon and black pepper-based beverages were prepared freshly in the morning of each trial day, approximately one-hour prior to being used in the meal intervention (**Papers I, II, III and IV**). A blend of tap water with non-caloric (4 kJ per 100 ml) strawberry marula flavoured squash concentrate (FunLight, Procordia-Orkla, Sweden) with a proportion 5:1 was used as a formula of flavoured water. The flavoured water itself was the basis of both the control and test beverages to standardized and enhance the taste of the drink. In order to make the spice-based beverages, an amount of 20 g of the respective spices (turmeric, star anise, ginger, cinnamon or black pepper) was extracted with 200 ml of heated flavoured water (90°C in 3 min). The sediment was discarded and only the liquid

part (extract) was used for the human trial. The total polyphenol content of each extract was measured with the Folin-Ciocalteu method and expressed as gallic acid equivalents (GAE) (120). Thereafter, the extract was adjusted to reach a standardized serving of 220 ml with a 185 mg GAE content (**Table 2**). The latter benchmark dose was based on the total polyphenol provided by 6 g (dry weight) of cinnamon, which was the highest dose previously used in the postprandial setting (58).

**Table 2**  
Composition of the intervention beverages (Paper I,II, III, IV and V).

Composition	Intervention beverages					
	Control <i>Paper I, II, III, IV and V</i>	Turmeric <i>Paper I, III, IV and V</i>	Star anise <i>Paper I</i>	Ginger <i>Paper I</i>	Cinnamon <i>Paper I</i>	Black pepper <i>Paper II</i>
Extract, mL	–	220	138	137	60	100 ml : 900 ml dilution between extract and flavoured water, then 220 ml of mixed was used and intervention beverage
Added flavoured water <sup>2</sup> , mL	220	–	82	83	160	
Total polyphenols, mg GAE	–	185	185	185	185	20
Total volume beverage, mL	220	220	220	220	220	220

<sup>1</sup> Total polyphenol concentration estimated from direct analysis of extract by Folin-Ciocalteu method

<sup>2</sup> Flavoured water made by blending tap water with non-caloric (4kJ per 100 ml) strawberry marula flavoured squash concentrate (FunLight, Procordia-Orkla, Sweden) in dilution 5:1.

## Meal challenges

### *White wheat bread (WWB) challenge (Papers I and II)*

One batch of WWB (JätteFranska, Pågen, Malmö, Sweden) was purchased from a local supermarket. Portions of WWB containing 50 g of available carbohydrate were packed in aluminium foil and frozen at  $-20^{\circ}\text{C}$ . The WWB portions were thawed overnight prior to being used in the morning of the meal study.

### *Medium and high fat meals (Papers III and IV)*

Medium (MF) and high fat (HF) meals used in the study presented in **Papers III** and **IV** contained the same amount of energy (423 kcal), but differed in macronutrient composition as presented in **Table 3**. Food items such as butter (Svenskt Smör, Arla, Sweden), cream cheese (Cantadou Naturell, Fromageries Bel, France), gouda cheese (ICA Basic Goudaost, ICA AB, Sweden) and WWB (JätteFranska, Pågen, Sweden) were purchased from the same batch at a local supermarket. All food items were weighed in advance and stored at  $-20^{\circ}\text{C}$ . The meals were prepared freshly in the morning of the meal study.

### *High fat meal with or without mixed spices (Paper V and VI)*

High fat meals (948 kcal), either enriched with mixed spices (HF-Spices) or without mixed spices (HF) were made in the form of tacos, and used as the meal challenge for the postprandial study presented in **Papers V** and **VI**. The macronutrient composition of food items and mixed spices are presented in **Table 4**. Food items such as ground beef meat (ICA AB, Solna, Sweden), tortilla bread (Santa Maria AB, Mölndal, Sweden), sweet corns (Green Giant, Uxbridge, UK) and coconut milk (ICA AB, Solna, Sweden) were purchased from the same batch at a local supermarket. Spices (turmeric, black pepper, cinnamon, ginger and clove) were obtained as a kind donation from Santa Maria AB, Mölndal, Sweden. All meal ingredients were weighted in advance and stored at  $-20^{\circ}\text{C}$  until used. The meals were prepared by thawing the ingredients for 1 hour. Oil was preheated for 1 min followed by 3 min medium heat cooking of the ground beef alone (HF) or blend with mixed spices (HF-Spices), salt, sweet corns and coconut milk. The cooked mix was then wrapped in a tortilla bread prior to serving.

**Table 3**  
Composition of high fat (HF) and medium fat (MF) meal challenge (Paper III, IV and V) either consumed after control beverage (CON) or turmeric-based beverage (TUR).<sup>1</sup>

Variables	CON-MF	TUR-MF	CON-HF	TUR-HF
Total calorie (kcal)	423.8	423.8	423.1	423.1
Protein (g, %E)	15.7, 14.8%	15.7, 14.8%	13, 12.3%	13, 12.3%
Carbohydrate (g, %E)	49.5, 46.7%	49.5, 46.7%	12.4, 11.7%	12.4, 11.7%
Fat (g, %E)	18.1, 38.5%	18.1, 38.5%	35.7, 76%	35.7, 76%
SFA (g, %E)	9.7, 20.6%	9.7, 20.6%	22.7, 48%	22.7, 48%
MUFA (g, %E)	4.9, 10.6%	4.9, 10.6%	8.6, 18.2%	8.6, 18.2%
PUFA (g, %E)	1.3, 2.7%	1.3, 2.7%	1, 2.1%	1, 2.1%
Food items consumed	White bread, 105 g Gouda cheese, 30 g Butter, 5 g Cream cheese, 5 g Control beverage, 220 ml	White bread, 105 g Gouda cheese, 30 g Butter, 5 g Cream cheese, 5 g Turmeric beverage, 220 ml (185 mg GAE)	White bread, 25 g Gouda cheese, 42 g Butter, 20 g Cream cheese, 19 g Control beverage, 220 ml	White bread, 25 g Gouda cheese, 42 g Butter, 20 g Cream cheese, 19 g Turmeric beverage, 220 ml (185 mg GAE)

<sup>1</sup> %E, energy percent; CON-MF, medium-fat diet plus control beverage; TUR-MF, medium-fat diet plus turmeric-based beverage; CON-HF, high-fat diet plus control beverage; TUR-HF, high-fat diet plus turmeric-based beverage; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid

**Table 4**Composition of high fat (HF) and high fat enriched with mixed spices (HF-Spices) meal challenges (Papers V and VI).<sup>1</sup>

Variables	HF	HF-Spices
Total calorie (kcal)	948	948
Protein (g, %E)	42.4, 17.9%	42.4, 17.9%
Carbohydrate (g, %E)	48.8, 20.6%	48.8, 20.6%
Fat (g, %E)	64.8, 61.5%	64.8, 61.5%
SFA (g, %E)	20.9, 19.8%	20.9, 19.8%
MUFA (g, %E)	18.6, 17.7%	18.6, 17.7%
PUFA (g, %E)	9, 8.5%	9, 8.5%
Food items consumed	Tortilla bread, 80 g Coconut milk, 50 ml Oil, 30 ml Ground beef, 180 g Sweet corn, 40 g	Tortilla bread, 80 g Coconut milk, 50 ml Oil, 30 ml Ground beef, 180 g Sweet corn, 40 g
Spices	No spices added	Black pepper, 3 g Cinnamon, 2 g Cloves, 2 g Ginger, 2 g Turmeric, 3.5 g

<sup>1</sup> %E, energy percent; HF, high fat meal; HF-SPICES, high fat meal enriched with mixed spices; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acid

## Chemical characterization of spices

### Total polyphenols and antioxidant capacity in spices

Total polyphenol content in the spice-based extracts used in **Papers I, II, III** and **IV** was measured with Folin-Ciocalteu method (120) based on a previous report (121). Briefly, the Folin-Ciocalteu reagent was added into the spice-based extract to which  $\text{Na}_2\text{CO}_3$  was added after 1 min and incubated for 2 h at room temperature. The absorbance of the mixture was then measured at 760 nm, with results expressed in mg GAE/100 mL spice-based extract. Prior to the determination of total polyphenols and antioxidant capacity in the mixed spices used in **Papers V** and **VI**, a hot pressurized liquid extraction was performed to get an extract of mixed spices according to optimal conditions suggested for extraction of phenolic compounds (122-124). In the hot pressurized liquid extraction, water and ethanol were separately used as extraction solvents. Extractions were performed using a Dionex ASE 150 instrument (Thermo Fisher, Germering, Germany) with 10 mL extraction cells that were filled with 1 g of mixed spices, at 125°C for 3 min (10.34 MPa). Prior to each experiment, the cell was pre-heated for 6 min and samples were run in triplicates. To prepare the sample solutions, the ethanol and water extracts were

diluted in 25 mL ethanol and 20 mL water, respectively. The obtained extracts were protected from light and stored at  $-20^{\circ}\text{C}$  until analysis. Total polyphenol content in the mixed spices used in **Papers V** and **VI** was determined with the Folin-Ciocalteu method as presented above (122). Furthermore, total antioxidant capacity in the mixed species was determined with the DPPH radical scavenging assay (122) and the Trolox equivalent antioxidant capacity (TEAC) assay (122). In the DPPH radical scavenging assay, the percentage of remaining DPPH against the extract concentration was then plotted to obtain the amount of antioxidant needed to decrease the initial DPPH concentration by 50% or EC<sub>50</sub>. Therefore, the lower the EC<sub>50</sub>, the higher the antioxidant activity. However, in the TEAC assay, Trolox was used as a reference standard, and results were expressed as TEAC values (mmol Trolox/g of sample). All analyses were done in triplicates for each extract.

### **Bioactive compounds characterization in spices**

Bioactive compounds contained in the spice-based beverages (**Papers I, II, III** and **IV**) were characterized using ultra-high-performance liquid chromatography coupled to electrospray quadrupole time-of-flight mass spectrometry (UHPLC-DAD-ESI-QTOF-MS). The UHPLC (Waters Acquity UPLC chromatographic system, Waters Corp., Manchester, UK) with a photodiode array detector (DAD) and a quadrupole and orthogonal acceleration time-of-flight tandem mass spectrometer Xevo G2 qTOF with electrospray ionization (ESI) (Waters MS Technologies, Manchester, UK) was used. The system was controlled by Waters® Empower™ Chromatography software, while MassLynx™ (V 4.1, SCN 779, Waters Corp., Manchester, UK) was used for the MS data acquisition. Five microliters of extracts were injected onto a Waters Acquity UPLC BEH-C18 column ( $2.1 \times 100$  mm,  $1.7 \mu\text{m}$   $110 \text{ \AA}$ ; Waters Corporation, Milford, MA). The mobile phase consisted of (A) water and (B) methanol, both containing 0.5% (v/v) formic acid. The column temperature was  $50^{\circ}\text{C}$  and the flow rate  $300 \mu\text{L}\cdot\text{min}^{-1}$ . Phenolics were eluted according to the following: 0 min, 5% B; 1 min, 5%B; 7 min, 50% B; 11 min, 50% B; 11.5 min 5% B; 15 min, 5% B. The diode array detector recorded the spectra from 200 to 500 nm. The mass spectrometer was operated in both ionization modes, negative and positive ESI interface using the following parameters: capillary voltage, 3 kV; cone voltage, 30 V; source temperature,  $120^{\circ}\text{C}$ ; and desolvation temperature,  $400^{\circ}\text{C}$ . Nitrogen was used as both cone gas ( $50 \text{ L}\cdot\text{h}^{-1}$ ) and desolvation gas ( $1000 \text{ L}\cdot\text{h}^{-1}$ ). Full-scan UHPLC-qTOF-MS spectra were obtained by scanning in the range  $m/z$  50-1200. The mass spectrometer was calibrated using a solution of sodium formate. Data were collected in centroid mode and all the analyses were performed with leucine-enkephalin ( $10 \mu\text{L}\cdot\text{min}^{-1}$ ,  $2 \text{ ng}\cdot\mu\text{L}^{-1}$ ) in the lockspray. MS<sup>E</sup> was performed with the low collision energy set off and the high collision energy ramped from 15 to 60 V, in order to obtain a full scan of

accurate mass fragment, precursor ion and neutral loss information. The tentative identification of phenolic compounds was performed by matching the obtained accurate mass values and the theoretical mass values (error less than 3.3 ppm) in the database FooDB (<http://foodb.ca/>). The experimental MS/MS spectra obtained for each phenolic compound were compared to those described both in FooDB database and literature, and/or predicted MS/MS spectra obtained in CFM-ID ([cfmid.wishartlab.com](http://cfmid.wishartlab.com)).

## Human clinical studies

### **Ethical approval, participants and recruitment process**

Study protocols were approved by the Regional Ethical Review Board in Lund, Sweden with identification numbers 2013/862 (**Papers I and II**), 2015/207 (**Papers III and IV**), 2016/483 (**Papers V and VI**), respectively. Human trials were registered at <https://clinicaltrials.gov> as NCT02035241 (**Papers I and II**), NCT02479334 (**Papers III and IV**) and NCT02931643 (**Papers V and VI**), respectively. Studies were performed at the Food for Health Laboratory at Food for Health Science Centre, Lund University, Sweden and conform to the principles outlined in the Declaration of Helsinki.

Participants were recruited from the Lund city area by advertisements posted in public and sport centre notice boards, and via online advertisement in social media such as Facebook. The inclusion criteria were: healthy individuals (aged 18-55 years) with body mass index (BMI) 20-28 kg.m<sup>-2</sup> (**Papers I, II, III and IV**) or 20-33 kg.m<sup>-2</sup> (**Papers V and VI**). To be eligible, they should also comply with the following criteria: normal range of haemoglobin (**Papers I, II, III, IV, V and VI**), ferritin (**Papers I, II, V and VI**), FBG (**Papers III, IV, V and VI**), fasting insulin (**Papers III, IV, V and VI**), TG (**Papers III, IV, V and VI**), total cholesterol (**Papers III, IV, V and VI**), creatinine (**Papers III, IV, V and VI**), ASAT (**Papers III, IV, V and VI**) and ALAT (**Papers III, IV, V, and VI**). Candidates were excluded if they were regular smokers or used other forms of tobacco (snuss), followed vegetarian or vegan diets, or had difficulties with or being stressed by cannulation, were receiving any drug treatment or taking dietary supplements, had aversion or intolerance to specific foods and spices included in the study, were excluded. In addition, pregnancy or breastfeeding were also exclusion criteria.

Candidates were invited for a screening process and an introductory meeting. During the introductory meeting, candidates were informed on the general objective of the study and the procedures which they would have to follow. In addition, body



composition was measured and venous blood samples were drawn for the evaluation of the inclusion criteria. Furthermore, potential participants were also required to fill out a health-related questionnaire. Based on the results of the screening process, selected participants were invited to join the study. Written consent was obtained from participants, both for the screening process and the intervention. Baseline characteristics for the participants in each of different studies are presented in **Table 5**.

## **Experimental design**

A randomized, single-blind, crossover design with at least a one-week interval between experimental visits was used for all of studies. In all of the studies – despite mainly maintaining the normal diet, participants were subjected to a low-phenolic diet and with no alcohol consumption for 48 h prior to the experimental visit. A list of foods (**Table 6**) that could be consumed during the low-phenolic diet was provided to the participants who were free to tailor their own diet. The low-phenolic diet consisted of unflavored dairy products, white wheat bread, red meat, fish, eggs, pasta (not whole grain), potatoes (without peel) and white rice. Participants were asked to avoid fruits, vegetables, nuts, seeds, chocolate and whole grain products as well as beer, wine, cider, coffee, tea and milk chocolate. They were also recommended to use butter instead of vegetable oil and avoid onions and garlic when cooking. Salt and small amounts of black or white pepper were allowed for flavoring. Participants were also instructed to avoid alcohol. No probiotics nor antibiotics were allowed two weeks prior to and during the entire experimental period. Adherence to the low-phenolic diet was assessed with their food records using online food record from Livsmedelsverket Sweden. Furthermore, participants were instructed to avoid excessive physical exercise during 48 h prior to each experimental visit. Likewise, adherence to the normal physical activity was evaluated by two-day physical activity questionnaire.

Participants arrived at the Food for Health Laboratory research facility in the morning after having fasted overnight for ~10 hours. Upon arrival, 10 min supine rest was allowed for participants to be in relaxed condition prior to the cannulation process by a registered nurse. An indwelling catheter was inserted into a forearm vein and a fasting blood sample was drawn as a baseline. Saline solution was used to flush in between each blood withdrawal. In **Papers I, II, III and IV**, the test or control beverage was then served to be consumed within 5 min. Afterwards, 10 min later, a meal challenge was served and the meal should be consumed within 15 min. A timer was started when participants took their first bite. The WWB was used as a meal challenge in **Papers I and II**, while isocaloric MF and HF were used in **Papers III and IV** as a meal challenge. In **Papers V and VI**, following cannulation, participants received a meal challenge either HF or HF-Spices immediately and

instructed to be consumed within 15 min. Subsequently, series of blood samples were drawn and appetite sensations as well as gastrointestinal well-being evaluations were performed over the following 3 (**Papers I and II**) or 4 hours (**Papers III, IV, V and VI**). The experimental design of studies presented in **Papers I, II, III, IV, V and VI** is depicted in the **Figure 2**.

**Table 5**  
Baseline characteristics of participants in Paper I, II, III, IV, V and VI. <sup>1</sup>

Variables	Paper I	Paper II	Paper III and IV	Paper V and VI
Gender, men / women	11 / 7	10 / 6	5 / 7	8 / 10
Age, years	25.6 ± 0.8	26 ± 0.9	26.5 ± 1.1	26.2 ± 1.05
BMI, kg.m <sup>2</sup>	22.8 ± 0.5	22.9 ± 0.5	23.8 ± 0.6	25.7 ± 0.6
BMR, kcal	1657.5 ± 68.3	1667 ± 74.4	1615 ± 109	1623 ± 72.6
Fat, %	20.1 ± 2.1	20.2 ± 2.3	24 ± 2.4	29.03 ± 1.37
Ferritin <sup>2</sup> , µg.L <sup>-1</sup>	99.4 ± 22.1	105.5 ± 24.3	NA	80.11 ± 15.35
Hemoglobin <sup>2</sup> , g.L <sup>-1</sup>	144 ± 3.3	144.5 ± 3.4	146.7 ± 4.7	141.2 ± 3.7
FBG <sup>2</sup> , mmol.L <sup>-1</sup>	NA	NA	5.0 ± 0.09	5.16 ± 0.09
Insulin <sup>2</sup> , pmol.L <sup>-1</sup>	NA	NA	67.1 ± 9.3	65.6 ± 6.8
Triacylglycerol <sup>2</sup> , mmol.L <sup>-1</sup>	NA	NA	0.85 ± 0.11	1.08 ± 0.13
Total cholesterol <sup>2</sup> , mmol.L <sup>-1</sup>	NA	NA	4.4 ± 0.3	4.4 ± 0.18
HDL-c, mmol.L <sup>-1</sup>	NA	NA	NA	1.48 ± 0.09
LDL-c, mmol.L <sup>-1</sup>	NA	NA	NA	2.75 ± 0.18
Apo-A1, g.L <sup>-1</sup>	NA	NA	NA	1.44 ± 0.05
Apo-B, g.L <sup>-1</sup>	NA	NA	NA	0.83 ± 0.05
Creatinine <sup>2</sup> , µmol.L <sup>-1</sup>	NA	NA	77.3 ± 3.4	80.4 ± 3.4
ASAT <sup>2</sup> , µkat.L <sup>-1</sup>	NA	NA	0.38 ± 0.03	0.37 ± 0.02
ALAT <sup>2</sup> , µkat.L <sup>-1</sup>	NA	NA	0.38 ± 0.07	0.30 ± 0.04

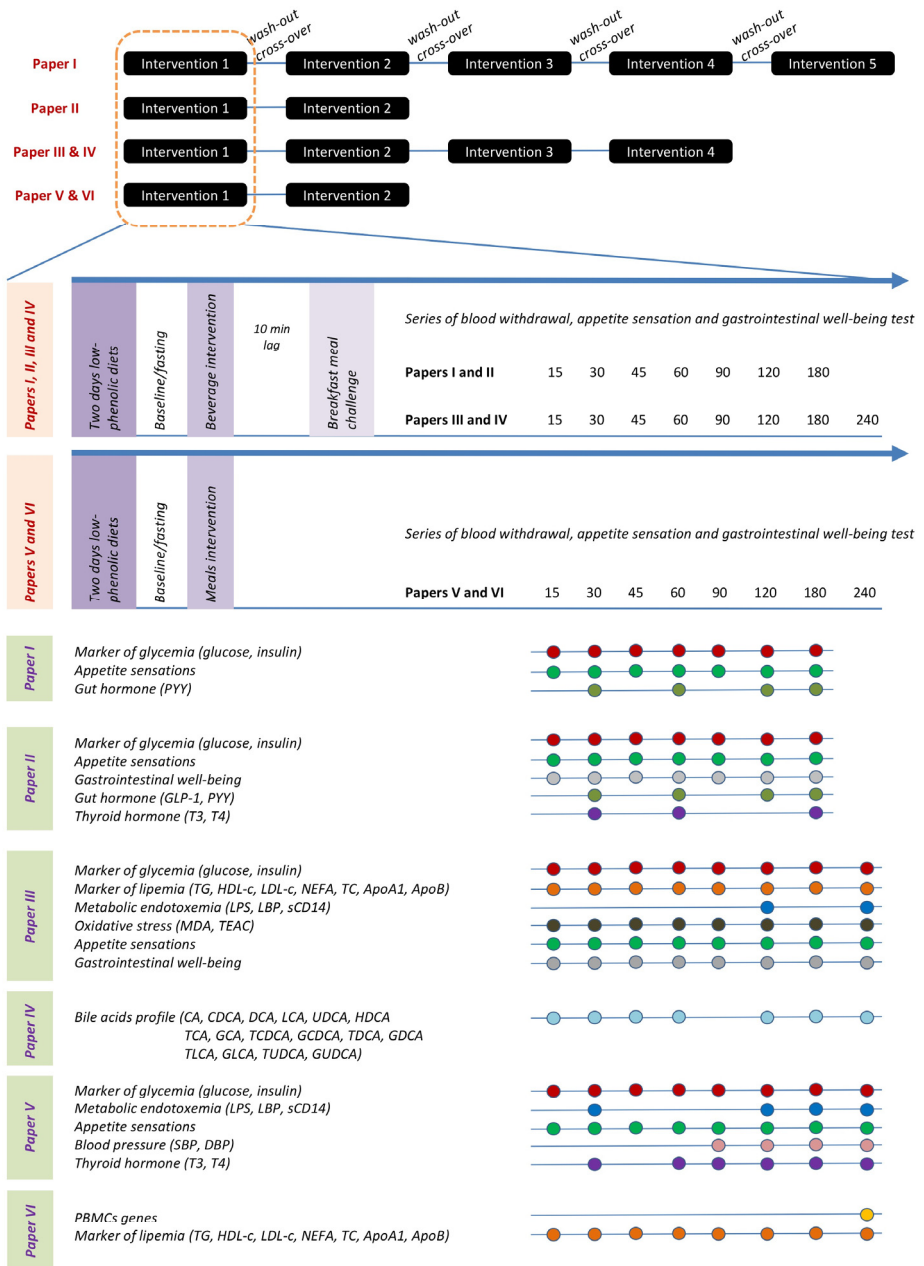
<sup>1</sup> Apo-A1; apolipoprotein-A1, Apo-B, apolipoprotein-B, ASAT, alanine aminotransferase; ALAT, aspartate aminotransferase; BMI, body mass index; BMR, basal metabolic rate; FBG, fasting blood glucose; HDL-c, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; NA, not analyzed.

<sup>2</sup> Reference range for FBG for male and female (4.2-6.3 mmol.L<sup>-1</sup>), triacylglycerol for male and female (0.4-2.6 mmol.L<sup>-1</sup>); total cholesterol for male (3.3-6.9 mmol.L<sup>-1</sup>) and female (2.9-6.1 mmol.L<sup>-1</sup>); creatinine for male (60-105 µmol.L<sup>-1</sup>) and female (45-90 µmol.L<sup>-1</sup>); ALAT for male (0.15-1.1 µkat.L<sup>-1</sup>) and female (0.15-0.75 µkat.L<sup>-1</sup>); ASAT for male (0.25-0.75 µkat.L<sup>-1</sup>) and female (0.25-0.6 µkat.L<sup>-1</sup>); hemoglobin for male (134-170 g.L<sup>-1</sup>) and female (117-153 g.L<sup>-1</sup>); ferritin for male (27-365 µg.L<sup>-1</sup>) and female (13-148 µg.L<sup>-1</sup>).

**Table 6**

List of food items allowed to be consumed during low-phenolic diet.

<b>Category</b>	<b>Food items</b>
Fruits	Banana, pear (ripe and without peel)
Vegetables	Bamboo shoots, white cabbage, parsnip, celery root, cucumber (without peel)
Beverages	Milk (plain without flavoring), yoghurt (plain without added fruits), buttermilk (plain without added fruits)
Meat and other protein sources	Beef meat, pork, chicken and other poultry, lamb, shellfish, fish, egg, ham
Main carbohydrate	White bread, pasta, white rice, par-boiled rice, potatoes (without peel)
Others	Cheese, cream / sour cream, butter, salt, sugar



by Y.C. Zanzer

Figure 2  
Study design used in Papers I, II, III, IV, V and VI

## Physiological, appetite and gastrointestinal well-being tests

Venous blood samples were collected in BD Vacutainer® SST™ (Becton Dickinson, Plymouth, UK) for serum and BD Vacutainer® K2 EDTA (Becton Dickinson, Plymouth, UK) for plasma separation. To obtain serum, the collected blood sat for 30 min at room temperature to clot before being centrifuged at 3000 rpm for 10 min at 4°C. To obtain plasma, blood was immediately centrifuged at 4000 rpm for 10 min at 4°C. Plasma was used for biomarker analysis of total cholesterol (mmol.L<sup>-1</sup>), TG (mmol.L<sup>-1</sup>), LDL-c (mmol.L<sup>-1</sup>), HDL-c (mmol.L<sup>-1</sup>), NEFA (ng.mL<sup>-1</sup>), Apo-A1 (g.L<sup>-1</sup>), Apo-B (g.L<sup>-1</sup>), FRAP (μmol.L<sup>-1</sup> Trolox equivalent), GLP-1, PYY, LBP (μg.mL<sup>-1</sup>), LPS (EU.mL<sup>-1</sup>), sCD14 (μg.mL<sup>-1</sup>), T3 (nmol.L<sup>-1</sup>), T4 (nmol.L<sup>-1</sup>) and TEAC (μmol.L<sup>-1</sup> Trolox equivalent), while serum was used for determination of insulin (pmol.L<sup>-1</sup>), bile acids (ng.mL<sup>-1</sup>) and TBARS (nmol.mL<sup>-1</sup> MDA equiv.). For the purpose of gut hormones analysis, inhibitor of dipeptidyl peptidase IV (Diprotin A/ile-pro-ile, Sigma Aldrich, St. Louis, MO, USA) and Pefabloc (Roche Diagnostics, Mannheim, Germany) were pre-added to the plasma collection tubes.

### *Markers of glycemia*

Blood glucose (mmol.L<sup>-1</sup>) was measured from a finger-prick blood samples using commercial kit (HemoCue® blood glucose, HemoCue AB, Ängelholm, Sweden). In **Papers I** and **II**, serum insulin was measured using an enzyme immunoassay (Insulin ELISA, Mercodia, Uppsala, Sweden), while in **Papers III** and **VI**, insulin analysis was performed by a certified clinical chemistry laboratory (Lund University/Skåne Hospital) using Cobas®6000 analyzer (Roche Diagnostics, Mannheim, Germany).

### *Markers of lipemia*

Measurements of plasma levels of total cholesterol, TG, LDL-c, HDL-c, Apo-A1 and Apo-B in **Papers III** and **VII** was performed as routine analysis by the certified clinical chemistry laboratory at Lund University/Skåne Hospital using Cobas®6000 analyzer (Roche Diagnostics, Mannheim, Germany). NEFA was measured using an enzymatic colorimetric method (NEFA, ACS-ACOD Wako Chemicals GmbH, Neuss, Germany).

### *Markers of oxidative stress and plasma antioxidant capacity*

Lipid oxidation (**Paper III**) was measured as serum TBARS according to a previously described method with a slight modification (125). Antioxidant capacity in plasma was measured with the trolox-equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) according to the previous report (126).

### *Gut hormones*

In **Papers I and II**, quantitative determination of plasma PYY was performed with an enzyme immunoassay (Human PYY EIA YK080, Yanaihara Institute Inc., Shizuoka, Japan) according to the manufacturer's instruction. In **Paper II**, plasma GLP-1 was quantitated using a commercial kit (GLP-1 Total ELISA kit, Millipore Co., St Charles, MS, USA).

### *Thyroid hormones*

Total triiodothyronine (T3) and total thyroxine (T4) in plasma (**Papers II and VI**) were measured by the certified clinical chemistry laboratory (Lund University/Skåne Hospital) using Cobas®6000 analyzer (Roche Diagnostics, Mannheim, Germany).

### *Metabolic endotoxemia*

Metabolic endotoxemia (**Papers III and VI**) which correspond to plasma LPS (HIT320 Hycult Biotech, Uden, The Netherlands), LBP (EU.mL<sup>-1</sup>) (HK315 Hycult Biotech, Uden The Netherlands) and sCD14 (HK320 Hycult Biotech, Uden, The Netherlands) were respectively measured using a commercial kit and according to the manufacturer's instruction. Plasma was diluted 1:10 with endotoxin-free water for LPS, 1:1000 with supplied wash/dilution water for LBP and 1:80 with supplied dilution buffer for sCD14 prior to the respective analysis. Pre-heating (70°C for 1 min in water bath) and ultrasonication (37°C for 10 min) treatments were required for diluted plasma prior to LPS analysis.

### *Bile acids (BAs)*

Sixteen BAs consisting of primary [cholic acid (CA) and chenodeoxycholic acid (CDCA)], secondary [deoxycholic acid (DCA), lithocholic acid (LCA), ursodeoxycholic acid (UDCA), hyodeoxycholic acid (HDCA)] bile acids and their conjugated forms [tauro-cholic acid (TCA), glyco-cholic acid (GCA), tauro-chenodeoxycholic acid (TCDC), glyco-chenodeoxycholic acid (GCDCA), tauro-deoxycholic acid (TDCA), glyco-deoxycholic acid (GDCA), tauro-lithocholic acid (TLCA), glyco-lithocholic acid (GLCA), tauro-ursodeoxycholic acid (TUDCA) and glyco-ursodeoxycholic acid (GUDCA)] were analyzed using UHPLC-QTOF-MS. The results of BAs profile are presented in **Paper IV**.

### *Blood pressure*

Systolic (SBP) and diastolic (DBP) blood pressures (**Paper V**) were measured using electronic sphygmomanometer OMRON Model M3 Intelligence (OMRON Healthcare Co., Ltd., Kyoto, Japan).

### *Subjective appetite rating*

Appetite sensations (**Papers I, II and V**) were measured using 100 mm visual analogue scales (VAS) on a personal laptop with adaptive visual analogue scales (AVAS) software (127). Appetite-sensation measurements included ratings of 'hunger' (*How hungry do you feel?*), 'desire to eat' (*How strong is your desire to eat?*), 'satiety' (*How satiated [i.e. pleasantly satisfied] are you?*), 'fullness' (*How full do you feel?*) and 'prospective consumption' (*How much food do you think you could eat right now?*) anchored by the terms 'not at all' and 'extremely' (128, 129).

### *Gastrointestinal well-being measurement*

Gastrointestinal well-being (**Papers II and III**) was measured using 100 mm VAS which included ratings of 'abdominal pain' (*Do you experience any abdominal pain?*), 'stomach rumbling' (*Do you experience any rumbling noises in your stomach?*), 'flatulence' (*Do you experience any flatulence [generation of excessive gas]?*), 'abdominal bloating' (*Do you experience any bloated [swollen] feeling in the abdomen?*), 'acid reflux' (*Do you experience any acid reflux [heartburn]?*), 'nausea' (*Do you experience nausea?*) and 'urge to vomit' (*Do you experience an urge to vomit?*) anchored by the terms 'not at all' and 'extremely' (130). The test was performed in individual laptops using AVAS software (127).

## **Transcriptomics**

In **Paper VI**, peripheral blood mononuclear cells (PBMCs) were isolated immediately after blood collection using 8 ml vacutainer Cell Preparation Tube containing sodium citrate (Becton Dickinson, Plymouth, UK). The collected PBMCs were washed with filtered phosphate-buffered saline and pelleted cells were retained. The buffer RLT was added into the pelleted cells for cell lysis and subsequently transferred into QIAshredder (QIAGEN, Hilden, Germany) and subjected to centrifugation (full speed centrifugation for 2 min) to further obtain the homogenized lysate.

### *mRNA extraction and cDNA generation*

The mRNA from PBMCs was extracted and purified using RNEasy (QIAGEN, Hilden, Germany) according to manufacturer's instruction. RNA quantity and quality were measured using a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and Agilent Bioanalyser (Agilent Technologies Inc., Santa Clara, CA, USA), respectively. All samples had RNA integrity (RIN) score above 8.7. The cDNA was generated using an iScript Advance cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). The cDNA samples were sent to SCIBLU Genomics microarray facility at Lund University, Sweden for further process of microarray hybridisation using a Illumina HumanHT-



12 v4 Expression BeadChip (Illumina Inc., San Diego, CA, USA) and scanned using a Illumina HiScan (Illumina Inc., San Diego, CA, USA).

### *Quantitative real-time polymerase chain reaction (RT-qPCR)*

Selected genes that were found to be differentially expressed from microarray results were validated with RT-qPCR. Pre-designed primers PrimePCR™ (Bio-Rad Laboratories Inc., Hercules, CA, USA) were used for IL-8, TNF- $\alpha$ , ATP-binding cassette subfamily A member 1 (ABCA1), prostaglandin-endoperoxide synthase 2 (PTGS2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The GAPDH was used as a housekeeping gene, and gene expression levels were normalized by measuring the cycle threshold ratios between candidate genes and the housekeeping gene. The real-time PCR data was analyzed by the comparative C<sub>T</sub> method using CFX Manager™ Software (Bio-Rad Laboratories Inc., CA, USA).

## Calculations and statistical analysis

### Calculations

The total, incremental and net area under the curve (t, i, netAUC) of the metabolic parameters were calculated using trapezoid method (131). The incremental peak (iPeak) of the metabolic parameters were calculated as the maximum postprandial increase from the baseline. The C-max was defined as the highest observed concentration of the metabolic parameters in a concentration-time profile, while T-max was time to reach C-max. The estimated metabolic clearance of glucose (MCR<sub>est</sub>) and the estimated insulin sensitivity index (ISI<sub>est</sub>) were calculated to represent insulin sensitivity (132). In addition, the insulinogenic index (IGI), the estimated first phase (FP-IS) and second phase (SP-IS) insulin secretion were measured to reflect  $\beta$ -cell function (132). Furthermore, the atherogenic index of plasma was calculated as log(TG/HDL-c) (133), while postprandial metabolic load index was calculated according to Emerson *et al.* (134).

### Univariate analysis

The mixed model analysis of covariance (ANCOVA, PROC MIXED procedure, SAS Institute) with repeated measures and either first order autoregressive (**Papers I and II**) or spatial power (**Papers III, IV, V and VI**) covariance structures was performed to evaluate the effect of treatment, time, gender, BMI and their interaction on dependent variables, followed by Tukey-Kramer's *post-hoc* test. The Kenward-Roger correction was applied for reducing small sample bias. The fixed

effects included in the model were corresponding baseline (fasting values) of respective markers, treatment (**Papers I, II, III, IV, V and VI**), time (**Papers I, II, III, IV, V and VI**), time  $\times$  treatment (**Papers I, II, III, IV, V and VI**), gender  $\times$  treatment (**Papers III, IV, V and VI**), BMI  $\times$  treatment (**Papers V and VI**). All data obtained for univariate analysis are presented as least square mean (LSM)  $\pm$  standard errors of the mean (SEM), unless otherwise specified.

## Multivariate analysis and modelling

In order to investigate the relationships and contributions of the observed variables in the treatment, principal component analysis (PCA) was performed in **Paper II**. The result was visualized in a bi-plot showing both loading and score plots. The PCA analysis was performed within R-environment ver. 3.2.4. using *FactoMineR*, *factoextra* and *ggplot2* packages. An imputation of the missing data was performed with *missMDA* package. Modelling with orthogonal projection to latent structure discriminant analysis (OPLS-DA) was performed to discriminate whether there were gender (**Papers II, III and V**) and/or BMI (**Paper V**) effects were present and to identify associated variables responsible for the cluster separation. The OPLS-DA modelling was performed using *ropls* package, while the Pearson's correlation and correlation matrix were analysed with the *corrplot* package.

## Bioinformatics analysis

### *Transcriptomics data processing*

In **Paper VI**, raw microarray data generated from Illumina HumanHT-12 v4 Expression BeadChip (Illumina Inc., San Diego, CA, USA) were quantile-normalized with Illumina GenomeStudio prior to downstream analyses. The microarray HumanHT-12 v4 Expression BeadChip presented total 47231 probes and only probes which had signal intensity with  $P < 0.05$  in all of the the group (HF0h, HF4h, HF-Spices0h and HF-Spices4h) of at least 17 out of 18 participants were considered for further moderated *t*-test (HF4h vs HF0h and HF-Spices4h vs HF-Spices0h) using the R/Bioconductor '*limma*' package. Differentially expressed genes from each of comparison (HF4h vs HF0h and HF-Spices4h vs HF-Spices0h) were defined as those with a  $P < 0.05$  and  $FDR < 0.05$ .

### *Pathway and process network analysis*

Functional enrichment pathway and process network analysis from differentially expressed genes was performed with MetaCore™ (Clarivate Analytics, Thompson Reuters, Philadelphia, PA, USA) (**Paper VI**). The score of significantly enriched

pathway and process network which uniquely corresponds to a particular treatment were presented as  $-\log P$ value with a  $FDR < 0.05$ .

# Results and Discussion

## Part I. Bioactive compounds in spices

### Characterization of spice-based beverages

Distinct bioactive compounds were represented in the different types of spice-based beverages used in this dissertation (**Table 8**). The cinnamon (*C burmannii*)-based beverage used in **Paper I** contained polyphenol subclass flavanols, identified as procyanidin A-type-trimer (cinnamtannin), procyanidin B-type-dimer and procyanidin C-type-trimer. In addition, kaempferitrin, 2-hydroxycinnamaldehyde, cinnamic acid and cinnamaldehyde were also characterized in the cinnamon-based beverage. Another study using a different type of cinnamon species (*C zeylanicum*), with 50% ethanol extraction, yielded additional bioactive compounds such as apigenin, caffeic acid, caffeic acid hexoside and its isomers, carnosic acid, catechin, chlorogenic acid, coumaric acid-*O*-hexoside 1, *p*-coumaric acid, 4-*O*-*p*-coumaroylquinic acid, cryptochlorogenic acid and dicaffeoylquinic acid (135).

On the other hand, flavonols such as rutin, quercetin glycoside, avicularin (quercetin 3-arabinofuranoside), nicotiflorin (kaempferol 3-rutinoside), isorhamnetin 3-rutinoside and isorhamnetin 3-glycoside were identified in star anise beverage as presented in **Paper I**. Compounds such as isorhamnetin 3-rutinoside and isorhamnetin 3-glycoside were observed in star anise, which has never been reported previously. The turmeric-based beverage which was used consistently in **Paper I**, **III** and **IV**, contained curcuminoids such as curcumin, demethoxycurcumin and bisdemethoxycurcumin. The main active compounds of ginger were not identified in the ginger-based beverage used in **Paper I**, possibly due to short extraction time (10 min) and low (90°C) temperature which limits gingerols and shogaols extraction (136). The black-pepper based beverage used in **Paper II** contained flavones such as apigenin and its derivatives. In addition, other compounds characterized include dihydroxybenzoic acid hexoside-pentoside, decaffeoyl-acteoside, cynaroside A, luteolin 6-*C*-hexoside-8-*C*-rhamnoside and kaempferol 3-rhamnoside-4'-xyloside. Similar to the case with gingerols and shogaols in ginger, it was not possible to identify predominant compounds in black pepper such as piperine and its derivatives due to its low water solubility and higher melting point

## Total polyphenol content and antioxidant capacity

The antioxidant capacity (DPPH and TEAC) and total phenolic content of the mixed spices (**Papers V and VI**) samples extracted with either water or ethanol are presented in **Table 7**.

**Table 7**

The antioxidant capacity (DPPH, TEAC) and total phenolic content (TPC) in mixed spices extracted with either water-based or ethanol-based (Papers V and VI).

Extraction type	DPPH (EC <sub>50</sub> , µg/mL sample)	TEAC (mmol trolox/g sample)	TPC (mg GAE/g of sample)
Water-based extract	2483 ± 191	0.470 ± 0.008	1.87 ± 1.05
Ethanol extract	688 ± 12	0.136 ± 0.013	20.48 ± 1.47

**Table 8**  
Composition of bioactive compounds in the spice-based beverages (Papers I,II, III and IV).

RT (min)	Compounds identified	UV-vis maxima (nm)	M-H, MF, mDa	Main fragments detected	Spice
1.80	Procyanidin C-type (Trimer isomer)	270.8	865.2174, C <sub>46</sub> H <sub>37</sub> O <sub>16</sub> , -2.1	713.17, 575.12, 289.07	Cinnamon
4.11	Procyanidin B-type (Dimer)	236.8, 279.8	577.1320, C <sub>30</sub> H <sub>25</sub> O <sub>12</sub> , -2.6	425.08, 409.08, 289.07	Cinnamon
4.42	Cinnamtannin (Procyanidin A-type, trimer isomer)	242.8, 278.8	863.1780, C <sub>46</sub> H <sub>35</sub> O <sub>16</sub> , -4.3	711.13, 573.10, 411.07, 289.07	Cinnamon
4.70	Cinnamtannin (Procyanidin A-type, trimer isomer)	239.8, 278.8	863.1780, C <sub>46</sub> H <sub>35</sub> O <sub>16</sub> , -4.3	711.14, 573.10, 411.07, 289.07	Cinnamon
4.70	Dihydroxybenzoic acid hexoside-pentoside	230.8, 275.8, 310.4	447.1139, C <sub>18</sub> H <sub>23</sub> O <sub>13</sub> , 0.0	152.0151, 108.0163	Black pepper
5.30	Decaffeoyl-acteoside	228.8	461.1653, C <sub>20</sub> H <sub>29</sub> O <sub>12</sub> , -0.6	315.1653, 298.0974, 165.0699, 135.0401	Black pepper
5.45	Cinnamtannin (Procyanidin A-type, trimer isomer)	237.8, 278.8	863.1840, C <sub>46</sub> H <sub>35</sub> O <sub>16</sub> , -1.7	711.13, 573.10, 411.08, 289.07	Cinnamon
5.74	Cynaroside A	229.8, 265.81	443.1884, C <sub>21</sub> H <sub>31</sub> O <sub>10</sub> , -3.3	375.0570, 236.5559	Black pepper
5.92	Cinnamtannin (Procyanidin A-type, trimer isomer)	236.8, 278.8	863.1780, C <sub>46</sub> H <sub>35</sub> O <sub>16</sub> , -1.7	-	Cinnamon
6.85	Rutin	255.8, 353.8	609.1422, C <sub>27</sub> H <sub>29</sub> O <sub>16</sub> , -3.4	300.3	Star anise
7.13	Rutin isomer	255.8, 353.8	609.1422, C <sub>27</sub> H <sub>29</sub> O <sub>16</sub> , -3.4	300.3	Star anise
7.30	Apigenin 6,8-di-C-hexoside	229.8, 269.8, 329.8	593.1503, C <sub>27</sub> H <sub>29</sub> O <sub>15</sub> , -0.3	575.1471, 503.1240, 473.1147, 383.0761, 353.0683, 297.0753	Black pepper
7.31	Quercetin glycoside	266.8, 353.8	463.0858, C <sub>21</sub> H <sub>19</sub> O <sub>12</sub> , -1.9	300.03	Star anise
7.58	Quercetin glycoside isomer	256.8, 353.8	463.0902, C <sub>21</sub> H <sub>20</sub> O <sub>12</sub> , 2.5	300.03	Star anise
8.17	Kaempferitin	331.8	577.1604, C <sub>27</sub> H <sub>29</sub> O <sub>14</sub> , 4.7	285.05	Cinnamon
8.24	Luteolin 6-C-hexoside-8-C-rhamnoside	229.8, 269.8, 329.8	593.1553, C <sub>27</sub> H <sub>29</sub> O <sub>15</sub> , 1.1	473.1058, 429.0855, 357.0617, 327.0517, 309.0399	Black pepper
8.35	2-Hydroxycinnamaldehyde	277.8, 308.8	147.0477, C <sub>9</sub> H <sub>7</sub> O <sub>2</sub> , 3.1	-	Cinnamon

**Table 8 continued**  
Composition of bioactive compounds in the spice-based beverages (Paper I, II, III, IV, V, VI).

RT (min)	Compounds identified	UV-vis maxima (nm)	M-H, MF, mDa	Main fragments detected	Spice
8.46	Avicularin (Quercetin 3-arabinofuranoside)	255.8, 353.8	433.0751, C <sub>20</sub> H <sub>17</sub> O <sub>11</sub> , -2.0	300.03	Star anise
8.53	Nicotiflorin (Kaempferol 3-rutinoside)	238.8, 346.8	593.1514, C <sub>27</sub> H <sub>28</sub> O <sub>15</sub> , 0.8	285.04	Star anise
8.55	Apigenin 8-C-hexoside-C-deoxyhexoside	229.8, 267.8, 331.8	577.1540, C <sub>27</sub> H <sub>28</sub> O <sub>14</sub> , -1.7	457.1078, 413.0857, 311.0534, 293.0448, 283.0529, 175.0001, 117.0317	Black pepper
8.58	Isorhamnetin 3-rutinoside	238.8, 353.8	623.1595, C <sub>28</sub> H <sub>31</sub> O <sub>16</sub> , -1.7	315.05	Star anise
8.64	Isorhamnetin 3-rutinoside isomer	239.8, 353.8	623.1595, C <sub>28</sub> H <sub>31</sub> O <sub>16</sub> , -1.7	315.05	Star anise
8.73	Isorhamnetin 3-glycoside	238.8, 352.8	477.1021, C <sub>27</sub> H <sub>27</sub> O <sub>12</sub> , -1.2	315.05	Star anise
8.78	Cinnamic acid	292.8, 339.8	147.0476, C <sub>9</sub> H <sub>7</sub> O <sub>2</sub> , 3.3	-	Cinnamon
9.82	Cinnamaldehyde	310.8	133.0686 [M+H] <sup>+</sup> , C <sub>9</sub> H <sub>9</sub> O, 3.3	115	Cinnamon
9.96	Bisdemethoxycurcumin	239.8, 360.8, 420.0	309.1218 [M+H] <sup>+</sup> , C <sub>19</sub> H <sub>17</sub> O <sub>4</sub> , 9.1	170.15	Turmeric
10.01	Kaempferol 3-rhamnoside-4'-xyloside	229.8, 349.8	563.1402, C <sub>26</sub> H <sub>27</sub> O <sub>14</sub> , 0.1	417.0728, 285.0423	Black pepper
10.07	Demethoxycurcumin	240.8, 356.8, 421.0	339.1333 [M+H] <sup>+</sup> , C <sub>20</sub> H <sub>19</sub> O <sub>5</sub> , 10.1	309.12, 235.18, 201.17	Turmeric
10.17	Curcumin	365.8, 262.8, 426.0	369.1437 [M+H] <sup>+</sup> , C <sub>21</sub> H <sub>21</sub> O <sub>6</sub> , 9.9	309.12, 235.18	Turmeric
10.20	Apigenin 7-neohesperoside	266.8, 338.8	577.1540, C <sub>27</sub> H <sub>28</sub> O <sub>14</sub> , -1.7	269.0434	Black pepper
10.47	Curcumin isomer	239.8, 416.8	369.1425 [M+H] <sup>+</sup> , C <sub>21</sub> H <sub>21</sub> O <sub>6</sub> , 8.7	309.12	Turmeric
10.88	Apigenin 8-C-arabinopyranoside-2'-rhamnoside	266.8, 338.8	547.1443, C <sub>26</sub> H <sub>27</sub> O <sub>13</sub> , -0.9	269.0434	Black pepper

## Part II. Spices in short-term appetite regulation

*A balanced state between energy intake and expenditure is pivotal to avoid positive energy balance. By controlling the short-term appetite regulation, it might beneficially enhance and prolong the perceived satiety and might further decrease the inter-meal consumption during the day. To which extent individual spices or mixed spices can modulate perceived appetite sensations, gut and thyroid hormones in different type of meal challenges will be discussed in the following section.*



### **Appetite sensations and gut hormone release**

In **Paper I**, hot water extracts of individual spices (cinnamon, turmeric, ginger and star anise) in the form of pre-meal beverages were tested against a control beverage and using WWB (295 kcal) as a meal challenge. Overall, a main effect of treatment was observed for '*desire to eat*' ( $P=0.048$ ) and '*prospective consumption*' ( $P=0.031$ ). While time effects were observed for all appetite ratings ( $P<0.0001$ ), a tendency of a time  $\times$  treatment interaction was only observed for '*prospective consumption*' ( $P=0.054$ ). Further observations showed that '*desire to eat*' was  $-26.8\%$  ( $P=0.0035$ ) and  $-21.3\%$  ( $P=0.019$ ) lower, respectively, following turmeric and star anise beverages while there were only tendencies observed for cinnamon ( $-16.3\%$ ,  $P=0.073$ ) and ginger ( $-15.7\%$ ,  $P=0.084$ ) compared to control. In addition, lower '*prospective consumption*' was seen after turmeric ( $-19.3\%$ ,  $P=0.0052$ ), star anise ( $-18.8\%$ ,  $P=0.0065$ ) and cinnamon ( $-15.5\%$ ,  $P=0.023$ ) compared to control. Based on the results of appetite sensations, PYY was measured only after consumption of turmeric, star anise and control beverages. Main effects of treatment ( $P=0.007$ ) and an increased iAUC<sub>0-30</sub> ( $P=0.049$ ) for PYY were evident. Relative to control beverage, a greater increase in PYY level (11%,  $P=0.004$ ) after turmeric-based beverage was observed. However, no significant results on PYY were observed after the star anise-based beverage compared to control.

### ***Cinnamon***

The appetite-suppressing effects of cinnamon intake indicated by subjective rating of '*prospective consumption*' found in **Paper I** was discordant with previous postprandial studies (58-60). In one study, gastric emptying but not perceived satiety was affected by adding 6 g of cinnamon to 330 kcal rice pudding (58). However, a corresponding effect in gastric emptying was not observed with a lower dose (1 or 3 g) of cinnamon added to the same meal (59). Yet, another study showed no differences in gastric emptying or appetite sensations after ingestion of 3 g cinnamon with a 632 kcal high fat meal (60). It is known that gastric motility plays



an important role in the appetite control, both during and after food intake (137), which activates satiety circuitry in the brain (138). Gastric distension and accommodation are major determinants in the regulation of satiation during food intake, while gastric emptying and intestinal exposure of the nutrients are important factors in the regulation of satiety. Although gastric emptying was not measured in **Paper I**, a recent animal study reported that supplementation with one of the active compounds in cinnamon, cinnamaldehyde was able to substantially reduce cumulative food intake and gastric emptying rates (139). In **Paper I**, a slight difference in the method of introducing treatment/meal is present compared to other studies (58-60). While cinnamon was mixed with a meal challenge (58, 59) or consumed as a capsule form immediately prior and after the meal challenge (60), in the current study reported in **Paper I**, a preload of a hot water extract, which was given to be consumed within 5 min, followed by a WWB challenge 10 min later. The rationale for giving 15 min prior to the WWB challenge was to ensure the intestinal exposure of the bioactive compounds contained in the tested beverages (140). Furthermore, while cinnamon-based beverage was used as mode of intervention in **Paper I**, other postprandial studies in humans used either ground powder (58, 59) or capsulated (60) cinnamon. Among other bioactive compounds, cinnamaldehyde was one of the main constituents in the cinnamon-based beverage used in **Paper I**. One possible mechanism by which cinnamon interacts with the appetite-associated gut/intestinal environment might be explained by the interaction of cinnamaldehyde or other bioactive compounds with the transient receptor potential (TRP) channels. Furthermore, pungency and astringency elicited by certain bioactive compounds present in spices such as cinnamon, ginger, chili pepper and black pepper might act as an agonist of TRP channels. Recent observations revealed that cinnamaldehyde was able to reduce ghrelin secretion mediated by TRPA1 both *in vivo* and *in vitro* in a ghrelin-producing cell line derived from stomach (MGN3-1 cells) (139). In humans, cinnamaldehyde has effectively increased energy expenditure and promoted fat oxidation in the postprandial setting (141).

### ***Star anise***

Star anise and its bioactive constituents (*e.g.* kaempferol, quercetin and respective glycosides) are known to confer anti-microbial (142), anti-inflammatory (143) and antioxidant (144, 145) activities which predominantly investigated in animal or *in vitro* studies (146). Tested in this setting for the first time, it was shown that consumption of star anise-based beverage prior to WWB challenge led to significant reductions in '*desire to eat*' and '*prospective consumption*' compared to the control beverage. However, measurement of the gut hormone PYY showed no difference between star anise and the control beverage, suggesting that appetite sensations evoked by star anise might be mediated by other factors or pathways. Quercetin,

isorhamnetin and kaempferol derivatives in the star anise-based beverage (**Paper I**) might be involved in the appetite-suppression effect. Consequently, an *in vitro* study has shown that quercetin was able to modulate GLP-1R-mediated signalling (147). Another recent study reported that the highly enhanced bioavailable quercetin known as  $\alpha$ -glucosyl-isoquercitrin increased transient stimulation of GLP-1 both in murine enteroendocrine L-cell lines (GLUTag cells) and in rats (148).

### ***Ginger***

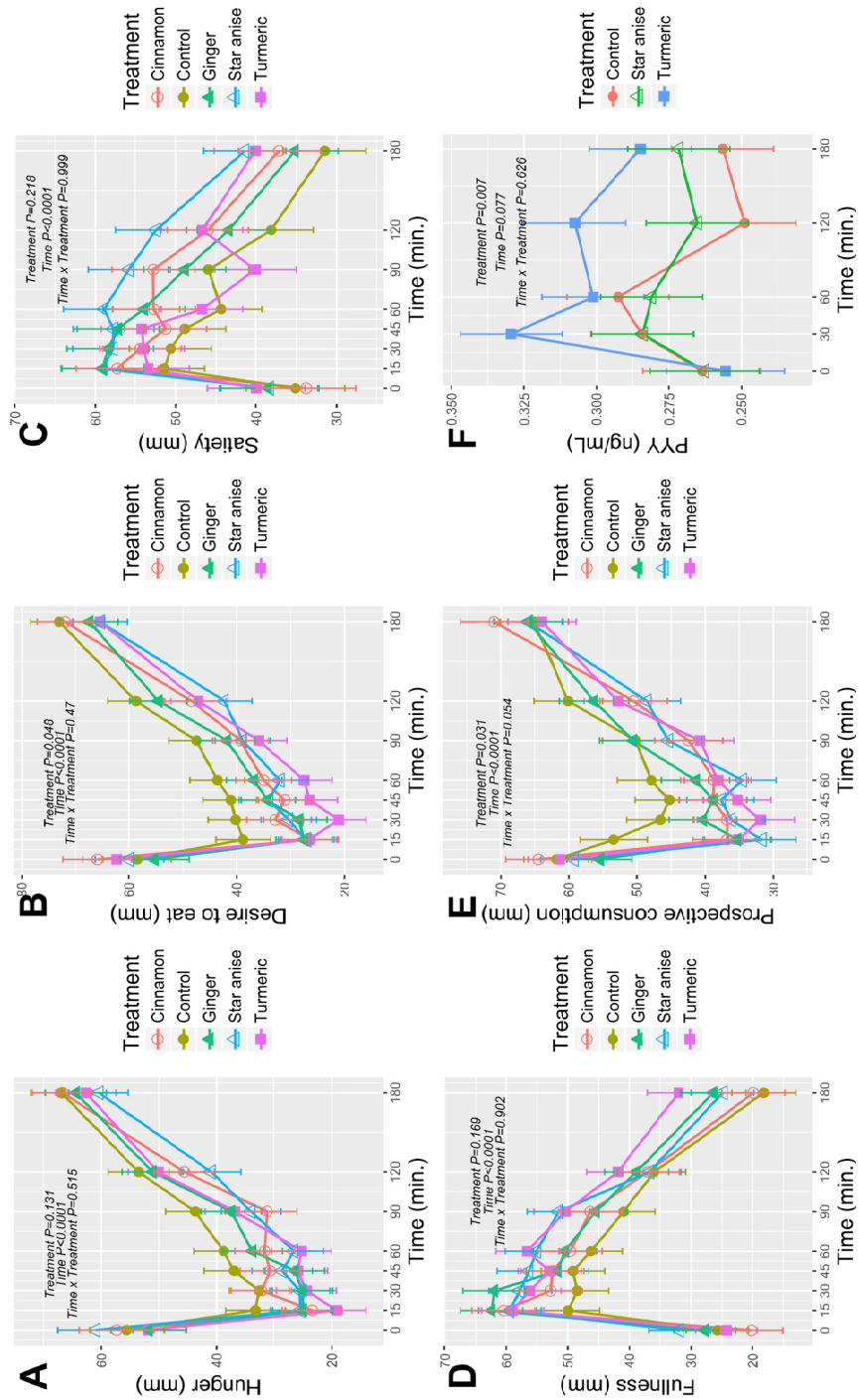
In **Paper I**, there was a tendency that ginger-based beverage could lower '*desire to eat*'. Others have reported that adding 2 g of ginger powder to hot water and consuming it with a 525 kcal meal challenge exhibited lower '*prospective consumption*' and '*hunger*' ratings as well as tended to promote greater '*fullness*' (61). It is commonly known that delayed gastric emptying has been associated with the increase of satiety-related measures (149) or anorexigenic hormone and reduced orexigenic hormone secretion (150). In contrast with the latter statement, Wu *et al.* interestingly reported that a ginger intervention (1.2 g) significantly accelerated gastric emptying and stimulated antral contractions, despite a marked increase in '*fullness*', a decrease in '*hunger*' and '*desire to eat*' (151). In patients with functional dyspepsia, ginger administration (1.2 g) also stimulated gastric emptying and antral contractions, while no effects on gastrointestinal symptoms and gut hormones (GLP-1 and ghrelin) were observed (152). Even longer-term intervention (12 weeks) with 2 g of ginger powder per day could lower the total appetite score (153). However, there are also studies of ginger in association with gastric emptying (154) and appetite (155) with conflicting results. A recent study in a type-2 diabetic animal model reported that [6]-gingerol, one of ginger active compounds significantly induced GLP-1 secretion (156). Although the main active compounds in ginger such as gingerols and shogaols were not detected in the ginger-based beverage in **Paper I**, the pungency perceived by ginger-based beverage might act as a noxious stimulus which further interact with TRP channels, similarly as observed in other pungent spices such as oregano, thyme and cloves (157). One study showed that gingerol was able to induce TRPA1-mediated calcium influx and subsequently release cholecystokinin and serotonin levels (158). However, the extent to which this compound acts in appetite-associated effect mediated by TRP channel activation needs to be further elucidated.

### ***Turmeric***

No previous postprandial human intervention has studied the association of single intake of turmeric with appetite-related outcomes. Instead, turmeric was rather used in combination with other spices in the appetite-associated studies in humans (159, 160). The turmeric-based beverage given prior to a WWB meal in **Paper I** showed

a profound reduction in 'desire to eat' (-26.8%) and 'prospective consumption' (-19.27%). Furthermore, the results showed a 113.9% increase in early iAUC<sub>0-30</sub> ( $P=0.049$ ) and an overall treatment effect ( $P=0.007$ ) of plasma gut hormone PYY after the turmeric-based beverage compared to control. The mechanism behind this might be explained by the interaction of bioactive compounds present in turmeric such as curcumin, demethoxycurcumin and bisdemethoxycurcumin with enteroendocrine cells, which are located throughout the gastrointestinal tract. In an *in vitro* study by Takikawa *et al.*, an increase in GLP-1 level was observed after addition of curcumin into murine enteroendocrine L-cell lines (GLUTag cells) (161). The proposed mechanism involved Ca<sup>2+</sup>/calmodulin-dependent kinase II (CaMKII) (161). To confirm that finding, an *in vivo* study was performed by Kato *et al.* showing that a curcumin intervention acutely induced GLP-1 secretion (162). In **Paper I**, only PYY, but not GLP-1, was measured, but it could be speculated that GLP-1 was co-secreted with PYY leading to a reduction in postprandial appetite sensations.

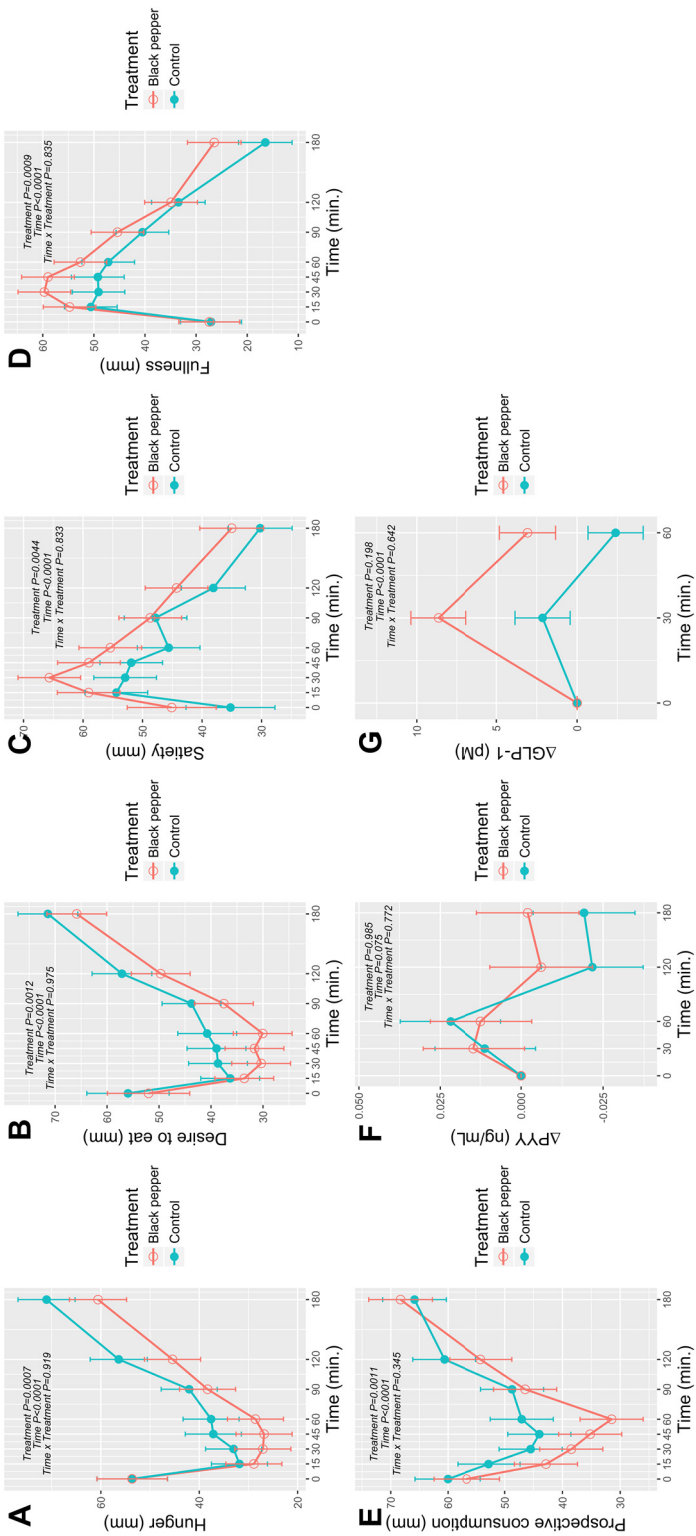
Based on the appetite suppressing effect and increase in PYY levels after the turmeric-based beverage presented in **Paper I**, another human study (**Papers III and IV**) was performed where appetite-related sensations were measured as secondary outcomes. Instead of WWB, an isocaloric (423 kcal) mixed meal formulated as medium fat (MF) and high fat meal (HF) was used as a meal challenge. Furthermore, turmeric-based (TUR) or control (CON) preload were given in the same manner as in **Paper I**. It was noted that postprandial 'satiety' was higher (18%,  $P=0.039$ ) after CON-MF compared to CON-HF, while no other appetite sensations such as 'hunger' ( $P=0.32$ ), 'desire to eat' ( $P=0.19$ ), 'fullness' ( $P=0.11$ ) and 'prospective consumption' ( $P=0.56$ ) were affected. With relatively comparable protein content (13 g or 12%E in HF and 15.7 g or 15%E in MF), the MF contained four times more carbohydrates (49.5 g, 47%E) compared to the HF (12.4 g, 12%E) meals, and conversely, the fat content was double in HF (35.7 g, 76%E) compared to the MF (18.1g, 38%E) meals. In concordance with previous studies, an isocaloric meal with high carbohydrate content suppressed appetite and increased satiety more than a fatty meal (163-165). Likewise, reduced ghrelin (163) and increased PYY (166) were observed in the high carbohydrate meal compared to other meals which were predominated by either protein or fat. Surprisingly, turmeric-based beverage, when consumed prior to the meal challenge (TUR-MF or TUR-HF) did not significantly affect appetite sensations. It could be assumed that the macronutrients composition overrides the effect of the turmeric-based beverage in the postprandial setting, but to understand the mechanism behind, further investigations are needed. Another obvious reason is the dose of extract beverage given itself might be important in eliciting the appetite-associated effects.



**Figure 3** Perceived 'hunger' (A), 'desire to eat' (B), 'satiety' (C), 'fullness' (D) and 'prospective consumption' (E) after either cinnamon, turmeric, star anise, ginger or control beverages. Postprandial response in plasma PYY (F) after either turmeric, star anise or control beverages.

## ***Black pepper***

Given the very limited number of studies involving black pepper in humans (155, 167), the study with black pepper-based beverage with appetite-related outcomes was performed and presented in **Paper II**. After having black pepper-based beverage followed by WWB challenge, reductions in '*hunger*' (-17%,  $P=0.0007$ ), '*desire to eat*' (-15%,  $P=0.0012$ ) and '*prospective consumption*' (-17%,  $P=0.0011$ ) were observed, while '*satiety*' (14%,  $P=0.0044$ ) and '*fullness*' (16%,  $P=0.0009$ ) were clearly increased as shown in **Figure 4**. The appetite sensations shown in **Paper II** diverge from others using 1.5 g black pepper incorporated into a mixed meal containing other phenolic-rich foods such as raspberry, apple, beetroot and multi-fruit juice (155). Despite the appetite suppressing effect, PYY was not affected ( $P=0.98$ ) and the 23% increase in GLP-1 observed after black pepper-based beverage consumption did not reach statistical significance ( $P=0.2$ ). Hence, the mechanism behind the appetite suppressing effect induced by black pepper might not relate to a gut hormone-associated mechanism. Indeed, piperine and its derivatives were not identified in the black pepper-based beverage, but other polyphenols were present as discussed in Part 1. Thus, the effects on appetite sensations might be induced by other compounds such as apigenin and its derivatives. One report showed that apigenin administration affects both short- and long-term food intake in an animal study (168). After all, inter-individual variability in the response of metabolic markers and appetite sensations was evident showing a cluster separation between men and women (**Figure 5**). The orthogonal projection to latent structure discriminant analysis (OPLS-DA) showed that the described response variation ( $R^2Y=0.53$ ) and predicted response variation ( $Q^2Y=0.33$ ) were comparable and accounted for 14% of total variation explained. In appetite sensations, the responsible driving forces for gender separation were '*hunger*', '*desire to eat*' and '*prospective consumption*' for women while '*satiety*' and '*fullness*' were associated with men.



**Figure 4** Perceived 'hunger' (A), 'desire to eat' (B), 'satiety' (C), 'fullness' (D) and 'prospective consumption' (E) after either black pepper-based or control beverages. Postprandial response in plasma PYY (F) and GLP-1 (G) after either black pepper-based or control beverages.

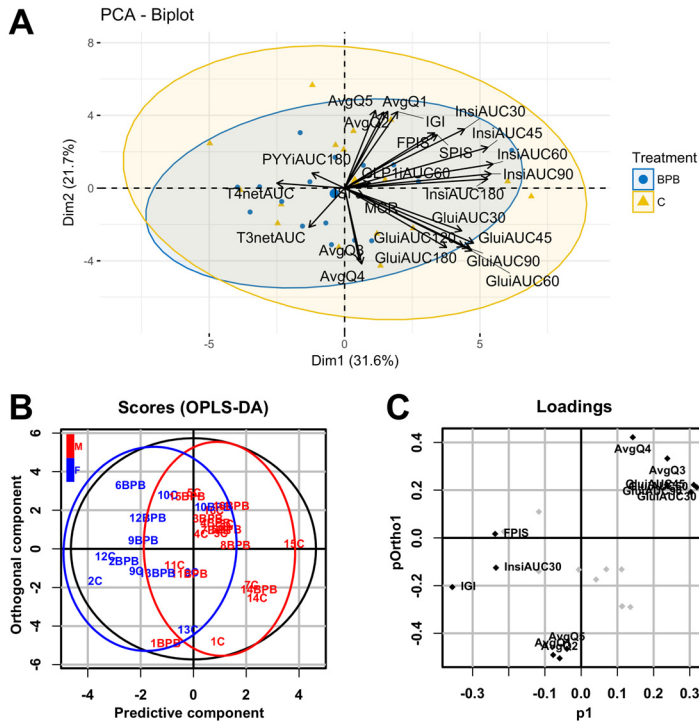
## **Mixed spices**

As individual spices showed a distinctive effect on appetite regulation, the last study was conducted with mixed spices being incorporated into a high fat meal (**Paper V**). A mixed spices-enriched high fat meal (HF-Spices) induced higher '*satiety*' (8.7%,  $P=0.048$ ) and '*fullness*' (13.3%,  $P=0.0041$ ) ratings compared to a corresponding high fat meal without mixed spices (HF). Inter-individual responses in term of gender and BMI status were also evident. As opposed to HF, HF-Spices consumption induced a stronger feeling of '*satiety*' in men (27.4%,  $P=0.0006$ ,  $n=8$ ) and overall normal weight participants of both genders (31.2%,  $P=0.0005$ ,  $n=7$ ). In contrast to the reduced '*prospective consumption*' experienced by overweight participants (-25.6%,  $P=0.0084$ ,  $n=11$ ), the normal weight participants showed the opposite effect in '*prospective consumption*' after HF-Spices relative to HF. In line with a recent report where mixed spices formulated as a curry meal showed that greater suppression in '*hunger*' and '*prospective consumption*' (160) was followed by an increase in postprandial GLP-1 (159), but not ghrelin (160) levels. Further investigations are needed to evaluate whether the perceived appetite sensations are positively correlated with the actual food intake shown by *ad libitum* energy intake during the whole day.

## **Modulation of thyroid hormones by spices**

Although some disagreement exists, a body of evidence on the effect of pungent spices, such as red chili pepper shows their involvement in modulation of the regulation of energy expenditure (169). Thyroid hormones, which have never been previously investigated, were chosen as exploratory markers in **Papers II** and **V**. In **Paper II**, a time effect ( $P<0.0001$ ) was found showing that T3 was reduced irrespective of treatments (black pepper-based or control beverages) across the 3 h time course. Nevertheless, a principal component analysis showed that postprandial T3 level was clustered after black-pepper based beverage and negatively correlated with postprandial insulin, insulinogenic index, estimated first- and second-phase of insulin secretion, sensation of '*hunger*', '*desire to eat*' and '*prospective consumption*'. In addition, postprandial T4 levels were negatively correlated with postprandial glucose and metabolic clearance rate of glucose (**Figure 5A**). In **Paper V**, incorporation of mixed spices (cinnamon, turmeric, black pepper, ginger and clove) into a high fat meal significantly increased T4 (2.7%,  $P=0.002$ ) but not T3 levels. It also observed that irrespective of treatments (HF-Spices or HF), normal weight participants ( $n=7$ ) had higher T3 level (5.8%,  $P=0.028$ ) compared to those overweight ( $n=11$ ). The clinical relevance of this observation associated with food intake and energy expenditure deserves further investigation. Since thyroid hormones were postprandially affected after meal intake, the inclusion on these

markers in future studies associated with appetite and energy balance might give an additional perspective.



**Figure 5** Principal component analysis bi-plot based on the tested physiological parameters after either black pepper-based or control beverages (A). Orthogonal projection to latent structure discriminant analysis (OPLS-DA) score plot separating based on gender (B). Loading plot showing the driving forces for the observed separation (C). BPB, black pepper-based beverage; C, control beverage; M, men; F, women; AvgQ1, average ‘hunger’; AvgQ2, average ‘desire to eat’; AvgQ3, average ‘satiety’; AvgQ4, average ‘fullness’; AvgQ5, average ‘prospective consumption’; FPIIS, estimated first phase insulin secretion; GluiAUC, incremental area under the curve of glucose; GLP1iAUC, incremental area under the curve of glucagon-like peptide 1; IGI, insulinogenic index; InsiAUC, incremental area under the curve of insulin; ISI, insulin sensitivity index; PYYiAUC, incremental area under the curve of peptide tyrosine-tyrosine; MCR, estimated metabolic clearance rate; SPIS, estimated second phase insulin secretion; T3netAUC, net area under the curve of triiodothyronine; T4netAUC, net area under the curve of thyroxine.



Individual spices such as turmeric, cinnamon, star anise and black pepper were shown to modulate appetite sensations and to some extent affect appetite-related and thyroid hormones when given prior to WWB. Adding mixed spices into high fat meal also showed to modulate appetite sensations and observed potential inter-individual effect based on gender or metabolic status. Further step to understand the holistic picture of appetite regulation such as measuring ad-libitum food intake and elucidating the underlining mechanisms is needed.



## Part III. Spices in glycemic regulation

*Postprandial glucose response is considered as independent risk factor for CVD in individuals with T2D (63) and risk factor for mortality in non-diabetic men (170). Thus, a tight management in postprandial glycemia is pivotal to avoid exacerbation in metabolic diseases-associated complications. Current knowledge on the beneficial effects of spices in glycemia was mainly investigated in longer-term intervention but to a limited extent in postprandial setting. To which extent individual spices or mixed spices are able to modulate postprandial glycemia in a different type of meal challenges are addressed below.*



### **Turmeric**

In **Paper I**, both turmeric and cinnamon showed early reductions in blood glucose after the WWB challenge. The decrease in postprandial glycemia was observed from 0-45 min ( $-34\%$ ,  $P=0.035$ ) after the turmeric-based beverage compared to control. Conversely, no changes in the insulin sensitivity or  $\beta$ -cell function were noted after the turmeric-based beverage intervention. Furthermore, the glucose reducing effect was discordant with other studies (171, 172). The discrepancy might be caused by some factors such as different methods of intervention (whether turmeric is ingested together with a meal challenge or as a preload prior to the meal challenge, as in this study) and the form of turmeric administration *per se* (in capsules form or as in the current study, as an extract-based beverage). In **Paper I**, the turmeric-based beverage containing 185 mg GAE was used while capsules containing a total of 6 g of turmeric powder was given in the study by Wikenberg *et al.* (171) and 0.5 g of turmeric powder incorporated in fruit juice was used by Oza *et al.* (172). Furthermore, WWB containing 50 g of available carbohydrate was used as a meal challenge in **Paper I**, while Wickenberg *et al.* (171) used 75 g glucose/250 mL of a standard oral glucose tolerance test (OGTT) and Oza *et al.* (172) used a meal containing 516 kcal consisting of mashed potatoes and fruit juice. Furthermore, giving the turmeric-based beverage 10 min prior to the meal challenge might allow the interaction of turmeric or its active compounds with intestinal glucose sensing located in the intestinal mucosa of the small intestine. Interestingly, a recent study reported that curcumin exhibits immediate inhibitory effect on basal glucose uptake of glucose transporter-1 (GLUT1) in L929 fibroblast cells, HCLE cells and HK2 cells in a dose dependent manner and the inhibition is reversed within an hour (173). Using similar study design as in **Paper I**, turmeric-based beverage in **Paper III** was ingested prior to either MF or HF. However, neither glucose nor insulin levels were modulated following ingestion of turmeric-based beverage which was given prior

to isocaloric medium (MF) or high fat (HF) meals. In comparison with **Paper I** that used WWB (50 g available carbohydrate) as meal challenge, MF and HF in **Paper III** contained 49.5 g and 12.4 g carbohydrates, respectively. An obvious reason why the turmeric-based beverage was able to modulate postprandial glycemia in **Paper I** but not in **Paper III** might be caused by different calories contained in different meal challenges used in the respective study. Although the carbohydrate content was similar between WWB (50 g CHO) and MF (49.5 g CHO), it is still obvious that MF contained higher calories than WWB. In addition, the dose of turmeric-based beverage given might also contribute in modulating postprandial glycemia, particularly when meal challenges are varies in calories and macronutrient composition. However, several human studies have shown that longer-term turmeric or curcumin interventions were able to reduce fasting glucose (174), HbA1c (174) and HOMA-IR (174, 175) in overweight or obese individuals with T2D. An earlier report showed that nine months of intervention with 1.5 g curcuminoids/day in a capsule form was able to prevent the development of T2D in prediabetic individuals as well as improved  $\beta$ -cell function (176). Besides direct inhibition of the active transport of GLUT1 in the intestine, other mechanisms involving suppression of hepatic glucose production could be behind these beneficial effects of curcumin (177, 178). In addition, other reports have shown that curcumin may stimulate glucose uptake into skeletal muscle (179, 180).

### **Cinnamon**

With regards to cinnamon, it was shown that *C. burmannii* based beverage in **Paper I** could reduce the early (0-30 min) postprandial blood glucose ( $-38.6\%$ ,  $P=0.046$ ) after the WWB challenge, while no change was found in insulin secretion or sensitivity. These results were in agreement with previous postprandial human studies using cinnamon of different dosage and type of meal challenge in either healthy (58, 181-183), impaired glucose tolerance (184) or obese (185) individuals. Incorporation of 6 g of cinnamon into rice pudding (330 kcal) markedly reduced early blood glucose 0-45 min by  $-55\%$  in healthy individuals (58). Likewise, 6 g of cinnamon (*C. cassia*) added to farina cereal (50 g CHO) significantly lowered glycemia up to 2 hours by  $-23.6\%$  (182). However, a lower cinnamon dose (3 g) in rice pudding (330 kcal) reduced insulin levels by  $-55.4\%$  (59). Another study administrating 5 g of cinnamon as capsules with a 75 g dextrose challenge showed a reduction in the glucose response and improved insulin sensitivity, even though the cinnamon was given 12 h prior to the OGTT (183). Mettler *et al.* reported that 4 g of cinnamon (*C. cassia*) alone or combined with acetic acid consumed with milk rice (75:5:7 g of CHO, F and P, respectively) significantly reduced early blood glucose up to 15 min (186). An extract of 5 g cinnamon (*C. zeylanicum*) consumed with 50 g WWB appeared to lower blood glucose ( $-36.9\%$ ) up to 2 hours in healthy individuals (187). A similar study using 1 g hydro-alcoholic extract of cinnamon (*C.*

*zeylanicum*) consumed with 103 g WWB was also noted to lower glucose response (−14.8%) up to 2 hours in apparently healthy individuals (188). Bernardo *et al.* reported that 100 ml extract of 6 g cinnamon (*C. burmannii*) given with 75 g of bolus dextrose significantly reduced the glucose peak by −30% in healthy individuals (189). Even adding 3 g cinnamon (*C. burmannii*) into 60 g custard tart significantly lowered 2 hour glucose by −7.2% in healthy individuals (190). Further investigation showed that intervention of 5 g cinnamon (*C. cassia*) together with 75 g dextrose challenge in individuals with impaired glucose tolerance significantly lower blood glucose response by −14.8%. Others have shown that giving 5 g of cinnamon (*C. cassia*) with 75 g glucose challenge markedly reduced the glucose peak (10.3%) in obese women. Besides this body of evidence on the beneficial effect of cinnamon in postprandial state, others have found no significant results either in healthy individuals (60, 181) or individuals with impaired glucose tolerance (191). Several longer-term interventions with different types of cinnamon ranging from 2 weeks to 3 months showed that cinnamon remarkably improved glucose homeostasis both in healthy and impaired individuals. The discrepancies on the magnitude of glucose lowering effects might be caused by different factors such as type of meal challenge given, type of cinnamon being used for intervention, metabolic status of participants as well as differences in study design, *i.e.* whether cinnamon is consumed with- or prior- to a meal challenge.

### ***Star anise and ginger***

In contrast to the beneficial effect on glucose reduction exhibited by the hot water extracts used in the turmeric and cinnamon-based beverages, the star anise and ginger-based beverages in **Paper I** did not significantly modulate postprandial glycemia, insulin sensitivity or  $\beta$ -cell function. Although no effect exerted after star anise-based beverage (**Paper I**), this is the first report involving star anise effect and its effect on glucose metabolism in humans. The only existing study with star anise in association with glycemia was found in an animal study showing that ethanolic extract of star anise (200 mg/kg) into streptozocin-induced diabetic rats markedly reduced postprandial blood glucose response compared to diabetic control rats (192).

Ginger was previously reported to have the ability to inhibit glucose diffusion *in vitro* (193). Other *in vitro* studies showed that ginger exhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities (194, 195), as well as possessed inhibitory activity of angiotensin I-converting enzyme which has been linked to hyperglycemia-associated hypertension (196). Opposed to the ginger results shown in **Paper I**, another postprandial study with ginger beverage containing 2.5% ginger concentration marked a −27% reduction in glucose response during 2 hours (187). Others have reported that longer-term ginger interventions varying between 1-3

months with a dose ranging from 1-3 g per day either in obese women (197, 198), T2D (199-206), peritoneal dialysis (207) or non-alcoholic fatty liver disease patients (208) significantly reduced fasting blood glucose levels (198-204, 206, 207) and improved insulin sensitivity (197, 199, 200, 204, 205, 208).

### ***Black pepper***

A large-scale longitudinal epidemiological study in China showed that habitual chili consumption is associated with improved insulin sensitivity measured as reduced HOMA-IR (209). Furthermore, in a randomized clinical study, adding 30 g per day of freshly chopped chilli blend for 4 weeks significantly reduced insulin response when tested in a postprandial setting (210). Yet, less is known about the effect of other pungent spices such as black pepper on glucose metabolism. In **Paper II**, the black pepper-based beverage tended to reduce early insulin level from 0-30 min ( $-18.3\%$ ,  $P=0.07$ ), but no other markers of glycemia were affected compared to the control. Similar to others, by adding 1.5 g of black pepper into mixed meal resulted only a trend in lowering insulin level compared to control (167). One animal study showed that longer term piperine intake significantly reduced blood glucose levels relative to control (211). However, piperine was not able to be identified in the black-pepper based beverage of **Paper II** due to its properties, such as lower water solubility and higher melting point (212).

### ***Mixed spices***

Accumulating human studies reported that combining different polyphenol-rich foods might synergistically result on more beneficial outcomes. For instance, combining black pepper and curcumin significantly increased bioavailability of curcumin compared with curcumin alone in both animal and human studies (213). In **Paper V**, a mixture of different spices consisting of turmeric (3.5 g), cinnamon (2 g), black pepper (3 g), ginger (2 g) and cloves (2 g) were incorporated into a high fat taco meal. The spice-enriched high fat meal (HF-Spices) resulted in the improvement of insulin sensitivity as measured by  $MCR_{est}$  ( $1.3\%$ ,  $P=0.032$ ) and  $ISI_{est}$  ( $1.5\%$ ,  $P=0.029$ ), respectively. Inter-individual variability was noted for treatment  $\times$  BMI interaction showing that normal weight participant had higher  $MCR_{est}$  ( $2.7\%$ ,  $P=0.0082$ ) and  $ISI_{est}$  ( $3\%$ ,  $P=0.0075$ ) after HF-Spices compared to HF alone. In addition, postprandial insulin response was significantly lower ( $-14.1\%$ ,  $P=0.04$ ) after HF-Spices compared to HF. Reported for the first time, multivariate analysis by OPLS-DA for gender separation showed that  $ISI_{est}$  and  $MCR_{est}$  were clustered together and associated with women ( $n=10$ ) while FP-IS and SP-IS were associated with men ( $n=8$ ). Furthermore, BMI status separation showed that glucose  $iAUC_{0-240}$ , FP-IS, SP-IS,  $MCR_{est}$  and  $ISI_{est}$  were associated with normal weight participants. Our results in postprandial glycemia were in concordance with

others (214-216). For instance, Skulas-Ray *et al.* reported that a spices blend added to meal (1200 kcal) significantly reduced postprandial insulin response by -21% (214). Similarly, a postprandial study adding curry to chapatti (-40%,  $P=0.002$ ) or pilau rice (-31%,  $P=0.001$ ), respectively reduced postprandial blood glucose response compared to chapatti or pilau rice alone (215). Likewise, a recent study by Haldar *et al.* showed that a curry meal (~600 kcal) significantly attenuated postprandial glucose (-32%,  $P<0.05$ ) and tended to reduce insulin (-14.5%,  $P=0.089$ ) levels compared to control (216). Conversely, others found no significant effects in postprandial glycemia and insulinemia in healthy men after a curry meal (~400 kcal) intake (217), in T2D individuals after spices mix was added to high fat hamburger meat (250 g ground beef, 10% fat) (218) or in healthy overweight individuals after a spices mix was added into high fat meal (1000 kcal, 45 g fat) which was concomitantly followed by a physiological stress test (219).



*Consumption of individual spices such as turmeric and cinnamon formulated as an extract-based beverage significantly reduced early blood glucose response when given prior to a WWB challenge. Furthermore, a turmeric-based beverage alone was not strong enough to improve postprandial glycemia when consumed prior to MF or HF challenges, but a mixture of spices added into HF significantly lowered postprandial insulin levels and improved insulin sensitivity. An inter-individual effect in estimated insulin sensitivity and  $\beta$ -cell function based on gender and metabolic status (BMI) were also evident. Whether the effects of spices in healthy normal to overweight participants in the present study holds true in other individuals suffering with T2D or others metabolically-impaired conditions warrant further investigation.*

## Part IV. Spices in postprandial lipemia, oxidative stress, blood pressure and metabolic endotoxemia

*Postprandial dyslipidemia is considered as a risk factor for CVD (220, 221) and has been shown to be associated with atherosclerosis (222). Irrespective of the type of fat, consumption of a fatty meal initiates an activated state of cellular adherence and an atherogenic milieu (71). Emerging evidences showed that fatty meal consumption promoted low-grade inflammation which, among other effects, provoked oxidative stress and metabolic endotoxemia (100, 223). Thus, a good management in postprandial lipid handling, oxidative stress, vascular health and metabolic endotoxemia is essential to avoid atherogenic milieu and further prevents CVD progression. To which extent individual spices or mixed spices are able to modulate postprandial lipid handling, oxidative stress and metabolic endotoxemia in a different types of meal challenges, was the question addressed below.*



### Spices in the regulation of postprandial lipemia

In **Paper III**, it is shown that turmeric-based beverage consumption prior to a high fat meal (TUR-HF) significantly reduced postprandial TG level by 12% ( $P=0.003$ ) relative to the control (CON-HF). An increase in HDL-c levels was observed both after TUR-HF (3%,  $P=0.0014$ ) compared to CON-HF; and TUR-MF (1.6%,  $P=0.02$ ) compared to CON-MF. Further *post-hoc* analysis showed a treatment  $\times$  gender interaction ( $P=0.018$ ) and that consumption of TUR-HF significantly lowered TG level ( $-24.2\%$ ,  $P=0.0001$ ) over 4-h postprandial time course in men relative to CON-HF. Furthermore, TUR-HF significantly reduced AIP ( $-63.3\%$ ,  $P=0.0017$ ) compared to CON-HF. Treatment  $\times$  gender interaction was also observed for AIP, showing that men significantly reduced AIP ( $-176\%$ ,  $P<0.0001$ ,  $n=5$ ) after TUR-HF compared to CON-HF. There was a main effect of treatment on MLI ( $P=0.0002$ ) showing that CON-MF had significantly higher MLI (7%,  $P=0.0099$ ) compared to CON-HF, but no differences when turmeric-based beverage was consumed either prior to MF or HF. Only tendencies were observed for the main effect of treatment on Apo-A1 ( $P=0.099$ ) and Apo-B ( $P=0.069$ ) but no significant effect on either total cholesterol ( $P=0.39$ ), LDL-c ( $P=0.27$ ) or LDL/HDL ratio ( $P=0.15$ ). In addition, a treatment  $\times$  gender interaction was observed for both Apo-A1 ( $P=0.0004$ ) and Apo-B ( $P=0.003$ ), respectively. Men in particular, exhibited higher Apo-A1 levels (3%,  $P=0.019$ ) after TUR-MF compared to CON-MF. There is no other study evaluating turmeric or its curcuminoids in postprandial lipemia except one study by Schiborr *et al.* which primarily aimed to investigate the kinetics of different curcuminoid formulations (native powder,

micronized powder and liquid micelles) in human plasma (224). The different design followed in the latter study which makes it difficult to compare their results with the findings in **Paper III**. For instance, Schiborr *et al.* did not include arm of intervention without curcuminoids as a control, and no meal challenge was introduced to perturb the metabolic homeostasis. In addition, markers of lipemia were only measured before the meal (baseline) and after 4 and 12 hours, which makes it difficult to compare with our results presented in **Paper III**.

Despite very limited investigation of turmeric or its curcuminoids in postprandial lipemia (224), a number of longer term studies were performed in healthy or impaired such as obese or T2D individuals. For instance supplementation with either turmeric powder (1.5-2.8 g) or pure curcuminoids (0.02-6 g) varying in dose and durations (7 days to 6 months) significantly improved lipid metabolism in healthy adults (225-227), elderly (228), overweight/obese (174, 229) and T2D (174, 175) individuals. A mechanism behind the improvement of lipemia after turmeric/curcuminoids might be explained by its ability to increase lipoprotein lipase (LPL), which acts as rate-limiting enzyme that hydrolyses circulating TG (230). In line with that, one study reported that curcuma oil significantly increased hepatic expression of LPL and concomitantly improved lipid metabolism in animal study fed with fat-rich diet (231). Although LPL was not measured in **Paper III**, it is tempting to speculate that involvement of LPL might have contributed to the improvement in lipid metabolism such as TG reduction found in **Paper III**. Indeed, a longer term study with 300 mg curcuminoids/day for 3 months in overweight/obese with T2D exhibited higher LPL and significant reductions in TG and NEFA levels (174). Other potential mechanisms might involve the activation/suppression of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), which mediates multiple metabolic pathways such as lipid signalling, inflammation (232), immunity (233), energy metabolism and metabolic diseases (234). Pan *et al.* reported that 8 weeks supplementation with curcumin significantly reduced body weight and fat mass, and improved lipid profile in male obese C57BL/6J mice (235). Further observations by Pan *et al.* showed that curcumin induced lipolysis and improved glycolipid metabolism through upregulating the expression profile of adipose triglyceride lipase and hormone-sensitive lipase, PPAR $\gamma/\alpha$  and CCAAT/enhancer binding protein alpha (C/EBP $\alpha$ ) in mice adipose tissue (235). However, whether curcumin is a true ligand for the PPAR $\gamma$  receptor is still debatable (236, 237).

To study potential synergistic effects, the blend of spices that were previously investigated in **Papers I, II, III and IV** were used and incorporated into a high fat meal (HF) as presented in **Paper VI**. Over the 4 h postprandial course, an increase in HDL-c (1.5%,  $P=0.048$ ) level was observed after HF-Spices compared to HF. No significant main effect of treatment in LDL-c ( $P=0.056$ ), TG ( $P=0.88$ ) Apo-A1

( $P=0.37$ ), Apo-B ( $P=0.97$ ), NEFA ( $P=0.35$ ) and total cholesterol ( $P=0.79$ ) were found. There were treatment  $\times$  BMI interactions found in Apo-B ( $P=0.0012$ ) and LDL-c ( $P=0.047$ ). Normal weight participants had lower levels of Apo-B ( $-2.7\%$ ,  $P=0.03$ ) after HF-Spices compared to HF in the 4 h postprandial course. In contrast, overweight participants experienced higher ( $2.9\%$ ,  $P=0.0038$ ) Apo-B levels after HF-Spices compared to HF. In line with the Apo-B results, overweight participants experienced higher LDL-c levels ( $3.2\%$ ,  $P=0.0002$ ) after HF-Spices compared to HF alone. The reason for this contradicting effect is not known and needs to be further investigated. To compare with other studies with the outcome on postprandial lipemia, there were limited human trials incorporating blend of spices into meals (214, 216, 219). In contrast to the unchanged TG response found in **Paper VI** ( $1.2\%$ ,  $P=0.88$ ) and other studies (217, 218), Skulas-Ray *et al.* (214) and McCrea *et al.* (219) reported a significant reduction in postprandial TG ( $-31\%$  for both studies) after mixed spices enriched high fat meal relative to high fat meal alone. However, a contrasting observation was made by Haldar *et al.* (216) showing that postprandial TG response was significantly  $78.7\%$  higher after higher dose of curry meal compared to control meal. Of note, total calories of the meal challenge used in six different studies was as follow: **Paper VI** (948 kcal; 64.8 g fat), Nakayama *et al.* (481 kcal; 10 g fat), Li *et al.* (236.3 ground beef patty, 23.6 g fat), Skulas-Ray *et al.* (1200 kcal; 49 g fat), McCrea *et al.* (1000 kcal; 45 g fat) and Haldar *et al.* (600 kcal; 19 g fat). It could be argued that the discrepancy in TG response between the four different studies might be caused by different factors, such as the differences in the composition of spices mixture and others might relate with total calories, quality and quantity of the fat contained in the meal challenge. However, to which extent of those individual factors are contributing need to be further investigated.

### **Effect of spices on oxidative stress, plasma antioxidant capacity and blood pressure**

In **Paper III**, TUR-HF significantly reduced TBARS level by  $-9.6\%$  ( $P=0.0045$ ) over the 4 h postprandial time course compared to CON-HF. Only tendency in the main effect was observed in TEAC ( $P=0.077$ ). Although no other postprandial study after turmeric or curcuminoids intake to compare with, our result in MDA reduction was concordance with longer term curcumin intervention showing a reduction in MDA levels in healthy individuals (225, 238) or with T2D (239). Following HF-Spices or HF, there were treatment  $\times$  gender ( $P=0.0021$ ) and treatment  $\times$  BMI ( $P=0.019$ ) interactions for SBP, while only treatment  $\times$  gender ( $P=0.036$ ) interaction was observed for DBP. Relative to HF (**Paper V**), consumption of HF-Spices has shown to lower SBP ( $-4.5\%$ ,  $P=0.0021$ ) in men and also markedly reduce SBP ( $-11\%$ ,  $P=0.019$ ) in normal weight participants for both



gender. Previously, cinnamon (240, 241) has shown to modulate BP in longer term studies with healthy individuals (241), prediabetic (242), T2D (243-245), MetS (246) and women with rheumatoid arthritis (240). Indeed, cinnamaldehyde as main compounds in cinnamon inhibited L-type  $\text{Ca}^{2+}$  channels, suggesting to contribute the vasorelaxing action (247).

### **Modulation of metabolic endotoxemia by spices**

As presented in **Paper III**, a repeated measures mixed model analysis showed that a turmeric based-beverage preload prior to both MF or HF did not significantly modify postprandial metabolic endotoxemia as shown by LPS ( $P=0.57$ ), LBP ( $P=0.16$ ) and sCD14 ( $P=0.26$ ). However, multivariate analysis by OPLS-DA showed that LPS and LBP were among other markers contributing to the cluster separation for gender. In particular, men were shown to be associated with LPS, while women were associated with LBP. The mixed spices study, **Paper V** highlighted for a treatment  $\times$  time interaction in plasma LBP ( $P=0.025$ ); showing that the HF-Spices significantly lowered LBP levels ( $-11\%$ ,  $P=0.036$ ) at 2 h compared to HF. Neither the main effect of treatment for LPS ( $P=0.72$ ) nor sCD14 ( $P=0.18$ ) were significant. There might be several factors involved in determining postprandial changes in metabolic endotoxemia. There were reports showing that composition of dietary fat might acts as determining factor for differences in postprandial metabolic endotoxemia (92, 93), rather than energy percentage from fat in the meals (93). However, a recent statement concerning the amount of fat might not be relevant for elderly individuals since both quality and quantity are more important factors in determining postprandial metabolic endotoxemia (248). In particular, meals rich in saturated fats can profoundly increase or exacerbate the postprandial metabolic endotoxemia (92, 93) and are further associated with inflammatory responses (92). Whether or not gender plays an important factor in metabolic endotoxemia is not currently known. Yet, multivariate data analysis by OPLS-DA in **Papers III** and **V** showed that LPS was one of the markers responsible for cluster separation and that was associated with men (**Papers III** and **V**) and overall in normal weight participants for both gender (**Paper V**). However, the extent to which this observation is relevant to the health-associated outcomes or pertaining as a certain gender characteristic, needs further investigation. It is well known that aging is a process characterized by alteration of overall metabolic processes. In relation to that, one interesting finding showed that irrespective of age, both young and healthy older adults without apparent metabolic dysfunctions had comparable postprandial endotoxemia and inflammatory responses when challenged with the fatty meal (1080 kcal, 62.2 g fat) (72). Until now, no human studies have addressed the effect of spices on metabolic endotoxemia. Some polyphenol-rich foods namely Haas avocado oil (249), grape (250) and pomegranate

(251) extracts have been investigated to a limited extent. Concerning the study design, only the Haas avocado oil (249) and grape extract (250) used postprandial setting while longer term intervention was investigated in pomegranate extract (251). Interesting findings from *in vitro* studies showed that proanthocyanidins own the ability to bound with LPS (250, 252). However, the mechanism behind the beneficial effect elicited by polyphenols on metabolic endotoxemia is still unclear.



*Turmeric-based beverage was shown to significantly reduce TG, AIP and MDA, while increased HDL-c when consumed prior to a fatty meal challenge. An increased in HDL-c levels was also observed after a high fat meal enriched with mixed spices. Mixed spices incorporated into a high fat meal were shown to modulate postprandial metabolic endotoxemia through significantly lowered LBP levels. Nevertheless, inter-individual variability concerning metabolic status and gender in postprandial lipemia, oxidative stress and metabolic endotoxemia were evident.*

## Part V. Turmeric in postprandial bile acids metabolism

*Emerging evidence suggests that BAs are important actors in the postprandial lipid and glucose metabolism. There is a substantial lack of studies on the role of dietary factors in modulating BA profile with particular interest of bioactives-rich plants incorporated into meals. Owing the beneficial effects of turmeric-based beverage in postprandial glycemia and lipemia, a further insight to which extent turmeric-based beverage is able to modulate BA profile in meals with different carbohydrate-to-fat ratio is addressed below.*



In **Paper IV**, BA profiles covering primary BAs (CA and CDCA), secondary (DCA, LCA, UDCA and HDCA) and their conjugated forms (TCA, GCA, TCDCA, GCDCA, TDCA, GDCA, TLCA, GLCA, TUDCA and GUDCA) were analysed postprandially during 4 hours after either turmeric-based beverage or control followed by MF or HF. Referring to the regulation of BAs synthesis, BA levels are fluctuating in cycles of fasting and refeeding. Except LCA (time effect,  $P=0.22$ ) and HDCA (time effect,  $P=0.85$ ), there were postprandial changes from baselines for all BAs in all of treatments (time effect,  $P<0.05$ ). Main effect of treatments was observed in GCA ( $P=0.0001$ ), TCA ( $P=0.016$ ), GCDCA ( $P=0.026$ ), TCDCA ( $P=0.01$ ), UDCA ( $P=0.022$ ), GUDCA ( $P=0.0038$ ), TUDCA ( $P=0.027$ ), GDCA ( $P<0.0001$ ), TDCA ( $P<0.0001$ ) and TLCA ( $P=0.02$ ), while only tendencies were found in CA ( $P=0.054$ ) and DCA ( $P=0.051$ ). As shown in **Paper IV**, a meal with similar protein content but different in carbohydrate-to-fat ratio, significantly induced higher GCA (49.2%,  $P=0.019$ ), GCDCA (55.2%,  $P=0.0062$ ), TCDCA (24.9%,  $P=0.0099$ ), GUDCA (60.6%,  $P=0.0006$ ), GDCA (114%,  $P<0.0001$ ) and TDCA (82.7%,  $P<0.0001$ ) in CON-HF relative to CON-MF. However, only tendencies were observed in TCA (17.6%,  $P=0.07$ ) and TUDCA (5%,  $P=0.078$ ) after CON-HF compared to CON-MF. Conversely, a reduction after CON-HF relative to CON-MF was only observed in TLCA (-8.1%,  $P=0.0088$ ). In concordance with others, the majority of primary postprandial BA responses are in the glycine-conjugated form (253). BA profile in **Paper IV** also resemble those found in another study with isocaloric OGTT, low fat (LF), medium fat (MF) and high fat (HF) meals showing that overall higher BAs levels depend on the meal fat content (OGTT<LF<MF<HF) (254). In addition, the study by Sonne *et al.* showed that BA responses were higher in T2D relative to healthy individuals (254). They reported that differences are reflected mainly in unconjugated and glycine-conjugated forms of DCA and to a lesser extent in CA and UDCA, but comparable in conjugated and unconjugated CDCAs between T2D and healthy individuals (254). In a similar study but using glucose challenge, also was shown to increase

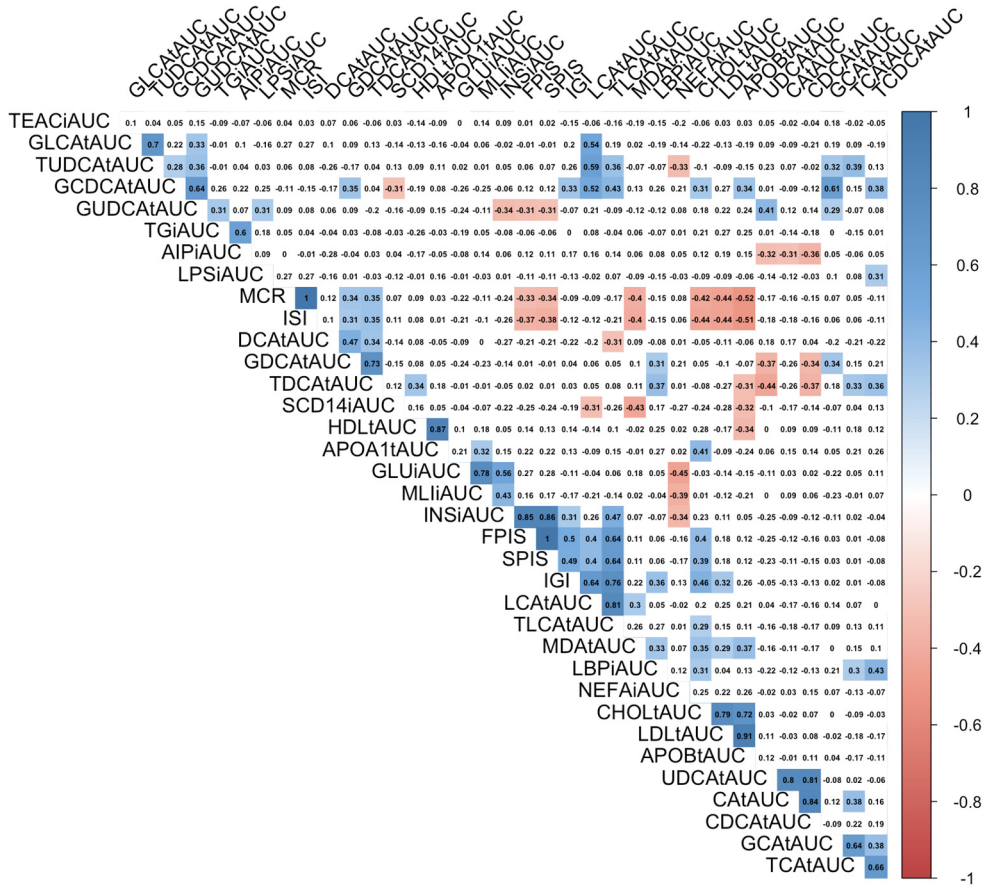
GCA, GCDCA and TCDCA levels compared to water as control during 2 hours (255). Along with conjugated BAs, other metabolites were also measured in the latter study and concluded to be in association with glucose homeostasis/insulin sensitivity (255). In another experiment with mixed meals, there was a marked increase in BA profile of obese compared to non-obese individuals and this response was associated with BAs synthesis marker 12 $\alpha$ -hydroxylated (256). In non-obese but not in obese individuals, BA levels were reduced after insulin infusion, suggesting that obese individuals were suffered in BA fluctuations (256).

Consumption of turmeric based-beverage prior to HF meal significantly increased GCA (46.6%,  $P=0.014$ ) and TLCA (7.54%,  $P=0.017$ ), but reduced GDCA (-29.24%,  $P=0.0003$ ) and TDCA (-38.58%,  $P<0.0001$ ) levels compared to CON-HF over the 4 hours. On the other hand, consumption of turmeric-based beverage prior to MF markedly increased GCA (60.64%,  $P=0.0044$ ), TCA (53.65%,  $P=0.0042$ ), TCDCA (26.42%,  $P=0.014$ ), UDCA (88.64%,  $P=0.0066$ ), GUDCA (25.18%,  $P=0.035$ ), TUDCA (9.57%,  $P=0.0029$ ) and GDCA (44.84%,  $P=0.01$ ). The Pearson's correlation between BAs and other measured biomarkers are presented in **Figure 6**, while the composition of BAs across the time after treatments were presented in **Figure 7**. A positive correlation was observed between TLCA and postprandial insulin ( $r=0.47$ ,  $P=8.55e-04$ ), FP-IS ( $r=0.64$ ,  $P=9.96e-07$ ), SP-IS ( $r=0.64$ ,  $P=1.13e-06$ ) and IGI ( $r=0.76$ ,  $P=3.88e-10$ ). Likewise, LCA was also positively correlated with  $\beta$ -cell function as indicated by FP-IS ( $r=0.4$ ,  $P=0.0044$ ) and SP-IS ( $r=0.4$ ,  $P=0.0046$ ), but negatively correlated with marker of endotoxemia sCD14 ( $r=-0.31$ ,  $P=0.032$ ). An earlier study in human islets reported that LCA was able to induce insulin secretion mediated by TGR5 involving classical Ca<sup>2+</sup>-dependent pathways (257). Indeed, TLCA levels were increased in TUR-HF compared to CON-HF. Similarly, another intervention study with acarbose in T2D patients showed a positive association of TLCA and GLCA with C-peptideAUC, insulinAUC, HOMA- $\beta$  and HOMA-IR (258). In addition, it was found that GDCA and TDCA were positively correlated with insulin sensitivity as indicated in MCR<sub>est</sub> ( $r=0.34$ ,  $P=0.019$ ;  $r=0.35$ ,  $P=0.013$ , respectively) and ISI<sub>est</sub> ( $r=0.31$ ,  $P=0.03$ ;  $r=0.35$ ,  $P=0.015$ , respectively). Furthermore, TDCA was positively correlated with HDL-c ( $r=0.34$ ,  $P=0.019$ ) but negatively correlated with Apo-B ( $r=-0.31$ ,  $P=0.03$ ). Further observation showed that GDCA also positively correlated with LBP ( $r=0.31$ ,  $P=0.032$ ). Indeed, a previous study showed that DCA and TDCA were able to activate glycogen synthase, which might aid the liver in the storage of glucose (259). Consistent with **Paper IV**, Gu *et al.* also showed a positive correlation between GDCA and TDCA with C-peptide, insulinAUC and HOMA-IR after acarbose treatment in T2D patients (258). In contrast, supplementation with DCA exacerbated glucose regulation by disrupting hepatic ER homeostasis (260). Others reported that DCA was positively correlated with the intestinal hyper-permeability and glucose intolerance in mice fed with high fat diet (261) and

confirmed at a dose-dependent manner in an *in vitro* study (262). Interestingly, TDCA was increased in TUR-MF relative to CON-MF, but was markedly reduced in TUR-HF compared to CON-HF (**Paper IV**). The extent of these opposite observation with TDCA is unknown. TCDCA was found to be positively correlated with LBP ( $r=0.43$ ,  $P=0.002$ ) and LPS ( $r=0.31$ ,  $P=0.033$ ). Furthermore, UDCA ( $r=-0.32$ ,  $P=0.027$ ), CA ( $r=-0.31$ ,  $P=0.033$ ) and CDCA ( $r=-0.36$ ,  $P=0.011$ ) were negatively correlated with AIP. Moreover, GCDCA was shown to be negatively correlated with sCD14 ( $r=-0.31$ ,  $P=0.033$ ) but positively correlated with IGI ( $r=0.33$ ,  $P=0.021$ ), total cholesterol ( $r=0.31$ ,  $P=0.031$ ) and Apo-B ( $r=0.34$ ,  $P=0.017$ ) levels. Pearson's correlation showed that GUDCA was positively correlated with TG ( $r=0.31$ ,  $P=0.032$ ) and LPS ( $r=0.31$ ,  $P=0.035$ ) and negatively correlated with insulin ( $r=-0.34$ ,  $P=0.019$ ), FP-IS ( $r=-0.31$ ,  $P=0.03$ ) and SP-IS ( $r=-0.31$ ,  $P=0.033$ ). In line with a marked increase of UDCA after TUR-MF compared to MF, UDCA administration in cystic fibrosis patients markedly enhanced lipid digestion and absorption (263). Furthermore, improvement in markers of lipemia and glycemia were also observed in non-alcoholic steatohepatitis (NASH) patients under UDCA intervention for 6 months (264). Pre-clinical studies have shown that UDCA administration diminished atherosclerotic plaques in ApoE<sup>-/-</sup> (265) and streptozocin-induced diabetic (266) mice, dampened NLRP3 inflammasome activation (265), increased Nrf2 level (266) as well as reduced ER stress (266). Moreover, UDCA also upregulated ATP-binding cassette (ABC) transporters, ABCA1 and ABCG1 which are known to be responsible for cholesterol efflux and reverse cholesterol transport activities (266). Furthermore, in preclinical study with experimental model of Crohn's disease, UDCA counteracted intestinal barrier dysfunction and oxidative stress (267). The conjugated form of UDCA, TUDCA attenuated the progression of high fat-induced non-alcoholic fatty liver disease (NAFLD) in mice by improving intestinal barrier function and modulating intestinal microbiota composition, while intestinal inflammation was inhibited (268). Other reports showed that intestinal permeability was evident after high fat consumption in mice but inversely correlated with the proportion of UDCA (269) and UDCA *per se* promoted colonic epithelial wound healing, while DCA posed the opposite effect (270).

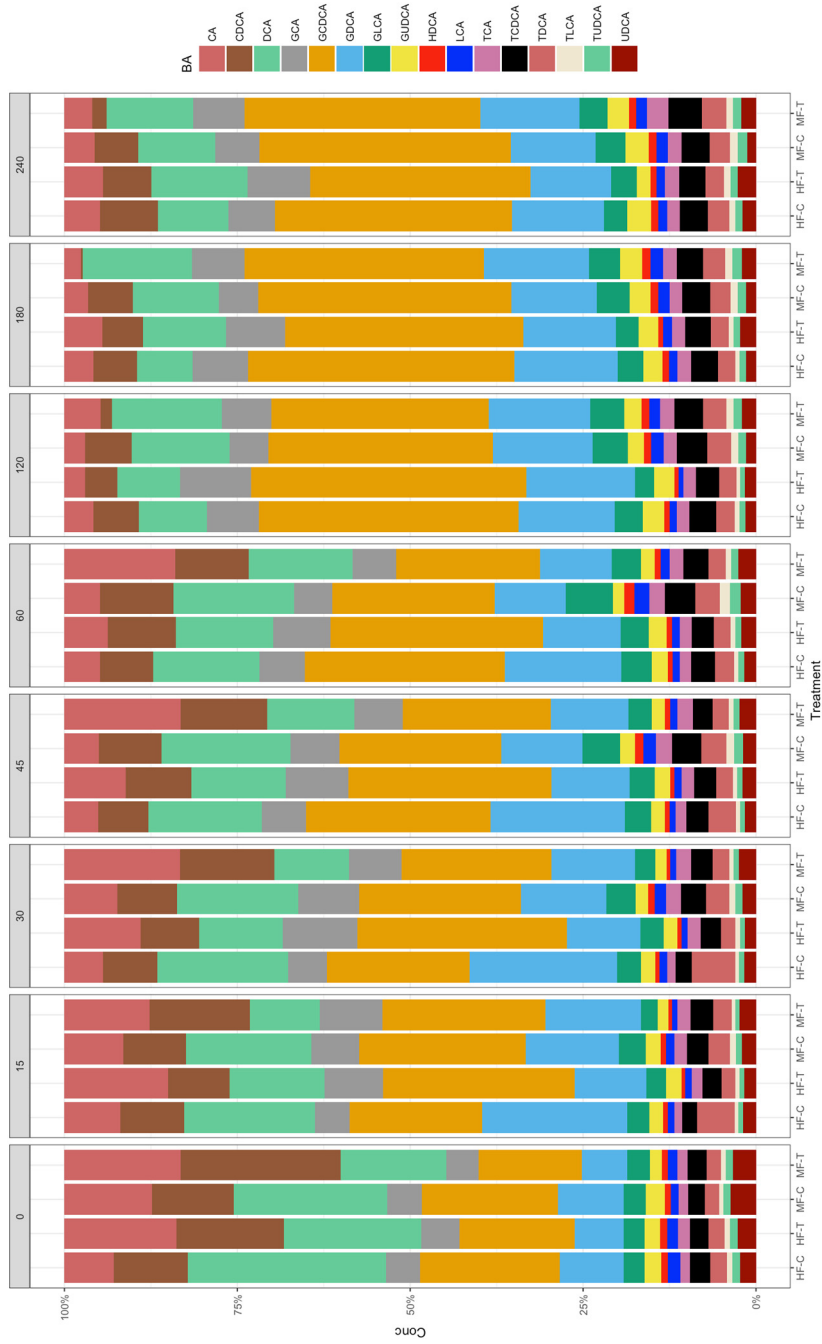


*Consumption of turmeric-based beverage preload prior to a different carbohydrate-to-fat ratio fatty meal has been shown to modulate BA profile in postprandial setting, leading to a possibly healthy one. Additionally, an association was found between BA profile and clinical markers associated with CVD risk factors, glycemia and metabolic endotoxemia. These results pave the way on how bioactive-rich spices can modulate BA profile and help unravel the molecular and physiological mechanisms on how spices or plant-derived bioactive-rich meals interact with BAs.*



**Figure 6**

The Pearson's correlation coefficients between BAs and other biomarkers evaluated is shaded blue (positive) and red (negative) with non-significant correlation are left with blank. Strength of the correlation is indicated by blue or red color saturation. (i; t) AUC, (incremental; total) area under the curve; AIP, atherogenic index of plasma; APO-(A1; B), Apolipoprotein-(A1; B); (G; T)CA, (glyco-; tauro-conjugated) cholic acid; (G; T)CDCA, (glyco-; tauro-conjugated) chenodeoxycholic acid; sCD14, soluble cluster of differentiation 14; CHOL, total cholesterol; (G; T)DCA, (glyco-; tauro-conjugated) deoxycholic acid; FPIS, estimated first phase insulin secretion; GAE, gallic acid equivalent; GLU, glucose; HDCA, hyodeoxycholic acid; HDL, high-density lipoprotein cholesterol; HF, high fat meal challenge; IGI, insulinogenic index; ISI, estimated insulin sensitivity index; LDL, low-density lipoprotein cholesterol; LBP, lipopolysaccharide binding protein; (G; T)LCA, (glyco-; tauro-conjugated) lithocholic acid; LPS, lipopolysaccharide; MCR, estimated metabolic clearance rate of glucose; MDA, malondialdehyde; MF, medium fat meal challenge; MLI, metabolic load index; NEFA, non-esterified fatty acids; SPIS, estimated second phase insulin secretion; TBARS, thiobarbituric acid reactive substances; TEAC, trolox-equivalent antioxidant capacity; TG, triacylglycerol; (G; T)UDCA, (glyco-; tauro-conjugated) ursodeoxycholic acid.



**Figure 7** Composition of BAs across 4 hours treatment (in % of concentration from total of 16 BAs measured). HF-C, control beverage + high fat meal; HF-T, turmeric-based beverage + high fat meal; MF-C, control beverage + medium fat meal; MF-T, turmeric-based beverage + medium fat meal; (G; T)CA, (glyco-; tauro-conjugated) cholic acid; (G; T)CDCA, (glyco-; tauro-conjugated) chenodeoxycholic acid; (G; T)DCA, (glyco-; tauro-conjugated) deoxycholic acid; (G; T)LCA, (glyco-; tauro-conjugated) lithocholic acid; (G; T)UDCA, (glyco-; tauro-conjugated) ursodeoxycholic acid

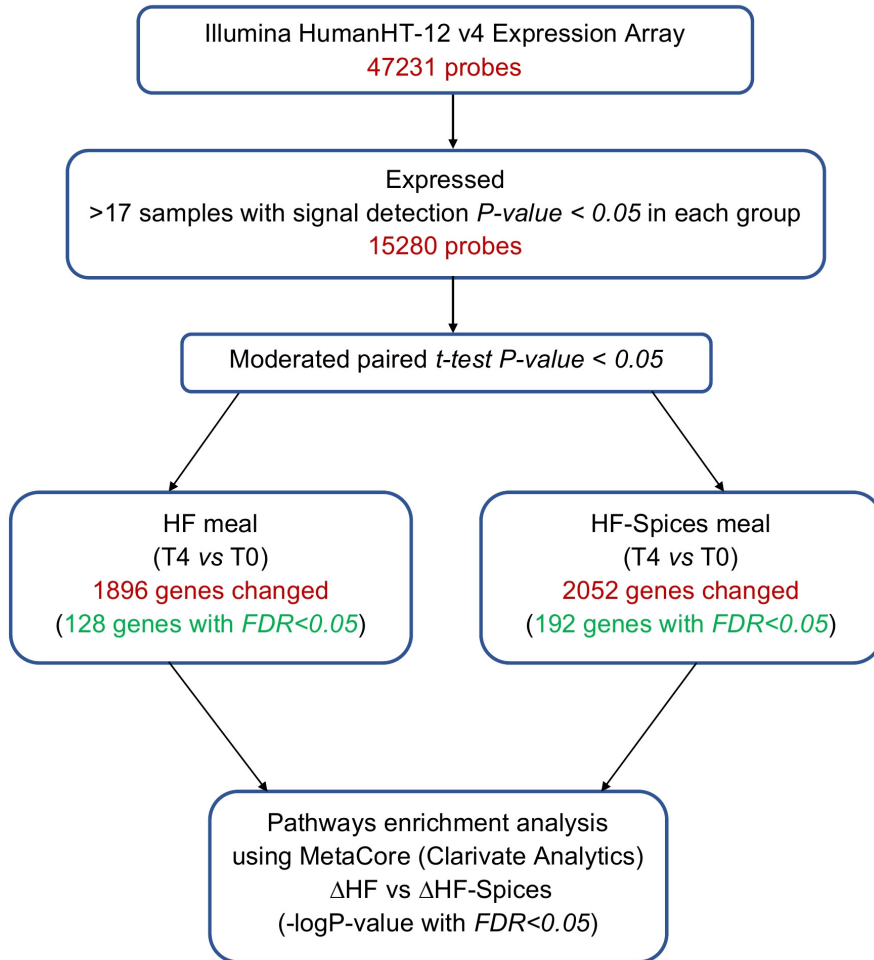
## Part VI. Metabolic pathways and process network associated with mixed spices intake

*In healthy individuals, clinical markers which are associated with inflammation often show rather subtle changes. Furthermore, a combination of a small sample size with high variability in the biomarkers observed after dietary modifications might override the plausible effects of the intervention, leading to a non-significant results. Thus, it is a challenge to measure diet-induced changes in clinical markers which are often smaller than the individual variation within a healthy phenotype or even in individuals at an early stage of metabolic derangement (e.g. overweight). PBMCs are a population of circulating immune cells (i.e., lymphocytes, monocytes and macrophages) that is easily accessible through venepuncture and is non-invasive compared to a white adipose tissue biopsy, particularly when it comes to human studies. PBMCs were previously investigated and shown to reflect the immune component of the white adipose tissue (271) and associated with genes related to metabolism and inflammation (272). Combining pathways derived from PBMCs gene expression analysis and clinical markers will allow to get a broader picture on the possible mechanism(s) involved in the effect of mixed spices intake when combined with a fatty meal challenge. To which extent mixed spices are able to modulate PBMCs gene expression when consumed with fatty meal challenge is the topic addressed below.*



In **Paper VI**, transcriptomics analysis using microarray was performed on PBMC's of 18 participants at baseline and 4 hours after either HF-Spices or HF meals. Prior to downstream analysis, microarray data was quantile normalized with GenomeStudio. The microarray HumanHT-12 v4 presented total 47231 probes, of which 15280 probes were defined as expressed after strict pre-processing selection criteria which considered only probes that had signal intensities with  $P < 0.05$  in all of the group (HF0h, HF4h, HF-Spices0h and HF-Spices4h) of at least 17 out of 18 participants. Following moderated *t*-tests with R/Bioconductor 'limma' package and considering  $P < 0.05$  as significant level, 1896 (of which 128 with  $FDR < 0.05$ ) gene transcripts were differentially expressed in HF4h vs HF0h– and 2052 (of which 192 with  $FDR < 0.05$ ) gene transcripts were differentially expressed in HF-Spices4h vs HF-Spices0h. The outline of above processes is depicted in flow diagram presented in **Figure 8**.





**Figure 8**  
Genes selection procedure in microarray processing and pathway enrichment analysis.

Functional enrichment pathway and process network analysis were performed with MetaCore (Clarivate Analytics, Thompson Reuters) in order to understand the relevancy of differentially expressed genes found after moderated  $t$ -test in physiological processes. A biological pathway is defined as a series of actions among genes or molecules in a cell which act together by chemical reactions, molecule modifications or signalling transduction to carry out such functions (273, 274). In addition, process network based on processes, disease biomarkers and metabolic pathways is giving additional perspective for functional enrichment on

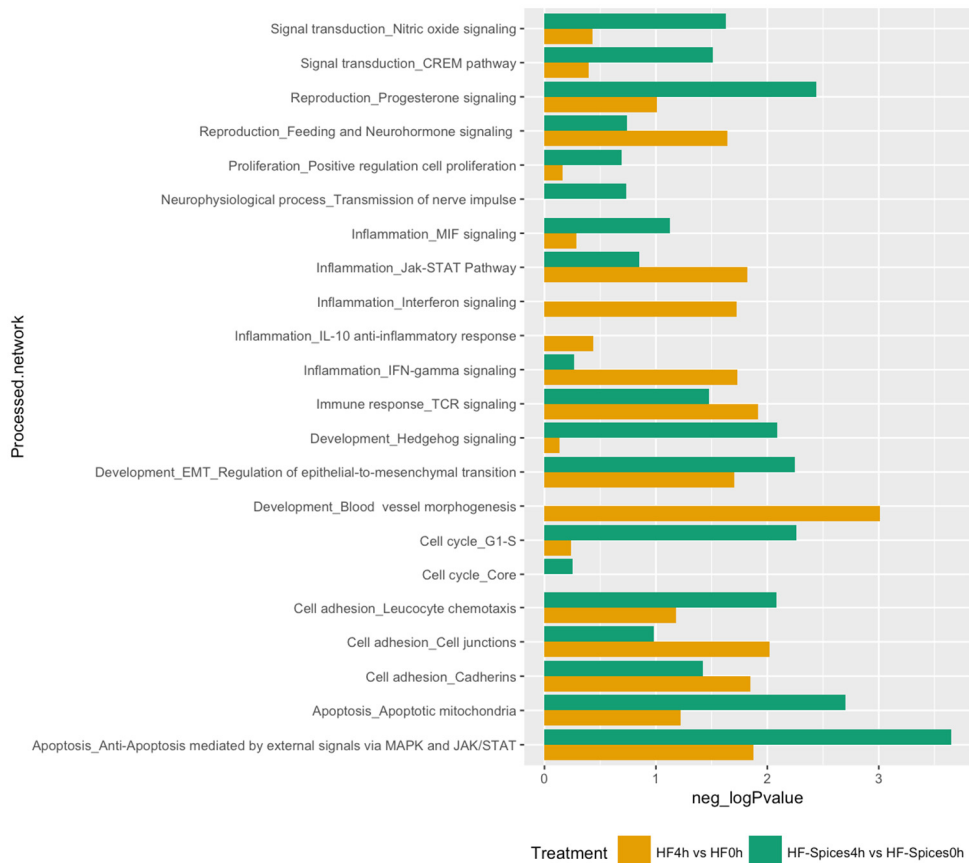
differentially expressed genes. Overrepresented metabolic pathways are depicted in **Figure 9** which covered diverse pathways associated with inflammatory responses, metabolic-associated diseases and lipid metabolism. In addition, a broader context of interaction from differentially expressed genes depicted as process networks presented in **Figure 10** covered inflammation, signal transduction, immune response, development, cell cycle, cell adhesion, apoptosis, neurophysiological and feeding and neurohormone signaling processes. In **Figures 9 and 10**, the  $-\log P$  value score of each specific terms of the ontology corresponds to pathway and process networks are represented with orange (as uniquely enriched in HF4h vs HF0h) and green (as uniquely enriched in HF4h vs HF0h) coloured histogram chart. Further observation showed that HF consumption was linked with the up-regulation of inflammatory-associated pathways, while the intake of HF-Spices reduced the magnitude as represented by common inflammatory-associated genes such as TNF- $\alpha$ , IL-8 and PTGS2 which were chosen for validation with qRT-PCR with GAPDH as a housekeeping gene. As shown in **Figure 11**, the comparison of inflammatory-associated gene expressions from microarray and qRT-PCR were consistent. The upregulated of inflammatory-associated genes in the current study (**Paper VI**) were also in concordance with other investigations evaluating the effect of fat quality on postprandial metabolic regulation (275) or testing different type of metabolic challenge (glucose, fat, mixed meal and water) in healthy individuals (276). Although controversies are existed with regards to the effect of fat quality in PBMC's genes expression (277-279), predominated saturated fatty acid (SFA)-rich meal was evidently shown to induce higher inflammatory-related genes compared to other type of fatty acids (280). Yet, Esser *et al.* reported that irrespective of the fat quality, fatty meal consumption triggers an activated state of cellular adherence and an atherogenic milieu (71). Concordance with Esser *et al.*, several pathways associated with inflammatory state, immune response and cell adhesion shown in **Figure 9** are uniquely enriched particularly after HF0h vs HF4h. Moreover, an earlier study showed that fatty meal consumption might contributed to endothelial dysfunction by recruitment of neutrophils and lymphocytes accompanied by a significant increase of hydroperoxides, IL-8 and impaired flow-mediated vasodilation in postprandial setting (276). Indeed process network built upon differentially expressed genes shown in **Figure 10** exhibited an enriched network associated with cell adhesion, oxidative stress and inflammatory process network.



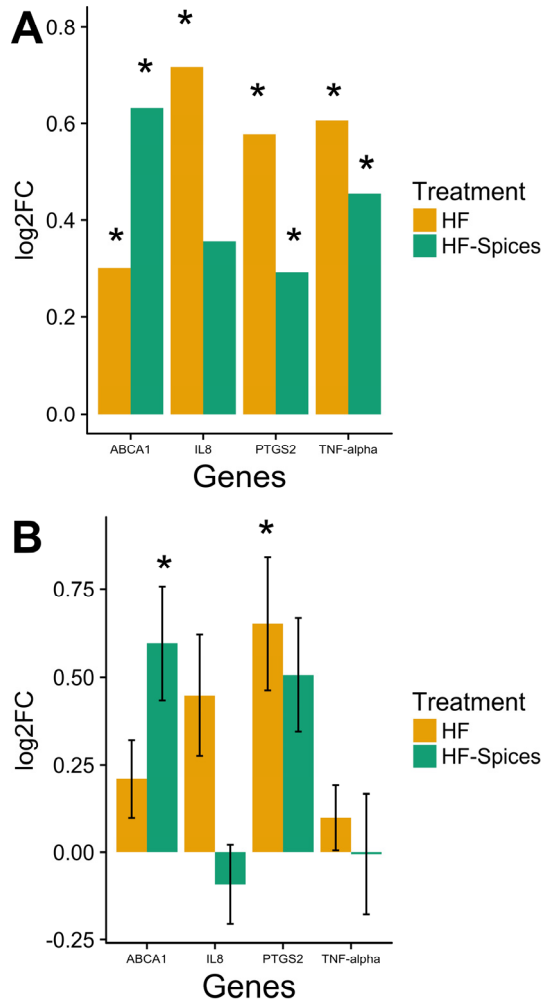
**Figure 9**  
Metabolic pathways associated with HF and HF-Spices. The  $-\log P$ value score of each pathway are represented with orange (as uniquely enriched in HF4h vs HF0h) and green (as unique enriched in HF4h vs HF0h) coloured bar chart.

Further observation revealed that ABCA1, which is known as a mediator of both cellular cholesterol and phospholipid, was significantly up-regulated following HF-Spices compared to a baseline. The relative levels of ABCA1 were also consistent, both after microarray and qRT-PCR, as shown in **Figure 11**. Yet, circulating HDL-c is pivotal in the process of reverse cholesterol transport by promoting the efflux of excess cholesterol from peripheral tissues and return it to the liver for biliary excretion, in which among others mediated by ABCA1 (281). Indeed, HDL-c levels

significantly increased following HF-Spices compared to HF as shown in **Paper V**. Polyphenols-rich coffee has previously shown to modulate HDL-c-mediated macrophage cholesterol efflux by ABCG1 and scavenger receptor class B type 1 (SR-B1). However, cholesterol efflux mediated by HDL-c and ABCA1 was for the first time reported after mixed spices intake in postprandial setting.



**Figure 10** Process network built upon differentially expressed genes both in HF and HF-Spices which represented by  $-\log P$ value in orange (as uniquely enriched in HF4h vs HF0h) and green (as unique enriched in HF4h vs HF0h) coloured bar chart.



**Figure 11**

Relative levels of selected gene expression pattern (shown as log<sub>2</sub>-transformed fold change, 4h vs 0h) after intervention meals (HF or HF-Spices) in microarray (A) and validated with quantitative real-time PCR results (B). The star notation (\*) showed significantly different compared to baseline, *P*-value < 0.05. TNF, tumor necrosis factor; IL8, interleukin 8; ABCA1, ATP-binding cassette subfamily A member 1; PTGS2, prostaglandin-endoperoxide synthase 2.



*The use of pathway and process network analysis combined with clinical biomarkers allowed to delineate the beneficial effect of mixed spices when consumed with a high fat meal; showing promoted cholesterol efflux, reduced inflammatory responses and suppressed pathways associated with inflammation and metabolic diseases. Further insight to understand on how metabolites derived from metabolic processes, clinical markers and genes expression profile are interacting require further investigation.*

# General Discussion

Chronic non-communicable diseases including obesity, T2D, CVD and some types of cancer, are becoming significant causes of morbidity and mortality in the developed and developing world, posing a massive burden on their economies and national health budgets (282). Changes in diet and lifestyle have led to overall energy imbalance becoming commonplace and the emergence of obesity and its comorbidities (283). Thus, consumption of foods or ingredients that have a metabolic advantage by inefficient absorption or metabolism or increased satiety could regulate energy intake and cardiometabolic risk factors. One group of plant derived components that display a metabolic advantage is spices, containing an array of bioactive compounds that could improve diet quality and contribute towards an improved cardiometabolic profile (5). Although there were several studies that epidemiologically or mechanistically examined the association or effect of consumption of spices on diseases outcomes including obesity and appetite regulation, there is limited data in adequately designed postprandial human intervention studies (5). Using a series of studies incorporating spices either as preload (**Papers I, II, III and IV**) or blended with a meal challenge (**Papers V and VI**) were explored.

Introducing preload beverages 15 min prior to a meal challenge as seen in **Papers I, II, III and IV**, aimed to give time for the beverage components to interact with the intestinal environment (140). Consequently, in **Papers I and II** where spice-based beverage was ingested prior to a carbohydrate rich WWB, appetite sensations were seen to be modulated by the turmeric ( $\downarrow$ '*desire to eat*',  $\downarrow$ '*prospective consumption*')-, star anise ( $\downarrow$ '*desire to eat*',  $\downarrow$ '*prospective consumption*')-, cinnamon ( $\downarrow$ '*prospective consumption*')- and black pepper ( $\downarrow$ '*hunger*',  $\downarrow$ '*desire to eat*',  $\downarrow$ '*prospective consumption*',  $\uparrow$ '*satiety*' and  $\uparrow$ '*fullness*')-based beverages. However, only turmeric-based beverage which shown to stimulate PYY release to a greater extent than in the control, postprandially. Neither PYY nor GLP-1 were modulated by star anise- or black pepper-based beverages. Thus, the appetite-associated effects pertained by star anise-, cinnamon- and black pepper-based beverages might be related to other mechanism(s) involving, for instance, TRP channels. However, findings in TRP-associated effects both in animal and *in vitro* studies cannot be easily extrapolated into humans (284, 285). Embarking on a hypothesis in activating intestinal TRPA1 receptor, Keszthelyi *et al.* administered

cinnamaldehyde, one of main compounds in cinnamon as TRPA1 agonist into duodenum over 30 min using nasoduodenal catheter in healthy individuals. In the latter study, neither intestinal permeability nor serotonin release as surrogate markers associated with TRPA1 were affected (284). In contrast to the observed effects of turmeric-based beverage in appetite sensations when consumed prior to WWB (**Paper I**), no effects on appetite sensations were seen when turmeric-based beverage ingested prior to either HF or MF (**Paper III**). Rather, MF which had four times higher carbohydrate content than HF, conferred higher satiety compared to HF which was also in line to other published reports (164). Although curcumin in turmeric was shown to induce gut hormones release shown in **Paper I** and other reported animal (162) and *in vitro* (161) studies, it seems that macronutrient composition might be more important to the appetite sensations than turmeric-based beverage *per se* when the meal challenge is a complex matrix of food that varies in macronutrient composition. In that sense, it is tempting to speculate that gastric emptying might actually override the turmeric effect, and thus consequently elicited the perceived appetite sensations. Similar to what we observed in **Paper III**, by controlling protein at the same amount but modifying carbohydrate-to-fat ratio in similar total calories, Marciani *et al.* observed that gastric emptying rate was slower after a high carbohydrate meal compared to a high-fat meal (286). On the other hand, other factors such as the dose given in the turmeric-based beverage might obviously influence the outcome. Given differences in the study design and type of intervention between **Papers I, II, III** and **IV** relative to **Papers V** and **VI**, the beneficial effect on appetite sensations perceived after spices consumption (HF-Spices relative to HF, as shown in **Paper V**) is regain as shown by increased feeling of '*satiety*' and '*fullness*'. In that sense, it could be assumed that mixed spices *per se* induced the effect on appetite sensations when consumed together with the meal challenge, not as a preload. This implicates that in a such complex matrix as food is, spices should be consumed together with a meal challenge. Inter-individual variability of the physiological response between men and women exist, however to what extent it pertained to is arguable. In **Paper V**, irrespective of treatment, women had lower feeling of '*hunger*', '*desire to eat*' and '*prospective consumption*' compared to men. In this context, it could be caused by the fact that women had longer small intestinal transit time relative to men (287, 288). Since prolonged small intestinal transit is correlated with higher satiety and increased anorexigenic gut hormones (289), this might be associated to the pertained effects in women participants shown in **Paper V**. Gender differences were also clearly observed in another study showing that women had lower '*desire to eat*', '*hunger*' and '*appetite*' sensations compared to men (290), but so far only few studies putting an effort when reporting the appetite-related results (290-292).

Although the studies conducted in this dissertation follow the well-established and validated preload paradigm, appetite assessment was a secondary outcome and more

adequately designed, controlled and powered studies are needed. Some of the considerations that should be taken into account in future studies include how the food should be consumed (as a preload- or together with a meal challenge), considering different type of macronutrient composition, the total calorie content, and also testing in both gender and considering gender in the model analysis.

The beneficial effects of plant bioactives in glucose regulation are, among others mediated by reduction and inhibition of  $\alpha$ -glucosidase and pancreatic  $\alpha$ -amylase as well as glucose transporters such as Na<sup>+</sup>/glucose cotransporter (SGLT1) and glucose transporter type 2 (GLUT2) found in the small intestinal epithelium (293). Turmeric- and cinnamon-based beverages in **Paper I** showed a significant reduction in early blood glucose response when given prior to WWB, but turmeric-based beverage did not show any effect on glycemia when given prior to either isocaloric MF or HF (**Paper III**). Rather, MF induced higher glucose and insulin levels compared to HF. In **Paper I**, it could be argued that bioactive compounds contained in both beverages had interacted with glucose transporter in the intestine, which leads to a reduced glucose absorption. But this argument might not be valid when the meal challenge is in a form of a complex matrix food such as in **Paper III**. Consequently, when a preload is used as the type of intervention, it is most likely that meal challenge with a complex matrix food is the determinant factor for the putative effect on postprandial glycemia. Previously, an *in vitro* study showed that blending spices resulted in a higher total polyphenol content compared to individual spice (294). Hence, it could be hypothesized that blending of spices might elicit a synergistic effect in the postprandial glycemia. Indeed, reduced insulin secretion and increased insulin sensitivity were observed when mixed spices consumed together with HF meal compared to HF meal alone as presented in **Paper V**. It is most likely that bioactives in the mixed spices had interacted with a cellular mechanism(s) associated with glucose regulation, posing an insulin mimetic effects. As a consequence, it could be argued that mixed spices did not burden the pancreatic  $\beta$ -cell to produce more insulin in compensating the circulating glucose. Instead, mixed spices help to accelerate the glucose uptake as shown by increased estimated metabolic clearance rate of glucose and insulin sensitivity index as shown in **Paper V**.

A balance state in chylomicron clearance is essential for lipid metabolism. The intake of turmeric-based beverage in **Paper III** clearly showed a reduction on TG levels. One may speculate that turmeric or its curcuminoids might have interacted with LPL as a TG-rate limiting enzyme, leading to a reduced TG levels. Indeed LPL levels were elevated after a longer-term study with curcumin (174). In connection to that, preclinical studies have shown that curcumin also contributed to a cholesterol efflux mechanism mediated by ABCA1 (295-300) and SR-B1 (295, 299) on macrophages (295, 296, 299, 300) and adipocytes (297). It has been known the



ATP-binding cassette (ABC) transporters, such as ABCA1 and ABCG1, play crucial roles in the efflux of cellular cholesterol to HDL and its apolipoproteins (301). Indeed, in **Paper III**, HDL-c and Apo-A1 (particularly in men) levels were elevated postprandially after consuming turmeric-based beverage. Hence, turmeric-based beverage consumption, as it shown in **Paper III**, could be beneficially improved lipid metabolism in postprandial setting.

Thyroid hormones regulate energy expenditure through both central and peripheral pathways (302). Given the fact that hypothyroidism is marked by a reduced chylomicron remnants clearance (303) and elevated TG levels (304), it is tempting to speculate that TG-lowering effect induced by turmeric-based beverage in **Paper III** might be associated with the enhanced chylomicron remnants clearance, and that might be linked to the thyroid hormones and energy expenditure. Indeed, a replacement therapy with T4 in a normolipidemic patients with hypothyroidism markedly showed an increase in a chylomicron remnants clearance after acute fat loading test (305). Although T4 was only measured in **Paper V** but not in **Paper III**, it is tempting to speculate that T4 might have contributed to the lipid-associated effects in **Paper III**.

Earlier study revealed that BAs administration in mice significantly induced energy expenditure by promoting intracellular thyroid hormone activation (119). The suggested underlining mechanisms are mediated by the increased cAMP production that stems from the binding of BAs with the G-protein-coupled receptor TGR5 (119). Certain BA species such as TCA and TCDCA which were found to be a potent activator of TGR5 shown in previous studies (119, 306), were also elevated after turmeric-based beverage in **Paper III**. In **Paper V**, T4 levels were significantly increased after HF-Spices relative to HF. Studies have shown that T4 administration could increase BMR and/or daily energy expenditure (307, 308). It could be assumed that elevated level of T4 after HF-Spices might have contributed to the energy expenditure. Further studies with doubly labelled water assessment are needed to confirm its effect on energy expenditure. If so, mixed spices could be used as a potential way of natural approach to increase energy expenditure. Interestingly, previous study showed that kaempferol was able to induce cellular energy expenditure and thyroid hormone activation (309). Kaempferol and its derivatives were also identified in the cinnamon (**Paper I**), star anise (**Paper I**) and black pepper (**Paper II**), and those spices were also included in the mixed spices used in **Paper V**.

Traditional biomarkers associated with inflammation which linked to a certain disease are commonly used to measure the effects of dietary interventions. However, when using dietary intervention with a preventive approach, it is warranted to be able to quantify phenotypic changes which are very close to or even

within the range of healthy state on healthy subjects (3). In fact, healthy subjects have the ability to rapidly re-establish normal homeostasis. Thus, it is a challenge to measure diet-induced changes in the phenotype which is smaller than individual variation within a healthy phenotype. Using only the “traditional biomarker” approach will not provide a whole mechanistic picture of diet-induced phenotypic changes as faced in several human studies (310). In this context, PBMCs serve as a great potential as a non-invasive and technically feasible approach that enable us to evaluate gene expression which might reflect metabolic and immune responses. By combining microarray transcriptomics and clinical markers approach in **Paper VI**, mixed spices consumption showed to improve maker of lipemia such as increased HDL-c and reduced LDL-c levels which might be mediated by cholesterol efflux, reduced inflammatory responses and suppressed pathways associated with inflammation and metabolic diseases.

In conclusion, this dissertation brings novel findings and sheds light on the beneficial effect of spices on human metabolism. This offer new evidence for advocating the inclusion of bioactive-rich spices in meals in order to mitigate the alteration cardiometabolic risk-associated markers induced by the diet.



# Conclusions and Future Perspectives

In this dissertation, different spices were studied either individually or as a mixture, and their effects were evaluated in a postprandial setting in healthy humans within the context of MetS prevention. Markers associated with MetS such as glycemia and lipemia were evaluated. In addition, appetite regulation and thyroid hormones which also closely linked to energy balance were also explored. In addition, analysis on the bile acids and transcriptomics were performed to give an expanded insight into the role of spices in the context of MetS. The main findings from this dissertation, including future perspectives are summarized as follow:

- Acute consumption of formulated extract-based beverages from individual spices such as turmeric, cinnamon, star anise and black pepper, when given prior to WWB meal challenge, significantly modulated appetite sensations. The appetite-related hormone PYY increased after turmeric-based beverage. Adding spice blend into high fat meal also shown to modulate appetite sensations (increased '*satiety*' and '*fullness*'), effects that varied depending on gender or metabolic status. A further step to a holistic picture of the influence of spices on appetite regulation would be the inclusion of measures of *ad-libitum* food intake on subsequent meals. Since overall appetite sensations elicited by black pepper-based beverage were evident and do not seem to involve changes in gut hormones (PYY and GLP-1) release, it would be interesting to investigate other potential mechanisms, such as those involving TRP receptors. It is also important to further investigate on the type of presentation of the spices showing more powerful effects that may be more effective, either as an extract or as isolated specific bioactive compounds.
- Consumption of turmeric- and cinnamon-based beverages prior to WWB challenge significantly reduced the early postprandial blood glucose response. The intake of mixed spices incorporated into high fat meal significantly lowered postprandial insulin and improved insulin sensitivity. Inter-individual variation in the effects on estimated insulin sensitivity and  $\beta$ -cell function based on gender and metabolic status (BMI) were also evident. Whether the effect of spices in healthy normal to overweight participants hold true in other metabolically-impaired individuals warrant

further investigation. Since bioactives are metabolized and transformed into either glucuronated, sulphated and methylated metabolites, displaying either higher or lower bioactivities; thus, it is an urge to investigate whether the putative effects elicited are caused by parent compounds of the bioactives or by metabolically/microbiota-derived compounds.

- Turmeric-based beverage was shown to reduce TG, AIP and MDA, while promoting increased HDL-c levels when consumed prior to a fatty meal challenge. An increase in HDL-c levels was also observed following a high fat meal enriched with mixed spices. Mixed spices incorporated into high fat meal was also shown to modulate postprandial metabolic endotoxemia by lowering LBP levels. Inclusion of mixed spices in a high fat meal was also shown to reduce postprandial SBP. Nevertheless, metabolic status- (BMI) and gender-dependent inter-individual variability in the effect of spices on postprandial lipemia, oxidative stress and metabolic endotoxemia were evident.
- Consumption of a turmeric-based beverage prior to a different carbohydrate-to-fat ratio in a fatty meal was shown to modulate BA profile in postprandial setting, leading to a possibly healthy one. Additionally, an association was found between BA profile and clinical markers related to cardiometabolic risk factors. These results pave the way on how bioactive-rich spices can modulate BA profile and help unravel the molecular and physiological mechanisms on how spices or plant-derived bioactive-rich interact with BAs.
- Pathway and process network analyses, combined with clinical biomarkers, were able to delineate the beneficial effect of mixed spices when consumed with a high fat meal, by promoting cholesterol efflux, reduced inflammatory responses and suppressed pathways associated with inflammation and metabolic diseases. Further insight to understand on how metabolites derived from metabolic processes, clinical markers and gene expression profiles are interacting deserves future investigation.

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*-Ali Ibn Abu Talib-*

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This dissertation explores the effects of spices (turmeric, cinnamon, black pepper, star anise, ginger and cloves) on the metabolic regulation of healthy humans. Different cardiometabolic risk factors such as glycemia and lipemia, as well as metabolic endotoxemia and appetite regulation were explored in the postprandial setting. In addition, profiling of postprandial bile acids response provided additional insight on the impact of spices intake on glucose and lipid metabolism. The mechanism behind such effects, including modulation of pathways and network processes associated with inflammatory responses, lipid metabolism and cholesterol efflux, were explored by combining high-throughput gene expression array in peripheral blood mononuclear cells (PBMCs) with clinical markers outcomes. In conclusion, this dissertation brings novel findings and sheds light on the beneficial effect of spices on human metabolism. This offer new evidence for advocating the inclusion of bioactive-rich spices in meals in order to mitigate the alteration cardiometabolic risk-associated markers induced by the diet.

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